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Faculty of Landscape Architecture, Horticulture and Crop Production Science

The Potential of Breeding Perennial Grain Legumes for Swedish Climates

– A literature study and a pilot experiment on polyploidy

Potentialen för förädling av perenna baljväxter för svenska klimat

– En litteraturstudie samt ett pilotexperiment om polyploidi

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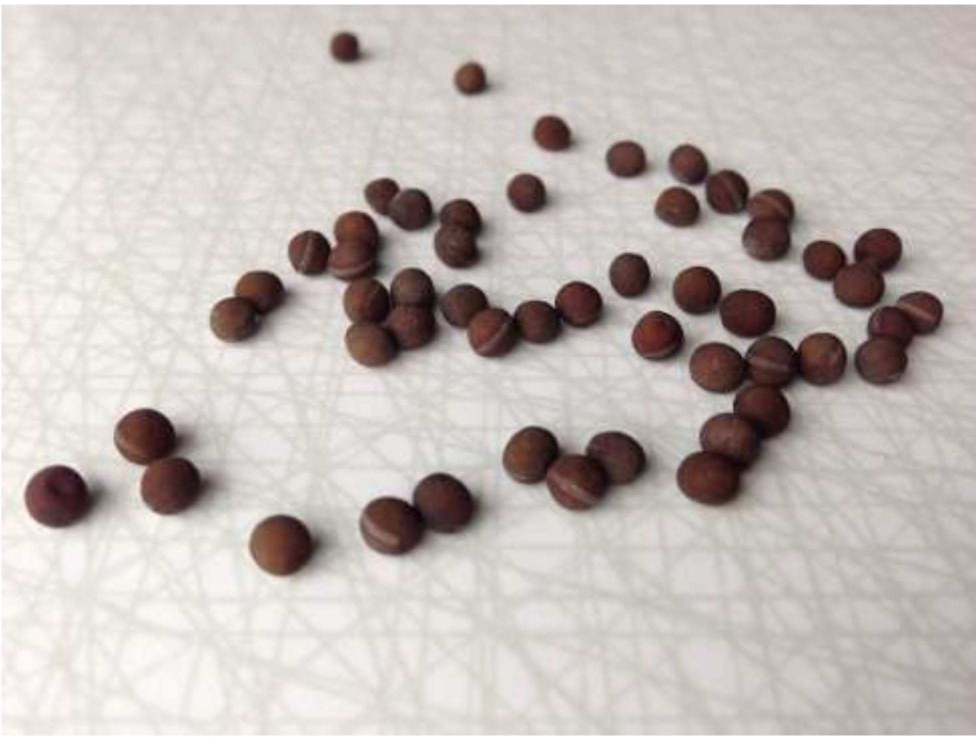

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Summary

In consideration of both the struggle facing smallholders and rural societies in Sweden and the environmental crisis, the potential of breeding and cultivation of perennial legumes in Sweden has been examined. The essay is comprised of a literature study and a pilot experiment.

In the literature study, focus have been given to pea vetch, *Vicia pisiformis* L., Siberian pea shrub, *Caragana arborescens* Lam., thicket bean, *Phaseolus polystachios* L. and some species of lupins, *Lupinus* sp. Additionally, the potential of using polyploidization and the prospects of achieve interspecific hybridization with the aim of increasing seed size and yield have been examined. In conclusion, the most promising path to a perennial legume crop for large parts of Sweden appears to be lupin species of the *Platycarpos* subgenus, i.e. hybrids between *L. mutabilis* Sweet. and either *L. polyphyllus* Lindl. or *L. perennis* L.

In the pilot experiment, solutions containing either 0,1% or 0,2% colchicine plus 0,2% dimethyl sulphoxide (DMSO) were applied to lateral buds of *Caragana arborescens*, as well as to apical meristem of *Vicia pisiformis*, in an attempt at inducing tetraploidity. However, no differences between treated plants and the control could be identified.

Sammanfattning

Med svårigheterna för småbrukare och svensk landsbygd och den stundande klimatkrisen i åtanke har potentialen för att förädla och odla perenna baljväxter i Sverige undersökts. Arbetet består dels av en litteraturstudie och dels av ett pilotexperiment.

I litteraturstudien har fokus legat på ärtvicker, *Vicia pisiformis* L., sibirisk ärtbuske, *Caragana arborescens* Lam., vild kidneyböna, *Phaseolus polystachios* L. samt några arter av lupin, *Lupinus* sp. Dessutom har potentialen att använda popyploidisering samt att ta fram interspecifika hybrider i syfte att öka fröstorlek och skörd undersökts. Samanfattningsvis verkar den mest lovande tillvägagångsättet att ta fram en perenn baljväxt för odling i större delarna av Sverige vara att utgå ifrån lupinarter från undersläktet *Platycarpos*, mer specifikt hybrider mellan *L. mutabilis* Sweet. och antingen *L. polyphyllus* Lindl. eller *L. perennis* L.

I pilotexperimentet applicerades lösningar med antingen 0,1% eller 0,2% colchicin samt 0,2% dimetylsulfoxid (DMSO) på lateralknoppar av sibirisk ärtbuske samt apikalmeristemet på ärtvicker. Detta i syfte att inducera tetraploidi. Dock kunde inga skillnader mellan de behandlade plantorna och kontrollerna indentifieras.

Table of Contents

1. Introduction

1.1 Past and future food production and consequences for rural Sweden

The prevailing trends for agriculture the last century have been industrialisation, specialisation and geographical concentration of agricultural and horticultural production. The rationalisation of production system has led to fewer farmers cultivating larger areas being able to support more people (Świergiel et al., 2018); Flygare & Isacson, 2003). But as stated in Wolfenson's (2013, p. 21) preparations and outcomes of the 2012 United Nations Conference on Sustainable Development:

> *"The neoliberal model of industrialised agriculture has not 'trickled-down' into more social well-being. In contrast, it has contributed to the global environmental and employment crisis and disconnection from local realities (White, 2011)."*

The consequence has been a haemorrhagic depopulation of rural Sweden, especially in the northern regions. Food production has been concentrated to the plains in the south, making way for production of timber, primarily spruce (*Picea abies* L.) and fir (*Pinus sylvestris* L.), which is a major export goods in Sweden.

Three periods of intensive depopulation have been identified by Torgny Östling (personal communication) of the smallholder organisation NOrdbruk- which is linked to the global peasant movement La Via Campesina. The first period, which mainly affected the inlands of Norrland and northern Svealand, occurred in the late 1900th century, when forestry companies bought up farms to get hold of the farmers timber (Stadling, 1894). The companies reaped vast profits and often paid unreasonably low prices to the farmers, who were regularly intoxicated with alcohol by the buyers in order to be persuaded to sell their land. These affairs, which are commonly referred to as 'Baggböleri', ended in 1906 when forest companies were prohibited to buy private land. The consequences remain; in 2012, 25 % of Sweden's total forest land is owned by privately owned forestry companies, and this number is significantly higher in the north, e.g. 48 % of Jämtland's forest land is held by privately owned forest companies (Swedish Forest Agency, 2014). Additionally, the state-owned company Sveaskog owns 14 % of Sweden's forest land, and large portions of the privately-owned forest land have owners living outside the municipality, which further decreases local sovereignty over resources.

The second period of intensive depopulation of rural Sweden followed the agricultural policy of 1967. The level of self-sustainability was set to decrease from close to 100 % to less than 80 %, financial support for small holders were limited and the ‘income goal’, stating that farmers should earn as much as industrial workers, was abandoned. This affected large parts of the country and in e.g. Småland the topsoil of fields was removed and sold in the cities to hobby gardeners, while the former fields were planted with spruce (Jonsson, 1971). Food prices went up when production was lowered, and massive protests lead to a price freeze on milk and cheese.

The third period began during 1990 when the Swedish agricultural policy first shifted towards total deregulation and then disappeared in 1995 when it was incorporated in the Common Agricultural policy, CAP, of the European Union, EU. The initial deregulation and free trade were in accordance with the General Agreement on Tariffs and Trade, GATT (Flygare & Isacson, 2003). Food was to be regarded as one commodity amongst others, especially after the World Trade Organisation, WTO, came to include agriculture. The EU’s directive on free movement of capital motivated a deregulation of land acquisition which resulted in an increased competition between farmers and speculators and thus higher prices on land (Świergiel et al., 2018). This fuels the ongoing rapid decline of numbers of farms in the competition with both domestic industrially produced food and cheap imported goods. In the adaptation to joining what became the EU, self-sufficiency has decreased drastically. Conventional sources claim a self-sufficiency level of 50 %, while more critical voices state that the level is about 25 % when fodder is included (Östling, personal communication). If fuel, fertilizers and pesticides used in production and transport are included, Sweden’s self-sufficiency level is about 0 %.

While costs for e.g. land and fuel have increased, prices paid to farmers and foresters for their products have stagnated or decreased from the prices of 1980. Urbanisation, putting an increasing strain on housing and infrastructure in and around cities (which together with free speculation fuels exploitation of arable land), and erosion of rural communities, with a decline in job opportunities and social services like hospitals and schools, are two sides of the same coin.

1.2 Agroecological approaches

The environmental effects of industrial agriculture like climate change, biodiversity loss, eutrophication, disturbance of the nitrogen and phosphorus cycles, ocean acidification etc. are well documented. Different production systems have been designed to decrease the amount of inputs needed and thus decreasing the environmental impact of the production.

Agroforestry and alley-cropping are new words for including trees in agriculture, which in actuality stems from ancient practices of e.g. windbreaks or simply cultivation on small fields naturally surrounded by multifunctional trees and shrubs. Alley-cropping decreases nutrient leakage due to deeper roots and leads to better utilization of phosphorus due to symbiosis with mycorrhizal fungi. Evapotranspiration decreases since the trees act as windbreaks but competition for water can occur between the trees and the field crops (Jose et al., 2000).

The no-till systems of conservation agriculture and restoration agriculture have been developed to decrease soil erosion and nutrient leakage while increasing the amount of soil carbon (Montgomery, 2017). By avoiding tillage, soil biota such as microorganisms and earthworms can thrive which increases the infiltration capacity which makes better use of precipitation while sharply decreasing erosion and occurrence of standing water.

Transition to cultivation of perennial crops may further decrease environmental impact by reduced need for irrigation, fuel and agrochemicals. Perenniality leads to a decreased need for soil preparation, Generally, perennials emerge faster and earlier in the growing season which can lead to a better utilization of the solar energy available in spring. With a well-developed root system present in spring, there is a decreased need for irrigation, which is especially practical since the spring generally is the period of the growing season with the least amount of precipitation in Sweden (Wastenson, Raab, & Vedin, 1995). As Westerbergh et al. (2018, p. 1) encapsulate, the “perennial growth habit is associated with reduced soil erosion, more efficient use of mineral nutrients, higher soil quality and carbon sequestration”. Yet annuals grow faster and invest all their energy into seed production each year which result in a higher average yield of seeds (Rasche et al., 2017), which explains how much of human societies came to depend on annual crops instead of perennials. Perennials on the other hand generally grows more slowly and invests more of their energy into their root system and are more resource efficient and tolerant of stress. The fact that planting perennials by definition is a more long-term procedure since the plants take longer time to come into full production, limits the adaptability of the farmer. Additionally, perennials generally take longer time to breed since many does not flower in their first year. Hopefully, the pros will outweigh the cons when sufficient breeding efforts have been conducted.

1.3 Legume crops

Legumes crops, species of the *Fabaceae* family, are in several ways important in agricultural and horticultural cultivation. Cultivation of legumes often requires less or even no fertilizers, especially of nitrogen (N), since most species of *Fabaceae* have a symbiotic relationship with certain strains of N-fixing soil bacteria (Fig. 1), commonly of the *Rhizobium* or *Bradyrhizobium* genuses. The protein rich seeds of numerous crops are important in feeding the world and contribute to a nutritious diet.

Perennial growth habit may reinforce the benefits of legume crops by extending the period of N-fixation. With decreased need for ploughing and increased permanent root mass, a smaller amount of the fixed nitrogen is lost through run-off and leeching. This aspect would make perennial legume crops more resource efficient, and subsequently more economically and environmentally viable.

1.4 Plant breeding

Plant breeding is the procedure of improving the genetic material of crops. Most domesticated legume crops display some similar traits that human selection have favoured including non-dormant seeds/soft coated seeds, reduced toxicity and non-dehiscent pods. This together with the crucial factors of higher yield and adaptation to the system of cultivation and to local environmental conditions.

Different methods of breeding are used depending on species, e.g. if the plant is cross-pollinated or self-pollinated, and the aim of the breeding effort, e.g. selection of superior genotypes in a diverse open-pollinated population for vegetative propagation or introduction of a suitable genes in a variety through controlled crossings between superior plant material. A multitude of techniques have been developed for introduction of variation such as mutation breeding, e.g. radiation breeding, and recently biotechnological tools such as transformation and gene editing methods has been developed and used to induce new traits (Xiong, Ding, & Li, 2015). Whole genome changes such as polyploidization occurs sometimes spontaneously and is also possible to achieve through breeding. Polyploidization is the method in focus in this essay, since it is an interesting method that could provide rapid results and because of the prospects of attaining larger seeds through the 'Gigas effect' which will be described further below.

1.4.1 Polyploidization

1.4.1.1 *Description and methods*

Polyploidization has, through studies on stomatal size in fossil plants, been estimated to have occurred to up to 70 % of all angiosperms historically. There are numerous examples of polyploid crops originating from more or less recent polyploidization events, e.g. allohexaploid bread wheat, *Triticum aestivum* L., and autotriploid apple cultivars like the large-fruited *Malus domestica* Borkh. 'Gravensteiner'. The prevalence of these and many other crops in cultivation shows how human selection has, at first unknowingly, favoured polyploidy. However, the larger genome and higher number of alleles makes marker assisted selection, MAS, such as identification of single nucleotide polymorphism, SNP, more difficult (Hayward et al., 2014).

There are different types of polyploids, autopolyploids and allopolyploids. Autopolyploids are created through doubling of the same genome. Allopolyploids are hybrids of distantly (true allopolyploids) or closely (segmental allopolyploids) related species (Sattler, Carvalho & Clarindo, 2016). Autopolyploidy can occur somatically by e.g. endomitosis, incomplete cell duplication leading to cells with a different number of chromosomes, or endoreduplication, doubling of genome without cell division. It can also occur through fusion of unreduced reproductive cells, which can also result in allopolyploids, depending on the cross.

Polyploidy is most often induced in plants by applying a substance "causing depolymerization of the microtubular cytoskeleton in early phases of metaphase" (Pavlíková et al., 2017, p. 828). The effect is that the chromosomes are prevented from separating during cell division, which results in a cell with twice as many chromosomes.

Different chemicals can be used to induce polyploidy in plants. Three of the most commonly used substances are colchicine derived from the autumn crocus, *Colchicum autumnale* L. (Limera, 2016), and the two herbicides oryzalin and trifluralin (Ebrahimzadeh, 2018). Colchicine is lethally poisonous to animals since it also has an affinity to animal tubulin, even higher than for plant tubulin, which makes it dangerous to handle. Oryzalin has no affinity for animal tubulin and is effective in lower concentrations compared to colchicine. Trifluralin requires a shorter exposure time (about 30 min) than colchicine (3-8 h) when creating double haploids of *Brassica napus* L. from anther culture (Zhao & Simmonds, 1995).

There are several ways of application of the substances, young seedlings (Pavlíková et al., 2017) or seeds can be immersed in a solution (Xing et al., 2011), the solution can be applied to the meristem (Limera, 2016) or explants can be treated in vitro (Podwyszynska, 2012). The application method, and the corresponding substance used varies with the aim of the procedure, and the optimal conditions for inducing polyploidy are often specific depending on the plant material used (Sattler, Carvalho & Clarindo, 2016).

1.4.1.2 *Physiological effects*

Polyploidisation is most commonly associated with the ‘Gigas effect’; with an increase of number of chromosomes, an increase of the nucleus and thus the cell follows, which leads to larger organs such as leaves, fruits and flowers (Sattler, Carvalho & Clarindo, 2016). However, polyploidy can lead to a lower cell number, which means that the expected larger organ size is sometimes absent. Another factor affecting growth, is that the contents of hormones have been found to decrease with an increase of ploidy level (Levin, 1983). The growth rate often suffers a decrease (Sattler, Carvalho & Clarindo, 2016), and the plant often becomes more compact e.g in loquat, *Eriobotrya japonica* (Thunb.) Lindl. The reduced growth rate has been attributed to the slower cell division of larger cells, and thus a consequence of the ‘Gigas effect’ (Ramsey & Schemske, 2002). (Blasco et al., 2015). Polyploidy can also alter the life cycle of plants, e.g some tetraploid perennials are more long lived than their diploid counterparts (Levin, 2002).

The type of polyploid is of significance to what extent the chromosomes can pair and recombine (Bourke et al., 2018). Diploids are disomic, with only two of each chromosome that can pair. Autopolyploids have more than two homologous chromosomes that all can pair and are thus called polysomic. True allotetraploids on the other hand are, like diploids disomic, since homologous chromosomes can pair and recombine, while homoeologous chromosomes, more distantly related that is, cannot. Lastly, segmental allopolyploids are mixosomic which is an intermediate state.

Newly formed polyploids, neopolyploids, display a more or less reduced fertility and it is commonly stated to reduced more so in neoautopolyploids (Ramsey & Schemske, 1998; 2002). But as Ramsey & Schemske (2002) conclude in an excellent review covering pollen and seed fertility in neopolyploids, that there was no significant difference in reduction of fertility between different types of neopolyploids.

The reduction in fertility can be caused by meiotic aberrations (Ramsey & Schemske, 2002). In polyploids, irregularities during chromosome pairing can lead to univalents and multivalents, i.e. unpaired, or association of more than two chromosomes. E.g. univalents and trivalents often result in aneuploid gametes i.e gametes with an irregular number of chromosomes, which more seldom result in viable seed. Contrastingly, quadrivalents have been associated with increased fertility in some autotetraploids, but a decreased fertility in other cases.

Fertility in neopolyploids is to some extent determined by genetic factors (Ramsey & Schemske, 2002). It has been shown that some strains of a specie, after polyploidization, displays higher fertility than other strains. Furthermore, fertility can increase when two different neopolyploid strains of the same species, each with low fertility, is crossed. Lastly, seed set in neopolyploids can be affected by phenotypic factors. Since e.g. ovules, pollen and seeds often are larger in polyploids, fewer are produced.

More importantly, fertility can be restored, and of the 12 studies quantifying changes in fertility in neopolyploids over 2 to 19 generations that Ramsey & Schemske found, 11 reported increases. Combining the data resulted in an average seed fertility increase of 39,7%, per generation, ranging from -0,8% to 104,1%. Still, their analysis proposes that polyploids has a slightly lower upper limit of fertility than their diploid counterparts. Fertility is positively correlated with bivalent and quadrivalent pairing of chromosomes. An important factor is the frequency of chiasmata formation, which is inheritable. High frequency of chiasmata formation was found to be correlated with quadrivalent pairing and seed fertility.

Polyploidy result in a decrease in the density of stomata while the stomatal size increases, which can lead to tetraploid plants having a lower transpiration rate than their diploid counterparts. The density of stomata constitutes a means to detect polyploidy. In Levin's (1983) review, some induced autotetraploids where found to have a lower CO_2 fixation rate than diploid counterparts. Simultaneously, some naturally occurring tetraploids had a higher photosynthetic rate than their diploid counterparts, indicating that post-polyploidisation evolution is important for the survival of tetraploids.

Levin (1983) found that polyploidisation can make plants either less or, perhaps more often, more resistance to low temperatures. In some species, the optimal temperature of photosynthesis has decreased, leading to a higher growth at low temperatures (Eagles & Othman, 1978). Tolerance of salt stress can increase in tetraploids compared to diploids, such as in black locust, *Robinia pseudoacacia* L., (Wang et al., 2016) and sugar beet, *Beta vulgaris* L. (Wu et al., 2019).

Synthetic tetraploids of *Solanum commersonii* Dunal. had, compared to diploids, an increased content of phenylpropanoids, but a decreased content of glycoalkaloids (Caruso et al., 2011). Autotetraploid plants of devil's snare, *Datura stramonium* L., had higher alkaloid content than their diploid counterpart (Philipov, 2002).

1.5 Aim

Considering the socioeconomic situation for small holders and rural Sweden, while acknowledging the low level of self-sufficiency due to industrialisation of production, free trade and free speculation, plant breeding must aim at easing a reversion of the situation. New crops selected should not be limited to the current centre of production i.e. the southern plains and thus continue to discriminate large parts of rural Sweden, but rather utilise all different cultivation regions of the country. The new crops should not be high price products aimed at niche markets; they should focus on satisfying local needs. At the same time, major environmental challenges must be addressed by decreasing the amount of inputs needed to produce food. Therefore, the aim of this essay is to examine the prospects of breeding perennial legume crops adapted to different regions of Sweden.

These two questions will be given main focus and divide the essay into two parts; the literature study and the experiment.

1. A literature study: What potential is there for breeding of perennial legumes for cultivation in all of Sweden?
2. A pilot experiment: Is it possible to induce tetraploidy in *Caragana arborescens* Lam. and *Vicia pisiformis* L. for breeding purposes?

2. What potential is there for breeding of perennial legumes for cultivation in all of Sweden? – a literature study

2.1 Method

The methods of acquiring information on the literature study were using different search engines, mostly SLU Primo, Web of Science and Google Scholar. This was combined with use of the library at SLU Alnarp and contacting a few people with experience of the studied species via email.

Perennial legume species of special interest were selected because of their hardiness and other interesting traits. Thus, information on *Vicia pisiformis, Caragana arborescens, Phaseolus polystachios* L. and some species of *Lupinus* in the subgenus *Platycarpos* were compiled for further examination.

The essay was for practical reasons limited to legumes grown for their dry seed, which unfortunately leaves out *Apios americana* Medik., which is a perennial vine cultivated for its tubers. Alex Wenger, plant breeder in Pennsylvania, is currently conducting breeding efforts and trials for machine harvesting (Wenger, personal communication. It originates from eastern North America and displays a high adaptability to different regions, currently grown in e.g. Iowa, South Korea and Japan.

2.2 Results

2.2.1 Pea vetch, *Vicia pisiformis*

The pea vetch, *Vicia pisiformis*, (placed in the Vicilla section of the *Vicia* genus) is a herbaceous perennial spread throughout Europe but is most common in the southeast. The northernmost populations grow in southeast Norway and south

Sweden (Gustafsson, 1991). Each plant produces multiple shoots growing to 0,5 - 1,5 m, using tendrils on its leaves to climb other vegetation (Gustafsson, 1991). The 1 cm large pale-yellow flowers are produced in a one-sided raceme (Knutsson, 2016) from middle of June to September (Gustafsson, 1991). The pods, which thus ripen over a long period of time, are up to 4 cm long and contain at most 6 - 7 (Gustafsson, 1991) small, dark brown spherical seeds (Fig. 2). The plant can to a lesser extent spread vegetatively by rhizomes (Black-Samuelsson, 1997). Gustafsson describes the plant as easily cultivated, referring to a vigorous example in the Botanical Garden in Uppsala.

Figure 1-2. *Left:* Small seedling of *Vicia pisiformis* with root nodules. *Right:* Seeds of *Vicia pisiformis*. Pictures: Maximilian Isendahl.

The seeds of pea vetch are reportedly used as lentils (Kunkel, 1984), although the original source is not cited. In contemporary popular literature, young shoots and pods can be used as vegetables (Weiss & Sjöberg, 2018). Seeds of several species in the *Vicia* genus contains toxic compounds, e.g. L-canavanine in *V. villosa*, a species found in section Cracca (Tate & Enneking, 2006). Studying the seed content to quantify eventual toxic compounds was an initial aim in this thesis but limited time and resources made this not possible and remains an important issue.

In Sweden, the pea vetch most commonly grows on south or south-west facing slopes in broad-leaved forest. Gustafsson (1991) reports that pea vetch grows under canopy cover of 20 -80 % of mostly *Quercus robur* L., *Pinus sylvestris* L., *Acer platanoides* L., *Betula pendula* Roth. and larger shrubs like *Corylus avellana* L. and

Lonicera xylosterum L. The soil is dry, rocky, humus rich and often calcareous (Knutsson, 2016).

The pea vetch is classified as endangered (EN) in Sweden as it is rare and in decline, mainly due to habitat destruction and grazing (Knutsson, 2016). During the 19th century, a decline in the Swedish population is attributed to overgrazing with livestock (Gustafsson, 1991). Today, the largest threat is habitat destruction through rational forestry with conifers, particularly with spruce, *Picea abies* L.*,* which is detrimental to the pea vetch due to acidification of the soil and shading (Gustafsson, 1991). Black-Samuelsson (1997) cites grazing by roe deer and seed predation by beetles as other negative factors on the population. Gustafsson (1991) also reports low seed set as factor limiting spread.

Pea vetch is self-compatible which result in a low genetic variation by decreasing the amount of recombination, but it can limit impact of deleterious genes and be favourable to seed set in small populations with insufficient pollinators (Black-Samuelsson, 1997).

Black-Samuelsson (1997) examined the effect of temperature on number of flowers and dry weight of pea vetch, and found that low night temperatures were limiting to both and especially to the number of flowers. However, the impact of low temperatures varied between populations of different origin. When studying plants grown from seed originating from different populations, a significant difference in numbers of flowers could be shown.

Favourable traits common for domesticated legumes like non-dehiscent pods, large seed size, high yield etc. is present in the cultivated relative broad bean, *V faba* L. Both *V. pisiformis* and *V. faba* have chromosome count 2n = 2x = 12. Interspecific crosses between *V. pisiformis* and *V. faba* would thus be desirable to introduce these traits. However, as Ramsay et al. (1984) shows through crossing experiments conducted with 127 accessions of *V. faba* and 37 accessions of wild relatives in *Vicia* section faba (*V. pisiformis* not included), barriers to prevent hybridisation are present leading to abortion of embryos at an early stage.

Li et al. (2001) has studied the genetic relationships of different subspecies of *V. amoena* Fisch., a specie native to China that, like *V. pisiformis*, is part of the Vicilla section of the *Vicia* genus. The study shows that *V. amoena* var *amoena* most likely is an allotetraploid originating from an interspecific hybridisation between the diploids *V. amoena* var. *sericea* and *V. pseudorobus* Fisch. The geographical distribution of the tetraploid *V. amoena* var *amoena* is broader than its diploid originators.

Experiments with naturally occurring tetraploid and diploid *V. cracca* L. (found in section Cracca of the *Vicia* genus), showed that diploids were more drought resistant than the tetraploid counterparts, since the tetraploids aborted more seeds during drought stress (Eliášová, 2017). The tetraploids had a are found in north-western Europe, while the diploids, were more concentrated to the drier southern parts of Europe.

2.2.2 Siberian pea shrub, *Caragana arborescens*

The Siberian pea shrub or pea tree, *Caragana arborescens*, is a large vigorous shrub, native to eastern Russia and northern China. Moore cites it in 1965 to be of great economic value to the Canadian prairies as a shelterbelt plant, and it is used in revegetation programmes (Dietz et al, 2008) and to stabilize soils (Shortt & Vamosi, 2012). However, the specie considered invasive in USA (Dietz et al, 2008) and Canada (Henderson & Chapman, 2006) and can negatively affect forest areas in North America (Shortt & Vamosi, 2012).

Figure 3-4. *Left:* Green pods of *Caragana arborescens*. *Right:* Two plants of *Caragana arborescens* in Alnarp, Sweden. Pictures: Maximilian Isendahl.

The foliage of *Caragana* species contain almost no alkaloids and is food for deer (Shortt & Vamosi, 2012) and livestock in China (Meng et al., 2009). The flowers of several species, especially *C. sinica* (Buc'hoz) Rehder are used as vegetables. The seeds are regularly stated to be used as feed for chickens. Seeds and young pods are commonly cited as edible (e.g. Meng et al., 2009), however the primary source appears questionable, with no thorough study on toxicity in the seeds.

Studying the seed content was an initial aim in this thesis but limited time and resources made this impracticable.

The utility of the Siberian pea shrub comes from its hardiness and vigour. It tolerates very low temperatures, windy sites and infertile or saline soils, as well as drought and high sun exposure (Shortt & Vamosi, 2012). It is fast growing, and can reach heights of 7 m (Dietz et al, 2008) and live at least 90 years (Hederson & Chapman, 2006).

The nitrogen-fixing ability of the Siberian pea shrub has been compared to that of buffaloberry, *Shepherdia argentea* Nutt., and sea buckthorn, *Hippophae rhamnoides* L., under greenhouse conditions (Issah et al., 2013). Siberian pea shrub has found to have the highest fixation ability, equal to 15-73 kg N / ha compared to 11-67 kg N / ha in sea buckthorn, and 16 kg N /ha in buffaloberry. Nitrogen-fixation begins at 3-5°C, which is lower than several other species (Shortt & Vamosi, 2012).

Dietz et al. (2008), in a paper regarding nursery production, state that the Siberian pea shrub bear their first “commercial” seed crops at age 3-5 years. The harvest period is reported to be less than two weeks and harvest is must be carried out carefully by hand before the seeds are dispersed, which makes harvest difficult. The pods, which are 2,5-5 cm long and contain about 6 red-brown seeds, are then dried and easily threshed and cleaned. Self-incompatibility factors are present in the species, although this trait is highly variable and ranges from completely self-sterile to almost completely self-pollinating (Cram, 1969).

The Siberian pea shrub has, to a varying degree, protective spines to ward of herbivores, but this defensive strategy is cited to be energy demanding and result in a trade-off in seed production (Shortt & Vamosi, 2012). A major disease attacking Siberian pea shrub is powdery mildew, *Erysiphe palczewskii*, which attack leaves and shoots.

Cram (1969) found a high variation in numerous characteristics, including vigour, growth habit and seed yield. A more recent source, Wenger (personal communication), affirms the genetic diversity between plants and differences in yields and fruiting habits.

Induced tetraploid *C. arborescens* are found to be more tolerant of drought than diploids, by attaining a higher water-retaining capacity (Borodina, 1981). Autotetraploid plants of Siberian pea shrub have been produced with the aim of attaining superior shelterbelt plants (Moore, 1965). Average seed weight of tetraploid

Siberian pea shrub was 32,8 mg, compared to 19,6 mg per seed in diploid Siberian pea shrub. However, pod length was the same and seed set was reduced, and number of seeds per pod was 1,8 in tetraploid plants compared to 3,6 in open pollinated diploid plants. Tetraploid seed was reported to germinate well, but growth rate was reduced; height after three months growth was 7 cm in tetraploid plants, 13 cm in diploid plants from self-pollinated seed, and 17,2 cm in diploids from open-pollinated seed. This reduction in vigour remained with age, and tetraploids were thus considered inferior to diploids in the purpose of shelterbelt plants.

2.2.3 Lupins, *Lupinus* sp.

Several annual species of lupins are cultivated as grain legumes, e.g. *Lupinus angustifolius* L., *L. albus* L., *L. luteus* L. and *L. mutabilis* Sweet. The first three species belong to the *Lupinus* subgenus which contain mostly annuals native to Eurasia and North Africa. *L. mutabilis* belong to subgenus *Platycarpos*, which contain mostly perennial but also annual herbs native to the Americas (Kurlovich & Stankevich, 2002). The subgenus *Platycarpos* will be given focus here due to the content of both perennial species that are adapted to the Swedish climates and cultivated annual species. Several species of lupins have been recommended as substitute for soy in livestock feed (Keeler & Gross, 1980; Kurlovich, Stoddard, & Earnshaw, 2008). Quinolizidine alkaloids are commonly present in lupins, and are a means to protect the plants against insects (Frick et al., 2017) Nonetheless, they are major antinutritional substances present in lupins, which may affect the nervous system (Keeler, 1980), and a threshold of 0,02 % has been set (Frick et al., 2017). Some species of lupins from the *Lupinus* subgenus are known to contain the alkaloid anagyrine (Keeler, 1980). This substance can cause congenital deformities, e.g. crooked calf disease, when present in feed. The three species that will be mentioned here, *L. perennis* L., *L. mutabilis* and *L. polyphyllus* Lindl., which are all from subgenus *Platycarpos* (Kurlovich & Stankevich, 2002) and have chromosome number, 2n=48 (Kurlovich, Stoddard, & Earnshaw, 2008), and does not contain anagyrine (Keeler, 1980).

Figure 5. *Lupinus polyphyllus* growing in an abandoned field in Vesljunga, Sweden. Picture: Christian Isendahl.

Lupinus perennis is a long-lived herbaceous perennial native to infertile sandy soils in the oak savanna of eastern North America (Michaels, Shi & Mitchell, 2007). It emerges early in the growing season and can spread both by seed and vegetatively (Halpern, 2005). The plant is self-compatible (Halpern, 2005) and produces numerous inflorescences, each with 30-50 protandrous flowers (Michaels, Shi & Mitchell, 2007) that result in pods with up to 7 seeds (Halpern, 2005). Alkaloid content from one accession was 1,28 % of dry weight (Keeler, 1980). In its native environment, they flower from early May to mid-June and are mainly pollinated by bumblebees (Michaels, Shi & Mitchell, 2007).

L. perennis is susceptible to inbreeding depression owing to deleterious genes (Michaels, Shi & Mitchell, 2007). Destruction of the oak savanna habitat has resulted in fragmentation of the population. This leads to smaller populations where inbreeding and thus genetic erosion results in an additional decline of the wild population of the specie. The plants are also susceptible to deer browsing (Halpern, 2005). There is a significant variation in seed size in the specie and in individual plants, with weights ranging from 8 mg to 41 mg per seed (Halpern, 2005). Larger seed size resulted in increased fitness in seedlings. Environmental conditions were found to affect variation of seed size, e.g. higher interspecific competition increased variation, however, mean seed size per plant was not affected by this.

The Andean lupin, *L. mutabilis* Sweet, is a herbaceous annual cultivated in South America as a grain legume, traditionally in e.g. the Inca culture (Galek et al., 2017). The lupin is often cultivated at 3000 m above sea level, in monocultures on plots of up to 0,2 ha. Given a long history of cultivation and breeding of the species, it has several agriculturally important traits such as non-dehiscent pods, non-dormant seeds and improved yield. The seeds contain about 42 % protein (Gross et al., 1988) and up to 20 % oil (Galek et al., 2017) which makes it a valuable food. However, the pods ripen non-uniformly and the seeds contain up to 5 % (Galek et al., 2017), on average 3 % (Gross et al., 1988) alkaloids. The high alkaloid content is traditionally addressed by a debittering process where the seeds are put in sacks in streaming water. Alkaloid content of traditionally debittered seed (in this case more precisely cooked for half an hour, then placed in streaming water for three days) was reduced from 3,17 % to 0,003 % of dry weight (Keeler & Gross, 1980). However, as reviewed by Keeler & Gross (1980) this can result in a loss of 25 - 60 % of dry matter. A variety of *L. mutabilis*, 'Inti', with an alkaloid content of 0,0075 % and a protein content of over 50 % has been selected (Gross et al., 1988).

The large-leaved lupin, *L. polyphyllus* Lindl., a herbaceous perennial originating from western North America, is classed as invasive in several countries in Europe, including Sweden, commonly growing on e.g. roadsides (Fig. 5). The potential for agricultural cultivation of this specie in Finland and Russia has been investigated, aiming mainly at fodder production (Kurlovich, Stoddard, & Earnshaw, 2008). Kurlovich, Stoddard, & Earnshaw are breeding with the aim at obtaining perennial lupins with a stable low alkaloid content of less than 0,02 %. Plants of *L. polyphyllus* with low alkaloid content (0,2 %) owing to a mutant recessive gene, have been identified by use of the Dragendorff test which is a fast method for determining the alkaloid content. A low alkaloid cultivar, 'Pervenec', that is adapted to northwest Russia has been released (Kurlovich & Kartuzova, 2002). Two different breeding methods were used in producing high yielding low alkaloid cultivars: The first method consisted of crossing different low alkaloid plants with resulting progeny cultivated separately according to the cross. This allows for creation of stable low alkaloid populations and selection of plants with even lower alkaloid content. The second method consisted of crossing low alkaloid plants with plants that perform well, which is a means of raising the productivity in low alkaloid populations. Since *L. polyphyllus* is cross-pollinated and frequently visited by insects, and since naturalized populations of the species commonly occurs on e.g. roadsides, there is high risk of reintroduction of the high alkaloid-trait, which mean that nurseries must eliminate all wild bitter lupins in the proximity (Kurlovich & Heinänen, 2002). Kurlovich &

Heinänen (2002, p. 69) state that their breeding methods “may be applied to other cross-pollinating lupin species such as *L. arboreus* Sims., *L. perennis* L., *L. nootkatensis* Donn., *L. elegans* H.B.K., *L. hartwegii* Lindl., with promising prospects for agricultural production.”

L. polyphyllus has been crossed with *L. mutabilis* and perennials with larger seed size (26-58 mg compared to 21-25 mg in *L. polyphyllus* ‘Pervenec’) and higher seed yield than *L. polyphyllus*, while showing “good seed composition, shattering resistance, frost resistance and high vigour” (Kurlovich, Stoddard, & Earnshaw, 2008, p. 306). However, it was made clear that pure *L. mutabilis* and annual hybrids had still higher seed size (160-165 mg for the relevant strain of *L. mutabilis* and 24-105 mg for the annual hybrids) and seed yield than perennial hybrids.

2.2.4 Thicket bean, Phaseolus polystachios

The thicket bean, *Phaseolus polystachios*, is a perennial herbaceous vine native to eastern North America, and was once spread from Florida to Ontario and Minnesota (Allard, 1947; Lorz, 1952). A more recent report (Egan, 2016) states that it is in decline, being ranked possibly extirpated in two of the 13 studied states and critically imperilled in four states, both in its northernmost and southernmost range. The decline is reportedly due to habitat destruction, spread of urban areas and competition from non-native plants (Dohle et al, 2013) and its survival is further endangered by seed predation by weevils (Allard, 1947; Egan, 2016). Different accessions are being collected, and “[g]enetic diversity studies are currently underway by Kisha and Egan” (Dohle et al., 2013, p. 117).

Figure 6. Seedlings of *Phaseolus polystachios* showing hypogeal germination. Picture: Maximilian Isendahl.

It is found in rocky and sandy forests and requires well-drained soil (Allard, 1947), favourably on south facing slopes with sparse canopy cover or full sun (Dohle et al., 2013). They emerge rather late in the season, in June, presumably due to frost sensitivity.

The thicket bean grows vigorously and bears flowers in 10-30 cm long racemes that flower from July to September (Santanna, 2013). The pods are 3-8 cm long and contain 4-6 (Santanna, 2013) small seeds that can be cooked and eaten like common beans (Schaefer, 2012). Archaeological evidence suggests that plants of this specie with non-dehiscent pods had been selected by the indigenous in North America. Toensmeier (personal communication) state that it is slow to germinate and somewhat difficult to transplant and that yield is low due to the small size of the seeds. Nonetheless, he also states that it is easily grown once established, tastes good and that his forms have pods that to a large extent are non-dehiscent.

The growth and flowering of thicket bean is strongly affected by day length (Allard, 1947). Plants given less than 12,5 hours of light exhibits a dwarfed bushy habitus and does not flower, while plants given over 16-18 hours of light had the usual vigorous twining growth habit, but did not flower. Thus, optimal day lengths are about 14 hours for growth and flowering and subsequent seed production.

The chromosome number of thicket bean is 2n=22, (Allard, 1947) and it is related to several commonly cultivated species with the same chromosome number, e.g. the common bean, *P. vulgaris* L., runner bean, *P. coccineus* L. and lima bean, *P. lunatus* L. It is more similar to *P. coccineus* (which also is a perennial but less hardy originating from Central America, and commonly used as an annual) than to the other two species in that it has a hypogeal germination, where the cotyledons stay below ground (Fig. 6). However, the lima bean is cited to be its closest cultivated relative (Dohle et al., 2013).

Wild species often show higher resistance to diseases and pests than their cultivated counterparts, and thicket bean is regarded as resistant to white mold, *Sclerotinia sclerotiorum* (Lib.) de Bary, which is a significant pest attacking lima bean (Dohle et al., 2013).

Crosses between the thicket bean and lima bean have resulted in amphidiploid (allotetraploids with one complete diploid genome from each parent) hybrids with 2n=44 (Wyatt, 1970): *P. polystachios x lunatus*, sometimes referred to as *P. lunastachios.* The crosses were originally conducted in Florida with the aim at improving the lima bean by solving the problem with breaking of the hypocotyl during epigeal germination through introduction of hypogeal germination, as well as potential resistance genes (Lorz, 1952). The F_1 hybrid plants produced large amounts of flowers but rarely formed seed, about 1 seed per 1000 flowers, due to high pollen sterility owing to meiotic irregularities (Dhaliwal & Pollard, 1962). Wyatt (1970) investigated the development of the F_{12} to F_{15} generation hybrids resulting from the cross and stated that amphidiploids experience a period of sterility due to pairing issues with heterogenetic chromosomes. This form of sterility can be overcome after sufficient recombination and selection of genetically compatible strains. Although fertility had increased from the F_1 hybrids, the breakthrough had not yet occurred in the case of *P. polystachios x lunatus*. However, Wyatt concluded that “[w]ith the number of phenotypes available for selection, their chances of survival to become a fully fertile form appear good.”

3. Is it possible to induce tetraploidy in *Caragana arborescens* and *Vicia pisiformis* for breeding purposes? - a pilot experiment

Caragana arborescens and *Vicia pisiformis* were chosen as study species for the pilot experiment. The aim was to investigate the possibility to increase the seed size by exploiting any ‘Gigas effect’ and thus increase the yield and make harvest easier and more worthwhile. Further experiments may also include *Phaseolus polystachios* for the same reason.

3.1 Materials and method

13 plants of *Caragana arborescens*, three years old, with about x branches, each about x long, were acquired from Splendor Nursery. When the shoots were about 5-15 cm long, the apical meristem was pinched of, and each branch of the plant were either kept as control or treated with colchicine (Fig. 8). Two different treatments were used with either 0,1 % (solution 1) or 0,2 % colchicine (solution 2), and 0,2 % dimethyl sulphoxide (DMSO) as a wetting agent. Between 2 - 9 lateral buds per shoot of this year’s growth were selected, and 10 microliters of the solution was applied to each bud. Due to variation in development between the plants, 7 plants were treated on the 8/4 and 5 plants were treated on 12/4.

Seeds of *Vicia pisiformis* were acquired from a private collection in Dalarna, originating from a nursery in Germany. 120 seeds were scarified using sandpaper and were sown in trays indoors. After 6 and a half weeks (on the 6/5 2019), the plants were transplanted into bigger pots and the treatment was carried out. Two groups of 53 plants each were formed while 11 plants were kept untreated as control (Fig. 7). The apical meristem of the plant (sometimes two apical meristems were treated, as the plants fairly quickly send up a second shoot), were dipped into either solution 1 or 2, with the same concentrations of colchicine and DMSO as above (Fig. 9).

The plants were examined visually to detect polyploidy through screening for larger leaves, higher number of leaflets. The density and size of stomata of leaves about three nodes above the treatment of some plants were examined. Although a chromosome count would be more precise, this was not possible.

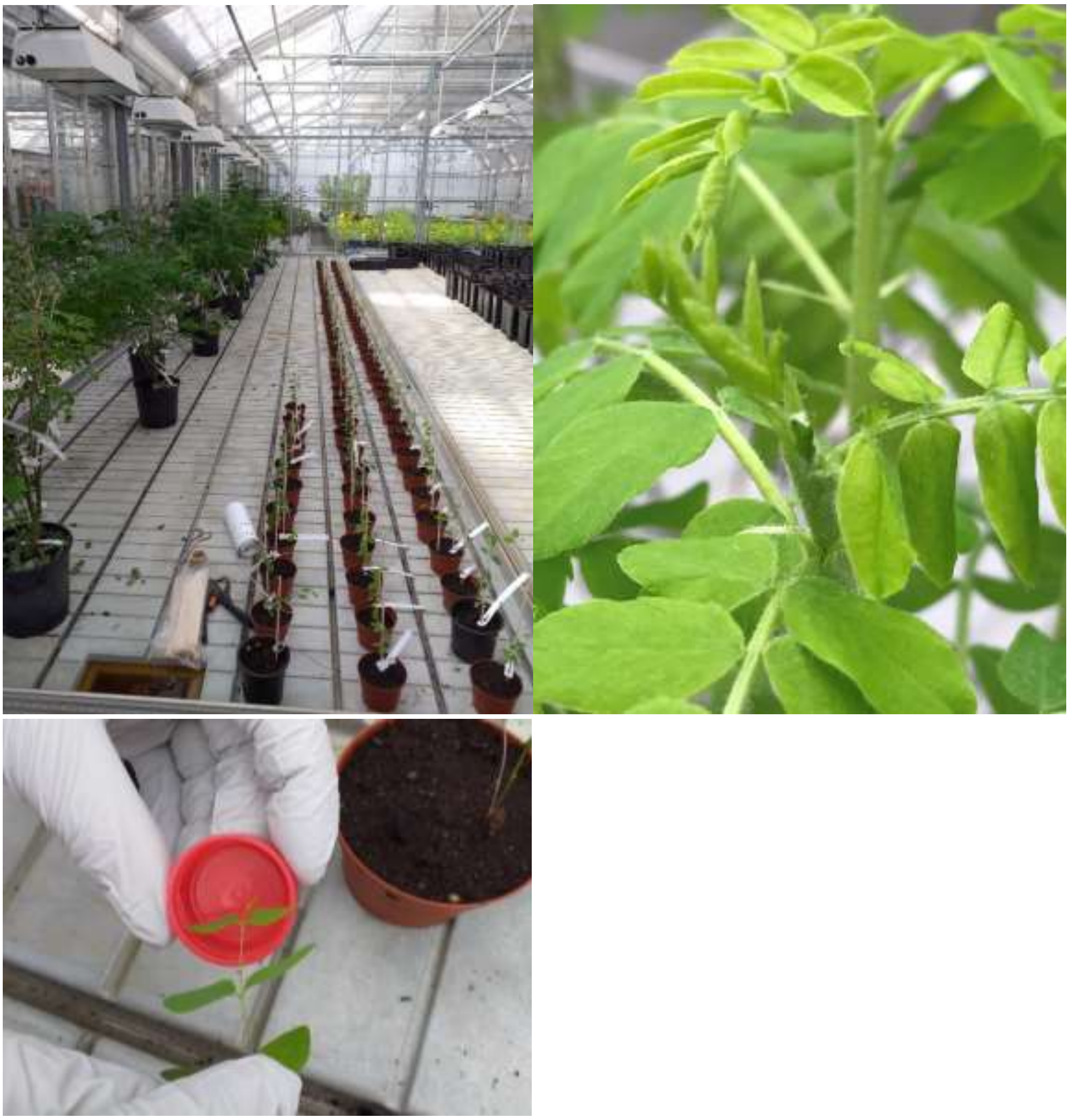

Figure 7-9. *Top left:* Experimental plants, from left to right; *Caragana arborescens*, control of *Vicia pisiformis*, treatment 1 and treatment 2. *Top right:* Shoots of *Caragana arborescens*. *Bottom:* Treatment of apical meristem of *Vicia pisiformis* with colchicine. Pictures: Maximilian Isendahl.

3.2 Results

3.2.1 Polyploidization of Siberian pea shrub

1-3 of the treated lateral buds per shoot developed within a few weeks after the treatment. There were no obvious effects of the treatment, like shorter nodes and larger leaves, on the new growth. Watering was accidentally skipped for one week which had detrimental effects, and much of the new growth dried down and died. New growth sprouted from the treated shoots, but no effects of the treatment could be noticed.

3.2.2 Polyploidization of Pea vetch

The seeds of pea vetch germinated fairly quickly and germination percentage was high. After three weeks, 72 % had germinated and when the seedlings were transplanted after six and a half weeks, 97,5 % of the seeds had germinated. At this time, nodules were present on most roots which indicate that symbiosis with N-fixing bacteria was initiated (Fig. 1).

After transplantation and the colchicine treatment, the plants grew vigorously and had a high tendency to send new shoots from the base. These shoots were removed to promote the treated meristem to grow. Yet, no effects of the treatment were visible. The density of stomata of some leaves from treated plants were compared with the control, but no difference could be identified.

4. Discussion

4.1 Evaluating the potential of the aforementioned species

The evaluation will start with a brief summary on the prerequisites for growing of each specie and comments on the potential of cultivation in Sweden. A discussion on the possibilities of breeding will follow, commenting previous studies as well as the pilot experiments on *V. pisiformis* and *C. arborescens.* An overview of some of the traits in the discussed species is found in the Appendix (Table 1.)

4.1.1 Evaluating the potential of the pea vetch

The pea vetch grows vigorously and the seeds germinates easily after scarification. Reports on wild populations of the pea vetch in Sweden indicate that they may suit alley-cropping systems since they tolerate partial shade and naturally coexist with e.g. hazel and berry bushes. Although they can handle full sun, they are likely not very efficient in shading out weeds, which may result in large problems with especially perennial weeds. Since they favour calcareous soils and since flower formation is adversely affected by low night temperatures, they geographical application of the specie is limited. However, it may still be suitable to larger parts of the country than its current distribution, which mean that it perhaps should not be completely overlooked for this reason.

The fact that the plant is readily consumed by livestock may be turned to an advantage. If tetraploid pea vetch produces less seed, it may produce more biomass which can be used as nutritious livestock feed. Its value would lie in its adaptability to semi-shade which may make it suitable for intercropping in fruit or nut orchards.

As Black-Samuelsson (1997) have shown, there is variation in temperature tolerance and seed set within the species, which may provide a basis for breeding. Since the fertility of has been found to increase when crossing neopolyploids of different accessions, this may be one way of improving the productivity in the species. Since the pilot experiment could not show positive results, more effort will be needed to examine the prospect of this project.

Reports of different naturally occurring tetraploid *Vicia* species are ambiguous and cannot be directly translated into how tetraploid *V. pisiformis* will perform. Nevertheless, these reports may offer a glimpse of what benefits and problems tetraploid pea vetch can have. Tetraploid *Vicia* species have a geographical distribution that differs from diploid counterparts and in some cases are greater, which shows definite benefits of tetraploidy. At the same time, decreased drought tolerance in tetraploids is a clear weakness.

Crossing experiments indicate that crossing barriers are present between *V. faba* and *V. pisiformis*. Being a rare species, prospects of finding sufficient intraspecific diversity to breed sufficiently productive varieties of pea vetch are small. It might therefore be interesting to study the possibility to use embryo rescue to facilitate viable *V. pisiformis* x *faba* hybrids. This to address important problems in *V. pisiformis* such as low yield, non-uniform ripening, small seed size and non-dehiscent pods.

4.1.2 Evaluating the potential of the Siberian pea shrub

The Siberian pea shrub is already in cultivation, as a hedge plant, in large parts of the country and is also suitable for the northern regions because of its hardiness. Although it favours calcareous soils, it also tolerates infertile soils, which gives it a wider application. As a shrub, it can be applied to alley cropping systems, especially because of its effective N-fixing ability, which commences early in the season even in low temperatures. Seed ripening is uniform, which is favourable since it can make harvest more efficient.

There appears to be a high variation in important characteristics such as seed yield, fruiting habit and growth habit. This indicates potential for selection on superior plants, with which further breeding efforts can be derived. Since the Siberian pea shrub does not have any closely related species cultivated for their seed, there are no obvious shortcuts in breeding superior varieties, except for polyploidization should it prove fruitful. Unfortunately, no tetraploids could be identified from the pilot experiment, which means that more effort is needed to examine this possibility. Collection of different accessions and identifying favourable traits could then be the first steps.

Previous trials with tetraploid Siberian pea shrub (Borodina, 1981; Moore, 1965) show are highly interesting and show both positive and negative sides of the polyploids. Clearly, increased drought stress tolerance is favourable, especially considering the effects climate change is assumed to have in the region. Vigour was reduced in tetraploids, which might result in a longer time until the plants come in to production. On the other hand, a more compact growth habit, which regularly is seen in tetraploids, might be desirable since it can result in more easily managed plants. Larger seed size, almost the double weight per seed was found in the tetraploids compared to the diploid counterparts. Since increasing seed size was the main aim of the experiment conducted for this essay, this rapport is promising. As could be expected due to the common incidence of meiotic irregularities in autotetraploids, seed set was reduced in tetraploid Siberian pea shrub. To address this issue, several accessions of Siberian pea shrub would be collected and treated to acquire a high diversity within the tetraploid gene pool. This would allow genetical recombination and selection of superior genotypes.

4.1.3 Evaluating the potential of the lupins

Naturalized populations of *L. polyphyllus* grows are present in every county of Sweden, indicating that the specie is highly adapted to the Swedish climates with short seasons and cold winters. The plants can thrive on dry, acidic infertile soils, which enables a broad use of the species. Since they emerge early in the spring, they effectively shade out weeds and can utilise the solar energy during the sunny spring which may translate into a higher potential yield.

Breeding perennial lupins for food production in large parts of Sweden appears to be a viable, yet complicated project. Perennial species of the *Platycarpos* subgenus like *L. polyphyllus* or *L. perennis* (as well as other species not discussed further here, e.g. *L. nootkatensis)* may be crossed with the annual *L. mutabilis*. The major drawback with lupins, the alkaloid content, can be dealt with through breeding, as has been done with several lupin species. Alkaloid concentrations should be reduced to less than the threshold of 0,02 %. Since the lupins have evolved to synthesize alkaloids to ward of pests such as herbivorous insects and seed predators, a decreased alkaloid content can be expected to result in larger problems with various pests. This must be taken into consideration both in breeding and in subsequent cultivation, to ensure that the sustainability of lupin cultivation is retained. One objective in breeding of lupins is selecting plants with high alkaloid content in vegetative parts, while keeping the concentration low in the seeds (Frick et al., 2017).

A significant question is what perennial specie to work with. Great progress has been made with *L. polyphyllus*, resulting in hardy perennial hybrids with non-dehiscent pods and higher yield as well as larger seed size. Nonetheless, the fact that these species are cross pollinated *L. polyphyllus* commonly grows along roads and fields result in a high risk of reintroduction of the high-alkaloid trait together with other undesired traits. This can be tackled with the establishment of special lupin nurseries, where naturalized lupins have been removed from the vicinity. This would still mean that farmers cannot use their own seed (unless naturalized lupins are dealt with), which limits local adaptation and in the long run, intraspecific crop diversity. For this reason, it might be more practical to use *L. perennis* instead of *L. polyphyllus*, but studies on how easily these two species hybridize in natural setting should first be examined to clarify however the assumed benefit of *L. perennis* holds true. Additionally, *L. perennis* is easily affected by inbreeding depression, which does not seem to be a problem in *L. mutabilis*. This is another factor of significance, since it indicates presence of deleterious genes.

One way to effectively separate cultivated and naturalized lupins genetically is by inducing polyploidy, since e.g. tetraploids and diploids rarely form fertile offspring. This would require further breeding and testing to ensure that the concentration of alkaloids does not rise, since inducing tetraploidy is known to interfere with synthesis of secondary metabolites (Caruso et al., 2011; Philipov, 2002). Additionally, caution should be taken to ensure that tetraploid lupins does not naturalize and contribute to the problem with invasive species. If not for biodiversity then for the sake of production, since the high alkaloid trait may return and spread rapidly in naturalizing populations when natural selection favours the most resistant and unpalatable plants.

4.1.4 Evaluating the potential of the thicket bean

The thicket bean has a historic distribution that extends into Minnesota and Ontario, which indicates that great cold hardiness is present in a specie of the *Phaseolus* genus which otherwise is confined to the subtropics and tropics of Central and South America. Nonetheless, the plants emerge late in the season which indicate that they require warm summers and are sensitive to late frost. The late emergence coupled with its habitat in sunny stony forest slopes also indicate that the plants have a limited ability to compete with weeds. They also require a well-drained soil, without which they apparently are prone to rot in the winter. Considering these factors, its use is geographically limited in Sweden. It could however be valuable in horticultural production in at least some of the warmer parts of the country, given sufficient research and breeding.

Since the natural distribution of the thicket bean is decreasing, the prospects of acquiring a satisfyingly high variation from which to conduct breeding is diminishing, but efforts of collecting plant material for diversity studies are carried out (Dohle et al., 2013). The fact that flower production in thicket bean is dependent on day-length is of substantial significance. Sweden, being at higher latitude than the native range of the thicket bean, has longer days than what the species is adapted to. This factor should be taken into consideration in potential breeding programmes and depending on how it affects production in the region, the sensitivity to day-length may be altered or removed.

Interspecific hybridization is of great interest in this case, since highly domesticated species are closely related to the thicket bean. The *P. polystachios* x *lunatus* crosses that were conducted in 1951 resulted in highly infertile plants, that gave rise to allotetraploid offspring. After generations of selections, fertility has improved, but is not fully restored. No official sources on the fate of the hybrids have been found after 1981, which is troublesome. Further recombination and selection will be needed to increase fertility and thus yield. Since the original hybrids originate from a limited number of crosses which all involve a *P. lunatus* parent which related to the cultivar ‘Fordhook’ (Lorz, 1952), it may be interesting to use other neopolyploids, to further examine how genetic factors may affect fertility. Also, the objective in this essay is to study potential for perennial legumes for the Swedish climates, which is in contrast with the objectives for the original crosses carried out in Florida, which were to improve the lima bean by introducing genes for hypogeal germination together with resistance to various diseases and pests. Therefore, it may be crucial to conduct breeding trials where also cold hardiness and perenniality are prioritized from the beginning.

4.2 Potentials of polyploidization

Doubling the number of chromosomes in a plant can have numerous additional effects than the commonly known ‘Gigas effect’. Polyploidization can change an ability in either direction, depending on the plant material used. One trait that is highly significant in this essay is the reduced fertility induced by autopolyploidization. Since the aim of the experiment is achieving a higher yield of seeds, inducing polyploidy in the studied species is likely counterproductive in the short term. However, the reduced fertility in neopolyploids can be restored, since it is governed by meiotic, genetic, and phenotypic factors which can be selected upon. Additionally, studies on naturally occurring polyploids displays a high productivity in e.g. tetraploids compared to diploid counterparts. In other words, post-polyploidization recombination and selection are crucial steps in attaining superior autopolyploids when breeding for seed production.

The pilot experiment was not successful and did not deliver any tetraploids. The study on the plants will continue, since the effects of the treatment may become visible at a later stage.

The results show that successfully inducing of polyploidization can be difficult, and strengthens the statement that the optimal procedure for polyploidization of a certain species can be quite specific. Better result may have been acquired by using different methods. Treating lateral buds may not be optimal, since active cell-division is needed for colchicine to work. The method described by Moore (1965) which resulted in one stable tetraploid branch, could be used on *Caragana arborescens*. This procedure comprised of placing agar-colchicine capsules on swelling terminal buds. Successful results with some species have been acquired be immersing seeds or small seedlings in colchicine solutions. Although this reportedly has failed with

Caragana arborescens (Moore, 1965), it may prove more successful on e.g. *Vicia pisiformis*.

In further experiments, it would be interesting to also attempt polyploidization of *Phaseolus polystachios,* as this species has the same drawbacks as the previous two. Creating tetraploid *P. polystachios* would be especially interesting since there are amphidiploid hybrids with *P. lunastachios* that may benefit from recombination.

4.3 Potential of hybridization

The most common approach in the scenario of breeding perennial counterparts to annual crops appears to be interspecific hybridization, as exemplified in breeding of perennial lupin (Kurlovich, Stoddard, & Earnshaw, 2008) and barley (Westerbergh et al., 2018). This method allows for a potentially rapid introduction of crucial traits such as high yield, non-dehiscent pods and large seed size into a non-domesticated specie. Hybridization also results in high diversity progeny from which selection can be made and adaptation to local conditions can be derived. Interestingly, hybridization can also result in polyploidization; allopolyploids like the amphidiploid *P. polystachios* x *lunatus.* The species discussed, except for Siberian pea shrub, has cultivated relatives that displays the aforementioned traits. Yet, obtaining viable progeny from interspecific hybridization is of varying difficulty depending on the species involved.

5. Conclusion

5.1 What potential is there for breeding of perennial legumes for cultivation in all of Sweden?

The studied species have different potentials for breeding and cultivation in Sweden. Given their different habitats and requirements, they may altogether make breeding and subsequently cultivation of perennial legumes for human consumption possible in large parts of Sweden. Development of resilient, soil enriching and less capital and resource intensive crops may be valuable to farmers and help increase the self-sufficiency level.

The most promising, especially considering parts of Sweden outside the current centres of agricultural production, are the lupins of the *Platycarpos* subgenus which have several crucial traits. The perennial species *L. polyphyllus* and *L. perennis* express cold hardiness, adaptability to short growing seasons, early emergence in the spring and tolerance of dry, infertile soils. Combined with the larger seed size, non-dehiscent pods and higher seed yield of the annual *L. mutabilis* constitutes a promising future crop for large parts of Sweden. The main drawback – the alkaloid content, can and has already been dealt with to a large extent. Until reliantly stable low-alkaloid cultivars for human consumption are selected, the crop can at least suit as substitute for soy in livestock feed.

The thicket bean, especially hybrids with the lima bean, is also of interest, expressing cold hardiness and high vigour. This specie likely requires warmer summers than e.g. lupins, which may give it a more southern limit than does its cold hardiness. Nonetheless, research on its adaptability to the Swedish growing season and further breeding, hybridization and perhaps polyploidization is crucial to making cultivation of this species viable.

Breeding of Siberian pea shrub and pea vetch appears more complicated since interspecific hybridisation with domesticated crops appears unfeasible (unless embryo rescue would permit *V. pisiformis* x *faba* hybrids). Improvement of these species consequently relies on acquiring diverse genetic material and subsequent crossing and selection, as well as polyploidization.

5.2 Is it possible to induce tetraploidy in *Caragana arborescens* and *Vicia pisiformis* for breeding purposes?

From the literature study on polyploidy, the conclusion can be drawn that it is possible to induce tetraploidy in Siberian pea shrub and pea vetch. The resulting neotetraploids would likely express decreased fertility, which could be regained through sufficient recombination.

It has not been possible to find tetraploidy in the treated plants of the pilot study, although the plants will be examined further to determine if any signs of polyploidy arise. In conclusion, more effort has to be made to successfully acquire tetraploid strains of Siberian pea shrub and pea vetch to evaluate how well procedure works in the aim of attaining plants with larger seeds and higher yield that are easier to harvest.

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Appendix

Table 1. Compilation of traits of the discussed species.

	Caragana arborescens	*Vicia pisiformis*	Perennial *Lupinus* sp.	*Phaseolus polystachios*
Uniform ripening	Yes	No	No	No
Non-dehiscent pods	No	No	In some hybrids	Somewhat
Sun exposure	Full sun	Part shade to full sun	Full sun	Slight shade to full sun
Soil acidity	Calcareous	Calcareous	Acidic	Acidic
Soil moisture	Medium	Well drained	Medium	Well drained
Growth form	Large shrub	Herbacious perennial	Herbacious perennial	Herbacious perennial
Related to widely cultivated species	No	Yes	Yes	Yes
Hybridizing with related cultivated species	-	Probably very difficult	Yes	Yes, difficult
Cold tolerance	High	Medium	High	High
Heat demanding	No	Yes	No	?