EUCARPIA 2023

10TH Eucarpia Leafy Vegetable Conference

August 28 - 31, 2023

Utrecht, the Netherlands

BOOK OF ABSTRACTS

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Welcome

Dear colleagues,

On behalf of The European Association for Research on Plant Breeding (EUCARPIA) and Utrecht University, host and organizor of this conference, we welcome you to the EUCARPIA Leafy Vegetable Conference. The conference is held from 28 to 31 August 2023 in Utrecht, the Netherlands. This event is part of a series of conferences organized by the European Association for Research on Plant Breeding (EUCARPIA), and welcomes the participation of researchers, practitioners, administrators, professionals, and their organizations.

This conference provides a platform for scientists, practical plant breeders, and professionals to engage in thought-provoking discussions. You will have the opportunity to explore the latest research and developments in leafy vegetable quality, covering topics such as genetic resources, breeding, production, and postharvest handling. The program aims to address general and specific problems in plant breeding and genetic research and explore future challenges and innovations. As a result, you will be able to exchange specialized knowledge and methodologies among industry experts. Ultimately, the conference aims to initiate international cooperation, facilitate knowledge transfer, and promote scientific and technical co-operation in the field of plant breeding to support its continued development.

We wish you an interesting, engaging conference with plenty of inspiration!

The Organizing Committee

Sponsors

We would very much like to thank all our sponsors for their contribution to the Eucarpia 2023 conference:

SPONSORS PLATINUM

- Gautier Semences
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- Enza Zaden (*also sponsor of the conference reception)*
- Takii

Sponsors

We would very much like to thank all our sponsors for their contribution to the Eucarpia 2023 conference:

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- Pop Vriend Seeds
- Syngenta

SPONSOR IN KIND

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Committees

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- Werner Most, Utrecht University
- Johan Schut, Rijk Zwaan
- Monique van Vegchel, Plantum
- Hester Pruiksma, Utrecht University (conference assistant)

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- Katherine Denby, University of York
- Anneke Horstman, Wageningen University & Research
- Dmitry Lapin, Utrecht University
- Aleš Lebeda, Palacky University, Czech Republic
- Ilja Roobeek, Enza Zaden

General information

Venue

The 10th EUCARPIA Leafy Vegetable International Conference will take place at the Utrecht University, Science Park Utrecht, in the Victor J. Koningsberger building, Budapestlaan 4a-b, 3584 CD in Utrecht. (See the map on the next page).

Registration

You can find the registration desk on the ground floor of the V.J. Koningsberger building, next to the entrance. The registration desk will be open on Monday 28 August from 9:00 to 16:00. During the conference, there will be a person from the organisation to whom you can address your questions. Participants arriving after Monday can find instructions and the name and number of the contact person at the registration desk.

Oral lectures

The lectures will be held in room Cosmos of the V.J. Koningsberger building on the 1st floor. Please contact Werner Most at the registration desk or in the Cosmos Lecture Hall for uploading the presentation file on the laptop in time (if possible, the day before your presentation). Please contact the session moderator before the beginning of your session. Please note, that the total time of presentations include the technical starting and the discussion.

Poster Sessions

Posters will be displayed on the first floor, in the hall of the Minnaert building. This building can be reached from the 1st floor of the V.J. Koningsbergergebouw. You will find the display number of your poster in the abstractbook and at the poster wall. Please fix your poster on the numbered display before lunchtime, Monday, 28th of August. Posters should be on display until Thursday. Authors are expected to be present at their poster during the respective poster sessions. Please remove your poster Wednesday 30th of August, end of the afternoon at 15:30.

Coffee breaks and lunches

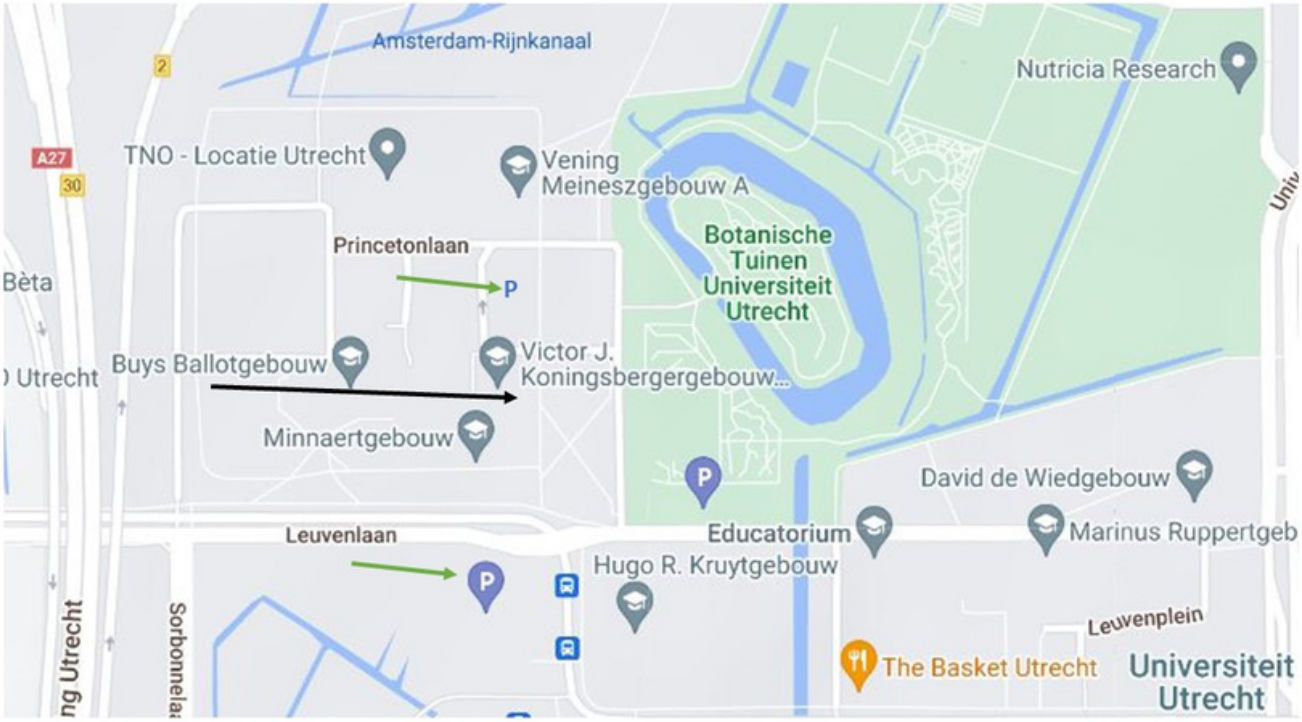
Between Monday and Wednesday, all coffee breaks and lunches will be served in the hall of the Minnaertbuilding. This building can be reached from the V.J. Koningsbergergebouw. On Thursday 31st of August, coffee and lunches will be served near the lecture room Cosmos in the V.J. Koningsbergergebouw.

Social program

- The Welcome Reception takes place on Monday 28th of August as of 16:00 in the Botanical Gardens, Budapestlaan 17 in Utrecht. This is opposite the V.J. Koningsbergerbuilding. We gather on the ground floor of the V.J. Koningsberger and walk together. It wil be a 5 min walk.
- The Conference Dinner will take place in the Utrecht city center at restaurant De Utrechter, Vredenburg 40, Utrecht, on Tuesday 29th of August, starting at 17:00.
- On Wednesday 30th of August at 16:00, we have planned visits to the UU NPEC (Netherlands Plant-ecophenotyping Centre) or the University Botanical Gardens. The NPEC visit includes two guided tours ~30 minutes with one group each (30 persons per group). The tour of the Botanical Gardens will take 1 hour and is available for 15 people. The tour will be in the Evolution Garden and focus on wild (native) plants. You can find sign-up sheets at the registration desk during the conference.
  If both tours are full, you can also walk through the Botanical Gardens by yourself with one of the Eucarpia hosts.

Website of the Eucarpia 2023 Conference

www.uu.nl/en/research/eucarpia-leafy-vegetable-conference-28-31-august-2023-in-utrecht-the-netherlands



Route Science Park Utrecht

The black arrow on the map below indicates the location of the V.J. Koningsberger building. The green arrows are nearby parking places. The map of the Science Park can also be found on the website.

Public transport

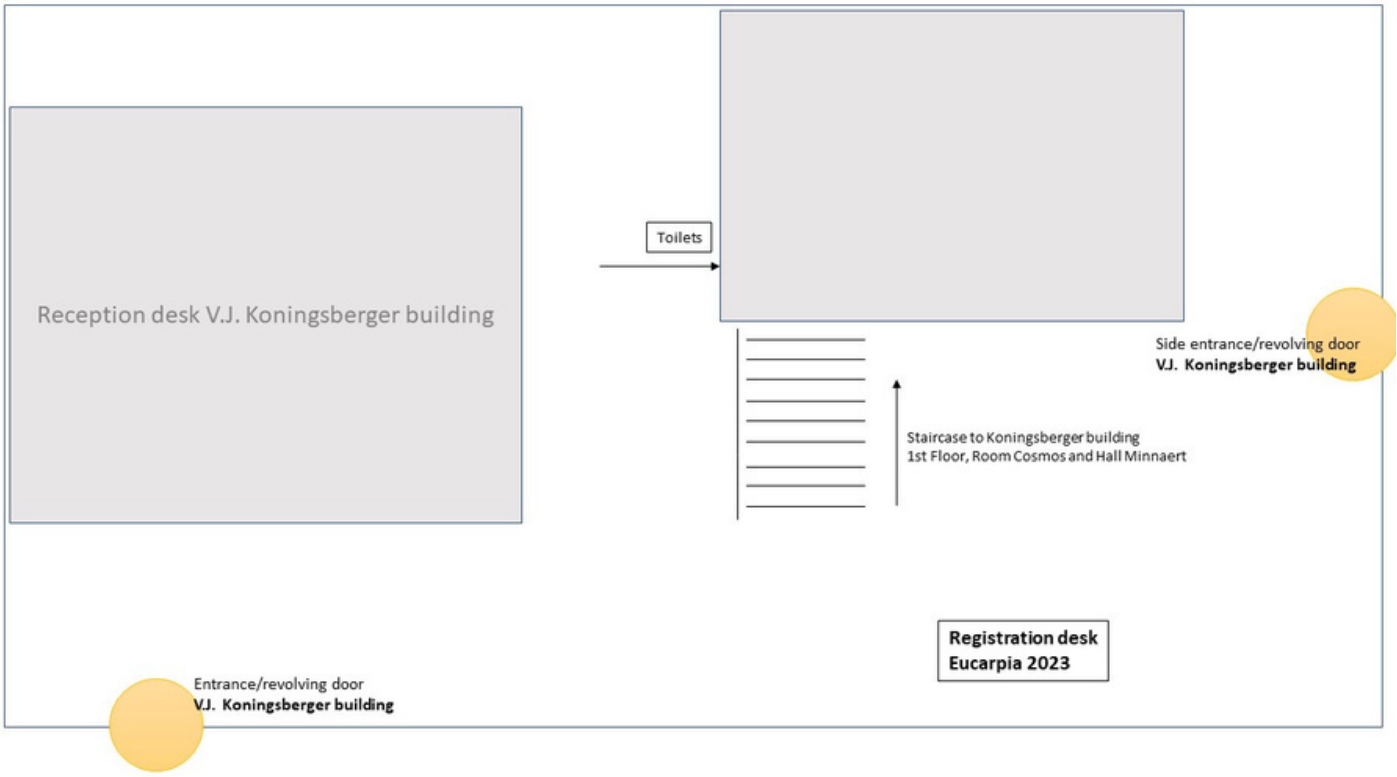
The Utrecht Science Park can be reached by public transport via an extensive network of bus lines and tram 22 (10 min from Utrecht Centraal to the Utrecht Science Park). You can plan your journey by public transport via the NS travel planner or 9292ov.

Parking

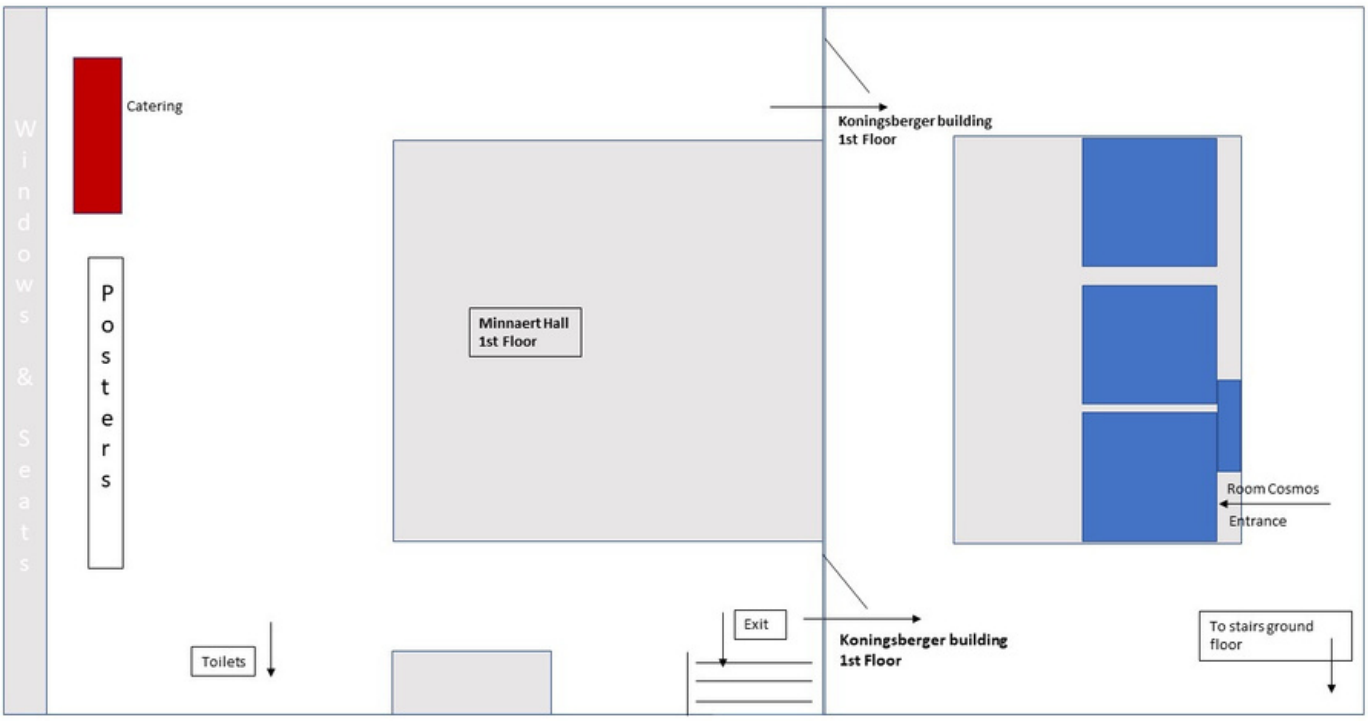
Visitors and guests can park in the Utrecht Science Park (USP) for a fee in various parking lots. Parking areas close to the Minnaert Building are: P Padualaan (opposite the Minnaert Building) and P Budapestlaan (opposite the Botanical Gardens, behind the V.J. Koningsberger Building).

You can also park in the P&R Utrecht Science Park, located at Uppsalalaan 6, 3584 CT Utrecht. From the A28 motorway take exit 2 (Utrecht Science Park). Then follow the signs to P&R Utrecht Science Park.

Map V.J. Koningsberger building, ground floor:



Map hall Minnaert building & V.J. Koningsberger building, first floor:



Scientific program

Monday 28 August 2023

9:00-9:45 Registration and coffee (put the posters up)

9:45-10:00 General opening by the local organizing committee and Eucarpia.

10:00-10:45 Opening lecture: **Guido van den Ackerveken**, Utrecht University (NL) *What's growing in my leafy greens? A story of pathogens, disease and immunity*

10:45-11:15 Coffee break

Genetic and genomic resources

11:15-11:45 **Eric Schranz**, Wageningen University and Research (NL) (keynote) *Comparative Genomics of Lettuce, its wild relatives and beyond*

11:45-12:15 **Edouard Severing**, Wageningen University and Research (NL) (invited): *Chromosome level assembly of wild spinach provides insights into the divergence of homo- and heteromorphic plant sex-chromosomes*

12:15-12:45 **Ales Lebeda**, Palacký University of Olomouc (CZ): *Prickly and opium lettuces in Central Chile and Mendoza district (Argentina*)

13:00-14:00 Lunch break

Development

14:00-14:30 **Rik op den Camp**, Keygene (NL) (keynote): *Apomixis: Breakthrough breeding technology transforming agriculture*

14:30-15:00 **Matthias Berens**, Keygene (NL) (invited): *Inactivation of a receptor kinase at the S-locus results in self-compatibility in chicory (Cichorium intybus, Asteraceae)*

Scientific program

15:00-15:20 **Celia Anton Sales**, Wageningen University and Research (NL): *PHYTOCHROME C mutations decelerated the circadian clock in cultivated lettuce while breeding for delayed bolting time*

15:20-15:40 **Athanasios Koukounaras**, Aristotle University of *Thessaloniki (GR): Factors affecting the agronomic performance of a underutilized leafy green: Sonchus oleraceus*

16:00 Welcome reception, Botanical Gardens, Utrecht University

Tuesday 29 August 2023

Stress resillience

9:30-10:00 **Christa Testerink**, Wageningen University and Research (NL) (keynote)

10:00-10:20 **Germán Sandoya,** University of Florida (US): *Heat tolerance in lettuce cultivated in the subtropics, from germplasm identification to molecular mechanisms*

10:20-10:40 **Ivan Paponov**, Aarhus University (DK): *Decoding Phytochemical Accumulation: Dissecting the Influence of Jasmonic Acid, Light, and Nutrient Variances*

10:40-11:00 **Alan Pauls**, Wageningen University and Research (NL): *Predicting the unpredictable: A novel approach to screening for inner leaf tipburn*

11:00-11:40 Coffee break

Trends in phenomics, biotech and other technologies

11:40-12:10 **Kim Boutilier**, Wageningen University and Research (NL) (keynote): *New Tools for an Old Problem*

Scientific program

12:10-13:10 Lunch break

13:10-13:40 **Theo van der Lee**, Wageningen University and Research (NL) (invited): *Phenotyping for biotic and abiotic stress*

13:40-14:00 **Martin Ganal**, SGS Institut Fresenius (DE): *A 40K SNP genotyping array for lettuce*

14:00-14:20 **Sandra Goritschnig**, ECPGR Secretariat c/o Alliance of Bioversity International and CIAT (IT): *Development and application of single primer enrichment technology (SPET) SNP assay for population genomics analysis and candidate gene discovery in lettuce*

14:20-16:00 **Poster session** with coffee break

17:00 **Social event and conference dinner**, Utrecht city center

Wednesday 30 August 2023

Resistance to aboveground diseases

9:30-10:00 **Katherine Denby**, Centre for Novel Agricultural Products, University of York (UK) : *Identification of lettuce transcription factors impacting resistance to Botrytis cinerea through* predictive network inference.

10:00-10:20 **Diederik Smilde**, Naktuinbouw (NL), *IBEB's monitoring of Bremia lactucae, the perspective of Naktuinbouw*

10:20-10:40 **Myluska Caro**, Rijk Zwaan (NL): *Identification, mapping and introgression of TSWV and INSV resistance leads to successful launching of resistant lettuce varieties*

10:40-11:10 Coffee break

Scientific program

11:10-11:30 **Bas ter Riet**, Enza Zaden (NL): *MACPF, a novel Bremia resistance gene in lettuce*

11:30-12:00 **Jim Correll**, University of Arkansas (USA): *Spinach downy mildew – mitigating a continual conundrum*

12:00-13:00 Lunch break

Resistance to belowground diseases

13:00-13:40 **Olga Scholten**, Wageningen University and Research (NL) (keynote): *Seed characteristics in relation to damping off tolerance in spinach*

13:40-14:10 **Kelley Richardson**, US Department of Agriculture (US): *Detecting and characterizing a new variant of lettuce Fusarium wilt in California*

14:10-15:00 **Poster session** with coffee break

Pathogen Biology

15:00-15:30 **John Clarkson**, University of Warwick (UK) (keynote): *Identification, pathogenomics and resistance to Fusarium oxysporum f.sp. lactucae causing Fusarium wilt in lettuce*

15:30-15:50 **Melanie Mendel**, Utrecht University (NL): *Shedding light on effector functions in spinach by employing a bacterial Type III Secretion System*

16:00-17:00 **Visit to UU NPEC or Botanical Gardens**

Thursday 31 August 2023

Postharvest Traits & market/consumer trends

9:30-10:00 **Leo Marcelis**, Wageningen University and Research (NL) (keynote)

Scientific program

10:00-10:20 **Gail Taylor**, University of California (US): *Indoor farming: changing the paradigm of leafy green breeding for cleaner, safer, more nutritious and sustainable crops*

10:20-10:40 **Aurora Diaz**, Agrifood Research and Technology Centre of Aragon (ES): *Genetic dissection of commercial and traditional lettuce varieties characterized for vitamin C content*

10:40-11:10 Coffee break

11:10-12:00 **Richard Michelmore**, University of California – Davis (US) (closing lecture): *Present and future genomics of lettuce improvement*

12:00-12:30 **Closing discussion and closing of the conference**

12:30-13:30 Lunch

Satellite meetings after the conference (not in parallel):

13:30-15:15 IBEB (International Bremia Evaluation Board) meeting, (Johan Schut) open to public

15:15-15:30 Coffee break

15:30-17:30 ILGC3 (Richard Michelmore), invitees only

Oral sessions

O1 Opening Lecture

What's growing in my leafy greens? A story of pathogens, disease and immunity

Guido van den Ackerveken

Translational Plant Biology, Dept. Biology, Utrecht University, Utrecht, The Netherlands

Leafy greens are grown to provide mankind with nutritious vegetables. However, they are also an attractive food source for pathogens and parasites that thrive on the leaves. The research in my group aims to understand what makes plants susceptible to microbial pathogens and how these microbes can successfully colonize leaves and cause disease. Through our studies, we aim to uncover biological processes that can be tailored to make our crops more disease resistant.

In my presentation, I will highlight results from our work on the downy mildews of Arabidopsis, lettuce, and spinach. Downy mildews are obligate biotrophic pathogens belonging to the oomycetes. They are among the most serious pathogens of leafy greens. On the microbial side, I will report on (1) the pangenome analysis of the spinach downy mildew Peronospora effusa that has revealed dynamic genomic regions in which new effectors genes can evolve, and (2) the microbiome that is associated with downy mildew infection. During infection, bacterial taxa become enriched in the phyllosphere microbiome that act antagonistically thereby reducing downy mildew infection.

On the plant side, our work focuses on susceptibility (S) genes and quantitative disease resistance. Mutation of the S genes DMR6/DLO1 results in growth reduction. Understanding the mechanisms driving this trade-off is necessary to reduce possible pleiotropic effects of deploying S-gene-based resistance. On the other hand, quantitative disease resistance (QDR) is becoming more and more important in disease resistance breeding, thus one also needs methods to quantify diseases. I will report on novel approaches to assess disease severity and resistance levels using advanced imaging techniques. We are also exploring natural variation within Lactuca to identify QDR traits by association studies. Research on lettuce QDR to downy mildew and basic principles of the lettuce immune system is carried out as part of the LettuceKnow program.

O2 Keynote - Genetic and genomic resources

Comparative Genomics of Lettuce, its wild relatives and beyond

Eric Schranz

Wageningen University and Research

O3 Invited - Genetic and genomic resources

Chromosome level assembly of wild spinach provides insights into the divergence of homo- and heteromorphic plant sex-chromosomes.

Edouard I. Severing[1], Edwin van der Werf[1], Martijn P.W. van Kaauwen[1,2], Linda Kodde[1], Chris Kik[3], Rob van Treuren[3], Richard G.F. Visser[1], Richard Finkers[1,2] and Yuling Bai[1]*

1) Wageningen University and Research. Droevendaalsesteeg 4, 6708 PB Wageningen, the Netherlands.
2) Current address: GenNovation B.V. Agro Business Park 10, 6708 PW Wageningen, the Netherlands.
3) Centre for Genetic Resources, the Netherlands (CGN), Wageningen University and Research, P.O. Box 16, 6700 AA Wageningen, the Netherlands
* Corresponding author: bai.yuling@wur.nl

Cultivated spinach (Spinacia oleracea) is a highly nutritional crop species of great economical value that belongs to a genus of dioecious plant species with both homomorphic and heteromorphic sex chromosomes. The wild spinach species Spinacia turkestanica and Spinacia tetrandra are import genetic sources for improvement of cultivated spinach and excellent material for studying sex chromosome evolution in plants. However, until now there were no publicly available genome assemblies for these species.
In this study we generated chromosome level assemblies of S. turkestanica and S. tetrandra and performed a tri-way comparative analysis together with S. oleracea. We show that many gene clusters related to abiotic- and biotic stress have expanded through tandem duplication in S. tetrandra after it diverged from the lineage leading to S. turkestanica and S. oleracea. Focussing on the sex chromosomes we found that the previously identified structural variation that distinguishes the male- and female- SEX DETERMINING REGION (SDR) in S. oleracea is conserved in S. turkestanica. Although, the SDR of these two species coincides with the PSEUDO AUTOSOMAL REGION of S. tetrandra the gene content is only marginally conserved and the genetic factors determining sex in these species probably differ. Finally we show that Y-chromosome of S.tetrandra is highly degenerated and recombination suppression with the X-chromosome potentially likely started before the species diverged from S. turkestanica and S. oleracea.
We believe that the wild spinach genomes produced in this study will be verry valuable for spinach breeders and evolutionary biologist alike.

O4 Genetic and genomic resources

Prickly and opium lettuces in Central Chile and Mendoza district (Argentina)

Aleš Lebeda* and Eva Křístková

Palacký University in Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 27, 783 71 Olomouc – Holice, Czech Republic; (* corresponding author: ales.lebeda@upol.cz)

The distribution and character of populations of Lactuca serriola L. and L. virosa L. were studied in Central Chile in 2016 and 2017, and in Mendoza district (Argentina), in 2020. Mountains of Andes strongly influence differences in climate between these geographically close areas.

In Central Chile, the occurrence of a single L. serriola, was recorded on 144 sites, and L. virosa was observed on 16 sites. Presence of both species was observed on 14 sites. L. serriola was recorded equally in urban areas and along transport corridors outside of the cities. Population sizes varied from few (cca 5 to 10) plants to hundreds of individuals. L. virosa was most frequent along roads outside of cities, with several individuals to high population densities. The potential for species succession was observed along roads with differing elevational gradients: L. serriola was recorded until an elevation of 2 235 m a.s.l.; dense populations of L. virosa were continuously at higher elevations, up to 2 148 m a.s.l. n Argentina, the occurrence of a single L. serriola was observed on 50 sites in altitudes of 504 - 2 726 m a.s.l., L. virosa was observed on 7 sites in elevation of 900 – 2 770 m a.s.l., both species were recorded on three sites.

These observations documented that both allochthonous (Eurasian) wild Lactuca species occur and regenerate in both South American countries and can expand to high elevations and extreme habitats. Complex study of both species populations could elucidate their history, biogeography, ecology and genetic variation.

This research was supported by the following grants: MSM 6198959215 (Ministry of Education, Youths and Sports, Czech Republic) and Internal Grant Agency of Palacký University in Olomouc (IGA_PrF_2023_001).

O5 Keynote - Development

Apomixis: Breakthrough breeding technology transforming agriculture

Rik H.M. Op den Camp

KeyGene N.V. Wageningen

Apomixis is clonal reproduction through seeds, producing offspring genetically identical to the mother plant. This reproductive system occurs in some 300 wild plant species but not in major crops. Introducing apomixis in crops can yield significant benefits for plant breeding and seed production. The most obvious is the perpetual fixation of heterosis, but in principle, any genetically determined trait can be fixed, regardless of its complexity. In order to introduce apomixis into sexual crops, genes that skip meiosis and fertilization must be identified and brought together. There are two main approaches to making apomictic crops. The first is the knock-out mutations in known meiotic genes combined with genes causing parthenogenesis (synthetic apomixis). The second is cloning naturally dominant apomixis genes and modifying the sexual orthologs (copy nature apomixis). Recently, remarkable progress in apomixis research has been made. Synthetic apomictic rice with high apomixis penetrance has been produced, suggesting that this application may be close to the market. In addition, the first naturally dominant parthenogenesis genes have been isolated (BabyBooMLike in Pennisetum and PARthenogenesis in Taraxacum). Apomixis genes are not completely new but modifications of sexual genes. In the presentation, I will discuss how we identified the PAR gene in Taraxacum and the possible future application of apomixis in plant breeding programs. Concerning the application in breeding, the dominance of apomixis genes and the female specificity of apomeiosis is essential. The recent breakthroughs in apomixis research justify the expectation that apomictic crops will be a reality before the end of this decade.

O6 Invited - Development

Inactivation of a receptor kinase at the S-locus results in self-compatibility in chicory (Cichorium intybus, Asteraceae).

Theo Hendriks[1], Maghsoud Pazhouhandeh[1,2], Paul Bundock[3], Robert Sevenier[3], Amélie Carré[1], Vincent Castric[1], and Marie-Christine Quillet[1]

1) Université de Lille, Villeneuve d'Ascq, France; 2) Azarbaijan Shahid Madani University, Tabriz, Iran ; 3) KeyGene, Wageningen, The Netherlands

Chicory is self-incompatible (SI) and the S-locus controlling its sporophytic self-incompatibility (SSI) system was mapped. A combination of map-based cloning and transcriptomic analyses of stigma-style tissue led to identification an S-locus gene encoding a LRR-RLK that might function as stigma receptor of a self-pollen ligand. The validity of this hypothesis was comforted by high allelic diversity of this gene in natural populations, with highly variable and strongly selected codons (dN/dS >1) in the region encoding the extracellular LRR domain. Functional validation of the gene, named SIRK for Self-Incompatibility Receptor Kinase, was undertaken in the context of the European research and innovation project CHIC, aiming at improving the potential of chicory as a multipurpose crop by new plant breeding techniques. CRISPR-Cas9 editing by the DNA-free method developed in the CHIC project was used to create InDel mutations that would inactivate alleles of SIRK. Protoplasts of two SI chicory cultivars were transfected by Cas9 enzyme-guide RNA ribonucleoproteins and about 140 simple and double mutant plants regenerated from protoplasts were sequenced and characterized. Self-compatibility (SC) of the mutant plants was evaluated by seed set determinations (number of full seeds per number of ovules) in capitula after autonomous self-pollinations. Plants with InDel mutations in both alleles of SIRK or only in the dominant allele were self-compatible while plants mutant for the recessive allele only retained SI. The applied interest of the results and perspectives for studies on the occurrence, evolution, and origin of SSI in Asteraceae will be addressed.

O7 Development

PHYTOCHROME C mutations decelerated the circadian clock in cultivated lettuce while breeding for delayed bolting time.

Celia Anton Sales[1], Joseph DiPalma[3], Chengcheng Cai[1], Robertson McClung[2], Marieke Jeuken[1], Guusje Bonnema[1]

1) Plant Breeding, Wageningen University and Research, Wageningen, The Netherlands. 2) Department of Computer Science, Dartmouth College, Hanover, NH, USA. 3) Department of Biological Sciences, Dartmouth College, Hanover, NH, USA

The circadian clock is an endogenous tim1
ekeeping mechanism that enables plants to synchronize their metabolic and physiological processes with the daily light and dark cycle of the Earth. It influences many aspects of plant biology; also, agronomic traits and it has recurrently been targeted through artificial selection during the domestication and improvement of crops. In this study, we performed a species-wide screening of the circadian clock in lettuce, by time-lapse tracking the circadian leaf movements of 184 lettuce accessions, including the cultivated species Lactuca sativa (n=126), its wild ancestor Lactuca serriola (n=35), and the distantly related Lactuca saligna (n=23). Our findings indicate that during the domestication of lettuce, the circadian clock period was altered, resulting in a shift from a 24-hour cycle in L. serriola to a 27- hour cycle in most cultivars of L. sativa. Through genome wide association studies (GWAS) we have identified mutations in the locus of PHYTOCHROME C (PHYC) that may be responsible for this change. PHYC is a protein that plays a key role in the plant's circadian clock and flowering processes. We propose that a slower clock in lettuce is a result of breeding efforts aimed at delaying bolting, a classical breeding target in lettuce. By selecting for PHYC allelic variation, it might have been possible to modify the timing of bolting and delay flowering, while indirectly slowing the plant's circadian clock and increasing its yield under long days. While further functional validation is required, these findings suggest that PHYC might promote photoperiodic flowering in lettuce and provide another example of the importance of the circadian clock in plant biology and the development of crops. Besides, it opens new possibilities for cultivating larger lettuce in controlled environments such as vertical farming. Keywords: PHYTOCHROME C, circadian clock, bolting time, flowering, lettuce, Lactuca sativa, vertical farming, GWAS.
Acknowledgements We thank Fiona E Belbin, Yakun Zhang and Lou Ping for experimental assistance, and Johan Bucher for technical support. We acknowledge the Dutch Research Council (NWO) for funding this work through the Sky-high Consortium.

O8 Development

Factors affecting the agronomic performance of an underutilized leafy green: Sonchus oleraceus

Athanasios Koukounaras[1], Daniele Rabboni[2], Anna Gkotzamani[1], Filippos Bantis[1], Eleni Papoui[1], Lorenzo Barbanti[2], Silvio Salvi[2], Andreas Katsiotis[3], Konstadinos Mattas[4]

1) Laboratory of Vegetable Crops, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
2) Department of Agricultural and Food Sciences, University of Bologna, 40127 Bologna, Italy
3) Department of Agricultural Sciences, Biotechnology and Food Science, Faculty of Geotechnical Sciences and Environmental Management, Cyprus University of Technology, 50329 Limassol, Cyprus
4) Department of Agricultural Economics, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

Emerging research documents establish the strict connection between biodiversity and diverse dietary behaviour. The benefits of the Mediterranean diet for human health and the critical role of underutilized leafy greens as an important nutritional ingredient are well known. However, a lack of cultivation knowledge inhibits the entrance of such vegetables' entrance into modern food supply chains and offers consumers a more nutritional and healthy food dish. The aim of this study was to evaluate different preharvest factors (growing season, plant density) on yield and quality of an underutilized leafy green common in the Mediterranean region, Sonchus oleraceus L. Therefore, an experiment was conducted in an open field with 2 growth periods, (15/9/22 to 28/3/23, 17/10/22 to 24/4/2023) with two plant densities (10 plants m-2 and 20 plants m-2), while a second one was conducted in a plastic non-heated greenhouse with the above plant densities. In the first experiment yield data was recorded while in the second experiment chemical composition (nitrates, phenolics, antioxidant capacity) of the plants was added. Both experiments show that the high density achieved a higher yield ranging from 47,0% to 61,5%. The later crop establishment resulted in a similar cultivation period compared to the earlier establishment, 189 to 194 days, but significantly better production 34.5% despite the plant density. The study clearly demonstrates that Sonchus oleraceus can be easily cultivated and produced commercially enriching leafy vegetable production and its use in today’s diets.

O9 Keynote - Stress reslience

Salt stress resilience; think global, act local

Christa Testerink

Wageningen University and Research

Salinity of the soil is highly detrimental to most plant species. Recently we have identified several molecular and cellular pathways in the model species Arabidopsis that contribute to finetuning of root development to better deal with salt stress. I will highlight what we can learn from these pathways and their regulation at the tissue-specific level in our new approaches to improve salt tolerance of crop species. Yet while we are sometimes successful in translating our findings from Arabidopsis to crops, at the same time we must invest in acquiring knowledge on the molecular basis of stress-induced developmental responses in crops.

O10 Stress reslience

Heat tolerance in lettuce cultivated in the subtropics, from germplasm identification to molecular mechanisms.

Germán V. Sandoya[1], Keving Begcy[2], Hannah M. Mather[1,2]

1) Everglades Research and Education Center/ Horticultural Sciences Department. Belle Glade, Florida, USA 33430
2) Environmental Horticulture Department. University of Florida – IFAS. Gainesville, Florida, USA 32611
(gsandoyamiranda@ufl.edu)

The subtropic of Florida are the southernmost place in the United States where lettuce (Lactuca sativa L.) is cultivated. The crop faces lack of germination at planting due to increased soil temperatures. Moreover, physiological disorders (bolting and tipburn) and unmarketability could be consequences of the lack of heat tolerance in mature plants. A research program was designed to identify germplasm (at germination and at mature stages) and to understand physiological, biochemical, and molecular mechanisms related to heat tolerance. Extensive laboratory and field screenings resulted in the identification of germplasm germinating at warm temperatures (32 to 34 ° °C) and to have an acceptable head weight and marketability with less bolting and tipburn. A lack of correlation between germination and good performance of lettuce in fields under warmer conditions indicates that heat tolerance mechanisms depend upon developmental stages. High night temperatures are detrimental when lettuce is cultivated in warmer environments. Simulation of different stress scenarios (day and night temperatures) were investigated in thermotolerant and thermosensitive germplasm. Lettuce head formation was unsuccessful while tipburn was highest under night stress (20/25°C), and lowest in the 35/25°C and 35/20°C regimes in the thermosensitive germplasm. Persistent day and night stress (35/25°C) significantly increased leaf starch, and imposed differential changes in sucrose, glucose, and brix values between thermotolerant and thermosensitive lettuce. The transcriptional expression of gene members in the energy biosynthesis pathway showed higher expression in thermotolerant lines. The molecular, biochemical, and physiological data could facilitate the breeding process for thermotolerant germplasm for the subtropics.

O11 Stress resilience

Decoding Phytochemical Accumulation: Dissecting the Influence of Jasmonic Acid, Light, and Nutrient Variances

Martina Paponov[1] and Ivan A. Paponov[2]

1) Division of Food Production and Society, P.O. Box 115 NO, 1431 Ås, Norway
2) Department of Food Science, Aarhus University, 8200 Aarhus, Denmark

Phytochemical accumulation in high-value plant species is intricately controlled by a convergence of hormonal responses, light signaling, and nutrient distribution dynamics. Specifically, the interplay of Jasmonic Acid (JA) and far-red (FR) light dictates growth-defense balance in Hypericum perforatum. JA acts as a growth inhibitor while concurrently boosting the build-up of secondary metabolites. Conversely, FR light facilitates growth without compromising on secondary metabolite production. Interestingly, these processes occur independently, and their combined effect amplifies secondary compound production, avoiding a decline in growth. Simultaneously, in the context of nutrient distribution, Artemisia annua adapts to nutrient deficiency by enhancing artemisinin root exudation, particularly under heterogeneous nutrient conditions. Conversely, H. perforatum, under uneven nitrogen distribution, bolsters bioactive compound accumulation in leaves, without significantly impacting root exudation. Our findings underscore a species- and compound-specific response to nutrient discrepancies, driving plant adaptation and potential rhizosphere interactions. This research reveals promising strategies to optimize phytochemical production in high-value leafy plants, effectively breaking through the growth-defense trade-off paradigm.

O12 Stress resilience

Predicting the unpredictable: A novel approach to screening for inner leaf tipburn

Alan Pauls*, Kiki Spaninks#, Remko Offringa#, Mark Aarts*

*Wageningen University and Research
#Leiden University

In cultivated lettuce(Lactuca sativa), the necrosis of leaf tips in younger developing leaves is what is commonly referred to as inner leaf tipburn, hereafter referred to as tipburn. It is associated with the inadequate supply of calcium(Ca) to the growing tips of young leaves, especially in lettuce grown in summer/spring when high growth rates are observed. It is a big dilemma because in heading lettuce varieties such as 'icebergs' the younger leaves are hidden and therefore tipburn isn't caught early on. Symptoms usually manifest only a few days prior to harvest by when the economic damage to growers and lettuce packing companies from tipburn can be devastating. Thus the importance of screening for tipburn resistance cannot be overstated. However, present screening methods for tipburn require extensive field trials across multiple test field locations where the lettuce plants need to be grown until maturity to score for tipburn severity. This is an expensive and time consuming undertaking. In this study, we introduce a novel approach to screening for tipburn severity using a hydroponics based growth system that induces tipburn at an early developmental stage(pre-heading) using a systemic low Ca approach as the tipburn pressure inducer. We screened the LK200 lettuce diversity panel using this approach to identify sensitive and tolerant lettuce varieties with a five point scale qualitative tipburn scoring system. Furthermore a correlation analysis was done with a field trial using the same population to cross-validate the tipburn severity observed in the hydroponics screen. Our results show that tipburn can be reliably induced at an early stage provided enough tipburn pressure is applied. If successfully implemented, this screening approach cuts screening time from months to weeks providing a fast and predictable solution to phenotyping the tipburn trait thereby aiding in quickening breeding and QTL identification strategies that aim to unravel the genetic architecture of this complex trait.es

O13 Keynote - Trends in phenomics, biotech and other technologies

New Tools for an Old Problem

Kim Boutilier

Wageningen University and Research

Somatic and gametophytic embryogenesis are two examples of induced totipotency, where embryos develop in vitro without fertilization. These two types of in vitro embryogenesis form the basis for a number of plant breeding and biotechnology applications, including clonal propagation, doubled-haploid production and regeneration after transformation, but are also used as model systems to understand how plant cells are reprogrammed to follow a new developmental pathway. Many plant species and genotypes are recalcitrant for in vitro regeneration through embryogenesis. One approach to overcome recalcitrance is to empirically identify the explant and tissue culture parameters that contribute to efficient regeneration. Almost all of the major breakthroughs in plant tissue culture have been achieved in this way, and although successful, this approach is often time consuming and inefficient, as only a few parameters can be tested at one time.
Our lab is using a chemical screening approach to better understand the mechanistic basis for induced totipotency and in turn, to identify small molecules that can be used in a tissue culture pipeline to overcome recalcitrance for in vitro embryogenesis in crops. In this approach, individual compounds from commercial targeted or non-targeted compound libraries are screened for their ability to enhance embryogenesis, while keeping the tissue culture conditions constant. I will highlight the general concepts behind chemical screening and demonstrate how we have used this approach to identify competence factors and new enhancer molecules for gametophytic and somatic embryogenesis.

O14 Invited - Trends in phenomics, biotech and other technologies

Phenotyping for biotic and abiotic stress

Theo van der Lee, Marie Duhamel, Laura Groenenberg

Wageningen University and Research

To assess the quality of seeds, plant, plant performance and produce in various steps in the production chain as well as to accelerate resistant variety development in plant breeding programs or to select for beneficial micro-organisms, non-destructive and quantitative methods for digital phenotyping are crucial. These novel methods are required to improve the monitoring of quality and disease and facilitate timely decisions and management. In the era of genomics, plant phenotyping, especially for quality control, and diseased plants is critically lagging behind. Current methods, rely mostly on visual estimation by trained experts. This is time-consuming and subjective, making it expensive, difficult to quantify or even to integrate in scientific studies where use of such subjective data is troublesome. In addition, many important aspects of quality and disease cannot be observed by eye and the latency period before disease symptoms are expressed can be very long. Innovative, sensor-based methods for the detection and evaluation of plant diseases have been developed. Their application to quantify disease and to detect non-symptomatic stages of the infection has been demonstrated. Using the new facilities such as NPEC we aim to generate experimental procedures and protocols, data-sets, and analysis pipelines that are FAIR by design with a linked data structure. To efficiently work with these complex datasets requires transition from expert observations to computation analysis supervised by features indicated by experts or unsupervised analysis. Several examples will be shown for different applications in seed quality, abiotic and biotic stress and quality of the produce.

O15 Trends in phenomics, biotech and other technologies

A 40K SNP genotyping array for lettuce

Martin W. Ganal, Joerg Plieske , Eva Grafahrend-Belau , Andreas Polley , Eva Graner1, Thomas Gross1 and Heike Gnad1

1SGS Institut Fresenius GmbH TraitGenetics Section, Am Schwabeplan 1b, 06466 Seeland OT Gatersleben, Germany

Single Nucleotide Polymorphism (SNP) markers are now the most widely used molecular marker type for plant breeding including marker assisted selection or backcrossing (MAS, MAB), genetic relationship analysis, marker/trait association studies and other applications such as Genomic Selection. We have developed an Axiom SNP genotyping array containing 41,975 markers selected from a public database (http://lgr.genomecenter.ucdavis.edu) based on polymorphisms in 281 sequenced lines and genome distribution. Through the analysis of the array on a set of lettuce lines, more than 26,000 good and polymorphic markers have been identified. This set of validated SNP markers will provide a large resource of high quality SNP markers for use in lettuce breeding (e.g. for the development of optimized smaller arrays) and genetic analyses in research.

O16 Trends in phenomics, biotech and other technologies

Development and application of single primer enrichment technology (SPET) SNP assay for population genomics analysis and candidate gene discovery in lettuce

Pasquale Tripodi[1], Massimiliano Beretta[2], Damien Peltier[3], Ilias Kalfas[4], Christos Vasilikiotis[4], Anthony Laidet[5], Gael Briand[5], Charlotte Aichholz[6], Tizian Zollinger[7], Rob van Treuren[8], Davide Scaglione[9], Sandra Goritschnig[10]

1) CREA Research Centre for Vegetable and Ornamental Crops, Via dei Cavalleggeri 25, 84098, Pontecagnano Faiano, SA, Italy; 2) ISI Sementi SpA, 43036 Fidenza (PR), Italy; 3) LIMAGRAIN - Vilmorin-Mikado, Route du Manoir, 49250 La Ménitré, France; 4) American Farm School, Thessaloniki, Greece; 5) Gautier Semences, Route d'Avignon 13630 Eyragues, France; 6) Sativa Rheinau AG, Rheinau, Switzerland; 7) Zollinger Conseilles Sarl, Les Evouettes, Switzerland; 8) Centre for Genetic Resources, the Netherlands (CGN), Wageningen University and Research, P.O. Box 16, 6700 AA Wageningen, the Netherlands; 9) IGA Technology Services Srl, Udine, Italy; 10) ECPGR Secretariat c/o Alliance of Bioversity International and CIAT, Via dei Tre Denari 472A 00054 Maccarese (RM)Via di San Domenico 1, 00153 Rome, Italy

Recent years witnessed astonishing advancements in the development of cutting-edge next-generation sequencing technologies to dissect the genetic basis of crops and discover genes of interest. Among these, SPET (single primer enrichment technology) is a novel high-throughput genotyping method based on short-read sequencing of specific polymorphic genomic regions, overcoming the limits of other reduced representation library sequencing methods based on a random sampling of genomic loci and allowing the discovery of closely linked, novel SNPs. Here we report the design and application of the first SPET panel in lettuce, consisting of 41,547 probes spanning the whole genome and designed to target both coding (~96%) and intergenic (~4%) regions. A total of 81,531 SNPs were surveyed in 160 lettuce accessions from the ECPGR EVA Lettuce network, originating from 10 countries and representing ten different horticultural types. Model ancestry population structure clearly separated the cultivated accessions (Lactuca sativa) from accessions of its presumed wild progenitor (L. serriola), revealing six genetic subgroups that reflected a differentiation based on cultivar typology. To determine the potentiality of SPET for gene discovery, we performed genome wide association analysis for main agricultural traits in L. sativa. Robust associations were detected for seed colour, outer leaf colour, leaf anthocyanin and bolting in chromosomal regions previously reported to hold candidate genes for these traits. Our research demonstrates the reliability of SPET for detecting polymorphisms to dissect the genetic diversity of lettuce collections and identify candidate genes for traits of agricultural interest, enabling a better use of lettuce germplasm.

O17 Resistance to aboveground diseases

Identification of lettuce transcription factors impacting resistance to Botrytis cinerea through predictive network inference

Harry Pink[1], John Clarkson[2], Frances Gawthorp[3] and Katherine Denby[1]

1) Centre for Novel Agricultural Products (CNAP), Biology Department, University of York, York, YO10 5DD, UK
2) University of Warwick, School of Life Sciences, Coventry, United Kingdom
3) Tozer Seeds, Cobham, United Kingdom

Resistance against Botrytis cinerea and Sclerotinia sclerotiorum is quantitative, governed by multiple small-medium impact loci, with plant responses involving large-scale transcriptional reprogramming. We aim to identify transcriptional regulators of the lettuce defence response against these generalist necrotrophic pathogens to use as targets for the development of cultivars with reduced susceptibility.
We have taken a systems biology approach to predict regulators and understand the complex networks in which they function. We profiled transcriptomes after infection with B. cinerea or S. sclerotiorum in 21 diverse lettuce accessions with varying levels of pathogen susceptibility (Pink et al, 2022). This identified >5000 genes whose expression (positively or negatively) correlated with disease severity. These genes are not differentially expressed during infection, suggesting that their basal level of expression impacts disease outcome.
Time series transcriptome data from lettuce after B. cinerea and S. sclerotiorum inoculation captured the dynamics of the transcriptional response to infection and identified a core set of 4362 genes similarly differentially expressed in response to both pathogens.
Using all the expression data for these core genes, we inferred a gene regulatory network (GRN) underlying the lettuce defence response. Using the GRN, we have predicted and validated key regulators of lettuce immunity, as well as their downstream target genes. These data provide a high level of detail about defence-induced transcriptional changes for a crop species and identify transcription factors that mediate disease resistance both in lettuce and other species.
Pink et al. (2022) Theoretical and Applied Genetics doi.org/10.1007/s00122-022-04129-5

O18 Resistance to aboveground diseases

IBEB's monitoring of Bremia lactucae, the perspective of Naktuinbouw

Diederik Smilde

Naktuinbouw

Bremia resistance breeding creates more and more genetic variation for resistance in commercial varieties of lettuce of different size and shape. All new varieties need a description of their characteristics before commercialisation, and growers expect reliable information about resistances. So, understandably, lettuce variety descriptions are not only based on morphology, but also on resistance, and all relevant isolates and differentials for checking the validity of a resistance claim must be available to official examination offices like Naktuinbouw.

This is not a trivial task for Bremia lactucae, so, in spite of competing interests, breeding companies are eager to work together in the International Bremia Evaluation Board (IBEB) and to share virulence patterns of isolates found in breeding material or in commercial fields, with great benefit to market communication in the lettuce industry.

Since 1999, IBEB has denominated 25 Bremia isolates for Europe. Since 2016, IBEB is split up in regional groups for EU and US isolates, using the same differential set to determine virulence patterns. A subset of the IBEB-denominated isolates, listed at worldseed.org/../ibeb, is still relevant today

The annual evaluation sessions of IBEB-EU are always focused on the most recent data and little attention is paid to the past. Maybe a retrospective analysis of all data can help to improve the efficiency of IBEB. Can we prevent wrong conclusions from necessarily incomplete monitoring data? Can we ever win the struggle with Bremia lactucae?

O19 Resistance to aboveground diseases

Identification, mapping and introgression of TSWV and INSV resistance leads to successful launching of resistant lettuce varieties

Myluska Caro*, Arnaud Thabuis*, Marc Villevieille*, Christophe Thomas*, Lisa Mulder*, Johan Schut*

*Rijk Zwaan Breeding B.V

Impatiens necrotic spot virus (INSV), a member of the Tospovirus family, affects a large number of plant species, including several horticultural crops of economic importance. In recent years, INSV has become an important disease in lettuce, with economic losses up to U$100 million in some production areas. Tomato spotted wilt virus (TSWV), another Tospovirus closely related to INSV, greatly affects lettuce production in some countries as well.

INSV and TSWV are transmitted by different thrips species. Methods to reduce the virus incidence and spread mainly focus on controlling the thrips vector population. The use of resistant varieties poses a more efficient, sustainable approach to control INSV/TSWV. Rijk Zwaan performed a large screen of Lactuca wild relatives in the quest for TSWV resistance. A wild accession with a high level of resistance was identified, and a population derived from a cross with a susceptible variety was developed. Using internal bioassays, such mapping population was tested for resistance/susceptibility to TSWV. Initial genetic studies pointed the resistance to inherit as a monogenic, dominant trait. Subsequent mapping studies located the genetic determinant of the TSWV resistance to chromosome 2, in the vicinity of one of the major Bremia resistance clusters (MRC2). The gene proved to also provide a high level of resistance to INSV.

Introgression of the resistance into Rijk Zwaan's lettuce varieties successfully followed using MAS (marker-assisted selection), aided by the use of a tightly-linked marker. The first two INSV/TSWV-resistant romaine lettuce varieties were successfully launched in 2023, and introduction of new varieties from a broad range of lettuce types will follow.

O20 Resistance to aboveground diseases

MACPF, a novel Bremia resistance gene in lettuce

Bas ter Riet, Ilja Roobeek, Kim Lakeman, Mathieu Pel and Gert-Jan de Boer

Enza zaden Research & Development

At Enza zaden we identified a novel Bremia resistance gene in lettuce for the priority 1 disease downy mildew. The resistant and susceptible alleles of this gene are determined and this gene is currently in a patent application (WO2020035145A1). Interestingly this novel Bremia resistance gene codes for membrane attack complex/perforin (MACPF) and is not a typical plant resistance gene as described in literature so far. MACPFs are playing critical functions in innate and adaptive immunity in human and are known to participate in lytic pore formation. Especially from a protein structure point of view a lot of knowledge is now at hand to study this gene for plant based resistance to pathogens in general.

O21 Resistance to aboveground diseases

Spinach downy mildew: mitigating a continual conundrum

James C. Correll[1], Braham Dhillon[2], Ainong Shi[1], Kurt H. Lamour[3], Peter Tandy[3], Burt Bluhm[1] Steve Klosterman, Kelley Clark, Hannah V. Zima[1], and Maria Villarroel-Zeballos[1].

1) Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas USA
2) University of Florida, Fort Lauderdale, Florida USA
3) Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, Tn,
4) United States Department of Agriculture, Agricultural Research Service, Salinas, Ca USA

Spinach continues to increase in popularity as a healthy nutritious leafy green vegetable in the human diet. However, downy mildew, caused by Peronospora effusa [=P. farinosa f. sp. spinaciae (Pfs)] is the single most important constraint to sustainable production, particularly for increasingly important organic markets. Up to 19 races of Pfs have emerged in commercial production over the past 20 plus years and races continue to emerge that overcome newly deployed resistance genes. In an effort to address knowledge gaps in our understanding of the host-pathogen interaction to improve the use of resistance as a management strategy, and evaluate the overall epidemiology of the pathogen, a number of approaches are being pursued. The approaches being used include the use of molecular markers linked to major genes (RPF genes) and QTL for resistance, the identification of RPF genes and QTL, the examination of functionality of RPF genes using VIGs, CRISPR/Cas editing, plant transformation with RPF genes, gene expression profiles with NILs containing or lacking RPF genes using RNAseq, and sequencing of resistant and susceptible lines including target regions on chromosome with known RPF genes. In addition, various aspects of the pathogen are being examined. The molecular arsenal of the pathogen is being characterized by examining effector genes, mutations affecting AVR genes in Pfs and thereby altering virulence, genome sequencing of various races of Pfs and mutants with and without altered virulence, and examination of fitness of virulence altered mutants. A number of well-characterized SNPs are being used to examine the regional and global population diversity and plasticity of the whole genome under controlled and field conditions as well as the impact of oospores as primary inoculum.

O22 Keynote - Resistance to belowground diseases

Seeds characteristics in relation to damping off tolerance in spinach

Kim J.H. Magnée[1,2], Olga E. Scholten[1], Jan Kodde[1], Joeke Postma[1], Gerrit Gort[3], Edith T. Lammerts van Bueren[1], Steven P.C. Groot[1]

1) Wageningen Plant Research, Wageningen University & Research, P.O. Box 16, AA, Wageningen 6700, the Netherlands
2) Certis Belchim B.V., P.O. Box 607 AP, Utrecht 3500, the Netherlands,
3) Biometris, Wageningen University & Research, PO Box 16 HB, Wageningen 6700, the Netherlands

Spinacia oleracea L. (spinach) is an economically important, highly nutritious leafy vegetable. Production is threatened by the occurrence of damping off, that includes non-emergence of seedlings (pre-emergence damping off) and wilting of seedlings or plants after emergence (post-emergence damping off). Damping off can be caused by multiple soilborne pathogens, including Pythium spp. We studied the relationship between spinach cultivars, seed vigour and germination, and pre-emergence damping off caused by Pythium ultimum to identify seed traits involved in tolerance to damping off. Variation in tolerance levels was observed, but could not be confirmed among cultivars due to a larger variation in emergence among seed lots of the same cultivars. Since speed of emergence could play a role in tolerance to damping off, priming and dehulling (removal of the pericarp) of seeds was applied. Priming resulted in faster seedling emergence and improved tolerance levels, while dehulling only resulted in some seed lots with increased seedling emergence and improved tolerance levels. As a next step, untreated seeds were individually measured for morphological and spectral seed traits, including seed size and maturity. In general, seeds with higher maturity levels showed higher P. ultimum tolerance than less mature seeds. Of those seeds, the smaller seeds and those with a darker pericarp were more tolerant than the larger or lighter coloured seeds. Further studies are needed to investigate the association between pericarp colour and secondary metabolites, and to understand their role in spinach damping-off tolerance.

O23 Resistance to belowground diseases

Detecting and characterizing a new variant of lettuce Fusarium wilt in California

Kelley L. Richardson[1] , Santosh Nayak[1], James D. McCreight[1], Patti Fashing[1], Frank N. Martin[1] , Ningxiao Li[1], Nick LeBlanc[1], and Alex Putman[2]

1) U.S. Department of Agriculture, Agricultural Research Service, 1636 E. Alisal St., Salinas, CA, 93905
2) University of California, Department of Microbiology and Plant Pathology, 238 Fawcett Lab, Riverside, CA, 92521

Lettuce Fusarium wilt (FW) is caused by Fusarium oxysporum f. sp. lactucae (Fol) and was first reported in Japan in 1955. The disease is now distributed worldwide, and four pathogenic races are known. Race 1 has been observed in the U.S.A. since 1990 and became a serious threat in 2001 when it caused significant losses in low desert production areas of California and Arizona. Central coastal California lettuce growers have experienced increased severity and incidence of FW since 2020, where some race 1-resistant cultivars now exhibit susceptible reactions, and some susceptible cultivars exhibit resistant reactions, or reduced symptom severity. Reduced resistance could indicate an increase in inoculum density, but unexpected resistant reactions suggest changes in the Fol population or possible interactions with other soilborne pathogens. We isolated Fol from FW symptomatic plants (Fol321 and Fol621) collected from two Salinas fields showing unexpected FW reactions in 2021. We conducted multiple controlled inoculation greenhouse tests to characterize the isolates on Fol race differential hosts 'Patriot', 'Costa Rica #4', 'Banchu Red Fire', and 'Romabella'. Reactions to Fol321 matched previously reported race 1 reaction patterns. In contrast, reactions to Fol621 are novel. A new Fol race could significantly impact lettuce production. We also 1) assessed reactions of locally (Salinas Valley) important cultivars to assess risk and utility of current international Fol race differentials, 2) characterized Fol321 and Fol621 via molecular sequencing, and 3) conducted field surveys to determine incidence of Fol621 in order to develop FW management strategies including resistance breeding

O24 Keynote - Pathogen Biology

Identification, pathogenomics and resistance to Fusarium oxysporum f.sp. lactucae causing Fusarium wilt in lettuce

John Clarkson[1], Andrew Legg[1], Jamie Pike[1], Nicole Pereira[1], Sascha Jenkins[1], John Connell[2], Helen Bates[2]

1) School of Life Sciences, University of Warwick, Wellesbourne, Warwick, CV35 9EF UK
2) NIAB, Cambridge CB3 0LE UK

Fusarium wilt of lettuce caused by Fusarium oxysporum f.sp. lactucae (FOL) results in severe losses in protected and field grown lettuce. The most significant races of FOL are race 1 (FOL1) which generally affects crops in warmer climates (e.g. USA / Spain) and race 4 (FOL4) which is newly emerged in Northern Europe primarily affecting protected crops. Currently there is little information on the genetic basis for FOL virulence while a lack of multiple resistance sources may lead to the emergence of new races and threaten future lettuce production.

We collected FOL1 and FOL4 isolates from different countries and confirmed identity through sequencing part of the TEF gene, race-specific PCR and presence of certain Secreted in Xylem (SIX genes) often associated with virulence in F. oxysporum. Following nanopore genome sequencing of FOL1 / FOL4 isolates, contigs associated with core genome and pathogenicity chromosomes were identified and putative effectors identified and compared with other F. oxysporum f.spp. This revealed common and unique effectors associated with FOL1 and FOL4. RNA seq identified 12,000 genes in FOL4 upregulated during early lettuce infection of which approx. 150 were identified as putative effectors including SIX8, SIX9, SIX14 and other candidates including some previously identified in F. oxysporum f.sp. apii affecting celery. Resistance to FOL1, FOL4 or both races was identified within a lettuce diversity and mapping populations produced for future analysis.

Overall, this research provides essential information on the genetics of FOL and lettuce resistance to enable durable strategies for control in the future.

O25 Keynote - Pathogen Biology

Shedding light on effector functions in spinach by employing a bacterial Type III Secretion System

Melanie Mendel[1,2], Xander Zuijdgeest[1], Femke van den Berg[1], Leroy van der Meer[1], Joyce Elberse[2], Petros Skiadas[2,3], Michael Seidl[3], Guido Van den Ackerveken[2], Ronnie de Jonge[1]

1) Plant-Microbe Interactions, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH, Utrecht, Netherlands
2) Translational Plant Biology, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH, Utrecht, Netherlands
3) Theoretical Biology and Bioinformatics, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH, Utrecht, Netherlands

Effectors play a critical role in determining the outcome of host-pathogen interactions. In many non-model crops, including spinach, we lack the tools to study the role and function of these effectors. While Agrobacterium-based effector assays are common, we ran into several challenges with this assay on spinach, limiting its use. Therefore, we are exploring alternative strategies for effector-screens on spinach. We established a Type III Secretion System-dependent bacterial effector-delivery platform based on the effectorless Pseudomonas syringae pv. tomato DC3000 mutant D36E. D36E lacks T3SS effectors. We observe no symptoms and limited proliferation in spinach compared to wild type DC3000. Using D36E, we are now able to screen for alterations in disease symptoms, bacterial proliferation and production of reactive oxygen species (ROS), allowing us to assess the impact of effectors on pathogen virulence, immune activation and suppression in spinach. Our results studying native DC3000 effectors show that single effectors can have dramatic effects. AvrE1 and HopM1 alone can largely restore DC3000 virulence, while HopAD1 suppresses ROS-bursts without promoting virulence or causing visible cell death. Overall, our results provide support for the fit of D36E as an effector-delivery platform on spinach. Further development of this tool paves the way for effector screens of spinach pathogens with the goal to identify novel and more durable resistance traits in spinach.

O26 Keynote - Postharvest Traits & market/consumer trends

Vertical farming: Moving from genetic to environmental modification of quality and yield of vegetables?

Leo F.M. Marcelis

Wageningen University, Horticulture and Product Physiology, Wageningen, The Netherlands. Leo.Marcelis@wur.nl

Vertical farming represents a production system where plants are grown under fully controlled conditions. All above- and below-ground conditions can be controlled and light is provided by LEDs, allowing dynamic manipulation of light spectrum and intensity. This enables high yield and quality, using very little use of land area, water, nutrients, and pesticides. However, energy use can costs are high. To compensate for high costs, the quality of vertical farming products should substantially exceed that of conventionally grown crops.

In this talk I will show some examples of how modification of environmental conditions can be used to control the quality of vegetables. To optimise both yield and quality, growth conditions during the initial stages of crop development can focus on yield, while during the last phase the focus can be on quality. For instance increasing the light intensity shortly before harvest, increases vitamin C and carbohydrate content in lettuce, resulting in enhanced shelf life. Anthocyanin accumulation may reduce plant growth, but it is often a desirable quality attribute for consumers. Manipulating the blue fraction of LED light allows to control the anthocyanin concentration in plants.

So far, there has been hardly any breeding for vertical farming. Breeding targets for vertical farming may deviate quite a bit from production in the field or in greenhouse, as the growth conditions are quite distinct. Not much focus is needed on breeding for (a)biotic stress resistance. Thus the trade-off between growth versus defence dilemma is eliminated. Last but not least, vertical farming might offer a tool to breeders for speed breeding.

O27 Postharvest Traits & market/consumer trends

Indoor farming: changing the paradigm of leafy green breeding for cleaner, safer, more nutritious and sustainable crops

Gail Taylor , Yufei Qian, Lauren Hibbert, Guy Robinson

Department of Plant Sciences, University of California, Davis

Indoor farming has the potential to enable cropping in extreme environments, including urban food deserts, arid environments and places with limited natural sunlight and thus, can assist in delivering global food security. They promise a dramatically reduced land and water footprint. Although currently, energy consumption limits sustainability, there seems little doubt that they will have a future role in the complex jigsaw of global food production systems. Leafy greens have been grown extensively in multiple indoor systems, including large warehouse farms already in commercial production and yet, there has been little dedicated plant breeding that targets these indoor environments, which are uniquely different to their outdoor counterparts or to intensive greenhouses using natural light. This talk will consider the changing paradigm of leafy green breeding for indoor growth, in particular how new speed breeding can deliver a number of plant traits of value to the indoor environments including changed architecture, nutrition, flavor and shelf-life. In addition, the way the use of specific wavelengths of light as a driver for this accelerated breeding for leaf quality, enhanced nutrition and shelf-life will be considered. Using watercress as an exemplar, we show that significant natural genetic variation exists for a variety of quality traits in a wild population, when grow in contrasting indoor light regimes, alongside the production of a novel chemo preventative glucosinolate. These data illustrate the significant potential of indoor farming to deliver better quality food for the future.

O28 Postharvest Traits & market/consumer trends

Genetic dissection of commercial and traditional lettuce varieties characterized for vitamin C content

Inés Medina-Lozano[1,3]. Juan Ramón Bertolín[2,3], Aurora Díaz[1,3]

1) Department of Plant Science. Agrifood Research and Technology Centre of Aragon (CITA). Avda. Montañana 930, 50059, Zaragoza, Spain.
2) Department of Animal Science. Agrifood Research and Technology Centre of Aragon (CITA). Avda. Montañana 930, 50059, Zaragoza, Spain.
3) AgriFood Institute of Aragon – IA2 (CITA-University of Zaragoza), Zaragoza, Spain.

Traditional varieties could be valuable resources in breeding programmes as they frequently retain nutrients that could have become “diluted” in the commercial varieties, as is the case in the most consumed lettuce (Lactuca sativa L.) varieties. Furthermore, they are also expected to harbour a high genetic diversity that grants them a great plasticity to face environmental changes.
The genetic variability, structure, and relationships of 23 cultivated lettuces, including green and red commercial varieties and green and semi-red traditional varieties ranging over a broad diversity in vitamin C content, were explored using an Axiom SNP genotyping array with more than 20,000 polymorphic markers.
The genetic differentiation among groups was also calculated using the Wright’s FST statistic and the AMOVA, the last one allowing us to assess the variance not only among groups but also within them.
The correlation matrix of the allele frequencies together with the vitamin C content were used to carry out a PCA to determine the associations among the samples.
In conclusion, traditional varieties could fuel the improvement of the nutritional value of food crops, like lettuce, especially when compared to their wild relatives, as they combine good values of agronomic and quality traits without the undesirable characteristics of undomesticated species. This use of traditional varieties is expected to save time and resources in breeding programmes which explains the pique in interest of those genetic resources by agrifood scientists and breeders.

O29 Postharvest Traits & market/consumer trends

Present and future genomics of lettuce improvement

Richard Michelmore

The Genome Center, University of California, Davis, CA 95616, USA

We are at an inflection point in our ability to analyze and manipulate variation for lettuce improvement.We can now accumulate unprecedented amounts of phenotypic, genetic, DNA sequence, gene expression, proteomic, and metabolomic data of lettuce and its pathogens. We have increasingly powerful computational tools to analyze and visualize this data as well as make predictions and decisions. We also have an increasing array of opportunities for editing the genome to complement traditional breeding approaches. The lettuce genome is becoming increasingly populated with loci determining numerous qualitative and quantitative traits, particularly disease resistance genes. These provide the basis for improving the accuracy of trait selection and marker-assisted selection to accelerate breeding programs. Recent advances in long-read sequencing technologies allow the routine generation of near-gapless, telomere-to-telomere genome assemblies of numerous accessions in the primary, secondary, and tertiary genepools of lettuce. These will form the foundation of a rich pan-genome for lettuce and provides a window into the allelic diversity of horticulturally important genes.Cloning such genes is becoming easier but can still be time-consuming. CRISPR-mediated gene knock-outs for gene validation and creation of recessive alleles is routine; base editing and sequence insertion technologies are still being developed. Utilization of high-throughput phenotyping, spatial genomics, and multi-omic approaches will allow deep insights into lettuce biology that can be harnessed for lettuce improvement. Exploiting the wealth of data will be enhanced by the collaborative spirit that has characterized the lettuce research and breeding community.

Posters

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Poster abstracts

P1 *Loss-of-function of a DMR6 ortholog in lettuce confers broad-spectrum disease resistance*

Michela Appiano*, Kees van Dun*, Yvette van Aesch*, Benoit Copin*, Nathan Jansen*, Johan Schut*

*Rijk Zwaan Breeding B.V

It is widely known that the use of impaired plant susceptibility (S-) genes offers an alternative to the more common practice to introduce single resistant (R-) genes. The benefit of the use of S-genes consists of a more durable (although often partial) resistance against a wider plethora of pathogens. For a sustainable lettuce breeding strategy, this makes them valuable when stacked behind R-genes, which are prone to be rapidly broken.
In Arabidopsis, the gene Downy Mildew Resistance 6 (AtDMR6) encodes for an enzyme identified as a susceptibility factor to bacterial and oomycete pathogens.
In a simple model-to-crop translation performed at Rijk Zwaan, we have identified one major ortholog of AtDMR6 in lettuce located on chr 9.
Following the screening of an EMS mutagenized population, we found a mutant carrying a C/T substitution within the coding sequence of the LsDMR6 allele, which leads to an earlier stop codon (Q54S-->STOP).
This mutant was crossed in eight lettuce backgrounds carrying a wild-type LsDMR6 allele. After the obtainment of NIL lines that differ for the presence/absence of the mutation, we tested them with multiple pathogens and recorded their phenotypes. We noticed in several backgrounds an increased resistance of the NILs carrying the mutated LsDMR6 towards Golovinomyces bolayi, Xanthomonas campestris pv vitians and Fusarium oxysporum f.sp lactuce race 1, which are the causal pathogens of powdery mildew, bacterial leaf spot and fusarium wilt.
As trade-off for the increased resistance, we observed a penalty growth of the lettuce heads, which is a common phenomenon for the impairment of DMR6 also in Arabidopsis.

P2 *Exploring natural variation in lettuce root system architecture*

Anneke Horstman[1], Sieme Pelzer[1], Valerian Meline[2], Renze Heidstra[1]

1) Cell- and Developmental Biology, Wageningen University and Research, The Netherlands
2) Netherlands Plant Eco-phenotyping Centre (NPEC), Utrecht University, The Netherlands

The proper architecture of a lettuce plant (Lactuca sativa) is crucial for maximizing resource acquisition from the roots and forming a healthy shoot while avoiding leaf malformations caused by nutrient deficiencies. In this study, the focus is on identifying key genes that regulate lettuce root system architecture (RSA).
To achieve this, a collection of 500 domesticated lettuce (L. sativa) varieties and wild Lactuca species (L. serriola, L. virosa, and L. saligna) are analyzed to explore natural variation. A genome-wide association study (GWAS) is conducted in hydroponics and plate-grown Lactuca to map loci associated with various root traits, including root/shoot ratio, primary root length, and lateral root length and distribution. The identified genes, along with known root architecture genes from other model species, are genetically modified and functionally characterized in lettuce.

P3 *Towards understanding the growth tradeoff of loss-of-susceptibility to downy mildew by dmr6 mutation*

Tijmen van Butselaar, Dmitry Lapin, Guido van den Ackerveken

Translational Plant Biology, Utrecht University, The Netherlands

The durability of disease resistance is an important trait in plant breeding, particularly for downy mildew resistance in leafy vegetables. Loss-of-susceptibility genetic technologies offer a complementary approach to increase disease resistance. They include modifying host genes used by pathogens for accommodation or to increase access to nutrients, e.g. sugar transporter genes. In other cases, knocking out loss-of-susceptibility loci heightens the level of plant immunity. An example is the DOWNY MILDEW RESISTANT 6 (DMR6) technology where knocking out DMR6-like hydroxylases of the defense hormone salicylic acid leads to increased resistance to downy mildew pathogens. Unfortunately, the use of this technology is, in some cases, associated with growth reduction. Here, we present an approach to understanding this tradeoff. We developed genome-edited mutants of DMR6 and its close homolog DMR6-LIKE OXIDOREDUCTASE 1 (DLO1) in four different Arabidopsis accessions. The double mutants in two genetic backgrounds were resistant to downy mildew but small. Surprisingly, the F2 population from the cross of the two mutants showed plants with recovered rosette growth. These phenotypes were reproduced in the F3 generation, and importantly some F3 lines remained resistant to downy mildew. We are identifying the underlying genes to increase our understanding of the mechanisms behind the epistatic control of the dmr6 growth-defense trade-off in plants.

P4 *Study of powdery mildew distribution on wild Lactuca spp. and lettuce (L. sativa) and their taxonomic identification*

T. Křivánková, M. Kitner, B. Mieslerová, E. Křístková, A. Čurná and A. Lebeda*

Palacký University in Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 27, 783 71 Olomouc, Czech Republic (*corresponding author: ales.lebeda@upol.cz)

Wild Lactuca spp. and cultivated lettuce (Asteraceae) are often infected by three genera of powdery mildews (order Erysiphales), Golovinomyces, Podosphaera, and Leveillula. In the period of 1995-2021 we collected 201 Lactuca spp. leaf samples with symptoms of powdery mildew in 23 countries from four continents. The aims of our study were to identify the powdery mildew species on herbarized leaf samples, to verify the species specification of the Lactuca – powdery mildews pathosystem, and to verify the level of intraspecific variability of pathogens.

Preliminary results show a strong prevalence of Golovinomyces bolayi among the analysed samples. Based on both morphological and molecular data, G. bolayi was identified on 190 samples, whereas Podosphaera xanthii was identified only on 11 Lactuca spp. samples (from China, Japan, South Korea). We identified a single P. xanthii ribotype (present in samples from Asian countries) and four G. bolayi ribotypes, however without a specific geographical pattern. Obtained morphological and molecular data are analysed statistically.

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P5 *Identification of senescence regulators in lettuce using a population of commercial varieties*

Jelmer van Lieshout[1], Thalia Luden[1], Karin Verkerk[2], Sarah Mehrem[3], Basten Snoek[3], Kiki Spaninks[1], Remko Offringa[1]

1) Plant Developmental Genetics, Institute of Biology Leiden, Leiden University, Sylviusweg 72, 2333 BE, Leiden, the Netherlands
2) Green Biotechnology, InHolland Amsterdam, De Boelelaan 1109, 1081 HV Amsterdam, the Netherlands
3) Theoretical Biology and Bioinformatics, Biodynamics and Biocomplexity, Utrecht University, Padualaan 8, 3584 CH Utrecht, the Netherlands

Leaf senescence is a major developmental process that determines the consumer value and shelf life of lettuce. Nevertheless, little is known about its genetic mechanisms. Multiple phenotyping methods have been used to map the variety of leaf senescence susceptibility in a population of 200 L. sativa accessions. Phenotyping was done on adult plants in the field, as well as on young lettuce plants in controlled conditions. We have shown that chlorophyll loss due to dark-induced senescence correlates highly with the green to red ratio of RGB images. Therefore, we developed automated scripts in Fiji to enable high-throughput phenotyping. Initial results indicate a wide variety in the degree of senescence susceptibility between lettuce accessions, as well as between different sub-types. A preliminary GWAS showed multiple associated SNPs, indicating the presence of genetic regulators. Future research will focus on further unravelling the genetic mechanisms of leaf senescence in lettuce. This will be done by comparing expression profiles of senescence-associated genes in resilient and susceptible accessions, as well as verifying the role of candidate genes in an Agrobacterium-mediated transient expression assay.

P6 *Dynamic control of LED-light intensity in vertical farming: growth, metabolic compounds, and electricity savings in lettuce (Lactuca sativa L.) cultivation*

Arttu Mäkinen[1], Hirofumi Ishihara[2], Nina Sipari[2], Paula Elomaa[1], Saijaliisa Kangasjärvi[1,2]

1) Department of Agricultural Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Finland
2) Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Finland
3) Viikki Metabolomics Unit, Organismal and Evolutionary Biology Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Finland

In highly advanced indoor vertical farming (VF) systems, where artificial lighting consumes 70–80% of electricity costs, modern control and automation systems present attractive opportunities for manipulating the light environment and designing lighting regimes. These systems enable growers to plan their lighting regimes in ways that simultaneously facilitate sufficient crop growth and economical electricity consumption through appropriately timed adjustments to light intensity.
Photobiological responses to changing light intensity conditions have mostly been studied in Arabidopsis thaliana, but less so in agricultural crops. In the present study, we tested three dynamically changing lighting regimes where periods of high intensity light were scheduled to occur at distinct times of the photoperiod. Growth and metabolite responses of lettuce (Lactuca sativa L. cv. 'Katusa') as well as production cost-efficiency were studied. The results indicated that moderate light intensity fluctuations may be incorporated into lighting regimes without major disruptions to growth in lettuce cultivation. Most of the primary metabolite compounds accumulated during high light intensity periods as expected. In terms of secondary metabolites, mostly flavonol derivatives accumulated towards the end of photoperiods regardless of changes in light intensity, implying that flavonol biosynthesis could be controlled by the circadian clock. The results suggested that in a scenario where electricity consumption is charged by the hour according to the daily/seasonal electricity market price fluctuations, up to 20–30% reductions in electricity costs (total electricity consumption remaining the same) could be achieved with dynamically adjusting lighting regimes.

P7 *Petromarula pinnata: a threatened local Greek endemic species with potential use as leafy vegetable*

Kapagianni Pantelitsa[*1], Ipsilantis Ioannis[*2], Papanastasi Katerina[*1], Sarropoulou Virginia[*1], Eleni Maloupa[*1], Grigoriadou Katerina[*1]

1) Hellenic Agricultural Organization-DEMETER, Institute of Plant Breeding and Genetic Resources (HAO-DEMETER, IPGRB)– P.C. 570 01 Thermi, Thessaloniki, P.O. Box 60458, PC 57001, Greece
2) Aristotle University of Thessaloniki, Faculty of Agriculture, Soil Science Laboratory – University Campus, 54124 Greece
*Corresponding author's e-mail: kpapanastasi@elgo.gr

Petromarula pinnata (L.) A. DC. is a threatened local Cretan endemic with potential agro-alimentary use. The plant in the wild was highly mycorrhizal, but little is known about its response to fertilization. One year-old seedlings were transplanted in 2.5 L pots with peat (Klasmann KTS2):perlite 3:1 and inoculated with Rhizophagus intraradices, an arbuscular mycorrhizal fungal (AMF) assemblage from P. pinnata rhizosphere soil that was propagated in corn (mix), or were non-inoculated. Plants were monthly treated with nutrient solution containing: (a) an inorganic fertilizer (19-19-19 NPK), (b) a plant-extract based organic fertilizer (3% N, 1.5% organic matter, 2.5% Cu) (c) control. The stems and leaves fresh (SFW) and dry (SDW) weight, the %AMF and tissue N, P, K, Na were assessed after five months.
Interactions were found between fertilization and inoculation treatments for fresh and dry biomass, AMF colonization and N (%). P. pinnata responded to inorganic fertilization when non-inoculated, or inoculated with the mix giving on the average 76.9 (FW) and 72.6 (FW) and 10.8 (DW) and 9.8 gr/plant (DW) respectively. Organic fertilization increased R. intraradices root length colonization, which resulted in a negative balance of mycorrhizal and therefore to smallest plants. N (%) was only higher for the non-inoculated inorganic fertilizer treatment, while P and K were higher overall for the inorganic fertilization. There were no differences for Na. Overall, mycorrhizal inoculation was not beneficial to P. pinnata growth.

P8 *Development and application of single primer enrichment technology (SPET) SNP assay for population genomics analysis and candidate gene discovery in lettuce*

Pasquale Tripodi[1], Massimiliano Beretta[2], Damien Peltier[3], Ilias Kalfas[4], Christos Vasilikiotis[4], Anthony Laidet[5], Gael Briand[5], Charlotte Aichholz[6], Tizian Zollinger[7], Rob van Treuren[8], Davide Scaglione[9], Sandra Goritschnig[10]

1) CREA Research Centre for Vegetable and Ornamental Crops, Italy; 2) ISI Sementi SpA, Italy; 3) LIMAGRAIN - Vilmorin-France; 4) American Farm School, Greece; 5) Gautier Semences, France; 6) Sativa Rheinau AG, Switzerland; 7) Zollinger Conseilles Sarl, Switzerland; 8) Centre for Genetic Resources, the Netherlands (CGN); Wageningen University and Research, the Netherlands; 9) IGA Technology Services Srl, Udine, Italy; 10) ECPGR Secretariat c/o Alliance of Bioversity International and CIAT, Italy

Recent years witnessed astonishing advancements in the development of cutting-edge next-generation sequencing technologies to dissect thegenetic basis of crops and discover genes of interest. Among these, SPET (single primer enrichment technology) is a novel high-throughput genotyping method based on short-read sequencing of specific polymorphic genomic regions, overcoming the limits of other reduced representation library sequencing methods based on a random sampling of genomic loci and allowing the discovery of closely linked, novel SNPs. Here we report the design and application of the first SPET panel in lettuce, consisting of 41,547 probes spanning the whole genome and designed to target both coding (~96%) and intergenic (~4%) regions. A total of 81,531 SNPs were surveyed in 160 lettuce accessions from the ECPGR EVA Lettuce network, originating from 10 countries and representing ten different horticultural types. Model ancestry population structure clearly separated the cultivated accessions (Lactuca sativa) from accessions of its presumed wild progenitor (L. serriola), revealing six genetic subgroups that reflected a differentiation based on cultivar typology. To determine the potentiality of SPET for gene discovery, we performed genome wide association analysis for main agricultural traits in L. sativa. Robust associations were detected for seed colour, outer leaf colour, leaf anthocyanin and bolting in chromosomal regions previously reported to hold candidate genes for these traits. Our research demonstrates the reliability of SPET for detecting polymorphisms to dissect the genetic diversity of lettuce collections and identify candidate genes for traits of agricultural interest, enabling a better use of lettuce germplasm.

P9 *Far red light regulates shoot and root architecture in Lettuce*

Siddhant Shetty[1], Leonardo Jo[1], Basten Snoek[2] and Ronald Pierik[1]

1) Plant-Environment Signaling, Department of Biology, Utrecht University
2)Theoretical Biology and Bioinformatics, Department of Biology, Utrecht University

Increasing demand necessitates growing crops in high density planting systems. In high dense planting systems, plants receive light reflected from their proximate neighbors that is strongly enriched in the far red (FR) part of the spectrum. Plants detect FR light with phytochrome photoreceptors and respond with so-called shade avoidance responses. These responses include elongated hypocotyl and internodes and a more upward orientation of leaves (hyponasty), but FR enrichment aboveground also has effects belowground where it inhibits lateral root formation primary root elongation. Under experimental conditions, these phenomena can be studied by exposing plants to additional FR light from dedicated LEDs as addition to a standard white light background. We study, as members of the LettuceKnow consortium, how FR enrichment regulates Lettuce (L. sativa) shoot and root growth and architecture. To this end we combine genetic variation screens using different lettuce varieties, RNA sequencing of shoot and root tissues of contrasting lettuce varieties and dedicated physiological and molecular biology approaches. These integrated approaches will provide new insights into our understanding developmental plasticity in lettuce under varying light quality regimes.

P10 *The International Working Group on Peronospora in spinach*

D. Smilde

Naktuinbouw, Sotaweg 22, 2371 GD Roelofarendsveen

Downy mildew in spinach, caused by the Peronospora effusa, is the common enemy of spinach breeders and growers. The success of resistance breeding depends on a harmonious collaboration between breeders and scientists for monitoring of the disease.

The International Working Group on Peronospora in spinach (IWGP), consisting of spinach breeders, Naktuinbouw (responsible for official variety registration) and an independent scientist, monitors the spinach downy mildew population by taking samples of the pathogen from breeders' or growers' fields, usually from varieties with known resistance. These isolates are analysed on a set of differential varieties, representing different resistances that are available in commercial varieties. The virulence patterns of the isolates are compared, and any new pattern that has a significant impact on the spinach industry qualifies as a candidate for a denomination of a new race.

A new race will be denominated when the virulence pattern is persistent and re-occurring in different spinach growing regions. Additionally, a stable type-isolate should be available. This procedure allows clear, unambiguous, consensus-based communication about new resistances in the market.
Before 2003, four races of spinach downy mildew were generally accepted by scientific researchers and breeders. IWGP was established in 2003, in response to the need to define type isolates of Pe: 5 , 6 and 7. Annual evaluations have resulted in more denominations. Pe: 18 and 19 were denominated in 2021.

P11 *Identifying Genes Linked to Rapid Rooting in Lettuce*

C. Wharton [1], M. Gifford [2], A. Beacham [1], J. Monaghan[1]

1) Centre for Crop and Environmental Science, Harper Adams University, TF10 8NB, UK.
2. School of Life Sciences, University of Warwick, CV4 7AL, UK

Lactuca sativa (lettuce) is a high-value vegetable grown predominantly for its edible leaf. However, in efforts to breed high yield of quality leaves, root vigour has been overlooked. A rapid-rooting system, with increased primary root length, increased total lateral root length and increased number of lateral roots, could enhance overall plant health, yield and tolerance to stresses including root pruning which occurs during transplantation in horticulture. Previous work by Roberts et al. (2020) identified L. sativa cv. Iceberg to be rapid-rooting and L. sativa cv. Saladin to be slow-rooting. In addition, root phenotyping of RILs (Recombinant Inbred Lines) from an intra-specific cross between Saladin and Iceberg was performed. This study used bioinformatic tools to compare RNA sequencing data, from the roots of Iceberg and Saladin parent cultivars, as well as two rapid-rooting RILs and two slow-rooting RILs. This revealed 21 genes differentially expressed in both parents and RILs. These are likely gene candidates for rapid-rooting involvement and would make interesting targets for future research and perturbation studies.
This work was supported by MIBTP, BBRSC and Syngenta.

P12 *Pan-genomics uncover mechanisms behind the rapid evolution of spinach downy mildew*

Petros Skiadas[1,3], Sofía Riera Vidal[1], Melanie Mendel[2,3], Joyce Elberse[3], Ronnie de Jonge[2], Guido Van den Ackerveken[3], Michael F. Seidl[1]

1) Theoretical Biology and Bioinformatics
2) Plant-Microbe Interactions
3) Translational Plant Biology, Utrecht University, Padualaan 8 3584 CH, Utrecht, The Netherlands

The extensive deployment of genetic disease resistances in crops exerts strong selective pressure on pathogens, which can rapidly break resistances. This rapid evolution is driven by the diversification of effectors, secreted proteins that are employed by plant pathogens to establish host colonisation. In contrast to the hundreds of predicted effector proteins very few have been functionally characterised, and consequently their function, genetic diversity, and the evolutionary processes that contributes to their diversification are largely unknown. Here, we generated complete and fully phased diploid genome assemblies of six racesof Peronospora effusa, an obligate biotrophic oomycete that causes downy mildew on spinach, which is the economically most important disease in cultivated spinach worldwide. Each P. effusa race encodes almost 400 predicted host-translocated effectors belonging to the RXLR and Crinklier families. Using Alphafold2 for computational structural genomics, we uncovered conserved structural folds in the N-termini of Crinkler and in the C-termini of Crinkler and RXLRs effectors. We furthermore described the genomic organisation of P. effusa effectors and observed effector genes that cluster in few distinct genomic regions. These physically clustered effectors also cluster based on protein sequence and fold. A pan-genomic analysis of the six races revealed a highly conserved chromosome structure with few highly variable regions enriched in repetitive elements and clustered effector genes. The diversification of these regions is driven by retroduplication, which results in a high number of pseudogenes. Summarizing, we here demonstrate how pan-genomics complemented with computational structural genomics can uncover the evolution of P. effusa and provide the framework for further research into the molecular mechanisms underlying the interactions between P. effusa and spinach.

P13 *Now you see me: UV light reveals hidden symptoms of lettuce downy mildew*

Sebastian Tonn[1], Mon-Ray Shao[1], Jos de Wit[2], Andrew Pape[1], Rami Mousa[1], Iñigo Bañales Belaunde[1], Roy van Beekveld[3], Arjen N. Bader[4], Henriette D. L. M. van Eekelen[5], Ric C. H. de Vos[5], Eefjan Breukink[3], Jeroen Kalkman[2], Guido van den Ackerveken[1]

1) Translational Plant Biology, Utrecht University, Utrecht, The Netherlands
2) Department of Imaging Physics, Technical University Delft, Delft, The Netherlands
3) Membrane Biochemistry and Biophysics, Utrecht University, Utrecht, The Netherlands
4) Laboratory of Biophysics, Wageningen University & Research, Wageningen, The Netherlands
5) Business Unit Bioscience, Wageningen Plant Research, Wageningen, The Netherlands

Lactuca sativa (lettuce) is a high-value vegetable grown predominantly for its edible leaf. However, in efforts to breed high yield of quality leaves, root vigour has been overlooked. A rapid-rooting system, with increased primary root length, increased total lateral root length and increased number of lateral roots, could enhance overall plant health, yield and tolerance to stresses including root pruning which occurs during transplantation in horticulture. Previous work by Roberts et al. (2020) identified L. sativa cv. Iceberg to be rapid-rooting and L. sativa cv. Saladin to be slow-rooting. In addition, root phenotyping of RILs (Recombinant Inbred Lines) from an intra-specific cross between Saladin and Iceberg was performed. This study used bioinformatic tools to compare RNA sequencing data, from the roots of Iceberg and Saladin parent cultivars, as well as two rapid-rooting RILs and two slow-rooting RILs. This revealed 21 genes differentially expressed in both parents and RILs. These are likely gene candidates for rapid-rooting involvement and would make interesting targets for future research and perturbation studies.
This work was supported by MIBTP, BBRSC and Syngenta.

List of participants

Name	Organisation	E-mail	Abstract page
Aarts, M.	Lab of Genetics, Wageningen University & Research	mark.aarts@wur.nl	27
Ackerveken, van den, G.	Utrecht University	g.vandenackerveken@uu.nl	6, 40, 49
Alcubierre, L.	Ramiro Arnedo SA	administracion@ramiroarnedo.com	
Anton Sales, C.	Wageningen University & Research	celia1.antonsales@wur.nl	22
Appiano, M.	Rijk Zwaan Breeding	b.schouw@rijkzwaan.nl	47
Bañales, I.	Utrecht University	i.banalesbelaunde@uu.nl	
Bai, Y.	Wageningen University & Research	bai.yuling@wur.nl	6
Beharav, A.	Institute of Evolution	abeharav@univ.haifa.ac.il	35
Bergh, van den, E.	Utrecht University	e.s.vandenbergh@uu.nl	
Berens, M.	Keygene	matthias.berens@keygene.com	21
Bijlmer, F.	Utrecht University	f.a.bijlmer@uu.nl	6
Blom, D.	Enza Zaden	d.blom@enzazaden.nl	
Bonnema, G.	PlantBreeding, WUR	Guusje.Bonnema@wur.nl	22
Boutilier, K.	Wageningen University & Research	kim.boutilier@wur.nl	28
Bragalini, C.	ISI Sementi Spa	marketing@isisementi.com	
Caro, M.	Rijk Zwaan B.V.	m.caro@rijkzwaan.nl	33
Clarkson, J.	University of Warwick	john.clarkson@warwick.ac.uk	38
Corral Martínez, P.	BASF Nunhems Netherlands	patricia.corralmartinez@vegetableseeds.basf.com	
Correl, J.	University of Arkansas, USA	jcorrell@uark.edu	
Damen, P.P.	Rijk Zwaan Breeding B.V.	p.barendse@rijkzwaan.nl	
Daverdin, G.	Enza zaden	g.daverdin@enzazaden.nl	
Deleu,W.	Ramiro Arnedo SA	administracion@ramiroarnedo.com	
Denby, K.	University of York	katherine.denby@york.ac.uk	6, 32
Díaz, A.	1) Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA); 2) Instituto Agroalimentario de Aragón (IA2)	adiazb@cita-aragon.es	43
Donati, F.	ISI Sementi	marketing@isisementi.com	
Ganal, M.	SGS Institut Fresenius GmbH TraitGenetics Section	martin.ganal@sgs.com	30
Garcia, A.	Meridiem Seeds	Alfonsogarcia@meridiemseeds.com	
Geraats, B.	BASF (Nunhems Netherlands BV)	bart.geraats@vegetableseeds.basf.com	
Giordani, G.	Enza Zaden	g.giordani@enzazaden.nl	
Goritschnig, S.	ECPGR	s.goritschnig@cgiar.org	31,53
Haanstra, J.	Rijk Zwaan Breeding	j.haanstra@rijkzwaan.nl	
Haverkorn, I.	Bejo Zaden B.V.	iris.haverkorn@bejo.nl	
Heidstra, R.	Wageningen University & Research	renze.heidstra@wur.nl	48
Helderman, T.	Takii Europe B.V.	the@takii.eu	
Hendriks, A.	Plant Breeding, Wageningen University & Research	anouk1.hendriks@wur.nl	

List of participants

Name	Organisation	E-mail	Abstract page
Hoff, B.	Incotec Europe BV	liesbeth.henningheim@incotec.com	
Hoogendam, A.	BASF Vegetable Seeds	annick.hoogendam@vegetableseeds.basf.com	
Horstman, A.	Wageningen University and Research	anneke.horstman@wur.nl	6, 48
Huizinga, S.	University of Amsterdam	s.huizinga@uva.nl	
Jeuken, M.	Plant Breeding group, Wageningen University	marieke.jeuken@wur.nl	22
Jong, de, E.	Bejo Zaden	eliza.de.jong@bejo.nl	
Kamp, L.	Bejo Zaden	laurens.kamp@bejo.nl	
Kokkinos, K.	Enza Zaden	k.kokkinos@enzazaden.nl	
Kollerie, N.	Bejo Zaden	nicole.kollerie.bejo.nl	
Koukounaras, A.	Aristotle University of Thessaloniki	thankou@agro.auth.gr	23
Krivánková, T.	Rijk Zwaan Breeding	t.Krivankova@rijkzwaan.nl	
Krysiak, M.	Sakata UK Ltd.	magdalena.krysiak@sakata.eu	
Kuijpers, R.	Enza Zaden	R.Kuijpers@enzazaden.es	6
Kuin, P.	KWS Vegetables	pkuin@popvriendseeds.nl	
Laan, R.	Bejo Zaden	raimon.laan@bejo.nl	
Lapin, D.	Utrecht University	d.lapin@uu.nl	6, 49
Lebeda, A.	Palacký University in Olomouc	ales.lebeda@upol.cz	19, 35, 50
Lee, van der, T.	Wageningen Plant Research	theo.vanderlee@wur.nl	29
Leeuwen, van, I.	Breedwise BV	ivleeuwen@breedwise.nl	
Leijen, M.	Rijk Zwaan Breeding	m.leijen@rijkzwaan.nl	
Lighthart, J.D.	Bejo Zaden	jan.dick.lighthart@bejo.nl	
Lieshout, J.	Leiden University	j.van.lieshout@biology.leidenuniv.nl	51
Lisi, L.	Bejo Zaden	lorenzo.lisi@bejo.nl	
Lokossou, A.	Syngenta	anoma.lokossou@syngenta.com	
Mäkinen, A.	University of Helsinki	arttu.t.makinen@helsinki.fi	52
Marcelis, L.	Wageningen University	Leo.marcelis@wur.nl	41
Masse, C.	Gautier Semences	clemence.plissonneau@gautiersemences.com	
Mendel, M.	University Utrecht	m.n.mendel@uu.nl	
Michelmore, R.	University of California, Davis	rwmichelmore@ucdavis.edu	44
Monaghan, J.	Harper Adams University, UK	jmonaghan@harper-adams.ac.uk	57
Most, W.	Utrecht University	w.w.most@uu.nl	6
Musseau, C.	Syngenta	constance.musseau@syngenta.com	
Nadiradze, K.	Association for Farmers Rights Defense, AFRD	nadiradzekakha@gmail.com	
Nganga, h, J.	Bontat company limited	Info@bontatcomltd.com	
Op Den Camp, R.	KeyGene	rca@keygene.com	20

List of participants

Name	Organisation	E-mail	Abstract page
Papanastasi, K.	Hellenic Agricultural Organization-DIMITRA, Inst. Of Plant Breeding and Genetic Resources	kpapanastasi@elgo.gr	53
Paponov, I.	Aarhus University	ivpa@food.au.dk	26
Pauls, A.	Wageningen University	alan.pauls@wur.nl	27
Pel, M.	Enza Zaden Research & Development B.V.	m.pel@enzazaden.nl	35
Peltier, D.	Limagrain	damien.peltier@limagrain.com	54
Peña, J.	Ramiro Arnedo SA	administracion@ramiroarnedo.com	
Perrot, S.	GEVES	sophie.perrot@geves.fr	
Pezard, J.	Vilmorin-Mikado	jade.pezard@vilmorinmikado.com	
Plissonneau, C.	Gautier Semences	clemence.plissonneau@gautiersemences.com	
Poluzzi, G.	ISI Sementi	marketing@isisementi.com	
Prakash, G.	BASF Vegetableseeds	gowtham.prakash@vegetableseeds.basf.com	
Proveniers, M.	Utrecht University	m.proveniers@uu.nl	
Richardson, K.	US Dept of Agriculture, Agricultural Research Service	kelley.richardson@usda.gov	38
Riet, ter, B.	Enzazaden	b.terriet@enzazaden.nl	35
Rijk, J.	Pop Vriend Seeds	jrijk@popvriendseeds.nl	
Roobeek, I.	Enza zaden	i.roobeek@enzazaden.nl	6, 35
Sandoya, G.	University of Florida	gsandoyamiranda@ufl.edu	25
Schachtschabel, J.	Bejo Zaden	Joelle.Schachtschabel@bejo.nl	
Scheurwater, T.	Bejo Zaden	tom.scheurwater@bejo.nl	
Schimmel, B.	Utrecht University	b.c.j.schimmel@uu.nl	
Scholten, O.	Wageningen University & Research	olga.scholten@wur.nl	36, 37
Schranz, E.	Wageningen University & Research	eric.schranz@wur.nl	17
Schreurs, D.	BASF (Nunhems Netherlands BV)	daan.schreurs@vegetableseeds.basf.com	
Schut, J.	Rijk Zwaan Breeding	j.schut@rijkzwaan.nl	34, 47
Sengers, M.	KeyGene	ms@keygene.com	
Shetty, S.	Utrecht University	siddhantsshetty@gmail.com	55
Simons, P.	BASF (Nunhems Netherlands BV)	philip.simons@vegetableseeds.basf.com	
Severing, E.	Wageningen University & Research	edouard.severing@wur.nl	18
Smilde, D.	Naktuinbouw	d.smilde@naktuinbouw.nl	33, 56
Smit, F.	Bejo Zaden	femke.smit@bejo.nl	
Taylor, G.	University of California, Davis	gtaylor@ucdavis.edu	42
Testerink, C.	Wageningen University & Research	christa.testerink@wur.nl	24

List of participants

Name	Organisation	E-mail	Abstract page
Tommasi, S.	Gautier Semences	clemence.plissonneau@gautiersemences.com	
Treuren, van, R.	WUR-CGN	robbert.vantreuren@wur.nl	18, 31, 54
Truong, L.	Rijk Zwaan Breeding	l.truong@rijkzwaan.nl	
Veenstra, R.	Bejo Zaden	r.veenstra@bejo.nl	
Vegchel, van, M.	Plantum	m.vanvegchel@plantum.nl	6
Wharton, C.	Harper Adams University	CWharton@live.harper.ac.uk	57
Zagajeski, R.	3 Star Lettuce, LLC	Rzagajeski@3starlettuce.com	