Adapting *Watsonia pillansii* as a new bedding, container, and perennial garden crop plant: Determine vernalization length and establish a cleaning and micropropagation protocol

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Work Location: Plant Growth Facilities, University of Minnesota, 1552 Gortner Ave, St. Paul, MN 55108

Focus on socially disadvantaged/limited resource farmers/ranchers: No

Project Category: Crop Production

Commodity category: Ornamentals & Turf

Summary

This proposal focuses on adapting *Watsonia pillansii* as a new bedding, container, and perennial garden crop plant through determining its vernalization length and establishing a cleaning and micropropagation protocol. *Watsonia pillansii* is a versatile South African cormous perennial geophyte that can potentially be grown as a new crop plant for home gardens as well as for the floriculture industry because of its attractive, orange-yellow to red flowers. *Watsonia pillansii* has a wide range habitat and is well-adapted to Mediterranean-type climates and tolerant of low-moisture, sandy conditions, which make it a unique addition to any garden.

This project aims at determining the length of vernalization required for this species to respond to flowering cues, the minimum age at which an individual can respond to vernalization as well as establishing a preliminary cleaning protocol and a feasible micropropagation protocol. Vernalization length requirements and minimum plant age for vernalization will be tested by creating different age groups of plants based on corm weight and treating groups to different lengths of vernalization. Successful vernalization will be evaluated as plants developing flowers after the treatment; data such as size of the inflorescence, time to blooming from end of treatment, and flower size as well as plant morphological characteristics (plant height, leaf number) will be recorded. After a preliminary cleaning process is developed, a micropropagation protocol will involve bud tissues extracted from cleaned corms and grown on different combinations of plant growth regulators such as auxins and cytokinins. Successful micropropagation will be evaluated as successful regeneration of new, genetically identical plantlets from bud tissue grown in a stable sterile environment. Number of new shoots, callus formation, size of plantlets, and the amount of time taken for generation of these characters will be recorded.

For garden centers and the floriculture industry, growing *Watsonia pillansii* successfully in tissue culture will allow cheap, efficient, and fast propagation of the plant, while determining the vernalization and age requirements the plant needs to flower will speed up seed generation as well as production of flowering-size plants. The realization of both goals will be the first steps to establishing *Watsonia pillansii* as a new garden perennial. Flower and bulb farmers will benefit from the addition of this new crop to their expertise as it is a novel, beautiful plant that can fit in a unique garden niche and it can also serve as a good pollinator plant providing ecosystem benefits.

Description

This project aims at determining the proper vernalization length and plant age for vernalization for *Watsonia pillansii* and establishing a preliminary cleaning and micropropagation protocol for this species. The experiments will justify the plant's potential to become a new horticultural and floricultural crop, and will ultimately benefit garden centers and flower farmers by including this unique plant in the floriculture industry in North Central region.

Outcomes

The target audience of this project involves flower farmers, nurseries, and other growers, gardeners, plant collectors, etc. Both beneficial learning and action outcomes for the North Central region will be generated from this project. Successful outreach of this project will allow the target audience and the general public to gain a better understanding of *W. pillansii*, its growth requirements, and their merits as a novel perennial garden and cut-flower potential. Consumers may ask for this plant by-name in garden centers or specialty plant stores if outreach is successful. The public will also gain an understanding of *Watsonia pillansii* as a pollinator plant, such as how it can attract bees and butterflies, as well as its benefits to the ecosystem by providing a continuous green cover. This may be indicated by the recognition of the name *Watsonia* by gardeners and other members of the public, and formal and informal meetings with growers and gardeners, along with surveys can be conducted to gauge the target audience's knowledge on the plant.

Along with the learning outcomes, the target audience and the public's willingness to purchase, grow, and propagate this plant will also indicate successful outreach. The success of this project will motivate gardeners and growers to incorporate *Watsonia pillansii* as a novel plant that fulfills an ecological niche into their gardens and landscapes. The publications and outreach

activities based on this project will enable growers to achieve fast propagation of the plant and speed up seed generation as well as production of flowering-size plants. *W. pillansii* can be marketed and offered for sale in large retail stores or smaller plant businesses. It can also be distributed throughout a gardening community on a friendly, non-professional basis. Increasing demand for growing and purchasing the plant and the observation of increasing instances of *W. pillansii* in gardens are indicators of the successful outcomes of this project.

Statement of Problem, Background, Justification

Background of *Watsonia*

Watsonia, or bugle lily, is a genus of cormous, showy perennials native to southern Africa (South Africa, Lesotho, Swaziland) in the Iridaceae family, closely related to garden favorites such as *Crocus, Freesia, Sparaxis, Ixia,* and Gladiolus. There are 52 accepted *Watsonia* species, all of which grow from underground corms and produce long inflorescences of showy flowers. As such, they have potential to become cut-flower or landscape crops (Thompson et al., 2011). Because of the colorful flowers of most species of the Iridaceae family, many of them are found outside their native range for horticultural purposes (Manning et al., 2002; van Kleunen et al., 2007). *Watsonia* was first introduced as garden ornamentals in perennial gardens and rock gardens in Australia in the mid-19th century owing to the similarities in climate between certain regions of southwest/western Australia and South Africa, and they became widely cultivated during the 1940s. Nowadays, *Watsonia aletroides, W. borbonica, W. marginata,* and *W. meriana* have been naturalized outside its native range (van Kleunen et al., 2007). Generally, *Watsonia* enjoys Mediterranean-type climates, while some are adapted to low-moisture, sandy conditions or marshy areas. Several species eventually became naturalized after escaping cultivation, establishing themselves near areas of high moisture such as riverbanks, wetlands, and anywhere

that has seasonally wet soil. When this occurs, *Watsonia* often displaces native flora in the understory that they inhabit, becoming invasive as they form competitive, dense clumps. Some species, such as *W. meriana* var. *bulbillifera*, has now become a weed in New Zealand, Réunion, Mauritius and the northern coast of California due to its exceptional asexual propagation ability, as it forms many bulbils in clusters in the axils of bracts as well as nodes along the peduncle of the inflorescence (Cape Bugle LILY, n.d.). In their native range, *Watsonia* are ecologically valuable as they provide a reliable, widely available food source for animals.

Watsonia pillansii

Also called Pillans' *watsonia*, *summer watsonia*, or *Beatrice watsonia*, *W. pillansii* is found in the eastern half of southern Africa, ranging from the Langeberg near Swellendarm and Outeniqua Mountains of George in the Western Cape, as well as the coastal mountain ranges of the Eastern Cape. It is commonly found at around 2400m altitude in grassy areas as well as rocky conditions (Batten et al., 1975). Although *Watsonia pillansii* is a species with a wide range, it doesn't commonly show geographical variation (Goldblatt, 1989). Like many other species in the genus, *W. pillansii* grows in full-sun in well-drained, well-composted soil with plenty of moisture year-round (especially during summer months) and produces tall spikes of orange flowers during the late spring and summer. It is evergreen with broad, grey-green strap-shaped leaves, growing to 120cm tall, and does not die back at the end of its growing season or during winter unlike other species (See Fig. 1-3). *Watsonia* keeps its foliage in winter, it requires protection from hard frosts and is half hardy. These characteristics lends it well to being a component of coastal gardens (Foden et al., 2005). As *W. pillansii* propagates itself readily via offsets (cormels) underground, it forms large clumps and may potentially become invasive. It is commonly propagated via seed, but seeds have a very long germination time (varying from 3 to

6 weeks) and requires at least 3 years to flower. It does not have known medicinal uses and is not a valuable food source for human consumption.

Fig. 1. *Watsonia pillansii* growing in South Africa
(Images from http://www.africanplants.senckenberg.de/)

Fig.2 *Watsonia pillansii* showing its tolerance of harsh growing conditions in the wild.
http://www.africanplants.senckenberg.de/

Fig. 3 Blomming *Watsonia pillansii*
https://www.gardenia.net/plant/watsonia-pillansii

This proposal focuses on the adaptation of *Watsonia pillansii* as a new bedding, container, and perennial garden crop plant. The background essentials for *Watsonia pillansii* include the following:

Taxonomy/Taxonomic Description: *Watsonia pillansii, Iridaceae* (Pillans' watsonia, summer watsonia, Beatrice watsonia) *Watsonia* is a monophyletic genus in the family Iridaceae, closely related to genera such as *Thereianthus*, *Micranthus*, *Savannosiphon*, and *Lapeirousia*. A sister tribe is *Ixieae* (See Fig. 4). *Watsonia* are characterized by specialized floral style branching, deeply divide for half of their length. This is also found in other genera of *Ixieae* such as *Freesia* and *Anomatheca*, presumably the product of convergent evolution. Another unique trait possessed by *Watsonia* is its absence of flavone o-glycosides in their tissues, compounds which are found in *Iridaceae* and Ixieae. *Watsonia pillansii* is diploid, with 2n=18 (Goldblatt, 1989).

Geographic range: Eastern half of southern Africa (from the Langeberg near Swellendarm and Outeniqua Mountains of George in the Western Cape, coastal mountain ranges of Eastern Cape. Found at 2400 m altitude (Batten et al., 1975))

Climatic conditions: Coastal grassy areas as well as rocky conditions, full sun, well-drained, well-composted soil with widely available moisture

Tendency to become invasive: Some species have been reported as invasive in New Zealand, Réunion, Mauritius and the northern coast of California; no information regarding invasiveness known for this species

Native habitat: Southern/Coastal Africa

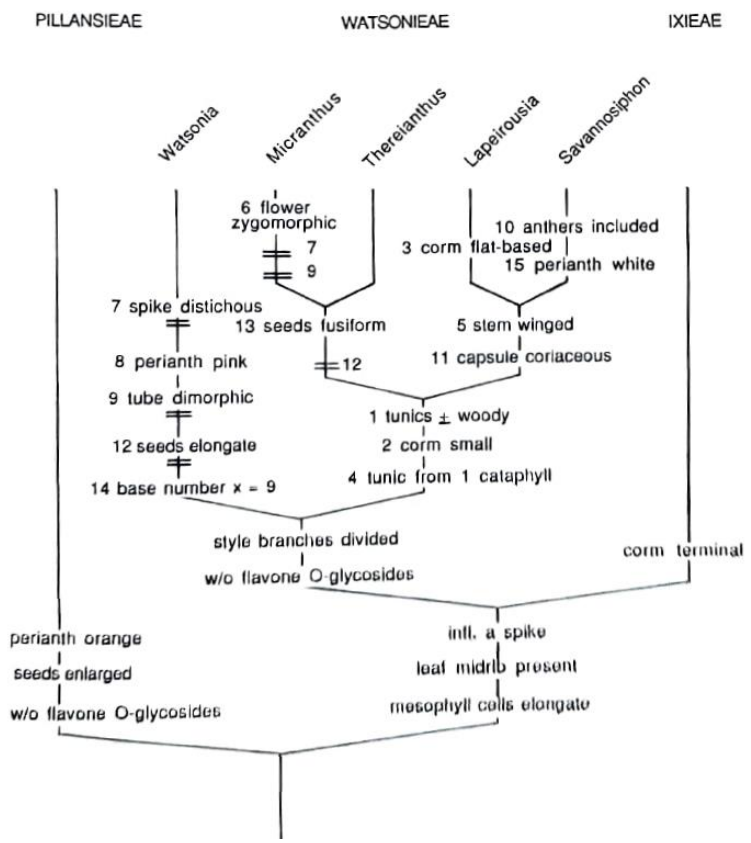

Figure 4 Cladogram of the genus *Watsonia* (Goldblatt, 1989).

Like other *Watsonia* species, *W. pillansii* produces tall, erect foliage that is light green around 120cm tall, as well as inflorescences which reach the same height. It grows from a subterranean corm that reaches around 10cm in diameter. Unlike other *Watsonia*, this species is evergreen, though some leaves may die back during the cold season, but there are always a few leaves that remain green. It produces a raceme of orange, orange-yellow, or pink flowers in the late spring or early summer, lasting until late summer or early fall (Goldblatt, 1989).

Uses by indigenous people(s): None

Edibility: Not used as a food or forage crop, though corms act as food sources for native animals

Useful medicinal properties: None

Sustainable production: Care should be taken to prevent possible naturalization or invasiveness.

New crop species history and potential uses

The first collection of *Watsonia pillansii* was collected in the 1820s and placed in *W. angusta* or *W. meriana*; it was only in 1921 when it was recognized as distinct by Pillans in Montagu Pass, George (Goldblatt, 1989). There is not much information about when *Watsonia pillansii* entered cultivation, and there does not seem to be any cultivars of the species. It is one of the few *Watsonia* that are cultivated as a species rather than as a cultivar or hybrid. This plant is currently most commonly seed propagated, but for a large-scale production scheme (i.e. for growers or farmers), asexual propagation (such as through division of cormels and tissue culture) will most likely be the fastest method. For the consumer, both seeds and divisions may be desirable. One reason why micropropagation should be the primary way of propagation for commercial growers is its theoretical speed compared to seed propagation, which may take up to 6 weeks for germination then a subsequent 3 years for first flowering. Micropropagation is an important tool to facilitate research and development in plant science, and successful tissue culture protocols have been established for relatives of *Watsonia* such as gladiolus. Micropropagation will allow environmental conditions affecting physiological development of the plant to be controlled, thus allowing faster maturity and growth. Four species of *Watsonia* (*W. gladioloides, W. lepida, W. laccata, and W. vanderspuyiae*) have been successfully grown *in vitro* (Ascough et al., 2007). Ultimately, this species may be used as an evergreen perennial in coastal climate gardens or rock gardens in mild, Mediterranean climates to provide dark green foliage accents and bright orange flowers (Proches et al., 2006). Application of dwarfing chemicals can also allow this species to become a suitable container and bedding plant; this method has been shown to be successful to

convert other tall species into suitable pot plants (Thompson et al., 2005)

Problem and justification

Although *Watsonia* has become a somewhat popular plant in Australia, it is not as common in other areas, and not much information is known about lesser-grown species such as *Watsonia pillansii*. Because of its numerous showy flowers per plant along with its tendency of blooming and growing well at cooler temperatures, *Watsonia pillansii* is a great potential for the ornamental market in North America. Due to its herbaceous perennial nature, *W. pillansii* can be a semi-hardy garden or potted perennial. In addition to its elegance show of color and form, Wastonias make superb garden subjects because of their tough, hardy nature, and proven ease of cultivation and low maintenance in parks and private gardens in southern Africa, Australia, western North America, especially California, and in southern Europe (Goldblatt, 1989). *W. pillansii*'s production information can be summarized as following:

Life cycle: Herbaceous perennial in nature; *W. pillansii* can be a semi-hardy garden or potted perennial.

Plant characteristics: Grows up to 120 cm tall without PGRs, has long, light green foliage reminiscent of Gladiolus and other related plants; may be reduced to dwarf size with paclobutrazol. Has a tall, slightly curved inflorescence with orange-red flowers that may be suitable as a cut-flower. Remains evergreen in winter.

Winter hardiness: Hardy from USDA Hardiness zones 6 to 7, may survive in zone 5 with protection; USDA Heat zones 8 to 9.

Potential Production Environment

If started from seed, plants can be sown in spring or autumn 3-4mm deep in small pots (at least

10cm in height) of well-drained compost. Pots should be covered with a thin cover of sand and kept at 15-20C; germination should occur in 5 to 6 weeks. Light should be kept at long day durations (12 to 14 hours); direct sunlight or full-spectrum light is preferred. After germination, temperature should be raised to 16-21C with nighttime temperatures being cooler (14-16C). Osmocote fertilizer (15-9-12) will be given when the plants are in their first growing season. As plants near the end of the growing season, day length should be shortened accordingly and plants will be exposed to a vernalization period at 4.5C for 1 month (Due to the evergreen nature of this species, vernalization should be done in a lighted growth chamber set to short days). Some leaves may die back and those should be removed. The plant is naturally pest and disease free, but aphids and similar insects may occasionally occur; these can be controlled with biological controls such as parasitic wasps or predatory nematodes. In the next season, plants may begin flowering around late spring or early summer. When this occurs, deadheading should be done to encourage more flowers. Fertilization should be switched to one with higher concentrations of phosphorous to encourage larger corms as well as replenish corms after flowering (Gardening in South Africa, n.d.).

Marketing story

From the cape of southern Africa comes a new *Watsonia* that provides long-lasting, spectacular sprays of bold pink or orange-yellow flowers from spring to fall. Native to mountains and coastal areas of southern Africa, the Beatrice watsonia makes a great addition to temperate, coastal perennial and rock gardens where it can be used as a striking highlight. Unlike other species of *Watsonia* that you may be familiar with, the Beatrice watsonia is evergreen, allowing gardeners to enjoy their long, strap-shaped light green foliage year-round. Because of the numerous flowers per plant, one planting can produce color for as much as four weeks! Other related and beloved

plants of the Beatrice watsonia include gladiolus and crocosmia, but unlike them, the Beatrice watsonia offers a unique, bugle-shaped blooms that come in orange, yellow, or pink. In addition, it is proven to be tough and hardy (down to zone 6) with ease of cultivation and low maintenance. Enjoy this glory of southern African flora as a superb new bedding, container, and perennial garden crop plant, and see how it performs in comparison to its tender relatives.

Impact on sustainable agriculture in the North Central Region

Watsonia pillansii is a perennial, clump-forming plant that may provide ecosystem benefits such as granting constant green cover because of its evergreen nature as well as its usefulness as food for pollinators such as bees and butterflies. When clumps are large, this species may crowd out weeds and hold down soil with its rhizomatous root system, reducing erosion as well as water inputs and nutrient run-off. However, care should be taken to not let the corms expand past garden boundaries in order to avoid the possibility of become naturalized. As a new crop that can serve in the coastal/rock garden niche, flower/bulb growers stand to profit by offering this new plant to gardeners who may have not tried *Watsonia* in their gardens yet. When incorporated in a pollinator garden, this species will likely perform well alongside other classics of similar color and stature such as bee balm and cardinal flower. As a new perennial crop, the plant can improve soil health by reducing compaction through its root systems and decreasing topsoil fertilizer/pollutant runoff, and provide food for native pollinators and animals; thus if managed properly, the plant is sure to have positive impact on surrounding communities and sustainable agriculture through performing beneficial services.

Approach and methods

This proposal aims to determine the minimum corm size required for flowering, assess the optimal age and length for vernalization, as well as establish a micropropagation protocol to

allow mass-production at a commercial scale. Ultimately, this proposal will help in aiding growers (both professional and private) in producing this species at a large scale through micropropagation and growing the plant to be a new bedding, container, and perennial garden crop plant.

Many *Watsonia* species are facing habitat loss in their natural environments due to encroachment of agriculture (Gardening in South Africa n.d.), so being able to grow these plants successfully outside of their native range in gardens will allow the preservation of the species. These objectives will allow the conservation of this species and the guarding of its biodiversity by allowing an inventory of germplasm to be preserved *in vitro* based on the methods of micropropagation. In addition, these methods may be able to be applied to other species in the genus to preserve their biodiversity.

Determine the vernalizaion length

To determine the length of vernalization required for plants to respond to flowering cues (i.e. long days), corms (or seeds) will be sourced from commercial growers and grown in the greenhouse. Plants will be divided into "age" groups based on the size of the corm (less than 10g, 11-20g, 21-40g, and 41-60g) and planted into a sterile, well-drained growing media (adapted from Thompson et al., 2011) in pots at least 10cm deep and 10cm in diameter. Each group will be replicated 8 times and each rep exposed to 0, 1, 2, 3, 4, 5, 6, and 7 weeks of vernalization at 4.4° C and dark conditions in a controlled growing environment (such as that of a climatic growth chamber), with 0 being the control. Humidity will be maintained at 30% to simulate humidity in the plants' native winter environments. Once their respective treatment of vernalization is concluded, they will be moved to the greenhouse and grown in 25% shade, average day/night temperatures of 23/14C, and gradually lengthening photoperiods(to simulate

the transition of spring to summer, when they usually flower). They will then be grown for 3 months and date of inflorescence appearance, number of inflorescences, number of flowers, and flowering time will be documented. When growing, plants will be fertilized with a slow-release fertilizer high in nitrogen and phosphorus. The smallest corm that is capable of flowering can be assumed to be the youngest physiological age at which this species will be able to respond to vernalization and subsequently flower. Growers and private gardeners are invited to participate in their own gardens or plant growth facilities so that they may get a chance to be familiarized with the plant as well as allowing additional environments to gauge potential genotype by environment effects. This may be done via extension services to recruit willing participants who have ample room in their gardens or growth spaces to accommodate reps of the experiment; surveys can be used to take data on how growers feel about their plants.

Establish a cleaning and micropropagation protocol

Establishing a micropropagation protocol requires a sub-experiment to determine the proper sterilization or cleaning process. A preliminary cleaning protocol may start with initial washing of corms to remove plant debris, dead material, dirt, etc. in soapy water. Then an instrument (such as the blunt edge of a scalpel) can be used to remove buds as well as any remaining debris or dirt on the surface of the buds. Spray buds with 70% alcohol then place them into sterile water. After that, buds are placed into containers of 10% hydrogen peroxide for 10 minutes; agitate container every 2 minutes to ensure even coverage of the buds. After removing materials from hydrogen peroxide solution, place them into 10% bleach, soak for 10 minutes, and agitate container every 2 minutes to ensure even coverage of buds. After that, remove materials from bleach and place into sterile water. Treatment media may need to be supplemented with plant

preservatives such as PPM biocide and antibacterials/antifungals such as gentamicin and captan, respectively.

For the establishment of a micropropagation protocol, plant tissues used will be buds excised from cleaned corms. Plant tissues will be sterilized and grown in MS (prepared based on the recommended dosages). Treatments will include different auxins and different cytokinins at different concentrations and the response of the plant (such as callus formation, root formation, leaf/stem formation, as well as the length of formed tissues) will be recorded. After the auxin and cytokinin that stimulated the most plant tissue regeneration is determined, they will be combined together in different ratios to determine how they affect plant tissues in tandem. Tissue culture may also give rise to new unique mutants (such as through somaclonal variation common in callus generation) which may be of horticultural interest (such as variegated plants, white flowers, unique leaf morphologies, genetic-based dwarfing, etc.).

Outputs

This research project can be a thesis project for a Master's student, which will be used to fulfill the degree requirements in horticulture. Experience with this project will be shared in academic conferences to distribute knowledge gained; a manuscript based on this research will be submitted to an academic horticulture journal such as *Journal of the American Society for Horticultural Science* (ASHS). Micropropation protocols and growing experiences will be shared with potential growers through extension publications or services.

Outreach

This project may be brought to public attention via extension programs from universities as well as formal and informal meetings with growers, instructional videos, demos, and public participation studies hosted by universities. Farmers and growers both professional and private

stand to benefit from the successful introduction of this new perennial as farmers can benefit from a novel perennial with potential ecosystem benefits and growers can enjoy a beautiful rock garden plant or cut flower. Potential publications based on this research will benefit the larger scientific community in terms of creating and distributing knowledge on exploring the adaptation of a South African native plant as a new bedding, container, and perennial garden crop plant in North Central Region.

Evaluation plan

Engaging growers and the public to learn about their attitude and perspective on *Watsonia pillansii* will be key in evaluating the success of this project and its outreach. The University of Minnesota can help gauge the public perception of this plant by holding formal and informal meetings with growers and gardeners, plant tours, and conducting demos and workshops to help stimulate awareness and interest in the potential crop and their market possibilities. Plant materials can be provided for volunteers to grow at their own gardens/nurseries, who then answer survey questions and provide feedback on growing and cultivating *W. pillansii* as a crop potential. Not only can this help with gauging public opinion, it can also provide valuable data regarding how *Watsonia pillansii* performs in different environments. Efforts can also be made to ask a broader audience (not growers or gardeners) what they think of *Watsonia pillansii* as an addition to public spaces or public pollinator gardens. This may be done via a public survey.

Summary table of Outcome, Outputs, Activities, Inputs, and Evaluation

Expected Outcomes	**Inputs and Activities** (What you're investing)	**Outputs** (What's produced—information products)	**Evaluation/Monitoring Plan; Measurement Methods**
Learning Outcomes: Action Outcomes:	To determine the length of vernalization required for plants to respond to flowering cues; establish a preliminary cleaning protocol and a feasible micropropagation protocol. Public should know about its garden merits, floriculture desirability and ecosystem benefits; recognize the name *Watsonia*. Gauge public opinion by asking growers/gardeners to grow and evaluate plant; send surveys to public asking their opinion Need at least 1 greenhouse (30'x16'x30') of growing space, controlled environment growth chambers, initial plants for research; may include expedition to native habitat (Africa) to observe conditions, outreach to farmers/growers, use of various fertilizers and micropropagation materials. Learn the proper ways to grow, vernalize, and micropropagate this species and integrate it into a production environment and garden setting; educate public on benefits of this species as well as its cultural aspects; growers and public should be willing to grow this plant and share it with others; increased consumer demand for plant	Publishable academic papers on how to grow this species in a greenhouse setting as well as publications regarding its proper vernalization and how to micropropagate it; provide extension guidelines, workshops for greenhouse production for growers; guidelines for home gardeners on how to grow and propagate the plant	For the target audience, meetings, workshops can be arranged to help stimulate awareness and interest in the potential crop and their market possibilities; plant materials can be provided to volunteers to grow; surveys, interviews can be conducted to gather feedback on adopting the plant as a crop potential. For the project itself, daily/weekly daily/weekly measurements of plant characteristics such as # of inflorescences, # of flowers per inflorescence, flowering duration, flowering date, corm size, overall plant size; daily monitoring of tissue culture plates/test tubes for tissue regeneration, callus formation, growth of new leaves, stems, etc. Regarding the process of the project, research plan, research process, data analysis and final results will be monitored and advised by faculty advisor. Manuscript will be shared in conferences to seek feedback.

Experience and roles

This project will be supervised by Dr. Neil Anderson of the University of Minnesota. As Dr. Anderson has much experience in floriculture, horticulture and production of greenhouse crops as well as geophytes, he will be able to co-advise staff of the project on how *Watsonia* pillansii (a geophyte) can be grown in a greenhouse environment as a horticultural crop for either the cut-flower market or as an intact, flowering plant for box office stores. His work with winter-hardy herbaceous perennials (which *Watsonia pillansii* is classified) and preventing invasiveness of ornamental crops makes him very qualified to supervise this project, as other species of *Watsonia* have shown the tendency to escape cultivation and compete with native vegetation. Dr. Anderson's experience with tissue culture, plant breeding, domestication of plants as ornamentals as well as his work with other members of the Iris family will be valuable to this project. My role as a researcher for the vernalization experiment and micropropagation experiment will be aided by my experience in working with the vernalization and tissue culture of another hardy herbaceous perennial, *Silphium* (a sunflower relative) which is also a candidate for domestication. I have designed successful vernalization experiments for *Silphium* and took data on key developmental milestones such as appearance of inflorescence, time to first flower, plant height, internode length, etc. which will help with data collection for vernalizing of *Watsonia pillansii*. I have also had experience with developing successful micropropagation protocols for *Alstroemeria* and *Impatiens*; I've also developed successful sterilization protocols for *Silphium* to prepare tissues for a sterile environment and working in said sterile environments to subculture and propagate plants grown *in vitro.* Having visited Chile to study geophytes and production of various crop plants, I am familiar with growing geophytes in a greenhouse setting. In addition, through part time jobs, internships, and outreach activities during my undergraduate years, I have gained the experience in designing pollinator gardens and engaging the community and the public such as participating in Cornell horticulture center open house days, leading garden tours, engaging with horticulture center volunteers. This experience will help me gauge public response to the plant in small gardening communities. As my own thesis has to do with the vernalization and micropropagation of

Silphium, insights I've gain from vernalization and micropropagation of *Watsonia pillansii* (such as various vernalization temperatures, compounds used for sterilization of plant tissues, etc.) can be incorporated into my research as well.

For this project, I will require access to at least 1/4th of a standard greenhouse (10,000 sq. ft, so 2500 sq. ft) for the growing plants involved in the vernalization experiment after their treatments have ended. I will also need a walk-in lighted growth chamber to conduct the vernalization experiment. For the micropropagation experiment, I will require a wet lab and autoclave to create sterile growth media as well as a laminar flow hood to conduct aseptic work, such as sterilizing plant tissues and transferring them onto sterile agar nutrient media.

Budget and budget justification

The total budget for the project is $14, 171, including the following categories:

Personnel involvement will require 10 to 40 hours of work per week at $20 per hour. Labor will include taking care of stock plants, selecting plants for experiments and extracting plant bud tissues when needed, taking care of plants in cold chambers and tissue culture, taking care of plants in greenhouse after vernalization treatments conclude, conducting micropropagation and sterilization protocols. In addition, there will be field work during the summer such as maintenance of plants transplanted into the field. This will cost $5700.

Supplies and materials will include all materials relating to field work, lab work, and greenhouse work. Shovels and trowels along with watering hoses and fertilizer may already be present. The bulk of this cost will be to purchase laboratory materials such as magenta boxes which will be necessary for micropropagation along with agar, pH regulators, Murashige & Skoog basal media, and PGRs such as various synthetic/natural auxins and cytokinins. Particularly, PGRs will be the most expensive from my own experience with working with PGRs for micropropagation. In total, supplies and materials will total to $2000.

Greenhouse space will require at least 250 sq.ft with supplemental lighting and watering for growing seedlings as well as plants that have finished their respective treatments, which will cost $5621, assuming $0.0308 per sq.ft. per day multiplied by 2 years. For growing space that is open-field (such as when plantlets will be transplanted outside for summer growing), 1 acre of research plot land from UMN's St. Paul research field will be needed costing about $250, as there is suitable soil (not too sandy or clay) for growing *Watsonia pillansii.*

Travel expenses will be for outreach visits to various growers and gardeners who are growing *Watsonia pillansii* as well as to visit gardening communities and events to raise awareness of this plant and gauge public perception of it. As travel will be limited to within Minnesota, travel by car is logical and will cost around $300. Finally, submission of research data, papers, and conclusions to journals as well as the printing of flyers, brochures, creation of digital information for public education, and conducting workshops and surveys will cost $300.

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