



**ABSTRACT BOOK / LIBRO DE RESÚMENES**

**XV**

**INTERNATIONAL LUPIN  
CONFERENCE 2019**

*DEVELOPING LUPIN*

*CROP INTO A MODERN AND SUSTAINABLE FOOD AND FEED SOURCE*

**XV**

**CONFERENCIA INTERNACIONAL  
DE LUPINO 2019**

*DESARROLLO DEL LUPINO,*

*UNA FUENTE MODERNA Y SOSTENIBLE PARA LA ALIMENTACIÓN*

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## **XV INTERNATIONAL LUPIN CONFERENCE 2019**

Developing lupin crop into a modern and sustainable food and feed source

## **XV CONFERENCIA INTERNACIONAL DE LUPINO 2019**

Desarrollo del Lupino, una fuente moderna y sostenible para la alimentación

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## PREFACE

The International Lupine Conference (ILC) has been carried out predominantly in Europe, where most of the researchers of lupin are found. In this XV ILC version, the International Lupine Association has decided to move the Conference to the other hemisphere, to the Andes of South America, the origin center of many species, where *Lupinus mutabilis*, known as tarwi or chocho, has originated. In Europe, it predominates *L. albus* and in Australia *L. angustifolius*. *Lupinus mutabilis* is grown mainly in Ecuador, Peru and Bolivia. It is an ancestral product, consumed by the original cultures of the Andes. However, due to different factors, it is still a marginal crop, except in Ecuador, where it has gained importance in the urban diet.

The XV ILC is the right moment to have the state-of-art of tarwi. It is one of the first opportunities, where people from South America, dedicated to the production, research, processing, nutrition, health and trade of tarwi, will meet. Also, they will be reinforced by the participation of European professionals, who are bringing their experiences in other lupin species, progresses in nutritional studies, non-food uses, etc. The results and advances in the research and promotion of lupins have been organized in seven technical sessions: 1) Food, feed and non-food uses, 2) Benefits to health, 3) Biochemistry & Biotechnology, 4) Physiology and Protection, 5) Taxonomy, Biodiversity and Agroecology, and 7) Agronomy, Farming. This book presents the summaries organized by session of the contributions of the participants to the XV International Lupin Conference.

The XV ILC also brings us four lectio magistralis that expose the potential and challenges in research and use of lupins from four different angles: 1) Lupin's situation and Potential, a critical look from the early years of the ILC to the present, 2) Barriers to the commercialization of lupin in Europe, 3) Challenges for the use of lupin in Bolivia from the perspective of PROINPA, and 4) Medical advances in the consumption of tarwi in Ecuador. This document also presents the summaries of these presentations.

At the Conference, three symposiums have been proposed: 1) Food, feed and non-food uses; 2) Agronomy and 3) Emerging markets. The first one brings us to discussing on the uses of lupin, within the context of the new world trends: non-communicable diseases, malnutrition, obesity, diabetes, etc. The second one addresses an issue not yet solved, particularly with *L. mutabilis*, the agronomic factors that restrict its productivity and competitiveness. And, the third symposium is referred to on how to address an international market for lupins, where the demand for vegetable protein is growing, its opportunities and its limitations. Each symposium is powered by a pair of key notes in order to stimulate reflection and debate of participants. The book presents their summaries.

A field day is planned, consisting of a trip from the valleys to the highlands of Cochabamba, arriving at the town of Colomi, an important center of the Bolivian agro-biodiversity, where tarwi is opening space, little by little. We will visit plots cultivated with Lupines and most importantly, we will have interaction with farmers in the area and with ladies who will offer us a banquet with local dishes based on tarwi.

We hope that the XV ILC marks a turning point in the promotion of this neglected crop and that many collaborative networks, common agendas and commercial relationships are generated.

Finally, the XV ILC is hosted in the valley of Cochabamba, the Bolivian capital of the gastronomy, slow food. We hope that this Conference can be the moment in which tarwi becomes one more ingredient of Cochabamba and Bolivian diet, enriching it not only in flavor but in nutritional value.

## PREFACIO

La Conferencia Internacional del Lupino (ILC por su sigla en inglés), se ha realizado predominante en Europa, donde se encuentran la mayoría de los investigadores de los lupinos. En esta XV versión, la Asociación Internacional de Lupinos ha decidido mover la Conferencia al otro hemisferio, al centro de origen de muchas especies, a los Andes de Sudamérica, donde se ha originado el *Lupinus mutabilis*, conocido como tarwi o chocho. Mientras en Europa predomina *L. albus* y en Australia *L. angustifolius*, el *L. mutabilis* se cultiva principalmente en Ecuador, Perú y Bolivia, es un producto ancestral de las culturas originarias de los Andes. Sin embargo, por diferentes factores, sigue siendo un cultivo marginal, excepto en Ecuador, donde ha tomado importancia en la dieta urbana.

La XV Conferencia es el momento propicio para conocer el estado de arte del tarwi, es una de las primeras oportunidades donde se reúnen personas de Sudamérica dedicadas a la producción, investigación, procesamiento, nutrición, salud y comercio del tarwi; que además se contará con la participación de profesionales de Europa, que traen experiencia en otras especies de lupinos, los avances en estudios nutricionales, médicos, usos no alimentarios, etc. Los resultados y avances en la investigación y promoción de los lupinos se han organizado en siete sesiones técnicas: 1) Alimento, forraje y otros usos, 2) Beneficios a la salud, 3) Bioquímica & Biotecnología, 4) Fisiología y Protección, 5) Taxonomía, Biodiversidad y Agroecología, y 7) Agronomía. En este libro se presentan los resúmenes de las contribuciones de los participantes al XV ILC organizados por sesión.

La XV Conferencia nos trae además cuatro charlas magistrales que exponen las potencialidades y desafíos en la investigación y aprovechamiento de los lupinos desde cuatro ángulos diferentes: 1) Situación y Potencial del Lupino, una mirada crítica desde los primeros años del ILC a la actualidad, 2) Las barreras para la comercialización del lupino en Europa, 3) Desafíos para el aprovechamiento del lupino en Bolivia desde la perspectiva de PROINPA, y 4) Avances médicos en el consumo del tarwi en el Ecuador. En este documento se presentan también los resúmenes de estas exposiciones.

En la Conferencia se han planteado además el desarrollo de tres simposios: 1) Alimento, forraje y otros usos, 2) Agronomía y 3) Mercados emergentes. El primero, nos lleva a la discusión sobre los usos del tarwi, en el contexto de las nuevas tendencias mundiales: enfermedades no transmisibles, desnutrición, obesidad, diabetes, etc. El segundo, aborda un tema aún no resuelto, particularmente con el *L. mutabilis*, los factores agronómicos que restringen su productividad y competitividad. El tercer simposio, referido a cómo abordar un mercado internacional para los lupinos, la demanda por proteína vegetal creciente, sus oportunidades y sus limitantes. Cada simposio es alimentado por un par de charlas claves con el fin de estimular la reflexión y debate de los participantes. El libro expone los resúmenes de estas charlas.

Se tiene previsto un día de campo, un viaje de los valles a las alturas de Cochabamba, a la localidad de Colomi, un importante centro de agrobiodiversidad boliviana, donde el tarwi se abre espacio poco a poco. Se visitarán parcelas cultivadas con lupinos y lo más importante, se tendrá la interacción con agricultores de la zona y con señoras que nos ofrecerán un banquete con platos locales basados en tarwi.

Esperamos que la XV Conferencia, se constituya en el punto de inflexión en la promoción de este cultivo postergado, que se generen muchas redes de colaboración, agendas comunes y relaciones comerciales. Finalmente, la XV Conferencia, es acogida en el valle de Cochabamba, la capital boliviana de la gastronomía, del *slow food*. Aspiramos que el XV ILC, sea el momento para el tarwi y que se constituya en un ingrediente más de la dieta cochabambina y boliviana, enriqueciéndola no solo en sabor sino en valor nutritivo

## NOTE OF HOMAGE TO GEORGE HILL

Presented by Ana María Planchuelo with contributions from Roberta Hill

Professor George Hill was a young Englishman and a university student in Perth, Western Australia, when in 1960 he met Roberta and later married her. They had two children, Alexandra and Andrew and, in 1972, the family established their residence in Christchurch, New Zealand, where he served for 40 years as Associate Professor at the Plant Science Department of Lincoln University.

During his academic career, Professor Hill supervised numerous students at undergraduate and graduate levels and was an advisor professor for a number of PhD theses in different areas of agriculture and livestock sciences.

In 1984, George Hill presented for the first time his research work at the III International Lupin Conference in La Rochelle, France. Since then he never stopped participating in all the following Lupin Conferences, presenting the results of his research as a main speaker and in posters, in areas of human and animal nutrition, all in relation to *Lupinus angustifolius* (narrow leaf lupin), *Lupinus albus* (white lupin) and the hybrids Russell lupins in New Zealand. Also, he was one of the editors of the conference proceedings of the XI Lupin International Conference which took place in Guadalajara, Mexico in May 2005.

As a member of the International Lupin Association (ILA), George Hill became part of the Scientific Committee and then he had the responsibility of being the General Secretary of ILA, an honorary position that he held until the last moment of his life in July 2017.

Those who had the opportunity to know George have very pleasant memories, not only for his contribution to the knowledge of lupins, beyond his academic and scientific activities, but most of all for his always present affability and cordiality.



## NOTA DE HOMENAJE A GEORGE HILL

**Presentado por Ana María Planchuelo con aportes de Roberta Hill**

El Profesor George Hill era un joven inglés, que durante su período de estudiante universitario en Perth, Australia Occidental, conoció en 1960, a Roberta con quién luego contrajo matrimonio. Ellos tuvieron dos hijos, Alexandra y Andrew, y en 1972 la familia estableció su residencia en Christchurch, Nueva Zelanda, en donde él se desempeñó como Profesor Asociado en el Departamento de Ciencias Vegetales de la Universidad de Lincoln, por más de 40 años.

Durante su carrera académica de Profesor supervisó a numerosos estudiantes de pregrado y postgrado y fue director de varias tesis doctorales en diversas áreas de las ciencias agrícolas y ganaderas.

En 1984 George Hill presenta por primera vez sus trabajos científicos en la III Conferencia Internacional del Lupino en La Rochelle, Francia. Desde entonces, nunca dejó de participar activamente en todas las siguientes conferencias de lupino, presentando los resultados de sus investigaciones en conferencias y paneles en las áreas de la nutrición humana y animal, todas relacionadas con *Lupinus angustifolius* (lupinos de hojas angostas), *Lupinus albus* (lupino blanco) y los lupinos híbridos Russell en Nueva Zelanda. Además, fue uno de los editores de las actas de las XI Jornadas de Internacionales de Lupino, que tuvo lugar en Guadalajara, México en mayo de 2005.

Como miembro de la Asociación Internacional de Lupino (ILA), George Hill fue desde un comienzo miembro del Comité Científico y luego tomó la responsabilidad de ser el Secretario General de ILA, un cargo honorario que desempeño hasta el último momento de su vida, en julio de 2017.

Quiénes tuvimos la oportunidad de conocer a George tenemos los más gratos recuerdos, no sólo por su contribución al conocimiento de los lupinos sino también, más allá de la parte académica y científica, por la afabilidad y cordialidad que siempre lo caracterizó.

## GENERAL PROGRAM

March 18 (Monday)		March 19 (Tuesday)		March 20 (Wednesday)	March 21 (Thursday)	
08:00 –	Participants	08:00 –	Registration		08:00 –	Registration
09:00	registration and posters location	08:30			08:30	
09:00 –	LLALLAGUANI ROOM	08:30 –	LLALLAGUANI ROOM		08:30 –	LLALLAGUANI ROOM
10:00	Opening ceremony and recognition to Prof. George D Hill	09:15	Lectio magistralis: Challenges for the use of lupin in Bolivia from the perspective of PROINPA (A. Gandarillas)		10:00	Session 5: Health benefits (5 presentations)
10:00 –	Coffee break	09:15 –	LLALLAGUANI ROOM		10:00-	Coffee break
10:15		10:15	Session 1. Food, feed and non-food uses (3 presentations)	07:30 –	10:15	
				17:00		
10:15 -	LLALLAGUANI ROOM	10:15-	Coffee break		10:15 –	LLALLAGUANI ROOM
11:00	Lectio magistralis: Lupin's situation and potential (E. von Baer)	10:30		Field day & Andean gastronomy	12:00	ANGOSTURA ROOM
						Symposium 1. Food, feed and non-food uses
11:00 –	LLALLAGUANI ROOM	10:30 –	LLALLAGUANI ROOM			Facilitator: A. Planchuelo
12:30	Session 1. Food, feed and non-food uses (5 presentations)	11:15	Lectio magistralis: Medical advances in the consumption of <i>Lupinus mutabilis</i> Sweet, chocho / tarwi, in Ecuador (Dr. Baldeón)			Key Note 1: E. Villacrés
						Key Note 2: P. Eisner
		11:15 –	SALA ANGOSTURA			
		12:30	Presentation of posters			
	Lunch		Lunch			Lunch
14:00 –	LLALLAGUANI ROOM	14:00 –	LLALLAGUANI ROOM	ANGOSTURA ROOM	14:00 –	LLALLAGUANI ROOM
14:45	Lectio magistralis: The barriers to the commercialisation of lupins in Europe (D.McNaughton)	15:15	Session 2. Taxonomy, Biodiversity & Ecology (5 presentations)	Session 4: Agronomy, Farming (5 presentations)	15:45	Symposium 3. Emerging markets
						Facilitator: D. McNaughton
						Key Note 1: U. Prins
						Key Note 2: E. von Baer

<b>March 18 (Monday)</b>		<b>March 19 (Tuesday)</b>		<b>March 20 (Wednesday)</b>	<b>March 21 (Thursday)</b>	
14:45 –	LLALLAGUANI ROOM	15:15 –	LLALLAGUANI ROOM	ANGOSTURA ROOM	15:45 –	Coffee break
15:45	Session 1. Food, feed and non-food uses (3 presentations)	16:00	Session 3. Genetics, Genomics, Molecular Breeding & Biochemistry (3 presentations)	Session 4: Agronomy, Farming (3 presentations)	16:00	
15:45 –	Coffee break	16:00-	Coffee break		16:00 –	LLALLAGUANI ROOM
16:00		16:15			17:00	ILA Meeting
						ANGOSTURA ROOM
						Andean network Chocho/Tarwi Meeting
16:00 –	LLALLAGUANI ROOM	16:15 –	LLALLAGUANI ROOM		17:00 -	LLALLAGUANI ROOM
17:00	Session 1. Food, feed and non-food uses (3 presentations)	17:30	Session 3. Genetics, Genomics, Molecular Breeding & Biochemistry (5 presentations)	ANGOSTURA ROOM	17:30	ROOM
				Session 4: Agronomy, Farming (6 presentations)		Closing session
17:00 –	ANGOSTURA ROOM	17:30 –	ROOM LLALLAGUANI ROOM			
18:00	Presentation of posters	18:30	Session 4: Agronomy, Farming (3 presentations)			
19:00 –	PAIRUMANI ROOM			19:00 -	GLORIETA ROOM	
20:30	Welcome toast			23:00	Gala dinner and folkloric show	



# PROGRAM OF ORAL PRESENTATIONS

March 18 (Monday)

## LLALLAGUANI ROOM

### Session 1. Food, feed and non-food uses

Time	Title	Authors (presenter)
11:00 – 11:15	DESIGN AND ELABORATION OF A HEALTHY BREAD WITH INCORPORATION OF LUPIN FLOUR ( <i>Lupinus mutabilis</i> Sweet)	<u>Elena Villacrés</u> , Paúl Cueva, Cristina Rosell, Milene Díaz
11:15 – 11:30	OBTAINING A CRISPY SNACK OF LUPINE ( <i>Lupinus mutabilis</i> Sweet) BY APPLYING DIFFERENT DEHYDRATION PROCESS	<u>María Quelal</u> , Gina López, Elena Villacrés, Edwin Vera
11:30 – 11:45	DEVELOPMENT OF LUPIN-BASED YOGURT ALTERNATIVES – CHALLENGES REGARDING PROCESSING AND FERMENTATION	<u>Andrea Hickisch</u> , Ute Schweiggert-Weisz
11:45 – 12:00	MODIFICATION OF LUPIN PROTEIN ISOLATES – INFLUENCE OF ENZYMATIC HYDROLYSIS AND FERMENTATION ON CHANGES IN THE MOLECULAR WEIGHT DISTRIBUTION, TECHNOFUNCTIONAL AND SENSORY PROPERTIES	<u>Katharina Schlegel</u> , Ute Schweiggert-Weisz, Peter Eisner
12:00 – 12:15	LUPIN CELL WALL POLYSACCHARIDES –INFLUENCE OF SEQUENTIAL EXTRACTION ON THE INTERACTION WITH BILE ACIDS	<u>Susanne Naumann</u> , Ute Schweiggert-Weisz, Peter Eisner
14:45 – 15:00	CHEMICAL CHARACTERIZATION OF <i>Lupinus mutabilis</i> Sweet AND <i>Lupinus angustifolius</i> SEEDS AND OIL	<u>Repo-Carrasco-Valencia, R.</u> , Chamorro, R., Delgado, V, Ollennu-Chuasam, P., Suomela, JP.
15:00 – 15:15	DEVELOPMENT OF GLUTEN-FREE BREAD WITH QUINOA ( <i>Chenopodium quinoa</i> Willd.) AND TARWI ( <i>Lupinus mutabilis</i> Sweet) FLOURS	<u>Julio Vidaurre-Ruiz</u> , Genny Luna-Mercado, Regine Schonlechner, Ritva Repo-Carrasco-Valencia
15:15 – 15:30	NUTRITIONAL CHANGES IN ANDEAN LUPIN ( <i>Lupinus mutabilis</i> ) AFTER PROCESSING	Javier S. Córdova-Ramos, <u>Patricia Glorio Paulet</u> , Andrea Brandolini, Alyssa Hidalgo
16:00 – 16:15	INFLUENCE OF TARWI ( <i>Lupinus mutabilis</i> Sweet) HUSK FIBER ON BREAD MOLD TEXTURE DURING STORAGE	<u>Sumire-Quenta Daniel</u> ; Glorio-Paulet Patricia; Repo-Carrasco Ritva; Silva-Paz Reynaldo; Camarena Felix
16:15 – 16:30	<i>Lupinus mutabilis</i> SWEET (TARWI) CONSUMPTION IN THE PERUVIAN MARKET: CHALLENGES AND THREATS	<u>Rendón Schneir Eric</u> , Camarena Mayta Felix, Mostacero Elvia, Huaranga Amelia

**March 19 (Tuesday)**

**LLALLAGUANI ROOM**

**Session 1. Food, feed and non-food uses**

Time	Title	Authors (presenter)
09:15 – 09:30	PROTEIN CONCENTRATES AND ISOLATES FROM LUPIN CULTIVARS ( <i>Lupinus mutabilis</i> AND <i>Lupinus albus</i> )	<u>Carmen Carla Quiroga Ledezma</u>
09:30 – 09:45	DESAMARGADO DE TARWI ( <i>Lupinus mutabilis</i> ) ASISTIDO POR MICROONDAS Y ULTRASONIDO	<u>José Manuel Prieto</u> ; Yakelin Eliana Yucra Mamani; Olivia Magaly Luque Vilca
09:45 – 10:00	DETERMINACIÓN DE LAS PROPIEDADES FÍSICAS, QUÍMICAS Y NUTRICIONALES DE HARINA INSTANTÁNEA DE TARWI ( <i>Lupinus mutabilis</i> Sweet)	Evelyn Tintaya, <u>Carmen Apaza</u> , Enrique Mamani

**Session 2. Taxonomy, Biodiversity & Agroecology**

Time	Title	Authors (presenter)
14:00 – 14:15	SPECIES REVIEW OF <i>LUPINUS</i> GROWING IN BOLIVIA	<u>Ana María Planchuelo</u>
14:15 – 14:30	TOWARDS A ROBUST SYSTEM FOR THE TAXONOMY OF MEXICAN LUPINS: A COMPARATIVE MORPHOANATOMIC STUDY	Aurora Isabel Cantor del Angel, Arianna Michelle Hernández Sánchez, María Concepción Guzmán Ramos, Estela Sandoval Zapotitla, <u>Kalina Bermúdez Torres</u>
14:30 – 14:45	BIODIVERSITY OF THE <i>Lupinus</i> GENUS IN LOS ANDES	Mario E. Tapia
14:45 – 15:00	IDENTIFICACIÓN DE PADRES DONANTES DE GENES PARA PRECOCIDAD Y MADURACIÓN UNIFORME EN EL BANCO DE GERMOPLASMA DE <i>Lupinus mutabilis</i> Sweet DE ECUADOR	<u>Laura Vega</u> , Nelson Mazón, Ángel Murillo, Diego Rodríguez
15:00 – 15:15	USO DE ECOTIPOS SILVESTRES DE LUPINUS EN LA RECUPERACIÓN DE SUELOS DEGRADADOS	<u>Alejandro Bonifacio</u>

**Session 3. Genetics, Genomics, Molecular Breeding & Biochemistry**

Time	Title	Authors (presenter)
15:15 – 15:30	GENOMIC SELECTION IN WHITE LUPIN	<u>Paolo Annicchiarico</u> , Nelson Nazzicari, Barbara Ferrari
15:30 – 15:45	<i>Lupinus luteus</i> BREEDING IN CHILE: AN OPPORTUNITY FOR SUSTAINABLE AGRICULTURE AND THE DEVELOPMENT OF NEW PRODUCTS FOR HEALTHY FOOD AND FEED INDUSTRIES	Nicole Lichtin Schythe, Annally Rupayan Riquelme, Fernando Westermeyer Figueroa, Bradley Till and <u>Haroldo Salvo-Garrido</u>
15:45 – 16:00	LUPIBREED: IMPROVING NARROW-LEAFED LUPIN – NOVEL GENETIC RESOURCES FOR HIGHER YIELD AND YIELD STABILITY PART I: YIELD POTENTIAL AND YIELD STABILITY	<u>Helge Fließ</u> , Steffen R. Roux, Kristin Fischer, Hans Ulrich Jürgens, Regine Dietrich, Ulrike Lohwasser
16:15 – 16:30	LUPIBREED - FROM BREEDING RESEARCH TO NEW CULTIVARS IN GERMANY PART II: PROTEIN AND ALKALOID CONTENT	<u>Anne Zaar</u> , Hans-Ulrich Jürgens, Gisela Jansen, Sylvia Seddig, Ulrike

Time	Title	Authors (presenter)
		Lohwasser, Regine Dieterich, Steffen Roux, Brigitte Ruge-Wehling
16:30 – 16:45	QUINOLIZIDINE ALKALOID BIOSYNTHESIS: INSIGHTS FROM TRANSCRIPTOME ANALYSES (RNASeq)	<u>Michael Wink</u>
16:45 – 17:00	QUINOLIZIDINE ALKALOIDS IN <i>LUPINUS</i> : WHEN AND HOW ARE THEY SYNTHESIZED?	Astrid Pamela Ramírez Betancourt, Arianna Michelle Hernández Sánchez, Macdiel Emilio Acevedo Quiroz, Guadalupe Salcedo Morales, <u>Kalina Bermúdez Torres</u>
17:00 – 17:15	OBTAINING SIMILAR PHENOTYPES TO THE DOMESTICATED TARWI FROM ITS WILD RELATIVES	<u>Alejandro Bonifacio</u> , Miriam Alcon
17:15 – 17:30	NUEVOS CULTIVARES DE TARWI ( <i>Lupinus mutabilis</i> Sweet) OBTENIDOS POR SELECCIÓN MASAL ESTRATIFICADA	Juan Vallejos, <u>Julio Gabriel</u> , Ada Angulo, Pablo Mamani, Ximena Cadima

#### Session 4: Agronomy, Farming

Time	Title	Authors (presenter)
17:30 – 17:45	POTENTIAL FOR LUPINS IN RELATION TO OTHER GRAIN LEGUMES AS A SOURCE OF PLANT PROTEIN FOR HUMAN CONSUMPTION	<u>U. Prins</u> , W.J.M. Cuijpers
17:45 – 18:00	EVALUATION OF TARWI ( <i>Lupinus mutabilis</i> SWEET) ADAPTABILITY TO CULTIVATION UNDER MEDITERRANEAN CLIMATE CONDITIONS	Norberto Guilengue, Sofia Alves, Pedro Talhinas, <u>João Neves-Martins</u>
18:00 – 18:15	INVESTIGATION OF THE RELATIONSHIP BETWEEN THE LEVEL OF ALKALOIDS AND DEGREE OF PEST DAMAGE IN <i>Lupinus polyphyllus</i> LINDL	Boguslav Kurlovich , Gavin Loxton, <u>David Mc Naughton</u>

## ANGOSTURA ROOM

### Session 4. Agronomy, Farming

Time	Title	Authors (presenter)
14:00 – 14:15	LUPIN A POTENTIAL CROP SPECIES FOR PLANT-BASED PROTEIN FOOD FOR DENMARK	<u>Gabriela Alandia</u>
14:15 – 14:30	PRODUCTION AND MARKETING MODEL FOR SWEET LUPIN IN CHILE	<u>Ricardo Agustín Anríquez Lara</u>
14:30 – 14:45	EL CHOCHO ( <i>Lupinus mutabilis</i> Sweet) EN ECUADOR: ESTRATEGIA DEL INIAP PARA EL FOMENTO DE LA PRODUCCIÓN Y EL CONSUMO	<u>Nelson Mazón</u> , Elena Villacrés, Ángel Murillo, Diego Rodríguez, Laura Vega
14:45 – 15:00	STRATEGIES FOR THE INTEGRATED MANAGEMENT OF 'CHOCHO' ( <i>LUPINUS MUTABILIS</i> ) PESTS IN SOCIO-ECOLOGICAL LANDSCAPES	<u>Vidal Toro</u> Diego Mina, Quentin Struelens Marco Rivera, Diego Muñoz, Andrea Guapi, Marco Vivar, Carlos Carpio, Olivier Dangles
15:00 – 15:15	THE RESEARCH ADVANCES OF THE PRODUCTIVE CHAIN OF THE LUPINE ( <i>Lupinus mutabilis</i> SWEET) IN ALLIANCES WITH PRODUCERS AND LOCAL ENTITIES IN THE PERUVIAN NORTHERN HIGHLANDS	<u>Félix Camarena Mayta</u> , Amelia Huaranga Joaquín, Elvia Mostacero de Bustillos, Patricia Glorio Paulet, Gloria Pascual Chagman, Jorge Tito
15:15 – 15:30	PHENOTYPIC EVALUATION AND PRELIMINARY YIELD OF LUPIN ECOTYPE ( <i>Lupinus mutabilis</i> Sweet) GROWN IN CARHUAZ, ANCASH	<u>Amelia Huaranga</u> , Néstor De la Cruz, Félix Camarena, Pilar Caycho, Elvia Mostacero, Miguel Patricio
15:30 – 15:45	VALIDACIÓN Y DIFUSIÓN DE ALTERNATIVAS DE MANEJO DEL CULTIVO DE CHOCHO ( <i>Lupinus mutabilis</i> Swett) EN LOCALIDADES DE LAS PROVINCIAS CHIMBORAZO Y COTOPAXI, ECUADOR	<u>Diego Rodríguez</u> , Nelson Mazón, Ángel Murillo, Laura Vega, Fausto Yumisaca
15:45 – 16:00	SELECCIÓN DE CULTIVARES NACIONALES DE TARWI ( <i>Lupinus mutabilis</i> Sweet.) POR RENDIMIENTO, PRECOCIDAD, CONTENIDO DE ACEITE Y PROTEÍNA EN PUNO, PERÚ	<u>Ángel Mujica</u> , E. Chura, J. Apaza, D. Chuquimia, G. Moscoso, E. Calandri, P. Montoya, F. Grasso, C. Guzmán
16:15 – 16:30	AGROECOLOGICAL CONDITIONS OF THE ARGENTINE PAMPAS FOR NARROW LEAF LUPIN CROPPING	<u>Andrés Carlos Ravelo</u>
16:30 – 16:45	EL CULTIVO DE TARWI ( <i>Lupinus mutabilis</i> Sweet) EN EL ALTIPLANO NORTE DE BOLIVIA: AVANCES DE INVESTIGACIÓN	<u>Hugo Bosque</u>
16:45 – 17:00	EVALUACIÓN DE DOS ESPECIES DE LUPINUS EN DIFERENTES AMBIENTES DE LAS ZONAS DE VALLES Y ALTOANDINAS DE BOLIVIA	<u>Juan Vallejos</u> , Pablo Mamani, Antonio Gandarillas
17:00 – 17:15	EFFECTO DE LA INCORPORACIÓN DE RESIDUOS DE COSECHA DE TARWI ( <i>LUPINUS MUTABILIS</i> SWEET) EN EL SISTEMA DE CULTIVOS Y LA RECUPERACIÓN DE SUELOS DE REGIONES ANDINAS SEMIÁRIDAS DE COCHABAMBA	<u>Pablo Mamani</u> ; Juan José Calisaya
17:15 – 17:30	DISEASES AFFECTING TARWI ( <i>Lupinus mutabilis</i> Sweet) CROP IN BOLIVIA	<u>Giovanna Plata</u> ; Antonio Gandarillas
17:30 – 17:45	PESTS OF TARWI AND ITS CONTROL	<u>L. Crespo</u> , A. Gandarillas, Alejandro Bonifacio, Reinaldo Quispe

**March 21 (Thursday)**

**LLALLAGUANI ROOM**

**Session 5: Health benefits**

<b>Time</b>	<b>Title</b>	<b>Authors (presenter)</b>
08:30 – 08:45	ESTIMATES OF TOTAL PHENOLICS CONTENT IN 52 GENOTYPES OF BITTER AND DEBITTERED LUPIN FLOUR ( <i>Lupinus mutabilis</i> )	Zuly Sabelino-Francia, <u>Patricia Glorio-Paulet</u> , Luis Rodriguez-Saona, Felix Camarena
08:45 – 09:00	EVALUATION OF NOCICEPTIVE TOLERANCE, TOXICITY AT REPEATED DOSE OF TARWI (LUPINUS MUTABILIS SWEET) IN ANIMALS OF EXPERIMENTATION	<u>Gamarra-Castillo Fabricio Paúl</u> ; Chirinos-Arista Rosario del Pilar; Salazar-Granada Alberto
09:00 – 09:15	THE USE OF WHITE LUPINE AND OTHERS SEEDS AS MEDICINAL SUPPLEMENTS AT BREAKFAST TIME	<u>Ana María Planchuelo</u>
09:15 – 09:30	$\beta$ -CONGLUTIN PROTEIN FROM <i>LUPINUS ALBUS</i> IMPROVES ORAL GLUCOSE TOLERANCE IN HEALTHY RATS AND REDUCES CIRCULATING CHOLESTEROL LEVELS IN STREPTOZOTOCIN-INDUCED DIABETES	Tereso J. Guzmán, Irma Catalina Soto-Luna, Pedro M. García-López, Alma L. Martínez-Ayala, <u>Carmen M. Gurrola-Díaz</u>
09:30 – 09:45	PREVALENCE OF LUPIN SENSITIZATION IN WESTERN MEXICAN CHILDREN POPULATION	<u>Carmen Magdalena Gurrola-Díaz</u> , Gloria Estefania Aguirre-Sandoval, Guadalupe Irma Catalina Soto-Luna, Pedro Macedonio García-López, Ma. Guadalupe Alcalá-Padilla

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# TECHNICAL SESSIONS

## FOOD, FEED & NON-FOOD USES

Several species of *Lupinus* have a record number of uses since ancient times as human food and animal feeding by different European and American civilizations. The scientific and technological documents show the high nutritional quality of their beans and the different food and non-food applications that are being incorporated to their physico-chemical qualities are recognized. Since the first International Lupin Conference (ILC) in Lima-Cuzco, Peru in 1980, subjects related to human foods and animal feeds were the subject of numerous high-level scientific presentations. In this new XVILC there were 31 summaries dealing with topics of current interest to empower the lupins as food and raw material for industrial products.

### ALIMENTOS, FORRAJES Y USOS NO ALIMENTARIOS

Varias especies de *Lupinus* tienen un sin número de antecedentes de haber sido usadas desde la antigüedad como alimento humano y animal por distintas civilizaciones europeas y americanas. Los documentos científicos y tecnológicos demuestran la alta calidad nutricional de sus granos y los diversos usos alimentarios, y no alimentarios que se van incorporando a medida que se reconocen sus cualidades fisicoquímicas. Desde la primera Conferencia Internacional de Lupinos (ILC) en Lima-Cuzco, Perú en 1980, los temas relacionados con alimentos humanos y animal fueron objeto de numerosas presentaciones de alto nivel científico. En esta XV ILC se recibieron 31 resúmenes que versan sobre temas de intereses actuales para potenciar a los lupinos como alimentos y materia prima para productos industriales de valor nutricional.

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## HEALTH BENEFITS

Although traditionally the health care have been based on numerous herbs, lupines species have gained interest in recent times by the health benefits which is provided by the nutritional components of its grain that were not identified previously. New research on proteins and alkaloids have shown that the concepts of toxicity that is attributed to the bitter lupins have some uses that are not harmful as they were previously considered. In the XV International Lupin Conference 14 research summaries related to issues of health, pain relief and medical issues are presented in where the species *Lupinus albus* and *L. mutabilis* play an important role in these areas of research. There is no doubt that the research work presented in this Conference will serve as a basis for new research and it will demonstrate that the lupins are plants that can be used in the modern health care practices.

### BENEFICIOS PARA LA SALUD

Si bien tradicionalmente los cuidados de la salud se han basado en numerosas plantas consideradas medicinales, las especies de lupinos han cobrado interés en tiempos recientes por los beneficios que proveen para la salud por los componentes nutricionales de sus granos que no fueron identificados con anterioridad. Nuevas investigaciones sobre proteínas y alcaloides han demostrado que los conceptos de

toxicidad que se le atribuía a los lupinos amargos tienen algunos usos que no son perjudiciales como se consideraban anteriormente. En la XV Conferencia Internacional del Lupino se presentan 14 resúmenes de investigación que están relacionados con temas que hacen a la salud, al alivio del dolor y a cuestiones médicas en donde las especies de *Lupinus albus* y *L. mutabilis* juegan un papel importante en estos temas de investigación. Es indudable que los trabajos de investigación que se exponen en esta Conferencia van a servir de base para nuevas investigaciones que demostrarán que los lupinos son plantas que pueden ser utilizadas en las prácticas médicas y los cuidados de la salud.

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## **GENETIC, GENOMICS & MOLECULAR BREEDING**

Conventional breeding of lupin was concentrated in past decades, particularly in *L. albus* and *L. angustifolius*, achieving sweet materials widely used especially in Europe and Australia. In the XV International Lupin Conference, 10 abstracts are presented, exposing new tools for the improvement and selection of lupins in Europe, and also important advances developed in the countries of South America using molecular and conventional tools. The works highlight that there is still much to investigate in the world of lupins.

## **GENÉTICA, GENÓMICA & MEJORAMIENTO MOLECULAR**

El mejoramiento convencional del lupino se concentró en décadas pasadas particularmente en *L. albus* y *L. angustifolius*, logrando materiales dulces de amplio uso especialmente en Europa y Australia. En la XV Conferencia Internacional del Lupino se presentan 10 resúmenes que exponen nuevas herramientas para el mejoramiento y selección de los lupinos en Europa, y también avances importantes desarrollados en los países de Sudamérica con herramientas moleculares y convencionales. Los trabajos resaltan que aún hay mucho por investigar en el mundo de los lupinos.

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## **BIOCHEMISTRY & BIOTECHNOLOGY**

The four contributions presented in this thematic area at the XV International Lupin Conference were focused on the use of new tools for the extraction and determination of lupin alkaloids, and protein content, and also to elucidate when and how alkaloids are synthesized in lupins.

## **BIOQUÍMICA & BIOTECNOLOGÍA**

Los cuatro trabajos presentados en esta área temática en la XV Conferencia Internacional del Lupino estuvieron enfocados en el uso de nuevas herramientas para la extracción y determinación de alcaloides del lupino, y del contenido de proteína, y también en dilucidar cuándo y cómo se sintetizan los alcaloides de los lupinos.

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## **PHYSIOLOGY & PROTECTION**

Considering the potential of the lupine as a product for food, feed and other non-food uses, it is important to evaluate different aspects that condition its success as a commercial crop. In the area of physiology, a research paper is presented to determine the best methods to evaluate the quality of lupine seeds, and in the protection area the evaluation of the response to anthracnose in a collection of tarwi germplasm under European conditions is presented.

### **FISIOLOGÍA & PROTECCIÓN**

Considerando el potencial del lupino como producto en la alimentación humana, animal y para otros usos no alimentarios, es importante evaluar diferentes aspectos que condicionen su éxito como cultivo comercial. En el área de fisiología se presenta un trabajo de investigación para determinar los mejores métodos para evaluar la calidad de las semillas de los lupinos, y en el área de protección se expone la evaluación de la respuesta a la antracnosis en una colección de germoplasma tarwi bajo condiciones de Europa.

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## **TAXONOMY, BIODIVERSITY & ECOLOGY**

The taxonomy of the genus *Lupinus* is very complex due to the large number of specific names that have to be validated and which in many cases do not correspond to real species. Over the years in all International Lupin Conferences several topics related to taxonomy and biodiversity of native species from different regions of the world were considered, but there are still many unknown aspects to clarify. In this XV International Lupin Conference, 11 summaries are presented mainly dealing with morphological aspects and environmental adaptations of Andean and Mexican species. These contributions provide new knowledge for interpreting the relations of kinship among relatives of cultivated and wild species.

### **TAXONOMÍA, BIODIVERSIDAD Y ECOLOGÍA**

La taxonomía del género *Lupinus* es muy compleja debido a la gran cantidad de nombres específicos que tienen que ser validados y que en muchos casos no corresponden a verdaderas especies. A través de los años en todas las Conferencias Internacionales de Lupinos se trataron temas relacionados con la taxonomía y la biodiversidad de especies nativas de distintas regiones del mundo, y aún quedan muchas incógnitas por dilucidar. En esta XV Conferencia Internacional del Lupino se presentan 11 resúmenes de trabajos que versan principalmente sobre aspectos morfológicos y de adaptaciones ambientales de especies andinas y mexicanas. Estas contribuciones aportan nuevos conocimientos que sirven de base para interpretar las relaciones y parentesco entre las especies silvestres y los parientes de las cultivadas.

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## **AGRONOMY & FARMING**

The expansion of lupin´ crops species depends on the knowledge of specific requirements of species and variety requirements and the appropriate agronomic management to allow the germplasm express its full potential performance as forage or grain crop. The agricultural aspects and the lupin crop were always taken into account in the 14 international Lupin Conferences (ILC). This is the reason that in this XV ILC, 24 abstracts were presented dealing with current issues in these areas of research and agricultural technology.

## **AGRONOMÍA Y CULTIVO**

La expansión de los cultivos de las especies de lupinos depende de un muy buen conocimiento de los requerimientos específicos y varietales y de un manejo agronómico adecuado para que el germoplasma exprese su máximo potencial de rendimiento en forraje y/o grano. Todos los temas relacionados con los aspectos agronómicos y de cultivo de los lupinos siempre fueron tenidos en cuenta en las 14 Conferencias Internacionales del Lupino (CIL). Es por esa razón que en esta nueva Conferencia se presentaron 24 resúmenes que tratan temas actuales en estas áreas de investigación y tecnología agropecuaria.

## **SYMPOSIUM SESSIONS**

### **FOOD, FEED & NON-FOOD USES**

The XV International Lupin Conference considered opportune to promote a symposium for establishing the "State of the art" on food, feed and non-food items and the inclusion of the species of lupines in the world of the nutritional request that society is expressing around the world. The oral presentations in this opportunity deal with technological innovation and the opportunities and challenges that lupins have at present time. This Symposium opens a debate for participants to express their knowledge, ideas and perceptions which jointly can draw new lines of research and applications of the species of lupines. The symposium is expected the participation of everyone able to promote a commercial takeoff at the international level of the species of lupines being cultivated in several countries and which can extend its cultivation to the five continents.

### **ALIMENTO, FORRAJE Y OTROS USOS**

En la XV Conferencia Internacional del Lupino se consideró oportuno propiciar un simposio para establecer el "estado del arte" sobre temas alimenticios, forrajeros y no alimenticios y la inserción de las especies de lupinos en el mundo de los reclamos alimenticios que la sociedad está haciendo a nivel mundial. Las presentaciones orales en esta oportunidad tratan sobre la innovación tecnológica y las oportunidades y desafíos que presentan los lupinos en la actualidad. Este simposio abre el debate para que los asistentes expresen sus conocimientos, ideas y percepciones para que en forma mancomunada se puedan delinear nuevas líneas de investigación y de aplicaciones de las especies de lupinos. Se espera que con la participación de todos se logre propiciar un despegue comercial a nivel internacional de las especies de lupinos que están siendo cultivadas en varios países y que pueden extender su cultivo a los cinco continentes.

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### **AGRONOMY**

Frequently, it is believed that problems of primary sector are solved, that raw material is always available in quality and quantity; but this is far from the truth. Many businesses fail because of the difficulties in obtaining raw material. In this XV Conference, a large number of specialists in agronomy, from different countries and working with different lupines species have gathered. Therefore, this is the best time to discuss in this Symposium the difficulties faced by each country in production; to analyze together the solution options for generating a joint agenda of technology development needs.

### **AGRONOMIA**

Frecuentemente, se cree que los problemas del sector primario están resueltos, que la materia prima siempre está disponible en calidad y cantidad. Nada más falso, muchos negocios fracasan por las dificultades en la obtención de la materia prima. En esta XV Conferencia, se han congregado un importante número de especialistas en agronomía, de diferentes países y que trabajan con diferentes especies de lupinos. Por ello, este resulta el mejor momento de debatir en este Simposio las dificultades que enfrenta cada país en la producción, analizar juntos las opciones de solución y generar una agenda conjunta de necesidades de desarrollo de tecnología.

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## **EMERGING MARKETS**

The new feeding currents in the world, that tending to increase a diet based on vegetable protein and to reduce the consumption of animal protein; the global warming concern, in part, caused by the cattle breeding for human consumption, generating large volumes of greenhouse gases; and the production systems that tend to include more and more a legume, improving sustainability and reducing the use of fertilizers in agriculture; all these points show that we are in a good moment to increase the production and human and animal lupines consumption. All these subjects will be addressed in the symposium, with the aim of giving greater lights of real possibilities of a growing market for lupines.

## **MERCADOS EMERGENTES**

Las nuevas corrientes de alimentación en el mundo, que tienden a incrementar una dieta basada en proteína vegetal y reducir el consumo de proteína animal; la preocupación del calentamiento global, en parte, causado por la cría de ganado destinado al consumo humano, que genera importantes volúmenes de gases de efecto invernadero; los sistemas de producción que tienden a incluir cada vez más una leguminosa, mejorando la sostenibilidad y reduciendo el uso de fertilizantes en la agricultura; estos puntos muestran que estamos en un buen momento de incrementar la producción y consumo humano y animal de los lupinos. Toda esta temática será abordada en el simposio, con la intención de dar mayores luces de las reales posibilidades de un mercado creciente para los lupinos.



**ABSTRACTS  
OF LECTIO MAGISTRALIS**



## LUPIN'S SITUATION AND POTENTIAL

Erik von Baer

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It has been thirty eight years since the first congress of lupin in Lima when we founded the ILA, summarizing the background of this noble cultivation from the Egyptians and Tiahuanaco.

Where are we? Where do we now go? Has increased demand and production been based on accumulated knowledge?

The answer is that only where demand has been created production has followed.

Unlike that, the SOY has become one of the most important crops in the world; only in Bolivia 1.3 million hectares are cultivated.

The announcement at ILA Poland 2.011, was that in 10 years soybean production will go to India and China. This soybean will mainly be transgenic.

In spite of this we remain optimistic in this changing world, the lupine with its different sweet and bitter species, will satisfy a non-transgenic protein niche.

How do we carry on? We create demand and satisfy it with constant, growing production and controlled quality. Our world is not the past, but it's tomorrow's! It's not just the publication; it's the application of innovation!

This is how we see successful initiatives on all continents that we hope will be consolidated over time. We have to apply and lead to the reality of what we know under a system of contracted production and prior assurance of demand.

If markets do not exist, let's create them!

## SITUACION Y POTENCIAL DEL LUPINO

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Ahora llevamos treinta y ocho años desde Lima cuando tuvimos el primer Congreso del Lupino, fundamos ILA, resumiendo los antecedentes de este noble cultivo desde los egipcios y Tiahuanaco.

¿Dónde estamos? ¿Dónde vamos? ¿Ha aumentado la producción y la demanda en base a todos los conocimientos acumulados?

La respuesta es que solo donde se ha creado la demanda, hay producción.

A diferencia de ello, la SOYA se ha convertido en uno de los cultivos más importantes del mundo; solo en Bolivia se cultivan 1,3 millones de hectáreas.

El pronóstico en ILA Polonia 2.011, era que en 10 años la producción de soya se iba ir toda a China e India, no se ha dado hasta ahora, y eso que es transgénica.

A pesar de ello seguimos siendo optimistas en este mundo cambiante, donde el Lupino con sus diferentes especies dulces y amargas, debe salir de su nicho de planta de estudio y producción.

¿Que demos hacer? Crear demanda y satisfacerla con producción constante, creciente y de calidad controlada. ¡Nuestro mundo no es el de ayer, es el de mañana! ¡No es solo la publicación, es la aplicación de la innovación!

Así vemos iniciativas exitosas en todos los continentes que esperamos que se consoliden en el tiempo. Tenemos que aplicar y llevar a la realidad de lo que sabemos bajo un sistema de contrato de producción y previo aseguramiento de la demanda.

¡Si los mercados no existen, hay que crearlos!

# THE BARRIERS TO THE COMMERCIALISATION OF LUPINS IN EUROPE

## An update on our ongoing search for a market

David McNaughton

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State of art and aim: The UK currently imports 3.4 million tonnes of soya and soya products per annum with most of it (3.2 million tonnes) being used directly in the animal feed industry. In total, soya accounts for around two-thirds of the supplemental protein fed to all livestock in the UK, and this figure is almost identical in the rest of the European Union. On paper, lupins would be the perfect solution to the problem to Europe's over-reliance on imported soya and European-grown lupins could theoretically replace much of this imported tonnage. Despite the efforts by various seed companies across Europe, the area of lupins grown in Europe remains relatively small, and broad uptake of the crop remains as elusive as ever. The author is a director of Soya UK, which is one of the few seed companies producing larger tonnages of certified lupin seed in Europe. This paper examines the various reasons why lupins have so far failed to take off as a major crop, and what the prospects are for commercial breakthrough.

Results and discussion: Whilst the UK has a well-established area of lupin production, that area has remained fairly static for the last 15 years with the annual area never less than 1000Ha but never rising above 3000Ha. Uptake has been much slower than may have been expected given the steady rise in the price of other protein sources within the same time frame. In addition, almost all of this area is grown on livestock farms with 80% of it being grown in mixture with cereals (usually spring triticale), and cut as forage. The remaining 20% will be grown as a pure stand of lupins and cut with a combine harvester, but will be retained on the farm for home-feeding as a high protein feed. As a result, very low tonnages of lupins are traded in the UK and there has been little or no uptake from the commercial feed sector. Despite the best efforts to introduce lupins into this sector, the current area almost entirely being grown on livestock units for home use with little commercial tonnage being grown.

The author has attempted to identify and address the factors that have been holding back the uptake of lupins. Major factors identified include;

- Confusion over the true feed value of lupins, and their feeding performance in comparison to established protein sources like soya.
- Confusion over the best type of lupin to grow, which are the best varieties to grow, and which geographic areas are most suitable for each type.
- Agronomic questions, including a lack of effective herbicides making weed control difficult.
- Lack of uptake from the feed sector. This has meant there is no volume market for lupins in the UK and has restricted lupins to relatively low volume niche markets.
- Concerns over allergenicity.
- Inconsistent yields and quality, which undermine confidence in the crop among both growers and potential end-users.

The author has also provided a speculative view of the future prospects for lupins in Europe and what can be done to take lupins to the next level of commercial uptake. Recent changes to the oilseeds and grain markets have not favoured lupins, as have other factors including changes to the EU support mechanisms. Contrary to this, however, there have been positive developments in the proteins markets which will favour lupins.

As a result, there are mixed prospects for lupins in 2019, but there is much work to be done in developing new markets. The search continues....

## CHALLENGES FOR THE USE OF LUPINE IN BOLIVIA FROM THE PERSPECTIVE OF PROINPA

Antonio Gandarillas

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As it is known in the small world of people working with lupins and particularly with *L. mutabilis*, the tarwi or chocho has a great number of attributes, superior to almost all crops of Andean origin and introduced crops. This makes that one falls in love quickly with tarwi, working under the illusion for having in our hands a super food that solves soil problems and improves production systems in the field; nutrition and health for society, etc. However, one quickly faces several problems, which have discouraged several initiatives for promoting tarwi in Bolivia. This leads us to the question: ¿what are the limiting factors for the production and tarwi consumption to increase in Bolivia? and ¿what should we do to overcome those limitations?

The limiting factors can be divided into: 1. Agronomy, 2. Industry and 3. Marketing. Regarding the Agronomic aspects, the tarwi crop cycle is very long (eight months) and rainfall regime in the Andes runs from October to March. Tarwi is still a semi-domesticated crop; its plants are very tall, the beans ripening is uneven and with a high alkaloid content. The Industry starts with an expensive raw material and not well processed. The cost of removing alkaloids is high; a lot of water is consumed and it takes several days. Once the alkaloid is eliminated, the life time of the grain is very short (2 to 3 days); it becomes very susceptible to rot by bacteria. This requires a cold chain for commercialization. In the Market, the final tarwi product is expensive compared to other Andean or legume products. This is most evident with flour; that of tarwi is worth several times more than those of cereals and quinoa.

### **What is the PROINPA strategy to overcome the limitations?**

Work has been done in selecting new varieties, of short cycle, uniform growth, and greater yield. We have studied pests and diseases, designed controlling strategies, both chemical and organic. Different varieties and lupin species have been evaluated, among them *L. mutabilis* with lower alkaloid content, sweet *L. albus* and sweet *L. angustifolius*. We are developing a cropping areas strategy of each species and variety, and their best uses for local consumption and for industry.

The creation of a SRL (limited liability company) company specializing in the processing and marketing of tarwi, called PANASERI, has been favored. Here we have worked on making industrial processes efficient, such as the reduction and recycling of water use; products development: mote, dehydrated, roasted, flour, etc. PANASERI commercializes mote, toasted and tarwi flour, with the TARWIX brand. The volume of grain processed in 2018 was four tons, by 2019 it is planned to process more than seven tons. On the other hand, thanks to the promotion of tarwi (mote) in the media of Cochabamba, PROINPA has led to an increase in the informal marketing of tarwi and local consumption, from 655 tons in 2015 to more than 1,200 tons in 2019.

In short, we can say that it is achievable to solve tarwi limitations. In the case of agronomy, it is crucial to select short cycle varieties, low alkaloid content and mechanize the harvest. Tarwi should be included as part of an improved fallow. The industry must have low alkaloid or sweet varieties. This significantly reduces the cost production. In the market, it is essential to work in an aggressive promotion by passing on information about its properties, to the general public and to decision makers.

## DESAFÍOS PARA EL APROVECHAMIENTO DEL LUPINO EN BOLIVIA DESDE LA PERSPECTIVA DE PROINPA

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Como es de conocimiento en el pequeño mundo de las personas que trabajamos con Lupinos y particularmente con *L. mutabilis*, el tarwi o chocho tiene un gran número de atributos, superior a casi todos los cultivos de origen andino y los cultivos introducidos. Esto hace que uno se enamore rápidamente del tarwi, trabaje con ilusión de tener en las manos un súper alimento que resuelva problemas de suelos y mejore los sistemas de producción en el campo; la nutrición y la salud para la sociedad, etc. Sin embargo, uno rápidamente enfrenta varios problemas, que han desanimado a varias iniciativas de promoción del tarwi en Bolivia. Esto lleva a preguntarse: ¿cuáles son los factores limitantes para que la producción y el consumo del tarwi se incrementen en Bolivia? y ¿qué debemos hacer para vencer esas limitantes?

Los factores limitantes se pueden dividir en 1. Agronomía, 2. Industria y 3. Mercado. En cuanto a los aspectos Agronómicos, el ciclo de cultivo del tarwi es muy largo (ocho meses) y el régimen de lluvias en los Andes va de octubre a marzo. El tarwi todavía es un cultivo semidomesticado, sus plantas son de porte muy alto, la maduración de los granos es desuniforme y tienen alto contenido de alcaloides. En la industria, se empieza con una materia prima cara y no bien procesada. El costo de eliminar los alcaloides es alto, se consume mucha agua y toma varios días. Una vez eliminado el alcaloide, el tiempo de vida del grano es muy corto (2 a 3 días), se torna muy susceptible a pudriciones por bacterias, esto exige tener una cadena de frío para su comercialización. En el Mercado, el producto final del tarwi es caro comparativamente con otros productos andinos o leguminosas. Esto es muy evidente con la harina, la del tarwi vale varias veces más que las de los cereales y la de la quinua.

### ¿Cuál es la estrategia de PROINPA para vencer las limitantes?

Se ha trabajado en seleccionar nuevas variedades, de ciclo corto, de crecimiento uniforme, de mayor rendimiento. Hemos estudiado las plagas y enfermedades, diseñado estrategias de control, tanto químico como orgánico. Se han evaluado diferentes variedades y especies de Lupinos, entre ellos *L. mutabilis* con menor contenido de alcaloides. *L. albus* dulce y *L. angustifolius* dulce. Estamos desarrollando una estrategia de zonas de cultivo de cada especie y variedad, y de sus mejores usos para el consumo local y para la industria.

Se ha propiciado la creación de una empresa SRL especializada en el procesamiento y comercialización del tarwi, PANASERI. Aquí se ha trabajado en hacer eficientes los procesos industriales, como la reducción y reciclado del uso del agua; desarrollo de productos: mote, deshidratado, tostado, harina, etc. PANASERI comercializa mote, tostado y harina de tarwi, con la marca TARWIX, el volumen de grano procesado el 2018 fue de cuatro toneladas, para el 2019 se tiene previsto procesar más de siete toneladas. Por otro lado, gracias a la promoción del tarwi (mote) en los medios de Cochabamba, PROINPA ha logrado que incrementen la comercialización informal del tarwi y el consumo local, se ha pasado de 655 toneladas el 2015 a más de 1.200 toneladas el 2019.

En resumen podemos decir que si es factible resolver las limitantes del tarwi. En Agronomía, es central seleccionar variedades de ciclo corto, bajo contenido de alcaloide y mecanizar la cosecha. El tarwi debe ser incluido como parte de un descanso mejorado. La Industria debe contar con variedades bajas en alcaloide, esto reduce significativamente el costo. En el Mercado es central trabajar en una promoción agresiva pasando la información de sus propiedades, al público en general y a los tomadores de decisión.

## MEDICAL ADVANCES IN THE CONSUMPTION OF *Lupinus mutabilis* Sweet, CHOCHO / TARWI, IN ECUADOR

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Type 2 diabetes mellitus (DMT2) is a growing public health problem that poses a great economic burden to health care systems around the world. Regular physical activity accompanied by a balanced-moderate diet can contribute to the prevention of T2DM and its complications. Scientific evidence shows that the consumption of protein-rich foods present in legumes could improve homeostasis in patients with cardio-metabolic problems. The consumption of legumes is safe and culturally accepted throughout the world. *Lupinus* is a legume rich in proteins that has been consumed for centuries in Europe, the Middle East and the Andean region. *Lupinus mutabilis* Sweet is a species native to the Andean region, whose grains are known as chocho in Ecuador. Chochos are sources rich in protein, fiber, unsaturated fat, vitamins and minerals. Our group has shown that consumption of protein-rich legumes decreases serum levels of glucose and glycosylated hemoglobin (A1C) in blood and improves insulin sensitivity in DMT2 patients. The possible components of the *Lupinus* grains and their mechanism of action to improve the metabolism of glucose in DMT2 have begun to be elucidated. Previous work has demonstrated the hypoglycemic effect of a preparation enriched with a protein of the *Lupinus albus* seed ( $\gamma$ C) in rats and humans both in the short and medium term. In-vitro studies also show that stimulation of C2C12 mouse muscle cells with  $\gamma$ C activates intracellular kinases, similar to that caused by insulin. These data indicate that the components of *L. albus* grains have important effects on the metabolism of insulin and glucose. Despite this progress with *L. albus*, very little is known about the components of Andean *lupinus* (*L. mutabilis*), responsible for the beneficial effects observed to lower blood glucose and insulin in T2DM. We have shown that hydrolysates of *L. mutabilis*, especially those produced by the enzymatic action of pepsin and pancreatin, on purified  $\gamma$ C affect the metabolism of glucose inhibiting the enzymatic activity of DPP4 (between 80 and 100% inhibition compared to the positive control sitagliptin); increasing the sensitivity of the insulin receptor in adipocytes with the consequent increase in glucose consumption (between 8 and 13 mM glucose / mg protein versus 15 mM glucose / mg with the positive control of insulin) and increase of the presence of GLUT-4 transporter in the cellular membrane; and inhibiting gluconeogenesis in liver cells (50% inhibition that is similar to the effect of the positive control metformin). Together, these data demonstrate that the *L. mutabilis* legume and its hydrolysates exert positive effects on the metabolism of patients with T2DM. Evidence generated by these research should contribute to promote consumption of Andean legumes as part of a healthy diet to prevent and treat metabolic diseases such as obesity, diabetes and cardiovascular diseases.

## AVANCES MÉDICOS DEL CONSUMO DE *Lupinus mutabilis* Sweet, CHOCHO/TARWI, EN ECUADOR

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La diabetes mellitus tipo 2 (DMT2) es un problema creciente de salud pública que representa una gran carga económica para los sistemas de atención médica en todo el mundo. Una actividad física regular acompañada de una dieta equilibrada-moderada puede contribuir a la prevención la DMT2 y sus complicaciones. La evidencia científica demuestra que el consumo de alimentos ricos en proteínas presentes en las leguminosas podría mejorar la homeostasis en pacientes con problemas cardio-metabólicos. El consumo de leguminosas es seguro y culturalmente aceptado en todo el mundo. El lupinus es una leguminosa rica en proteínas que se ha consumido durante siglos en Europa, Oriente Medio y la región andina. El *Lupinus mutabilis* Sweet es una especie originaria de la región Andina, cuyos granos son conocidos como chocho en Ecuador. Los chochos son fuentes ricas en proteína, fibra, grasa insaturada, vitaminas y minerales. Nuestro grupo de trabajo ha demostrado que el consumo de leguminosas ricas en proteína disminuye las concentraciones séricas de glucosa y hemoglobina glicosilada en sangre y mejora la sensibilidad a la insulina en sujetos con disglucemia o DMT2. Los posibles componentes de los granos de *Lupinus* y su mecanismo de acción para mejorar el metabolismo de la glucosa en DMT2 han comenzado a dilucidarse. Datos demuestran el efecto hipoglicemiante de una preparación enriquecida con una proteína de la semilla de *Lupinus albus* (gamma-conglutina ( $\gamma$ C)) en ratas y humanos tanto a corto como mediano plazo. Estudios in-vitro también muestran que la estimulación a células musculares de ratón C2C12 con  $\gamma$ C activan de quinasas intracelulares, de manera similar al provocado por la insulina. Estos datos indican que los componentes de los granos de *L. albus* tienen efectos importantes sobre el metabolismo de la insulina y la glucosa. A pesar de este progreso con *L. albus*, se sabe muy poco de los componentes del lupinus andino (*L. mutabilis*), responsable de los efectos beneficiosos observados para disminuir la glucosa en sangre y la insulina en la DMT2. Nuestro grupo de investigación ha demostrado *in-vitro* que los hidrolizados de *L. mutabilis* en especial los producidos por la acción enzimática, de la pepsina y pancreatina, sobre el purificado de  $\gamma$ C afectan el metabolismo de la glucosa mediante la inhibición de la actividad enzimática de DPP4 (entre el 80 y 100% de inhibición comparado con el control positivo sitagliptina); incrementando la sensibilidad del receptor de la insulina en adipocitos con el consecuente aumento del consumo de glucosa (entre 8 y 13 mM glucosa/mg proteína versus 15 mM glucosa/mg con el control positivo de insulina) y aumento del transportador GLUT-4 en la membrana celular; e inhibiendo la gluconeogénesis en células hepáticas (50% de inhibición que es similar al efecto del control positivo metformina). En conjunto estos datos demuestran que la leguminosa *L. Mutabilis* y sus hidrolizados ejercen efectos positivos en el metabolismo de pacientes con DMT2 que se explican por sus mecanismos de acción celulares y moleculares *in vitro*. La evidencia generada por estos trabajos de investigación deben contribuir en la promoción del consumo de leguminosas andinas como parte de una dieta saludable para prevenir y tratar enfermedades metabólicas como la obesidad, diabetes y enfermedades cardiovasculares.



## **ABSTRACTS OF KEY NOTES**



# TECHNOLOGICAL INNOVATIONS FOR THE LUPINE VALORIZATION IN ECUADOR

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In Latin America and the Caribbean, the number of undernourished people in the region increased by 2.4 million between 2015 and 2016, reaching 42.5 million in total, equivalent to 6.6% of the population. In South America there is a significant increase in the evolution of undernourishment between 2000 to 2016. On the other hand, the phenomenon of overweight and obesity is increasing in all countries of the region, in all age groups and regardless of their level of wealth or geographic location. In 24 countries the prevalence of obesity in the adult population is close to or greater than 20% of the population. Another indicator of malnutrition is the lack of micronutrients. In 2016, 22% of women of childbearing age in LAC were affected by iron deficiency or anemia. The lupine (*Lupinus mutabilis* Sweet) could respond to the nutritional and food requirements of many populations, due to its high content of nutrients, among which protein, fat and minerals stand out. The objective of this study was to valorise the grain, through the determination of its nutritional, functional components, its contribution in the nutrition and health of the population, as well as the development of new products, prior to grain technical debitter. This process was done by applying a thermal-hydric system. For the quantification of nutrients and functional compounds, the methods of A.O.A.C (1984-2002) were applied. Among the results is the high protein content of the grain, whose potential production is 765 kg per hectare. A fraction of lupine proteins is of special importance in the control of blood glucose level, this property is useful for diabetics and people concerned about their health care. This nutrient along with dietary fiber (> 35%) give the grain satiating properties, which is used in weight reduction diets. Another component of nutritional importance is oil, whose potential production is 300 kg per hectare. The quality of lupine oil is similar to soybean oil, with a low content of saturated fatty acids and 80% of unsaturated fatty acids. The crude oil of the Andino-451 variety presents an average of 746.95 ppm of  $\gamma$ -tocopherol, among the phytosterols the Stigmast-4eno-3-one stands out, with an average of 25 mg / 100 g. Preliminary studies show that the oil has lipid-lowering and hypoglycemic properties. Among the minerals, calcium (0.43-0.48% BS) and iron (1.5-2.0 mg / 100 g BS) stand out. Due to its high moisture content (> 60%) and nutrients, the useful life of the debitter grain is very short (two days) at a temperature of 17°C and 50% relative humidity, so techniques were developed to prolong its durability in different packages. In order to diversify the use and consumption of grain, new products were developed, especially adapted to the consumption pattern of children and young people, their physical, chemical, nutritional and sensory characteristics were determined. Its durability was evaluated in different packaging and storage conditions and its production cost was determined. The species *L. mutabilis* characterizes the high content of quinolizidine alkaloids (3-4%), which decreased to safe levels for human consumption (<0.07% W / V), by successive water washes. At the pilot plant level, an alkaloid recovery system was installed, its properties were studied and techniques were developed for its use.

# FOOD INGREDIENTS FROM LUPIN SEEDS – OPPORTUNITIES AND CHALLENGES

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The supply of a growing world population with high quality and affordable foods is one of the biggest challenges of the 21st century. The growing prosperity in emerging economies such as China has led to an increasing demand for high quality animal proteins such as meat, eggs or milk and in consequence to an increasing land requirement for feed cultivation. Additional to this fact the growing production of fuels from renewable resources leads to an increased demand in land for energy production. The competition between the production of food, feed, green chemicals or bio-energy for the available land has been developed to a serious area of conflict that has been responsible for a significant increase in volatility of food commodities prices in recent years.

Against this background plant proteins are becoming more and more important for human nutrition in the future. Besides the most common plant based protein ingredients from soybeans or peas lupin seeds might become a promising source for food ingredients due to their high protein content and their excellent functional properties of the proteins and the nutritional fibers. For example protein meals from *Lupinus angustifolius* or *Lupinus albus* show excellent emulsifying and foaming properties. But they also contain green and beany flavor notes that might limit their use in food applications. Thus strategies to reduce the off-flavors and to maintain the functional properties of the protein and fiber fraction are needed to fulfill the requirements of the consumers.

By the use of optimized extraction processes as a combination of solvent and aqueous extractions protein fractions can be obtained with outstanding sensory and functional properties that can be applied in almost all foods to replace animal protein. So protein isolates from *L. angustifolius* that are rich in  $\alpha$ - and  $\beta$ -conglutin and isolated from neutral protein solutions to so-called LupiE, enable a total substitution of egg yolk protein in bakery goods or food emulsions such as mayonnaise or dressings. Also a substitution of casein is possible by the use of LupiE to create products such as alternatives for milk, ice-cream, yoghurt, cheese or spreads with excellent texture, mouthfeel and sensory properties. The unique foaming properties and the high solubility of ultra-filtrated LupiF, mainly consisting of  $\gamma$ -conglutin is a highly functional ingredient that can be used to create whipped cream, foamed fine bakery goods or clear refreshing drinks for sports and nutrition. These findings underline the high potential of isolated lupin proteins for applications in food industry.

On the other hand the solvent based aqueous protein isolation is a very expensive process. Thus challenges for the use of these kinds of lupin ingredients might be based on economic limitations. Existing processes show that the required high effort for de-oiling, low yields of extraction based on low protein contents in the raw materials can lead to costs of the proteins that are not acceptable for the food industry. It will be crucial for the success of lupin proteins as food ingredients to optimize and simplify existing technologies and to increase the protein content in the kernels. As *L. angustifolius* is quite limited in this component, *L. albus* and *L. mutabilis* might be promising sources for future applications.

# AGRONOMIC AND MANAGEMENT CONSTRAINTS IN CHOCHO OR TARWI CROP IN ECUADOR

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The chocho, tauri o tarwi crop (*Lupinus mutabilis* Sweet), during centuries has maintained a low importance level as a crop by itself or as a food staple within the traditional agrosystems. Nevertheless, after two decades of researching and strengthening efforts done by the National Agropecuarian Research Institute of Ecuador (INIAP, 1977-2017), actually occupy a relevant participation not only in agri productive systems, but also in agroindustrial sector as a part of nutritional, health and food programs. As a consequence, these efforts has incentivized the cultivated areas, crop yielding, and product consumption and processing. But in contrast, unsolved important constraints has been detected at agronomic level and at the crop socioeconomic participation is concern. In one hand, at farm level biotic and abiotic hazards are permanently present affecting in numerous occasions the crop sanity; and at the other hand, problems of the crop socioeconomic participation are detected, such as crop zonification, planting dates, and climate changes incidence. The both increasing demand of the product and attractive market prices, are promoting to incorporate new productive areas located in non-optimal natural conditions, with almost monthly planting dates along cropping season, and at inconvenient altitude ranges out of recommended (2800 – 3400 m asl). Traditionally chocho crop is planted in sandy and mild sandy soils of Cotopaxi and Chimborazo provinces of Ecuador. These soils due to the volcanic origin have poor content levels of nitrogen, phosphorus, and organic matter. As not all farmers apply the recommended doses of fertilization yields subsequently are low. The productive parcels are located at slopping areas which are labored by tractors causing consecutive soil erosion problems. Another technical constraint is referring to seed quality and availability, which is produced mainly by non-conventional systems. Nowadays, nine insect plagues have been identified: bean seed fly (*Delia platura*), soil cut worm (*Barotheus castaneus*), cutworm (*Agrotis sp.*), apex stem borer (*Anthomyiidae*), lower stem borer (*Melanagromyza sp.*), bugs (*Proba sallei*), leafminer fly (*Liriomyza sp.*), trips (*Frankliniella sp.*), and moth (*Lepidoptera*). Their control is exclusively chemical. In terms of diseases the following fungus problems are detailed: anthracnose (*Colletotrichum acutatum*), ascochyta blight (*Ascochyta spp.*), ring spot (*Ovularia lupinicola*), cercospora leaf spot (*Cercospora spp.*), lupin rust (*Uromyces lupini*), rooting rust (*Fusarium oxisporum*), and rhizoctonia (*Rhizoctonia solani*). With this wide sanitary spectrum farmers have serious difficulties to achieve satisfactory economic results. Finally, at harvest time farmers have to face up scarcity of harvesting machinery (availability and harvest costs), and how to manage uncontrolled weeds to obtain efficient operation of harvest pieces of equipment.

## ***Lupinus albus* IS THE SPECIES THAT ACHIEVES GREATEST GRAIN AND PROTEIN YIELDS IN CHILE**

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Lupin species originated in the Mediterranean basin have shown to be the sound options to produce plant protein in Chile and it is desirable to gather more information in order to allocate future funds for breeding, research and development preferentially to the species with greater potential. During cropping seasons 2015-16 and 2016-17 we evaluated three species of *Lupinus*, under the agronomic management recommended for each one, at four locations in the southern zone, between parallels 38 and 40° S. Nine cultivars/lines were considered, four *L. albus* (three obtained in Chile and one in France), three *L. angustifolius* (two obtained in Chile and one in Australia) and two *L. luteus* (one obtained in Chile and one in Poland), in two sowing times (autumn and winter). *L. albus* achieved the highest grain and protein yields and, within this species, the Chilean materials out yielded the French cultivar. Autumn sowing was more favorable than winter sowing for *L. albus*, whereas the yields of *L. angustifolius* and *L. luteus* were virtually unaffected by sowing time. Sown in autumn, the Chilean materials of *L. albus* produced 5 Mg ha<sup>-1</sup> as average of sites and years, while *L. angustifolius* and *L. luteus* reached 78% and 53% of that, respectively. *L. albus* cv Alboroto-INIA achieved an average yield of 4.7 Mg grain ha<sup>-1</sup> in the first cropping season and 5.7 Mg grain ha<sup>-1</sup> in the second one. *L. luteus* accumulated the highest crude protein content in the whole grain (41.1% dry matter basis), followed by *L. albus* (34.4%) and then by *L. angustifolius* (29.0%). However, the greater productivity of *L. albus* positioned it as the most efficient species in terms of protein produced per hectare, exceeding 1.5 Mg protein ha<sup>-1</sup> and occasionally 2 Mg protein ha<sup>-1</sup> under favorable conditions for the crop. *L. angustifolius* y *L. luteus* produced at most 62% y 68%, respectively, of the protein produced by *L. albus*.

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## ***Lupinus albus* ES LA ESPECIE QUE PRESENTA MAYORES RENDIMIENTOS DE GRANO Y PROTEÍNA EN CHILE**

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Las especies cultivadas de lupino originadas en el Mediterráneo se han perfilado como buenas opciones para producir proteína vegetal en Chile y es deseable contar con más información para asignar futuros fondos para mejoramiento genético, investigación y desarrollo, preferentemente a la especie con mayor potencial. Las temporadas agrícolas 2015-16 y 2016-17 se evaluó el comportamiento de tres especies de *Lupinus*, con el manejo agronómico recomendado para cada una, en cuatro localidades de la zona sur, entre los paralelos 38 y 40° S. Nueve variedades y líneas fueron consideradas, cuatro de *L. albus* (tres obtenidas en Chile y una en Francia), tres de *L. angustifolius* (dos obtenidas en Chile y una en Australia) y dos de *L. luteus* (una obtenida en Chile y una en Polonia), en dos épocas de siembra (otoño e invierno). *L. albus* fue la especie que alcanzó los mayores rendimientos de grano y proteína, y, dentro de ella, los materiales chilenos superaron a la variedad francesa. La siembra otoñal fue más favorable que la invernal para *L. albus*, en tanto que los rendimientos de *L. angustifolius* y *L. luteus* fueron poco afectados por la época de siembra. Sembrados en otoño, los materiales chilenos de *L. albus* produjeron como promedio de sitios y años 5 Mg ha<sup>-1</sup>, en tanto que los de *L. angustifolius* and *L. luteus* alcanzaron 78% y 53%, respectivamente, de este valor. La variedad Alboroto INIA (*L. albus*) alcanzó un rendimiento promedio de 4,7 Mg ha<sup>-1</sup> de grano la primera temporada y 5,7 Mg ha<sup>-1</sup> la segunda. *L. luteus* concentró mayor porcentaje de proteína cruda en el grano entero (41,1% base materia seca), seguida de *L. albus* (34,4%) y luego de *L. angustifolius* (29,0%). No obstante lo anterior, la mayor productividad de *L. albus* la situó como la especie más eficiente en producción de proteína por hectárea, superando 1,5 Mg ha<sup>-1</sup> y ocasionalmente 2 Mg ha<sup>-1</sup> bajo condiciones favorables para el cultivo. *L. angustifolius* y *L. luteus* produjeron no más de 62% y 68%, respectivamente, del rendimiento de proteína de *L. albus*.

Reconocimientos: FONTAGRO, Proyecto FTG/RF-14893-RG; Ministerio de Agricultura de Chile, Proyecto 502610-70.

# FINDING A MARKET FOR (WHITE) LUPINS IN THE NORTH-WESTERN PART OF EUROPE

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The opportunities for the increase in cultivation of lupins and their use for food and feed already seem to be very favourable for some time now. The awareness within the EU of its high dependency on imported soya, the need for diversification of crop rotations with leguminous crops to reduce fertilizer input and increase of biodiversity and the growing realisation that a protein transition from animal based diets to plant based diets all seem to be working in favour of promoting the cultivation of grain legumes in general and lupins in particular. This increased attention for local grown grain legumes seem to have resulted in a considerable increase in area of most grain legumes since the low point of cultivation in 2008. Although the increase of other grain legumes like peas, broad beans and soy is found in several European countries, the increase of lupin cultivation is mainly found in Poland where 75% of the production takes place and the adjacent northeastern part of Germany with 11% of the area. In the rest of Europe it seems that lupins are not benefitting in the same way from the increased opportunities of EU grown plant protein.

The objective of this article is to identify opportunities and obstacles for increasing the cultivation and processing of white lupins in northern Europe. Experiences in The Netherlands and Germany with the promotion of these lupins among farmers and food/feed industry are gathered to find important factors for success.

For several decades efforts have been made in the The Netherlands and Germany to increase the area of cultivation of the three lupin species used in European agriculture. Although a substantial area of cultivation of narrow-leafed lupins for feed can be found in the northeastern part of Germany with a dominance of low fertile soils, this area does not seem to increase. Going west in Northern Europe, cultivation of lupins as a protein source for feed is largely unprofitable as other protein sources like faba beans and imported soy are more economical. The cultivation here is restricted to livestock farmers, mainly organic, that want to grow part of their own feed protein. Due to a growing interest in plant based foods attempts have been made to create a premium market for lupins as a food ingredient. Although lupins seem to be a perfect candidate for this, the industry has little experience with this new food ingredient. The reluctance has even increased since lupins have been added to the list of allergens in the EU in 2012. Despite these hindrances some new products based on narrow leafed lupin have been introduced to the market: plant based dairy (Prolupin) and meat replacers (Meatless, Vivera, Vegetarian Butcher). Narrow-leafed lupins are used for this as they can be produced relatively cheap in the extensive areas of eastern Europe and Australia and their round form makes them more suitable for processing (eg protein concentration). White lupins need more intensive care to grow successfully. The use of white lupins as a food ingredient however is different from narrow-leafed lupin as they need less processing due to their thinner seed coat. This makes white lupins more suitable for direct consumption and minimal processing. Both in The Netherlands and Germany efforts have been made to focus on the increase of white lupin production for human consumption. The market is found in the organic sector where there is a larger demand for non or minimally processed food items which can be regionally grown. Although this market is growing, a lot of unlocked potential is still waiting for this new 'superfood' to become more widely available.

## THE LUPIN MARKET: THE EXPERIENCE OF CHILE

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The climate of the long and narrow strip of Chilean territory in the portion of the Center-South of the country, is Mediterranean, with rainy winters and dry summers. Much of the soil is of volcanic origin and pH-neutral to acid. In ancient times only in the part of the ancient Inca Empire was produced and consumed *Lupinus mutabilis*, known with the name of "chocho" or "tarwi", and its cultivation did not extend to the South as the potato and maize did. The only native species in the latitudes of the Center-South of Chile is *Lupinus microcarpus*, but over time they were introducing other species as *L. angustifolius*, *L. arboreus* and *L. polyphyllus*. In 1949 it was introduced in the area the first sweet spring varieties of *L. luteus* and *L. albus*, primarily as forage resource. In 1969 was the first spring sowing of the variety Multolupa of *L. albus* which resulted from the cross of a bitter winter cultivar of lupine from Italy, with a sweet spring variety from Germany. A further selection of winter crops of this variety allowed to produce, under a system of "Contract with Disinfected Seeds ", up to 40,000 hectares in the country. On the basis of this germplasm have emerged new varieties which are currently being cultivated in Chile and other countries. The first massive demand for lupine grain in Chile was for poultry feeding, in replacement of fish meal. Subsequently, and in parallel with the plant breeding of Lupine, works of F. Monckeberg, E. Yañez and others, allowed the acceptance of sweet lupine flour, to be used in products for human consumption especially for infants and preschoolers. The lupins are in permanent competition with soy products, which currently have increased demand for protein-energetic complement food for salmon. Currently the lack of renewal of new "disinfected sweet seed" for sowing, mainly by indigenous farmers, produce a resurgence of bitter types, which together with larger calibers grain achievement, produced a derivation of the production of the lupines grown in Chile for export to Europe and Asia. Lupine human consumption of derived lupine products in Chile, is currently focused in the use as protein complements of food such as dried soups, cookies and desserts.

## EL MERCADO DEL LUPINO: LA EXPERIENCIA DE CHILE

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El clima de la larga y angosta franja del territorio chileno en la porción del Centro-Sur del país, es mediterráneo, con inviernos lluviosos y veranos secos. Gran parte del suelo es de origen volcánico y de pH neutro a ácido. En la antigüedad sólo en la parte del antiguo incanato se producía y consumía *Lupinus mutabilis*, conocido con los nombre de "chocho" o "tarwi", pero su cultivo no se extendió hacia el sur como lo hicieron la papa y el maíz. La única especie autóctona en las latitudes del centro-sur de Chile es el *Lupinus microcarpus*, pero con el tiempo se fueron introduciendo otras especies de *Lupinus* como el *L. angustifolius*, *L. arboreus* y *L. polyphyllus*. En 1949, se introdujeron en el área las primeras variedades primaverales dulces de *L. luteus* y *L. albus*, principalmente como recurso forrajero. En 1969, se realiza la primera siembra primaveral de la variedad Multolupa de *L. albus* que resultó de la cruce de un cultivar de lupino amargo invernal de Italia, con una variedad primaveral dulce de Alemania. Una posterior selección de siembras invernales de esta variedad permitió producir, bajo un sistema de "Contrato con Semilla Desinfectada", hasta 40.000 hectáreas en el país. Sobre la base de este germoplasma han surgido nuevas variedades que están actualmente siendo cultivadas en Chile y en otros países. La primera demanda masiva de grano de lupino en el país fue para la alimentación de aves, en reemplazo de la harina de pescado. Posteriormente, y en forma paralela al mejoramiento vegetal del lupino, los trabajos de F. Monckeberg, E. Yañez y otros, permitieron la aceptación de la harina de lupino dulce, para ser usada en productos de consumo humano, para lactantes y preescolares. Los lupinos están en competencia permanente con los productos de soya, que actualmente tienen la mayor demanda para la complementación proteica-energética de los alimentos para salmones. En la actualidad la falta de renovación de semilla "dulce desinfectada" para las siembras, principalmente por campesinos indígenas, produjo un resurgimiento de los tipos amargos, que junto con los mayores calibres de grano logrados, produjeron una derivación de la producción de los lupinos cultivados en Chile para la exportación a Europa y Asia. En Chile el consumo de derivados de lupinos para consumo humano en la actualidad está focalizado en el uso como complementos proteicos de alimentos como sopas deshidratadas, galletas y postres.



The image features abstract, layered geometric patterns in shades of purple, pink, and grey. These patterns consist of various lines, curves, and overlapping shapes, creating a sense of depth and movement. The patterns are most prominent in the top and bottom corners, framing the central text.

**ABSTRACTS  
OF TECHNICAL SESSIONS  
(in alphabetical order)**



**FOOD, FEED & NON-FOOD USES**

**ALIMENTOS, FORRAJES  
Y USOS NO ALIMENTARIOS**

## DETERMINATION OF THE PHYSICAL, CHEMICAL AND NUTRITIONAL PROPERTIES OF TARWI INSTANT FLOUR (*Lupinus mutabilis*)

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The objective of this article is to determine the physical, chemical and nutritional properties of tarwi flour; for which the tarwi bean was debittering by the "Cuzco" method recommended by Tapia (1981), then the tarwi flour was elaborated and the physical (solubility, absorption and granulometry), chemical and nutritional analyzes (index of acidity, peroxide index, alkaloids, humidity, fat, fiber, ash, carbohydrates, protein) were carried out. A factorial design 2x2 was applied, having as dependent variables: time and temperature, independent variables: solubility, absorption, acidity index granulometry, peroxide index, alkaloids, humidity, fat, fiber, ash, carbohydrates and protein. The adequate results were obtained for the preparation of tarwi flour in a parabolic oven at a humidity of 10.5% and temperature 130 °C x10 min approximately, then it was grinded in a hammer mill, obtaining a flour of 0.75 mm in diameter. Obtaining an average solubility of 67.56% to 80.20%, water absorption index 2.789% to 3.168%, granulometry 1.31mf to 1.46mf, acidity index from 0.04% to 0.10%, peroxide index 5.349mEqO<sub>2</sub>/Kg to 8.177mEq O<sub>2</sub>/Kg, alkaloid 0.04% to 0.06%, moisture 8.017% to 2.73%, fat 24.21% to 24.74%, fiber 8.53% to 9.91%, ash 1.383% to 1.92%, carbohydrates 3.772% to 9.164% and protein 54.03% to 57.06%, these data are considered acceptable to be within the range for instant meals, established by the Peruvian norms and CODEX. Instant flour of tarwi was elaborated and according to its physical, chemical and nutritional properties are suitable for human consumption.

## DETERMINACIÓN DE LAS PROPIEDADES FÍSICAS, QUÍMICAS Y NUTRICIONALES DE HARINA INSTANTÁNEA DE TARWI (*Lupinus mutabilis*)

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El objetivo del presente artículo es determinar las propiedades físicas, químicas y nutricionales de harina de tarwi; para lo cual se procedió a desamargar el grano de tarwi por el método “cuzco” recomendado por Tapia (1981), seguidamente se elaboró la harina de tarwi y se realizaron los análisis físicos (solubilidad, absorción y granulometría), químicos y nutricionales (índice de acidez, índice de peróxido, alcaloides, humedad, grasa, fibra, ceniza, carbohidratos, proteína). Se aplicó un diseño factorial 2x2, teniendo como variables dependientes: tiempo y temperatura, variables independientes: solubilidad, absorción, granulometría índice de acidez, índice de peróxido, alcaloides, humedad, grasa, fibra, ceniza, carbohidratos y proteína. Se obtuvo los resultados adecuados para la elaboración de harina de tarwi en un horno parabólico a una humedad de 10.5% y temperatura 130 °C x10 min, seguidamente se llevó a molienda en un molino de martillo, consiguiendo una harina de 0.75 mm de diámetro. Obteniendo una solubilidad promedio de 67.56% a 80.20%, índice absorción de agua 2.789% a 3.168%, granulometría 1.31mf a 1.46mf, índice de acidez 0.04% a 0.10%, índice peróxido 5.349mEqO<sub>2</sub>/Kg a 8.177mEq O<sub>2</sub>/Kg, alcaloide 0.04% a 0.06%, humedad 8.017% a 2.73%, grasa 24.21% a 24.74%, fibra 8.53% a 9.91%, ceniza 1.383% a 1.92%, carbohidratos 3.772% a 9.164% y proteína 54.03% a 57,06%, se consideran estos datos aceptables por estar dentro de rango para harinas instantáneas, establecidas por las normas peruanas y CODEX. Se elaboró harina instantánea de tarwi y de acuerdo a sus propiedades físicas, químicas y nutricionales son apropiadas para consumo humano.

## **OBTAINING A PROTEIN ISOLATE FROM LUPIN CAKE (*Lupinus mutabilis* Sweet) AND EVALUATION OF ITS TECHNO-FUNCTIONAL PROPERTIES**

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Lupin (*Lupinus mutabilis* Sweet) is a legume of Andean origin with strategic importance in nutrition because its high protein content for a population that develops rapidly, also this legume is rich in unsaturated oils. The oil industry obtains good quality oil from oilseeds, leaving as residue the cake.

The present research seeks to take advantage of the defatted cake, subjecting it to an extraction alkaline evaluating the parameters: extraction pH, raw material ratio: solvent of extraction, temperature and time of extraction; in such a way that with the best parameters determine their techno-functional properties.

The lupin grains were debittered by the aqueous method, dried, milled, sieved and the oil extracted by press Expeller defatting the cake later. The variables evaluated for protein extraction were: pH, defatted flour/ solvent, temperature and time where the best parameter was the one of the greater absorbance obtained by the spectrophotometric method of Lowry at 750nm; the techno-functional properties determined for protein isolate were: maximum solubility, water holding capacity, foaming capacity, foaming stability, emulsifying capacity and gelification capacity.

The best parameters for protein extraction were 10.5; 1/25; 50 °C and 60 minutes respectively. The techno-functional properties determined for lupin protein isolate were: maximum solubility (87.74% at pH 8) and minimum solubility (19.31% at pH 4); maximum water holding capacity (3.74 g of water / g of isolate at pH 2) and minimum water holding capacity (1.86 g of water / g of isolate to pH 4); foaming stability around 30 percent (1% concentration and after 120 minutes); gelification capacity of 15 %; emulsifying capacity 63.38 %; emulsion stability 50.51 % and whiteness index 59.59.

The extraction yield of protein isolate was 57.8 % with respect to the defatted flour of tarwi cake; with the established extraction parameters lupin protein had 91.79 % of protein content and the techno-functional properties with business ranges were solubility, water holding capacity, oil absorption capacity and emulsifying capacity.

## **EL TARWI (*Lupinus mutabilis*) Y SU IMPORTANCIA SOCIAL Y ECONÓMICA EN LAS FAMILIAS DEL ALTIPLANO NORTE DE BOLIVIA**

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El tarwi en Bolivia es cultivado en cuatro departamentos, con un rendimiento medio de 637 kg/ha, y una producción total anual de 1208 t en una superficie de 1895 ha. Investigaciones anteriores mencionan que en la comunidad de Carabuco del municipio de Puerto Mayor Carabuco existe una superficie cultivada de 17,8 ha de tarwi, representando el 21% del total de la superficie cultivada con rendimiento promedio de 1718 kg/ha cuyo mercado de comercialización está situado en las comunidades de Escoma y Korahuasi. Es por esta razón que el objetivo del presente estudio es evaluar la importancia del tarwi en las familias del municipio de Puerto Mayor Carabuco del altiplano Norte de Bolivia, desde el punto de vista productivo, de comercialización y de consumo. Para alcanzar tal fin se desarrollaron talleres participativos, encuestas a 105 agricultores de 22 comunidades del municipio y el seguimiento durante tres años y tres meses de los precios y cantidades comercializadas de tarwi. El tratamiento de la información fue realizado con el programa estadístico Stata ver 12.0, también se deflactaron los precios con el fin de obtener precios reales. Entre los principales resultados se tiene que en la gestión agrícola 2010-2011 y 2011-2012, el 25% de los agricultores sembraron tarwi con precios de comercialización diferenciados entre grano de buena y mala calidad, donde el consumo en la región es muy bajo, pudiendo señalar que el cultivo tiene importancia, debido a que los productores pueden almacenar el grano por largos periodos de tiempo entre uno a dos años ofertándolo en las ferias locales principalmente cuando tienen alguna necesidad económica o cuando los intermediarios asignan mayores precios por la compra de este producto.

## DETERMINACIÓN DE PARÁMETROS DE ELABORACIÓN DE BEBIDA PROBIÓTICA DE TARWI (*Lupinus mutabilis* Sweet) EMPLEANDO *Saccharomyces boulardii*

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El objetivo del presente trabajo fue determinar los parámetros para la elaboración de una bebida probiótica de tarwi (*Lupinus mutabilis* Sweet) empleando *Saccharomyces boulardii*. La bebida de tarwi, fue elaborada a partir de la obtención de un extracto acuoso con una relación de 1:2 (tarwi-agua). Seguidamente se preparó los sustratos para evaluar el crecimiento cinético de *Saccharomyces boulardii* de acuerdo al diseño factorial 2<sup>2</sup>, cuyas variables en estudio fueron el % de sacarosa (6-12 °Brix) y el % de inóculo, a una temperatura de 37°C y tiempo de fermentación de 24 horas. Se evaluó el recuento de levaduras (ufc/ml), °Brix, pH, acidez, densidad y viscosidad. Luego se procedió a elaborar la bebida probiótica de tarwi, y se realizó una evaluación sensorial utilizando una escala hedónica de 9 puntos, encontrándose que la mejor combinación fue 6% sacarosa y 4% de inóculo. Finalmente se analizó las propiedades físico-químicas de la muestra. Los datos obtenidos fueron: pH 5.40, materia seca 8.11%, humedad 91.99%, proteína 44.05%, grasas 15.32%, cenizas 3.50%, acidez 0.26%, alcohólico 0.98%, sólidos totales 8.11%, densidad 1.025g/ml. Este producto a base de tarwi (*Lupinus mutabilis*) presentó características potenciales de una nueva bebida probiótica dentro del grupo de productos no lácteos.

## NUTRITIONAL CHANGES IN ANDEAN LUPIN (*Lupinus mutabilis*) AFTER PROCESSING

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Andean Lupin (*Lupinus mutabilis*) is a high-nutrient food (especially rich in protein and lipid). Lupin requires an aqueous debittering during food processing, previous to consumption, due to its high level of bitter quinolizidine alkaloids. Lupin samples were processed in a pilot plant and laboratories of Universidad Nacional Agraria La Molina. Debittered lupin is suitable food products such as extruded flours, aqueous drinks or rehydratable powders. With the aim of the characterization of nutritional changes after food processing the composition, protein in-vitro digestibility, color and water activity ( $A_w$ ) were assessed in three different genotypes (Andenes, Altagracia and Yunguyo). After food processing, significant changes were observed ( $p < 0.05$ ) for protein and lipid content, color and in-vitro digestibility. These results were different to Europe lupins (*L. albus* and *L. angustifolios*). The untreated lupins had high protein and lipid content (47.4 and 16.2 g/100 g DM). The process of debittering and extrusion change the total content of nutrients of the Andean lupin. Protein digestibility was highest in samples of atomized lupin (72.8 – 74 %). Finally, nutritional content varied according with genotype and food process.

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## BIOCHEMICAL COMPOSITION OF LUPIN SEEDS FROM VIR COLLECTION

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The use of lupine as a raw material for industry is due to its chemical composition and biological value. Seeds of different species of lupine contain up to 50% of protein and up to 20% of oil. Lupine oil has a whole complex of useful properties, and the lupine meal remaining after seed processing is very rich in proteins and is valued in the preparation of feed. Lupine oil is widely used in the food industry, cosmetology, pharmacology. The VIR collection includes about 3000 samples and over 50 species of lupine of various origins. Collection samples are a valuable source of material for breeding. The collection contains samples of lupine, having a significant amount of oil with an optimal ratio of fatty acids. We have performed chemical analysis and determined the oil fatty acid profile of seeds from *L. elegans* Kunth., *L. nanus* Douglas ex Benth., *L. ornatus* Douglas ex Lindl., *L. pubescens* Benth. Samples were grown in Leningrad and Tambov region. The oil content in samples of lupine seeds was determined by the dry skim residue using Soxhlet equipment. Protein content was studied using the Kjeldahl method using KjelttecAuto 1030. The fatty acid composition was determined using gas chromatograph Agilent 6850. The content of the eleven fatty acids was determined: lauric (C 12: 0), myristic (C 14: 0), palmitic (C 16: 0), palmitoleic (C 16: 1), stearic (C 18: 0), vaccenic (C 18:1 s 11) oleic (C 18: 1), linoleic (C 18: 2), linolenic (C 18: 3), arachic (C 20), behenic (C 22: 0). Fat content ranged from 7.6% (*L. nanus*) to 10.6% (*L. pubescens*). The most variable on this trait was the *L. elegans*, whose oil content varied from 7.15 to 11.32%. High content of oil (9.88-11.81%) was noted in *L. pubescens*. All lupin oils contained a high concentration of linoleic, oleic and palmitic acid, ranging from 33 to 63%, 16 to 31 and 8 to 20%, respectively. From a dietetic point of view, oil quality is more important than oil quantity in lupin seeds. On average the fatty acid in examined accessions ranked in following order of abundance: linoleic acid (C 18: 2) > oleic (C 18: 1) > palmitic (C 16: 0) > linolenic (C 18: 3) > stearic (C 18: 0) > arachic (C 20) > behenic (C 22: 0) ≈ lauric (C 12: 0) ≈ myristic (C 14: 0) ≈ palmitoleic (C 16: 1) ≈ vaccenic acid (C 18:1 cis 11). It has been shown that in lupine seed oil the main fatty acids are linoleic (38-58%), oleic (14-37%), palmitic (8-19%) and linolenic (3-12%). The highest mean values of linolenic acid (53%) was found in samples of *L. elegans*. The average oleic acid content is about 27%. For *L. nanus* and *L. pubescens*, its high content was noted (31 and 32%, respectively). Linolenic acid content ranged from 3.7% (*L. pubescens*) to 9.5% (*L. nanus*). An important indicator of the value of oil when used in nutrition is the ratio of linoleic and linolenic acids. In the studied species of lupine, this ratio varied from 4.5 to 12.2. The most interesting in this respect is *L. nanus*, for which this indicator is 4.5. In the studied samples, the trace amount detected for the following unsaturated fatty acids palmitoleic (0.01-0.6%) and vaccenic (0.05-0.6%). In addition, in the studied samples, an insignificant content of such saturated acids as stearic (0.51-2.76), lauric (0.01-1.49%), myristic (0.04-0.13%), behenic (0.09-1.82%) and arachidic acid (0.19-1.49%). In addition to the oil content, the protein content was determined. In all studied samples, it exceeded 34%. Protein from lupin seeds ranged from 34 to 50%. The highest protein content in seeds was observed in *L. ornatus* 42.3-49.7% (46.1% on average). It should be noted that in some species, a high content of protein and oil are combined. In particular, *L. pubescens* has defined average protein levels at 45.6% and oils 11.1%. Sample (k.1322) combined high oil content (11%) and protein in seeds (44%). Thus, we conclude that the oil content and fatty acid profile of the 4 species of lupin studied are similar to those already reported for most lupin oils. These species are characterized by a high content of linoleic (31-59%) and oleic (15-48%) acids. Further study allows to identify samples that can be used in breeding and industrial production.

## PHYSICOCHEMICAL PROPERTIES OF PROTEIN ISOLATES FROM TWO MEXICAN NATIVE *Lupinus* SPECIES (*Lupinus mexicanus* AND *Lupinus exaltatus*)

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Proteins are used in the manufacturing of a variety of food products. As of today there are numerous studies that report the physicochemical qualities of vegetable proteins of domestic and semi-domestic species, of the genus *Lupinus*; however, few studies have investigated native species. In this work we determined the physicochemical properties of protein isolates from two Mexican native *Lupinus* species (*L. mexicanus* y *L. exaltatus*). The protein isolate was obtained by means of isoelectric precipitation and the tests determined included: solubility, gelling and emulsifying capacity, foaming, and water and oil retention in the 2-10 pH range. pH had an effect on all the physicochemical properties, especial at extreme pH values (2, 10). The water retention was 48.63% and 38.58% for *L. mexicanus* and *L. exaltatus*, respectively. However, at pH 4 and 7, water retention decreased. Oil absorption capacity for *L. mexicanus* was 23.66% while for *L. exaltatus* 24.63%. *L. mexicanus* protein isolate formed stable foam at pH 2 and 10. Conversely, *L. exaltatus* protein isolate formed weak foam at pH values of 2, 7, 8, and 9. In conclusion, protein isolate from native lupine species displayed different functional properties depending on pH and can be exploited in food systems.

# DEVELOPMENT OF LUPIN-BASED YOGURT ALTERNATIVES – CHALLENGES REGARDING PROCESSING AND FERMENTATION

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Interest of consumers in plant-based dairy alternatives has been rising in recent years, due to ethical and sustainability issues. So far, main research of dairy alternatives has been focused on soy-based products. However, lupin protein is another promising source for the development of yogurt alternatives. As lupin protein is considered as poor gelling agent in comparison to soy this study was focused on the factors influencing the texture of the lupin-based yogurt alternatives (LBYA). For the development of the LBYA, the traditional process for cow milk yogurt production was adapted. Therefore, a stable emulsion composed of lupin protein isolate from *Lupinus angustifolius* cv. Boregine, glucose and fat was homogenized and pasteurized (80 °C, 60 s) or ultra-high temperature heated (140 °C, 10 s). This milk-like substrate was inoculated with different lactic acid bacteria and respective products were evaluated after fermentation according to their aroma and texture, as well as to the acidification performance of the lactic acid bacteria. For LBYA production several differences to the production of cow milk yogurt production were obvious. A screening of 35 different lactic acid bacteria showed that the typical yogurt starter cultures *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* were not the most promising microorganisms for the production of LBYA. *Lactobacillus plantarum* TMW 1.460 and TMW 1.1468, *Pediococcus pentosaceus* BGT B34 and *Lactobacillus brevis* BGT L150, which are known from raw sausage fermentation and as beer-spoilage bacteria, were more promising regarding aroma and texture. In addition, quite high inoculation volumes of 8.0 log<sub>10</sub> colony-forming units per millimeter were needed to ferment the lupin-based milk alternative (LBMA) properly. In despite of these high inoculation volumes, quite long fermentation durations were obvious (14-35 h) to reduce the pH from 7.0 to 4.5, which might be attributed to the usage of non-adapted microorganisms. Back-slopping experiments confirmed that repeated re-inoculations of fresh LBMA reduced the fermentation duration of about 10 h as the lag-phase disappeared. Also process management was crucial for the texture of LBYA. The intensity of heat treatment of the LBMA prior to fermentation highly influenced the texture of respective products and growth performance of the used starters. A more intense heat treatment resulted in LBYA with better textural appearance, but fermentation times were significantly longer. It is supposed that with higher heat treatment prior to fermentation, proteins are unfolded to a higher extent. Reactive side chains, which were buried in the core and were exposed due to heat treatment, were more prone to cross-linking such as disulfide bondings. These higher amounts of linkages were visualized with microstructural observations. This study showed that lupin proteins despite of the poor gelling properties, are a promising ingredient for LBYA production. With respect to the texture of LBYA, the choice of microorganisms, as well as process management is different to the production of cow milk yogurt and influences the yogurt texture. Future approaches should be focused on the development of starter cultures for LBYA production with high adaption to the fermentation medium, in order to reduce fermentation durations and inoculation volumes by maintaining a promising yogurt and aroma.

## LA RED DE LUPINO PAISANO (COTOPAXI ECUADOR) Y SU RELEVANCIA PARA FORTALECER LA SOBERANÍA ALIMENTARIA

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El objetivo de este trabajo es explicar las características que tiene la producción, procesamiento, comercialización y consumo del lupino denominado en los páramos de la provincia de Cotopaxi (Ecuador) como “chocho paisano”. El “chocho paisano” desde la producción en los páramos hasta el procesamiento y consumo en las tierras bajas, facilita la formación de asociaciones de distinta índole. Tales vínculos generan relaciones horizontales entre los productores, procesadoras y consumidores, produce transformaciones en los significados de la semilla y vincula a las tierras altas con los valles. Todo lo cual promueve la soberanía alimentaria.

El análisis se centra en la red de participantes con el lupino paisano que, durante la investigación pudimos constatar, que funcionaba de manera diferente a otros cultivos debido a sus orígenes culturales y ecológicos. En esta red, la producción de semilla de lupino se origina en los páramos, siendo un cultivo cuya producción está destinada en su totalidad al mercado; durante la etapa de producción, la semilla es identificada por su nombre kichwa, "tawri". Luego, el tarwi es vendido en las pequeñas ciudades (Chugchilan, Zumbagua) o en centros urbanos como Latacunga y Saquisilí. Es en estos dos lugares donde, principalmente las mujeres mestizas, lo desamargan, y lo vuelven comestible para luego venderlo en los mercados. Una vez procesado, su nombre y calidad cambian y es llamado 'chocho'. El regreso de este grano alimenticio a las tierras altas de origen, se debe a que los indígenas lo compran en los mercados durante los días de feria para su consumo ocasional; es así como el originario “tawri”, convertido por las mujeres procesadoras en “chocho”, se transforma nuevamente en un regalo apetecido, que lleva el nombre kichwa de "wanlla". Allí, adquiere un significado cultural muy especial para ese grupo étnico que lo consume. El trabajo etnográfico en el que se basa el documento, involucró seguir y trazar los distintos enlaces de la red lupino paisano. El estudio se inició en Guayama San Pedro, una pequeña comunidad indígena (en su mayoría de habla kichwa con muy poco conocimiento del español) con una población de unas 150 familias, en los páramos de la provincia de Cotopaxi (3500 msnm) y termina a unos 80 km de distancia, en la ciudad de Latacunga (2784 msnm) y en la ciudad comercial de Saquisilí (2943 msnm), ambas ubicadas en un valle interandino ecuatoriano.

Siguiendo la producción, el procesamiento y la comercialización del lupino, concluyo que el lupino paisano es una “nature-culture-food network”. En contraste con la forma moderna de producir, procesar y consumir alimentos, en la red del chocho paisano la producción, procesamiento y consumo del lupino facilita la reproducción de la cultura andina de los páramos ecuatorianos y facilita vínculos más horizontales entre los participantes en la red.

# PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF BREADS WITH WHITE LUPINE AND QUINOA FLOURS

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Flour is the product of the milling of grains from various plant sources. Besides cereals, flours can be obtained from legumes species, nuts, and tubers, among others; however, the most common flour is of wheat. In the food industry, flours are used as ingredients or additives to improve the technological characteristics of many processed foods, because have the ability to retain water, and depending on the physical and chemical characteristics of the starch granules, may cause changes in the rheology of the final product. In particular, the white lupine (*Lupinus albus* L.) and quinoa (*Chenopodium quinoa* Willd), are ancient grains which in recent times have gained great significance by its remarkable nutritional qualities by the high proportion of protein with essential aminoacids and without gluten. According to the above statements, the physical, chemical and functional properties of the white lupine (LB) and quinoa (HQ) flours were determined in order to ascertain its usefulness to be incorporated in fermented gluten-free products. For this purpose, the chemical composition of macro and micronutrients; the physical parameters, such as the microstructure, properties of filling and heating of starch granule, were determined as well as functional properties, retention of water-oil, rate of solubility and swelling capacity. The results showed that the nutritional value of the LB and HQ stands out since it contains quantities of proteins, fats, minerals, and dietary fiber. In addition, their profiles of amino acids are suitable to cover the daily intake recommended for an adult person, excelling its content of lysine, cysteine and methionine (sulfur amino acids), which is deficient in grains such as wheat. Likewise, they presented a high proportion of polyunsaturated fatty acids similar to corn and soybean, among them the essential  $\omega$ -3 fatty acid (0.4%) and  $\omega$ -6 (39%). In terms of physical characteristics, the microstructure of the LB and HQ starches, presented large spherical aggregates where they were starch granules with size, whose average were 4.68 and 1.33  $\mu$ m, respectively; the LB are oval-shaped and HQ are polygonal. The filling temperatures reported were 89-79° C, respectively. The LB presented a low viscosity while HQ was high, this indicates that LB may be suitable for products like cookies, muffins or nutritional bars and HQ may be used for products such as bread to improve the quality of the texture. Functional properties are based on the contents of starch, which for LB and HQ were 1.52 and 16.46%, respectively; the high content of this component allows an increase in the capacity of water retention, being higher in the HQ. Furthermore, the capacity of oil retention did not have significant differences ( $p > 0.05$ ) between these two flours, and the swelling capacity was higher than 80% in both of them. However, the solubility of the starch was greater in LB (7.7%) than in the HQ (2.5%). From previous data, we propose to use both flour as an alternative for gluten-free bread for the bakery industry, aimed to provide a good nutritional product to people with disabilities such as celiac disease or gluten high sensitive.

# PROPIEDADES FÍSICO-QUÍMICAS Y TECNO-FUNCIONALES DE PANES CON HARINA DE LUPINO BLANCO Y QUINOA

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La harina es el producto de la molienda de granos de cereales y de otros granos ricos en almidón. Otras fuentes vegetales para obtener harinas son leguminosas, frutos secos, tubérculos, entre otras; sin embargo, la harina más habitual es la del trigo. En la industria de alimentos, las harinas son utilizadas como ingredientes o aditivos para mejorar las propiedades tecnológicas que caracterizan a muchos de los alimentos procesados, debido a que tienen la capacidad de retener agua, y por tanto provocar cambios en la reología del producto final, dependiendo de las características físicas y químicas que presenten los gránulos de almidón. En particular, el lupino (*Lupinus albus*) y la quinoa (*Chenopodium quinoa* Willd), son granos ancestrales que en tiempos recientes han cobrado gran relevancia por sus cualidades nutritivas, entre las cuales se destacan la calidad de su proteína, que además de no poseer gluten, presenta un alto valor nutricional, pues tiene todos los aminoácidos esenciales y algunos en elevada proporción. Por lo expresado, se determinaron las propiedades físicas, químicas y tecno-funcionales de la harina de lupino blanco (LB) y quinoa (HQ), a fin de conocer su utilidad para ser incorporadas en productos fermentados libres de gluten. Para tal fin, fueron determinadas la composición química de macro y micronutrientes; los parámetros físicos como son la microestructura, propiedades de empastamiento y térmicas del gránulo de almidón; y las propiedades tecno-funcionales como capacidad de retención de agua-aceite, índice de solubilidad y capacidad de hinchamiento. Los resultados mostraron que destaca el valor nutricional de LB y HQ, ya que contiene cantidades elevadas de proteínas, grasas, minerales y fibra dietaria. Igualmente, su perfil de aminoácidos es apropiado para satisfacer las necesidades de ingesta diaria recomendada para una persona adulta, sobresaliendo su contenido en lisina, cisteína y metionina (aminoácidos azufrados), deficitarios en los cereales como el trigo. Así mismo, presentaron elevada proporción de ácidos grasos poliinsaturados similar al de maíz y soja, entre ellos el ácido graso esencial  $\omega$ -3 (0,4%) y  $\omega$ -6 (39%). En cuanto a las características físicas, la microestructura del almidón de LB y HQ presentaron grandes agregados esféricos donde se alojaban los gránulos de almidón con tamaños promedios de 4,68 y 1,33  $\mu$ m, respectivamente; los de LB son de forma ovalada y los de HQ son poligonales. Las temperaturas de empastamiento reportadas fueron de 89 y 79°C, respectivamente. La LB presentó una baja viscosidad mientras que HQ fue alta, esto indica que LB puede ser adecuada para productos como galletas, muffins o barras nutricionales y HQ podría usarse para productos como panes para mejorar la calidad en la textura. De las propiedades tecno-funcionales se destaca, el contenido de almidón modificado de LB y HQ que fue de 1,52 y 16,46%, alto contenido en este componente permite un aumento en la capacidad de retención de agua, siendo mayor en HQ. Por su parte, la capacidad de retención de aceite no presentó diferencias significativas ( $p > 0,05$ ) entre estas harinas. Entretanto, la capacidad de hinchamiento fue superior al 80% en ambas. Sin embargo, la solubilidad del almidón fue mayor en LB (7,7%) que en HQ (2,5%). Por todas las características que presentan las harinas estudiadas, se proponen como una alternativa para la industria de panificación sin gluten, donde se muestra una posibilidad de desarrollar estos productos para un grupo de la población con necesidades especiales como los celíacos o sensibles al gluten.

## STAKEHOLDERS IDENTIFICATION OF THE TARWI (*Lupinus mutabilis* Sweet) HIGH VALUE CHAIN IN THE PERUVIAN MARKET

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The commercial chain of tarwi in Peru has different actors, starting from primary production to final consumers. This list includes farmers, research centers, intermediaries and distribution centers in the cities of Huaraz, Cusco and Lima mainly. The production of tarwi in Ancash is carried out by low-income farmers located in the Andean highlands, it does not cover the local demand, so it is important to know the actions of the actors in the chain to improve production and identify potential markets for the tarwi and its derivatives, is important also the role played by chefs and restaurants, taking into account the boom of Peruvian cuisine, which may include tarwi. Therefore, the objective of this investigation was the identification of actors of the tarwi chain in the Peruvian market. The methodology used was an exploratory analysis that allowed us to identify the forms of participation of the actors in the chain. This research was carried out in the Ancash region.

The results of this work show that in the Ancash Region, considered as the main consumer center of tarwi in Peru, the research centers as generators and diffusion of new products participate as actors in the Peruvian market, ONGs that consolidate the associations of producers and the private companies that promote the consumption of tarwi and its derivatives. The linkage and greater interaction between the different actors could strengthen the tarwi trade chain, and improve the supply and promotion of the consumption and commercialization of high quality products and tarwi derivatives.

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## IDENTIFICACION DE ACTORES DE LA CADENA DE TARWI (*Lupinus mutabilis* Sweet) EN EL MERCADO PERUANO

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La cadena comercial del tarwi en el Perú cuenta con diferentes actores, partiendo de la producción primaria hasta los consumidores finales. Esta lista incluye a los agricultores, centros de investigación, intermediarios y centros de distribución en las ciudades de Huaraz, Cusco y Lima principalmente. La producción de tarwi en Ancash es realizada por agricultores de escasos recursos ubicados en zonas altoandinas, ésta no cubre la demanda local por lo que es importante conocer las acciones de los actores de la cadena para mejorar la producción e identificar los potenciales mercados para el tarwi y sus derivados, es importante también el papel que juegan los chefs y restaurants, tomando en cuenta el boom de la gastronomía peruana, que puede incluir el tarwi.

Por lo mencionado el objetivo de esta investigación fue la identificación de actores de la cadena de tarwi en el mercado peruano. La metodología utilizada fue un análisis exploratorio que ha permitido identificar las formas de participación de los actores de la cadena. Esta investigación se realizó en la Region Ancash. Los resultados del trabajo muestran que en la Región Ancash, considerada como principal centro consumidor de tarwi en el Perú, participan como actores en el mercado peruano los centros de investigación como generadores y difusión de nuevos productos, ONGs quienes consolidan las asociaciones de productores y las empresas privadas que promueven el consumo del tarwi y sus derivados. La vinculación y una mayor interacción entre los diferentes actores podrían fortalecer la cadena comercial del tarwi, y mejorar el abastecimiento y promoción del consumo y comercialización de productos de alta calidad y derivados del tarwi.

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# LUPIN CELL WALL POLYSACCHARIDES –INFLUENCE OF SEQUENTIAL EXTRACTION ON THE INTERACTION WITH BILE ACIDS

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Hypercholesterolemia is one major risk factor for coronary heart disease—the most frequent cardiovascular disease in developed and developing countries. It has already been demonstrated *in-vivo* that the consumption of lupin fiber contributes to the decrease of blood cholesterol levels in hypercholesterolemic adults. Recent *in-vitro* findings suggest that the interaction of lupin fiber with bile acids redounds to these cholesterol-lowering effects. This interaction can be differentiated in adsorptive effects and the entrapment of bile acids in viscous chyme matrices. However, which lupin fractions contribute to these interactions remains unclear. In the present study, lupin hulls and cotyledons were sequentially extracted to examine the interactions of lupin fiber fractions with primary bile acids *in-vitro*. Cotyledons of *Lupinus angustifolius* L. cv. Boregine were deoiled and proteins were removed by pronase hydrolysis. Hulls and cotyledons were milled and extracted with boiling ethanol. Alcohol insoluble residues were sequentially extracted yielding three fractions: pectin-like substances, a hemicellulose fraction and a lignocellulosic fraction. Additionally, lignin contents of hulls and cotyledons were analyzed. To assess the interaction of the maintained fractions with conjugated primary bile acids (cholic acids and chenodesoxycholic acids), digestion was simulated *in-vitro*. The chyme was dialyzed and released bile acids were analyzed by high performance liquid chromatography. Parameters of release kinetics, which are linked with the viscosity and the adsorption of the fractions, were calculated by non-linear regression. Lupin hulls essentially contained lignocellulosic substances (80% cellulose, 5% lignin) as do other legume seeds. Accompanying hemicellulosic and pectic polysaccharides accounted for 9% and 2%, respectively. In contrast, hemicellulosic fraction represented with 60% the main fraction of lupin cotyledons, while pectic substances and cellulosic fraction amounted to 14% and 6%, respectively. The pectic fractions of lupin hulls and cotyledons showed a significantly slower release rate of all tested bile acids due to the formation of a viscous network. But due to the low pectic content, considerable bile acid retardation in presence of lupin hulls could not be observed. In the case of lupin cotyledons a viscous effect was notable due to the retardation of bile acids. Bile acid adsorption could not be measured for the lupin hull but for the lupin cotyledone with 11% for chenodesoxycholic acids. However, extracted fiber fractions showed no adsorption. Our results showed bile acid adsorption of lupin is not directly attributable to the dietary fiber fraction. However, sequential extraction revealed that mainly pectin-like substances are responsible for the formation of entangled networks. Due to the characterization of components accountable for the viscous effect on bile acid reabsorption our results could contribute to the elucidation of cholesterol-reducing mechanisms of lupin dietary fibers.

## **DETERMINATION OF THE USEFUL LIFE TIME OF THE TARWI SEED OIL (*Lupinus mutabilis*) OF THE ANDENES VARIETY ANALYZED BY THE RANCIMAT METHOD**

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In recent years there has been an unusual interest in the cultivation of tarwi (*Lupinus mutabilis* Sweet), due to the valorization of its components, especially from the nutritional point of view. A profitable way to use the tarwi is to transform it into oil since it constitutes 20% of the weight of each grain. Therefore, the objective of this research was to determine the maximum shelf-life storage time in tarwi oil by accelerated testing at different storage temperatures using the rancimat method.

The preparation of the raw material to be analyzed was carried out (debit of the tarwi). We used seeds with an approximate humidity percentage of 8%, cleaning and selection, grinding and sieving, particles of size between 0.85 to 2.0 mm. The equipment used for the extraction of the oil was the expeller or continuous pressing, at a temperature of 72°C and with a nozzle No. 6, the centrifugation was performed at 4,000 rpm for 15 minutes. To determine the shelf life of the tarwi oil, it was introduced to the equipment with a preliminary preparation such as centrifugation of freshly extracted oil and assembly of the equipment, with the exact weight of the samples with a current of 20 L / h a temperature of 110 °C in the first row which towards the three repetitions in simultaneous and 120 °C in the second row which also towards the three repetitions simultaneously.

Our results shows tarwi oil at T = 110 ° C, induction time with an air flow of 20L / h, 1st repetition, 2nd repetition, 3rd repetition, were 19.96 h, 19.88 h, 17.67 h and 17.67 h respectively, tarwi to T = 120 ° C, induction time with an air flow of 20L / h, 1st repetition, 2nd repetition and 3rd repetition were 8.86 h, 10.53 h and 9.06 h respectively. With these results, the team's software determined an equation that relates the time of oxidative stability with the temperature at which the oil is located in order to find the shelf life:  $t = A * e^{(B * T)}$   $t = 213822.191475259 * e^{(-0.0846676076631618 * T)}$ . Therefore, the lifespan of tarwi oil is 4.49 years exposed to an ambient temperature of 20°C and it has a similarity in its composition with soybean oil.

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## DESAMARGADO DE TARWI (*Lupinus mutabilis*) ASISTIDO POR MICROONDAS Y ULTRASONIDO

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La especie de leguminosa - *Lupinus mutabilis* (tarwi) es un grano andino considerado un super food por PROMPERU (Comisión de Promoción del Perú para la exportación y el turismo), denominación promovida gracias a la denominación del año internacional de las legumbres del 2016 de la FAO. El tarwi se cultiva tradicionalmente en los Andes, y ocupa uno de los primeros lugares entre los alimentos nativos con elevado contenido de proteína y aceites a nivel mundial. Sin embargo, el grano requiere un tratamiento previo para su consumo, siendo necesario eliminar las sustancias anti nutricionales, como los alcaloides formados por esparteína, lupinina, lupanina entre otros. Esta situación hace que el consumo y producción de este grano sea reducido, ya que la extracción de los alcaloides genera mucho tiempo. El objetivo de esta investigación fue estudiar la cinética de extracción de lupanina del tarwi utilizando métodos como microondas y ultrasonido. Las muestras de tarwi se obtuvieron del INIA (Instituto nacional de investigación Agraria Puno) que fueron hidratadas por 24 horas a 25 °C en una relación 1 de tarwi y 5 de agua, luego fueron tratadas con microondas (Samsung mod.342-2017 ap3, Tokyo) a una potencia de 6, 7 y 9 w/g, y con ultrasonido (Branson Model B-1200-R1) a una potencia de 20 y 40 KHz. También se realizó una combinación de los dos tratamientos microondas y ultrasonido a 9 w/g y 40 KHz de potencia de micro ondas y ultra sonido respectivamente. Se analizó el agua de la extracción y se secaron las muestras de tarwi tratadas para evaluar por HPLC (metodología INEN) el contenido de alcaloide extraído. El tiempo de proceso ha sido realizado de acuerdo a la metodología descrita por Wageningen University, Product Design and Quality Management Group. Los resultados mostraron una reducción del 97% del contenido de alcaloides iniciales en los granos de tarwi en todos los tratamientos con las metodologías introducidas, el mejor tratamiento de micro ondas fue 9 w/g a intervalos de 2 minutos con un tiempo total de 30 minutos. La extracción a 40 KHz de potencia de ultra sonido ha permitido reducir el contenido de alcaloides en un tiempo total de 40 minutos, la combinación de 9 w/g y 40 KHz de potencia ha reducido el 97% de alcaloides en un tiempo total de 25 minutos con intervalos de 2 minutos. La reducción de alcaloides fue cuantificada por HPLC, determinándose un contenido inicial de 80 mg/kg en base seca a 2.4 mg/kg de lupanina. La investigación muestra que a nivel experimental se ha podido evaluar la eficiencia de la combinación de micro ondas y ultra sonidos en el proceso de desamargado de tarwi, lo que sugiere que estructuralmente los alcaloides se encuentran en fase líquida intra celular y el proceso ha permitido romper la membrana de los tejidos internos del tarwi para facilitar la difusión del alcaloide.

## OBTAINING A CRISPY SNACK OF LUPINE (*Lupinus mutabilis* Sweet) BY APPLYING DIFFERENT DEHYDRATION PROCESS

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In Latin America, the animal proteins consumption is very limited because of the cost of this nutrient. It is even less than 10% of dietary intakes recommended. Ecuador's National Health and Nutrition Survey refers that there is a nutritional transition where the consumption of simple sugars and saturated fats has been prioritized, especially in marginal areas with low economic income. Poor nutrition is a risk factor for the appearance of non-communicable chronic diseases. However, legumes such as lupine have vegetal origin protein and are alternative for the nutritional security of the population.

Therefore, the objective of this research was diversify the use and consumption of lupine, through the application of osmotic dehydration, drying techniques and the use of a topping to obtain a lupine crispy snack. It was worked with INIAP-450 lupine variety. During the research process, it was made a physical-chemistry characterization of the bean. Then the osmotic dehydration with different solutions was applied and it was complemented with a preference test with consumers in order to determinate the most accepted treatment in this stage. The osmotic dehydration of the lupine was subjected to two drying methods (microwave and forced air) until obtain a 10% of humidity approximately. Physical analyses (texture, color, grain size, humidity and water activity), chemical (protein digestibility and bioavailability of minerals) and yield, helped for the best treatment. Also, there was identified the cover to apply in the dehydration process of the bean with an acceptability sensory test. The physical and nutritional profile characteristics were analyzed in the crispy cover lupine and they compared with fresh phase of the lupine. The best conditions for obtaining the snack were the application of a sodium chloride and microwave drying. The application of topping was in a relation 1:1.125 (grain: topping) with a baking time of 35 minutes.

The crispy product presented a higher hardness in relation to a fresh lupine bean, with a humidity content of 1.52%, water activity of 0.15. The protein was 37.50% with a bioavailability of 82.85%, fat content (9%), fiber (8%) and ash (8%). In the product, bioavailability of iron was improved from 3.38 to 4.80%, zinc (0.92 to 0.97%), calcium (0.94 a 3.55%) and phosphorus (1.74 a 1.98%). The application of dehydration process, constitute a technology alternative for the food industry. This technique is helpful in order to dabble in new markets, increase consumption of lupine bean and extend shelf life of the product.

# STUDY OF LUPINE PRODUCTIVE CHAIN (*Lupinus mutabilis* Sweet) IN THE URBAN DISTRICT OF QUITO, ECUADOR

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United Nations General Assembly declared 2016 the International Year of Pulses (IYP), in order to promote the sustainable food production and nutrition security. Lupine is a legume that has been cultivated for thousands years ago in the Andean region. Also, Pre-Inca Civilizations considered it as one basic food in their diet. However, over time there have been developed different transformations along this bean agroproductive chain. Therefore, the objective of this research was to know the dynamic of lupine productive chain: its environment, main actors and different channels of commercialization in the Urban District of Quito, Ecuador. Flexible partial mapping was carried out through interviews with main actors of the chain, direct observation and review of secondary information. The study took as reference Cotopaxi province, for being one of the most important flow of commercialization of lupine towards Quito city. The lupine bean farmers are one of the main actors in the chain. The production concentration of bitter bean is located in Sigchos and Saquisilí in Cotopaxi Province. Farmers are classified according to their production capacity. Small farmers ( $\leq 0,5$  t) have limit access to machinery, agricultural supplies, and national markets. The intermediation and gathering center continue to be one of the most accessible ways to market their harvest. The price is established by a businessman based on the quality and market demand. The group of small farmers is one of the weakest links in the chain. In relation to processing, the technify of debittering process highlights in agroindustrial factories; while in the commercialization, there are different commercialization and distribution flows in wholesale and retail markets and supermarkets chains, which sell the product to restaurants, roadside stands and as raw material for other factories until they reach the final consumer.

In summary, the agroproductive chain of lupine has had changes throughout history. First, the bean was associated with traditional production systems and low volumes of consumption. Then, the cultivated area has increased and the grain consumption has been promoted. That is why lupine bean has having an increasing demand that is not covered by the national production of grain.

## PROTEIN CONCENTRATES AND ISOLATES FROM LUPIN CULTIVARS (*Lupinus mutabilis* AND *Lupinus albus*)

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Worldwide, millions of people perform physical activity in order to prevent diseases, improve their physical condition or participate competitively in a sport. For this, many resorts to dietary supplements are use, because their diet is not enough to cover their nutritional requirement. Protein supplements that are marketed are mainly of animal origin (milk, egg and meat) and seldom of plant origin (soy) as an alternative for people intolerant to lactose. Protein concentrates (> 70 % protein content) and protein isolates (> 90 % protein content) are available on the market.

The objective of this work was to obtain protein concentrates and isolates from cultivars of the species *Lupinus mutabilis* (Jayata and Candela) and *Lupinus albus* (Victor/Jatunta). Jayata and Candela are Bolivian cultivars that were obtained through selection by the PROINPA Foundation, the latter, a cultivar with a lower alkaloid content than the traditional one, while Victor/Jatunta is a Chilean cultivar.

The lupin seed was characterized (via proximal analysis) according to AOAC standards. In this work we evaluated the effect of processing whole seed, dehulled seed and defatted seed in the performance of the protein extraction process. For this, each sample was ground to a particle size smaller than 0.210 mm (< 70 mesh), suspended in water in a 1:10 ratio at a pH of 8.5 and after an extraction time of 30 minutes at room temperature, the suspension was centrifuged at 3500 x g for 10 min and the precipitate washed once at a pH of 8.5. The process of extracting the protein continued with the supernatant collected from the previous step which was adjusted to a pH of 4.8 and centrifuged at 3500 x g for 10 min and the precipitate was washed twice at a pH of 4.8. In each sample of the experimental design, the content and yield of crude protein (N x 6.25) was determined.

The initial crude protein content of the whole seed of *Lupinus mutabilis* was between 39 - 42%, higher than that of *Lupinus albus*, which was about 30 %. As a result of the extraction process, products with a crude protein content of 63 – 79 % were obtained from the whole grain, 68 – 80 % from the dehulled seed and 94 – 97 % from the defatted seed, regardless of whether the seed was whole or dehulled. The yields of the extraction process were between 30 - 45%. In the samples of *Lupinus albus*, the crude protein content was higher than that of *Lupinus mutabilis*, however, the yields were the lowest. In this way, protein concentrates and isolates were obtained from the lupin cultivars studied.

## CONCENTRADOS Y AISLADOS PROTEICOS A PARTIR DE CULTIVARES DE LUPINOS (*Lupinus mutabilis* y *Lupinus albus*)

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A nivel mundial, millones de personas realizan actividad física con el fin de prevenir enfermedades, mejorar su condición física o participar de manera competitiva de una disciplina deportiva, para ello muchos recurren a la suplementación porque la dieta alimentaria no es suficiente para cubrir su requerimiento nutricional. Los suplementos proteicos que se comercializan son principalmente de origen animal (leche, huevo y carne) y escasamente de origen vegetal (soya) como alternativa para las personas intolerantes a la lactosa. En el mercado se ofertan concentrados (> 70 % de contenido de proteína) y aislados proteicos (> 90 % de contenido de proteína).

Por tanto, el objetivo de este trabajo fue obtener concentrados y aislados proteicos a partir de cultivares de las especies *Lupinus mutabilis* (Jayata y Candela) y *Lupinus albus* (Victor/Jatunta). Jayata y Candela son cultivares bolivianos que fueron obtenidos a través de selección por la Fundación PROINPA, siendo la última un cultivar con un contenido menor de alcaloides respecto al tradicional, y Victor/Jatunta es un cultivar chileno.

El grano de lupino se caracterizó (análisis proximal) de acuerdo con normas de la AOAC. En este trabajo se evaluó el efecto de procesar el grano entero, descascarado y desengrasado en el rendimiento del proceso de extracción de la proteína, para ello cada muestra fue molida a un tamaño de partícula menor a los 0.210 mm (< 70 mesh), suspendida en agua en una relación 1:10 a un pH de 8.5, un tiempo de extracción de 30 min y a temperatura ambiente, posteriormente la suspensión fue centrifugada a 3500 x g por 10 min y el precipitado lavado una vez a un pH de 8.5. El proceso de extracción de la proteína continuó con el sobrenadante colectado de la etapa anterior que fue ajustado a un pH de 4.8 y centrifugado a 3500 x g por 10 min y el precipitado lavado dos veces a un pH de 4.8. En cada muestra del diseño experimental se determinó el contenido y rendimiento de proteína cruda (N x 6.25).

El contenido de proteína cruda inicial del *Lupinus mutabilis* fue de 39 – 42 %, mayor al del *Lupinus albus* que fue de 30 %. Como resultado del proceso de extracción se obtuvieron productos con un contenido de proteína de 63 – 79 % a partir del grano entero, de 68 – 80 % a partir del grano descascarado y de 94 – 97 % del grano desengrasado, independientemente de si el grano era entero o descascarado. Los rendimientos del proceso de extracción estuvieron entre 30 – 45 %. En las muestras de *Lupinus albus* el contenido de proteína cruda fue mayor respecto al del *Lupinus mutabilis*, sin embargo, los rendimientos fueron los más bajos. Obteniéndose así concentrados y aislados proteicos a partir de los cultivares de lupinos estudiados.

# DEVELOPMENT OF AN EFFICIENT PROCESS FOR THE PREPARATION OF SPARTEINE

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During decades, (-)-sparteine has been the battle horse of the chiral chemical reagents, broadly used as a ligand in asymmetric synthesis and as catalyst in polymerization reactions by ring opening, furthermore, it has also been used as a potential drug precursor. Its capacity as a metal ligand has been extended to the use of (-)-sparteine copper complexes to catalyze nitro aldol enantioselective condensations. *Lupinus* is a plant genus of the Fabacea family that is characterized by the production of quinolizidine alkaloids. Sparteine represents between 70 and 80% of the alkaloids synthesized by *Lupinus montanus*. This species is widely distributed in Mexico and particularly in the Trans-Mexican Volcanic Belt. In the Iztaccíhuatl-Popocatepetl National Park, *L. montanus* shows a broad altitudinal distribution that goes from 3100 m a.s.l. to 4147 m a.s.l. In this work, an environmentally friendly and efficient process for the preparation of (-)-sparteine from *L. montanus* fresh material is presented. Therefore, the objective of this work was to use *Lupinus montanus* leaves as a sustainable source of sparteine. Leaves of in anthesis plants of *L. montanus* were collected at the Iztaccíhuatl-Popocatepetl National Park and stored immediately after arrival in the laboratory in polyethylene bags at -22 °C. The extraction procedure consisted of grinding one part of frozen leaves with three parts of 0.5 M sulfuric acid aqueous solution in a blender until achieving a homogeneous consistency, followed by heating at 50 °C during 72 hours with mechanical stirring. Then, the material was filtered through celite, washed with water, alkalized with ammonium hydroxide to pH=9 and finally extracted with chloroform. This crude extract of total alkaloids is free of chlorophylls, glucosides and some other neutral components, which was vacuum concentrated to allow the isolation of free alkaloids, comprising about 80% of (-)-sparteine. This oily material is transformed to crystalline (-)-sparteine sulfate through dissolution in isopropyl alcohol and the addition of an excess of 0.5 M sulfuric acid, or may be high vacuum distilled (0.066 mbar) to produce high purity (-)-sparteine.

A process for the preparation of crude alkaloid extract yielded 2.15 % in a scale of 200 g (wet material, 36 g dry material) and 3.9 % in a 1.4 kg (wet material, 252 g dry material) scale. Crystalline (-)-sparteine sulfate from a crude alkaloid extract was obtained with a final yield of 1.73-2.55 % (dry material). High purity (-)-sparteine was obtained in a yield of 1.5 %.

This work shows an efficient process for the preparation of (-)-sparteine sulfate and high purity (-)-sparteine free base from fresh material has been developed.

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## ***Lupinus mutabilis* Sweet (TARWI) CONSUMPTION IN THE PERUVIAN MARKET: CHALLENGES AND THREATS**

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Peru is the country in the Andean Region that has the biggest *Lupinus mutabilis* Sweet (tarwi) production, having increased its surface in the last decade. La Libertad and Cusco report today the most important producing areas of Peru, and sell their production mostly to the Lima, Ancash and Cusco markets, and a part to the Ecuador market. Since some years ago some private and public actions are being developed in order to promote the national tarwi consumption, and those actions are still in progress. The tarwi consumption is still restricted to some specific niches, with a traditional way of consumption, with a scarce added value development. Between the restrictions we can mention the limited supply of the crop because the sowing and the harvest is difficult, and also is a labor-intensive crop. Other disadvantage is the long vegetative cycle that last nine months, and the great water requirements of the plant; those factors has contributed to limit the production of the crop. On the other hand, the key factor that could lead to increase the tarwi consumption is its use as a natural medicine, because this is a market that is growing faster. Peru is a country that has many natural products, including the tarwi crop, which is a leguminous that could heal different illnesses like diabetes and kidney problems.

The following investigation shows some key factors that are limiting the tarwi production and consumption in the Peruvian market, also shows some opportunities that could allow a market growth not only as a fresh product, but mainly as processed food.

The methodology used is the high value chain analysis and the short food circuits, stressing the contribution of the crop to local food security. The Ancash Region has the biggest yearly per-capita consumption with 10 kg, followed by the Cusco Region with 5 kg. The Ancash Region is not self-supplied, and it is necessary to bring tarwi from La Libertad and Huanuco Regions. On the other hand, the great Lima Market, country's capital with 10 million people, has limited tarwi distribution points, like naturist weekend fairs, a few naturist shops, two regional restaurants and a couple of markets situated on the outskirts of the city. Tarwi is consumed mainly fresh, and marginally as flour, by Peruvian Highland's migrants. According to this research, Lima and Peruvian consumers are able to demand more tarwi, but they don't find a permanent supply in the market, specifically in Lima, that is the main factor that is limiting the consumption.

# EL CONSUMO DEL *Lupinus mutabilis* Sweet (TARWI) EN EL MERCADO PERUANO: DESAFIOS Y AMENAZAS

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Perú es el país de la región andina que presenta la mayor producción de *Lupinus mutabilis* Sweet (tarwi), habiendo incrementado su superficie en la última década. La Libertad y Cusco en la actualidad reportan las mayores áreas productoras de tarwi del Perú, y distribuyen su producción tanto a los mercados de Lima, Ancash y Cusco, y una parte se destina al Ecuador. Desde hace algunos años algunas acciones privadas y públicas vienen siendo desarrolladas para promover el consumo nacional de tarwi, ya que el consumo tradicional de tarwi está todavía restringido a nichos de mercado específicos, con escaso desarrollo de valor agregado. Entre las restricciones que podemos mencionar, están la limitada oferta dado que la siembra y cosecha es difícil, debido a que es un cultivo intensivo en mano de obra. Otra restricción para el desarrollo del cultivo es su largo período vegetativo de 9 meses, y el alto requerimiento de agua; estos factores son limitantes para expandir la producción del cultivo. De otro lado, los factores claves que podrían contribuir a incrementar su consumo es su uso como medicina natural, que es un mercado que está creciendo rápido en el Perú, y el tarwi, es una leguminosa que puede servir para curar diversas enfermedades como diabetes y enfermedades renales.

La presente investigación muestra algunos factores claves que están limitando la producción y consumo de tarwi en el mercado peruano, también muestra algunas fortalezas u oportunidades que podrían determinar el crecimiento del mercado de tarwi no solo en fresco, sino también con valor agregado.

La metodología usada fue el análisis de cadenas de alto valor, destacando los circuitos de valor cortos, y la contribución del cultivo a la seguridad alimentaria. La Región Ancash presenta el mayor consumo nacional de tarwi con 10 Kg/Habitante/año, seguida por la Región Cusco con un consumo aproximado 5 Kg/Habitantes/año; la Región Ancash, no se autoabastece de tarwi, debiendo traerlo de Las Regiones La Libertad y Huánuco. De otro lado, el gran mercado de Lima de 10 millones de consumidores, cuenta con limitados puntos de venta de tarwi, como son algunas ferias naturistas los fines de semana, tres galerías donde se venden productos naturales y dos restaurantes regionales. El producto se encuentra principalmente en fresco y de manera limitada como harina, en mercados de la periferia de la ciudad. El producto es consumido principalmente por inmigrantes de la Sierra. De la investigación realizada quedó en evidencia que la gente quisiera consumir volúmenes mayores de tarwi, pero no lo encuentran de manera regular en el mercado, lo que impide el crecimiento de la demanda interna.

## CHEMICAL CHARACTERIZATION OF *Lupinus mutabilis* Sweet AND *Lupinus angustifolius* SEEDS AND OIL

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Bitter lupin, also known as tarwi (*Lupinus mutabilis* Sweet), is widely cultivated in the Andean region of South America. In Europe, sweet species of lupins, such as *Lupinus angustifolius*, are grown for human consumption. The aim of this study was to carry out the chemical characterization of seeds and oil of these two lupin species. The tarwi samples originated from Peru and the *L. angustifolius* from Finland. The following analyses were carried out: proximate composition, dietary fiber, phenolic compounds, antioxidant activity, flavonoids, fatty acid composition and physicochemical characteristics of the oils. The tarwi seed had very high protein content (56 %) while *L. angustifolius* had 33 %. Oil and ash content were very similar in these two species. There were significant differences in carbohydrate and crude fiber content, with *L. angustifolius* having a higher content of these compounds. The total, soluble and insoluble dietary fiber content in *L. angustifolius* was significantly higher than the content of these compounds in *L. mutabilis*. The content of phenolic compounds, flavonoids and antioxidant activity in the two lupin species was similar. The analysis of fatty acid composition of the lupin oils demonstrated that the oil of *L. angustifolius* has a higher content of polyunsaturated fatty acids than the oil of *L. mutabilis*. Oleic acid content was higher in tarwi than in *L. angustifolius*. Both species of lupins are very nutritious crops, rich in protein, dietary fiber and bioactive compounds. They are good sources of high-quality oil, with an adequate composition of essential fatty acids.

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## PREPARATION AND EVALUATION OF A DIET FORMULATED WITH *Lupinus albus* AND HATCHERY WASTE FLOURS IN JUVENILE TILAPIA (*Oreochromis niloticus*)

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The use of alternative ingredients is an option in the formulations of diets for intensive fish exploitation. This study compared an isoproteic (37%) and isocaloric diet (~3520 kcal/kg) formulated with *Lupinus*, and hatchery waste flours against a commercial diet. Juvenile tilapia obtained from a local Aquaculture Center were assigned randomly to a control and an experimental group of approximately 200 g total mass each. Juveniles were fed twice a day with a quantity of food corresponding to 3% of their total mass weight. During the experimental trial (96 days), we recorded the weight and size of each fish every 14 days and monitored the water physicochemical parameters and temperature daily. At the end of the study the fish parameters, for the control and experimental groups were: total mass (2.99 kg and 1.91 kg), average weight (37g vs 27 g), average height (12.0 cm vs 10.8 cm) and average weight gain per period of evaluation (5.67 g and 3.97). Water physicochemical parameters met the Mexican norm for aquaculture exploitation. Results of the statistical analysis indicate that there were no differences between the two diets. Therefore, it is feasible to elaborate fish meals with *L. albus*, and hatchery waste flours for juvenile tilapia that performed similarly to commercial feeds.

# CARACTERIZACIÓN FÍSICO QUÍMICA Y BROMATOLÓGICA DE LICUADOS VEGETALES DE TRES VARIEDADES ANDINAS DE *Lupinus mutabilis* Sweet

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Los cultivos andinos tradicionales que se encuentran en el Ecuador, como el tarwi o chocho (*Lupinus mutabilis* Sweet), deben ser aprovechados mediante el fomento de su siembra y consumo. El chocho puede generar opciones de diversificación alimentaria y aportar con productos que pueden convertirse en alternativas al consumo tradicional. El valor nutricional del grano de chocho, una vez que ha sido sometido al proceso de extracción de alcaloides, es de 54.04% de proteína y 0.48% de calcio según reportes del Instituto Nacional de Investigaciones Agropecuarias del Ecuador. Por lo expuesto, los objetivos de este trabajo de investigación incluyeron, i) desarrollar la técnica para elaborar licuados de chocho a partir de tres variedades andinas (Andino INIAP 450, Guaranguito INIAP 451 y Criollo), que son los más consumidos en la Región Andina del Ecuador, ii) caracterizar físico química y bromatológicamente los licuados obtenidos, y iii) optimizar la formulación del licuado de chocho en base a sus mejores características nutricionales.

Se trabajó con 100 g de grano, dos cantidades de agua (100 ml y 200 ml) y dos velocidades distintas (20000 y 40 RPM) de la licuadora y la prensa para la extracción del licuado, obteniendo un total de 12 tratamientos con 3 repeticiones cada uno. En los licuados obtenidos se determinó el rendimiento, sólidos totales, proteína total, grasa, pH, color y cenizas. El mayor porcentaje de proteína se obtuvo con la variedad Andino 450, con 100 g de grano y 200 ml de agua, mediante la extracción utilizando 40 RPM. La variedad Guaranguito presentó mayor porcentaje de grasa bajo el tratamiento de 100 g de grano y 200 ml de agua, a una velocidad de 40 RPM. El pH más cercano a neutro se obtuvo a partir de la variedad Guaranguito con la relación de 100 g de grano con 200 ml de agua y a una velocidad de 40 RPM. El mejor rendimiento fue obtenido con la variedad Andino 450, con 100 g de grano y 200 ml de agua y 40 RPM. La variedad Criollo presentó mayor cantidad de sólidos totales, con la velocidad de 20000 RPM y una relación de 100 g de grano con 100 ml de agua. La variedad Guaranguito presentó mayor cantidad de cenizas, con el tratamiento de 100 g de grano con 100 ml de agua a 40 RPM. Entonces el licuado que presentó mejor rendimiento, mayor cantidad de proteína y grasa es aquel elaborado con la variedad Andino INIAP 450. La mayor cantidad de sólidos totales se obtuvo con la variedad Criollo con 100 g de grano y 100 ml de agua a una velocidad de 40 RPM. En la primera etapa experimental de esta investigación, se corroboró la información del valor nutricional del chocho con datos de investigaciones anteriores. Este trabajo forma parte de una Tesis Doctoral que se encuentra en desarrollo en la Universidad Autónoma de Barcelona–España, en colaboración con el Instituto Ecuatoriano de Investigaciones Agropecuarias del Ecuador.

# MODIFICATION OF LUPIN PROTEIN ISOLATES – INFLUENCE OF ENZYMATIC HYDROLYSIS AND FERMENTATION ON CHANGES IN THE MOLECULAR WEIGHT DISTRIBUTION, TECHNOFUNCTIONAL AND SENSORY PROPERTIES

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In recent years, the demand for high quality vegetable proteins for applications in the food and feed sector is rising. Lupins are widely cultivated in Europe and are rich in seed proteins, which exhibit valuable technofunctional properties and a well-balanced sensory profile. The most abundant storage proteins in lupin seeds are the globulins, namely  $\beta$ -conglutin,  $\alpha$ -conglutin,  $\gamma$ -conglutin and  $\delta$ -conglutin.  $\beta$ -Conglutin is identified as the major allergen (~55-61 kDa molecular weight) for *Lupinus angustifolius* L. and is classified by the recognized allergen nomenclature subcommittee as a recognized allergen (code: Lup an 1.0101). Moreover, lupin and its products have been included in Annex IIIa to Directive 2000/13/EC, which lists the ingredients to be indicated on the food label. With the increasing prevalence of food allergies and the sensitization of proteins, several attempts have been made to reduce the allergenic potential of food proteins to mitigate allergic reactions in susceptible individuals. This includes the process of extensive or mild hydrolysis to produce hypoallergenic foods.

The aim of our study was the depletion or elimination of the major IgE-reacting polypeptides in the *Lupinus angustifolius* cultivar Boregine protein isolate using enzymatic hydrolysis. Hydrolysis was performed with nine different protease preparations and combination of four of them in one-step and two-step processes to study their influence on technofunctionality such as protein solubility, foam activity and emulsify capacity, and the sensory and protein integrity to estimate the reduction in immunoreactivity. Analyzing SDS-PAGE showed that Alcalase 2.4 L, papain and pepsin were most effective in degrading  $\alpha$ - and  $\beta$ -conglutin, although the degree of hydrolysis increased only slightly. Most proteolytic enzymes improved the technofunctional properties of LPI, with the most prominent increases in foam activity from 980 % for LPI to 3614 % for pepsin hydrolysate, from 9.7 % protein solubility at pH 4.0 for LPI to 83.6 % protein solubility at pH 4.0 for Alcalase 2.4 L and papain combination. With the exception of the Alcalase 2.4 L hydrolysate, the other hydrolyzates did not show excessive bitterness compared to LPI. Other sensory attributes of the hydrolyzates were also very similar to the LPI. We also investigated the effect of fermentation on protein degradation and technofunctionality with the additional attempt to achieve a balanced sensory profile. For this purpose, growth kinetics (CFU/ml, pH, glucose) of 26 microorganisms were recorded and showed that all of them were able to grow in LPI. Based on the evaluation of the SDS-PAGE, eight microorganisms were proteolytic active, of which we investigated the effect of the fermentation on the technofunctional properties as well as the sensory profile in detail.

The results of this study have shown the potential of enzymatic hydrolysis of LPI to modify the IgE-reacting polypeptides. Enzymatic and fermentation treatments are able to improve the technofunctionality of the isolates and create a well-balanced sensory profile, therefore making them suitable to use as food ingredients for different kind of food products.

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## CHANGES IN AMINO ACID COMPOSITION IN THE PRODUCT (KERNEL) AFTER DEHULLING LUPIN SEEDS

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The lupin varieties Dieta, Amiga and Zulika were the object of the study. Each of the varieties was grown on the area of 10 ha in the land registry of Bartošovice village in the district Nový Jičín, under the identical soil and climate conditions (region where cereals are grown, 276 m above sea level, annual rainfall of 776 mm). The sowing occurred in the beginning of April 2017 in the amount of 200 kg/ha. The crop was harvested at the end of August 2017. On a day before the harvest, 10 samples were taken from each variety (0.5 kg) from various parts of the field stand. It resulted from the amino acid (AA) analysis in the lupin seeds that the kernel after manual dehulling had the higher content of all analyzed AA ( $P \leq 0,05$ ), except for methionine (Met), for which no change was observed. There were differences in the average values of individual amino acids as well as among the studied varieties. Higher differences in AA contents were shown among the individual varieties, the highest for Dieta, from 25.33 % (alanine) to 43.09 % (tyrosine, Tyr), lower for Amiga, from 14.33 % (valine, Val) to 24.33 % (arginine, Arg) and the lowest for Zulika, from 11.07 % (Val) to 17.68 % (Tyr). The results showed that the highest effect on the increase of AA content after dehulling was observed in the kernel of Dieta. The percent increase of the AA content in the final product – kernel of the studied varieties, as compared to their whole seeds, was from 11.1 (Zulika) to 43 % (Dieta). In comparison with the standard extracted soybean meal, the lupin kernel contained less lysine and Met, on the other hand, it contained markedly higher content of Arg, an essential AA especially for poultry. The results showed that the highest amount of total AA, as well as all essential AA, was present in the kernel, slightly lower amount in the whole seed and the lowest amount in the hull, for all the studied varieties. The results showed that the majority of AA is contained in the kernel and after dehulling, their content increased significantly as compared to the whole seed. Regarding individual varieties, the highest content of all AA in the kernel dry matter was observed for Zulika 427.1 g/kg, slightly lower amount for Dieta 424.6 g/kg and markedly lower content the variety Amiga 386.9 g/kg. In the whole seed, the highest amount of all AA was observed for Zulika 374.4 g/kg, lower for Amiga 324.6 g/kg and the lowest for Dieta 317.9 g/kg. Regarding the hull, the highest content of all AA was observed for Zulika 62.3 g/kg, lower for Amiga 58.9 g/kg and the lowest for Dieta 54.0 g/kg. The results suggested that the respective varieties differ in the content of all AA in the seed, kernel and hull. The results thus show the significant difference in the content of amino acids among the varieties. The study was financially supported as part of the grant project by MZe ČR NAZV QJ1510136.

# CHANGES IN FATTY ACID COMPOSITION IN THE PRODUCT (KERNEL) AFTER DEHULLING LUPIN SEEDS

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The study focuses on fatty acids (FA) in the seed fat of three white lupin varieties (Dieta, Amiga, Zulika) and on their content (expressed as g of FA/100g of fat) in the kernel after dehulling. Each of the lupin varieties was grown on the area of 10 ha in the land registry of Bartošovice village in the district Nový Jičín, under the identical soil and climate conditions (region where cereals are grown, 276 m above sea level, annual rainfall of 776 mm). The sowing occurred in the beginning of April 2017 in the amount of 200 kg/ha. The crop was harvested at the end of August 2017. On a day before the harvest, 10 samples were taken from each variety (0.5 kg) from various parts of the field stand. In a laboratory, seeds were manually dehulled to produce a kernel. The paper presents the results of comparing the content of individual groups of FA in relation to dehulling the lupin seed. For all three tested varieties, the resulting product – kernel contained a higher content of all groups of FA as compared to the whole seed, which proves that the majority of fat is contained in the kernel. The results show that after dehulling the seed, there is a higher proportion of the majority of individual FA, which is related to the higher crude fat content in the kernel in comparison with the whole seed. Regarding saturated fatty acids (SFA), their content (g of FA/100g of fat) increased in the kernel as compared to the whole seed for Dieta from 8.31 to 9.91, for Amiga from 8.39 to 9.74 and for Zulika from 4.37 to 8.91. Regarding monounsaturated fatty acids (MUFA), their content (g of FA/100g of fat) in the kernel increased as compared to the whole seed for Dieta from 24.53 to 28.59, for Amiga from 26.35 to 30.83 and for Zulika from 12.60 to 26.68. As for polyunsaturated fatty acids (PUFA) from the group *n*-6 PUFA, their content (g of FA/100g of fat) in the kernel increased as compared to the whole seed for Dieta from 13.89 to 15.40, for Amiga from 15.11 to 16.77 and for Zulika from 7.29 to 14.08. Concerning the group *n*-3 PUFA, their content (g of FA/100g of fat) in the kernel increased as compared to the whole seed for Dieta from 10.09 to 12.13, for Amiga from 10.85 to 12.74 and for Zulika from 5.43 to 11.24. The narrow *n*-3/*n*-6 PUFA, which decreased even more after dehulling, displays the high nutritional quality of the lupin oil, while its values in the whole seed were 1.38, 1.39 and 1.34 for Dieta, Amiga and Zulika, respectively. The high dietetic quality of lupin oil is also shown by the low content of SFA and the high content of unsaturated FA. From the point of view of diet, the quality of lupin fat is positive for its high content of unsaturated FA and a favourable proportion of individual groups of FA. In comparison with standard extracted soybean meal, we can conclude that the contents of majority FA are markedly higher in the lupin whole seed and lupin kernel. The study was financially supported as part of the grant project by MZe ČR NAZV QJ1510136.



## INFLUENCE OF TARWI (*Lupinus mutabilis* SWEET) HUSK FIBER ON BREAD MOLD TEXTURE DURING STORAGE

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Tarwi husk might become a subproduct from the industry of vegetable protein ingredients. Husks are full of fiber and can be incorporated in processed foods. The main objective of this research is to prepare bread enriched with a fiber ingredient prepared from Tarwi's husk and to determine the evolution of texture of this bread during storage. *Lupinus mutabilis* seeds commercially available, were processed by aqueous soaking, cooking and water lixiviation to remove toxic alkaloids (traditional food processing called "debittering" since Inca times at Peru). Dried husks free from alkaloids, were milled and separated on particle size basis: fine (<250 µm), medium (250-425 µm) and coarse (> 425 µm). A total of three treatments evaluated for each fiber size ingredient, the effect of adding a 1% level to bread on the formulation, water activity (Aw) and Texture (TPA) by Instron® of fiber enriched bread. Texture results indicated that before and after storage, the Hardness (mN) of bread was the lowest for samples containing fine particle tarwi fiber. This finding was consistent with a softer bread due to a better absorption of water, allowing Aw to be kept close to characteristic values during storage allowing a longer shelf life. A practical application for the food industry includes the reduction of bread aging due to the addition of fiber, mainly cellulose, a component of husk's fiber linked to an effective water retention and firmness.

We acknowledge to Bakery "Productos Unión"™ for the facilities provided in the preparation of bread and to the PNIA project for Tarwi directed by Felix Camarena Mayta.

# POTENCIALIDAD DEL TARWI (*Lupinus mutabilis* Sweet) Y FIBRA DIETARIA DE CASCARA: SUS PROPIEDADES FUNCIONALES Y TRANSFORMACIÓN EN LA INDUSTRIA ALIMENTARIA PERUANA

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El objetivo del presente trabajo es presentar las propiedades funcionales y productos agregados a partir de la cáscara de tarwi. Se visitaron ferias regionales comerciales (nacionales e internacionales) y se recopiló estudios y trabajos científicos donde se presenta los beneficios del producto integral y de la cáscara de tarwi para la salud y la economía. Se ha encontrado que existen aplicaciones en la pequeña empresa peruana donde se elaboran productos a base de tarwi (incluyendo cotiledón y cáscara) demostrando que tiene un gran potencial de aplicación en la industria alimentaria tales como: harina de tarwi y sus derivados, hojuelas de tarwi, helados, sopas, salsa, bebidas de tarwi, productos fermentados, productos horneados, conservas, guisos, etc. Como fuente de proteínas y aceites es muy conocido porque brinda toda una oportunidad para la luchar contra la desnutrición, sin embargo las aplicaciones de fibra dietaria proveniente del tarwi en los diferentes productos horneados para reducción de obesidad es importante. También se encontró múltiples aplicaciones gastronómicas según la región peruana. Llegamos a la conclusión de que el tarwi es un alimento con grandes propiedades y potencialidad en la agroindustria para el Perú y el mundo, debido a las propiedades que presenta, beneficiando a la salud previniendo enfermedades degenerativas, pero su consumo depende del control de los niveles de alcaloides que presente naturalmente al final del proceso de desamargado.

## OPTIMIZATION OF ELABORATION NUGGET ANALOGUE BASED ON GLUTEN WITH TARWI (*Lupinus mutabilis* Sweet) AND ANDEAN FLOUR

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Meat analogue products based on gluten and soy have been an alternative for people who cannot consume animal products. Even with these products, other protein sources are desired that have a high nutritional quality and digestibility to contribute to the amino acid requirements. The objective of this work was to elaborate a 'nugget' analogue using wheat gluten and Andean flours, such as tarwi, broad bean, quinoa and cañihua. Initially, based on texture, the best concentration of gluten was evaluated sensorially and the best was 60%. The other 40% consisted in the combination of a mixture of flours of quinoa, tarwi and cañihua with the best profile of limiting amino acids, taking into account the sensorial acceptance, and the chemical digestibility-corrected amino acid scores ('PDCAAS '), through a mix design. A sensory evaluation was performed on the nuggets analogues for attributes color, taste, general acceptance, texture and odor; the most preferred was its proximal composition. The analogue of 'nugget' made with 60% gluten with 40% in mixtures of Andean flours (broad bean 18.0%, quinoa 1.0%, tarwi, 20.0%, cañihua, 1.0%) was the one with the highest PDCAAS (0.43) and sensory preference and its protein content was 28.53%. We think that is a healthy product. We acknowledge to Universidad Peruana Union to finance this project.

## DEVELOPMENT OF GLUTEN-FREE BREAD WITH QUINOA (*Chenopodium quinoa* Willd.) AND TARWI (*Lupinus mutabilis* Sweet) FLOURS

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Peru has a growing market for gluten-free products due to the increase of persons with coeliac disease and people looking for healthier and more nutritious products. The gluten-free breads available in the market are generally made from starches and rice flour, showing poor quality characteristics in volume and texture, as well as a poor nutritional profile with respect to protein, fiber, and minerals. The whole flours of the Andean grains such as quinoa and tarwi are excellent providing raw materials for the development of gluten-free products, due to the content of natural emulsifiers and good nutritional profile. That is why the aim of this research was to develop a gluten-free bread containing whole-grain quinoa and tarwi flours as primary components. The Central Composite Rotatable Design (CCRD) was used to determine the optimum level of tarwi flour (range: 10 - 30%) and water (range: 75 - 160%) needed to imitate the textural characteristics (Firmness, Consistency, Cohesiveness and Viscosity index) of a control mass, which was composed of 53% of potato starch, 47% of quinoa flour, 75% of water, 0.5% mixture of xanthan gum and tara gum (ratio 1: 1) , 6% vegetable oil, 3% sugar, 2% salt and 3% yeast (Formulation developed in a previous work). The textural properties of the 13 treatments of the CCRD were measured using the Back Extrusion accessory of the Instron texturometer and the results were processed using the Design-Expert® 11 software. Quadratic models were obtained for all the textural parameters and the optimized formulation was: 12 % of tarwi flour, 47% of quinoa flour, 41% of potato starch, 102% of water and the rest of the components equal to the control formulation. Then 3 different fermentation times were evaluated (10, 20 and 30 min) and subsequently baked for 60 minutes at 200 ° C. The end-product was physically characterized, determining its specific volume, alveolar structure and crumb texture. As a conclusion, it was found that, due to the high concentration of water in the masses, the long fermentation time (30 min) is not favorable for this type of dough since it promotes the coalescence of the CO<sub>2</sub> cells generated during fermentation and short fermentation times (10 min) does not allow the correct development of the dough, producing loaves with low specific volume and hard crumbs. Therefore, the optimal fermentation time was 20 min, achieving gluten-free breads with a higher specific volume ( $2.01 \pm 0.04$  mL/g), good alveolar structure and smooth crumbs ( $223 \pm 36$  gf of Firmness;  $88 \pm 11$  gf of gumminess, and  $79 \pm 10$  gf of chewiness).

## DESIGN AND ELABORATION OF A HEALTHY BREAD WITH INCORPORATION OF LUPIN FLOUR (*Lupinus mutabilis* Sweet)

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The human body, in addition to other nutrients, requires the daily intake of protein to stay healthy. A source of this nutrient is the lupine, an Andean legume, which can help improve the nutrition and health of the population. The purpose of this study was to evaluate the rheological properties and the nutritional profile of wheat flour with partial substitution of debittered ground and dry lupine (DGDL). "Superior France" brand, commercial wheat flour, was used; INIAP-450 lupine variety was used. A part of the grain was dried and ground (DGDL), another part was fermented and then dried and ground (DFGDL). Wheat flour was substituted at levels of 10, 15 and 20%, for which the greatest changes were registered in the gluten (GI), viscosity ( $\mu$ l) and amylases (AI) indexes. Thus, the GI was increased to a value of 8, at three levels of substitution, which infers, that the DGDL, helps to increase the strength of gluten, strengthening the maintenance of the protein structure, possibly by an increase of hydrogen bonds. The ( $\mu$ l) decreased to a value of 2, due to the substitution with DFGDL, which will affect the physical characteristics of the bread. When the substitution was made with DFGDL, the  $\mu$ l increased to a value of 5, considered adequate for the baking process. While the AI, decreased to a value of 1, at a substitution level of 20%. In contrast, with DFGDL, diastasic activity decreased, which expressed in the highest value (8) of AI. Replacement with DGDL helped improve the nutritional profile of the wheat flour, expressed in the highest protein content (22.14%), total dietary fiber (12%), calcium (0.07%), phosphorus (0, 28%), potassium (1.27%) and iron (104 ppm).

## CHANGES IN BASIC NUTRITIONAL COMPOSITION OF THE PRODUCT (KERNEL) AFTER DEHULLING LUPIN SEEDS

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The objective of the study was to focus on lupin varieties which can be grown under the conditions of the Czech Republic and which have comparable or higher content of crude protein (CP) in their seeds than soybean meal. From this perspective, the optimum varieties used in the present study were white lupin varieties Dieta, Amiga and Zulika. Each of these varieties was grown on the area of 10 ha in the land registry of Bartošovice village in the district Nový Jičín, under the identical soil and climate conditions (region where cereals are grown, 276 m above sea level, annual rainfall of 776 mm). The sowing occurred in the beginning of April 2017 in the amount of 200 kg/ha; the crop was harvested at the end of August. On a day before the harvest, 10 samples were taken from each variety (0.5 kg) from various parts of the field stand. In a laboratory, seeds were manually dehulled to produce a kernel in order to increase the content of CP and the total nutritional value. After dehulling, the content of CP was significantly higher compared to the whole seed, from 19.89 (Zulika) to 27.14 % (Amiga). The product after dehulling lupin seeds contained from 426.2 (Amiga) to 461.1 g/kg (Zulika) nitrogen-containing substances, what is comparable to standard extracted soybean meals. The product contained the significantly higher crude fat content as compared to the whole seed, by 11.38 (Zulika) to 16.94 % (Amiga) which was related to its higher energy value (gross energy, GE) that increased by 3.35 (Zulika) to 4.11 % (Amiga). From the perspective of diet, the content of crude fibre decreased by 83.40 (Zulika) to 85.19 % (Amiga), the acid detergent fibre (ADF) decreased by 72.7 (Zulika) to 78.6 % (Dieta), the neutral detergent fibre (NDF) decreased by 70.9 (Amiga) to 77.3 % (Zulika) and the acid detergent lignin (ADL) decreased by 87.8 to 100 % (Zulika). The fibre and the mentioned fibre fractions sometimes can act as antinutrients, mainly in poultry diets. As compared to the standard soybean meals, the lupin kernel had the higher content of starch and nitrogen-free extract (NFE) and the lower content of crude ash. The analysis showed that the kernel after dehulling contained the higher content of CP, crude fat, crude ash, GE and the lower content of crude fibre, ADF, NDF, ADL compared to the whole seed ( $P \leq 0.05$ ). There were no significant differences between the whole seed and kernel for NFE, starch and organic matter. The study was financially supported as part of the grant project by MZe ČR NAZV QJ1510136.



**HEALTH BENEFITS**

**BENEFICIOS PARA LA SALUD**



# ANTI-INFLAMMATORY POTENTIAL OF *Lupinus mutabilis* SEEDS: ISOLATION OF DEFLAMIN, A POLYPEPTIDE WITH A POTENT INHIBITORY EFFECT ON MMP-9 AND MMP-2 ACTIVITIES

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Worldwide incidence and prevalence of inflammatory diseases have been dramatically increasing, evidencing their emergence as a global ailment. One important mediator in inflammation is a group of enzymes engaged in the remodeling of connective tissue, the matrix metalloproteinases (MMPs), particularly the gelatinases MMP-9 and MMP-2, which play important roles in inflammation and in several oncologic processes. Their inhibitors (MMPIs) have been shown to reduce or prevent inflammatory pathologies such as inflammatory bowel diseases, so in the last decade a substantial amount of research has turned towards discovering novel plant foods containing MMPIs, that can be used in preventive or curative diets against these ailments.

Previous studies by our work group have shown that the seeds of *Lupinus albus* contain a polypeptide with these protease inhibitor properties, which was named deflamin. This protein inhibits the activity of MMP-2 and MMP-9, and was shown to reduce inflammation, both *in vitro* and *in vivo*. As a natural inhibitor of MMPs, the study of this protein is very valuable. Deflamin also has the great advantage of withstanding boiling and to low pH values, and it is resistant to digestion, thus showing a great nutraceutical potential. However, although it was shown to be present in *L. albus*, it remains to be detected in other species such as *Lupinus mutabilis*. Hence, the present work aimed to isolate deflamin from different varieties of *Lupinus mutabilis* and evaluate their MMP-9 and -2 inhibitory activities.

Seeds from 11 different varieties of *L. mutabilis* were used and the presence of MMP inhibitory activity was determined by the fluorescent DQ-gelatin assay and also by reverse zymography. Deflamin was isolated through a sequential precipitation protocol developed in our group.

Results show that the protein extracts from the different varieties strongly differ in their MMPI activities, and reverse zymography revealed the presence of several MMPI proteins. Further isolation of deflamin demonstrated the presence of this polypeptide in several varieties, however, they were present in different amounts and with different levels of activity.

Overall, our results suggest the importance to search for deflamin in other *Lupinus* species and suggest that *L. mutabilis* can be a good candidate to be used in anti-inflammatory diets.

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## THE POTENTIAL USE OF *Lupinus montanus* AS ANTI-INFLAMMATORY

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*Lupinus montanus* is distributed in the Izta-Popo National Park, at altitudes between 2500 and 4200 m a.s.l. This species is characterized for synthesizing a great variety of metabolites such as flavonoids. Studies have demonstrated that these compounds have anti-inflammatory activity. The use of first instance anti-inflammatory drugs such as AINE's generates side effects, thus the search for new compounds and/or drugs is necessary. Natural compounds are an alternative treatment. However, their safe use has to be guaranteed before being commercialized, thus their harmlessness has to be demonstrated. The Organization for Economic Co-operation and Development (OECD) has established a set of guidelines to test the genotoxicity of new chemical or natural compounds by *in vivo* and *in vitro* assays. The aim of this project was to evaluate the anti-inflammatory effect of a fraction rich in flavonoids (FrFI) from *L. montanus* on a mouse auricular edema model as well as to evaluate its genotoxicity. A fraction rich in flavonoids (FrFI) from aerial parts of *L. montanus* was obtained by chemical fractioning of methanolic extracts through column chromatography (CC). Anti-inflammatory activity of FrFI was evaluated on a mouse auricular edema model induced with tetradecanoyl phorbol acetate (TPA). For the evaluation of the genotoxicity of FrFI two models were used: \* *in vitro*, in which human lymphocytes were treated with FrFI and/or mitomycin (positive control) during 24 hours. Lymphocytes were analyzed using two different assays: comet assay and micronuclei assay. \* *in vivo*, five groups of six mice each were randomly formed and orally administered with FrFI for 28 consecutive days, then blood samples were analyzed through comet and micronuclei assays.

FrFI had an anti-inflammatory effect similar to commercial indomethacin. The fraction was composed of luteolin (60%) and apigenin (40 %). The results of the evaluation of *in vitro* and *in vivo* genotoxicity by the comet assay and the micronucleus assay, did not show genotoxic damage by the FrFI on human lymphocytes and mouse erythrocytes.

The conclusions of this research are: FrFI of *L. montanus* composed of luteolin and apigenin has an anti-inflammatory effect similar to commercial indomethacin. FrFI was not genotoxic, so *L. montanus* could be an alternative anti-inflammatory treatment.

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## OPTIMIZATION OF FERMENTATION PARAMETERS FOR THE GROWTH OF *Lactobacillus plantarum* 299v IN LUPIN (*Lupinus mutabilis* Sweet)

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Legumes are an important component in the traditional diet of many regions of the world and are an excellent source of protein, as well as other macro and micronutrients. Among them lupin and its derivatives contain compounds of biological interest, like phenolics compounds and fatty acid. These compounds comprise a food matrix suitable for the incorporation of probiotic microorganisms. Therefore the objective of this study was to establish the optimum conditions of lupin-water ratio and sweetener concentration for the growth of *Lactobacillus plantarum* 299v, a probiotic used in vegetable matrices, using a response surface factorial design (RSM), during 12 hours of fermentation.

The obtained response surface model (RSM) produced a correlation coefficient of approximately 0.80. Optimal growth was given to conditions of 8 g of sweetener and water/raw material ratio of 31/100(w/v) resulted in an exponential grow from  $3.8 \times 10^{-7}$  cfu ml<sup>-1</sup> to  $1.39 \times 10^{-9}$  cfu ml<sup>-1</sup>. This study shows the capacity of growing *Lactobacillus plantarum* 299v in a plant matrix, which was not affected by the presence of some secondary metabolites of *Lupinus mutabilis*, therefore the formulated drink is suitable for lactic fermentation.

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## EVALUATION OF NOCICEPTIVE TOLERANCE, TOXICITY AT REPEATED DOSE OF TARWI (*Lupinus mutabilis* Sweet) IN ANIMALS OF EXPERIMENTATION

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Pain is the most frequent cause of patient visits to doctors, and their relief should be one of the main reasons for medicine. The World Health Organization and the International Association for the Study of Pain, consider the treatment of pain as one of the human rights of people. Nociception is the only common stage in all people who suffer pain. Highly toxic opioids are used in pain treatments whose therapeutic doses have been highly evaluated. In Peru, *Lupinus mutabilis* Sweet, known as tarwi or chocho, is a native species that contains quinolizidine alkaloids, mainly lupanin and spartein, with a total content ranging between 0.02% and 3.17%. Although it has been demonstrated that alkaloids are toxic, in Peru traditional knowledge considers the use of water of lupin for medicinal purposes, since the Inca period. Therefore the objective of this investigation was to evaluate sub acute toxicity, sub chronic toxicity and the antinociceptive effect of tarwi (*Lupinus mutabilis* Sweet) in rodents.

N = 84 mice and n = 110 albino rats were used. The Plantar Test and Tail Flick tests were carried out. The Kolmogorov Smirnov normality test, ANOVA and Tukey test were applied. Statistical significance was considered  $p < 0.05$  with 95% confidence interval. The nociceptive effect in albino mice using the Tail Flick test (fixed tail) was at a dose of 1250 mg / kg in the 15 min of latency, and the nociceptive effect in albino rats using the Plantar Test test (hot paw) was at the dose of 1250 at the 30 minute latency. It was observed that the highest nociceptive effect of lyophilized extract of *L. mutabilis* was obtained at the dose of 1250 mg / kg compared with Placebo; In addition, it was demonstrated that there was statistical significance at the dose of 1800 mg / kg at 30 and 60 min of reaction time. In the results of the experiments, both morphine 10 mg / kg and lyophilized extract of tarwi had a nociceptive effect, in morphine 10 mg / kg it was in 60 min (22.03 min reaction time) compared to placebo by the tail test in motion (Tail Flick). In the multiple comparison (Tukey test), for the reaction times, only the group that received the lyophilized extract of *L. mutabilis* with a dose of 1800 mg / kg at 30 and 60 minutes, compared with placebo, using the Plantar Test ( $P < 0.05$ , 95% CI). Previous investigations carried out with atomized extracts of tarwi in albino rats demonstrate their anti-inflammatory effect at a dose of 4000mg / kg (89%) of inhibition, which demonstrates that *L. mutabilis* has a significant anti-inflammatory effect compared to the control Diclofenac © 10mg / kg (93%). Conclusions of this research are: the lyophilized extract of *Lupinus mutabilis*, both in the acute toxicity test by the Probits and sub chronic toxicity by means of the administration of dose response, during 30 days in the exposed albino rats, its consumption is not toxic, so it is innocuous, according to the toxicity parameters of Williams. In the antinociceptive evaluation only an effect was found in the Plantar Test, since the effect of the 1800 mg / Kg dose at 30 and 60 minutes of administration was similar to that of Morfina ©.

## EVALUACION DE LA TOLERANCIA NOCICEPTIVA, TOXICIDAD A DOSIS REPETIDA DEL TARWI (*LUPINUS MUTABILIS SWEET*) EN ANIMALES DE EXPERIMENTACIÓN

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El dolor es la causa más frecuente de las visitas de los paciente a los médicos, y su alivio debería ser una de las principales razones de la medicina. La Organización Mundial de la Salud y la Asociación Internacional para el Estudio del Dolor, consideran el tratamiento del dolor, como uno de los derechos humanos de las personas. La nocicepción es la única etapa común en todas las personas que sufren dolores. En los tratamientos del dolor se utilizan opiáceos de alta toxicidad cuyas dosis terapéuticas han sido altamente evaluadas. En Perú el *Lupinus mutabilis Sweet*, conocido con los nombres de tarwi o chocho es una especie nativa que contiene alcaloides quinolizidínicos, principalmente lupanina y esparteína, con un contenido total que oscila entre 0.02% y 3.17%. Si bien está demostrado que los alcaloides son tóxicos, en Perú se usa, en forma empírica y tradicional, el agua de chocho con fines medicinales, desde la época incaica.

Por lo mencionado, el objetivo de esta investigación fue evaluar la toxicidad sub aguda, toxicidad sub crónica y el efecto antinociceptivo del tarwi (*Lupinus mutabilis Sweet*) en roedores.

Se utilizaron n=84 ratones y n=110 ratas albinas. Se realizaron los test de Plantar Test y Tail Flick. Se aplicó la prueba de normalidad de Kolmogorov Smirnov, ANOVA y prueba de Tuckey. Se consideró significancia estadística de  $p < 0.05$  con intervalo de confianza de 95%.

Los resultados muestran un efecto nociceptivo en ratones albinos utilizando el test Tail Flick (cola fijada) fue a la dosis de 1250 mg/kg en el min 15 de latencia, y el efecto nociceptivo en ratas albinas utilizando el test Plantar Test (pata caliente) fue a la dosis de 1250 en el minuto 30 de latencia. Se pudo observar que el mayor efecto nociceptivo de extracto liofilizado de *L. mutabilis* se obtuvo a la dosis de 1250 mg/ kg comparado con Placebo; además, se demostró que hubo significancia estadística a la dosis de 1800 mg/ kg a los 30 y 60 min de tiempo de reacción. En los resultados de los experimentos, tanto la morfina 10 mg/Kg como el extracto liofilizado de tarwi tuvieron efecto nociceptivo, en morfina 10 mg/ kg fue en 60 min (22.03 min de tiempo de reacción) comparado con el placebo mediante el test de cola en movimiento (Tail Flick). En la comparación múltiple (Prueba de Tuckey), para los tiempos de reacción, solo el grupo que recibió el extracto liofilizado de *L. mutabilis* con dosis de 1800 mg/ kg a los minutos 30 y 60, comparado con placebo, utilizando el Plantar Test ( $P < 0,05$ , IC 95%). Investigaciones previas realizadas con extractos atomizados de tarwi en ratas albinas, demuestran su efecto antiinflamatorio a la dosis 4000mg/ kg (89%) de inhibición, por lo que se demuestra que el *L. mutabilis*, manifiesta efecto antiinflamatorio significativo, comparado con el control Diclofenaco® 10mg/Kg (93%).

El Extracto Liofilizado del *Lupinus mutabilis*, tanto en el ensayo de toxicidad aguda por los Probits y toxicidad sub crónica mediante la administración de dosis respuesta, durante 30 días en las ratas albinas expuestas, no resulta tóxico su consumo, por lo que es inocuo, de acuerdo a los parámetros de toxicidad de Williams. En la evaluación antinociceptiva solo se encontró efecto en el Test Plantar, ya que el efecto de la dosis 1800 mg/Kg a los 30 y 60 minutos de administración era similar al de Morfina®.

## **$\beta$ -CONGLUTIN PROTEINS FROM NARROW-LEAFED LUPIN (*Lupinus angustifolius* L.) SEEDS WITH ANTI-COLORECTAL CANCER POTENTIAL: CURRENT EVIDENCES FOR THEIR MOLECULAR MECHANISMS**

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*Lupinus angustifolius* (narrow-leafed lupin, NLL) seeds are a valuable source of proteins, and an innovative source of functional food ingredients with different positive health aspects. Previous studies identified active plant secondary metabolites as isoflavones and cinnamic acid derivatives from *Lupinus albus* and *Lupinus angustifolius* seeds with antiproliferative activity in breast cancer cells. However, no information is known about how NLL seed proteins affect cancer (colorectal) cell at molecular level.

The aim of this study was to evaluate the effects of  $\beta$ -conglutin proteins on colon cancer cell *in vitro*.

We purified NLL recombinant beta-conglutin proteins ( $\beta$ 1,  $\beta$ 3, and  $\beta$ 6) using affinity-chromatography. We used *In vitro* cell culture of two colon cancer cell lines with different status of p53 gene: HCT-116 (p53 wild-type), and HCT-116 p53 null (p53 inactive). We performed MTT assay to analyze cells viability after treatments. Cell cycle and cell death were studied using commercial kits and flow cytometry.

Our results showed that  $\beta$ 1,  $\beta$ 3 and  $\beta$ 6 conglutins affect the viability by inhibiting the growth of colorectal cancer cells. This effect is achieved at a very low concentration for all proteins, where IC<sub>50</sub> values of HCT-116 cell line were 0.8, 5.8 and 30.1  $\mu$ g/ml for  $\beta$ 1,  $\beta$ 3 and  $\beta$ 6, respectively. Interestingly, when the HCT-116 p53 null line was treated, the IC<sub>50</sub> values were 3.0, 3.4 and 51.8  $\mu$ g/ml for  $\beta$ 1,  $\beta$ 3 and  $\beta$ 6, respectively. Thereby, in both cell lines, the higher effect of  $\beta$ -conglutin proteins was  $\beta$ 1> $\beta$ 3> $\beta$ 6.

$\beta$ 1,  $\beta$ 3, and  $\beta$ 6 conglutins decreased cell proliferation by inducing cell cycle arrest, independently of p53.  $\beta$ 1 and  $\beta$ 3 induced accumulation in G0/G1 phase, while  $\beta$ 6 induces, in addition, the accumulation in G2/M phase. These conglutins considerably increased the apoptosis, independently of p53.

NLL  $\beta$ -conglutin proteins may be potential chemotherapeutic agents with potential uses for treatment of human colon cancer.

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# PREVALENCE OF LUPIN SENSITIZATION IN WESTERN MEXICAN CHILDREN POPULATION

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Lupin foods have acquired scientific relevance and have now begun to be consumed in many countries due to its proven health benefits. Lupins are a rich and important protein's source. However, lupin safety inclusion in the human diet must be considered, since IgE binding proteins are present in their seeds and therefore allergenic responses are triggered. Mainly, lupin storage proteins have been identified as allergens. Although, Mexico is a country with a wide spectrum of natural resources including wild- Mexican lupin species these are not consumed due to their high alkaloid content and toxicity. Nevertheless, before consumption of domestic lupin products, either for health or nutritional benefits, the side effects as the allergenic potential, in the Mexican population, must be considered. In order to know, if the lupin could be safely consumed, we aimed in this cross-sectional study to assess the lupin sensitization in western Mexican children.

This study was conducted at the Immunology and Food Allergy Department of the Hospital Civil de Guadalajara, Guadalajara, Jalisco, México and involved 119 Mexican children (6 to 15 years of age) attended for the first time. The internal Ethics Committee of Hospital Civil de Guadalajara approved this study. Children and their parents or legal guardians were invited and if they agreed to participate in the study provided their informed consent. All participants and/or their parents or tutors answered questions regarding habits, clinical and genetic backgrounds. None of the participants was treated with antihistamines, homeopathy or was taking anti-inflammatory anti-viral or anti-flu drugs. Percutaneous of prick test was performed and interpreted by the same expertise and certified pediatric allergist. Negative (mineral oil as vehicle) and positive (histamine) controls were also simultaneously done in every test.

Results of this research have shown that the mean age  $\pm$  SD was  $9 \pm 2.34$  years old. The male-female ratio was 1.2:1 (65 boys and 54 girls). The most common diagnoses were bronchial asthma, allergic rhinitis, acute urticaria, rhinoconjunctivitis and food allergy. Almost 9% (8.6%) of the total number of Mexican pediatric children had positive skin reactivity against lupin in the prick test.

In Mexico, there are studies that report especially food allergy against soybean, and peanuts, among others. Worldwide, food allergy could affect up to 8% of children (ranging from 2 to 10 %). From these allergies, as far as we know, lupin allergy prevalence in Mexican population has not been evaluated or even reported. Therefore, we have firstly reported lupin sensitization in Mexican children. Taking together our results, we can propose and design the lupin products inclusion accordingly with our traditional Mexican diet enhancing its health benefits.

## **$\beta$ -CONGLUTIN PROTEIN FROM *Lupinus albus* IMPROVES ORAL GLUCOSE TOLERANCE IN HEALTHY RATS AND REDUCES CIRCULATING CHOLESTEROL LEVELS IN STREPTOZOTOCIN-INDUCED DIABETES**

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Diverse lupin seed components have shown advantageous effects on health. Around 40% of the lupin seeds corresponds to protein, mainly albumins and globulins. This latter fraction of globular proteins is composed in turn by  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -conglutins. Interestingly, proteins and peptides derived from this legume have been identified as biologically active constituents.  $\gamma$ -conglutin protein, the scarcest of the four globulins present in lupin seeds, has been reported to decrease glycaemia *in vivo* through the modulation of glucose metabolism. On the other hand, lupin purified protein isolate, containing mainly  $\alpha$ - and  $\beta$ -conglutins, either from white or narrow-leaf lupins have been reported to significantly reduce total cholesterol levels, LDL-cholesterol, and the atherogenic potential of cholesterol-rich diets. Additionally, *in vitro* experiments with recombinant  $\beta$ -conglutins have been found to promote the activation of the insulin signaling cascade whereas pro-inflammatory markers were reduced in peripheral cells from type 2 diabetes patients. Moreover, peptides derived from  $\beta$ -conglutin have been pointed out as modulators of crucial enzymes for glucose homeostasis such as the dipeptidyl peptidase IV. Therefore, we aimed to assess *in vivo* the acute and chronic effects of  $\beta$ -conglutin on glycaemia. Then, the objective of this investigation was to evaluate the acute effect of  $\beta$ -conglutin protein from *Lupinus albus* on the oral glucose tolerance test in healthy rats and the chronic effect of the protein on induced-to-diabetes animals. Lyophilized  $\beta$ -conglutin protein was obtained from certified *Lupinus albus* seeds. After an overnight fasting (12 h), male Wistar rats (200 – 250 g) received either vehicle (saline solution), 120 mg/kg or 240 mg/kg of  $\beta$ -conglutin *per os* 30 min before an exogenous oral load of glucose (2 g/kg). Using a glucometer, glycaemia levels were determined at 0, 30, 60, 90 and 120 min after glucose administration. For the chronic effect, male Wistar rats (200 – 250 g) were chemically induced to diabetes by a single intraperitoneal injection of streptozotocin (65 mg/kg). Fasting glycemia  $\geq$  200 mg/dl was the cut-off value for experimental animals to be considered as diabetic.  $\beta$ -conglutin (120 mg/kg) was administered by oral gavage for 7 consecutive days. Blood samples were obtained from retro-orbital plexus of fasted (12 h) and sedated animals at the beginning and the end of the trial. Two-tailed Student's *t* test was used to compare the results, *p* values  $<$  0.05 were considered as statistically significant differences. Results of this investigation have shown as an acute effect,  $\beta$ -conglutin protein (120 or 240 mg/kg) significantly reduced the curve of the oral glucose tolerance test in healthy rats as compared to control at 60 and 90 min ( $n = 3$ ,  $p < 0.05$ ). The effect of  $\beta$ -conglutin on glycaemia was not found to be dose-dependent since there was not significant difference when 120 vs 240 mg/dl doses were compared. Based on these results, the long-term effect of 120 mg/kg of  $\beta$ -conglutin protein was assessed. After a 7-day treatment, fasting glycaemia was not affected in diabetic rats by  $\beta$ -conglutin protein treatment ( $n = 4$ ,  $p = 0.45$ ). Similarly, serum levels of urea, creatinine, triglycerides, aspartate aminotransferase, and alanine aminotransferase remained unchanged after treatment ( $p > 0.05$ ); in contrast, total cholesterol serum levels were significantly decreased by 21.77% ( $n = 4$ ,  $p = 0.039$ ) after a 7-day administration of  $\beta$ -conglutin in diabetic rats. To the best of our knowledge, this is the first report of the effect of  $\beta$ -conglutin protein on glycaemia under healthy and disease-model animals. Acutely,  $\beta$ -conglutin significantly improves glucose tolerance in healthy rats whilst in the chronic effect, fasting glycaemia remained unchanged in diabetic rats, however levels of total cholesterol were significantly reduced in this state.

# SEED $\beta$ -CONGLUTIN PROTEINS FROM NARROW-LEAFED LUPIN (*Lupinus angustifolius* L.) AS FUNCTIONAL FOOD TO TACKLE INFLAMMATION AND INSULIN RESISTANCE: CURRENT INSIGHTS AND MOLECULAR MECHANISMS

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Sustained inflammation causes and advances many common diseases, i.e. obesity linked to metabolic disease, insulin resistance leading to Type 2 Diabetes (T2DM), dyslipidemia and cardiovascular diseases. Thus, it is of mayor urge to develop alternative and efficient new treatments and therapies to ameliorate the inflammatory processes. *Lupinus angustifolius* (Narrow-leafed lupin, NLL) seed  $\beta$ -conglutin proteins rise as an outstanding alternative to be used as functional food to tackle inflammatory-related diseases, on the basis of current ongoing research and its nutraceutical properties.

The objective of this investigation was the evaluation of anti-inflammatory and anti-diabetic effects of NLL  $\beta$ -conglutins at molecular basis.

$\beta$ -Conglutin isoforms were purified by using affinity-chromatography. Challenges with  $\beta$ -conglutins were performed in *ex vivo* (blood samples from T2DM subjects) and *in vitro* pancreatic cell model, and in an insulin resistance model. Biochemical and molecular approaches consist in immunoblotting and ELISA, enzymatic activities quantification, proteins posttranslational modifications (phosphorylation, carbonylation), RT-qPCR, PCR array, glucose uptake assay, pro-inflammatory molecules quantification.

Results of this research have shown that inflammatory and T2DM molecular effects are improved by NLL Conglutin  $\beta$ 1,  $\beta$ 3 and  $\beta$ 6 through promoting the attenuation of mRNA expression in key pro-inflammatory mediators of  $\beta$ -cell damage including IL-1 $\beta$ , CCL5, MCP-1, ICAM-1, cytokines (TNF- $\alpha$ , INF- $\gamma$ ), and mediator NF- $\kappa$ B1. The same conglutins significantly decline proteins production of pro-inflammatory cytokine TNF- $\alpha$ , INF- $\gamma$ , IL-1 $\beta$ , IL-2, IL-6, IL-8, IL-12, and IL-17, as well as inhibit NO production and mRNA and protein levels of iNOS.

In this regard, the ability of NLL  $\beta$ -conglutins to improve insulin resistance is due to the activation (phosphorylation) of insulin signalling molecular pathway genes IRS-1 and Akt, and downstream mediators (Cbl, Caveolin), up-regulation of IRS-1 and GLUT-4 genes expression and increasing p85-PI3K and GLUT-4 proteins levels, the enhancement of anti-oxidative cellular systems while reducing the ROS production, and balancing genes expression of T2DM metabolic as molecular makers.

Our results unveiled for the first time the molecular basis and mechanisms promoted by NLL  $\beta$ -conglutin proteins able to reverse the induced insulin resistance as potential T2DM treatment, as well as the amelioration of the underlying molecular effects happening during the inflammation.

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# FUNCTIONAL INTERPLAY BETWEEN CONGLUTIN PROTEINS MOBILIZATION AND OXIDATIVE-RELATED METABOLISM IN NARROW-LEAFED LUPIN (*lupinus angustifolius* L.) SEED GERMINATION AND SEEDLING GROWTH

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Oxidative metabolism plays a crucial role in seed germination since its activation allows from releasing the seed from dormancy to controlling the advance of the different germination stages. Seed storage mobilization together with oxidative metabolism, with ascorbate-glutathione cycle as crucial signalling and metabolic functional crossroad, are one of the main regulators of the control of cell morphogenesis and division, fundamental physiological processes driving seed germination and seedling growth.

The aims of this study are to characterize the cellular changes, composition and patterns of proteins mobilization and ROS-dependent genes expression of redox metabolism in *Lupinus angustifolius* (narrow-leaved lupin, NLL) cotyledons during germination and seedling growth.

We performed genes expression analyses by RT-qPCR for conglutinins  $\alpha$  (1, 2, and 3),  $\beta$  (1, 2, 5),  $\gamma$  (1,2),  $\delta$  (2, and 4), including ubiquitin gene as control; and for redox metabolism related genes; GADPH was used as control gene. Microscopy study was developed on cotyledon samples from different germination stages such as IMB (Imbibition), 2-5, 7, 9 and 11 DAI (days after imbibition), which were processed for light microscopy. SDS-PAGE and Immunocytochemistry assay were performed by using an anti- $\beta$ -conglutin antibody (Agrisera), and an anti-rabbit IgG Daylight 488-conjugated secondary antibody. The controls were made omitting primary Ab.

Our results showed that a large amount of storage proteins is accumulated in protein bodies (PBs), and mobilized during germination. Particular families of conglutinins ( $\beta$ , and  $\gamma$ ) may play important roles as functional (signalling) molecules (beyond the storage function) in intermediate steps of the germination progress. In this regard, metabolic activities are closely associated to the regulation of the oxidative homeostasis through ascorbate-glutathione activities ( $\gamma$ -L-Glutamyl-L-cysteine synthetase, NOS, Catalase, Cu/Zn-SOD, GPx, GR, GS, GsT) after imbibition of NLL mature seed, metabolism activation and dormancy breakage, which are key molecular and regulatory signalling pathways having a particular importance in morphogenesis and developmental processes.

The knowledge generated in this study provides evidence for the functional changes, cellular tightly regulated events occurring in the NLL seed cotyledon, orchestrated by the oxidative-related metabolism machinery involved in seeds germination advance.

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# THE USE OF WHITE LUPINE AND OTHERS SEEDS AS MEDICINAL SUPPLEMENTS AT BREAKFAST TIME

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Medicinal herbs are widely used both for culinary and therapeutic purposes from ancient times and they are part of the traditional medicine. The benefits of medicinal plants and seeds in illness treatment or as illness prevention are becoming more widely used as a regular health care practice in the last two decades. In Argentina the grains of bitter white lupines (*Lupinus albus*) are commonly used as medicine, also, in herbal and dietary shops and pharmacies, tablets obtained from pure extracts of grains of white lupines produced by laboratory Natufarma, are sold under the commercial name “Lupines with vitamin E”, as dietary complement to control gout and rheumatism. A comparative analysis was done between people ingesting bitter white lupines seeds, Lupines tablets and the ones that use other seeds as medicinal supplement meals at breakfast time, which give the idea of a “medicinal breakfast survey”. Plant ingredients used as part of the medicinal breakfast are denominated with the generic name of “seeds” (*sensus lato*) in concordance with names use in the food shops, although many are complexes seed dispersal organs. A transversal survey to collect information with a standardized procedure with predetermined answers that were chosen by marking a cross, was submitted for 30 days to the customers of mayor health or dietary food stores, in the city of Buenos Aires (25 shops), in the city of Córdoba (10 shops) and in the city of Alta Gracia (two shops). The first part of the questionnaire was to register sex and age using a scale of ages that began with less than 20 years and ended with more than 80. The second parte asked about the use of lupines or lupines tablets, the list of all seeds used in the breakfast meal, the way that are processes and ingested and the purpose of the intake. Results shows that most consumers of lupines seeds and Lupines tablet are ages between 45 and 80 years old (80% of total) with not significant different between females and males. The total number of people taking lupines seeds was 368 (272 Buenos Aires, 83 Córdoba, 13 Alta Gracia). The most common way of lupines intake are overnight soaked seeds: 3 seeds (80 %), 5 seeds (15 %) and variable number 1 to7 (5 %), which are taken each morning before the regular breakfast meal. The total number of Lupines tablet consumers was 54 (63 Buenos Aires, 16 Córdoba, 4 Alta Gracia). The most common way of taking them was one tablet a day at any time. In addition, 78 people in Buenos Aires and 9 in Córdoba mentioned that as well as taking lupines seeds in the morning they also take Lupines tablet at any time of the day. The main purposes of lupines or Lupines tablets were: to reduce uric acid 267 (72.55%), cholesterol 23 (6.25%), cholesterol and uric acid 78 (21.19%). The other part of the survey shows that only women between 40 and 70 years prepare a mixture of seeds, to be eaten every morning at breakfast time. The species most commonly used in seed mixtures were: chia, sesame, flax, sunflower. Other ingredients also used in fewer amounts were wheat germ, oat bran and almonds. The more common uses are mixing the seeds with yogurts and spreading the seeds over bread or cookies as toppings, only 15 are taking a teaspoon of the dry seeds with a sip of water. The main purposes of seeds intake were to lowering cholesterol, losing weight or being healthy. The surveys reveal that the number of people that prepared a mixing of seeds for medicinal breakfast are: In Buenos Aires, 423; in Córdoba, 210 and in Alta Gracia 40. This research reveals that people consider that ingesting seeds in the morning is a healthy habit for preventing illness which are giving credit to the denomination of medicinal breakfast. The contribution of the people and the personal of the shops that participate in the survey is greatly appreciated.

## EL USO DE SEMILLAS DE LUPINES BLANCO Y OTRAS SEMILLAS COMO SUPLEMENTOS MEDICINALES A LA HORA DEL DESAYUNO

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Las hierbas medicinales son ampliamente utilizadas desde la antigüedad tanto para fines culinarios como terapéuticos y forman parte de la medicina tradicional. En las últimas dos décadas son cada vez más ampliamente utilizados los beneficios de las semillas y de las plantas medicinales para tratamientos de enfermedades o como prevención de enfermedades. En Argentina los granos de los lupines blancos amargos (*Lupinus albus*) se utilizan como medicinales, y también, se expenden en las farmacias y negocios dietéticos las tabletas de extracto puro de semillas de lupines, producidas por el laboratorio Natufarma, que se venden como complemento dietético para el control de la gota y el reumatismo bajo la nombre comercial de "Lupines con vitamina E". Se realizó un análisis comparativo entre la gente que ingiere semillas de lupines blanco amargos, las que toman las tabletas de Lupines y aquellas que utilizan otras semillas como suplemento dietético medicinal a la hora del desayuno, lo que dio la idea de una "encuesta llamada desayunos medicinales". Los Ingredientes que forman parte del desayuno medicinal están denominados con el nombre genérico de "semillas" (*sensus lato*) en concordancia con el uso de nombres en los negocios, aunque muchos de ellos son órganos de dispersión de semillas más complejos. Para recoger información se realizó por 30 días una encuesta con los clientes de las principales herboristerías y dietéticas de la ciudad de Buenos Aires (25 negocios), de la ciudad de Córdoba (10 negocios) y de la ciudad de Alta Gracia (dos negocios). La encuesta tuvo un carácter transversal con un procedimiento estandarizado con respuestas predeterminadas que fueron elegidas marcando una cruz. La primera parte del cuestionario fue registrar el sexo y la edad utilizando una escala de edades que comenzó con menos de 20 años y terminó con más de 80. En la segunda parte se preguntó sobre el uso de semillas y de tabletas Lupines y la lista de todas las semillas utilizadas en el desayuno, la manera en que son procesadas e ingeridas y el propósito de la ingestión. Los resultados muestran que la mayoría de los consumidores de semillas y de tabletas tienen entre 45 y 80 años (80% del total) sin diferencias significativas entre hombres y mujeres. El número de personas que toman semillas de lupines fue 368 (272 Buenos Aires, Córdoba 83 y 13 Alta Gracia). La forma más común de la ingesta es tomar todas las mañanas antes de la comida del desayuno, 3 semillas (80%), 5 semillas (15%) o un número variable de 1 a 7 (5%) de semillas previamente remojadas durante toda la noche. El número total de consumidores de tabletas Lupines fue 54 (63 Buenos Aires, 16 Córdoba y 4 Alta Gracia). La forma más común de tomarlos es una tableta al día en cualquier momento. Además, 78 personas en Buenos Aires y 9 en Córdoba tomar semillas de lupines en la mañana y también tabletas Lupines en cualquier momento del día. Los propósitos principales de la toma de semillas o tabletas de Lupines fueron: para reducir el ácido úrico 267 (72.55%), el colesterol 23 (6,25%), y el colesterol y el ácido úrico 78 (21.19%). La otra parte de la encuesta muestra que sólo mujeres entre 40 y 70 años preparan una mezcla de semillas, para comer por las mañanas en el desayuno. Las especies más comúnmente en las mezclas son: chia, lino, sésamo, girasol. Otros ingredientes que también se utilizan en menor cantidad fueron el germen de trigo, el salvado de avena y almendras. Los usos más comunes son, mezclar las semillas con yogures y esparcirlas sobre pan o galletas; sólo 15 personas reportaron que toman una cucharadita de semillas secas con un sorbo de agua. Los propósitos principales de la ingesta de semillas fueron, para bajar el colesterol, perder peso y estar saludable. Las encuestas revelan que el número de personas que preparan una mezcla de semillas como desayuno medicinal es: en Buenos Aires, 423; en Córdoba, 210 y en Alta Gracia 40. Esta investigación revela que la gente considera que la ingestión de las semillas en la mañana es un hábito saludable para prevenir enfermedades lo que da crédito al término desayunos medicinales. Se agradece la contribución de la gente y el personal de las tiendas que participaron en la encuesta.

## ESTIMATES OF TOTAL PHENOLICS CONTENT IN 52 GENOTYPES OF BITTER AND DEBITTERED LUPIN FLOUR (*Lupinus mutabilis*)

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Phenolics are components that contribute with the scavenging of free radicals, specially mentioned are polyphenols and flavonoids in the protection against atherogenic LDL modifications. This work determines total phenolic content in different genotypes of Peruvian *Lupinus mutabilis*. Phenolics were determined on samples previously dried and grounded; from whole seeds and from water washed debittered seeds. Flour samples were extracted with ethanol:water 50:50 and sonicated in a water bath for 30 minutes. Centrifugation followed at 3000 RPM for 15 minutes at 5°C. Extraction was repeated twice. The reaction of the extracts with Folin-Ciocalteu and all reagents was followed using a microplate reader at 760nm. Gallic acid was used as standard. Results indicated that total phenolics for bitter flour of genotypes ranged from (mg AGE/100g)  $437.39 \pm 3.65$  (*Andenes UNALM* white grain) or  $428.49 \pm 1.79$  (*PLGO 3* white grain) for lower values to higher values of (mg AGE/100g)  $526.80 \pm 6.24$  (*Compuesto blanco semi precoz* white grain) or  $526.71 \pm 3.50$  (*CD Junín 2* white grain). On the other hand, for debittered flour of genotypes, total phenolic content ranged from (mg AGE/100g)  $53.03 \pm 0.99$  (*Patón grande* white grain) or  $55.15 \pm 1.03$  (*Chacas* white grain) for lower values, to higher values of (mg AGE/100g)  $193.70 \pm 7.40$  (*Yunguyo negro* black grain) or  $231.84 \pm 1.99$  (*Yana tarwi* black grain). A reduction in the amount of total phenolics was observed due to the process of water washed debittered of seeds for all genotypes.

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# DEFLAMIN ISOLATED FROM *Lupinus mutabilis* SEEDS INHIBITS COLON CANCER CELL INVASION

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Colorectal cancer (CRC) is the second most common cancer diagnosed worldwide and despite the significant advances in medicine, the overall long-term outcome in patients has not significantly changed. A subgroup of matrix metalloproteinases (MMPs) called gelatinases (MMP-2 and MMP-9) was found to be largely implicated in CRC and their inhibitors (MMPIs) were demonstrated to be effective in reducing cancer development and metastization and consequently, the search for novel food-borne MMPIs has become a major topic for research.

A natural protein inhibitor of MMPs called deflamin was found by our research group in *Lupinus albus* seeds. Deflamin is a low molecular weight protein (~17 KDa) that showed to have anti-inflammatory and anti-cancer activities *in vivo* and *in vitro* assays. Although the MMPI potential has been studied in other legume seed species, *Lupinus albus* was the one with highest activity. Thus, the search for this peptide in other lupin species and varieties holds great potential, particularly in *Lupinus mutabilis* since it is a species of lupin that is adapted to unique and harsh environmental conditions, which could be related to the presence of different bioactive compounds worth studying, including possible variations in deflamin. Hence our main goal was to attempt to isolate deflamin from *L. mutabilis* and determine its potential against colon cancer cell invasion.

Seeds of different *L. mutabilis* varieties were used and deflamin was extracted and isolated through a method developed in our laboratory adapted to this protein's features such as resistance to high temperature and low pH as well as a high water solubility. *Lupinus mutabilis* deflamin's polypeptide profile was identified through SDS-PAGE in reductive and non-reductive conditions and compared with *L. albus* deflamin in the same conditions as described. Anti-cancer activities were determined in colon adenocarcinoma cells (HT29) using the wound healing assay.

Results reveal that the MMP inhibitor deflamin is indeed present in different varieties of *L. mutabilis* species, which suggests that this peptide may be common within the *Lupinus* genus. The isolated deflamin also showed ability to effectively reduce colon cancer cell invasion *in vitro*. Further steps include selecting *L. mutabilis* deflamin to be sequenced and become novel candidates as metalloproteinase inhibitors with possible pharmacological interest.

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## UTILIZATION OF WHITE LUPINE SEEDS FOR DWARF RABBITS' NUTRITION – THEIR EFFECT ON GROWTH RATE AND BLOOD INDICATORS

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Recent studies on hybrid meat-type rabbits found that dietary inclusion of white lupin (WL) seeds had a favourable effect on the production and quality of milk in does, on growth performance of young rabbits and fattened rabbits, on mortality and other important productive traits. The aim of the present study was to assess the effect of inclusion of the meal from whole WL seeds in diets on growth and selected blood indicators of young dwarf rabbits. The control (C) group of the Dwarf Lop rabbit does and their litters was fed a commercial pelleted diet intended for nutrition of dwarf rabbits, when main protein sources used in this diet were alfalfa meal and malt sprouts. Dwarf Lop rabbit does and their litters in the experimental (E) group were fed a pelleted diet containing 25 % proportion of meal from whole WL seeds of *Amiga var.* as the main protein feed component. After weaning (7<sup>th</sup> week of age), young rabbits of the C group were fed the same commercial diet as before weaning, whereas young rabbits of the E group were fed the pelleted experimental post-weaning diet containing 20 % proportion of meal from whole WL seeds of *Amiga var.* At the age of 21 days, rabbit kits of the E group displayed a higher average body weight (BW) as compared to those in the C group (225 vs. 192 g, respectively;  $P < 0.05$ ). This finding indirectly indicates on higher milk production of does in the E group, which is in accordance with results recently observed in meat-type rabbit does, when they were fed a diet partially consisting of WL seeds. Afterwards, at the age of 35, 49, 63, 77 and 91 days, no significant differences were found in BW between the E and C groups. However, at the end of the experiment (105<sup>th</sup> day of age), rabbits of the E group showed a higher BW as compared to those in the C group (1179 vs. 1062 g, respectively;  $P < 0.05$ ). Blood examination in the 105-day old dwarf rabbits revealed that the E group showed the decreased level of albumin and the value of albumin/globulins ratio in the plasma as compared to the C group ( $P < 0.01$ ). In addition, rabbits of the E group displayed the lower plasma level of triacylglycerols (TAG) and the lower activity of alanine aminotransferase (ALT) in the blood plasma as compared to those in the C group ( $P < 0.05$ ). Inclusion of the 25 % proportion of meal from whole WL seeds in the diet intended for lactating does led to the favourable increase in BW of suckling dwarf rabbit kits. Feeding the experimental post-weaning diet with 20 % proportion of meal from whole WL seeds resulted in favourable growth intensity of raised dwarf rabbits, while at the end of the experimental period these rabbits gained advantageous higher BW. Moreover, the experimental post-weaning diet had the significant effect on protein metabolism and the significant TAG-lowering effect was observed in blood plasma of 105-day old rabbits. Also the positive effect of the experimental post-weaning diet on the activity of ALT in blood plasma was found in these rabbits. The study was financially supported by project no. 207/2017/FVHE IGA of UVPS Brno.

## REDUCTION OF *JNK-1* GENE EXPRESSION IN A DIABETIC RAT MODEL TREATED WITH CONGLUTIN GAMMA ISOLATED FROM TWO *Lupinus* SPECIES

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*Jnk-1* is a member of c-Jun-N-terminal-kinase family that increases in obesity and diabetes conditions and contributes to insulin resistance. Due *Jnk-1* is considered target for type 2 diabetes (T2D) treatment, recently, compounds that reduces its expression has been investigated. In this senses, conglutin gamma (C $\gamma$ ) of *Lupinus* is an interesting glycoprotein due their several beneficial effects on human health. It has been described that C $\gamma$  reduces blood glucose levels, diminishes insulin resistances and *G6pc* gene expression, besides, increases *Ins-1* gene expression, among other effects, suggesting its potential use to treat T2D. The *Lupinus* genus is composed by several wild and domesticated species. In the present study particularly two species, *L. albus* (domesticated specie) and *L. rotundiflorus* (wild specie) are of interest. The aim of this study was to evaluate the *Jnk-1* gene expression in a diabetic rat model treated with conglutin gamma isolated from two *Lupinus* species.

Conglutin gamma (C $\gamma$ ) was isolated from *Lupinus albus* (domesticated specie) and *L. rotundiflorus* (wild specie) seeds and characterized by SDS-PAGE. Male Wistar rats were induced to diabetes through streptozotocin intraperitoneal injection (65 mg/kg bw). Experimental animals were randomly divided into three groups and received orally (7 days) the corresponding treatment as following; HG- Ctrl (vehicle), HG-La (120 mg/kg, C $\gamma$  of *L. albus*) and HG-Lr (120 mg/kg, C $\gamma$  of *L. rotundiflorus*). Serum glucose levels were quantified after and before treatments in all groups. The rats were anesthetized, sacrificed and the liver tissue was obtained to extract RNA. The RNA was retrotranscribed into cDNA to quantified *Jnk-1* gene expression by real time-PCR.

In the present study, C $\gamma$  of a domesticated (*L. albus*) and a wild (*L. rotundiflorus*) *Lupinus* species was assayed. As expected, serum glucose levels in both C $\gamma$  groups (HG-La and HG-Lr) after treatment were diminished in 21.9 % and 23.4% respectively ( $P<0.05$ ). This reduction correlated with the C $\gamma$  hypoglycemic effect reported by other studies. In contrast serum glucose levels were increased in diabetic group without treatment (HG). On the other hand, it has been described that JNK is involved in insulin resistance representing a target for diabetes treatment. Interestingly, administration of C $\gamma$  of both *L. albus* (49.5%) and *L. rotundiflorus* (55.83%) reduced *Jnk-1* gene expression in experimental animals in comparison to control group (HG). This could be one of the diverse mechanisms of C $\gamma$  hypoglycemic effect. Further studies are necessary to investigate the effect of C $\gamma$  in molecules involved in JNK pathway.

In conclusions C $\gamma$  of *L. albus* and *L. rotundiflorus* diminish *Jnk-1* gene expression in diabetic Wistar rats.



**GENETIC, GENOMICS  
& MOLECULAR BREEDING**

**GENÉTICA, GENÓMICA  
Y MEJORAMIENTO MOLECULAR**





## GENOMIC SELECTION IN WHITE LUPIN

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Genomic selection, which estimates breeding values by large sets of molecular markers in a statistical model, represented a breakthrough for improving cattle production. Its use for plant breeding has been favoured by the development of Genotyping-by-Sequencing, which can generate large sets of SNP markers at a lower cost than array-based techniques. Genomic selection has shown promise for improving legume crop yield in pioneer studies on soybean, alfalfa and pea.

The main aim of this study was to investigate for the first time the application of genomic selection to lupins, by assessing its ability to predict the grain yield and 10 agronomic traits of white lupin (*Lupinus albus* L.) landraces and varieties grown in Northern Italy. A second aim was to report on on-going work aimed to develop genomic selection models for yield, stress tolerance and grain quality of inbred lines.

The study included 83 landraces from 9 major historical cropping regions (Italy, Egypt, Spain, Portugal, Turkey, Maghreb, Madeira-Canaries, Near East, East Africa), each represented by 3-4 individuals, and 8 French varieties, each represented by 2 genotypes. Phenotypic data of the 91 cultivars are described in Annicchiarico et al. (2010). GBS of the genotypes based on Elshire et al.'s (2011) protocol with modifications issued 6578 polymorphic SNPs. Genomic predictions based on SNP allele frequencies of the cultivars using Ridge regression BLUP or Bayesian Lasso models were assessed by cross validations.

The two models performed comparably. Genomic selection exhibited quite high predictive ability (>0.83) for grain yield, winter survival and onset of flowering, and moderately high predictive ability (0.49-0.63) for plant height, number of leaves on the main stem, leaf size, proportion of seeds on the main stem, number of seeds per pod, and individual seed weight. Only two traits with narrower genetic variation showed modest predictive ability. Our results encourage the genome-enabled identification of promising genetic resources within large germplasm collections. We describe the development a broadly-based sweet-seed reference population from crosses between elite landrace and variety germplasm and its exploitation for defining genomic selection models for inbred lines.

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## OBTAINING SIMILAR PHENOTYPES TO THE DOMESTICATED TARWI FROM ITS WILD RELATIVES

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The tarwi (*Lupinus mutabilis* Sweet) is native to the Andes. Its cultivation is in charge of families of producers with scarce technical assistance, so the planting areas are small. The limiting factors of the crop are the long growing cycle, the susceptibility to adverse abiotic factors and the lack of availability of improved varieties. Tarwi research focused on genetic diversity, crop agronomy, processing and nutritive quality of the grain. Research on the wild relatives of the tarwi in the altiplano is scarce and the ancestral progenitor of the domesticated lupine is still not known. So it has been proposed to collect wild lupine of phenotype closest to domesticated tarwi and study the segregation of its progenies toward the selection by favorable agronomic characteristics. The collection of wild species was carried out in the northern highlands of Bolivia, preferring those wild plants with the size of pods and seed relatively large in relation to wild populations. Once collected, the samples were planted in isolated plots at the Kiphakiphani Research Center (Viacha) and the Cota-Cota Experimental Station (UMSA) in the city of La Paz. The progenies were reproduced by five successive generations by open pollination and the selection started from the third reproduction cycle. As a result of the work, at least five samples of wild tarwi with pod length varying between 31.21 and 43.81 mm and the largest diameter of the seed between 5.19 to 6.28 mm have been collected. In families  $S_1$  and  $S_2$ , the most evident segregation was for productive cycle and grain size, while generations  $S_3$  to  $S_5$  showed a wide diversity in grain size between 5.61 and 9.65 mm, as well as, flower color between blue, purple and pink, while the grain colors were similar to either way the cultivated tarwi and the wild ones. The wild lupine grain varies between 3.03 and 4.66 mm in the largest diameter of the seed. In the cultivated lupine, the largest diameter of the seed is 9.60 mm. On the other hand, in the segregating progenies, the annual growing cycle has been selected. The results obtained lead us to propose that the genetic material collected corresponds to the ancestral relatives that gave rise to the domesticated tarwi. In conclusion it is reported to have collected wild relatives of bi-annual growing cycle (*Lupinus* aff. *mutabilis*.) with relatively large pod and seed size in relation to the common wild species (*Lupinus* ssp.). These germplasm reproduced during five generations of open pollination allowed to obtain phenotypes very similar to the domesticated tarwi in flower color, grain color and size of seed. In the segregating progenies, selection has been initiated by large grain size and annual production cycle. This result is evidence of having collected the probable ancestral progenitors of *Lupinus mutabilis*.

## OBTENCIÓN DE FENOTIPOS SIMILARES AL TARWI DOMESTICADO A PARTIR DE SUS PARIENTES SILVESTRES

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El tarwi (*Lupinus mutabilis* Sweet) es originario de los Andes. Su cultivo está a cargo de familias de productores con escasa asistencia técnica, por lo que las superficies de siembra son pequeñas. Los factores limitantes del cultivo son el ciclo largo, la susceptibilidad a factores abióticos adversos y la falta de disponibilidad de variedades mejoradas. Las investigaciones en tarwi se centraron en la diversidad genética, agronomía del cultivo, transformación y calidad nutritiva del grano. La investigación referida a los parientes silvestres del tarwi en el altiplano es escasa y no se conoce con certeza el progenitor ancestral del tarwi domesticado. Por lo que se ha propuesto recolectar lupinos silvestres de fenotipo más próximo al tarwi domesticado y estudiar la segregación de sus progenies con la finalidad de hacer una selección por características agronómicas favorables. La colecta de especies silvestres se realizó en el altiplano Norte de Bolivia dando preferencia a aquellas plantas silvestres con el tamaño de vaina y grano relativamente grande en relación a las poblaciones silvestres. Una vez recolectadas las muestras fueron sembradas en parcelas aisladas en el Centro de Investigación Kiphakiphani (Viacha) y Estación Experimental de Cota-Cota (UMSA) en la ciudad de La Paz. Las progenies fueron reproducidas por cinco generaciones sucesivas de polinización abierta y la selección se inició a partir del tercer ciclo de reproducción. Como resultado del trabajo se ha recolectado al menos cinco muestras de tarwi silvestre con longitud de vaina que varía entre 31.21 y 43.81 mm y el diámetro mayor de la semilla entre 5.19 a 6.28 mm. En las familias  $S_1$  y  $S_2$  la segregación más evidente fue para ciclo productivo y tamaño de grano, mientras que las generaciones  $S_3$  a  $S_5$  se ha evidenciado amplia diversidad en tamaño de grano entre 5.61 y 9.65 mm como así también en color de la flor entre azul, púrpura y rosado, mientras que los colores de grano fueron similares indistintamente al tarwi cultivado y a los silvestres. El grano del lupino silvestre varía entre 3.03 y 4.66 mm en el diámetro mayor de la semilla. En el tarwi cultivado, el diámetro mayor de la semilla es de 9.60 mm. Por otra parte, en las progenies segregantes se ha seleccionado aquellas de ciclo productivo anual. Los resultados obtenidos conducen a proponer que el material genético recolectado corresponde a los parientes ancestrales que dieron origen al tarwi domesticado. En conclusión se reporta haber colectado parientes silvestres del tarwi (*Lupinus* aff. *mutabilis*.) de ciclo bi-anual con tamaño de vaina y grano relativamente grande en relación al común de las especies silvestres (*Lupinus* spp.). Estos germoplasmas reproducidos durante cinco generaciones de polinización abierta permitieron obtener fenotipos muy similares al tarwi domesticado en color de flor, color de grano y tamaño de grano. En las progenies segregantes se ha iniciado la selección por tamaño de grano grande y ciclo productivo anual. Este resultado evidencia haber recolectado los probables progenitores ancestrales de *Lupinus mutabilis*.

# LUPIBREED: IMPROVING NARROW-LEAFED LUPIN – NOVEL GENETIC RESOURCES FOR HIGHER YIELD AND YIELD STABILITY: PART I: YIELD POTENTIAL AND YIELD STABILITY

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LupiBreed, a research project and name of a German consortium, aimed in improving the productivity of narrow-leafed lupin in terms of yield stability, grain-yield potential and seed composition through the use of novel genetic resources and accompanying breeding research. These novel genetic resources include an EMS-based mutant collection and a subset of different gene bank accessions. In terms of yield performance, in total a collection of 44 mutant lines derived from an EMS treatment have been examined in field, over three years and three locations. A set of mutant lines showed significant increases in kernel yield, protein content, and protein yield compared to the respective original cultivars (Boruta, Probor). Some of these lines turned out to be significantly improved in protein content and protein yield, as well as in earliness compared to the high-yielding cv. Boregine, which has been the dominating narrow-leafed lupin cultivar in Germany during the past ten years. With regard to yield stability, the focus was the improvement of (1) disease resistance against anthracnose and (2) early shattering resistance. Molecular markers were identified which are closely linked to the anthracnose resistance locus *LanrBo* and, thus, allow for marker-assisted selection of *LanrBo* carriers. In addition, these markers may be helpful for pyramiding the independent resistance loci *LanrBo* and *Lanr1*, in order to provide a genetically broad and hopefully durable basis for anthracnose resistance in narrow-leafed lupin. Furthermore, a marker-assisted selection for the non-pod shatter genes *lentus* and *tardus* in crossing programs with the shatter resistant mutant lines and breeding lines that carry the respective genes will improve yield stability. Among a sub-set of gene bank accessions that were analysed for the first time concerning protein and alkaloid contents, some lines were found to have high protein contents along with low alkaloid levels. These accessions may have potential for further breeding activities. Detailed results on protein and alkaloid contents are presented in part II of the LupiBreed project.

# GENETIC AND GENOMIC DIVERSITY IN A TARWI (*Lupinus mutabilis* Sweet) GERMPLASM COLLECTION AND PHENOTYPIC VARIABILITY UNDER MEDITERRANEAN CONDITIONS

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Tarwi (*Lupinus mutabilis* Sweet) exhibits key traits of domestication, including indehiscent pods and seeds with permeable tegument. As for other domesticated crops with little availability of wild specimens, the genetic diversity of *L. mutabilis* is relatively low, but concealing important morphological variability, relatable with the diverse agroecological conditions found across its native range. In an attempt to introduce the cultivation of tarwi in Mediterranean-climate regions, a tarwi germplasm collection was created focused on promising accessions. In this work we report the characterization of the genetic and genomic diversity of such collection using ISSR markers and flow cytometry genome size estimates, respectively, paralleled by the characterization of the phenotypic variability, including morphological/architectural traits. Results show narrow levels of genetic and genomic diversity, reflecting the domesticated nature of this collection, but reveal higher levels of morphological variability, suggesting some phenotypic plasticity and capacity of adaptation. [This project has received funding from the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme under grant agreement No 720726]

## IDENTIFICATION OF ANTHRACNOSE RESISTANCE AND FLOWERING TIME GENES IN YELLOW LUPIN (*Lupinus luteus* L.): A KEY COMBINING GENETIC TRAITS FOR SUSTAINABLE PRODUCTION

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Lupins have great potential to develop as a viable alternative crop as part of sustainable agricultural systems. *Lupinus luteus* (yellow lupin) has good nutritional qualities and protein content. The protein content in *L. luteus* has been reported as an enormous advantage in the aquaculture sector and is becoming the most prominent species for this sector. Furthermore, protein isolate of *L. luteus*, has functional and physicochemical properties suitable for the health food industry. In Chile, in the Agriaquaculture Nutritional Genomic Center (CGNA) there is an ongoing breeding program of yellow lupin oriented to develop more adapted cultivars in terms of agronomic, protein content and techno-functional protein properties. However, given its “orphan condition”, it requires improvement in terms of agronomic traits to become an acceptable protein source. *Lupinus luteus* lacks of genetic and molecular tools that can assist breeding programs of this species. In relation to flowering time and resistance to anthracnose, neither identification of genes nor markers have been achieved, despite the fact that this trait has strong impact on adaptation and yield. Nevertheless, some efforts have been under taken in terms of molecular studies and genetic platforms but more have been achieved in *L. angustifolius*. The identification of QTLs and candidate genes associated with these traits is a relevant challenge to assist *L. luteus* improvement. Thus, the main goal of this study was to identify *L. angustifolius* orthologous genes in the yellow lupin genome to these traits. Two contrasting genotypes for flowering time and anthracnose were crossed to develop two mapping populations for each trait. Flowering time was evaluated in field conditions and anthracnose resistance in growth chamber under controlled environment. Molecular markers were used to genotype the populations. Following the mapping and QTL studies in *L. angustifolius* some assumptions were made about the genetic control and the position of the orthologous genes in *L. luteus* genome. The sequence of the markers sca46508\_114 and sca82470 are homologous to *FT* and *LanR1* of *L. angustifolius*, respectively, and co-segregated with the phenotype in *L. luteus*. It is interesting that by using this approach, *L. luteus* recombinant lines tolerant to anthracnose were identify; same result was achieved for specific flowering times, which is relevant for the development of new cultivars adapted to specific environment. Thus, we can conclude that the *FT* and *LanR1* genes from *L. angustifolius*, are orthologous gene to *L. luteus* genome. One of these populations is being sequenced for GBS analysis and to develop a genetic linkage map for *L. luteus* genome and to identify more relevant QTLs/genes associated to important traits in this specie.

# ***Lupinus luteus* BREEDING IN CHILE: AN OPPORTUNITY FOR SUSTAINABLE AGRICULTURE AND THE DEVELOPMENT OF NEW PRODUCTS FOR HEALTHY FOOD AND FEED INDUSTRIES**

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Food security and sustainable production systems are global challenges. Protein demand will increase 40% for 2030, with 90g/capita/day; where plant protein will reach nearly 60 g/capita/day. Plant protein in 2020 is already reaching 53 g/capita/day. This increasing demand of plant protein is mainly due to lower cost of production, sustainability concern and positive impact on health. To address these global challenges, lupin species can play an important role in providing valuable protein and nutrition under sustainable production system, especially in cold temperate climates. Within lupins, *Lupinus luteus* (yellow lupin) has good nutritional qualities and protein content. Thus, it could be an alternative to soybean in cold temperate climates, like southern Chile. However, due its semi-domesticated condition, and lack of molecular tools, it requires genetic and molecular tools improvement in order to develop a more adapted cultivar to local conditions. In Chile, in the Agriaquaculture Nutritional Genomic Center (CGNA) there is an ongoing breeding program of *L. luteus* oriented to develop more adapted cultivars in terms of agronomic, protein content, biological and techno-functional protein properties, either for the food or feed industry, and to add more molecular tools to assist breeding. The center released the sweet variety *AluProt*-CGNA, with a commercial yield of 3 t/ha, with 60% of protein content (dry matter) in dehulled seed, well balanced in all essential amino acids and good digestibility of its protein. It is relevant to mention that unlike other lupins, no chemical application of Nitrogen and phosphate is used, which is essential in sustainable agriculture and small farmer producer. The center has also developed a range of technological products that are being used in the salmon cluster, egg producer, dairy substitute for calf, and in the food industry with several applications. To go further, different genetic strategies are being applied, which include, biparental mapping population and Next-RAD GBS approach in order to discover and map important QTL/genes associated to agronomic and feed and food quality traits. A MAGGIC population is under construction by using very contrasting parental lines. This RILs population will be evaluated for QTL analysis under different environmental conditions of southern Chile. A mutation breeding is also ongoing with 22,000 mutant lines in order to discover new genes for target traits under study, by using forward genetic and TILLING approach. Several advanced inbred lines are in trials evaluation in different environments, in order to release new varieties. Results achieved so far in this species, and its application in the food and feed industries will be presented in the conference.

## RECENT ADVANCES IN THE DEVELOPMENT OF MOLECULAR TOOLS FOR ANDEAN LUPIN (*Lupinus mutabilis* Sweet)

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*Lupinus mutabilis* Sweet, commonly known as tarwi, chocho or lupin, is an important neglected crop of the Andean region. Peru has the most diverse collection of tarwi in different germplasm banks with more than 2000 accessions. An efficient conservation and breeding activities of these crops are only possible by the use of modern tools. The main goal of the present study was the generation of molecular tools for genetic studies of *L. mutabilis*. This study is the first identification of simple sequence repeats (SSRs) markers derived from genomic resources from *L. mutabilis*. Assembled RNA-seq libraries from the Hi-Seq 2500 Illumina platform have been analyzed by using SSR locator and allowed us the identification of 7,540 simple sequence repeats. Forty-nine SSR primer pairs were randomly selected and compared with the genome of narrow leaf lupin (*L. angustifolius*) by using BLASTN. This has made possible the assignment of 31 SSR markers on the chromosomes of NLL. For the validation of the selected SSRs, DNA was isolated from 6-weeks old leaves of 32 accessions using the CTAB method and treated with RNase I. DNA quality and quantitation was done by agarose gel electrophoresis and readings in a nanodrop spectrophotometer, respectively. We have characterized a selected group of 24 SSRs by using the polymerase chain reaction (PCR) in a 20-microliter PCR reaction mix containing 10 ng of DNA, dNTPs (200  $\mu$ M), reverse primer (0.4  $\mu$ M) M13-forward primer (0.2  $\mu$ M), and Taq polymerase (0.75 Unit) in an thermal cyclers (Eppendorf Mastercycler). Labeling of PCR products was performed using the M13-tailed primer (0.2  $\mu$ M) with the following dyes 6-FAM, VIC, NED, and PET. PCR products were separated in a genetic analyser 3130xl (Applied Biosystems). The number of observed alleles for each SSR ranged from 1 to 4 with least 12 SSRs being polymorphic markers, whereas the rest of the SSRs were monomorphic or useless. Each accession has shown one or two alleles for each marker, which suggests a diploid genome of tarwi. In addition, we have also conducted a bioinformatic analysis to the annotation of the transcriptome of tarwi by BLASTX analysis against known protein sequences from narrow leaf lupin, soybean, and *Arabidopsis*. We have found important candidate genes associated with important traits of tarwi (alkaloid metabolism, anthracnose resistance). In this work we will be reporting the advances of the characterization of these genes. The outcomes of the present work will be useful in modern genetic studies of Andean lupin.



# QUINOLIZIDINE ALKALOID BIOSYNTHESIS: INSIGHTS FROM TRANSCRIPTOME ANALYSES (RNASeq)

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Quinolizidine alkaloids (QA) mainly serve lupins as defense compounds against herbivores and to a lesser degree against microbes. As mobile nitrogen storage compounds in seeds they have some importance for lupin physiology. QA are bitter-tasting neurotoxins and modulate the activity of acetylcholine receptors and of sodium and potassium channels. QA are synthesized in green parts of lupins (mainly leaves) and are stored in high concentrations in seeds. For animal and human use, plant material should contain none or very low levels of QA; for human consumption, a QA level of <0.02% has been declared as safe.

In order to breed for sweet lupins, we need to understand the genes and enzymes of QA biosynthesis, and also the mechanisms for transport from leaves to seeds and QA storage in vacuoles. Using classical biochemical approaches, we had problems to identify the corresponding genes. However, with the introduction of Next Generation Sequencing (NGS) during the last 10 years, a window of opportunity has opened. Using NGS, complete genomes and transcriptomes of organisms can be investigated.

We employed RNASeq (Illumina sequencing) to sequence the leaf and seed transcriptomes of several bitter and sweet lupins and related Fabaceae. The methodology will be introduced and first results for genes of QA biosynthesis and QA transport will be discussed.

## NEW TARWI (*Lupinus mutabilis* Sweet) CROP OBTAINED BY STRATIFIED MASAL SELECTION

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The tarwi (*Lupinus mutabilis* Sweet) is a native legume of the Andean region of South America, very important in human nutrition for its high nutritional value. However, a determining factor for the low productivity of tarwi is the lack of improved cultivars. The current ecotypes were not fully developed, because the crop was postponed and marginalized since ancient times. Native ecotypes are long cycle (8 to 10 months), their growth is excessively high and non-uniformed, which makes harvesting difficult; plants mature heterogeneously with high alkaloid content, low harvest index, difficult to harvesting with threshers combined.

The objective of this research was to obtain early crops, with better plant architecture and good yield. In 2013, the PROINPA Foundation initiated activities of tarwi plant breeding by evaluating local genotypes collected from various parts of the country. This initiative allowed to starting a stratified mass selection process to achieve new tarwi cultivars. That same year, in farmers' plots in the Anzaldo area, researchers began a process of selecting 2000 plants from 120 plots of tarwi (64 ha). The selection of the plants was made by characteristics of precocity, architecture and performance.

In the 2014-2015 year, the seeds of selected plants were sown in individual rows and evaluated by the same variables mentioned above. Additionally, in the same agricultural season in Tiraque area, 4,000 plants were selected from 115 farmers' plots, which were added to the material from the first year. The material of both zones was evaluated during three consecutive years in Tiraque, Anzaldo and Colomi (Cochabamba) zones. Farmers participated in all the selection stages in fields. In the 2013-2015 agricultural season, 6000 plants were selected in Anzaldo and Tiraque. From this total of plants, three lines were selected in Anzaldo and 10 lines in Colomi. These selected lines were subjected to yield trials at both sites. The performance of the different tarwi lines was different for each zone. They were superior for Colomi compared to Anzaldo; the Pr40 and Pr13 lines reached yields higher than 1.1 t / ha of grain with respect to the other lines. Plant height showed differences between the lines. The plants developed better in Colomi than in Anzaldo. The Pr40 line had a plant height of 110 to 130 cm, compared to the local ecotype that reaches higher heights of 180 cm, while the plant height for the Pr13 line was from 90 to 120 cm. Additionally, the plant architecture was improved, where the inflorescence of the main stem and the secondary branches was shortened substantially, observing a uniform flowering in the field. For the three years, in the two zones, significant differences were found for the variables number of pods per plant and number of grains per plant, for the Pr40, Pr22 and Pr13 lines. These lines had a higher number of pods per plant with respect to the others lines evaluated. Due to their good characteristics they presented, the Pr40 line was registered as Jayata.

## NUEVO CULTIVAR DE TARWI (*Lupinus mutabilis* Sweet) OBTENIDO POR SELECCIÓN MASAL ESTRATIFICADA

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El Tarwi (*Lupinus mutabilis* Sweet) es una leguminosa nativa de la región Andina de Sudamérica, muy importante en la alimentación humana por su alto valor nutritivo. Sin embargo, un factor determinante para la baja productividad del tarwi es la falta de cultivares mejorados. Los ecotipos actuales no fueron completamente desarrollados, debido a que el cultivo fue postergado y marginado desde las épocas antiguas. Los ecotipos nativos son de ciclo largo (8 a 10 meses), su crecimiento es excesivamente alto y desuniforme lo que dificulta su cosecha, las plantas maduran de manera heterogénea, tienen alto contenido de alcaloides, bajo índice de cosecha, difíciles de cosechar con trilladoras combinadas. El objetivo de esta investigación fue obtener cultivares precoces, con mejor arquitectura de la planta y de buen rendimiento. La Fundación PROINPA inició actividades de mejoramiento genético de tarwi en el año 2013, evaluando genotipos locales recolectados de varias partes del país. Esta iniciativa permitió iniciar un proceso de selección masal estratificada para lograr nuevos cultivares de tarwi. Ese mismo año en parcelas de agricultores de la zona de Anzaldo, los investigadores iniciaron un proceso de selección de 2000 plantas de 120 parcelas de tarwi (64 ha). La selección de las plantas se realizó por características de precocidad, arquitectura y rendimiento. En el año 2014-2015, las semillas de las plantas seleccionadas fueron sembradas en surcos individuales y evaluadas por las mismas variables antes mencionadas. Adicionalmente, en la misma campaña en la zona de Tiraque, fueron seleccionaron 4000 plantas de 115 parcelas de agricultores, que se sumaron al material del primer año. El material de ambas zonas se evaluó durante tres años consecutivos en las zonas Tiraque, Anzaldo y Colomi (Cochabamba). En todas las etapas de selección en campos participaron los agricultores. En la campaña 2013-2015 fueron seleccionados 6000 plantas en Anzaldo y Tiraque. De este total de plantas fueron seleccionadas tres líneas en Anzaldo y 10 líneas en Colomi. Estas líneas seleccionadas fueron sometidas a ensayos de rendimiento en ambos sitios. El rendimiento de las diferentes líneas de tarwi fue diferente para cada zona. Fueron superiores para Colomi respecto de Anzaldo, las líneas Pr40 y Pr13 alcanzaron rendimientos superiores a 1.1 t/ha de grano con respecto a las demás líneas. La altura de planta presentó diferencias entre las líneas. Las plantas desarrollaron mejor en Colomi que en Anzaldo. La línea Pr40, tuvo una altura planta de 110 a 130 cm, respecto al ecotipo local que alcanza alturas superiores de 180 cm, mientras la altura planta para la línea Pr13 fue de 90 a 120 cm. Asimismo, se mejoró la arquitectura de la planta, donde la inflorescencia del tallo principal y de las ramas secundarias se acortó sustancialmente observándose en el campo una floración uniforme. Para los tres años y en las dos zonas se encontraron diferencias significativas para las variables número de vainas por planta y número de granos por planta, para las líneas Pr40, Prar22 y Pr13, estas líneas tuvieron mayor número de vainas por planta respecto a las otras líneas evaluadas. Por las buenas características que presentaron, la línea Pr40 fue registrada como Jayata.

# LUPIBREED - FROM BREEDING RESEARCH TO NEW CULTIVARS IN GERMANY

## PART II: PROTEIN AND ALKALOID CONTENT

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LupiBreed was an initiative of a German lupin consortium from breeding research to new cultivars in Germany. The overall goal of the project was to improve productivity of narrow-leaved sweet lupin (*Lupinus angustifolius*) with regard to kernel yield, yield stability, and seed composition. The innovative approach of the project was the integration of new genetic variability from (I) a mutant collection and (II) genetic resources. Special attention was paid to high protein content and at the same time low alkaloid content. In order to determine the variation ranges of crude protein and alkaloid content in genetic resources, from a German gene bank (IPK) and 44 lines from a mutant collection were analysed for these quality parameters. Low alkaloid content of lupin seed is a main condition for human food and feed production. Selection of novel lines with low and stable alkaloid values may build the basis of novel successful varieties. Therefore, the content of the individual alkaloids was also characterized by GC-FID. After a correlation analysis of the analysed quality traits (protein and alkaloid content), several mutant lines of the mutant collection were clearly conspicuous. Noteworthy is, inter alia, the A4 line with a significantly higher protein content and significantly lower alkaloid content compared to its parent variety 'Boruta' as well as the variety 'Boregine'. In addition, there were other lines, which also had higher protein contents compared to their parent varieties. The average protein content of the 230 gene bank accessions was 31.8% of dry matter (dm) with a variation range of a minimum of 23.6% and a maximum of 38.8% dm. With an alkaloid content of up to 5000 µg·g<sup>-1</sup> dry matter, 29 accessions could be assigned to the blue sweet lupins (*L. angustifolius*) and 198 accessions to the bitter ones. From these 29, seven were in the crop year 2015, and 22 were cultivated 2016<sup>th</sup>. With a measured minimum value of less than 0.02% dm, this was within the recommended range for use/application in the food industry. With an average of 44% of the total alkaloid content 13-hydroxylupanin is the main alkaloid, with minimum values of 22% and maximum values of up to 73% were determined. Lupanin accounts for an average of 36% of the total alkaloid content. In addition, a wide range of variation of at least 0.5% and a maximum of 69% could be observed. This is also reflected in the content of angustifolin, which is predominantly present in blue lupins. A correlation between the alkaloid and the protein content was not recognizable after statistical evaluation by JMP. However, there were ten interesting accessions, which have a low alkaloid contents (≤ 0.2% dm) and at the same time high protein contents (≥ 33% dm). These are relevant for breeding and should be further characterized.



**BIOCHEMISTRY & BIOTECHNOLOGY**  
**BIOQUÍMICA & BIOTECNOLOGÍA**

## EXTRACTION OF QUINOLIZIDINE ALKALOIDS FROM *Lupinus* SPECIES BY TWO DIFFERENT METHODOLOGIES

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Species of the genus *Lupinus* are characterized by the presence of quinolizidine alkaloids (QA) as part of a defense strategy against herbivores, QA profiles are constant in the species and more than 170 QA have been identified. The big diversity of biological activities such as antiviral, antimicrobial, allelopathic, and insecticidal, make of these metabolites interesting compounds for the pharmaceutical and agricultural industries. However, current lab extraction protocols use expensive supplies and organic solvents that have a big impact on the environment. The aim of the present work was to compare the conventional protocol used in the lab with an alternative eco-friendly extraction protocol. Plant material consisted of leaves from plants of *L. montanus* Kunth, *L. aschenbornii* Schauer and *L. bilineatus* Benth. at the floral stage collected at the Iztaccíhuatl-Popocatepetl National Park, state of Mexico, in September 2018. Plant material was dried at 40°C during three days, ground until reaching a particle size of 0.5 mm<sup>2</sup> and stored at room temperature in paper bags until use. Specimens for Herbarium were collected as references. They were pressed, dried at 40°C and deposited in the MEXU National Herbarium. QA extraction was performed using the following protocols: Protocol I: 0.5g of ground plant material was homogenized in 20 mL of 0.1 M HCl and macerated for 24h at room temperature (23-25°C), then it was centrifuged for 10 min at 8500 rpm. Supernatant was alkalinized adding 3 M NH<sub>4</sub>NO<sub>3</sub> and run through an Isolute<sup>®</sup> column. Alkaloids were eluted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 36 mL), and finally solvent was evaporated at atmospheric pressure, the remanent which contains the QA was dissolved in 1mL of methanol and placed into an ambar vial until analysis. Protocol II: 0.5 g of plant material was homogenized in 0.5 M H<sub>2</sub>SO<sub>4</sub> during 72 h at 50 °C, then vacuumm filtrated and alkalinized at pH 9.3 with NH<sub>4</sub>OH, alkaloids were extracted with CHCl<sub>3</sub> and solvent evaporated under vacuum, remanent which contains QA was dissolved in 1mL of methanol and placed in an ambar vial until analysis. Extraction yields (p/p) using protocol I were 14.56% for *L. montanus*, 0.14% for *L. aschenbornii* and 1.46% for *L. bilineatus*, while the extraction yields (p/p) obtained with protocol II were 8.54% for *L. montanus*, 3.72% for *L. aschenbornii* and 5.94% for *L. bilineatus*. Comparing GC-MS analysis of the extracts, more components were observed in *L. bilineatus* and *L. montanus* extracts obtained with protocol II than in the extracts generated using protocol I. Relative ammount of specific QA was different in the extracts depending on the protocol used. For *L. bilineatus*, slightly more aphylline was extracted using protocol I (20.50%) than when protocol II was used (18.78%) and for *L. montanus*, the proportion of sparteine was heavily affected by the extraction protocol (100% for protocol I and 32.60% for protocol II). Regarding *L. aschenbornii*, the ammount of compounds extracted using protocol I was higher compared to the one obtained with protocol II and differences in the composition of the extracts were also observed. Protocol II, in which CH<sub>2</sub>Cl<sub>2</sub> is substituted by CHCl<sub>3</sub> and no Isolute<sup>®</sup> columns are used showed the best extraction yields and produced more complex extracts.

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## LUPANINE AND SPARTEINE DETERMINATIONS BY GC-FID ON AQUEOUS PROCESSED LUPIN (*Lupinus mutabilis*) FOR ALKALOIDS REMOVAL

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Tarwi or Chocho are common names in Peru for *Lupinus mutabilis*. Grains need processing before consumption. This process includes pre-cooking and water washing of bitter alkaloids. Residual levels of alkaloids had been traditionally checked only by sensory evaluation of bitter taste. Industrial production of edible Tarwi flour requires the implementation of a high number of analytical methods to assure an objective verification that the residual levels are below the level of 0.02% in agreement with international regulations. The objective of the study was verify the capability of the GC-FID for the determination of residual levels of alkaloids in 50 samples of Peruvian tarwi debittered and stablish the safety of the process for human consumption. A Gas Chromatography (GC-FID Agilent 7890A) method was implemented with a DB-1 column for the determination of residual levels of alkaloids for Peruvian genotypes of *Lupinus mutabilis*. Grains were subject to debittering techniques by soaking, pre-cooking and changing water daily during five days. For analysis, alkaloids were extracted with trichloroacetic acid, neutralized and re-extracted in dichloromethane, finally injected dissolved in methanol. The results show that alkaloids determinations in debittered samples was only possible when more than 1 gram of sample was extracted due to the limits of detection and quantification that in this GC systems were for lupanine 0.006284%: LOD and 0.007675%: LOQ and for Sparteine 0.01656% LOD and 0.01893% LOQ.

As a conclusions, the GC-FID is a valid methodology for the determination of residual levels of alkaloids in 50 genotypes of debittered tarwi. We acknowledge PNIA project for Tarwi directed by Felix Camarena Mayta for funding.

## QUINOLIZIDINE ALKALOIDS IN *Lupinus*: WHEN AND HOW ARE THEY SYNTHESIZED?

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Species of the genus *Lupinus* synthesize quinolizidine alkaloids (QA) as part of a defense strategy against herbivores. These compounds are synthesized in photosynthetic tissues and stored in seeds. QA are metabolized as a source of nitrogen in the cotyledons during germination and seedling development. The formation of cadaverine from L-lysine and its cycling to form sparteine are the initial steps in the biosynthesis of QA. *Lupinus* species show specific and diverse QA chromatographic profiles. Notably, there is little knowledge regarding the biosynthesis of QA and its regulation. Hence, the following questions arise: At which developmental stage does the synthesis of QA initiate? When does the diversification of QA in the species of the genus *Lupinus* occur? And is there a common basal profile from which the synthesis of QA begins and diversifies in the *Lupinus* species? and if so, which QA characterize it? *L. montanus*, *L. bilineatus* and *L. aschenbornii* produce distinct QA profiles. Thus, in this work, they were used as a model of study to determine the developmental stage at which synthesis of QA initiates and the basal QA profile from which QA diversification arises. Seeds of evaluated species were germinated onto filter paper, transferred to pots containing perlite and grown in a bioclimatic chamber under controlled conditions (20 °C, 60% RH and a photoperiod of 16/8 light/dark). Plantlet growth and development was monitored daily during 14 days to determine the start point and duration of each developmental stage. To determine the developmental stage at which QA synthesis initiates, plantlets in the following developmental stages: germination, hypocotyl elongation, emergence of first leaves and second leaf emergence, were harvest and QA extracted using the protocol reported by Montes Hernández *et al.* (2012). At the end of the 14-day monitoring period, plantlets of the three evaluated species had reached the emergence of second leaves stage. However, the duration of each developmental stage was variable among species. Germination (radicle of 5mm long), had a duration of 3-5 days for *L. montanus*, and 3-4 days for *L. bilineatus* and *L. aschenbornii*; hypocotyl elongation, had a duration of 3-7 days for *L. montanus*, 3-9 days for *L. bilineatus* and 3-5 days for *L. aschenbornii*. First leaf emergence, showed a duration of 6-7 days for *L. montanus*, 6-9 days for *L. bilineatus* and 6-8 days for *L. aschenbornii* and finally, emergence of second leaves had a duration of 3-4 days for *L. montanus*, 2-3 days for *L. bilineatus* and 2-6 days for *L. aschenbornii*. Results show that seed QA content decreased for all evaluated species, confirming previous reports indicating that QA are used as N source during germination. No QA total content increase was observed for evaluated species, suggesting that *de novo* synthesis starts on a developmental stage beyond the emergence of second leaves. In all evaluated species, seed QA profile (presence and abundance of QA) was complex and disappearance of several peaks was observed at germination. Up to the emergence of second leaves, no similar QA profiles were found among the three species. Our results suggests that QA synthesis in *L. campestris*, *L. bilineatus* and *L. montanus* initiates after plantlets have produced two leaves. Finally, the existence of a basal QA profile could not be determined since the onset of QA synthesis was not defined. A. Ramírez acknowledges the postgraduate scholarship granted by CONACyT-México. This research was carried out with the financial support of SIP-IPN (SIP-20180777).



## FT-IR SPECTROSCOPY IN THE ESTIMATION OF PROTEIN CONTENT OF BITTER AND DEBITTERED LUPIN (*Lupinus mutabilis*)

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Infrared spectroscopy is an instrumental technique that provides faster analysis and lower cost compared to others. The applications range from the identification, quantification and contrast of the authenticity of different chemical compounds. This investigation developed a calibration model to estimate the content of proteins in bitter and debitter tarwi (*Lupinus mutabilis*) using attenuated total reflectance mid-infrared spectroscopy (ATR-MIR). For this, 52 different ecotypes of Peruvian region were used. Partial Least Squares Regression (PLSR) was used for quantification analysis. From each sample, the spectrum measurement was taken, in duplicate, in a range of 4000-700 cm<sup>-1</sup>. For the calibration, spectrums were preprocessed using the second derivative, obtaining the representative bands of the protein in bitter and debitter tarwi centered in 1458 cm<sup>-1</sup> (amide II, CH<sub>3</sub> asymmetric bend), 1550 cm<sup>-1</sup> (amide II, N-H in plane bend and CN stretching) and 1691 cm<sup>-1</sup> (amida I). The model required 4 factors. The internal validation carried out can be used to predict the concentration with high reliability and the ATR-MIR can be used to differentiate the bitter and un-marred ecotypes. The results of the statistical indicators, the correlation coefficient ( $r^2 = 0.95$ ) and the standard error of cross-validation (2.17%) were satisfactory in the parameters evaluated. We acknowledge Dr. Luis Rodriguez-Saona for kindly provide the portable FT-IR (ATR-MIR Thermo) and to the PNIA project for Tarwi directed by Felix Camarena Mayta for funding.



**TAXONOMY, BIODIVERSITY  
& ECOLOGY**

**TAXONOMÍA, BIODIVERSIDAD  
Y ECOLOGÍA**

## PRODUCTIVE CYCLE AND VIABILITY OF SEED OF LUPINO SILVESTRE (*Lupinus ssp.*) IN THE ALTIPLANO OF BOLIVIA

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In the altiplano grows a diversity of wild species of the genus *Lupinus* that are known by different native names (q'ila-q'ila, sarqawi, salqa, salqiri). As a species of the Legume Family, the plants are in symbiosis with specific root-bacteria and fix atmospheric nitrogen. Therefore, these species are of interest for the sustainable management of soils, allowing to make rotation options with crops, use them as vegetable cover of the soil and production of green manure. However, there is no information on the productive cycle or on the viability of the seed of the wild species of lupine (*Lupinus ssp.*). For what has been proposed to investigate the productive cycle of wild lupins and the viability of their seed. The productive cycle of wild lupine was studied in natural populations of the altiplano and in populations under cultivation in the experimental center and in plots managed by producers. In these fields the date of emergence, the vegetative and reproductive phases were recorded. Seed characteristics and their viability were studied in the laboratory of the Kihakiphani Research Center using the seed from the sampling fields. As probable indicators of maturity of the seed, dehydrated seed, seed extracted from dehiscent pod (dry), from yellow pod and green pod was taken. In the seeds, the color and shape of the seed were registered, as well as the viability in different stages of maturity. The results show that the wild lupins (*Lupinus ssp.*) are of biannual cycle, whose emergence takes place in the months of December and January, the plants are established in February and March, they pass the autumn and winter in vegetative phase. The reproductive phase begins in October with profuse flowering between December and January. The maturation of the seed occurs between January to April. Then, the plant enters in gradual senescence drying in June. Plant senescence occurs as long as the plant has completed normal fruiting, if it has not successfully completed seed formation, the plant remains in a state of lethargy and regrowth in August, entering a second reproductive stage in October and forming seed between February and March. The maturation of the seeds is gradual in the plant and in the bunch of pods, but the physiological maturity begins much earlier of the state of yellow pod, that is to say, young seeds extracted from green pods have viability germinating between 87 and 98% with the only difference of greater time for the germination in relation to the scarified dry seed. In conclusion, the species and ecotypes of wild lupine studied have a bi-annual productive cycle with the exception of a sample that is perennial. In field conditions, the seed of these species germinates every four years, the germination tests in the laboratory using dehydrated seeds did not germinate, deducting the seed dormancy. The non-dehydrated seed from the yellow pod lacks dormancy since it germinates immediately when placed in a conditioned Petri dish. The seed obtained from green pod germinates in greater time compared to yellow pod seed and scarified dry seed, deducting that the physiological maturity of the seed takes place even in the green pod state. The information reported can contribute to the management of wild lupine species.

## CICLO PRODUCTIVO Y VIABILIDAD DE SEMILLA DE LUPINO SILVESTRE (*Lupinus ssp.*) EN EL ALTIPLANO DE BOLIVIA

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En el altiplano crece una diversidad de especies silvestres del género *Lupinus* que se conocen con diferentes nombres nativos (q'ila-q'ila, sarqawi, salqa, salqiri). Por ser una especie de la Familia Leguminosa, las plantas están en simbiosis con rizo-bacterias específicas y fijan el nitrógeno atmosférico. Por lo que, estas especies son de interés para el manejo sostenible de suelos, permitiendo hacer opciones de rotación con cultivos, usarlas como cobertura vegetal del suelo y producción de abono verde. Sin embargo, no se tiene información sobre el ciclo productivo ni sobre la viabilidad de la semilla de las especies silvestres de lupino (*Lupinus ssp.*). Por lo que se ha planteado investigar el ciclo productivo de los lupinos silvestres y la viabilidad de su semilla. El ciclo productivo del lupino silvestre se estudió en poblaciones naturales del altiplano y en poblaciones bajo cultivo en centro experimental y en parcelas manejadas por productores. En estos campos se registró la fecha de emergencia, las fases vegetativa y reproductiva. Las características de la semilla y la viabilidad de las mismas fueron estudiadas en el laboratorio de semillas del Centro de Investigación Kihakiphani empleando la semilla proveniente de los campos de muestreo. Como probables indicadores de madurez de la semilla se ha tomado la semilla deshidratada, semilla extraída de vaina dehiscente (seca), de vaina amarilla y de vaina verde. En las semillas se registraron el color y forma de la semilla como también la viabilidad en diferentes estados de madurez. Los resultados muestran que los lupinos silvestres (*Lupinus ssp.*) son de ciclo bianual, cuya emergencia tiene lugar en los meses de diciembre y enero, las plantas se establecen en febrero y marzo, pasan el otoño e invierno en fase vegetativa. La fase reproductiva se inicia en octubre con floración profusa entre diciembre y enero. La maduración de la semilla ocurre entre enero a abril. Luego la planta ingresa en senescencia gradual secándose en junio. La senescencia de planta ocurre siempre y cuando la planta haya completado fructificación normal, de no haber completado exitosamente la formación de semilla, la planta queda en estado de letargo y rebrota en agosto próximo ingresando en una segunda etapa reproductiva en octubre y formando semilla entre febrero y marzo. La maduración de las semillas es gradual en la planta y en el racimo de vainas, pero la madurez fisiológica se inicia mucho más temprano del estado de vaina amarilla, es decir, granos tiernos extraídos de vainas verdes tienen viabilidad germinando entre 87 y 98% con la única diferencia de mayor tiempo para la germinación en relación a la semilla seca escarificada. En conclusión las especies y ecotipos de lupino silvestre estudiados tienen ciclo productivo bi-anual con excepción de una muestra que es de ciclo perenne. En condiciones de campo, la semilla de estas especies germinan cada cuatro años, las pruebas de germinación en laboratorio empleando semilla deshidratada no germinaron, deduciéndose la dormancia de semilla. La semilla no deshidratada proveniente de vaina amarilla carece de dormancia puesto que germina inmediatamente cuando se puso en cápsula Petri acondicionado. La semilla obtenida de vaina verde germina en mayor tiempo frente a semilla de vaina amarilla y semilla seca escarificada, deduciéndose que la madurez fisiológica de la semilla tiene lugar aun en estado de vaina verde. La información que se reporta puede contribuir al manejo de las especies silvestres de lupino.

## USE OF LUPINUS WILD ECOTYPES IN THE RECOVERING OF DEGRADED SOILS

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The Bolivian Altiplano, located between 3600 and 3860 masl, is characterized by soils aridity and the regime of adverse climatic factors for agriculture. In these zones, numerous agricultural species are cultivated, being the main ones quinoa (*Chenopodium quinoa* Willd.), qañawa (*Chenopodium pallidicaule* Aellen), and potato (*Solanum tuberosum*, *S. juzepzucki*, *S. curtilobum* and *S. ajanhuiri*). The commercial production of these crops, especially quinoa, has led to the expansion of the agricultural frontier, which has resulted in the elimination of vegetation cover, leaving exposed to wind erosion. The direct consequence is the soil degradation, particularly in the Southern Altiplano of Bolivia, where Quinoa Real is grown for export. There are several proposals to recover degraded soils; however, under conditions of ecological fragility of the Southern Altiplano and climate change, there are few practical and efficient options for recovering degraded soils. Therefore, it has been proposed to take advantage of the wild lupine (*Lupinus* spp.) ecotypes for the recovery of degraded soils of the Southern Altiplano with a view to the sustainability of agricultural production. The work was carried out in the Southern and Central Altiplano of Bolivia, where samples from different ecological sites have been collected. Their reproductive cycle, methods of seed collection from natural fields and pilot production plots, seed physiology, dormant seed treatment methods, sowing methods, soil cover, green matter yield, have been investigated.

Results show, that ecotypes or morphotypes of the bi-annual productive cycle, generally exist in the Altiplano. These species germinate from the end of December to the month of February. Plants spend the winter with moderate growth providing soil covering and fixing atmospheric nitrogen. The plants mature gradually between January and March and seed can be harvested. Senescent plants can be incorporated into the soil as green manure with nitrogen fixed to the roots. The seed is dormant and, under natural conditions, the dormancy breaks in 3 to 4 years, but with scarification treatments, it has been possible to reach between 80 and 86% of germination. With the results of practical interest, a strategy for the use of wild lupine ecotypes in the recovering of degraded soils of the highlands has been designed and that can be used in quinoa rotation, improved soil fallow, planting cover, intercropping in tandem on quinoa. In conclusion, at least a dozen ecotypes of potential use have been identified in the recovering of degraded soils of the Southern Altiplano of Bolivia. As part of the technology, there is a group of wild legume plants of the genus *Lupinus*, the only ones that can grow in cold and dry winter. In addition, we have the methods of mechanized planting, techniques of seed collection in natural fields and production plot, dormant seed treatment (manual and mechanized), methods of incorporating green manure to recover degraded soils.

## USO DE ECOTIPOS SILVESTRES DE LUPINUS EN LA RECUPERACIÓN DE SUELOS DEGRADADOS

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El altiplano de Bolivia, ubicado entre 3600 y 3860 msnm se caracteriza por la aridez de los suelos y régimen de factores climáticos adversos para la agricultura. En estas zonas se cultivan numerosas especies agrícolas, siendo las principales la quinua (*Chenopodium quinoa* Willd.), la qañawa (*Chenopodium pallidicaule* Aellen), la papa (*Solanum tuberosum*, *S. juzepzucki*, *S. curtilobum* y *S. ajanhuiri*). La producción comercial de estos cultivos, especialmente la quinua, ha conducido a ampliar la frontera agrícola que ha derivado en la eliminación de la cobertura vegetal dejando expuesta a la erosión eólica. La consecuencia directa es la degradación de suelos particularmente en el Altiplano Sur de Bolivia donde se cultiva la quinua real de exportación. Existen varias propuestas para recuperar los suelos degradados, sin embargo, bajo condiciones de fragilidad ecológica del Altiplano Sur y el cambio climático, son pocas las opciones prácticas y eficientes para recuperar suelos degradados. Por tanto, se ha propuesto aprovechar los ecotipos de lupino silvestre (*Lupinus* ssp.) para la recuperación de suelos degradados del Altiplano Sur con miras a la sostenibilidad de la producción agrícola. El trabajo se realizó en el Altiplano Sur y Central de Bolivia, donde se ha recolectado muestras de diferentes sitios ecológicos. Luego se ha investigado su ciclo reproductivo, métodos de colecta de semilla de campos naturales y parcelas de producción piloto, la fisiología de semilla, métodos de tratamiento de semilla dormantes, métodos de siembra, cobertura del suelo, rendimiento de materia verde. Los resultados muestran que en el altiplano existen ecotipos o morfotipos de ciclo productivo bi-anual generalmente. Estas especies germinan desde fines de diciembre hasta el mes de febrero. Las plantas pasan el invierno con crecimiento moderado proporcionando cobertura al suelo y fijando nitrógeno atmosférico. Las plantas maduran gradualmente entre enero y mayo en las que se puede cosechar la semilla. Las plantas senescentes pueden ser incorporadas al suelo como abono verde con el nitrógeno fijado en las raíces. La semilla es dormante y bajo condiciones naturales la dormancia se rompe en 3 a 4 años, pero con tratamientos de escarificación se ha logrado alcanzar entre 80 y 86% de germinación. Con los resultados de interés práctico, se ha diseñado una estrategia de empleo de ecotipos de lupino silvestre en la recuperación de suelos degradados del altiplano, pudiendo emplearse en rotación con quinua, descanso mejorado del suelo, cobertura vegetal, siembra intercalar en tándem sobre quinua. En conclusión, se ha identificado al menos una docena de ecotipos de uso potencial en la recuperación de suelos degradados del Altiplano Sur de Bolivia. Como parte de la tecnología se tiene un grupo de plantas leguminosas silvestres del género *Lupinus*, las únicas que pueden crecer en invierno frío y seco. Además, se tiene los métodos de siembra mecanizado, técnicas de recolección de semilla en campos naturales y parcela de producción, tratamiento de semilla dormante (manual y mecanizado), métodos de incorporación de abono verde para recuperar suelos degradados.

# TOWARDS A ROBUST SYSTEM FOR THE TAXONOMY OF MEXICAN LUPINS: A COMPARATIVE MORPHOANATOMIC STUDY

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About 160 species of the genus *Lupinus* have been described in Mexico. Nonetheless, it is very likely that the number of species is overestimated since there is multiplicity in the names assigned to them. This is due not only to the great similarity in their floral structures but also to the high phenetic plasticity and hybridization they display. Moreover, a robust set of taxonomic keys for the identification of Mexican lupins is lacking, which has hindered the integral classification of the genus. Studies focused on the use of anatomical characters for species delimitation have been carried out in genera such as *Amaranthus*, *Moussonia* and *Hymenocallis*. Currently, there are a few anatomical studies on Mexican lupins but none of them focused on the identification of characters with taxonomical value. In an attempt to search for morpho-anatomical characters that facilitate the unequivocal classification of Mexican lupins, leaf morphology and leaf and petiole anatomy of *Lupinus bilineatus*, *L. campestris* and *L. montanus* were analysed at the inter-individual, inter-population and inter-specific level. This study is based on the constant properties of these characters at the inter-individual and inter-population level and their variability among species. Plant material was collected at the Iztaccihuatl-Popocatepetl National Park and at Laguna de Zempoala and Santiago Tianquistengo roads on June of 2016. Leaves were harvested from ten plants at anthesis from three populations of each species. Leaf area and perimeter, leaflet number, length and width, as well as petiole length of 120 leaves of each species were measured using Image J. For anatomical studies, leaflet and petiole sections (0.5x0.5 cm) were collected from three plants of each population and fixed with formaldehyde-acetic acid-ethanol on site. Stoma index, epidermal cells area, occlusive cells width and length, trichome density, number of socket cells and periclinal wall thickness were determined on both the adaxial and abaxial epidermis. Cross sections of leaf and petiole were generated and used to determine the length of adaxial and abaxial papillae, number of vascular vessels per row in the middle vein, vessel wall thickness, vessel diameter, lamina thickness, spongy and palisade parenchyma thickness, and area of the main vascular bundle of the petiole. Data statistical analysis was performed using an ANNOVA Tukey test  $p=0.05$  and cluster hierarchical analysis. Results showed that most of the morpho-anatomical characters were constant at the inter-individual level. *L. campestris* populations showed greater constancy in their structural characters than *L. bilineatus* and *L. montanus*. Finally, morpho-anatomical characters were variable at the interspecific level. The morpho-anatomical characters: leaf area, leaf perimeter, long leaflet number, petiole length, abaxial occlusive cells width, density of abaxial trichomes, adaxial socket cells, thickness of the external abaxial periclinal wall, vessel wall thickness and spongy parenchyma thickness had taxonomic value and allowed the delimitation of *L. bilineatus*, *L. campestris* and *L. montanus*. This work was supported by IPN (Grant SIP20150658, SIP20161150 y SIP20172240). A. Cantor was a recipient of a postgraduate scholarship (CONACYT-706695).

# MORPHO-ANATOMICAL REVIEW AS A TOOL TO IDENTIFIED BIODIVERSITY IN ANDEAN LUPINUS SPECIES COMPLEX

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The Argentine flora has 34 species of *Lupinus* distributed in two areas of speciation, where 10 species grown in the Atlantic Subregion and 24 in the Andean Subregion. In this Subregion, topography plays a fundamental role in creating microclimates and patches of related ecosystems, distributed along the mountain ranges. Among the species growing in these environments are: *L. grisebachianus* C.P.Sm., *L. subacaulis* Griseb. and *L. subinflatus* C.P.Sm., which are found in the high valleys and hillsides of Jujuy, Salta and Tucumán provinces, forming a morphologically complex of turf plants with embedded inflorescences between the leaves of the basal rosette. Planchuelo and Dunn clarified the nomenclatural problems regarding materials types in 1980, and since then the taxa are considered good species, with small morphological differences in vegetative and floral characters. As part of a critical review of Argentinean native *Lupinus*, key morphological characters that define species growing in the Northwest of the country were evaluated. The objective of this study is to present the results of a morpho-anatomical review of leaflets pubescence of the *Lupinus grisebachianus-subacaulis-subinflatus* complex, along with an overall analysis of vegetative and floral characters using numerical taxonomy. We worked with identified herbarium specimens from several herbaria (ACOR, CORD, F, LIL, MCNS, MO, SI, US) and authors personal plant collections. The density of the pubescence was encoded as 0=glabrous; 1=sparse trichomes, 2=trichomes scattered across the surface; 3=dense pubescence; 4=very dense pubescence. Epidermal extractions and cross-sections of the leaflets were analyzed from representative specimens of each species. A cluster analysis was based on 26 Operational Units (OTUs) using Euclidean Distance, and Principal Components based on a matrix containing 4 qualitative data and 19 quantitative data, with a total of 23 morphometry parameters, from which 11 were vegetative characters and 12 floral characters. The leaflet morpho-anatomical characters showed two different sizes of trichomes; short (200-600  $\mu\text{m}$ ) and long (600-800  $\mu\text{m}$ ). All the trichomes are appressed, simple and with three cells. A globose larger cell than the other epidermal cells is at the base, a second cell with thick wall that form an epitricopodium is in the middle and a third acicular cell in the apex. Big differences were found in leaflets pubescence. The abaxial side of the leaflets always has higher density of trichomes regarding adaxial face. Leaflets of *L. subinflatus* and *L. subacaulis* are glabrous in de adaxial face in contrast *L. grisebachianus* is woolly on both sides. The numerical taxonomy analysis presented several clusters, which separate the species in groups of OUT's by the combinations of similar and different morphological characters. The principal component shows that the variables of greater weight are the distribution of pubescence that is related with the energy balance and photosynthetic rate and flower characters such as the length of upper and lower lip of the chalice and the longitude and latitude of the banner. These studies support the definition of species and reaffirm the need of morpho-anatomical studies in germplasm complex such the ones that are relate with the wild relatives of *Lupinus mutabilis*. The authors thank the Universidad Nacional de Córdoba and the Universidad Nacional de Salta for the financial support.



# REVISIÓN MORFO-ANATÓMICA EN UN COMPLEJO DE ESPECIES DE LUPINOS ANDINOS COMO UNA HERRAMIENTA PARA IDENTIFICAR LA BIODIVERSIDAD

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La flora Argentina tiene 34 especies de *Lupinus* distribuidos en dos áreas de especiación, de las cuales 10 especies crecen en la Subregión Atlántica y 24 en la Subregión Andina. En esta Subregión, la topografía juega un papel fundamental en la creación de microclimas y parches de ecosistemas semejantes, que están distribuidos a lo largo de las cadenas montañosas. Entre las especies que crecen en estos ambientes están: *L. grisebachianus* C.P.Sm., *L. subacaulis* Griseb. y *L. subinflatus* C.P.Sm., que se encuentran en los altos valles y laderas de las provincias de Jujuy, Salta y Tucumán, formando un complejo morfológico de plantas cespitosas con inflorescencias incrustadas entre las hojas de la roseta basal. En 1980 Planchuelo y Dunn clarificaron los problemas nomenclaturales sobre materiales tipos y desde entonces los taxones se consideran buenas especies, con pequeñas diferencias morfológicas en caracteres vegetativos y florales. Como parte de una revisión crítica de *Lupinus* de Argentina, se evaluaron caracteres morfológicos que definen las especies que crecen en el noroeste del país. El objetivo de este estudio es presentar los resultados de un análisis morfo-anatómico de pubescencia foliar del complejo *Lupinus grisebachianus-subacaulis-subinflatus*, junto con un análisis de caracteres vegetativos y florales, mediante taxonomía numérica. Trabajamos con ejemplares de herbario identificados de varias instituciones (ACOR, CORD, F, LIL, MCNS, MO, SI, US) y con colecciones personales de las autoras. La densidad de la pubescencia fue codificada como 0=glabro; 1=escasos tricomas, 2=tricomas esparcidos por la superficie; 3=pubescencia densa; 4=pubescencia muy densa. Se realizaron extracciones epidérmicas y secciones transversales de los folíolos de muestras representativas de cada especie. Un análisis de conglomerados se basó en 26 unidades operacionales (OTUs) utilizando Distancia Euclidiana y Componentes Principales, basados en una matriz que contenía 4 datos cualitativos y 19 datos cuantitativos, con un total de 23 parámetros morfométricos de los cuales 11 eran caracteres vegetativos y 12 caracteres florales. Los caracteres morfo-anatómicos de los folíolos mostraron dos tamaños diferentes de tricomas; cortos (200-600  $\mu\text{m}$ ) y largos (600-800  $\mu\text{m}$ ). Todos los tricomas son adpresos, simples y tricelulares. En la base hay una célula globosa más grande que las otras células epidérmicas, en el medio una segunda con paredes gruesas que forma un epitricopodium y una tercera célula acicular en el ápice. Se encontraron grandes diferencias en la pubescencia de los folíolos. La pubescencia de la cara abaxial siempre tiene mayor densidad de tricomas que la cara adaxial. Los folíolos de *L. subinflatus* y *L. subacaulis* son glabros en la cara adaxial, en cambio los de *L. grisebachianus* son lanosos en ambas caras. El análisis de taxonomía numérica presenta varios conglomerados que separan las especies en grupos de OUT's por las combinaciones de similitudes y diferencias de caracteres morfológicos. Los componentes principales muestran que las variables de mayor peso son la distribución de la pubescencia, que está relacionada con el balance energético y el nivel de fotosíntesis y, los caracteres florales como la longitud del labio superior e inferior del cáliz y la longitud y latitud del estandarte. El análisis morfo-anatómico demuestra que estas especies comparten características de herencia genética y caracteres relacionados con las condiciones ambientales como la arquitectura de la planta y la pubescencia. Estos estudios apoyan la definición de especies y reafirman la necesidad de realizar análisis morfo-anatómicos en germoplasmas complejos tales como los parientes silvestres de *Lupinus mutabilis*. Los autores agradecen a la Universidad Nacional de Córdoba y a la Universidad Nacional de Salta por el apoyo financiero.

## ASSESSING PHENOTYPIC DIVERSITY OF LUPIN LANDRACES (*Lupinus mutabilis* Sweet)

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Lupins are known and cultivated by humans since antiquity. *Lupinus angustifolius* L. (narrow-leaf, blue lupin), *L. albus* L. (white lupin) and *L. luteus* L. (yellow lupin) are cultivated species of the Old World, and *L. mutabilis* Sweet (Andean lupin) is the New World cultivated species. Nowadays, *L. mutabilis* receives a renewed interest from breeders regarding breeding varieties adapted in South European edapho-climatic conditions, as it grows well in poor soils and is a good source of protein, oil and biomass. For this reason, we aimed to assess the diversity of five *L. mutabilis* populations and primarily evaluate them under a Mediterranean environment. The plant material used consisted of five populations of *L. mutabilis* (LIB201, LIB203, LIB206, LIB208 and LIB217) kindly provided by Instituto Superior de Agronomia (ISA), two commercial varieties (*L. angustifolius* cv. Polo and *L. albus* cv. Multitalia), as well as a breeding variety under registration process of white lupin (LIB224) originated from Portugal. Sowing took place in an experimental field of Agricultural University of Athens (N 37°59'10'', E 23°42'29'', 24 m above sea level) on February 9<sup>th</sup> 2017. The soil was characterized as Sandy-clay-loam (SCL) and alkaline (pH 7.9) with a CaCO<sub>3</sub> concentration of 17.46%. The experimental design followed was a Randomized Complete Block Design (RCBD) with three replications. Lupins' diversity was assessed through 48 agro-morphological traits and measurements were taken on 45 plants per population. All continuous traits were transformed to ordinal by dividing their range into equal classes and thus formed discrete ranks. The frequency of each rank within each trait was calculated. Data were used to characterize the entire collection and each population separately, as well as for the calculation of phenotypic diversities, based on Nei's genetic diversity index ( $H_e$ ). For each trait, total phenotypic diversity of the collection ( $H_t$ ), inter-population ( $G_{st}$ ) and intra-population phenotypic diversity ( $H_s$ ), as well as its average across all populations ( $\bar{H}_s$ ) were calculated. Mean phenotypic diversity within each population across all traits ( $\bar{H}_p$ ) was also calculated and used for the estimation of heterogeneity of each population. Comparisons of all populations  $\bar{H}_p$  were carried out applying Duncan's multiple range test ( $p \leq 0.05$ ). Principal component analysis (PCA) was also used in order to examine the contribution of each trait to the total diversity. Seed shape and number of seeds per pod were the traits that contributed most to the total phenotypic diversity of the collection ( $H_t = 0.726$  and  $0.766$ , respectively). Number of seeds per pod was also the trait that ranged the most within populations ( $H_s = 0.715$ ). The highest mean phenotypic diversity within each population across all traits ( $\bar{H}_p = 0.26$ ) presented by LIB201 and LIB203. The first three principal axes of PCA explained the 51.61% of the total phenotypic diversity. In the first principal axis were related traits such as petiole color and leaflet shape, while in the second, traits like seed shape, seed secondary color and leaf diameter. Seed yield traits were related mainly in the third axis. *L. mutabilis* populations discriminated from the other lupin species, with the exception of LIB208. These lupin populations can therefore be cultivated and be productive under a Mediterranean climate and alkaline, calcareous soil conditions. Furthermore, they are characterized by a remarkable amount of among and within diversity and therefore consist a valuable source of desirable traits for breeding.

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## ***Lupinus* SILVESTRES DEL ECUADOR**

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El presente trabajo representa una revisión de las especies silvestres del género *Lupinus* L. en el Ecuador. Desde tiempos preincaicos los ecuatorianos como otros pueblos andinos, han utilizado al chocho (*Lupinus mutabilis* Sweet) como base alimentaria de hombres y animales, por esta razón la mayoría de los estudios se basan en las especies domesticadas del género. En este sentido, investigaciones previas señalan que en los Andes hay muchos más nombres de especies de *Lupinus* que especies en sí, recalcando la evidente necesidad de una revisión de este género en el país. Para cumplir con este objetivo iniciamos un estudio para conocer las especies citadas, los patrones de diversidad, la riqueza y el área de ocupación de *Lupinus* en Ecuador, para ello se elaboró una base de datos con material de herbarios nacionales e internacionales, junto con información y material de campo. Hasta la fecha, se registran 71 especies de este género repartidas por todo el territorio nacional, aún se continúa con la revisión de material de herbario e identificación de ejemplares botánicos para finalizar la base de datos de este proyecto. Entre las especies citadas encontramos a: *L. alveorum*, *L. arvensis*, *L. brevecuneus*, *L. carchiensis*, *L. cotopaxiensis*, *L. couthouyanus*, *L. extrarius*, *L. foliolosus*, *L. goodspeedii*, *L. haughtianus*, *L. huigrensis*, *L. insulae*, *L. involutus*, *L. jamesonianus*, *L. kunthii*, *L. longilabrum*, *L. michellianus*, *L. nonoensis*, *L. nubigenus*, *L. pachanoanus*, *L. paniculatus*, *L. pasachoensis*, *L. paucovillous*, *L. penlandianus*, *L. pubescens*, *L. rupestris*, *L. semperflorens*, *L. smithianus* y *L. summersianus*. Entre los resultados esperamos generar información de análisis taxonómicos de las especies de *Lupinus* presentes en Ecuador, nuevas colecciones y análisis de regiones para la conservación de este importante genero con gran potencial agroeconómico.

## EVALUATION OF ANDEAN *Lupinus* SPECIES TO BE INCORPORATED IN THE IUCN RED LIST OF ENDANGERED SPECIES

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The Andean Lupin (*Lupinus mutabilis* Sweet), known by the common names tarhui, tarwi or chocho is a native species to the Central Andes. It was cultivated and improved by the Inca civilization and currently is cultivated in Ecuador, Peru and Bolivia, between 2500 and 3800 m above sea level, for its grain with high nutritional protein and oil contents. As its scientific name indicates, *L. mutabilis* has a great capacity to mutate; consequently, several wild breeds are part of a gene pool with random intercrossing. Within the framework of a program to assess the conservation status of South American crop wild relatives, a workshop was held in July 2018 with a panel of experts. They evaluated 29 species of *Lupinus* that are native to the tropical Andes of Bolivia, Peru, Ecuador and Colombia and that are considered wild relatives of tarwi or chocho. The aim of the workshop was to assess the species' risk of extinction using the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species. During the workshop, information on the distribution, population size and trends, habitats and ecology, use and trade, threats, and conservation measures was reviewed for each species. The IUCN Red List Categories and Criteria were used to assign the species to the different categories: Not evaluated (NE), Data deficient (DD), Least concern (LC), Near threatened (NT); Vulnerable (VU); Endangered (EN); Critically endangered (CR); extinct in the wild (EW); Extinct (EX). The species assessed were: *Lupinus arvensis* C.P.Sm.; *L. ballianus* C.P.Sm.; *L. bangii* Rusby; *L. bogotensis* Benth.; *L. chachas* Ochoa ex C.P.Sm.; *L. chrysanthus* Ulbr.; *L. ellsworthianus* C.P.Sm.; *L. huaronensis* J. F. Macbr.; *L. huigrensis* Rose ex C.P.Sm.; *L. lindleyanus* C.P.Sm.; *L. mantaroensis* C.P.Sm.; *L. microphyllus* Desr.; *L. misticola* Ulbr.; *L. mollendoensis* Ulbr.; *L. mutabilis* Sweet; *L. nubigenus* Kunth; *L. piurenensis* C.P.Sm.; *L. praestabilis* C.P.Sm.; *L. prostratus* J. Agardh.; *L. pubescens* Benth.; *L. pulvinaris* Ulbr.; *L. purosericeus* C.P.Sm.; *L. ramosissimus* Benth.; *L. semperflorens* Hartw., ex Benth.; *L. solanagrorum* C.P.Sm.; *L. tarapacensis* C.P.Sm.; *L. tomentosus* DC.; *L. tominensis* Wedd.; *L. weberbaueri* Ulbr. The results show that of the 29 species, none is considered Critically endangered (CR), one falls into the category of Endangered (EN); two species are considered Vulnerable (VU); two others fall into the category of Near threatened (NT) and the rest of species are Least concern (LC). Unfortunately, four species lacked enough data to be assessed and fall in the category of Data deficient (DD). This problem may occur because the species descriptions were made based on herbarium specimens collected at sites that have not been surveyed again. Another issue may be that the scientific names of the species have no taxonomic value and only reflect morphological differences that fall into the range of variability of other species and, therefore, may just be synonyms of other taxa. For these reasons, it is necessary to conduct further taxonomic studies, and new field surveys. The Andean ecosystems form patches of vegetation, and geographic barriers that can promote the generation of endemic species in relict locations, or support more scattered distribution of the species in remote areas with difficult access. This research helped establish the current conservation state of species that are considered secondary relatives of tarwi or chocho, and serves as a base for future studies of the Andean *Lupinus* germplasm. The authors are grateful to the International Union for the Conservation of Nature (IUCN) for the organization of the workshop, Rainforest Trust for travel aid support and to the Oak Spring Garden Foundation for the support and all the attentions received during the working week in its dependencies.

## EVALUACIÓN DE ESPECIES DE *Lupinus* ANDINOS PARA SER INCORPORADAS EN LA LISTA ROJA DE ESPECIES AMENAZADAS DE LA IUCN

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El lupino andino (*Lupinus mutabilis* Sweet), conocido con los nombres de tarhui, tarwi o chocho es una especie nativa de los Andes centrales que fue cultivada y mejorada por la civilización Incaica y que en la actualidad se cultiva por sus granos de alto valor proteino-oleaginosos en Ecuador, Perú y Bolivia, entre los 2500 y 3800 m s.n.m. Tal como su nombre científico lo indica *L. mutabilis* tiene una gran capacidad de mutar, consecuentemente varias razas silvestres forman parte de un pool genético con entrecruzamientos aleatorios. Dentro del marco de un programa de evaluación del estado de conservación de especies relacionadas con cultivos sudamericanos, para su incorporación en La Lista Roja de la Unión Internacional para la Conservación de la Naturaleza (UICN), se convocó a un grupo de expertos para evaluar entre otras, 29 especies de *Lupinus* nativas de la región de los Andes Tropicales de Bolivia, Perú, Ecuador y Colombia y consideradas parientes silvestres del chocho o tarwi. Durante el taller se revisaron las fichas y mapas de distribución de cada especie utilizando los criterios y categorías ya estandarizadas de la Lista Roja de la UICN. La información recabada fue a través de datos disponibles de distribución, tendencias de las poblaciones, requerimientos de hábitat y ecología. Las categorías consideradas para la evaluación (con el acrónimo en inglés) y de menor a mayor riesgo de extinción fueron: No evaluada (NE); Datos deficientes (DD); De menor preocupación (LC); Casi amenazada (NT); Vulnerable (VU), Amenazada (EN); En peligro crítico (CR); Extinguida en ambientes naturales (EW); Extinta (EX). Las especies evaluadas fueron: *Lupinus arvensis* C.P.Sm.; *L. ballianus* C.P.Sm.; *L. bangii* Rusby; *L. bogotensis* Benth.; *L. chachas* Ochoa ex C.P. Sm.; *L. chrysanthus* Ulbr.; *L. ellsworthianus* C.P.Sm.; *L. huaronensis* J. F. Macbr.; *L. huigrensis* Rose ex C.P.Sm.; *L. lindleyanus* C.P.Sm.; *L. mantaroensis* C.P.Sm.; *L. microphyllus* Desr.; *L. misticola* Ulbr.; *L. mollendoensis* Ulbr.; *L. mutabilis* Sweet; *L. nubigenus* Kunth; *L. piurenensis* C.P.Sm.; *L. praestabilis* C.P.Sm.; *L. prostratus* J. Agardh.; *L. pubescens* Benth.; *L. pulvinaris* Ulbr.; *L. purosericeus* C.P.Sm.; *L. ramosissimus* Benth.; *L. semperflorens* Hartw, ex Benth.; *L. solanagrorum* C.P.Sm.; *L. tarapacensis* C.P.Sm.; *L. tomentosus* DC.; *L. tominensis* Wedd; *L. weberbaueri* Ulbr. Los resultados muestran que de las 29 especies, ninguna es considerada En Peligro Crítico (CR), una sola entra en la categoría En Peligro (EN); dos especies son consideradas Vulnerables (VU); otras dos entran en la categoría de Casi Amenazadas (NT) y el resto está dentro de las especies de Menor Preocupación (LC). Lamentablemente cuatro de las especies carecían de datos suficientes como para ser evaluadas y establecer su estado de conservación (DD). Este problema puede surgir porque las descripciones fueron realizadas sobre la base de especímenes de herbarios coleccionados en sitios que no han sido nuevamente relevados o porque los nombres científicos de esas especies no están valorados taxonómicamente y reflejen características morfológicas que entran en el rango de variabilidad de otras especies y por lo tanto podrían ser simplemente sinónimos de otros taxones. Es por esas razones que se considera necesario ampliar los estudios taxonómicos y realizar nuevos relevamientos de campo, dado que los ecosistemas andinos forman parches de vegetación y la distribución de las especies puede verse afectada por barreras geográficas y crear endemismos en lugares relictos o puede estar más dispersa en lugares remotos aún no relevados. Esta investigación permitió establecer el estado actual de conservación de las especies consideradas parientes secundarios del tarwi o chocho y sirve de base para futuros estudios del germoplasma de los *Lupinus* andinos. Los autores agradecen a la Unión Internacional para la Conservación de la Naturaleza (UICN) por la organización del taller, a Rainforest Trust por el apoyo para viajes y a la Oak Spring Garden Foundation por el apoyo y las atenciones recibidas durante la semana de trabajo en sus dependencias.

## SPECIES REVIEW OF *Lupinus* GROWING IN BOLIVIA

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Bolivia is in the core of the Andes and the country in where most *Lupinus* species are growing in isolated patches at high altitudes. The taxonomic treatment of the genus for the country date from the review made by Charles P. Smith in his "Signatures in Species Lupinorum" published in different issues from 1940 to 1945. At that time, Smith published 41 taxa for Bolivia, from which 25 were newly described species and the rest were species from other authorships. In Smith's papers, written in Latin with some comments in English, he divided the 41 taxa growing in the country, in 14 groups, which are differentiating by vegetative and floral morphological characters. The descriptions were done over herbarium specimens from different herbaria which were collected mostly in the Department of La Paz (27 taxa), followed by Tarija (7 taxa), Cochabamba and Potosí (3 taxa), Oruro (2 taxa) and Chuquisaca (only one taxa), during late 1800 to 1935. Although the taxonomic monograph of *Lupinus* from Bolivia is still pending, the actual checklist considered 39 native species and one introduced or escaped from cultivation (*L. montanus* var. *austrovolcanicus* C.P.Sm.) which origin is Guatemala. Taking into consideration the new phylogenetic trees for *Lupinus*, it was found that the Andean species have the highest rate of diversification; with a greater variety of plant growth forms, which make the relationships among several species unresolved. Through an study of lupines from several herbaria and taking under consideration species distribution map it was possible to identified major species group and the status of the germplasm growing in Bolivia. The shrubby or bushy species described by Rusby before C. P. Smith publications are: *L. guggenheimianus*, *L. soratensis*, *L. sufferugineus*, *L. tatei*, all of them from Department La Paz. The list of lignose species described by Smith are: *L. alaristatus* (La Paz), *L. altimontanus* (La Paz, endemic), *L. cardenasianus* (Cochabamba, and Argentina & Chile), *L. celsimontanus* (La Paz), *L. erectifolius* (Tarija, Argentina & Chile), *L. imminutus* (Cochabamba), *L. mandonanus* (La Paz), *L. perissophytus* (Tarija), *L. rusbyanus* (La Paz & Cochabamba), *L. willamsianus* (La Paz). This type of species represents 35.89 % of the taxa in the country. In relation with life span, sometimes the identification of annual is difficult because, in some perennial the root and caulex remain alive and the aerial portion look as an herbaceous annual plant. In the annual category are: *L. mutabilis* Sweet which have several cultivated and wild races, and the closely related and endemic species *L. bolivianus* Rusby. The other probably annuals species are: *L. asplundianus* C.P.Sm., *L. bandeliera* C.P.Sm.; *L. cuspidatus* Rusby, *L. nubilorum* C.P.Sm., *L. pearceanus* C.P.Sm. (all, endemic in La Paz) and *L. tacitus* C.P.Sm. (La Paz & Oruro), *L. poopoenis* C.P.Sm. (Oruro, endemic) and *L. xenophytus* C.P.Sm., which were collected near the Bermejo river and can be related with the annual and perennial species of the Atlantic Subregion such the *L. gibertianus* C.P.Sm. complex. In conjunction, annual or possible annual species represent the 25.64% of the Bolivian *Lupinus* taxa. Other species are adapted to high elevation such as the dwarf prostrate plant are: *L. buchtienii* Rusby widely distributed in Bolivia, Perú and Argentina and the others with more restricted distribution such, *L. otto-buchtieni* C.P.Sm., *L. otto-kuntzeanus* C.P.Sm. and *L. tarijensis* Ulbr. Some species are small caulescent or acaulescent, forming a clump of long petiolate leaves in rosette at the base of the plant such as: *L. breviscapus* Ulbr., *L. chilense* C.P.Sm., *L. fiebrigianus* Ulbr., or with a discoid base such *L. pulvinaris* Ulbr. This group of dwarf plants represents the 20.51 % of Bolivian lupines. Several species present fistulose or subfistulose inflated stems, 2 cm or more in diameter such as *L. altiplani* C.P.Sm., *L. herzogii* Ulbr., *L. tominensis* Wedd., *L. umidicola* C.P.Sm., which represent only 10.25 % of Bolivian lupines, although this life form of *Lupinus* is highly represented in other Andean countries. The author is indebted to the curators of the herbaria for the specimens loan for this research.

## REVISIÓN DE LAS ESPECIES DE *Lupinus* QUE CRECEN EN BOLIVIA

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Bolivia está en el corazón de los Andes y es el país en donde más especies de *Lupinus* crecen en parches aislados y en elevadas altitudes. El tratamiento taxonómico del género hasta la fecha, es la revisión hecha por Charles P. Smith en sus "Signatures in Species Lupinorum" publicada en diferentes números de boletines, entre 1940 y 1945. En ese período Smith publicó 41 taxones para Bolivia, de los cuales 25 corresponden a nuevas especie y el resto a especies de otras autorías. Las publicaciones de Smith están en latín, con algunos comentarios en inglés, él dividió los 41 taxones que crecen en el país, en 14 grupos, que se diferencian por caracteres morfológicos vegetativos y florales. Las descripciones se realizaron sobre muestras de herbario de diferentes instituciones que fueron coleccionadas en su mayoría en el Departamento de La Paz (27 taxones), seguido por el de Tarija (7 taxones), Cochabamba y Potosí (3 taxones), Oruro (2 taxones) y Chuquisaca (sólo un taxón), durante fines del 1800 a 1935. Aunque la monografía taxonómica de *Lupinus* de Bolivia está aún pendiente, la lista actual considera 39 especies nativas y una introducida o escapada de cultivo (*L. montanus* L. var. *austrovolcanicus* C.P.Sm.) que es originaria de Guatemala. Teniendo en cuenta los nuevos árboles filogenéticos para *Lupinus*, se encontró que las especies andinas tienen la tasa más alta de la diversificación; con una mayor variedad de formas de crecimiento de las plantas, que hacen que las relaciones entre varias especies aún no esté resuelta. A través de un estudio de lupinos de varios herbarios y teniendo en cuenta el mapa de distribución de especies, fue posible identificar los grupos de especies más importantes y el estado del germoplasma que crece en Bolivia. Las especies arbustivas o matosas descritas por Rusby antes de las publicaciones de C. P. Smith son: *L. guggenheimianus*, *L. soratensis*, *L. sufferugineus*, *L. tatei*, todas ellas del Departamento de La Paz. La lista de especies leñosas descritas por Smith son: *L. alaristatus* (La Paz), *L. altimontanus* (La Paz, endémica), *L. cardenianus* (Cochabamba, Argentina y Chile), *L. celsimontanus* (La Paz), *L. erectifolius* (Tarija, Argentina y Chile), *L. imminutus* (Cochabamba), *L. mandonanus* (La Paz), *L. perissophytus* (Tarija), *L. rusbyanus* (La Paz y Cochabamba), *L. willamsianus* (La Paz). Este tipo de especies representa el 35,89% de los taxones del país. En relación al hábito, a veces es difícil identificar si la planta es anual o perenne porque, en algunas especies perennes la raíz y la corona basal siguen vivos y la porción aérea se comporta como una planta anual herbácea. En la categoría anual están: *L. mutabilis* Sweet que tienen varias razas cultivadas y silvestres y la especie estrechamente relacionada y endémica *L. bolivianus* Rusby. Las especies probablemente anuales son: *L. asplundianus* C.P.Sm., *L. bandeliera* C.P.Sm.; *L. cuspidatus* Rusby, *L. nubilorum* C.P.Sm., *L. pearceanus* C.P.Sm. (todas, endémica de La Paz) y *L. tacito* C.P.Sm. (La Paz y Oruro), *L. poopuensis* C.P.Sm. (Oruro, endémica) y *L. xenophytus* C.P.Sm., que fueron coleccionadas cerca del río Bermejo y que pueden estar relacionadas con especies anuales y perennes de la Subregión Atlántica, como lo es el complejo de *L. gibertianus* C.P.Sm. En conjunto, las especies anuales o posiblemente anuales representan el 25,64% de los taxones de *Lupinus* bolivianos. Otras especies adaptadas a gran altura como plantas enanas y postradas son: *L. buchtienii* Rusby ampliamente distribuida en Bolivia, Perú y Argentina y las otras con distribución más restringida como, *L. ottobuchtieni* C.P.Sm., *L. otto-kuntzeanus* C.P.Sm. y *L. tarijensis* Ulbr. Algunas especies son pequeñas caulescentes o formando un mazo de planta con hojas en roseta basal y largos pecíolos tales como: *L. breviscapus* Ulbr., *L. chilense* C.P.Sm., *L. fiebrigianus* Ulbr., o con una base discoide como: *L. pulvinaris* Ulbr. Este grupo de plantas enanas representa el 20,51% de los lupinos bolivianos aunque esta forma de vida de *Lupinus* esa altamente representadas en otros países andinos. La autora agradece a los curadores de los herbarios el préstamo de ejemplares para este estudio.

## SOIL REGENERATION, USING THE POTENTIAL OF THE GENUS *Lupinus*

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In Mexico, approximately over 100 species of *Lupinus* (Fabaceae) have been reported, growing in different habitats, from the sand dunes at 5 m above sea level to the subalpine vegetation at 4000 m a.s.l. Species of the Fabaceae family are widely used as green fertilizers due to their symbiotic association with nitrogen fixing bacteria (NFB) and to the fact that they improve the physical and chemical properties of soils. It has been reported that domesticated species of the genus *Lupinus* fixed between 13 and 40 g of N<sub>2</sub> per m<sup>2</sup> per year. The aims of the present work were to isolate, purify and characterize bacteria from the nodules of *L. montanus* plants and to evaluate *Lupinus* wild species as natural fertilizers in agricultural soils. The characterization of the isolated bacteria was performed using selective culture media, mobility tests and Gram staining. The identification was corroborated through sequencing of the 16S-rARN gen. Plants of *L. montanus*, *L. bilineatus* and *L. campestris* were collected in the surroundings of the Iztaccihuatl-Popocatepetl National Park, at 2500-3400 m a.s.l. Nitrogen content of the three species was determined. The species which showed the highest N content was used as green fertilizer in a pecan orchard located in Amecameca de Juárez. Seeds were scarified and inoculated before sowing. Plants in vegetative stage were ground and incorporated into the soil as green fertilizer. To assess the effect of native and wild populations of *Lupinus* on soil fertility in corn plots, soil physical and chemical parameters related to their fertility were analyzed. From the results of the morphological and physiological evaluation, the isolates Lm.P1.14, Lm.P2.9, Lm.P2.10, and Lm.P2.11 were identified as *Rhizobium*, while the isolate Lm.P2.8 was identified as *Bradyrhizobium*. The results of the sequencing of the gen confirmed the identification of isolate Lm.P1.14 as *Rhizobium*, while the isolates Lm.P2.8 and Lm.P2.10 were identified as *Brevibacillus* and Lm.P2.9, Lm.P2.11 as *Pseudomonas*, which have been previously reported in the rhizosphere associated with nitrogen fixing bacteria. Nitrogen percentage was 2.2% in *L. montanus*, 2.5% in *L. campestris* and 3.3% in *L. bilineatus*. A percentage of germination of 15-27% of *L. bilineatus* in the peanut orchard was achieved. According to the analysis of the physical properties, the soils of the evaluated plots were very homogeneous, presenting significant differences only in clay content and bulk density. The soil of D presented differences in both, while the soil of BG in apparent density. In terms of texture, the soils of the plots of D, DL and MI are sandy loam, while the soil of B is sandy loam. According to the results obtained, the nitrogen content varied from 0.155 to 0.17%. With respect to extractable phosphorus, the soils had a content of 422.80 to 489.00 ppm.

In conclusions *L. montanus* and its associated bacteria, could be considered as a viable alternative for its use as a natural fertilizer in agricultural soils and their remediation. This research was financed by IPN (SIP-20150658, SIP-20161150). M. Figueroa acknowledges CONACYT for a postgraduate scholarship (CONACYT-640583).



## BIODIVERSITY OF THE *Lupinus* GENUS IN LOS ANDES

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The information about biodiversity of the *Lupinus* genus is evaluated, presenting a list of wild species as possible ancestors of *Lupinus mutabilis*, due to their morphological similarities such as: erect growth of the plant, greater size of the grain, presence of white color in the seeds, larger size and shape of leaves, less presence of dehiscence.

Related species are *L. praestabilis*; *L. cuzcencis* in the valleys and *L. aridulus* for terrains above 4,000 m. in the regions of Cusco and Puno. For the northern highlands of Peru, other investigations suggest *L. piurensis*.

According to its regional name of "chocho", "tarwi" and "tauri" *L. mutabilis* shows different morphological characteristics such as, growth type, color of the grain and precocity, as relevant differentiation suggesting an arrangement of accessions to be needed in the current germplasm banks, for a better selection of new varieties.

Concentration of diversity of *L. mutabilis* is detected for regions of Colombia, Ecuador, Peru and Bolivia, where is necessary to complete the collections of wild and cultivated species, taking into account the region of origin. Including Rhizobium species and its nitrogen fixing capacity.

It is suggested to move forward in the arrangement of the current accessions in germplasm banks, according to their, differentiation in protein content (24.8-49.8%), fats (14.0-23.6%) and alkaloids (0.72-2.13%). ), precocity 170-230 days, grain yield, potential and tolerance to diseases mainly anthracnose and pests. including the classification of races

## BIODIVERSIDAD DEL GENERO *Lupinus* EN LOS ANDES

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Se evalúa la biodiversidad del género *Lupinus*, presentando una relación de especies silvestres como posibles ancestros de *Lupinus mutabilis*, por sus similitudes morfológicas que son crecimiento erecto de la planta, mayor tamaño y presencia de color blanco en las semillas, mayor tamaño y forma de hojas, menor presencia de dehiscencia.

Se mencionan como especies afines *L. praestabilis*; *L. cuzcencis* en los valles y *L. aridulus* para terrenos sobre los 4,000 m. presentes en la región del Cusco y Puno. Para la sierra norte del Perú, otras investigaciones sugieren *L. piurensis*.

Sobre *L. mutabilis* se propone, de acuerdo a su denominación regional de “chocho”, “tarwi” y “tauri” y características morfológicas: tipo de crecimiento, producción y color de grano del grano; precocidad y tolerancia a enfermedades como relevantes características de diferenciación, para su mejoramiento genético; sugiriéndose un ordenamiento y caracterización de las accesiones, en los actuales bancos de germoplasma, de manera que facilite la selección de nuevas variedades.

Se define la concentración de la diversidad de *L. mutabilis* en regiones de Colombia, Ecuador Perú y Bolivia, en las cuales será necesario completar las colecciones de especies silvestres y cultivadas, tomando en cuenta la región de procedencia, así como incluir la recolección de las especies de *Rhizobium* y evaluar su capacidad de fijación de nitrógeno.

Se sugiere avanzar en el ordenamiento de los actuales bancos de germoplasma incluyendo la clasificación de razas de las accesiones según la procedencia, diferenciación en contenido de proteína (24.8-49.8%), aceites (14.0-23.6%) y alcaloides (0.72-2.13%), precocidad 170-230 días, rendimiento de grano, potencial y tolerancia a enfermedades principalmente antracnosis y plagas.

# IDENTIFICACIÓN DE PADRES DONANTES DE GENES PARA PRECOCIDAD Y MADURACIÓN UNIFORME EN EL BANCO DE GERMOPLASMA DE *Lupinus mutabilis* Sweet DE ECUADOR

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En Ecuador, el cultivo de chocho (*L. mutabilis*) es importante sobre todo para la agricultura familiar de la Sierra y por su valor nutricional es estratégico para disminuir los índices de desnutrición y malnutrición en el país. Ante el incremento de la demanda del grano de chocho, a nivel nacional e internacional y en un contexto de variabilidad climática es urgente desarrollar variedades precoces, con maduración uniforme, amplia adaptación, alto rendimiento y que faciliten la mecanización del cultivo. El INIAP dispone de una colección de germoplasma de *Lupinus* de más de 500 accesiones, de las cuales 380 corresponden a *L. mutabilis*; cerca del 50% han sido colectadas en Ecuador, lo que representa la alta diversidad de este cultivo en la Sierra del país, y el otro 50% de accesiones han sido obtenidas por intercambio con otros países de la región y de otras latitudes. Para el mantenimiento de la colección de germoplasma de chocho, el Programa de Leguminosas y Granos Andinos del INIAP, de forma periódica realiza el refrescamiento y multiplicación de semilla, incluyendo también la caracterización y la evaluación agronómica. El objetivo del presente trabajo fue seleccionar accesiones precoces, con menor duración de floración, maduración uniforme y alto rendimiento para ser incluidas dentro del plan de mejoramiento del chocho. Actualmente, el mejoramiento genético del chocho está enfocado en generar líneas precoces, de maduración uniforme y altura de planta igual o inferior que la variedad vigente INIAP 450 Andino. La investigación se realizó en la Estación Experimental Santa Catalina del INIAP, ubicada al sur de Quito a 3050 m de altitud. Se evaluaron 227 accesiones, en parcelas de un surco de 3 m de longitud. Se tomaron datos de variables cuantitativas, cualitativas y se realizaron análisis de frecuencias y medidas de tendencia central (media, máximo, mínimo, desviación estándar). En general, se encontró amplia variabilidad para los descriptores cuantitativos. Analizando la variable días a floración, 48 accesiones (27%) se identifican como precoces (80 y 97 días a floración), mayormente provenientes de Perú, Bolivia y Ex URSS; en tanto que la mayoría de las tardías fueron colectadas en Ecuador. En cuanto a la duración de la floración, 26 accesiones provenientes de Ecuador y Perú, presentan valores entre 22 y 45 días, lo cual puede estar relacionado con mayor uniformidad a la cosecha. Para altura de planta, 26 accesiones, provenientes de Perú y Bolivia, presentaron la menor altura de planta (43 a 80 cm), característica que junto a la uniformidad de la maduración son claves para la mecanización de la cosecha. El rendimiento también es un aspecto fundamental y un componente del mismo es el número de vainas por planta, para cuya variable, la mayoría de las accesiones alcanzan promedios de hasta 80 vainas, pero las accesiones procedentes de Ecuador y unas pocas de Perú, superan dicho promedio. En peso de semilla (g/planta), menos del 5% de accesiones superaron la media más alta (139 g/planta); mientras que el 10% de las accesiones procedentes de Ecuador y Perú, presentaron un promedio situado entre 94 y 138 g/planta. En conclusión, en las accesiones originarias de Perú y Bolivia existen potenciales fuentes de genes para precocidad, uniformidad de cosecha y menor altura de planta, que combinando con accesiones con mayor potencial de rendimiento, pueden ser muy útiles para la generación de nuevas variedades que respondan a los desafíos actuales de productores y consumidores. Es importante continuar con las evaluaciones, en diferentes condiciones agroclimáticas y por diferentes ciclos de cultivo con el fin de confirmar y seleccionar progenitores para futuras cruces.



**PHYSIOLOGY & PROTECTION**  
**FISIOLOGÍA & PROTECCIÓN**

## EVALUATION OF ANTHRACNOSE RESISTANCE ON A TARWI (*Lupinus mutabilis*) GERMPLASM COLLECTION

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Lupin anthracnose, caused by *Colletotrichum lupini* (Bondar) Nirenberg, Feiler & Hagedorn, is known since the first half of the 20<sup>th</sup> century in North America, and was subsequently reported in Europe, losing importance afterwards. A second outbreak, presumably by a different and more virulent form of the pathogen, reappeared in South America and Europe towards the end of the 20<sup>th</sup> century, namely on tarwi (*Lupinus mutabilis*) and white lupin (*L. albus*), and subsequently spread to all parts of the world and to all cultivated lupin species, causing high yield losses or lesser profit due to the cost of protection measures. In some cases lupins cultivation was rendered inviable, particularly in wet climates. Most *Colletotrichum* pathogens are polyphagous, with the same genetic entity found on multiple hosts. Moreover, frequently the same host is affected by multiple *Colletotrichum* spp., with no clear differences on the symptoms caused. However, the lupin anthracnose pathosystem seems to be an exception to this common trend, as lupin anthracnose is caused solely by *C. lupini* and *C. lupini* seems to prefer *Lupinus* spp. Additionally, very little genetic diversity is recognized among *C. lupini* populations, with only two groups reported: one corresponding to the North American outbreak in the first half of the 20<sup>th</sup> century, and currently not occurring in the nature; the other corresponding to the contemporary outbreak, found across the world. In a context of near-clonality of the pathogen, finding resistant germplasm seems a promising strategy for sustainable disease control. Previous studies on tarwi showed little success, prompting the need for further studies on this species with a relatively limited genetic diversity pool. In this work we report the characterization of anthracnose response in a tarwi germplasm collection that is being studied for its adaptability to Mediterranean climate growing conditions, in Portugal. For such, a contemporary and local *C. lupini* strain was used. Results reveal diversity concerning anthracnose response, enabling to point out lines that endure less severe disease symptoms (LM268, among others), suggesting their suitability for downstream anthracnose resistance studies. [This project has received funding from the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme under grant agreement No 720726]

## ANALYSIS FOR THE QUALITY CONTROL OF LUPINE SEEDS

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The success of lupines as a commercial crop depends on the species and the variety use, which need to be the most suitable for the cropping area, but also on the quality of the seed sowed. Low seed quality never has a good crop performance and does not reach high yields. Before sowing the farmer must be sure that the seeds are viable and with good physiological properties that guarantee the germination and seedling establishment in the field. The International Rules for Seed Testing of the International Seed Testing Association, known as ISTA Rules, establish and provide definitions and standardized methods for testing seed lots that help the farmer to know the quality of his own seeds and to establish parameter in national and international seed trades. It is well known that seed deterioration processes can start immediately after the seed reaches the physiological maturity. One of the most common causes of seed deterioration is the loss of cell membrane integrity that involves the leakage of electrolytes, which may be reflecting low germination percentage. As part of a research projects several seed coat studies and physiological tests in species of *Lupinus* were conducted to establish the best methods to evaluated seed quality. The seed coat plays an important role in seed longevity since it is the first barrier against adverse environmental conditions, providing the embryo and other seed components (endosperm remnants layers) with physical and chemical protection against cellular rupture during imbibitions; since it also regulates water uptake rate. The quality of the seeds were evaluated by the physiological standard germination percentage test (GP), the topographic biochemical tetrazolium test (TT) and the electrical conductivity test (EC), following ISTA Rules. Besides, tetrazoliunn was used to seek the water entrance during imbibitions. The GP test revealed significant quality differences between seed lots in relation with the year in which the seeds were harvested. The TT trials showed that in *Lupinus albus* and *L. angustifolius* the water pathway at the very beginning of imbibition is related to the lens and the pores present in this region. Following the ISTA rules, which establish that the tissues stained carmine red were considered alive and vigorous, the tissues stained deep carmine red were considered to be deteriorated and the ones that remained colorless or milky white were regarded as dead, was possible to establish the seed vigor of several seed lots. The EC values correlated negatively with field emergence and correlate better than the GP test. Seed lots of white lupines with high EC values ranging between 112.7-307.5  $\mu\text{Simens cm}^{-1}$  showed lower GP than the ones with EC values between 67 and 73.5  $\mu\text{Simens cm}^{-1}$ . The higher EC values could be explain as a result of the deterioration process of the membranes that result in a poor seed lot quality. The results shows that between the different vigor tests evaluated, the EC test did provide consistent advantages in the discrimination between seed lots with similar GP values, but different physiological qualities consequently it showed to be reliable to predict field performance, in a quick way and at low-cost. The research allowed to establish that a value of  $162.5 \pm 1.5 \mu\text{Simens cm}^{-1}$ , is the highest one accepted in order to identify high performance seed lots. The authors are grateful for the economic support of the University of Córdoba and the laboratories facilities of the seed laboratory of the Facultad de Ciencias Agropecuarias and CREAN of UNC.

## ANÁLISIS PARA EL CONTROL DE CALIDAD DE SEMILLAS DE LUPINOS

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El éxito de los lupinos como cultivo comercial depende de la especie y de la variedad que se usa para la siembra, que deben ser las más adecuadas para el área de cultivo, como así también, de la calidad de la semilla sembrada. Las semillas de baja calidad nunca tienen un buen comportamiento de cultivo y no alcanzan altos rendimientos. Antes de la siembra el agricultor debe estar seguro de que las semillas son viables y con buenas propiedades fisiológicas que garanticen la germinación y establecimiento de plántulas en el campo. Las Normas Internacionales para el Control de Calidad de las Semillas de la Asociación Internacional de Semillas, conocidas como Reglas ISTA, establecen y proporcionan definiciones y métodos estandarizados para pruebas de lotes de semillas, que ayudan a los agricultores a conocer la calidad de sus propios lotes y a establecer parámetros para el comercio nacional e internacional de semillas. Es bien sabido, que los procesos de deterioro de la semilla pueden iniciarse inmediatamente después que la semilla alcanza la madurez fisiológica. Una de las causas más comunes de deterioro de las semillas es la pérdida de la integridad de las membranas de las células que implica la fuga de electrolitos, que puede estar reflejando un bajo porcentaje de germinación. Como parte de un proyecto de investigación se llevaron a cabo varios estudios de tegumento seminal y test fisiológicos de especies de *Lupinus* para establecer los mejores métodos para evaluar calidad de las semillas. El tegumento seminal juega un papel importante en la longevidad de la semilla, ya que es la primera barrera contra condiciones ambientales adversas, proporcionando protección física y química al embrión y otros componentes de la semilla (capas de restos de endosperma) a la ruptura celular durante la imbibición, ya que regula también la velocidad de entrada de agua. La calidad de las semillas fue evaluada por la prueba fisiológica estandarizada de porcentaje de germinación (GP), la prueba topográfica y bioquímica de tetrazolio (TT) y la prueba de conductividad eléctrica (CE), siguiendo las Reglas ISTA. Además, se utilizó tetrazolio para visualizar la entrada de agua en la semilla. La prueba GP reveló diferencias significativas de calidad entre lotes de semillas, en relación al año en que se cosecharon las semillas. Los ensayos de TT mostraron en *Lupinus albus* y *L. angustifolius* que el camino del agua al comienzo de la imbibición está relacionado con la lente y los poros presentes en esta región. Siguiendo las Reglas ISTA, los tejidos teñidos de rojo carmín se consideraron vivos y vigorosos; los tejidos teñidos de rojo carmín profundo fueron considerados deteriorados y los que continuaron incoloros o de color blanco lechoso fueron considerados muertos, así fue posible establecer el vigor de la semilla de varios lotes para la siembra. Los valores CE correlacionaron negativamente con la emergencia a campo y correlacionan mejor que la prueba GP. Muchas de las semillas de los lupinos blancos con altos valores CE entre 112.7-307.5  $\mu\text{Simens cm}^{-1}$  tuvieron GP más bajo que las que tenían valores de CE entre 67 y 73.5  $\mu\text{Simens cm}^{-1}$ . Los mayores valores de CE, se podrían explicar como resultado del proceso de deterioro de las membranas, que dan lugar a la pérdida de calidad de las semillas. Los resultados muestran que entre las distintas pruebas de vigor evaluadas, la prueba de CE presentó ventajas consistentes en la discriminación entre lotes de semillas con similares valores GP, pero diferentes cualidades fisiológicas de una manera rápida y de bajo costo. La investigación permitió establecer que un valor de 162.5-1.5  $\mu\text{Simens cm}^{-1}$ , es el más aceptado para identificar lotes de semillas de alto rendimiento. Los autores agradecen el apoyo económico de la Universidad de Córdoba y las instalaciones de los laboratorios de Semillas y del CREAN de la Facultad de Ciencias Agropecuarias, UNC.

The background features a teal color palette with a stylized landscape at the top, showing a horizon line and a field. Below this, there are several overlapping, curved, and straight lines in various shades of teal and blue, creating a sense of depth and movement. The overall design is modern and clean.

**AGRONOMY & FARMING**  
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# PHOSPHORUS SOLUBILIZATION CAPACITY AT DIFFERENT PH IN *Bradyrhizobium* sp. ISOLATED FROM *Lupinus ballianus* C.P. SMITH OF HIGHLAND ANDEAN SOILS

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Legumes are very extractive of phosphorus (P) from soil. The dynamics of microorganisms facilitate the absorption of P in plants where availability is limited mainly by extremely acid or alkaline pH. *Lupinus ballianus* C.P. Smith, "jera", is a wild relative of *Lupinus mutabilis* "tarwi" or "chocho" being extractive in phosphorus (46% proteins), the presence of the symbiont *Bradyrhizobium* sp. benefits the fixation of nitrogen of the air and intervenes in the solubilization of the soil P. The objective of this research was to evaluate the solubilization capacity of phosphorus at different pH in *Bradyrhizobium* sp. isolated from *Lupinus ballianus* C.P. Smith of highland Andean soils. The sampling areas were the slopes of the Parinacochas Lagoon (3750 masl), Ayacucho (Peru). The rhizospheres of lupin were sampled and the physicochemical and microbiological characterization analyses of these soils were determined in the Soil Analysis Laboratory of the Faculty Agronomy-UNALM. The results were: loamy sandy texture, 13% Gravimetric Moisture, pH 4.7, 5.23 % organic matter, 2.3 ppm available P, microbial populations: bacteria  $6.8 \times 10^6$  CFU / gram of dry soil (g.d.s); fungi  $1.2 \times 10^5$  CFU / g.d.s and actinomycetes  $1.65 \times 10^5$  CFU / g.d.s. *Bradyrhizobium* sp. from lupin nodules was isolated in medium Yeast, Mannitol and Agar (YMA) at pH 4.7. Subsequently, a complete randomized design was applied and the strain was sowed in four punctures per Petri dish and in three replicatons at different pH (4.5, 5.5, 6.5, 7.5 and 8.5) in both YMA and Pikoskaya (phosphate rock) media. The plates were incubated in an oven at 27 °C. After 10 days of sowing, the diameter of the colonies of the YAM medium was recorded. At 10, 16 and 26 days in the colonies of the Pikoskaya medium the solubilization index or halozones (halo diameter/colony diameter) was determined. The data analyses were processed with the SAS statistical software version 9.1 and were subjected to the Tukey test at  $\alpha = 0.05$ . The results showed in the YMA medium a positive effect for the increase of the diameter of the colony, being the optimum pH of 5.5 and 6.5, at lower or higher value it decreased. With respect to the P solubilization index, the colonies at pH 4.5 reached the highest value, at the same time it was evidenced that if they can solubilize P in a wide range from extremely acid to alkaline pH. The assessment of soil microbial biodiversity such as *Bradyrhizobium* sp. and native species of *Lupinus* in highland Andean soils are very important, being potential for the recovery of degraded areas or restoration of soils where the availability of P may be a limiting factor. Financing: AGROASESORIAS SAC, Laboratory of Soil Microbiology. Agronomy Faculty (LASPAF –UNALM).

# LUPIN A POTENTIAL CROP SPECIES FOR PLANT-BASED PROTEIN FOOD FOR DENMARK

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As the world population increases, the same happens with food consumption. Animal based food products have a high negative impact in the environment. Europe is a major gas emitter and their food sector needs to change in order to reduce 80-95% of the greenhouse gas emissions they have targeted for 2050. Hence their food systems need to change and become more efficient considering alternative sources of proteins such as plant-based proteins. PROTEIN2FOOD (2015-2020) is an EU project that has the objective of contributing to this target by generating innovative, high quality plant-protein food to sustain human and environment health as well as biodiversity. Lupin is one of the plant species that this project works with from different angles of the food chain (crop production, processing, marketing, dissemination and environment). The presentation will focus in the main results this project had with lupins in Denmark along four years of work (from 2015 to 2018) in the crop production area. The experiments for this project took place in the experimental station of Højbakkegaard, Taastrup, at the Faculty of Science of the University of Copenhagen in Denmark (55°40' 9" N, 12° 18' 35" E and an altitude of 28 m above sea level). The study site is characterized by sandy loam soils and has a typical coastal climate relatively warm for its latitude. The mean annual temperature ranges 7–9 °C and the annual precipitation is between 640–770 mm. In order to study the agronomic adaptability of the selected high protein crop, a screening trial and different sowing date trials were implemented from 2015 to 2017. In the screening, two cultivars of *Lupinus angustifolius* (Iris and Primadonna) and two lines of *Lupinus mutabilis* (27CA and 27CB) were grown under the described conditions. Results showed that *Lupinus angustifolius* has potential to be produced in Denmark: Iris and Primadonna cvs (with 36 and 41% protein respectively) had yields of 1.2 and 1.4 t/ha that respectively corresponded to 18% and 12.5% less than 2016 lupin average yield of Europe (1.6 t/ha). In contrast, the Andean lines with higher protein content yielded in average 0.86 t/ha or 43% less compared to the average yield of South America of 2016 (1.5 t/ha). The protein contents in the grain of the tested lines were in average 48% but with known high alkaloid levels. Sowing date trials in the same years of experimentation showed that a difference of 15 – 30 days in the date of sowing didn't impact the yields ( $p>0.05$ ) of Primadonna cv. In 2017, the amplification of this trial to four cultivars of *Lupinus angustifolius* showed different results that could be related to the specific characteristics of the cultivars used (branching and no branching). The last screening trial carried out in 2018, considered in Denmark as the driest year since 1874, showed an outstanding response of *Lupinus* species (both *L. angustifolius* and *L. albus*) to drought with yields that ranged from 1.2 to 2.9 t/ha with only 64 mm of rain during the growing season. Moreover the presentation will give brief highlights of important achievements and results from other areas of the PROTEIN2FOOD project (e.g. innovative products obtained, environmental impacts and projections estimated). These results show the great potential of lupins as an innovative plant-based protein alternative for the North of Europe and as a resilient species that can help to reach the environmental compromises assumed to mitigate climate change.

## **PRODUCTION AND MARKETING MODEL FOR SWEET LUPIN IN CHILE**

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The success of Lupin in Chile depends upon the chaining process between research-production and continuous uses of the generated products. An example of this production model is the one developed by the company Avelup Spa Nutritious Products. This company focus their model in a system of agriculture agreements, in which it take under consideration not only the hiring of the sowings plots, but also relevant aspects such the supply of quality seed and the recommendation of the need for renewal the sowing lot each year. Another factor is the technical assistance by a company consultant, which provides the necessary recommendations to get the crop to a good term. Finally, as the main focus of all the productive and commercial chain emphasizes are the strict quality controls starting at seed production, continues with the grain production process in the field and ending in the industrialization and commercialization of lupin products. The company Avelup markets under this system an average annual volume close to 10,000 tons of lupin. The production of lupin grits (peeled and split lupin) with 42% Protein and 9% Oil stands out, it is commercialized in bulk for companies that produce salmon feed. In the lupin husking process, lupin fiber called Vitafiver (commercial brand) is also obtained, with 86% of total dietary fiber and used to enrich the diet and foods with fiber. A percentage not less than 30% of lupin production is marketed with exporters for human consumption in the Middle East.

## MODELO DE PRODUCCIÓN Y COMERCIALIZACIÓN DE LUPINO DULCE EN CHILE

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El éxito del lupino en Chile depende del encadenamiento entre investigación -- producción y utilización continua de los productos generados. Un ejemplo de este modelo productivo es el desarrollado por la empresa Productos Nutritivos Avelup Spa. Esta empresa basa su modelo gracias a un sistema de agricultura de contrato, que no solamente consiste en la contratación de las siembras, sino que, además se consideran factores relevantes como: el suministro de semillas de calidad y la recomendación de la necesidad de renovación de los lotes de siembra todos los años. Otro factor importante es el seguimiento técnico del cultivo, en donde un asesor provee las recomendaciones necesarias para que el cultivo llegue a un buen término. Finalmente y como eje principal de toda la cadena productiva y comercial, se hace énfasis en los estrictos controles de calidad que van desde la producción de semilla, pasando por todo el proceso productivo en el campo y finalizando en la industrialización y comercialización de los productos de lupino. La empresa Avelup comercializa bajo este sistema un volumen promedio anual cercano a las 10.000 toneladas de lupino. Destaca la producción de grits de lupino (lupino pelado y partido) con un 42% de Proteína y un 9% de Aceite, se comercializa a granel para empresas que elaboran alimento para salmones. En el proceso de descascarado de lupino se obtiene además fibra de lupino denominada VitaFiver (marca comercial), con un 86% de fibra dietética total y utilizada para enriquecer la dieta y alimentos con fibra. Un porcentaje no menor cercano al 30% de la producción de lupino se comercializa con exportadores para el consumo humano en el medio oriente.

# IMPROVING NARROW-LEAFED LUPIN – NOVEL GENETIC RESOURCES FOR HIGHER YIELD AND YIELD STABILITY - LUPIBREED – A GERMAN CONSORTIUM FROM BREEDING RESEARCH TO NEW CULTIVARS IN GERMANY: INTERCROPPING STRATEGIES AND WEED CONTROL

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In this part of the project ‚LupiBreed‘ eight prebreeding lines of blue lupin (*Lupinus angustifolius*) and two varieties cv. Boregine (branched type) and cv. Boruta (terminated type) as reference were examined in field trials in the years 2016 and 2017 at the Thünen-Institute of Organic Farming in northern Germany for their competitiveness against weeds and their suitability for mixed cropping. Performance of the prebreeding lines, which were developed through artificially induced mutation at Julius Kühn Institute, was assessed against the reference varieties and between growth types of the prebreeding lines (2 terminated, 5 branched, 1 intermediate). Competitiveness against weeds was simulated at different levels of weed pressure by comparing yields under weed-free conditions, in competition with site-specific weeds and with a mixture of so called “artificial weeds“ consisting of rapeseed (*Brassica napus*), buckwheat (*Fagopyrum esculentum*) and phacelia (*Phacelia tanacetifolia*) at lower and higher seed density (60 and 120 seeds per m<sup>2</sup>). Suitability of blue lupin for mixed cropping was tested in additive mixtures with spring wheat (*Triticum aestivum*), oat (*Avena sativa*) and false flax (*Camelina sativa*) as partners at two reduced seed densities (wheat and oat: 60 and 120, false flax: 200 and 600 seeds per m<sup>2</sup>) compared to sole cropping seed density, while blue lupin was sown at 100 % sole cropping seed density (100 except Boruta with 130 seeds per m<sup>2</sup>). The aim was to identify prebreeding lines with good competitiveness against weeds, which also yield highly as sole and mixed crops, and to specify a suitable mixed cropping system for blue lupin. For that purpose yields of above ground biomass and grain yield were surveyed separately for lupin, partners and natural weeds three times throughout the growing season (stem elongation, flowering and ripeness) and relative total grain yields in the mixtures were evaluated. Variety-specific differences in weed tolerance were found at each sampling and were in addition dependent on weed abundance at final harvest (ripeness). However, weed suppression potential of varieties and prebreeding lines only differed at ripeness sampling and those differences were irrespective of weed abundance. Competitiveness of prebreeding lines was not generally superior to comparative varieties in sole and mixed cropping systems. Branched and terminated prebreeding lines varied when sole cropped concerning better weed suppression of branched types, but not regarding lupin yields. In mixed cropping systems branched and terminated prebreeding lines did not show those differences. There were positive effects of intercropped blue lupins in total yield. A higher seed density of the partners resulted in better weed suppression, but without affecting the yields. In addition, prebreeding lines with a good suitability for mixed cropping systems were identified.

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# INVESTIGATION OF THE RELATIONSHIP BETWEEN THE LEVEL OF ALKALOIDS AND DEGREE OF PEST DAMAGE IN *Lupinus polyphyllus* Lindl

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The first sweet forms of lupins were created almost 100 years ago by Reinhold von Sengbusch in Germany. The Russian Institute of Plant Industry (VIR) also developed its own research as well as an instruction to identify alkaloid-free lupins. It was immediately published with the foreword by N.I. Vavilov (Ivanov *et al.*, 1932). With the help of this method, the first sweet form of *Lupinus polyphyllus* was bred in 1932 at VIR. The above-mentioned publication of VIR as well as the discovery of German scientists was a cornerstone of the modern breeding work with low-alkaloid fodder (sweet) lupins in the whole world. We produced the first fodder commercial variety of *L. polyphyllus* Lindl. named '*Pervenets*' (the first sweet variety) for the conditions of the Northwest Russia. Variety '*Truvor*' was developed for the conditions of Ukraine. Now we are conducting research on sweet forms of the perennial Washington lupin in Finland, New Zealand and UK. We present some of our research results in this paper. The developed methods may also be applied to other cross-pollinated lupin species, such as *L. mutabilis* Sweet., *L. nootkatensis* Donn., *L. arboreus* Sims., *L. perennis* L., *L. elegans* H.B.K., *L. hartwegii* Lindl. and other forms promising for agricultural production. Accessions of *L. polyphyllus* with different alkaloid content from the collection of the N.I.Vavilov Institute of Plant Genetic Resources (VIR) and bitter samples from New Zealand were used in this investigation. The methodology included qualitative evaluation of alkaloid activity applying a conventional traditional method with the help of litmus paper impregnated with Dragendorff's solution. Gas chromatography was used for the quantitative analysis of alkaloids. The extraction and analyses were conducted following the procedure presented by Kamel *et al.* (2016). We used the scale of the descriptor list for lupin while measuring the degree of plant susceptibility to pests: 0 - No symptoms, 2 - Slight, 5 - Intermediate, 7 - Severe, 9 - Very severe. Our results shows harmful fauna of lupin is characterized by great diversity in the environments of Finland. Leaves and flowers are damaged by various leaf-eating insects and leaf rollers. Different accessions were affected by pests to varying degrees. Some of them showed no signs of damage, while others were strongly afflicted. We drew attention to the fact that the leaves of lupin severely affected by leaf pests had low content of alkaloids. The relationship between plant susceptibility to pests and the level of alkaloids appeared in our studies as follows: no symptoms of damage – 4.00% of alkaloids, slight – 2.00%, intermediate – 0.20%, severe – 0.09%, and very severe damage – 0.02%. We managed to collect a small amount of seeds from very severely affected plants whose alkaloid content was 0.02%. The residual composition of alkaloids in these seeds was as follows: 58% of 13-hydroxylupanine, 23% of lupanine, 4.5% of angustifoline, 1.2% of 11, 12-dehydrolupanine, 1% of ethyl-lupanine, and 12.3% of some other unidentified substances. We are trying to multiply these seeds. The revealed regularity has both positive and negative effects. On the one hand, the search for low alkaloid forms is simplified and can contribute to the creation of sweet varieties of lupins in the future. On the other hand, the low alkaloid forms revealed in this way have a low productivity and are not resistant to pests. There is a lot of work to improve them. In this regard, alkaloid forms of *L. polyphyllus* are successfully growing in New Zealand as a food for sheep. We believe that our observations will contribute to the development of new low-alkaloid (sweet) varieties of different American lupin species (subgen. *Platycarpus* (S. Wats.) Kurl.).

## EL CULTIVO DE TARWI (*Lupinus mutabilis* Sweet) EN EL ALTIPLANO NORTE DE BOLIVIA: AVANCES DE INVESTIGACIÓN

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En el marco del Proyecto ANDESCROP, ejecutado en el Altiplano Boliviano entre 2010 y 2014, se ha incorporado al tarwi (*Lupinus mutabilis*), como uno de los cultivos andinos subutilizados, y en ese contexto se presentan los avances de las siguientes investigaciones: **“La organización e integración vertical – horizontal de la cadena de valor del tarwi (*Lupinus mutabilis*), una legumbre andina”**. Este trabajo identifica y describe las características y los actores que intervienen en la integración de la cadena vertical y horizontal del tarwi, así mismo identifica sus limitantes y potencialidades. Se condujeron encuestas a 105 agricultores de 22 comunidades del municipio de Carabuco, mismas que se complementaron con entrevistas y talleres participativos con representantes de asociaciones de productores de tarwi. Los resultados muestran el desarrollo de tres tipos de integración en la cadena de valor del tarwi: integración vertical parcial, vertical horizontal y la vertical hacia atrás y adelante. El estudio concluye que el principal desafío para el desarrollo de la cadena se encuentra en gestionar la articulación entre diferentes instituciones como municipios, universidades, organizaciones gubernamentales y no gubernamentales entre otros, a fin de apoyar a los agricultores en la formación de microempresas industrializadoras de éste y otros cultivos andinos, siendo que éstos desconocen las posibilidades y políticas de apoyo al sector productivo de la región. Otra investigación bajo el título: **“Efecto de tres niveles de fertilizante fosfatado en el cultivo de tarwi (*Lupinus mutabilis* Sweet) en el Altiplano Norte de Bolivia”**, tuvo como objetivo evaluar el efecto de la aplicación de diferentes dosis de fosfato de amonio en la productividad del tarwi y la acumulación de fósforo disponible en el suelo. Esto porque diversos estudios mencionan que este cultivo es exigente en fósforo. En el trabajo se empleó un ecotipo de tarwi blanco y dosis de fertilizante de 0, 40, 60 y 80 kg ha<sup>-1</sup> de fosfato de amonio. Los resultados muestran que la mejor dosis de aplicación fue de 60 kg ha<sup>-1</sup>, por mayor altura de planta y rendimiento. El fósforo asimilable fue mayor en las dosis de 60 kg ha<sup>-1</sup> y 80 kg ha<sup>-1</sup>.

## **RESEARCH ADVANCES OF THE PRODUCTIVE CHAIN OF THE LUPINE (*Lupinus mutabilis* Sweet) IN ALLIANCES WITH PRODUCERS AND LOCAL ENTITIES IN THE PERUVIAN NORTHERN HIGHLANDS**

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According to the Ministry of Agriculture and Irrigation of Peru, the harvested area of lupin (*Lupinus mutabilis* Sweet) increased from 9,349 in 2010 to 11,327 hectares in 2016, as well as the production of 10,525 t/ha<sup>-1</sup> in 2010 to 14,281 t/ha<sup>-1</sup> in 2016. The average yield has not changed a lot and it is 1,200 kg/ha<sup>-1</sup>. Ancash is in the 10th place of 12 producing regions and has lower yields than the national average. The Leguminous Program of the UNALM, led by Dr. Felix Camarena and his team, with the participation of the National University Santiago Antúnez de Mayolo of Ancash and the Study Center for Development and Participation-Ancash in the period 2013 to 2018, has evaluated lupin ecotypes for Carhuaz conditions in small farmers' fields and found that some showed good adaptation, yields over 2,500 kg / ha<sup>-1</sup>, with high values of the protein content of 40.1 to 44.5% and oil between 11.2 to 18.6%.

The general objective of our work was to strengthen the technical capacities of stakeholders in the lupin chain of production in Carhuaz, and the specific ones, 1) Identify and characterize ecotypes with high productive capacity, 2) design and optimize the semi-continuous process of debittering of the grain of the lupin, and 3) evaluate grain quality in terms of bioactive compounds to add value to lupin.

The followed methodology was: 1) Evaluation of the lupin ecotypes at the field level with Carhuaz farmers 2) Design of a prototype for the debarking of the grain based on a series of pneumatic components that allow movement actions and semi-continuous operation tests, and 3) Use of an alkaline extraction and isoelectric precipitation method to obtain products for protein isolates.

The results of our work showed that the semi early ecotypes lupin La Molina I and lupin Abelino both of white matte grain exceeded in yield with 2,288 and 2,286 kg/ha<sup>-1</sup> to the local control with 1,539 kg /ha<sup>-1</sup>, both are now in process of diffusion. The prototype designed has three tanks for soaking and cooking grain, screw pumps that allow the transfer of grain from one tank to another and silos where the lupin rests. In the debittering cake of lupin obtained, 66.4% of protein was determined on a wet base and 70.2% on a dry base.

Some general conclusions of our research are that two lupin ecotypes adapted to the area have yields higher than the national average and have values of 40.5 and 44.5 per cent in proteins. The leaching system works with volumes of 100 kg of lupin per batch. In the established extraction parameters of the protein, lupin obtained 91.77% protein solubility.

The research included a thesis of pre-degree in agronomy, two thesis of pre-degree in food industries, one of masters and five of doctorates.



## TARWI PESTS AND ITS MANAGEMENT

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One of the main causes of low tarwi yields, which reaches an average of 555 kg/ha, is the attack of insects, being the main pests the tarwi fly (*Delia platura*) and the tarwi black weevil (*Apion* sp.) because they occur persistently year after year, with high populations, causing economic damage to the crop. There is another group of occasional pests and potential pests that if they occur in large populations, can cause significant economic losses. The increase of these pest populations is associated with climatic factors.

The *D. platura* fly occurs throughout the Altiplano and valley areas where tarwi is cropped, with important adult populations in Spring and Summer, but it is also possible to find it in Autumn and even in Winter. In the Central and Southern Altiplano, it is common to find it in wild lupine species. The larvae feed from the radicle and hypocotyl of tarwi seedlings, the seedlings die before emerging or in their first development stage. The hosts range is wide, being the species of *Lupinus* genus the most sensitive.

The black weevil of tarwi *Apion* sp. is apparently limited for the species of *Lupinus* genus, considering the numerous species of wild lupines in the Altiplano and valleys, where abundant hosts could be found. The most important damage is caused by larvae, which cut off the stems of small plants, causing their wilting and death. The damage is also observed in larger plants; adults place their eggs in the leaves armpits, newly emerged larvae enter the stems to feed on them until causing the plants death. In more advanced stages of the crop, symptoms can be observed in leaves, like small black spots, inside which are larvae and pupae of the insect. When the plague pressure is high, larvae can also be observed attacking the pods, feeding from the grains affecting their quality. When there are suitable conditions for the insect, such as high temperatures, low relative humidity, soils below field capacity, losses can reach up to 80%.

The seed treatment is very important for the control of these pests. For this purpose, the use of insecticides with Imidacloprid, an active substance, is recommended, at a rate of 45 g per 100 kg of seed, providing protection from the attack of these pests in the initial stages of the crop.

These pests should be monitored throughout the crop growing season. In case of attack, treatment with systemic insecticides with Imidacloprid or Thiamethoxam + Lambda-cyhalothrin, at 10 or 40 cc, respectively, is recommended per 20 liters of water. With the combination of seed treatment and foliage treatment, an efficiency control greater than 90% can be achieved in the case of the tarwi black weevil and 60% in the case of the seed fly.

PROINPA is tackling this problem with the searching of bio-controllers to control these pests for reducing the agrochemicals use and to be able to make a friendlier management with the environment.

## LAS PLAGAS DEL TARWI Y SU MANEJO

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El tarwi *Lupinus mutabilis* Sweet es un cultivo de poca importancia de la región andina boliviana, la superficie cultivada al 2013 apenas llega a 1.263 hectáreas. Sin embargo, se considera que presenta un enorme potencial en el nuevo contexto de la gastronomía nacional y mundial, que busca alimentos de alto valor nutritivo. Las principales áreas de cultivo en Bolivia se encuentran en el altiplano norte de La Paz y en los valles inter-andinos de Cochabamba, Chuquisaca y Potosí.

Una de las principales causas de los bajos rendimientos del tarwi, que alcanza a 555 kg/ha, es el ataque de insectos, siendo las principales plagas la mosca del tarwi (*Delia platura*) y el picudo negro del tarwi (*Apion* sp.), porque se presentan en forma persistente año tras año, con altas poblaciones, ocasionando daños económicos al cultivo. Existe otro grupo de plagas ocasionales y plagas potenciales, que si se presentan en grandes poblaciones, pueden causar pérdidas económicas importantes. El incremento de las poblaciones puede estar asociado con factores climáticos.

La mosca *D. platura* se presenta en todo el Altiplano y zonas de valle donde se cultiva tarwi, con importantes poblaciones de adultos en primavera y verano, pero también es posible encontrarla en otoño y aún en invierno. En el Altiplano Central y Sur, es común encontrarla en especies silvestres de lupino. Es atraída por la materia orgánica en descomposición y los suelos húmedos, donde se asientan para ovipositar. Las larvas se alimentan de la radícula y del hipocótilo de las plántulas de tarwi, las plántulas mueren antes de emerger o en su primera etapa de desarrollo. El rango de hospederos es amplio, siendo las especies del género *Lupinus* las más sensibles.

El picudo negro del tarwi *Apion* sp. aparentemente se encuentra limitado a las especies del género *Lupinus*. Considerando las numerosas especies de lupinos silvestres existentes en el altiplano y valles, se esperaría encontrar abundantes hospederos. El daño más importante lo producen las larvas, las cuales barrenan los tallos de las plantas pequeñas, ocasionando su marchitez y muerte. El daño también se observa en plantas más grandes, los adultos colocan sus huevos en las axilas de las hojas, las larvas recién emergidas ingresan a los tallos para alimentarse de ellos hasta causar la muerte de las plantas. En etapas más avanzadas del cultivo, se pueden observar síntomas en las hojas, como pequeñas manchas negras, en cuyo interior se encuentran larvas y pupas del insecto. Cuando la presión de la plaga es alta, también se pueden observar larvas atacando las vainas, alimentándose de los granos afectando su calidad. Cuando existen condiciones adecuadas como temperaturas altas, humedad relativa baja, suelos por debajo de capacidad de campo, las pérdidas pueden llegar hasta un 80%.

Para el control de estas plagas es importante el tratamiento de la semilla antes de la siembra. Para ello se recomienda el uso de insecticidas que tengan como principio activo el Imidacloprid, a razón de 45 g por 100 kg de semilla, con lo que se logra una protección del ataque de estas plagas en las etapas iniciales del cultivo. Estas plagas deben ser monitoreadas durante todo el periodo vegetativo del cultivo, en caso de que ocurra el ataque, se recomienda el tratamiento con insecticidas sistémicos que tengan como ingrediente activo Imidacloprid o Tiametoxam + Lambdacihalotrina, a razón de 10 g o 40 cc, respectivamente, por 20 l de agua. Con la combinación de tratamiento a la semilla y tratamiento en follaje, se puede lograr una eficiencia de control mayor al 90% en el caso del picudo negro del tarwi y un 60 % en el caso de la mosca de la semilla.

PROINPA está abordando este problema con la búsqueda de controladores biológicos para controlar estas plagas para reducir el uso de agroquímicos y poder realizar una gestión más amigable con el medio ambiente.

## PERFORMANCE EVALUATION OF TWO LUPIN SPECIES IN THE PAMPEAN REGION OF ARGENTINA

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Up to now, in Argentina there are no extensive crops of any of the *Lupinus* species, except experimental plots and small areas of bitter *L. albus* L. (white lupin), intended for local consumption in the form of pickles and medicinal grain. Through a research and technology transfer project of the Center for Survey and Evaluation of Agricultural and Natural Resources (CREAN), the optimal cultivation areas for white lupin were determined, but the agricultural areas that would allow the introduction into the country of the cultivation of *L. angustifolius* L. (narrow-leafed lupin) are still unknown. As part of a food development project that promotes the sustainable use of resources from a systemic point of view (ecological, social and economic), we are working to integrate two species of *Lupinus* – sweet narrow-leafed lupin and sweet white lupin- into the Argentine market, with the mission of producing food for human consumption based on these proteoleaginous species. In order to achieve this goal, our enterprise was vertically integrated into the production chain, from the field to the consumer, offering industrial products such as flours, lupin flakes and other derived products. The area of grain production is located in the southeast of Buenos Aires province, Argentina, considered as a zone with optimal conditions for the cultivation of lupins. In this place, during the 2018/19 season, we planted 8.5 ha of sweet narrow-leafed lupin, Lila Baer variety, and 0.1 ha of sweet white lupin, Rumbo Baer variety, as an experimental trial. The objective was to evaluate the productivity of the area for the production of these legume species, using a production method without the use of agrochemicals. Both varieties came from the establishment "Baer semillas", from Temuco, Chile. Planting was carried out on August 25, with a fine seed planter and three different planting densities of 140, 150 and 160 Kg / ha, and the seeding design was with two different inter-row spacing (17.5 and 35 cm). During the first evaluation campaign, it was not possible to grow under optimal conditions for various reasons, such as very late planting, underachievement of inoculation, and a totally external factor, as the herbicide drift of an application on a nearby sunflower plot. This last factor affected the lupin by killing 30% of the plant stock, and stopped the vegetative growth of the rest, damaging them in the competition with the spring weeds, which suffocated the crop produced without agrochemicals. In spite of all this, the results of the project were very positive from the point of view of the experience that allows an adequacy of the management of species never planted before. Although the narrow-leafed lupin grain yields were very low (300 Kg / ha), it is considered that the cultivation of this species has an interesting productive potential if the sowing conditions, the weed control and the environmental factor are optimized with respect to the management of other crops in the area. The results of the 2018/19 campaign allowed us to consider with enthusiasm that the cultivation of the two lupin species tested in the southeast of the province of Buenos Aires, Argentina, has a productive sustenance, and the lupins can emerge as a new alternative for agrifood development in the region.

## EVALUACIÓN DEL CULTIVO DE DOS ESPECIES DE LUPINOS DULCES EN LA REGIÓN PAMPEANA, ARGENTINA

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En Argentina hasta el momento no existen cultivos extensivos de ninguna de las especies del género *Lupinus*, salvo parcelas experimentales y pequeñas superficies de *L. albus* L. (lupino blanco) amargo, destinadas al consumo local en forma de pickles y grano medicinal. A través de un proyecto de investigación y transferencia de tecnología del Centro de Relevamiento y Evaluación de Recursos Agrícolas y Naturales, se determinaron las zonas de cultivo óptimas para el lupino blanco dulce, pero aún se desconocen las áreas agrícolas que permitirían la introducción en el país del cultivo de *L. angustifolius* L. (lupino de hoja angosta). Como parte de un emprendimiento de desarrollo de alimentos que promuevan el uso sustentable de los recursos desde un punto de vista sistémico (ecológico, social y económico), se está trabajando en la inserción en el mercado argentino de dos especies de *Lupinus* (lupino de hoja angosta dulce y blanco dulce), con la misión de producir alimentos de consumo humano a base de esas especies proteoleaginosas. Para poder lograr este objetivo, el emprendimiento se integró verticalmente en la cadena de producción, desde el campo hasta el consumidor, ofreciendo en diversos puntos de venta productos industriales tales como harinas, pre-mezclas y otros productos derivados. El área de producción de grano está localizada en el sudeste de la provincia de Buenos Aires, Argentina, considerada como la zona del país con óptimas condiciones para el cultivo de lupinos. En este lugar durante la campaña 2018/19 se cultivaron 8,5 ha de lupino dulce de hoja angosta variedad Lila Baer, y en forma de ensayo experimental 0,1 ha de lupino blanco variedad Rumbo Baer. El objetivo fue evaluar la productividad de la zona para la producción de esas especies de legumbre, utilizando un método de producción sin uso de agroquímicos. Ambas variedades provenían del establecimiento "Semillas Baer" de Temuco, Chile. La siembra se realizó el 25-08 con una sembradora de grano fino y tres densidades de siembra 140, 150 y 160 Kg/ha, y el diseño de sembrado fue con dos espaciamientos entre líneas diferentes (17,5 y 35 cm). Durante la primer campaña de evaluación no se pudo cultivar bajo óptimas condiciones por diversas causas, tales como, siembra muy tardía, bajo logro de inoculación, y un factor totalmente externo como fue la deriva de una aplicación de herbicida sobre un lote de girasol alledaño, que afectó al lupino matando parte del plantel de plantas (30%), y detuvo el crecimiento vegetativo de las restantes, perjudicándolas en la competencia con las malezas primaverales, que sofocaron al cultivo producido sin agroquímicos. A pesar de todo esto, los resultados del emprendimiento fueron muy positivos desde el punto de vista de la experiencia que permite una adecuación del manejo de estas especies nunca experimentadas anteriormente. Si bien los rendimientos en grano de lupino de hoja angosta fueron muy bajos (300 Kg/ha), se considera que el cultivo de esta especie tiene un potencial productivo interesante si se optimizan las condiciones de siembra, el control de malezas y el factor ambiental con respecto al manejo de otros cultivos de la zona. Los resultados de la campaña 2018/19 nos permitieron considerar con entusiasmo que el cultivo de las dos especies de lupinos ensayadas en el sudeste de la provincia de Buenos Aires Argentina, tiene un sustento productivo, y que los lupinos pueden surgir como nuevas alternativas de desarrollos agroalimenticios en la región.

## EVALUATION OF TARWI (*Lupinus mutabilis* Sweet) ADAPTABILITY TO CULTIVATION UNDER MEDITERRANEAN CLIMATE CONDITIONS

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Tarwi (*Lupinus mutabilis* Sweet) has been domesticated in the Andean region and cultivated for many centuries. This crop is regarded in Europe as a potential source of oil and protein that may reduce the regional dependency on the import of soybean from other continents. However, the cropping conditions in the Andean region, at high altitude and low latitude, are substantially different from the European conditions, requiring evaluation of the crop cycle and selection of suitable genotypes. Diverse Old World lupin species have been grown throughout Europe, either Autumn- or Spring-sown, depending on the region, and knowledge acquired provide a background for the study of this adaptation. The main difficulty found under central and northern European conditions was related to the indeterminate growth of the plants, preventing synchronized crop maturation in the absence of summer drought. Under Mediterranean conditions, the Autumn-sown crop completes its cycle imposed by the summer drought, mimicking the cropping cycle of native lupin species. This work reports the evaluation of the adaptability of tarwi to cultivation under Mediterranean climate conditions, in Portugal, analyzing a germplasm collection under diverse parameters, including agronomical, morphological, phytopathological and biological variables. Experimental plots show variable productivity (0.26 to 6.0 t/ha), suggesting that it should be possible to obtain lines with productivity levels high enough to make of this species a non-irrigated autumn-sown legume crop. Seed oil content is lower than under Andean conditions, presumably due to the lack of cold nights during seed filling and maturation, but is higher than under central European conditions. Resistance to anthracnose (caused by local *Colletotrichum lupini* isolates) is generally low, but some variability was found, suggesting potential sources of partial resistance/tolerance. Molecular markers and cytogenomic data corroborate low levels of genetic diversity within this germplasm collection, evidencing the domesticated nature of the crop, although morphological and agronomical parameters reveal high variability and plasticity, namely on cycle length (91 to 116 mean days to 1<sup>st</sup> flowering time), plant architecture (a V branching type) and yield components (TSW between 8.66 to 20.89 g/Acc. with a total seed number between 300 to 744/Acc./m<sup>2</sup>). The main challenges ahead for making tarwi an alternative crop for Mediterranean conditions include identifying sources of cold/frost resistance, to increase yield and seed oil content, to increase disease resistance and to optimize the plant architecture in order to maximize pod and seed set without compromising productive flexibility. The establishment of tarwi as a crop in Mediterranean countries will certainly contribute to make this crop better known internationally, enabling both Mediterranean and Andean growers to reach new markets, namely those eager for healthy food, namely from crops of South American origin. This project has received funding from the Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme under grant agreement No 720726.

## PHENOTYPIC EVALUATION AND PRELIMINARY YIELD OF LUPIN ECOTYPE (*Lupinus mutabilis* Sweet) GROWN IN CARHUAZ, ANCASH

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In Peru, Andean crops grown at altitudes above of 3,200 meters, one of them is the *Lupinus mutabilis* Sweet that is know as “tarhui or tauri” in the south, “tarwi or lupin” in the central zone and “chocho” in the northern zone of Perú. The producers of these three regions incorporate *L. mutabilis* en their rotation, production systems and they have been keeping it to this day. The mainly varieties cultivated in each region are local ecotypes in this sense the objective of the present research was to characterize the ecotypes that come from the production areas at a very late time and at lower levels of typical agroecosystem and to evaluate the preliminary yield of the ecotypes under conditions of Marcará located at the coordinates of 9 ° 16'59" South latitude and 7777 ° 36'00" West longitude, at 2726 meters above sea level.

The genetic material evaluated belongs to 30 lupin ecotypes cultivated in the highland in the Allpa Rumi farm of the Santiago Antúnez de Mayolo National University in Huaraz. The characterization was done using the descriptors of *Lupinus* (IBPGR, 1981). The yield trials of the lupin ecotypes from the center and the south region were sowing under the randomized complete block design with variable number of treatments and with three repetitions. Twenty-six qualitative and 16 quantitative descriptors were evaluated and their main morpho-agronomic components were taking under consideration for grain yield. When characterizing lupin ecotypes, high standard deviation was found for plant height, yield, length of main inflorescence, pods in lateral inflorescences, weight of grain of the main inflorescence, pods/main inflorescence, days to flowering and branches/plant. The yield, grain weight of the lateral inflorescences, lateral pods/inflorescences and plant height showed a high coefficient of variation. Shrub growth, stem not prominent, branching, glabrous stem and green color, green and elliptical leaflets, flowers with wings of mostly blue color, pods with central and short beak, grains of primary white color are predominant in the study. Grain yield was higher for ecotypes of the center region with yields of 3711; 3329 and 2974 kg/ha for Moteado beige, H6 INIA and Yanamucló PLGO with weights of 100 seeds of 26.6, 23.0 and 22.3 grams respectively; while southern ecotypes such as 03-10-214, Lupine Cuzco I and Apurímac I reached yields of 2341, 1864 and 1216 kg/ha and for the weight of 100 seeds the values are 17.4, 21.6 and 23.5 g respectively.

According to our results we concluded that all lupin ecotypes had shrub growth and the stem were not prominent with branching and without pubescence, green elliptical leaflets. The ecotypes Moteado beige, H6 INIA, Yanamucló PLGO, Yanamucló 008-1, White compound, 03-10-214 and Lupin Cuzco I, all were outstanding in performance. Lupin cultivation at low altitudes (2700 masl) with gravity irrigation at alternative times produced acceptable yields.

## EVALUACIÓN FENOTÍPICA Y PRUEBA PRELIMINAR DE RENDIMIENTO DE LOS ECOTIPOS DE TARWI (*Lupinus mutabilis* Sweet) CULTIVADOS EN CARHUAZ, ANCASH

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En el Perú los cultivos andinos se siembran en altitudes mayores a los 3,200 metros y entre ellos se encuentra el *Lupinus mutabilis* denominado como tarhui ó tauri en el sur, el tarwi en la zona central y el chocho en la zona norte del Perú. Los productores de las tres regiones lo incorporan en sus sistemas de producción en rotación y son los que han conservado hasta la actualidad. Las variedades cultivadas mayormente en cada región son ecotipos locales. En el presente estudio se tuvo por objetivo caracterizar los ecotipos provenientes de las zonas de producción en época muy tardía y a menores altitudes de su agroecosistema típico y evaluar el rendimiento preliminar de los ecotipos en condiciones de Marcará ubicado en las coordenadas de 9 ° 16'59 " LS y 7777 ° 36'00 " LO, a 2726 metros sobre el nivel del mar.

El material genético evaluado fue 30 ecotipos de tarwi cultivados en la sierra en el Fundo Allpa Rumi de la Universidad Nacional Santiago Antúnez de Mayolo de Huaraz. La caracterización se hizo utilizando los descriptores de *Lupinus* (IBPGR, 1981). Los ensayos de rendimiento de los ecotipos de tarwi del centro y del sur se sembraron bajo el diseño estadístico de bloques completo al azar con número variable de tratamientos y con tres repeticiones. Se evaluaron 26 descriptores cualitativos y 16 cuantitativos y para el rendimiento de grano se evaluaron sus principales componentes morfoagronómicos.

Al realizar la caracterización de los ecotipos de tarwi se encontró alta desviación estándar para altura de planta, rendimiento, longitud de la inflorescencia principal, vainas en las inflorescencias laterales, peso de grano de la inflorescencia principal, vainas/inflorescencia principal, días a floración y ramas/planta. El rendimiento, peso de granos de las inflorescencias laterales, vainas/inflorescencias laterales y altura de planta presentaron un alto coeficiente de variación. El crecimiento arbustivo, tallo no prominente, ramificación, tallo glabro y de color verde, folíolos verdes y elípticos, flores con alas de color mayormente azul, vainas con pico central y corto, granos de color primario blanco son predominantes en el estudio. El rendimiento de grano fue superior para ecotipos de la región del centro con rendimientos de 3711 3329 y 2974 kg/ha para Moteado beige, H6 INIA y Yanamucló PLGO con pesos de 100 semillas de 26,6, 23,0 y 22,3 gramos respectivamente; mientras que los ecotipos de la región sur como 03-10-214, Tarwi Cuzco I y Apurímac I alcanzaron rendimientos de 2341; 1864 y 1216 kg/ha y para el peso de 100 semillas 17,4; 21,6 y 23,5 g, respectivamente.

A manera de conclusiones vemos que todos los ecotipos de tarwi presentaron plantas de crecimiento arbustivo y tallo bajo con ramificación y sin pubescencia, y folíolos elípticos verdes. Los ecotipos Moteado beige, H6 INIA, Yanamucló PLGO, Yanamucló 008-1, Compuesto blanco, 03-10-214 y Tarwi Cuzco I tienen rendimientos aceptables. La siembra de tarwi a altitudes de 2700 msnm, bajo riego por gravedad y en periodo seco es factible.

# ***Lupinus mutabilis* GROWTH, SEED YIELD AND BIOLOGICAL NITROGEN FIXATION ABILITY UNDER DIFFERENT RHIZOBIA INOCULATION TREATMENTS IN COMPARISON TO OTHER LUPIN SPECIES**

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Lupins as grain legumes can offer ecosystem services, improve soil fertility and contribute to cropping systems diversification. Their ability to grow on soils which are unsuitable for other crops and their high potential as alternative protein source in animal feeding characteristics is the reason why nowadays there is a renewed interest in this species. However, a few studies have investigated biological nitrogen fixation ability under Mediterranean farming systems and edapho-climatic conditions. Therefore, in the present study, we aimed to study growth, seed yield and biological nitrogen fixation (BNF) ability of *Lupinus mutabilis* accessions in comparison to two native lupin species, with and without applying inoculation (*Bradyrhizobium lupini*). The plant material used included three accessions of *L. mutabilis* (LIB220, LIB221, LIB222), a commercial variety of *L. albus* cv. Multitalia and a commercial variety of *L. angustifolius* cv. Polo. A commercial variety of faba bean cv. Aguadulce and a commercial variety of durum wheat cv. Normano were also used as references of biological nitrogen fixation. The experimental layout followed was that of a split plot design, with main factor the rhizobia inoculation treatments while the varieties and accessions used sowed in a randomized complete block design (RCBD) with three replications. The experimental field (38°22'42", 23°33'72", 480 m a.s.l.) was characterized by a Clay Loam (CL) soil texture with 0.02% CaCO<sub>3</sub> content and a pH value of 7.7. Measurements were obtained from ten central plants in each experimental plot. The measurements taken were regarding plant growth such as plant height (cm), seed yield traits like seed weight per plant and number of seeds per plant, the nodules efficiency (visual observation) as well as their BNF ability by using N-balance method. Statistical analysis of variance was performed using a Generalized Linear Model (GLM). During the experiment, we noticed that in the non-inoculated plots, nodules were found in the lupin roots, indicating the presence of an indigenous rhizobia strain. The results showed that there was no interaction between the different treatments with regard to the majority of lupin plant growth traits. *L. angustifolius* cv. Polo and *L. albus* cv. Multitalia presented higher percentages of active-efficient nodules, that characterized visually by a red inside color, and resulted up to 19.6 and 17.2% while 33.2% and 30.7% for non-rhizobia and rhizobia treatments, respectively for the two species. Andean lupin accessions presented lower numbers of effective nodules with LIB220 to present under both treatments the less ones. Among *L. mutabilis* accessions, LIB222 fixed more nitrogen (59.81 kg ha<sup>-1</sup>) under inoculation, while among the non-inoculated plants was LIB221 that fixed more nitrogen (31.65 kg ha<sup>-1</sup>). In general, cv. Polo and cv. Multitalia fixed more nitrogen than *L. mutabilis* accessions. Rhizobia inoculation affected significantly and positively N<sub>2</sub>-fixation amounts of LIB222 and cv. Multitalia but not the other lupin varieties/accessions used. The results showed that *L. mutabilis* accessions have an N<sub>2</sub> fixation capacity under Mediterranean conditions and alkaline soils, while inoculation treatment with *Bradyrhizobium lupini* strain was not effective in enhance N<sub>2</sub>-fixation of *L. mutabilis* accessions except in the case of LIB222, indicated a native strain competitiveness. Further research is therefore needed to find effective lupin x rhizobia interactions under Mediterranean conditions.

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# INCORPORATION EFFECT OF TARWI (*Lupinus mutabilis* Sweet) CROP RESIDUES IN CROP SYSTEMS AND SOIL RECOVERY OF SEMI-ARID ANDEAN REGIONS OF COCHABAMBA

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The production systems of the semi-arid Andean regions of Bolivia are becoming increasingly vulnerable due to the productive pressures exerted by farmers on their soils and climate change. Crop management practices, such as tillage, crop rotation, incorporation of crop residues, can modify soil organic matter (SOM). In crop rotation, legumes play an important role in SOM, due to their effect on the C/N ratio. The value of lupin is recognized as N fixer and P solubilizer in the soil. The objective of the research was to determine the incorporation effect of tarwi crop residues, in the traditional crops rotation system and in soils recovery of semi-arid Andean regions of Bolivia.

During the 2014-2015 and 2015-2016 agricultural seasons, the study was carried out in the semi-arid Andean region of the Municipality of Anzaldo, Cochabamba. Trials were implemented to see the incorporation effect of tarwi crop residues into the soil on potato and quinoa crops. The design of Parcels Divided in Strips, in Complete Blocks at Random with three repetitions was used. The treatments were: T1 = With tarwi, without incorporation, without guano; T2 = With tarwi, without incorporation, with guano; T3 = With tarwi, with incorporation, without guano; T4 = With tarwi, with incorporation, with guano; T5 = Without tarwi and without guano and T6 = Without tarwi and with guano. Yield was evaluated, as well soil respiration (with Vernier LabQuest 2 equipment), SOM, N, P and K soil. The incorporation was made with stick plow and yoke of oxen.

The results show that there is a favorable effect on potato and quinoa development and yield due to the incorporation of tarwi crop residues into the soil, mainly when accompanied by guano. Soils, where these residues were incorporated, showed greater respiration, suggesting that the organic matter generated achieves a greater biological activity of the soils. On the other hand, soils where tarwi was cultivated, improved the productivity of potatoes and quinoa with respect to the soil where it was not cultivated, an aspect that shows the favorable effect of tarwi in the rotation system, even if its stubble is not incorporated into the soil. The chemical soils analysis also showed that residues incorporation managed to increase their content in SOM, N, P and K. Farmers of the Andean semi-arid regions do not know the benefits of tarwi residues incorporation; due to their woody condition, some farmers use it as firewood.

The study showed that, under these conditions, a time of 7 to 8 months was sufficient to decompose tarwi residues, which was estimated through increased crop yield, soil respiration, improved SOM and the nutrients release in favor of crops in the rotation system. The incorporation of tarwi stubble into the soil improved the productivity of the potato and quinoa crops, improved soil respiration, suggesting a higher microbial activity and increased of nutrients release in favor of crops in the rotation system.

## **EFFECTO DE LA INCORPORACIÓN DE RESIDUOS DE COSECHA DE TARWI (*Lupinus mutabilis* Sweet) EN EL SISTEMA DE CULTIVOS Y LA RECUPERACIÓN DE SUELOS DE REGIONES ANDINAS SEMIÁRIDAS DE COCHABAMBA**

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Los sistemas de producción de las regiones andinas semiáridas de Bolivia se tornan cada vez más vulnerables debido a las presiones productivas que ejercen los agricultores sobre sus suelos y al cambio climático. Las prácticas de manejo del cultivo, tales como la labranza, rotación de cultivos, incorporación de residuos de cosecha, pueden modificar la materia orgánica del suelo (MOS). En la rotación de cultivos, las leguminosas juegan un papel importante en la MOS, debido a su efecto sobre la relación C/N. Es reconocido el valor de los lupinus como fijador de N y solubilizador de P en el suelo. El objetivo de la investigación fue determinar el efecto de la incorporación de los residuos de cosecha de tarwi, en el sistema de rotación tradicional de cultivos y en la recuperación de los suelos de regiones andinas semiáridas de Bolivia.

El estudio se llevó a cabo en la región andina semiárida del municipio de Anzaldo, Cochabamba, durante las campañas 2014-15 y 2015-16. Se implantaron ensayos para ver el efecto de la incorporación al suelo de residuos de cosecha tarwi, sobre los cultivos de papa y quinua. Se utilizó el diseño de Parcelas Divididas en Franjas, en Bloques Completos al Azar con tres repeticiones. Los tratamientos fueron: T1 = Con tarwi, sin incorporación, sin guano; T2 = Con tarwi, sin incorporación, con guano; T3 = Con tarwi, con incorporación, sin guano; T4 = Con tarwi, con incorporación, con guano; T5 = Sin tarwi y sin guano y T6 = Sin tarwi y con guano. Se evaluó rendimiento, respiración del suelo (con el equipo Vernier LabQuest 2), MOS, N, P y K del suelo. La incorporación se realizó con arado de palo y yunta de bueyes.

Los resultados muestran que existe un efecto favorable en el desarrollo y rendimiento papa y quinua por la incorporación al suelo de los residuos de cosecha tarwi, principalmente cuando se acompaña con guano. Los suelos donde se incorporó estos residuos presentaron mayor respiración lo que sugiere que la materia orgánica generada logra una mayor actividad biológica de los suelos. Por otra parte, los suelos donde se cultivó tarwi mejoraron la productividad de papa y quinua respecto a los suelos donde no se cultivó, aspecto que muestra el efecto favorable del tarwi en el sistema de rotación, aún si su rastrojo no es incorporado al suelo. El análisis químico de los suelos también mostró que la incorporación de los residuos logró incrementar su contenido en MOS, N, P y K. Los agricultores de las regiones andinas semiáridas no conocen las bondades de incorporación de los residuos de tarwi, por su estado leñoso algunos buscan usarlo como leña.

El estudio demostró que, bajo estas condiciones, un tiempo de 7 a 8 meses fue suficiente para lograr descomponer los residuos de tarwi lo que fue estimado a través del incremento del rendimiento de los cultivos, la respiración del suelo, la mejora de la MOS y la liberación de nutrientes a favor de los cultivos en el sistema de rotación. La incorporación de rastrojo de tarwi al suelo, mejoró la productividad de los cultivos papa y quinua, mejoró la respiración del suelo lo que sugiere una mayor actividad microbiana e incrementó la liberación de nutrientes a favor de los cultivos en el sistema de rotación.

## EL CHOCHO (*Lupinus mutabilis* Sweet) EN ECUADOR: ESTRATEGIA DEL INIAP PARA EL FOMENTO DE LA PRODUCCIÓN Y EL CONSUMO

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Ecuador es el país de la Zona Andina con más alto consumo de chocho y entre otros factores, responde a la estrategia de investigación y desarrollo implementada por el INIAP alrededor de este rubro. Hasta finales de la década de los 90, el cultivo del chocho en Ecuador era marginal, tanto en la producción como en el consumo y actualmente es demandado en todos los estratos sociales y cada vez hay más interés en la producción y uso de este importante alimento andino. La estrategia del fomento de la producción y consumo del INIAP con enfoque de investigación participativa, incluye acciones para recuperar y conservar la variabilidad genética, selección de variedades precoces con alto potencial de rendimiento, desarrollo de alternativas tecnológicas para el manejo agronómico, cosecha, poscosecha, fomento de sistemas mixtos de producción de semilla, mejoramiento del proceso de desamargado, investigación del valor nutricional y usos en la alimentación y en la agroindustria; y promoción de su valor agronómico, nutricional y agroindustrial, a través de diferentes medios. Los principales resultados de los últimos 20 años de trabajo del INIAP se pueden resumir en el mantenimiento y caracterización de la colección de germoplasma de *Lupinus*, liberación de dos variedades precoces (INIAP 450 Andino es la más sembrada actualmente en el país), difusión y adopción de recomendaciones para el manejo del cultivo (época y densidad de siembra, labores culturales, fertilización, cosecha oportuna), manejo integrado de plagas y fomento de la producción y uso de semilla de buena calidad; lo cual se refleja en el incremento de la productividad (700 a 1000 kg/ha en zonas de intervención, frente a los 300 kg/ha reportados en el 2002). Por otro lado, como resultado de las actividades relacionadas con el mejoramiento del proceso de desamargado, concientización del valor nutricional y alimentario del grano de chocho y desarrollo de alternativas agroindustriales, se ha incrementado el consumo per-cápita a nivel nacional (principalmente en la región Sierra), implementándose iniciativas empresariales en los diferentes eslabones de la agrocadena del chocho, beneficiando a los productores (precios estables en el mercado), a los consumidores y a todos los involucrados en la cadena del chocho. El chocho sigue siendo un alimento popular, pero ha conquistado las mesas de todos los estratos sociales e inclusive es parte ya de la cocina gourmet. A pesar de los avances, aún persisten varios desafíos relacionados con la caracterización de los bancos de germoplasma (diversidad genética, contenidos nutricionales y funcionales), fitomejoramiento (uniformidad para la cosecha, resistencia a plagas, contenido de alcaloides), mejoramiento de la producción (amplia brecha entre los rendimientos actuales y el rendimiento potencial; alternativas para el manejo agroecológico del cultivo; mecanización), optimización del proceso de eliminación de alcaloides del grano (cantidad de agua, tiempo del proceso), generación de valor agregado, etc.

## EFFECTS OF PRECIPITATION ON THE PHENOLOGICAL AND PHENOMETRIC VARIABLES OF WHITE LUPINE (*Lupinus albus* L.) IN SERBIA

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The white lupine (*Lupinus albus* L.) is a native species of the European Mediterranean region and it is cultivated since antiquity as a green manure, fodder and food grain. Currently, commercial varieties with no alkaloids are cultivated in cold temperate regions and slightly acid soils. Plant breeding involves exchange of germplasm for disease resistance, higher yields, and adaptations to different environments. A cooperation agreement between the Institute of Field and Vegetable Crops" (IFVC) of Serbia and the Center for Surveying of Agriculture and Natural Resources (CREAN-CONICET/UNC) of Argentina was executed to conduct a joint research on germplasm of *Lupinus*. This presentation examines the relationship between precipitation and phenological and phenometric responses of three accessions of germplasm of white lupine grown in Serbia. The goal was to identify areas with similar rainfall conditions, which will allow the introduction of germplasm in Argentina. The trials were conducted in the experimental field of the IFVC (45° 15' N, 19° 49' E, 80 masl), in a Chernosoil soil with pH of 7.4-7.9; N=0.196%; CaCO<sub>3</sub>=5.60%; P<sub>2</sub>O<sub>5</sub> 17.99 mg/100 g; K<sub>2</sub>O=21mg/100g and humus 2.97%. The monthly average temperature (March to July) is 15°C and average precipitation accumulated for that period is 299 mm. Sowings started early in March for the period 2007-2016, in plots of 5 m<sup>2</sup>, with a density of 75-85 seeds/m<sup>2</sup>, and with three replications. The crop cycle was completed between mid and late July. The germplasm accessions were identified as: Bac (from Poland), BG002553 (from Portugal) and Vesnia (from Serbia). The averages of the following variables were obtained from 10 plants selected at random: length of main branches (cm), plant height (cm), number of fruits and grains counting separately from the main and secondary inflorescences, total dry weight of the plant (g) and grain yield for the entire plot (kg/ha). The mean and the standard deviation were obtained for the variables. The results showed that the germplasm have specific genetic characteristics for plant height, architecture and the quantity of grain. The average plant height and the average length of the main branches showed the following results: BAC=71±1.22 cm and 42±1.40 cm; BG-002553=118±1.84 cm and 102±1.95 cm; Vesnia=114±1.1 cm and 113±2.56 cm. The plant height and the lengths of the main branches are different in each germplasm; however, these characters remain more or less constant over the years in spite of precipitation variability, this means that weather conditions during the growing season had little influence on vegetative characters. Regarding plant total dry weight, the BAC variety has smaller size but greater dry weight with an average of 40.65±5.65 g, while BG-002553 and Vesnia came despite 27.63±1.53 g and 30.80±2.08 g, respectively. Grain yields for BAC germplasm was 7311±620 kg/ha, which is the higher average yield per plot responding to rainfall variability; it is followed by BG-002553 with 5168±517 kg/ha and finally Vesnia with only 3880±388 kg/ha which has the lower grain yield and it had the least response to variations in rainfall occurred during the period of crop development. It is evident that the three accessions of germplasm have different behaviors to environmental conditions, possibly since each germplasm is being improved for different uses, such as Vesnia for forage, BAC for grain production and BG-002553 for dual purpose. The authors are grateful to the Institute of Field and Vegetable Crops (IFVC) of Serbia and the Center for Surveying of Agriculture and Natural Resources (CREAN-CONICET/UNC) of Argentina for the support of this research.

## EFECTO DE LA PRECIPITACIÓN EN LAS VARIABLES FENOLÓGICAS Y FENOMÉTRICAS DE LUPINO BLANCO (*Lupinus albus* L.) EN SERBIA

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El lupino blanco (*Lupinus albus* L.) es una especie originaria de la Subregión Mediterránea Europea que se cultiva desde la antigüedad como abono verde, forraje y grano alimenticio. Actualmente variedades comerciales sin alcaloides son cultivadas en regiones, templado frías y suelos ligeramente ácidos. El mejoramiento vegetal contempla intercambio de germoplasma para lograr resistencia a enfermedades, mayores rendimientos y adaptaciones a diversos ambientes. Por un convenio de cooperación entre el “Institute of Field and Vegetable Crops” (IFVC) de Serbia y el Centro de Relevamiento y Evaluación de Recursos Agrícolas y Naturales (CREAN-CONICET/UNC), se realizan investigaciones conjuntas sobre germoplasma de *Lupinus*. Esta presentación analiza la precipitación y la respuesta fenológica y fenométrica de tres accesiones de germoplasma de lupino blanco (*L. albus* L.) cultivadas en Serbia, para identificar zonas homoclimáticas que permitan su introducción en Argentina. Los ensayos se llevaron a cabo en el campo experimental del IFVC (45° 15' N, 19° 49' E; 80 msnm), en un suelo es Chernosoil con pH 7.41-7.9, N=0.196%; CaCO<sub>3</sub>=5.60%; P<sub>2</sub>O<sub>5</sub> 17.99 mg/100 g; K<sub>2</sub>O=21mg/100g y humus 2.97%. La temperatura media mensual (marzo a julio) es de 15°C y la precipitación media acumulada para ese período es de 299 mm. Las siembras se realizaron a principio de marzo de 2007 al 2016, en parcelas de 5 m<sup>2</sup>, con una densidad de 75-85 semillas/m<sup>2</sup>, y con tres repeticiones y el ciclo de cultivo finalizó entre mediados y fines de julio. Las accesiones de germoplasma utilizadas se identificaron como: Bac (Polonia) BG 002553 (Portugal); y Vesnia (Serbia). Se obtuvieron los promedios de 10 plantas al azar de las siguientes variables: altura de planta (cm); longitud de las ramas principales (cm); número de frutos y de granos, en forma separada los de la inflorescencia principal y las secundarias; peso seco total de la planta y rendimiento en granos de toda la parcela (kg/ha). Se determinaron las medias y las desviaciones estándares de cada una de las variables consideradas. Los resultados muestran que los germoplasmas tienen características genéticas que los identifican, por la altura y arquitectura de la planta y por la cantidad de granos producido. El promedio de la altura de la planta y el promedio del largo de las ramas principales mostró los siguientes datos: BAC=71±1.22cm y 42.5±1.40cm; BG-002553=118±1.84cm y 102±1.95cm; Vesnia = 114±1,1cm y 113±2,56 cm. Como se puede observar la altura de las plantas y los largos de las ramas principales son diferentes en cada germoplasmas, y esas características se mantienen más o menos constante a lo largo de los años a pesar de que han tenido distintos valores de precipitación, de manera que las condiciones meteorológicas durante el período de cultivo no tienen influencia en estos caracteres vegetativos. Con respecto al peso seco total de la planta (g) en el período de máximo desarrollo vegetativo podemos ver que la variedad BAC si bien tiene menor porte es la que posee mayor peso seco con un promedio de 40.65±5.65 g, mientras que BG-002553 y Vesnia llegaron a pesar 27.63±1.53 g y 30.80±2.08 g respectivamente. En relación a los rendimientos en granos, se registró un mayor promedio de granos por parcela en el germoplasma BAC con 7311±620 kg/ha, que respondió mejor acorde con la variabilidad pluviométrica; seguido por BG-002553 con 5168±517 kg/ha y por último Vesnia con solo 3880±388 kg/ha que fue la que registra un menor rendimiento en granos y además fue la que menos respuesta tuvo a las variaciones de lluvias ocurridas durante el período de desarrollo del cultivo. Se pone en evidencia que las tres accesiones de germoplasma tienen distintos comportamientos a las condiciones ambientales, posiblemente debido a que cada germoplasma fue mejorado para distintos usos, como por ejemplo Vesnia para forraje, BAC para producción de granos y BG-002553 para doble propósito. Los autores agradecen al Institute of Field and Vegetable Crops (IFVC) de Serbia y al Centro de Relevamiento y Evaluación de Recursos Agrícolas y Naturales por el apoyo a esta investigación.

## SELECCIÓN DE CULTIVARES NACIONALES DE TARWI (*Lupinus mutabilis* Sweet) POR RENDIMIENTO, PRECOCIDAD, CONTENIDO DE ACEITE Y PROTEÍNA EN PUNO, PERÚ

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El tauri, tarwi o chocho, reviste enorme importancia en el Perú por ser alimento cotidiano de la población andina y por ser una Fabáceas que fija nitrógeno atmosférico, es apreciado en la rotación de cultivos ya que incorpora al suelo hasta 200 kg/ha, así como por su alto contenido de proteínas y aceites; sin embargo tiene largo periodo vegetativo, susceptible a las heladas en las primeras etapas del desarrollo y de mediana productividad, por ello se ha sometido a evaluación 14 cultivares procedentes de diferentes condiciones agroclimáticas del Perú, con el objetivo de seleccionar aquellos que tengan menor periodo vegetativo, mayor rendimiento, que resistan a las heladas en sus primeras etapas fenológicas y que tengan alto contenido de aceite y proteína, aunque en el altiplano peruano solo se espera producción del eje central y en el mejor de los casos ramas primarias. Los cultivares utilizados fueron: 1. Yunguyo (Puno), 2. Cholo fuerte (Huaraz), 3. Huancayo-6 (Junín), 4. SCG-22 (Cusco), 5. Alta gracia (La libertad), 6. Andenes-80 (Cusco), 7. Patón grande (Otuzco-La libertad), 8. SLP1- Puno (Puno), 9. UNCP (Junín), 10. Seccelambra (Ayacucho), 11. Sacatacani (Puno), 12. Vilquechico (Puno), 13. Yunguyo 1 (Puno), 14. Yunguyo 2 (Puno), los cuales se sembraron en el CIP, Camacani de la UNA-Puno, Perú a 3850 msnm, en la campaña agrícola 2017/2018, en un diseño de bloques completos al azar con cuatro repeticiones. Se han evaluado los siguientes caracteres: Altura de planta, N° de ramas primarias, N° de vainas por eje central, longitud de vaina, rendimiento de grano por eje central, diámetro de grano, peso de 100 granos, rendimiento de ejes primarios, adaptabilidad, días a floración, días a madurez fisiológica, color de grano, así como efectuado análisis químico de composición proximal con énfasis en proteína, grasas y carbohidratos, en el ICTA- Argentina de la Universidad Nacional de Córdoba. Los resultados indican que los cultivares: Vilquechico(Puno), Sacatani (Puno) y SLP1 (Puno), son los de mayor rendimiento en grano con: 4,363, 4,073 y 3951 kg/ha respectivamente; los cultivares SCG-22 (Cusco), Vilquechico (Puno) y Yunguyo 1 (Puno), son los más precoces con: 221, 225 y 235 días de período vegetativo y los cultivares: Yunguyo 2 (Puno), Andenes-80 (Cusco) y Huancayo 6 (Junín), tiene el mayor contenido de proteína con 48 % y los cultivares: Vilquechico (Puno), Patón grande (Otuzco-La libertad) y SLP-1 (Puno), con mayor contenido de aceite: 23.5, 21.5 y 20.2 % respectivamente. Por lo que se concluye que los cultivares: Vilquechico (Puno), con rendimiento de grano de 4,363 kg/ha, 225 días de periodo vegetativo, 23.5% de aceite y 45 % de proteína y el cultivar Sacacatani (Puno) con 4,073 kg/ha, 241 días de periodo vegetativo, 46 % de proteína y 16% de aceite, son los más recomendados para la zona altiplánica de Perú y que podrían tener el mismo comportamiento en zonas mucho más benignas.

## LIMITATIONS AND POTENTIAL FOR ANDEAN LUPIN (*Lupinus mutabilis*) IN BOLIVIA: A CASE STUDY FROM ANZALDO MUNICIPALITY

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*Lupinus mutabilis* Sweet, or Andean Lupin, is a legume native to the Andes, known in Bolivia as *tarwi*. Its use has declined to the point of becoming an underused crop. However, it has the potential to become an important source of plant protein in areas with poor nutrition. It is particularly significant given its high protein content (up to 51%) and its ability to grow in the harsh climates of the Andes. The study was undertaken in 2017 in collaboration with Fundación PROINPA in Cochabamba. It investigates the limitations to production of Andean Lupin in Bolivia and the potential it has to achieve the aims of improving nutrition, reducing poverty and thus improving livelihoods. This study uses a mixed methods approach that includes: key informant interviews and participatory observation; farmer questionnaires with *tarwi*-producers and non-*tarwi* producers; consumer questionnaires; and participatory rural appraisal (PRA) methods, including seasonal calendars, crop classifications and production problem rankings. The study sites were five small villages near to the town of Anzaldo, 60 km south of Cochabamba. Production in this area is dominated by 3 crops: wheat, potato and maize. Farmers rank *tarwi* as their 4<sup>th</sup> most important crop. However, yields are very low: the average yield is 19kg, which is only 1.7 times the quantity sown. The majority of *tarwi* is auto-consumed. The principal barriers for production are drought and hail; farmers rank *tarwi* as having high water requirements compared to other crops. Biotic factors, including insects and disease, entail a cost for treatment that is out of reach of most farmers. Consumption of *tarwi* is almost entirely in the form of *mote*, soaked and boiled seeds of *tarwi* eaten cold as a snack, favoured by 72% of consumers in the sample. *Tarwi* is consumed regularly, (at least monthly) by 87% of respondents, although in small quantities. Due to the high levels of alkaloids in *tarwi*, it must be washed thoroughly in order to become palatable. However there is limited water available for post-harvest processing. This limits the processing of *tarwi*, along with the limited awareness of its uses as food. Commercially, *tarwi* is limited by the lack of a stable market and fixed prices, and by the very small production quantities. *Tarwi* production can be improved with research into drought tolerance and insects, and by capitalizing on farmers' existing competences. Some farmers select seeds, use crop rotations or intercrop to prevent insects and disease. Farmer knowledge sharing can allow other farmers to benefit from these practices. It is important to encourage production of a wider range of crops to spread risk. *Tarwi* can be promoted for its soil-improving properties, and practices such as green manure can be encouraged. Consumption is largely dependent on production, as of those who consume *tarwi*, 82% are *tarwi* producers. Therefore it is important to encourage local consumption, in order to provide a market creating stable demand for producers. Water for processing is an issue that cannot be resolved internally. However, the development of sweet varieties of *tarwi* would prevent this problem, as well as reducing the labour required to produce *tarwi*. Furthermore, development of a narrow leafed *tarwi* could reduce hail damage. *Tarwi* is often endorsed as a crop that can withstand difficult climate conditions and that will produce even in poor soil. However, these farmers have found that *tarwi* is not a reliable crop and is susceptible to many problems. These limitations should be considered when planning new policy and projects. *Tarwi* can instead be promoted through a consumption-led approach, within the context of increasing agrobiodiversity, reducing poverty and improving nutrition.

## DISEASES AFFECTING TARWI (*Lupinus mutabilis* Sweet) CROP IN BOLIVIA

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Tarwi (*Lupinus mutabilis* Sweet) is a legume that is grown in different regions of Bolivia, on a small scale. Because of its capacity to fix nitrogen, it is an alternative to improve soils fertility, in those areas with low organic matter and low fertility. Like other crops, it is not exempt from the attack of fungal, bacterial and viral diseases, which, depending on environmental conditions, reduce the population of plants, from emergence, during development until harvest.

Due to its economic importance, the objectives of the present study were to identify, characterize and categorize the diseases affecting tarwi crop from sowing to harvesting in the different eco-regions of Bolivia.

We worked from samples collected from farmers' plots (7 to 10 plots per eco-region) at different crop stages. Samples, with different symptoms and/or signs were taken to the laboratory for processing and identification of the causal agent of the disease by using routine procedures (observation under a microscope, sowing in culture medium, etc.). Three to four field visits were made: one, during the emergency; two, during the development and one, before the harvest.

After the samples were processed, it was determined that during seeds germination, *damping off* (*Rhizoctonia solani*, *Fusarium* sp., and/or *Pythium* sp.) occurs as a consequence of the borers, cutters attack and flies damaging the root system, facilitating the entry of the fungi mentioned above. During the development of the plants until flowering, "anthracnose" occurs, a disease caused by *Colletotrichum gloesporioides*, affecting leaves, stems and pods. It annuls the main apex causing the emission of lateral shoots; therefore, it reduces the yield and seed quality. When sowing diseased seed, plants are affected from the emergency. For this reason, it is important the seed treatment before or during planting. Other diseases detected in the development phase were *Cercospora* sp, *Ascochyta* sp, *Sclerotinia esclerotiorum*, *Uromyces lupini*, *Peronospora trifoliorum*, *Pleiochaeta setosa* and *Phoma* sp. These are considered as diseases of secondary importance, although due to climate change, some of them can cause serious damage to the plant if proper management is not carried out. Another problem that has been observed in the development phase are symptoms caused by viral diseases that, due to the low incidence (less than 5%), have not yet been identified. Bacterial disease has not been observed.

Based on the proposed objectives, it is concluded that in Bolivia, diseases affecting tarwi crop are: *damping off*, anthracnose, rust, cercosporiosis, oidium, brown spot, ojival spot and virosis. The most economically important disease in tarwi cultivation is "anthracnose", caused by *Colletotrichum gloesporioides*. It occurs in all the producing areas of the country and is disseminated through the seed. The diseases of secondary importance are: rust, brown spot, ojival spot and *damping off*. Rust, like anthracnose, is a disease common to all growing regions, although it does not severely affect yield, but affects photosynthesis causing leaf fall when the severity is high. *Pleiochaeta setosa* and *Phoma* sp. are diseases requiring high relative humidity. The first one, causes necrosis and wilting of leaves and branches and the second one, the rupture of stems.



## ENFERMEDADES QUE AFECTAN AL CULTIVO DEL TARWI (*Lupinus mutabilis* Sweet) EN BOLIVIA

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El tarwi (*Lupinus mutabilis* Sweet) es una leguminosa que se cultiva en diferentes regiones de Bolivia en poca escala. Por su capacidad de fijar nitrógeno, es una alternativa para mejorar la fertilidad de los suelos, en aquellas zonas con poca materia orgánica y baja fertilidad. Al igual que otros cultivos no está exento del ataque de enfermedades fungosas, bacterianas y virósicas, que dependiendo de las condiciones ambientales reducen la población de plantas, desde la emergencia, durante el desarrollo y hasta la cosecha.

Los objetivos del presente estudio fueron identificar, caracterizar y categorizar por su importancia económica las enfermedades que afectan al cultivo de tarwi desde la siembra hasta la cosecha en las distintas eco-regiones de Bolivia.

Se trabajó a partir de muestras colectadas de parcelas de agricultores (7 a 10 parcelas por eco-región) en diferentes estadios del cultivo. Las muestras con diferentes síntomas y/o signos fueron llevadas al laboratorio para su procesamiento e identificación del agente causal de la enfermedad utilizando procedimientos de rutina (observación al microscopio, siembra en medio de cultivo, etc.). Se realizaron 3 a 4 visitas en campo: una durante la emergencia, dos durante el desarrollo y una antes de la cosecha

Después de procesadas las muestras, se ha determinado que durante la germinación de las plantas se presenta el *damping off* (*Rhizoctonia solani*, *Fusarium* sp. y/o *Pythium* sp.) como consecuencia del ataque de barrenadores, trozadores y moscas que dañan el sistema radicular, facilitando el ingreso de los hongos antes mencionados. Durante el desarrollo de las plantas hasta la floración se presenta “la antracnosis”, enfermedad ocasionada por *Colletotrichum gloeosporioides*, que afecta hojas, tallos y vainas. Anula el ápice principal ocasionando la emisión de brotes laterales, por tanto baja el rendimiento y la calidad de semilla. Cuando se siembra semilla enferma las plantas se ven afectadas desde la emergencia, por esta razón es importante el tratamiento de la semilla antes o durante la siembra. Otras enfermedades detectadas en la fase de desarrollo fueron *Cercospora* sp., *Ascochyta* sp., *Sclerotinia esclerotiorum*, *Uromyces lupini*, *Peronospora trifoliorum*, *Pleiochaeta setosa* y *Phoma* sp. Estas son consideradas como enfermedades de importancia secundaria, aunque por efecto del cambio climático, algunas pueden ocasionar serios daños en la planta si no se realiza el manejo apropiado. Otro problema que se ha observado en la fase de desarrollo son síntomas ocasionados por virosis que debido a la baja incidencia (menor al 5%) aún no han sido identificados. No se ha observado ninguna enfermedad bacteriana.

En base a los objetivos propuestos se concluye que en Bolivia, las enfermedades que afectan al cultivo del tarwi son: *damping off*, antracnosis, roya, cercosporiosis, oidium, mancha café, mancha ojival y virosis. La enfermedad económicamente más importante en el cultivo del tarwi es la “antracnosis”, ocasionada por *Colletotrichum gloeosporioides*, se presenta en todas las zonas productoras del país y se disemina mediante la semilla. Las enfermedades de importancia secundaria son: roya, mancha café, mancha ojival y *damping off*. La roya al igual que la antracnosis, es una enfermedad común a todas las regiones de cultivo, aunque no afecta severamente el rendimiento, afecta la fotosíntesis y ocasiona la caída de hojas cuando la severidad es alta. *Pleiochaeta setosa* y *Phoma* sp. son enfermedades que requieren de alta humedad relativa, la primera ocasiona necrosis y marchitez de hojas y ramas y la segunda la ruptura de los tallos.

## POTENTIAL FOR LUPINS IN RELATION TO OTHER GRAIN LEGUMES AS A SOURCE OF PLANT PROTEIN FOR HUMAN CONSUMPTION

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Due to the growing world population, the limitations of the world food production are becoming an increasing concern. There is a growing consensus on the need for the gradual transition of diets primarily based on animal protein to diets based on plant protein. At the moment a lot of the new plant protein foods are based on soy as this is the most extensively grown grain legume of the world. Many food producers are looking for alternatives for soy, powered by consumer concerns related to transgenic soy and large-scale deforestation in the Amazon. A growing part of food producers also want to incorporate more regionally produced ingredients as part of their social responsibility strategy. This increases the possibilities for locally produced grain legumes.

In a 5 year European project, Protein-2-Food, the most promising grain legumes in The Netherlands were investigated as a potential ingredient for protein rich food items, both for processed food items like meat replacers and plant based dairy products, but also as a whole grain food item. Narrow leafed and white lupins were extensively investigated.

Over four years (2015-2018) field trials have been conducted mainly on sandy soils in the northern parts of The Netherlands. The lupins that were included in these trails were *Lupinus angustifolius* (11 varieties and 11 breeding lines) and *Lupinus albus* (10 varieties and 11 breeding lines) and the results were compared to faba beans (both winter and summer) and soya beans. Field trial plots of approximately 3 x 10 m were used in a completely randomized design with three replicates. . Samples were analysed for protein contents (N-Kjeldahl) and alkaloids (GC-MS). The effect of several agronomic interventions was measured. Sowing density trials were conducted with two white and two narrow-leafed lupin varieties using 50%, 75%, 100% and 125% of the normal sowing density. The effect of fungicides on yield was tested in a split-plot design with three replicates.

Over the years both narrow leafed lupins and white lupins showed large variations in production ranging from a complete loss of production due to early infection of anthracnosis (*Colletotrichum lupini*) to production levels of 3.6 tonnes/ha. Overall both total grain production and protein production per hectare seemed to be slightly lower or similar to soy and significantly lower than faba beans, making it a more expensive source of protein than especially faba beans. Sowing density had no significant effect on lupin yields in most years, but the use of fungicides did increase average yields by 13% in 2015 and 18% in 2017 although the yield increase varied largely between varieties.

Although both narrow-leafed and white lupins form an interesting source of plant protein for human consumption, it is not the most productive one for The Netherlands. The large varietal differences in yields over the years still suggest a large potential for both total yield and protein yield improvement. In the meantime specific distinguishing qualities of lupins or their proteins need to be highlighted in finding a suitable market.

## INCREASING THE OPPORTUNITIES OF CULTIVATION AND PROCESSING OF ANDEAN LUPIN ON MARGINAL LANDS IN EUROPE

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Andean lupins (*Lupinus mutabilis*) have drawn the attention of researchers all over the world as the beans of this new world lupin have the rare quality of combining high protein contents (40-50%) with a substantial oil content (15-20%) and a complex carbohydrate fraction that is known for its probiotic effects. The main area of cultivation has always been the Andean region, where the growing of this type of lupin has been part of the agricultural tradition for many centuries. The lupins are grown in small-scale farming systems dominated by small fields and hand-labour. The crop can grow to substantial lengths and produce flowers and pods in several levels that ripen sequentially. The long period of flowering and sequential ripening in levels of branching make the crop less adapted to modern, mechanised agriculture. Although efforts have been made to understand these processes, determined growth varieties are not yet available, hampering substantial expansion of the area of cultivation outside the region of origin. Global increasing demand for plant proteins, both for food and feed, has motivated the European Union to invest in European grown plant protein which also re-entered Andean lupins.

In the 4 year European project, LIBBIO (*L*upinus *m*utabilis for *I*ncreased *B*iomass from marginal lands and value for *B*IOrefineries), we aim to develop Andean lupin varieties (*Lupinus mutabilis*) adapted to European farming conditions. These new varieties will provide food, feed and non-food products by applying bio-refinery cascading principles. The project aims to increase crop yield and harvest index and accelerate supply chain development via a consumer-driven approach for developing high value-added food and non-food products by applying state-of-the-art solvent-free technology for raw material processing.

The project is set-up in four work packages targeted at improvements in different parts of the production chain.

1. Crop improvement with the use of modern breeding (non-GM) tools for developing better adapted varieties with a determined growth type and low alkaloid levels.
2. Improved agronomic strategies to ensure more stable and higher yields focussing on the opportunities of this crop on marginal lands. Summer cropping is used in northern Europe and winter cropping in southern Europe. Both seed production and biomass production is considered.
3. Development of semi-industrial scale solvent-free extraction methods for lupin oils, proteins and alkaloids
4. Development of several new food, feed and non-food applications for the main components of the seeds: oil (food: mayonnaise, margarine, non-food: cosmetics, paints and coatings), protein (food: protein rich drinks and yoghurts, non-food: anti-ageing cosmetics, protein films, thickening agents and gelling agents), alkaloids (non-food: biobased insecticides) and other components (prebiotic rich food, lecithins for food and cosmetics). Additionally the use of green biomass for feed or lignocellulose or bioenergy (biogas) applications is looked into.

A fifth work package is looking at the viability and sustainability of this production chain.

## AGROECOLOGICAL CONDITIONS OF THE ARGENTINE PAMPAS FOR NARROW LEAF LUPIN CROPPING

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In Argentina soybean crop strengthened its leadership thanks to extensive research and experimentation for more than 50 years. Agro-climatic studies led to an optimum use of natural resources -weather and soil- and made it possible to find the appropriate ecological niche for a new crop. The first recognition of the lupin crop potential within an innovative agricultural frame in Argentina was made in 1987. The agro-economic analyses proposed lupin as a profitable alternative for winter cropping. The objective of this work was to determine the agro-ecological conditions of the Argentine Pampas adequate for narrow leaf lupin (*Lupinus angustifolios* L.) cropping with the goal of identifying suitable agricultural areas and making recommendations on appropriate varieties for each area. Meteorological data used were obtained from a network of weather stations located in the provinces of Buenos Aires, Córdoba, Entre Ríos, La Pampa and Santa Fe for 1980-2015. In each sites, the potential evapotranspiration (ETP) was calculated using Penman's method and the actual evapotranspiration (ETR) was estimated using a soil moisture balance. Monthly hydrologic deficiency was defined as the difference between ETP and ETR. The deficiencies were accumulated for the crop cycle (April-November) for each year of the series. The mean deficiency was calculated and used for drawing the lines of 50, 100 and 150 mm on a map of the region. A minimum threshold of 450 chilling hours was established to satisfy vernalisation requirements. Regional chilling hours were extracted from agroclimatic maps and the photoperiod duration was obtained from agro meteorological tables. Growing degrees started for the 5°C bio-period during the crop cycle. The soil characteristics were obtained from a soil atlas. Loam, silt-loam and sandy-loam texture were extracted as soils apt for the crop, and soils with other textures were grouped as non-apt soils. Digitalization of hydrologic deficiency and its cartographic representation were carried out and the overlay of cartographic information of meteorological and soil variables was completed. Thermo-hydrologic crop requirements, as defined by the plant breeder, were obtained from the scientific literature as well as the phenologic and phenometric responses of selected varieties to agro-meteorological conditions. Growing degree-days during crop cycle fluctuated from 2000 degrees-days in the South of the region up to 3300 degrees-days in the North. According to the variety response and the crop cycle total completion, it was determined that both the thermal condition and the photoperiod duration are adequate over the entire region. An overlay graph was constructed with climatic and soil information layers relevant to the agro-ecological zoning of lupin. Cartographic representation of hydrologic deficiency delimited those areas where lupin can grow under non-irrigated conditions and with eventual, partial or full irrigation. The final map was obtained by overlaying agro-climatic (hydrologic deficiencies and chilling hours) and soil texture information. The map shows eight regions to establish an adequate crop management in relation to variety and sowing time characteristics. According to these hydrologic thresholds, non-irrigated lupin crop is possible in all the territory of the provinces of Buenos Aires and Entre Ríos, in the Centre-South of Santa Fe, Southeast of Córdoba and a narrow strip toward the East of La Pampa. This region of non-irrigated crop is subdivided into two sub-regions: A Western, with a deficiency between 50 and 100 mm, and an Eastern, with deficiencies lower than 50 mm. The 450 chilling hour line indicates the North boundary of early sowings with varieties of the fall-winter cycle. In zones located between 800 and 1,500 chilling hours, vernalisation requirements of all varieties are completely satisfied. To the North of the 450 hours line, only winter-spring varieties can be cultivated.

# VALIDACIÓN Y DIFUSIÓN DE ALTERNATIVAS DE MANEJO DEL CULTIVO DE CHOCHO (*Lupinus mutabilis* Sweet) EN LOCALIDADES DE LAS PROVINCIAS CHIMBORAZO Y COTOPAXI, ECUADOR

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El cultivo del chocho (*L. mutabilis*), única especie del género *Lupinus* originaria y domesticada en los Andes, con alto valor agronómico (adaptación a condiciones marginales de clima y suelo) y nutricional (contenido de proteína, grasa, minerales, compuestos funcionales). Ecuador es el país de la zona Andina con mayor consumo de esta leguminosa y con un área sembrada de aproximadamente 8000 ha, mayormente cultivado por productores de la agricultura familiar, principalmente de las provincias Cotopaxi y Chimborazo, ubicadas en la Sierra Centro del país. En la provincia Cotopaxi, el cantón Sigchos lidera la producción del chocho y en Chimborazo, en la zona de la parroquia Ilapo, cantón Guano también es importante este cultivo. En las dos zonas, la productividad es baja (600 y 200 kg/ha, respectivamente) como consecuencia de la escasa tecnificación del cultivo y por las condiciones agroecológicas marginales. Para atender la creciente demanda del grano de chocho a nivel nacional, es importante incrementar la producción y la productividad, a través de la validación y difusión de las alternativas tecnológicas actualmente disponibles, como variedades, épocas, densidad y métodos de siembra, fertilización, manejo de plagas. El objetivo del presente trabajo es validar y difundir la tecnología del INIAP (variedad INIAP 450 Andino, densidad de siembra, fertilización y poda), en localidades del cantón Sigchos de la provincia Cotopaxi y del cantón Guano en la provincia Chimborazo. En el cantón Guano, el ensayo de validación incluyó la variedad INIAP 450 Andino, densidad de siembra y fertilización; comparada con la tecnología local; y en Sigchos, se validó dos fuentes de fertilizantes y la poda del eje central, frente a los testigos (sin fertilización y poda). En las dos zonas se trabajó en dos localidades, con tres repeticiones por localidad en un Diseño de Bloques Completos al Azar. Se registró datos de variables fenológicas (días a la floración y a la cosecha), altura de planta, número de ramas por planta y rendimiento en grano, con cuyos datos se realizó análisis de varianza y separación de medias con la prueba DMS de Fisher. De manera general, los resultados muestran que con fertilización al menos se duplica la productividad y también se gana en rendimiento con la menor densidad de siembra. En Sigchos, no se observó ningún efecto de la poda en todas las variables. En Ilapo, la variedad local presentó el rendimiento más alto y el mayor número de ramas por planta, pero el ciclo de cultivo duró dos meses más que la variedad del INIAP. Ahora, en las dos zonas se debe difundir las mejores prácticas, pero en función de las condiciones edafoclimáticas y sociales de una determinada localidad; pues, las tecnologías no deben ser consideradas como parte de un paquete, sino como opciones para los diferentes tipos de productores.

## STRATEGIES FOR THE INTEGRATED MANAGEMENT OF 'CHOCHO' (*Lupinus mutabilis* Sweet) PESTS IN SOCIO-ECOLOGICAL LANDSCAPES

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In the Ecuadorian highlands, there is a diverse entomofauna associated with the cultivation of lupin. Within this diverse entomofauna, there are at least five orders of insects (Coleoptera, Diptera, Lepidoptera, Hemiptera and Thysanoptera) that would be causing damage to the lupin. The LEGUMIP project, centered in the provinces of Cotopaxi and Chimborazo (Ecuador), is financed by the McKnight Foundation. The project aims to answer the following questions: 1. What are the pests of the lupin, its biology, its ecology? 2. What are the alternative solutions for the control of lupin pests? 3. How does the abiotic, biotic, and social landscape influence the level of pests? 4. How do typology, associations and farmers influence pest control? The following are the most important results of five studies that provide information to answer these questions: 1) Participatory insect monitoring with farmers: a tool for knowledge of the entomofauna of lupin (*Lupinus mutabilis*) in Ecuador. During one year, a participatory monitoring of the lupin pests was carried out. This monitoring has aroused much interest from farmers and their families. It made them reflect on the idea that not all insects are pests and that many can help their cultivation. The recognition of "good" insects is an additional argument for not using pesticides indiscriminately and conserving patches of natural habitats in their plots. 2) A meticulous analysis of hundreds of plants of lupin allowed to describe a new pest not registered in Ecuador, a coleopteran Cucurlionidae that attacks the crops in the upper part of Cotopaxi and in Chimborazo. The two other major pests are two Diptera, but they are not the same species in Cotopaxi (minor borer, Agromyzidae) and in Chimborazo (apex borer, Anthomyiidae). Based on this information, the first records of an entomological catalog of lupin cultivation (which will also include beneficial insects) are generated. 3) Pest control and pollination at the landscape level. Preliminary results suggest a counterintuitive relationship between landscape structure and pest control since landscapes with more complex forms are associated with fields of lupin more attacked by herbivores. Another important result at the landscape level: although the lupin has always been considered as a self-pollinated plant (autogamous), we show that the lupine plants pollinated by insects produce about 20% more of the grains than the plants that only make autogamy. It is important to have an Integrated Pest Management of lupine that integrates the conservation of pollinators at the landscape level. 4) Perception of the landscape and ecosystem services by farmers cultivating lupin. Participatory activities with some 60 farmers from several provinces indicated a large difference in landscape perception between indigenous and non-indigenous farmers. Non-indigenous farmers prefer diverse and less intensified landscapes, while indigenous farmers, mostly prefer little different landscapes. In general, very few farmers associate pest control as an ecosystem service that nature could provide, for example, natural predators. 5) Technical surveys provided information to understand how differences in social organization (e.g., between Cotopaxi and Chimborazo) affect the collaboration between farmers during the cultivation of lupin. Several important factors were identified (sociological, geographical, historical and psychological) to assess the barriers to cooperation for IPM within a community.

## ESTRATEGIAS PARA MANEJO INTEGRADO DE PLAGAS DE CHOCHO (*Lupinus mutabilis* Sweet) EN PAISAJES SOCIO-ECOLÓGICOS

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En la sierra ecuatoriana, existe una diversa entomofauna asociada al cultivo del chocho. Dentro de esta diversa entomofauna, se encuentran por lo menos cinco órdenes de insectos (Coleopteros, Dipteros, Lepidopteros, Hemipteros y Thysanoptera) que estarían causando daños al chocho. El proyecto LEGUMIP, centrado en las provincias de Cotopaxi y Chimborazo (Ecuador), es financiado por la Fundación Mcknight tiene como objetivo contestar a las siguientes preguntas: 1. Cuáles son las plagas del chocho, su biología, su ecología?; 2. Cuáles son las soluciones alternativas para el control de las plagas de chocho?; 3. Cómo el paisaje abiótico, biótico, y social influye en el nivel de plagas?; 4. Cómo influyen tipología, asociaciones y agricultores en el control de plagas? A continuación se presentan los resultados más importantes de 5 estudios que provén información para contestar estas preguntas: 1. El monitoreo participativo de insectos con agricultores: una herramienta para el conocimiento de la entomofauna de chocho (*Lupinus mutabilis*) en el Ecuador. Durante un año, se realizó un monitoreo participativo de las plagas del chocho. Este monitoreo ha despertado mucho interés por parte de los agricultores y sus familias. Les hizo reflexionar sobre la idea que no todos los insectos son plagas y que muchos pueden ayudar a su cultivo. El reconocimiento de los insectos "buenos" es un argumento adicional para no usar pesticidas de manera indiscriminada y conservar parches de hábitats naturales en sus parcelas. 2. Un análisis minucioso de centenas de plantas de chocho permitió describir una nueva plaga no registrada en el Ecuador, un coleóptero curculionido que ataca a los cultivos en la parte alta de Cotopaxi y en Chimborazo. Las dos otras plagas principales son dos dípteros, pero no son las mismas especies en Cotopaxi (barrenador menor, Agromyzidae) y en Chimborazo (barrenador del ápice, Anthomyiidae). En base a esta información, se genera las primeras fichas de un catálogo entomológico del cultivo de chocho (que incluirá también insectos benéficos). 3. Control de plagas y polinización a nivel del paisaje. Resultados preliminares sugieren una relación contra-intuitiva entre estructura paisaje y control de plagas ya que los paisajes con formas más complejas están asociados a campos de chocho más atacados por los herbívoros. Otro resultado importante a nivel del paisaje: aunque el chocho ha sido siempre considerado como una planta autógena (autofecundación) demostramos que las plantas de chocho polinizadas por insectos producen unos 20% más de granos que las plantas que solo hacen autogamia. Es importante tener una gestión MIP de chocho que integre la conservación de los polinizadores a nivel del paisaje. 4. Percepción del paisaje y servicios ecosistémicos por agricultores cultivando chocho. Actividades participativas con unos 60 agricultores de varias provincias indicó una gran diferencia de percepción del paisaje entre los agricultores indígenas y no-indígenas. Los agricultores no-indígenas prefieren paisajes diversos y poco intensificados mientras que los agricultores indígenas prefieren en su mayoría paisajes poco diversos. Por lo general, muy pocos agricultores relacionan el control de plaga como un servicio ecosistémico que podría proveer la naturaleza, por ejemplo, a través de depredadores naturales. 5. Encuestas técnicas proporcionaron información para entender como las diferencias de organización social (p. ej. entre Cotopaxi y Chimborazo) afectan la colaboración entre agricultores durante el cultivo de chocho. Se identificaron varios factores (sociológicos, geográficos, históricos y psicológicos) importantes para evaluar las barreras a la cooperación para el MIP dentro de una comunidad.

## EVALUATION OF TWO LUPINUS SPECIES IN DIFFERENT ENVIRONMENTS IN VALLEYS AND HIGHLANDS OF BOLIVIA

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During the agricultural campaign 2016-2018, in five municipalities of Cochabamba were evaluated two varieties of lupines of two different species. The objective was to evaluate the performance of the varieties 'Victor' (*Lupinus albus*) and 'Lila' (*Lupinus angustifolius*) in different Bolivian areas. In the first year were implemented 16 plots of Lila and 8 of Victor, while in the second year 10 plots of Victor and 9 of Lila. Sowing was carried out in farmers' plots. For performance evaluation samples were harvested by plot and by variety. In addition, the genotype x environment interaction was analyzed considering the variable yield (kg/ha). The variance analysis and means comparison were developed under a completely randomized blocks design with sub-sampling, where blocks were represented by locals. Also was preformed farmers participatory evaluations during pods formation. Performance analysis shows that in all the places *L. albus* species was higher in yield than *L. angustifolius*. The highest yield was obtained in Colomi for the two species, likewise, *L. angustifolius* had a different performance in each local, while *L. albus* seeks for better environments conditions to show its potential. According to the analysis of genotype x environment, *L. angustifolius* shows a stable performance in all assessed areas, despite the little performance difference between the two species; However, stability was not consistent due to the variation in the data from year to year. By other hand, *L. albus* has better yields in favorable environments, while in unfavourable environments has lower yields than the *L. angustifolius*. The participatory evaluation results, shows that women highlight the value of both lupins as food since they are not bitter and have large seeds as *L. albus*, and tarwi who know. They also highlight the fodder use with the stubble of these crops. Men highlighted the value in the soil improvement and in productive capacity of *L. albus*. Also highlight *L. angustifolius* precocity which allow escape to drought especially in semi-arid areas like Anzaldo.



## EVALUACIÓN DE DOS ESPECIES DE LUPINUS EN DIFERENTES AMBIENTES DE LAS ZONAS DE VALLES Y ALTOANDINAS DE BOLIVIA

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Durante la campaña agrícola 2016-2018, en cinco municipios de Cochabamba fueron evaluadas dos variedades de lupinus de dos especies diferentes. El objetivo fue evaluar el rendimiento de las variedades Victor (*Lupinus albus*) y Lila (*Lupinus angustifolius*) en diferentes zonas de Bolivia. En el primer año se implementaron 16 parcelas de la variedad Lila y 8 de Victor mientras en el segundo año 10 parcelas de Victor y 9 de Lila. La siembra se realizó en parcelas de agricultores y en franjas. Para la determinación de rendimiento se cosecharon muestras por parcela y por variedad. Asimismo, se analizó la interacción genotipo x ambiente considerando la variable rendimiento (kg/ha). El análisis de varianza y la comparación de medias se realizaron bajo un diseño de bloques completamente al azar con sub-muestreo, donde los bloques representaron las localidades. También se realizaron evaluaciones participativas con agricultores durante la formación de vainas. El análisis de rendimiento muestra que en todas las localidades, la especie *L. albus* fue superior en rendimiento que *L. angustifolius*. El mayor rendimiento se obtuvo en Colomi para las dos especies, asimismo, *L. angustifolius* tuvo un rendimiento diferente en cada localidad, mientras *L. albus* necesita ambientes con mejores características agroclimáticas para mostrar su potencial de rendimiento. De acuerdo al análisis genotipo x ambiente, *L. angustifolius* muestra un comportamiento estable en todas las localidades evaluadas, a pesar de la poca diferencia de rendimiento entre las dos especies; sin embargo, la estabilidad no fue consistente debido a la variación en los datos de año en año. Por otro lado, *L. albus* tiene rendimientos superiores en ambientes favorables, contrariamente en ambientes desfavorables, la especie tiene rendimientos inferiores a la de *L. angustifolius*. Los resultados de la evaluación participativa, muestra que las mujeres destacan el valor de ambos lupinus por su uso como alimento ya que no son amargos y de grano grande como el *L. albus*, y muy parecido al tarwi que conocen. También destacan el uso forrajero que podría tener el rastrojo de estos cultivos. Los hombres destacan el valor de estos cultivos en la mejora de los suelos y la capacidad productiva de *L. albus*. También destacan la precocidad de *L. angustifolius* que le permitió escapar a la sequía especialmente en zonas semiáridas como es el caso de Anzaldo.

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