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Introducing faba bean as a new multi-purpose crop for Northeast U.S.A.

Fatemeh Etemadi

INTRODUCING FABA BEAN AS A NEW MULTI-PURPOSE CROP FOR NORTHEAST U.S.A

A Dissertation Presented
by

FATEMEH ETEMADI

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INTRODUCING FABA BEAN AS A NEW MULTI-PURPOSE CROP FOR NORTHEAST U.S.A

A Dissertation Presented

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DEDICATION

Dedicated to my beloved parents for their love, endless support, encouragement, and sacrifices. They have given me love and a strong will to encounter the challenges of everyday life. I have been raised and taught the value of education since I was a child. Also, to my sisters, who have always encouraged me in my academic pursuits.

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As a final word, I would like to thank each and every individual who have been a source of support and encouragement and helped me to achieve my goal and complete my dissertation work successfully.

ABSTRACT

INTRODUCING FABA BEAN AS A NEW MULTI-PURPOSE CROP FOR NORTHEAST U.S.A

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Faba bean is a multi-benefit cool-season grain legume that can be integrated into cropping systems of a shorter-growing season regions such as New England. A comprehensive research study was conducted to explore the ecological, nutritional, medicinal, and financial benefits of faba bean as a new multi-purpose crop for Northeast U.S.A. It was revealed that the faba bean genotypes exhibited dramatic variations in thermal units required to reach various phenological stages. Using phenology, morphology and physiological growth pattern of six faba bean varieties showed that larger seeds and later maturity did not necessarily produced higher yield. Aquadulce, the earliest maturity with medium size seed out-yielded other genotypes thus providing the opportunity for double cropping. When planted in August 1, faba bean accumulated up to 192 kg N ha^{-1}. Nitrogen mineralization from faba bean residues had a better synchrony with sweet corn N uptake in no-till system than conventional tillage system. Sweet corn planted into faba bean residues produced higher ear yield and less unfilled ear tip compared with those grown in no faba bean plots. On average, sweet corn planted into faba bean residues responded positively to applications of supplemental N up to 60 kg ha^{-1}. Averaged over two years, sweet corn after faba bean and 50 kg N ha^{-1}, yielded similarly to those that received 100 kg N ha^{-1} without faba bean cover crop. Faba bean varieties exhibited considerable variations in their macro- and micronutrient contents. Averaged over six varieties, faba bean seeds contained over 19% protein, 0.76% P, 1.78% K, 0.19% Ca, and 0.20% Mg on dry weight basis.

Micronutrients content of seeds were Fe 71, Cu 20, B 25, Mn 16, and Zn 40 mg kg^{-1}. Results also revealed that faba bean leaves and pod walls were also rich in protein, macro and micro nutrients. The results of the experiments suggested that the highest L-Dopa concentration was detected at seedling stage however, to maximize the optimum L-Dopa yield, the crop should be harvested prior to the physiological maturity, roughly 70 days after transplanting. It was concluded that the amount of dry matter produced by faba bean was considerable enough to be grown as a viable source of natural L-Dopa that is comparable with synthetic sources. However, given that total extractable L-Dopa is a function of both concentration and biomass, the highest L-Dopa yield was extracted from mature plants. The highest L-Dopa content was detected in fresh leaves followed by flowers, young pods, mature seeds, and roots. It was concluded that all processing methods reduced the L-Dopa content. Among all tested processing methods, immediate freezing of plant tissues preserved more L-Dopa than oven drying, air drying, and boiling. Plants grown in drought stress condition synthesized higher L-Dopa however, their L-Dopa yield was significantly lower due to producing lower biomass. Supplemental N application did not influence L-Dopa synthesis, however L-Dopa yield increased with increased N rate, primarily because of higher biomass production.

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CHAPTER I
INTRODUCTION

AGRONOMY, NUTRITIONAL VALUE, AND MEDICINAL APPLICATION OF FABA BEAN (*VICIA FABA* L.); A REVIEW

Abstract

Faba bean (*Vicia faba* L.) is a cool season grain legume crop with the potential to be grown as multi-purpose crop in areas with short growing season. Faba bean is grown in many regions in the world due to its high nutritional value, medicinal effect, and effective biological nitrogen fixation. Diverse ecosystem benefits are expected from integrating faba bean in cropping systems. This paper specifically reviews the published work of the authors and other relevant published papers covering agronomic practices, nutritional values, medicinal benefits, and faba bean's capacity for nitrogen fixation, and its nitrogen contribution to the preceding crops.

Introduction

Faba bean is a cool-season grain legume crop that originated in the Middle East in the pre-historic times and traditionally has been used as a main source of protein for human and animal nutrition (Youssef et al., 1986; Multari et al., 2015). The genus Vicia is in the family Fabaceae, which is enormous in number of species with worldwide distribution. Current estimates are of 16,000 to 19,000 species in approximately 750 genera in this family (Chakraverty et al., 2013). The Fabaceae is second only to the grasses (Poaceae) in terms of economic importance. Various species of Fabaceae are currently growing in temperate areas, humid tropics, arid regions, highlands, savannas, and lowlands, and there are even a few aquatic legumes (Wrigley et al., 2015).

In optimum growing conditions, germination of faba bean seeds takes about 10 to 14 days (Etemadi et al., 2015a). However, it will take much longer in dry condition or when soil temperature is cold. On average, faba bean plant grows one node per week. Because faba bean stems are relatively strong and grows upright, the plant can grow about 90 to 130 cm tall, mainly depending on the genotype. At the 8-to-10 node growth stage, when the plant is around 30 cm tall, faba bean produces its first flowers, usually in the month of June in the Northeastern U.S.A. Flowers and pods appear about 20 cm above the ground. Roughly 25% of the flowers will produce pods, which usually contain three to six seeds (Etemadi et al., 2015a). Therefore, proper management practices including irrigation, soil fertility, and time of planting can significantly reduce the number of aborted flowers, thus improve final seed/pod yield.

Generally, seed germination of most legumes is sensitive to low soil temperature. However, faba bean is one of the few cool-season grain legumes and its germination can tolerate cold soil temperature better than most grain legumes. Attempts to select for improved seed germination at the soil temperatures below 15 ^{o}C have shown some degree of success (Singh and Jauhar, 2005). Better germination rate has been reported in large-seeded cultivars than in small-seeded beans at 12.5^{o}C (Kang et al., 2008).

Due to the superior nutritional values including protein, carbohydrates, B-group vitamins, and minerals (Crépon et al., 2010), faba bean is considered as one of the most important pulse crop in the world. Faba bean is a staple food for many ethnic groups, such as Portuguese-speaking, Middle Eastern, as well as many Asians. In recent years, cultivation of faba bean has received large attention in other countries including U.S.A, Canada and Europe (Etemadi et al., 2014a; 2015a; 2016; 2018a). As a cool-season legume, faba bean can be included in various copping systems in shorter-growing season regions such as Northeast U.S.A. However, currently only two varieties of faba bean are available to the growers in the Northeast U.S.A. The current available varieties demonstrated some drawbacks such as high seed cost due to the large seed size and relatively low pod yield (Etemadi et al., 2017).

Faba bean is used not only as staple food but also integrated into various crop rotation systems to minimize the occurrence of cereal cyst nematode (*Heterdera avenae*) (Sattell et al., 1998) and soil-borne pathogens (Landry et al., 2016). Faba bean is partially a self-pollinating plant however flowers attract various pollinators, specifically honey bees. Current reports indicated that honey bees and other natural pollinators can increase the pollination incident and thus grain yield in faba bean (Musallam et al., 2004; Marzinzig et al., 2018).

Faba bean has been identified for its efficient N fixation capacities which is highest among the cool season legumes (Herridge et al., 1994; Mekkei, 2014). Reports indicated that faba bean can fix 50 to 330 kg N ha^{-1} (Galloway et al., 2004; Visalli, 2015; Etemadi et al., 2018d) depending on cultivation management and environmental conditions (Hu and Schmidhalter, 2005).

Legumes have a substantial role in maintaining soil fertility, not only through biological N fixation but also by solubilizing insoluble phosphorus (P) in soil, improving the soil physical environment, and increasing soil microbial activity (Rashid et al., 2016). In legumes, the development of effective root nodules with the high capacity for symbiotic N fixation involves a complex series of interactions between the host plant and the symbiotic bacteria (Guinel, 2009). Genetic variation for N fixation has been identified in faba bean (Graham and Vance, 2000). Other factors such as nodule efficiency and earliness and duration of nodulation may also play important role (Martinez-Romero, 2003). It is well known that legumes generally respond to existing N concentration in the soil (Graham, 2008). In soils with relatively high N, legumes prefer using soil N rather than to engage in symbiosis with rhizobia (Divito and Sadras, 2014). However, minimal application of N may stimulate N fixation by improving early plant growth until N fixation provides adequate N for plant growth and development (Muehlbauer et al., 2006; Huang et al., 2017; Abdul Rahman et al., 2018). On the other hand, fertilizer application rates exceeding the minimal "starter-N" generally suppress nodulation and N fixation (Kinkema et al., 2006).

Variation in time to reach specific phenotypic stage is related to the sensitivity of the genotypes to variations in the photo-thermal regimes, thus affecting days to maturity, adaptation to the environment, and crop's yield in diverse growing conditions (Peng et al., 2004; Aghaalikhani et al., 2012). Different types of faba bean, including spring, winter, and Mediterranean, exhibit various phenological characteristics; therefore, induction and maintenance of flowering differ among these classes (Patrick and Stoddard, 2010).

Agronomic practices

Traditional agronomic practices in faba bean include certain steps for best incorporating the crop into farming systems to improve soil quality, enhance water use, manage crop residues, and improve the environment through better fertilizer management. Some of the most important agronomic practices in faba bean cultivation described below.

Seed size

Faba bean generally considered as a large-seed crop however, its seed size varies greatly among varieties and cultivars (Table 1). Within each cultivar, the seed size also varies greatly depending on pod location. Faba bean seeds are classified as large (>1.0 g $seed^{-1}$), medium (0.5-

1.0 g seed^{-1}) and small (<0.5 g seed^{-1}) (Etemadi et al., 2015a). Traditionally at the farm level, the desired population density is determined by the seeding rate, which is primarily determined by the number of seeds per unit weight (kg) (Gezahegn et al., 2016). Plant breeders have recognized the importance of larger seeds in production of food crops and have been breeding for the trait for years. The general conception is that large seeds have more food reserves thus germinate faster, have better vigor and higher yield than smaller seeds within the same cultivar (Gan et al., 2004; Harker et al., 2015; Patel et al., 2016). However, the use of smaller seeds can significantly reduce the production costs by lowering the amount of seeds needed per unit area (Etemadi et al., 2017). For example, for cultivars presented in Table 1 (Etemadi et al., 2017) the seeding rate varies from 32 to 244 kg $^{-1}$ which dramatically influences the seed cost. Seed cost is especially important when faba bean is grown as cover crop or as dual-purpose cash and cover crop. When used as cover crop, it is highly recommended to select small seeded varieties.

Seed Treatment

As a legume crop, faba bean is able to fix atmospheric N through a symbiotic relationship with rhizobium bacteria (Herridge et al., 2008). Maximum benefit from the symbiotic relationship occurs when soil available N is low and soil moisture and temperature are adequate for normal seedling development from the time of seeding until seedlings are well established. Rhizobia can live in the soil for a number of years; however, the most efficient N-fixing bacteria may not be among those that survive. Although rhizobium bacteria remain alive for about 3-5 years in soils, it is recommended to inoculate the seeds every year for achieving maximum N fixation. Faba bean seeds should be inoculated with "R. leguminosarum bv. viciae" (Abd-Alla et al., 2014). Inoculants should be kept dry and fresh and stored properly (Rodelas et al., 1999). Seeds should be inoculated just before planting (Etemadi et al., 2015a) so that large numbers of rhizobia will be ready to start the infection process as soon as the roots emerge. These rhizobia can infect the roots quickly and start the process of nodulation (Makoudi et al., 2018).

Seeding rate

For most legumes, the choice of population is an important agronomic practice that not only affects their final yield but also influences the amount of biologically N fixation. When used as cover crop, a legume needs to produce at least 4,000-4,500 kg ha^{-1} of biomass to provide sufficient available N to meet the N demand of succeeding crop (Teasdale et al., 2004; Parr et al., 2011).

The optimum plant population of faba bean for maximum pod and grain yield as well as N fixation is not well documented. A wide range of densities of faba bean is commonly used, from 45,000-60,000 plants per ha, depending on the cultivar (seed size) and the region.
Like other grain crops, the response of faba bean to increasing population density is parabolic. However, due to high seed cost the economic population density should be considered for recommendation to the growers. Regardless of seed size, faba bean yield reaches its maximum yield at different plant populations depending on the size of the seed; the smaller the seed, the higher the plant population is needed to reach the yield peak at around 60,000 plants per ha. It must be noted that faba bean tends to produce more lateral branches when grown at below optimum populations. The lateral branches often are not as productive as the main stem, therefore, seeding rates should be adjusted for lowering the number of lateral branches and limit them to 2-3 per plant (unpublished data). There is a correlation between high seeding rate and high humidity within the canopy. Given the fact that faba bean is more sensitive to diseases in high humidity, high populations should be evaluated not only for higher yields but also susceptibility to diseases**.** Also, when possible, west –east planting pattern is preferable for faster drying by sunlight.

Variety

Similar to other crops, variety or cultivar selection requires achieving a balance between adaptability to a specific environment, disease tolerance, purpose of cultivation, and marketability (Zandvakili et al., 2018). Faba bean varieties vary significantly in seed size and colour. Small-seeded types (*Vicia faba* L. subsp. *minor*) also called faba bean or tick bean are commonly used for feeding animals and cover cropping. Medium and large size seed types (V. *faba* subsp. *major* Harz) are called broad bean and commonly used as dry and green beans (Crépon et al., 2010). Although modern cultivars dominate in Australia, Europe and Canada, traditional landraces are grown widely in many countries and a mix of traditional and modern varieties are grown in other countries. Germplasm with best adaptation in southern Australia originated from the Mediterranean Basin and was the basis for the commercial varieties Fiord and Fiesta (Gnanasambandam et al., 2012). In 2014, three cultivars of high-yielding faba bean were released in Sudan which can tolerate temperatures up to 35°C – ‘Hudeiba93’, ‘Basabeer’ and ‘Ed-Damer’. These cultivars have increased faba bean yields in Sudan by 40% and currently are dominating the farmers’ fields in the country. The cultivars released in Egypt in 2011, ‘Nubaria 3’ are drought tolerant (ICARDA, 2018). In New England, the variety Windsor is currently the dominant cultivar available to the growers (Etemadi et al., 2017). Thus, evaluating more varieties for high potential yield and suitable morphological characteristics can provide valuable information to the researchers and faba bean growers. In addition, introducing faba bean with smaller seed size may result in adoption of faba bean as an efficient N-fixing cover crop.

Ideal soil conditions

Faba bean grow best on fine-textured soils but tolerate nearly any soil type (Jensen et al. 2010). The ideal soil pH for growing faba bean is ≥ 7 (Köpke and Nemecek, 2010). In areas with relatively high precipitation such as Northeast U.S., the soils tend to be acidic (New England vegetable guide, 2018). Therefore, liming is required for faba bean cultivation when soil pH level is below 6. Although sandy loams are suitable for growing faba bean but more frequent irrigation will be required. Faba bean has relatively shallow roots, thus the crop may suffer from water stress in soils that dry quickly. Faba bean seems to be tolerant to short period of water-logging (Tekalign et al., 2016).

The optimal temperature for plant growth is 18 to 27 °C (65-85°F) (Jensen et al., 2010; Link et al., 2010), especially during the stages of flowering and pod development. Faba bean flowers and young pods abort if temperatures exceed 27 °C. This is because flowers and pods are particularly sensitive to hot and dry conditions during pod development stage. Prolonged cool weather in the spring is ideal for development and survival of flowers and pods.

Nitrogen

In general, legumes are known as self-sustaining crops regarding N. However, the efficiency and magnitude of biologically fixed N varies greatly among the legumes. Legume species that meet 50 to 90% of their total N requirements from symbiotic N fixation are considered effective N fixer (N’Dayegamiye et al., 2015). Faba bean and soybean with 75 and 68%, respectively are ranked as high capacity legumes for N fixation (Herridge et al., 1994; Peoples et al., 2009).

Arguably, the most important contribution of faba bean to agricultural ecosystems is the substantial amount of atmospheric N that can be fixed by the hosting legume and its associated rhizobia. Depending on the growing conditions, faba bean is capable of fixing up to 200 kg N ha^{-1} (Cline and Silvernail, 2002; Hoffmann et al., 2007; Horst et al., 2007; Etemadi et al, 2018d). However, the amount of biologically N fixation by legumes is influenced by many factors. Variations in soil pH, water and nutrient availability, and soil temperature, are among factors greatly influence the growth, survival, and metabolic activity of biological N fixation (Werner and Newton, 2005; Mohammadi et al., 2012). Legume plants generally respond to existing N in the soil and often reject symbiosis with rhizobia when sufficient N is available in the soil (Kellman et al., 2006; Sinclair, and Nogueira, 2018). This effect of plant available soil N is greatest during early vegetative growth, where N fixation by faba bean is low (Vance et al., 1988; Kinkema et al., 2006).

There has been some controversy about the requirement for sowing grain legumes with low levels of ‘‘starter N’’ to overcome N-limitations during early growth stages, however in general positive yield response is seldom observed when the soil contains >20–30 kg N ha^{-1} in the plow layer (Roman et al., 2004). Interestingly, faba bean differs from many other legumes and maintains its N-fixing capabilities, even if soils are relatively rich in N (Peoples et al., 2009; Kőpke and Nemecek, 2010). Since the host legume provides carbohydrates to the rhizobia, optimum photosynthesis is required to support maximum derivation of atmospheric N to meet N requirement of the legume host plant. It is well documented that the magnitude of agronomic services from legume cover crops are largely dependent on the amount of biomass they can produce at harvest (Finney et al., 2016; Mirsky et al., 2017). Early planting is considered as one major factor that influences legume biomass yield (Komainda et al., 2016; Etemadi et al, 2018d) especially when faba bean is used for cover cropping purpose.

Phosphorous

Phosphorous plays important roles in nodulation and biological N fixation, photosynthesis, and nutritional values of legumes including faba bean (Kőpke and Nemecek, 2010; Sulieman and Tran, 2015; Haling et al., 2016; Makoudi et al., 2018). Phosphorus application often results in increased yield and biomass of faba beans in P-limited soils (Agegnehu and Fessehaie, 2006; Daoui et al., 2012), indicating that P fertilizer is essential for grain production in faba beans (Bolland et al., 2000; Nebiyu et al., 2016). Also, it has been shown that the induction of plant histidine acid phytase HAP gene depends on P sufficiency in faba bean and significant induction of phytase activities in nodules has been reported under P deficiency (Nasto et al., 2017; Makoudi et al., 2018). Makoudi et al. (2018) concluded that under P deficiency condition, more P is allocated to the nodules of faba bean than shoot, presumably to maintain atmospheric N fixation which requires significant amount of energy. Under P deficiency legume reduce their N fixation in exchange for a greater preference for soil available N uptake by roots (Magadlela et al., 2014; Valentine et al., 2017). Although faba bean yield may be compromised by low availability of soil P, it has been demonstrated that faba bean has the capacity of mobilizing soil P by secretion of acids from its rhizosphere (Nuruzzaman et al., 2005; Nelson and Janke, 2007). When soil P level is high, faba bean can still benefit from application of starter P fertilizer (Selles et al., 2011; Rosolem et al., 2014; Gelfand and Robertson, 2015), especially when the soil temperature at the planting time is low in which N mineralization and biological N fixation is also limited (Agehara and Warncke, 2005; Osborne and Ridell, 2006).

Potassium

Potassium is an essential nutrient and is involved in plant metabolism by activating many enzymes and also plays important role in water management of plants (Zörb, et al., 2014). Faba bean often responds positively to K application, especially in dry growing seasons (Barłóg et al., 2018). Potassium deficiency results in reduced rate of photosynthesis which in turn results in impaired ATP production and reduced transports of assimilates towards roots. Therefore, availability of adequate K to faba bean plants has a positive effect on N fixation (Sangakkara et al., 1996; Kurdali et al., 2002; Römheld and Kirkby, 2010; Gucci et al., 2019). Increased biomass and grain yield production have been reported when K fertilizer was applied to faba bean (Fan et al, 1997; Mona et al, 2011; Grzebisz et al, 2013). It is shown that the symbiotic system in faba bean is less tolerant to limiting K supply that the plant itself, but when nodulation occurs, the assimilation of N from the symbiotic source will not be selectively affected by K as opposed to N assimilation from fertilizer (Sangakkara et al., 1996). Furthermore, application of organic fertilizers such as poultry manure, composted rice straw with oilseed rape cake (Abdelhamid et al., 2004) and/or combination of mineral and organic fertilizers such as olive pomace combined with half of conventional N, P, K dose of mineral fertilization resulted in the same faba bean productivity as full mineral fertilization (Gucci et al., 2019).

Magnesium

Magnesium (Mg) deficiency can reduce faba bean yield (Hariadi and Shabala, 2004). Availability of Mg to plants is affected by various factors including soil moisture deficit or high level of other cations in soil which can inhibit Mg uptake by the crop (Marschner et al., 1995). Although the influence of Mg on biological fixation of atmospheric N in faba bean is not well documented, Peng et al. (2018) reported that application of Mg improved nodule growth and formation of large nodules in soybean. Reports indicated that there is a relationship between Mg supply and the efficiency of N use since the stabilization of RNA depends on Mg with its major function for the activation of glutamine synthetase (Marchner, 1995; Grzebisz et al., 2010). Magnesium application often results in higher concentration of chlorophyll and subsequently higher photosynthesis rate which improves pod yield (Neuhaus et al., 2014). The low Ca an Mg concentration in the seed has been associated with poor germination and weak seedlings in legumes (Keiser and Mullen, 1993).

Sulfur

There is a growing evidence that application of sulfur (S) to various crops resulted in their yield improvement due to reduction in industrial emissions and the associated decrease in deposition of S in soils (Scherer, 2009; Pötzsch et al., 2018). Other reasons for S deficiency in soils could be cultivation of high yielding varieties and use of S-free fertilizers. Sulfur is essential for legume-rhizobium symbiotic system, thus application of S fertilizer to the deficient soils may result in increased number and weight of the nodules, total dry matter, grain yield and total amount of N fixed by faba bean specially in soils with low organic matter (Pasricha and Fox, 1993; Scherer and Lange, 1996; Scherer, 2001; Habtemichial et al., 2007, Scherer, 2008, Cazzato et al., 2012). In general, legume crops require higher amount of S compared with cereals due to their higher amount of proteins and S-containing amino acids including methionine and cysteine in their seeds (Gaylor and Sykes, 1985; Elsiddig and Abdulhafize, 1997; Barłóg et al., 2018). Pötzsch et al. (2018) reported that concentration of S in faba bean shoots was similar to those of peas but lower than soybean and the absorbed S was mainly accumulated in the seeds of faba bean. Although the benefit of S application in improving faba bean yield may have been due to various reasons, in general positive yield responses have been reported when faba bean was fertilized with S (Zhou et al., 2009; Cazzato et al., 2012; Barłóg et al., 2018).

Molybdenum

Nitrogen fixation in faba bean is strongly associated with the Molybdenum (Mo) status of the shoot tissue (Van Zwieten et al., 2015). The availability of Mo in acidic soils is limited mainly due to sorption to Fe and Al-oxides with the maximum sorption is soils with the pH of 4 to 5 (Smith et al., 1997). Therefore, application of lime to the acidic soils can significantly enhanced Mo availability in soils and higher Mo content in faba bean (Van Zwieten et al., 2015).

Date of planting

In temperate regions faba beans are grown either as winter crop or as spring crop where temperature and day-length are significantly different than winter conditions in Mediterranean-type environments (Luna-Orea et al., 1996). In long growing season conditions, plants often compensate for low plant populations density by producing larger number of lateral branches (Mirshekari et al., 2012; Etemadi et al., 2015a). Therefore, the crop cycle i.e. autumn–winter sowing versus spring sowing, greatly influence the length of the vegetative period, thus the final dry matter. The biomass and grain yield benefits from early planted legumes have been documented (Thalji, 2006; Keneni et al., 2012; Etemadi et al., 2018d). In general, delayed in faba bean sowing results in seed yield reduction (Marcelos and Constable,1986; L´opez-Bellido et al., 2005). In New England, delay in spring planting of faba bean results in coincidence of flowering stage with high temperatures in summer which not only increase the number of aborted flowers and pods, but also intensifies the incident of chocolate spot bacteria disease (Etemadi et al., 2015a).

Method of planting

Traditionally faba bean is seeded directly into the soil. However, to ensure early sowing in shorter-season areas, faba bean may benefit from transplanting compared with direct seeding (Etemadi et al., 2015a). In shorter-growing season region such as Northeast U.S., transplanting faba bean provides the opportunity for double cropping and avoids incident of some diseases such as chocolate spot (*Botrytis fabae)* (Etemadi et al., 2018e). Moreover, in such areas, wet soil and weather conditions in early spring may not allow early planting, thus transplanting faba bean can be considered as an alternative method to direct seeding. Transplanting seedlings also offers some other potential benefits including higher yield, survival rate, early flowering, and earlier harvest (Kim, 2006; Lee et al., 2018). Transplanting faba bean requires an indoor sowing in late

March and transplanting into the field when seedlings are approximately 15 cm tall. Research indicated that the optimum temperature for growing faba bean in the greenhouse is 15 ^{o}C and seedlings will be ready for transplanting within 12-15 days (Etemadi et al., 2015a). Pro-mix media in a 3.2 cm cell size tray is enough for this short growing period in the greenhouse.
In the field condition, faba bean seeds should be planted about 2.5 cm deep and the distance between plants on planting rows is 15 cm when row width is 76 cm and 23 cm when row spacing is reduced to 38 cm. This planting arrangement provides approximately 48,000 to 60,000 plants ha^{-1} (Etemadi et al., 2015a).

Diseases

Some of the serious diseases of faba bean are discussed below:

Chocolate spot (*Botrytis fabae*) is one of the most important economic disease that damages the foliage, limits photosynthetic activity, and limits faba bean production (Torres et al., 2004). Long periods of high humidity and high temperature promote the switch from a non-aggressive phase to an aggressive phase of the disease (Terefe et al., 2015). The aggressive phase of the disease is favored also by low levels of K and P in the soil and high plant population density, which leads to more humid conditions within the plant canopy. Symptoms for non-aggressive chocolate spot include small red-brown lesions on leaves of the plant and sometimes on stems and pods (Haile et al., 2016). Under high temperature and humidity, the disease moves to the aggressive stage, and lesions coalesce and become covered by fluffy mycelia, where large patches of tissue can become necrotic. Prevention is the most effective management strategy. Early planting is recommended to avoid high humidity and high temperatures in late spring and early summer (Kora et al., 2017). Appropriate plant spacing to encourage air circulation within the canopy also considered as an effective management tool. Foliar fungicide application throughout the season can be effective (Hawthorne, 2004).

Leaf blight (*Xanthomonas campestris*, syn. *Xanthomonas axonopodis*) is another bacterial disease that can be introduced by contaminated seeds and over-wintering of bacteria in crop residues (Belete and Bastas, 2017). This disease is favored by warm temperatures and wet, humid conditions. Water-soaked spots on leaves which enlarge and become necrotic. Spots may be surrounded by a zone of yellow discoloration, and lesions can coalesce and give the plant a burnt appearance (Harveson, 2009; OMAFRA, 2009; Buruchara et al., 2010). Dead leaves remain attached to the plant and circular, sunken, red-brown lesions may be present on pods. The lesions on pod may leak during humid conditions. Use of clean seeds (Tar'an et al., 2001; Gillard et al., 2009), resistant varieties, seed treatment with an appropriate antibiotic prior to planting and spraying plants with an appropriate protective, copper-based fungicide before appearance of symptoms are used as effective methods of treatments (Schwartz et al., 2005; OMAFRA, 2009; Buruchara et al., 2010).

Bacterial brown spot (*Pseudomonas syringae*). The bacteria over-winter in crop residues and is more severe when the foliage is wet for a long period (Harveson, 2009). Small, dark-brown necrotic spots on leaves may be surrounded by an area of yellow tissues. Water-soaked spots on pods turn brown and necrotic, and pods may twist and distort in the area of infection (Schwartz et al., 2005). Use of clean seeds, proper crop rotation, and the removal of crop residues from the field after harvest are considered as preventive measures (Harveson and Schwartz, 2007).

Powdery mildew (Erysiphe cichoracearum). This fungus overwinters on plant residues or alternate hosts. Disease emergence is favored by warm, dry weather with cool nights that result in dew formation. Yellow spots on upper surface of leaves, and powdery gray-white areas coalesce to cover entire plant. If the plant is infected heavily, it may appear light blue or gray in color (Trabanco et al., 2012). Use of resistant varieties and when possible use of overhead irrigation that washes the fungus from leaves and reduces its viability are preventive practices. Also, recommended is early planting to avoid high air temperature and humidity. Application of S-based pesticides may be required to control heavy infestations (Van Emden et al., 1988).

Fusarium root rot (*Fusarium solani*). Damage caused by the emergence of the fungal disease is worsened by warm, compacted soils, limited soil moisture, and poor soil fertility. The disease is noted by stunted plant growth and yellowing, necrotic basal leaves, brown, red, or black streaks on roots that coalesce as they mature. Lesions may spread above the soil line (Abdel-Kader et al., 2011). The use of clean seed and avoiding planting faba bean in the same area for more than five years in the same field are preventive practices (Anonymous, 2006).

Insects

Black bean aphid ***(****Aphis fabae****).*** Black bean aphids can cause severe damage to faba bean (Webster et al., 2010). When using seedlings, check for aphids before transplanting. Reflective mulches such as silver-colored plastic can deter aphids from feeding on plants by limiting landing of the pest. Sturdy plants can be sprayed with a strong jet of water to knock aphids from leaves. Insecticides are required generally only to treat aphids if the infestation is very high since plants generally tolerate low or medium leaf infestation. Insecticidal soaps or oils such as neem or canola are usually the best insecticides for control (Birch, 1985).

Biomass, Seed, and Pod yield

Faba bean is an important source of plant protein for human and animal consumption and is harvested as fresh pods with immature seeds or dried seeds (Crepon et al., 2010; Singh et al., 2013). General conception is that the larger seeds may have more food reserves thus germinate faster, establishes more successfully and out yield smaller seeds within the same variety/cultivar (Gan et al., 2004). In faba bean, the induction of flowering, retention of flowers and development of seeds are all critical processes determining the final yield (Patrick and Stoddard, 2010; Etemadi et al., 2018e). It has been suggested that higher biomass accumulation is required to obtain higher seed yield (McClean et al., 2008; Gallagher and Biscoe, 1978; Mortimer et al., 2012; Rondon et al., 2007). A high positive correlation between seed yield and biomass production has been reported for faba bean (Kubure et al., 2016). However, faba bean as a cool-season grain legume is sensitive to high temperature, low moisture availability, and days to flowering (L'opez-Bellido et al. 2005) therefore earlier maturity may be beneficial for producing optimum yield. For example, Etemadi et al. (2018e) reported that in shorter-season conditions, Aquadulce with less GDD requirement out-yielded the late maturity faba bean cultivars. They concluded that the number of days to reach maximum CGR is highly correlated with the seed yield. Seed yield is a function of biomass production and partitioned assimilates into the seeds (Ricciardi et al., 2001; Tilman et al., 2002).

In most legumes, dry matter accumulation reaches a maximum around the start of seed filling. In faba bean, root, petiole, stem, and pod weights then remain constant. However, a report noted that as seed weight increased, leaf weight decreased by about 45% (Singh and Schwartz, 2010). Total DM accumulation in faba bean is quite variable, but Croser et al. (2003) reported that field beans accumulated two-thirds of their overall DM before the end of flowering. In other legumes, soybean accumulates 30-67% before flowering, and lentils accumulated16-30% before flower initiation with the majority of the DM being produced after anthesis (Ayaz et al., 2004). In most grain legumes, total DM production often is underestimated due to the loss of leaves and petioles after physiological maturity at harvest time and flower loss, and pod abortion (L'opez et al., 2005). Ayaz et al. (2004) with pinto beans (*Phaseolus vulgaris* L.), in Canterbury, New Zealand, found seed yield was correlated highly with total DM with a stable harvest index. He considered that a high total DM yield was probably a prerequisite for high pinto bean seed yield as was found in chickpea (*Cicer arietinum* L.) by Russo (2003). By comparison, with DM accumulation in soybean (*Glycine max* Merr.), indeterminate plants produced more vegetative growth during flowering and pod set than determinate plants (Eckert et al., 2011).

The yield component approach has been used extensively to study the physiology of seed yield of legumes and other crops (Greven, 2004). The relationship among yield components has been described as compensatory or mutually interdependent (Mhamdi et al., 2002). Consequently, a significant change in one yield component may not necessarily affect the final seed yield (Muehlbauer et al., 2006). Nevertheless, highest seed yields are often obtained when all of the yield components are maximized (Gomez-Paniagua and Wallace, 1992; Mirshekari et al., 2013). Because of considerable variations in faba bean seed size (Etemadi et al., 2017), lack of recommended population density, and also whether the seed is being harvested for fresh or dried purpose, results of seed yields are dramatically different. For example, Coelho (1987) reported that the seed yield of faba bean planted in various population densities varied from 4,750 to 10,950 kg ha^{-1}. Fresh Pod yield of eight faba bean varieties in Etemadi et al. (2015a) study varied from 2,052 to 17,038 kg ha^{-1}.

Faba bean in cropping systems

Crop rotations have been represented as agro-ecosystem diversification "in time" whereas intercropping systems have been practiced providing diversity "in space" (Tosti and Guiducci, 2010). Diverse ecosystem benefits are expected from integrating faba bean in cropping systems

(Kőpke and Nemecek, 2010). In general legumes are traditionally used in cropping systems due to their contribution to the N need of proceeding crops (Hardarson and Atkins, 2003; Zandvakili et al., 2012; Jahanzad et al., 2014; N'Dayegamiye et al., 2015; Denton et al., 2017; Ntatsi et al., 2018). This is even more practiced in organic operations which are heavily rely on non-fossil-based N. In recent years, some economic measures including soil health as well as environment concerns due to the application of commercial N fertilizer resulted in reassessment of the use of legumes in various cropping systems. The capacity of faba bean in biologically fixing N has not been conclusively determined. However, faba bean has been grouped as a high efficient source of N. Thus, whether as cash crop, cover crop, or dual-purpose cash/cover crop, faba bean can be used in rotational systems with other non-legume crops. Other benefits from using faba bean in cropping systems includ2325e (i) break crop in a rotational program (Kőpke and Nemecek, 2010; Abera et al., 2015; Landry et al., 2015), (ii) feed source for pollinators and beneficial insects (Somerville, 2002; Etemadi et al., 2015a), (iii) enhanced soil microbial activity (Wang et al., 2007; Van der Putten et al., 2013; Wahbi et al., 2016), (iv) control of soil-borne diseases (Jensen et al., 2010), and (v) a rich source of L-DOPA (Etemadi et al., 2014b; 2015b; 2018b; 2018c).

Traditionally faba bean is used in rotational program with cereals (Ashraf et al., 2004). The benefits of faba bean to the proceeding cereal include N contribution (Etemadi et al, 2018d; Kaci et al., 2018), enhanced soil biological characteristics (Bu¨nemann et al., 2004; Aschi et al., 2017), and break crop to control plant diseases. When faba bean is grown primarily for its N contribution, the maximum benefit is obtained when the succeeding crop has relatively long growth period to make most efficient use of N mineralization occurs later in growing season (Jensen et al., 2010). A Canadian five-cycle rotation-study comparing a faba bean–barley–wheat and a barley–barley–wheat rotation showed that faba bean enhanced the average yield in the subsequent barley and wheat crops by 21 and 12%, respectively which was equivalent to providing the cereals with around 120 kg N ha^{-1} (Xinyou and Van, 2005). The residues of shoot and the root of faba bean generally have a low C/N ratio therefore, N mineralization often takes place very fast (Shi, 2013). This fast release of N may not be well synchronized with the N uptake of the succeeding crop, thus may results in increased risk of N loss to the environment (Jensen et al., 2010), more so in conventional tillage system (Etemadi et al., 2018d). Improving the synchrony of N release from faba bean residues can enhance the N uptake/utilization efficiency of the succeeding crop, thus reducing potential environmental hazards. Etemadi et al. (2018d) reported that in no-till system, 50% N release from faba bean residues delayed by approximately one month, compared with conventional tillage system.

The complementarity multispecies agroecosystems (intercropping), especially those that include legumes, are more productive and resource-efficient in comparison with monoculture systems. Intercropping faba bean with various cereal grains including barley (Getachew et al., 2006), wheat (Tosti and Guiducci, 2010), and maize (Song et al., 2007) is widely practiced because of their complementarity in the use of N resources and other nutrients. The results from almost all intercropped faba bean with various cereal crops indicated that while the yield of grain cereals was improved, natural soil fertility including soil organic matter and microbial activity greatly enhanced.

Dietary Nutrients in Faba Bean

Mineral nutrients

Faba bean is rich in proteins, complex carbohydrates, dietary fiber, choline, lecithin, minerals and secondary metabolites including phenolic compounds (Mohseni and Golshani, 2013; Etemadi et al., 2018c). Also, faba bean seeds are low in fats, cholesterol-free, and low sodium (Gepts et al., 2008; Adamu et al., 2015). Globally, faba bean is recognized as one of the most important staple legumes for human consumption (López-Pedrouso et al., 2012). The high protein content of faba bean makes it a cheap alternative protein source compared to the highly priced animal or meat-protein sources in developing countries (Adamu et al., 2015).

Diversification in the use of beans, e.g., soybean, garden bean, faba bean, can stimulate an increased cultivation and supply of these crops. Furthermore, the increased utilization of these legumes in human nutrition may contribute to achieving the targeted daily dietary protein intake recommended by the World Health Organization (WHO, 2007), especially in developing nations. This is essential to attaining sustainable food security in terms of nutritional quality (Jahanzad et al., 2014). Micronutrient deficiency is recognized as the “hidden hunger” in the world. Legume seeds are typically rich in micronutrients such as Fe and Zn (Khazaei et al., 2017). Iron and Zn are essential for sustenance and optimal physiological function of all forms of life on the planet (Bailey et al., 2015). Two billion people around the world suffer from micronutrient malnutrition

(WHO, 2007; Gupta et al., 2016). Nearly one-third of the population of the world is Fe-deficient, and one-fifth is Zn- deficient (de Benoist et al., 2008).

Phosphorus and potassium contents

Among the different edible parts of faba bean, P content is in the order of seed > pod wall > leaf (Etemadi et al., 2018a). Similar to N mobilization, P in leaves and pods are mobilized into other pods and seeds (Hanway and Weber, 1971; Bender et al., 2015). Faba bean varieties differ with regard to nutrient accumulation. For example, Etemadi et al. (2018a) reported a significant variation among the faba bean varieties in terms of P content. Unlike N and P, which were more concentrated in the seed part, the highest K concertation were detected in pod wall with 38% and 44% higher than seed and leaves, respectively (Etemadi et al., 2018a). In Mediterranean diets, pods are edible part and are considered as important nutrient source when consumed with the immature seeds; hence, accumulation of K in pods can be an important dietary source of K (Sing et al., 2013).

Nitrogen or protein content

Nitrogen accumulation in various parts of faba bean is in the order of seed > pod wall >leaf (Etemadi et al., 2018a). Higher accumulation of N in seeds than in other parts is expected since N is mobilized from the leaf and pod wall to the seeds during the seed-filling period (Hanway and Weber, 1971; Pazdernik et al., 1997; Bender et al., 2015). However N content in various varieties differ significantly. For example, Etemadi et al. (2018a) compared eight faba bean varieties for their N content and reported that the highest N concentration was measured in Windsor with 3.47%, followed by Delle Cascine with 3.43%, whereas the lowest N concentration in seeds was detected in Early Violletto with 2.71%.

Calcium and Magnesium Contents

Unlike N, P, and K, with the lowest accumulation in the leaf, Ca mainly accumulates in faba bean leaves. Concentration of Ca in the faba bean leaves was more than three-fold than its concentration in the pod wall and eight-fold in the seed (Etemadi et al., 2018a). Similar to Ca the highest concentration of Mg was in the order of leaf > pod wall > seed. They also concluded that the difference between plant parts in regard to Mg accumulation was more pronounced than the difference amongst the varieties. Magnesium content in the leaf was more than two-fold than in the seeds. Calcium in an immobile element while Mg is a mobile nutrient (Zandvakili et al., 2017); however, the similar pattern of partitioning of these nutrients in plant organs may indicate that mobilization of Ca and Mg from vegetative plant parts to seeds and pods is minimal. The high Ca and Mg in the leaf tissue could be important from the nutritional point of view for human diet in areas that leaves are also consumed.

Iron, Copper, Zinc, and Manganese Contents

Research showed that the concentration of Fe in different organs of faba bean was significantly different and in the order of leaf > pod > seed. The accumulation of Fe in the leaf component of the plants was 8.7-fold and 3.2-fold higher than in seeds and pod walls, respectively (Etemadi et al., 2018a). Iron deficiency is one of the major nutrition problems in developing countries. Generally, legumes are considered as a major source for essential elements, including Fe and Zn. However, the high amount of Fe concentration in leaves of all faba bean varieties may also be important in human diets. This plan, however, should be practiced cautiously since it is reported that some legumes, like faba bean, can contain high amounts of Fe binding polyphenols that inhibit Fe absorption (Sandberg, 2002). In Etemadi et al. (2018a) report, the highest and lowest Fe concentration was detected in Delle Cascine leaves at 804 mg kg^{-1} while the lowest Fe concentration was measured in the seeds of Early Violletto with 59 mg kg^{-1}. Mahmoud et al. (2006) reported a similar result on the accumulation of Fe in the leaf component of faba bean. However, in general faba bean seeds could be considered as an important dietary source to alleviate Fe deficiency in humans. Similarly, there is a difference among plant organs in Zn and Mn accumulation. The concentration of these two elements in various parts of faba bean were in the order of seed > leaf > pod wall while Cu distribution was in the order of seed=leaf > pod. (Etemadi et al., 2018a). Faba bean leaves can be consumed as a good source Cu in human diets. Medicinal value

Faba bean accumulates a large amount of L-Dopa (L-3,4-dihydroxyphenylalanine) in its various parts (Holden, 2006; Etemadi et al., 2015b). L-Dopa (3, 4-dihydroxyphenyl-L-alanine), a precursor of dopamine currently used as a major ingredient in treating Parkinson’s disease (PD) and hormonal imbalance (Ali et al., 2005; Rani et al., 2007; Surwase et al., 2012; Inamdar et al., 2013; Hu et al., 2015; Etemadi et al., 2018b).

<u>Faba Bean as a medicinal plant</u>

L-Dopa as main medicine for Parkinson

In the 1960s, major improvement in PD was made when researchers identified the low level of dopamine in the brain of Parkinson disease patients (Davie, 2008). L-Dopa, is an amino acid, naturally isolated from various legumes (Chattopadhyay et al., 1994; Inamdar et al., 2013) and other crops such as banana (Musa spp.) (Bapat, 2000; Rani et al., 2007; Gautam et al., 2012). L-Dopa is synthesized from the amino acid L-tyrosine in the mammalian body and brain (Randhir and Shetty, 2004; Haq and Ali, 2006; Miller et al., 2009). Earlier reports (Nagatsu et al., 1964; Peaston and Weinkove, 2004; Miller et al., 2009) indicated that L-Dopa is an important precursor for the various neurotransmitters like dopamine (Upadhvay et al., 2012), noradrenaline and adrenaline collectively known as catecholamines (Gautam et al., 2012). Commonly PD patients are treated with synthesized L-Dopa. Synthesized L-Dopa is expensive and often related to a variety of side effects including nausea, vomiting, low blood pressure, drowsiness, and restlessness (Lee et al., 1996; Knowles, 2003; Patil et al., 2013). Therefore, consuming natural sources of L-Dopa to avert potential side effects has been recommended (Singh et al., 2012; Patil et al., 2013).

It is estimated that the annual world demand for L-Dopa is about 250 tons (Katayama and Kumagai, 2010) with a market value of about $101 billion (Koyanagari et al., 2005; Inamdar et al., 2013; Patil et al., 2013). Cultivation of crops that are rich in natural L-Dopa to overcome side effects and the high cost of production of synthetic L-Dopa thus seems justifiable (Singh et al., 2012; Patil et al., 2013).

L-Dopa content in faba bean

Faba bean is ranked after velvet bean (*Mucuna Pruriens* L.) in terms of L-Dopa content (Soares et al., 2014). The amount of L-Dopa in faba bean, however, is dependent on genotypes (Lorenzetti et al., 1998; St. Laurent et al., 2002; Etemadi et al., 2014b), environmental conditions (Goyoaga et al. 2008; Multari et al. 2015) plant's growth stage (Geng, 2012), and organs (Etemadi et al., 2014a). Cao (2010) reported a large variation of L-Dopa content among faba bean genotypes including in flowers across 197 cultivars, seedlings across 32 cultivars, seeds across 52 cultivars with the green seed coat. Similarly, Hu et al. (2015) reported a significant variation in L-Dopa concentration among six faba bean lines that differed in flower color.

Distribution of L-Dopa in various parts of faba bean is not uniform, and different organs accumulate L-Dopa at different rates (Geng, 2012). However, many earlier reports have focused on L-Dopa content in seeds, and less attention has been given to the amount of L-Dopa in other parts of the plant. In general, L-Dopa concentration in various faba bean organs reaches its peak concentration at early stages of growth (Etemadi et al., 2018b). However, L-Dopa yield is a product of concentration and plant biomass (Etemadi et al., 2018b) thus highest L-Dopa yield can be obtained at late stages of growth when biomass is maximized.

Plants exposed to some degree of drought stress may accumulate higher concentrations of secondary metabolites (Selmar and Kleinwächter, 2013). Such enhancement is reported to occur in nearly all classes of natural products including simple and complex phenols, numerous terpenes, and also N-containing substances such as alkaloids, cyanogenic glucosides, and glucosinolates. Owing to the beneficial effects of drought stress on accumulation of secondary metabolites, especially phenolic compounds, exposing plants to some degree of water stress has been suggested as a potential approach for increasing the production of these active substances (Bettaieb et al., 2009). However, there is limited information available on the response of L-Dopa content of faba bean to drought stress.

Availability of N and P to faba bean plants may influence the L-Dopa synthesis. Ramakrishna and Ravishankar (2011) reported that deficiencies in N and P directly influenced the accumulation of phenylpropanoids. Although faba bean is an N-fixing plant, the effect of supplemental N fertilization on L-Dopa concentration is not well documented. Etemadi et al. (2018c) concluded that N stress had no significant influence on the amount of accumulated L-Dopa.

Because fresh faba bean is not available at all times in all locations, Parkinson patients relying on fresh natural source of L-Dopa should have stored relatively large amounts of plants for consumption. Therefore, naturally L-Dopa rich plants may be processed by various methods including chopped frozen tissues or dried powdered plant parts. Processing plant parts may negatively influence the L-Dopa concentration. Earlier reports (Echeverria and Bressani, 2006; Vadivel and Pugalenthi, 2010) indicated that L-Dopa in legumes can be destroyed by cooking and soaking in alkaline solutions. Dahouda et al. (2009) concluded that the L-Dopa in velvet bean was degraded by cooking but not by roasting. Recently, Etemadi et al. (2018c) studied the influence of various processing methods on L-Dopa content of faba bean leaves and seeds. In this study the L-Dopa content influenced by different processing methods were in the order of: Fresh material > frozen > oven-dried > air dried (2 days) > air dried (4 days) > air dried (7 days) > boiled.

Conclusion

Faba bean is a valuable multi-purpose crop which can be grown for its nutritional and medicinal values. Faba bean seeds, pods, and leaves are rich in protein and almost all elements required for human diets. The biological N fixation by faba bean is considered high among the grain legumes. Integrating faba bean in various cropping systems such as crop rotations and intercropping improves natural soil fertility and reduce the consumption of commercial N fertilizer.

Future Research

To maximize the ecological benefits from faba bean efficient nodulations and biological N fixation is required. Therefore, one important area of research should be focused on introduction of elite strains of N-fixing bacteria, especially in regions with lack of faba bean cultivation history. In shorter-season regions, faba bean can be used to extend growing season therefore integrating faba bean into various cropping systems including various forms of intercropping and crop rotations in these areas should be explored. Also, introducing genotypes with winter hardiness capacity are useful for areas with harsh winter condition. Since chocolate spot bacteria is a limiting factor in faba bean cultivation, more research should be applied to overcome chocolate bacteria by introducing resistance cultivars. Since we found several benefits from transplanting faba bean in contrast to the conventional direct seeding, the authors believe that transplanting faba bean as an alternative planting method should be explored further.

Table 1. 1 Seed size of some faba bean varieties (Etemadi et al., 2017)

Cultivar	**Seed Size (g/100 seeds)**	**Seeding rate (kg ha^{-1})**
Aquadulce	265	165
Bell Bean	51	32
D'Aquadulce	392	244
Delle Cascine	335	208
Early violletto	280	174
Early white	253	157
Sweet Lorane	68	42
Windsor	311	193

Table 1.2 Recommendation for phosphorus fertilizer for faba bean (New England vegetable guide, 2018)

	Nitrogen Kg/ha	Phosphorus P_2O_5 kg/ha				Potassium K_20 kg/ha			
Soil test result		Very Low	Low	Optimum	Above Opt.	Very Low	Low	Optimum	Above Opt.
Rate recommended	50	100	75	0-50	0	100	75	0-50	0

CHAPTER II

PHENOLOGY, YIELD AND GROWTH PATTERN OF FABA BEAN VARIETIES

Abstract

Faba bean seeds are generally large which limits its adoption as cover crop and/or dual-purpose cover crop/cash crop due to the high seed cost. The purpose of this study was to evaluate six faba bean varieties for their yield and yield components, using phenology, morphology and physiological growth pattern. Faba bean varieties included Early Violletto, Aquadulce, Delle Cascine, Windsor, Early White, and D'Aquadulce, which studied in 2015 and 2016. Aquadulce produced the highest grain yield followed by Delle Casine. These two varieties also produced the highest biomass, which potentially can provide more N to the succeeding crop. Aquadulce and Delle Cascine had the highest HI (40.1 and 41%, respectively) indicating an efficient distribution of assimilates to their seeds. Delle Cascine produced the highest number of seeds plant^{-1} and seed weight, respectively. Plant height variation also revealed that varieties Early White was the shortest (40.6 cm) and the tallest (76.0 cm), respectively. Significant differences were observed in regard to the growing degree days required to reach flowering and pod formation stages. Aquadulce and Delle Cascine ranked early among the tested varieties, providing the opportunity for dual purpose and double cropping in short-season areas.

Introduction

Faba bean is a cool-season grain legume crop with high nutrition value. Recently more attention has been made on using faba bean as a multi-purpose legume crop (L'opez- Bellido et al. 2005; Etemadi et al. 2015; Landry et al. 2015) and/or in intercropping systems (Zhang et al. 2004; Song et al. 2007; Li et al. 2009). Faba beans fit nicely into various crop rotations, including double cropping with other vegetables and grains (Turk and Tawaha 2002; Agegnehu et al. 2006). Due to the diverse and significant ecological services (Köpke and Nemecek 2010) faba bean has increasingly received attention. Currently, faba bean is widely cultivated in many regions of the world as a source of food and as a break crop in cereal production to minimize the occurrence of cereal cyst nematode (*Heterdera avenae*) (Sattell et al. 1998) and some soil-borne pathogens (Landry et al. 2016).

In legumes, the total biomass production plays an additional role in sustainability of cropping systems (Zandvakili et al. 2012; Etemadi et al. 2018). Arguably, the most important contribution of faba bean to agricultural ecosystems is its high capability of fixing atmospheric nitrogen (Cline and Silvernail 2002; Hoffmann et al. 2007; Horst et al. 2007; Ruisi et al. 2017; Etemadi et al. 2018). It is well documented that the magnitude of N contribution to the soil nitrogen largely depends on the amount of final biomass of the legume crop (Finney et al. 2016; Jahanzad et al. 2016; Mirsky et al. 2017).

Integrating faba bean into the crop rotation systems requires some information about the growth pattern and morphological characteristics in addition to the potential yield of various genotypes (Agegnehu et al. 2006). Etemadi et al. (2015) recommended that faba bean should be sown in spring as early as late March to provide the opportunity for successful double cropping in areas with relatively short growing season. In cold areas, faba bean can also be grown in summer by mid-July as dual-purpose cash/cover crop (Etemadi et al. 2015; Landry et al. 2015). Domestication of faba bean began approximately 4000 years ago through selection among wild ecotypes for larger seeds and plants. The selection for larger plants resulted in higher potential ecological benefits including N fixation (Köpke and Nemecek 2010). The ability of faba bean to produce large quantity of biological N fixation is well documented (Unkovich and Pate 2000; Jensen et al. 2010; Dayoub et al. 2017; Denton et al. 2017; Barłóg et al. 2018; Gebremariam and Assefa 2018). Therefore, faba bean can significantly contribute to the soil N that lowers the need for the N by the subsequent cash crop (Etemadi et al. 2018).

Faba bean is a new crop in New England and Windsor is currently the dominant variety available to the growers however, Windsor has not performed well in our previous studies and out-yielded by several other varieties (Etemadi et al. 2017). Thus, evaluating more varieties for their potential yield and suitable morphological characteristics can provide valuable information to the researchers and growers. Seed yield is a function of DM accumulation and partitioned assimilates into the seeds (Ricciardi et al. 2001). Phenology is the study of the timing of biological events, the cause of their timing with regards to biotic and abiotic forces, and the interrelation among phases of the same or different species (Hunt and Yamada 2003). Phenological development described as the rate of progress through growth stages. Temperature and photoperiod can control phonological growth stages, more specifically the reproductive growth (Peng et al. 2004; Wilczek et al. 2010). Therefore, variation in time to reach each phenotypic stage relates to the sensitivity of genotypes to changes in the photo-thermal regimes thus affecting days to maturity, adaptation to the environment, and yield in divers growing conditions (Peng et al. 2004; Etemadi and Hashemi 2012). Different types of faba beans including spring, winter and Mediterranean exhibit various phenological characteristics therefore induction and maintenance of flowering differs among these classes (Patrick and Stoddard 2010). Plant breeders have recognized the importance of larger seed in the production of food crops and have been breeding crops for the trait. General conception is that the larger seeds may have more food reserves thus germinate faster, establishes more successfully and out yield smaller seeds within the same variety/cultivar (Gan et al. 2004). The desired population density traditionally determined by seeding rate, which primarily relate to the number of seeds per unit weight (kg). Therefore, seeding rate of cultivars with smaller seed size can be significantly lower than larger seeds. Since the cost of faba bean seeds is relatively high, smaller seeds with high yield potential are preferable and can significantly reduce the production costs.

Despite the significant economic and ecological importance of faba bean, the relationship between various phenology, morphology, and growth pattern characteristics of various faba bean varieties in short growing season regions is not extensively investigated.

The present study aimed to assess selected traits that are important for adaptation of faba bean in short-season areas. The phenology, morphology, and seed yield of six faba bean varieties studied in a two-year field experiment.

Material and Method

Experimental site and weather condition

A 2-year field experiment was conducted in 2015 and 2016 at the University of Massachusetts Amherst Agricultural Experiment Station, Crops and Animal Research and Education Farm in South Deerfield (42°28′37″N, 72°36′2″W). Weather condition including growing degree-days (GDD) and precipitation throughout the experiment period in both years and the norm (average of 20 years) for the region presented in Table 2.1. The soil type at the experimental site was a Hadley fine sandy loam (nonacid, mesic TypicUdifluvent). Composite soil sample (0–0.2 m depth) taken prior to planting indicated soil pH (1:1, soil/H2O) was 6.6, cation exchange capacity (CEC) was 8.1 meq/100 g and available P, K and Mg were all in the optimum range thus no fertilizer was applied to the experimental plots in both years. Throughout the experiment, weeds periodically removed by hand when necessary. No irrigation used in this experiment, as irrigation is not a common practice for agronomic field crops in Massachusetts.

Experiment layout and Measurements

Six faba beans varieties including; Aquadulce, Early Violletto, Delle Cascine, Early White, D'Aquadulce, and Windsor, were hand seeded on April 16 in 2015 and April 14 in 2016. A complete randomized block design experiment with four replications used in this study. Each plot consisted of three rows, 76 cm apart and 15 cm space between the plants, which is equivalent to a population of 60,000 plants ha^{-1}. Morphological characteristics including height of plant, number of lateral branches per plant, height of the first pod from the ground measured on five randomly tagged faba bean plants in each plot. Plant height was determined by measuring the distance between the ground and the terminal growing point. Number of lateral branches counted at the harvest. First pod height was determined by measuring the distance between the soil surface and the first pod presence in the plant before the harvest. The major phenological stages of the varieties were determined when 50% + 1 of plants in the center row reached the corresponding stage. The recorded phenological stages included the days required to reach three leaf stage, flowering, first pod formation, and pod maturity. The number of Growing Degree Days (GDD) accumulated for reaching each phenological stage was calculated using Eq. 1:

$$GDD = (T_{max} + T_{min})/2 - T_{base} \quad (1)$$

where Tmax and Tmin are the maximum and the minimum daily air temperature (°C), Tbase is the base temperature equal to 4 °C. Crop Growth Rate (CGR) was calculated (Eq. 2) for entire growth period (Aghaalikhani et al. 2012). At each harvest, five plants from first and third rows of each plot cut at the soil surface and oven dried at 50 °C for 72 h in a forced-air oven to reach a constant weight.

$$CGR = (W_2 - W_1)/SA\ (t_2 - t_1) \quad (2)$$

Seed yield (kg ha^{-1}) and seed size (100-seed weight) were determined based on harvesting pods from 2 m2 from the middle row. Seed yield is corresponding to the total seed weight of multiple harvests and seed size is the average weight of multiple groups of 100 seeds. At maturity, all plants from the 5 m2 of the center row harvested manually by cutting at the soil surface for determination of biological yield. Harvest index (HI) derived as the proportion of grain yield to total biological yield.

Statistical analysis

Data subjected to analysis of variance using Proc GLM procedure of SAS software (SAS institute 2003). Data shown in figures are the arithmetic means of four replicates of each treatment. Effects considered significant at $P \leq 0.05$ by the F test, and when the F test was significant, Fisher's Least Significant Difference Test (LSD) was used for mean separations. Regression equations (averaged of 2 years) for faba bean CGR based on days after planting was determined by fitting the data to a quadratic trend using SAS linear regression procedure (PROC REG). The relationships between the traits analyzed using PROC CORR of SAS software.

Results and Discussion

Weather

Cumulative growing degree days (GDD 4 °C) at Orange Airport, MA (roughly 27 km away from the research site), during the growth period of faba bean (April–July), were 2484 and 2461 in 2015 and 2016, respectively which were similar to the norm for this location (Table 2.1). Since irrigation is not a common practice for field crops production in Northeast U.S., plants are depending on the amount of precipitation they receive during the growing season. In 2016, faba bean plants received almost 50% less precipitation than in 2015 during April through July, the months that spanned the crop growth period. More specifically, lower moisture availability in the month of June 2016 when pods and seeds are normally formed, resulted in 28 and 29% reduction in biomass and seed yield, respectively when averaged across all faba bean varieties (Table 2.3).

Phenology

The life cycle of grain legumes identifies by several major phenological stages. These stages include seed emergence, vegetative growth, flowering, pod set, pod filling, and physiological maturity. In faba bean, the induction of flowering, retention of flowers and development of seeds are all therefore critical processes in final yield determination. Figure 2.1 illustrates the mean thermal units (above 4 °C) of the varieties that needed to reach different phonological stages. While the varieties did not show significant differences in required GDD to reach their 3-leaf stage, large variations recorded for other stages of growth among the varieties. Aquadulce and Delle Cascine required 534 GDD to produce their first flower, which was almost 25% lower than the Early White, which flowered last. Interestingly, the flowering period of Aquadulce and Delle Cascine was longer than other varieties except in Windsor. Aquadulce matured earlier than all other varieties and required 33% less GDD than the second earliest variety, Delle Cascine. Considering that faba bean is sensitive to high temperature and low moisture availability, days to flowering and maturity is very important (L'opez-Bellido et al. 2005). Earlier maturity potentially considered as a positive characteristic for the varieties grown in short growing season areas such as New England. This is because firstly, the plant will not coincide with high temperature in summer, which makes faba bean plants sensitive to chocolate spot bacteria. Secondly, earlier harvest provides the opportunity for successful double cropping which is crucial to the growers' income. However, early maturity varieties must demonstrate an efficient distribution of assimilates to the economic sinks to produce high pods/ seeds yield. Other reports indicated that faba bean varieties exhibited large variations in terms of maturity (Tafere et al. 2012; Talal and Munqez 2013; Mitiku and Wolde 2015). Our results, however, does not confirm the earlier report by Girma and Haila (2014) who concluded that physiological maturity of faba bean varieties was not different in irrigated and rain fed conditions.

Morphology and growth

Growing season had a significant influence on all measured morphological and growth indices (Table 2.2). As mentioned earlier, the amount and distribution of precipitation was more suitable in 2015 than 2016 (Table 2.1). As a result, average all varieties, plant height, height of first pod from the soil surface, number of lateral branches, and maximum CGR were higher in 2015 compared with 2016. However, the interaction of year and variety was not statistically significant therefore; an average of the 2 years of experiment presented in Table 2.2. Varieties used in this experiment showed significant differences in terms of plant height, height of first pod from the soil surface, and number of branches (Table 2.2). Windsor was the tallest variety followed by Aquadulce, while the Early White was the shortest variety. Except in Early Violletto, the height of the first pod from the ground ranged from 15 to 19 cm. The height of the first pod is important for the ease of fresh pods hand harvesting and for mechanical harvesting of dry seeds. Moreover, the lowest pods may contaminate with soil particles due to the splash of raindrops. Number of lateral branches per plant varied between 2–4 and the difference between the varieties was significant. Number of branches is mainly influenced by agronomic management practices including plant density (Loss et al. 1998a, b; Gezahegn et al. 2016), time of planting (Badran and Ahmed 2010) and environment conditions such as moisture availability (Toker 2004; Hegab et al. 2014). Number of lateral branches per plant can also differ based on the differences in the genotype (Mwanamwenge et al. 1998; Sozen and Karadavut 2016). In the current experiment, since a uniform population density and environmental condition existed, the difference in the number of branches primarily related to the genotypic differences rather than agronomic practices and/ or environmental conditions. In general, producing several branches per plant should not be considered as a good characteristic since lateral branches are not as productive as the main stem. Therefore, optimum canopy closure should be reached through increasing population density rather than the capacity of varieties for branching capability.

The maximum CGRs of the varieties tested in this study were considerably different and varied between 2.7 g m^{-2} day^{-1} in low yielding variety, D'Aquadulce, and 6.6 g m^{-2} day^{-1} in high producing varieties, Delle Cascine and Aquadulce (Table 2.2). The highest value obtained in this study was significantly lower than the maximum CGR recently reported by Barłóg et al. (2018). The lower maximum CGR in our study could be attributed in part to the growing conditions in the Northeast U.S., which usually has more cloudy days, and due to the lack of irrigation practice. The time to reach the peak CGR was also significantly different among the varieties and ranged from 62 days in Aquadulce to 75 days in Windsor. Intensive growth period of faba bean occurs during flowering stage, when the crop reaches its highest leaf area index (LAI) and CO2 fixation rate (Coelho and Pinto 1989; Pilbeam et al. 1991). Our results indicated that the days to reach the maximum CGR in all varieties, regardless of their maturity, coincided during the flowering period. Interestingly it turned out that the earliest (Aquadulce) and latest (Windsor) maturity varieties required the fewest and the highest days to reach their maximum CGR, respectively (Fig. 2.1). As the time passed, dry matter accumulation rate decreased with the aging of the leaves, thus CGR reduced at final stages of growth.

Biomass, seed yield, and harvest index

Biomass yield was significantly different in the two growing seasons and averaged all varieties was 28% higher in 2015. Biomass also varied greatly among the faba bean varieties and ranged from 2162 kg ha^{-1} in D'Aquadulce in 2016 up to 7970 kg ha^{-1} in Aquadulce in 2015 (Table 2.3). Biological yield is one of the most sensitive traits to environmental conditions and varies greatly among the faba bean genotypes (Toker 2004; Abdalla et al. 2015; Flores et al. 1996; Gasim et al. 2015; Mitiku and Wolde 2015). Final biological yield represents the photosynthetic efficiency of the genotypes whereas the seed yield primarily related to the effective translocation of assimilates to the economic sinks. However, often a high correlation exists between the total biomass and final seed yield (Losset al. 1998b).

In current study, we found similar ranking of biomass and seed yield among the varieties (Table 2.3). For example, Aquadulce with highest biological yield out-yielded other varieties and produced the maximum seed yield. However, Lo'pez-Bellido et al. (2003) reported that although there is a certain correlation in faba bean crops between biological yield and seed yield, the high biological yield does not necessarily correspond with the high seed yield.

Statistical analysis indicated that varieties of faba bean tested in this experiment produced significantly ($P < 0.05$) different seed yield (Table 2.3). Aquadulce and Delle Cascine, which produced the highest biomass, also ranked top as the most productive varieties and yielded 2756 and 2352 kg seed ha^{-1}, respectively. Depite of being late maturity varieties, D'Aquadulce and Windsor performed poorly and produced only 900 and 993 kg seed ha^{-1}, respectively. Interestingly Windsor is the dominant variety currently available to the growers in New England. The results of this study further confirmed the necessity of varieties/cultivars testing trials for each region. Final seed yield also depends on distribution efficiency of genotypes to translocate assimilates to the target sinks. The harvest index is a physiological criterion that represents the assimilation distribution efficiency and influenced by both the growing season and the genotype (Table 2.3). The harvest index range obtained in the current study (35–41%) is in the range of several other reports (Agung and McDonald 1998; Gebremeskel et al. 2011; Abdalla et al. 2015; Gezahegn et al. 2016). Agung and McDonald (1998) who stated that the HI of faba beans differed little among different genotypes have reported contrasting result.

The 100-seed weight was significantly different among the varieties (Table 2.3). The average seed size of Aquadulce, as the highest yielding variety, was 265 g 100-$seed^{-1}$ which was the smallest among the tested varieties. Seed size of faba bean varieties varies greatly (Duc 1997; Landry et al. 2016; Etemadi et al. 2017) and such significant variation makes faba bean a unique legume crop when it comes to the cultivar evaluation. Faba bean varieties are classified as large-seeded (> 1.0 g seed^{-1}), medium-seeded (0.5–1.0 g seed^{-1}) and small seeded (< 0.5 g seed^{-1}) (Etemadi et al. 2015). Regardless of the seed size, faba bean yield generally increases with increased population density and reaches its peak at different plant populations (L'opez-Bellido et al. 2005). Growers commonly use seed weight per unit land area to achieve the recommended population density. Therefore, in large seed crops such as faba bean, the seed size plays a significant role in determining the seed cost. Aquadulce not only out-yielded other varieties, also because of its smaller seed size, requires lower seeding rates. Lower seed cost promotes faba bean cultivation as cash/cover crop dual purpose.

Conclusion

The present study provides evidence that the faba bean genotypes exhibited dramatic variations in thermal units required to reach various phenological stages. This variation allows growers to select the proper genotype that favors their environmental conditions. The current study revealed that late-maturity varieties are not necessarily superior over early-maturity genotypes. Thus, in shortseason regions, high yielding early maturity varieties such as Aquadulce provide the opportunity for double cropping. Moreover, our findings confirm the existence of high correlation between biomass and seed yield. This correlation is very important in cultivation of legumes since production of higher biomass additionally provides higher N yield, which benefits the succeeding crop.

Table 2. 1 Precipitation and GDD during faba bean growing season in 2015, 2016 and norm (20 years average) for the experimental site.

	Precipitation (mm)			**GDD[a]**		
Month	**2015**	**2016**	**20-year mean**	**2015**	**2016**	**20-year mean**
April	51	53	80	157	185	204
May	26	65	88	674	530	515
June	192	34	116	714	765	767
July	84	43	98	938	980	942
Total	353	195	382	2484	2461	2428

[a]GDD = (Tmax+ Tmin)/2 – Tbase where Tbase is 4^{o}C

Table 2. 2 Mean values of morphological and physiological traits of different faba bean varieties.

	Height (cm)	1st Pod Height (cm)	Branch #	Max CGR (g m^{-2} d^{-1})	Days to Max CGR
Year					
2015	63	19	3.3	56.3	-
2016	45	14	2.4	40.7	-
Variety					
Aquadulce	57	16	3.0	6.6	62
D'Aquadulce	46	18	4.0	2.7	70
Delle Cascine	56	19	3.1	6.7	63
Early Violletto	53	11	2.0	5.3	65
Early White	41	15	2.2	5.0	65
Windsor	71	18	3.1	2.9	75
F test					
Year (Y)	*	**	*	*	*
Variety (V)	*	*	*	*	*
Y*V	ns	ns	ns	ns	ns

*Significant at P≤0.05, **Significant at P≤0.01, ns= non-significant

Table 2. 3 Mean values of seed yield, biological yield, harvest index and some morphological traits of different faba bean varieties.

	BY (kg ha^{-1})		Seed yield (kg ha^{-1})	HI (%)	100-Seed Fresh weight (g)
Year					
2015	5271		2057	39.0	355
2016	3818		1470	38.5	257
Variety	**2015**	**2016**			
Aquadulce	7970	5774	2756	40.1	265
D'Aquadulce	2985	2162	900	35.0	392
Delle Cascine	6648	4816	2352	41.0	335
Early Violletto	6256	4532	2109	39.1	280
Early White	4509	3266	1473	37.9	253
Windsor	3257	2360	993	35.4	311
F test					
Year (Y)	**		**	*	*
Variety (V)	*		*	*	*
Y*V	*		ns	ns	ns

*Significant at P≤0.05, **Significant at P≤0.01, ns= non-significant

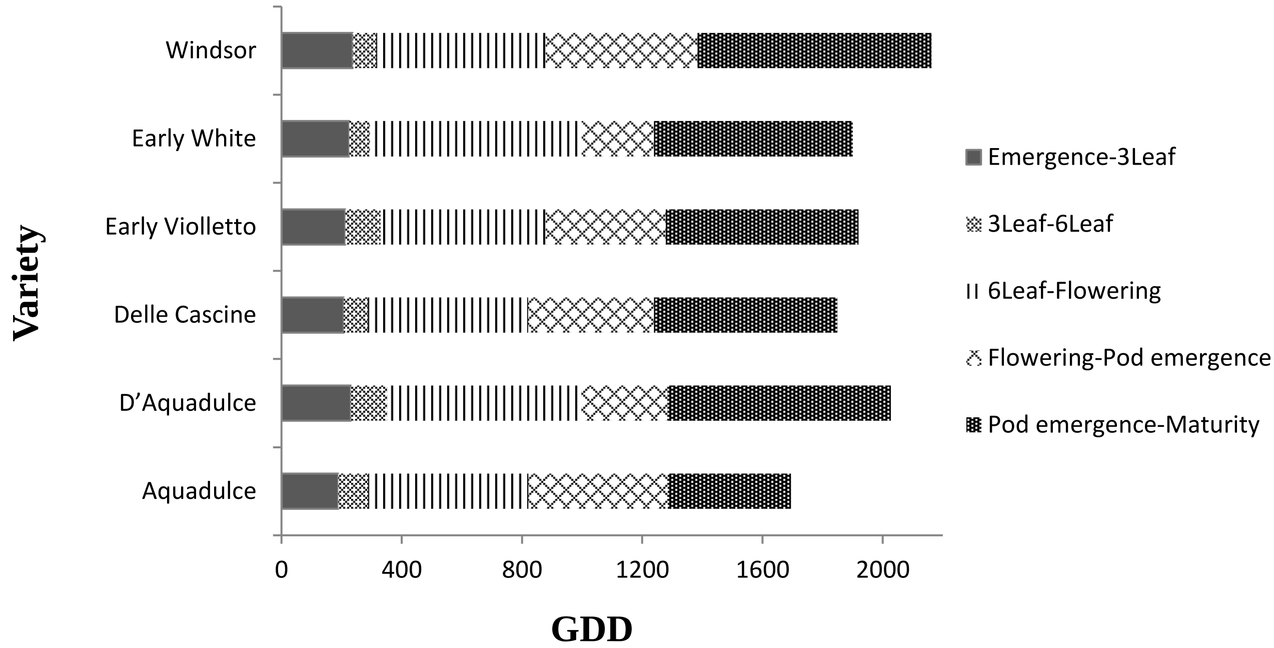

Figure 2.1 Growing degree days required to reach various growth stages of six faba bean varieties.

CHAPTER III

NITROGEN CONTRIBUTION FROM WINTERKILLED FABA BEAN COVER CROP TO SPRING-SOWN SWEET CORN IN CONVENTIONAL AND NO-TILL SYSTEMS

Abstract

The N release trend of winter-killed faba bean (*Vicia faba* L.) residues has not been previously investigated. A 2-yr experiment was conducted in 2013–2015 to investigate potential N accumulation in fall-grown faba bean as cover crop and N contribution to subsequent sweet corn under notill (NT) and conventional tillage (CT) systems. Faba bean biomass prior to winter-kill was reduced linearly with delayed planting. The amount of reduced biomass estimated approximately 180 and 210 kg ha^{-1} d^{-1} in 2013 and 2014, respectively. Faba bean sown on 1 August accumulated as much as 192 kg N ha^{-1} vs. 67 kg N ha^{-1} when planted on 14 August. Under CT, 50% of N was released from residues by the end of May however NT system delayed 50% N release until end of June, thus providing better synchrony with N uptake by sweet corn. Averaged over two years, sweet corn planted into the residues of the earliest sown faba bean produced 19% more marketable ears, 23% higher fresh ear weight, and 39% less unfilled ear tip compared with sweet corn grown in plots lacking a prior faba bean cover crop. Both number of marketable ears and fresh ear yield of sweet corn were significantly higher in NT compared with CT systems. On average, sweet corn seeded in faba bean residues and amended with an additional 50 kg N ha^{-1}, yielded similarly to sweet corn received 100 kg N ha^{-1} with no prior faba bean cover crop.

Introduction

Legumes can potentially replace, or significantly contribute to, the N fertilizer requirements of N demanding crops such as sweet corn (Zandvakili, et al., 2012; Jahanzad, et. al., 2014; Hardarson and Atkins, 2003; N'Dayegamiye et al., 2015). Additionally, grain legumes serve as an excellent protein source in human and livestock diets (Huang et al., 2016; Ito et al., 2016). Faba bean is known as one of the oldest crops in the world, and it is the third most important grain legume after soybean (*Glycine max* L.) and pea (*Pisum sativum* L.) (Mihailovicet et al., 2005; Daur et al., 2011; Singh et al., 2013).However, faba bean is not currently grown as a cover crop mainly due to its large seed size and relatively low biomass production that makes it non-competitive compared with other legume cover crops (Etemadi et al, 2017).

Diverse ecosystem services are expected from growing faba bean (Ko¨pke and Nemecek, 2010), and in recent years increasing attention has been given to using faba bean as a multi-purpose legume cover crop (L´opez-Bellido et al., 2005; Etemadi et al, 2015; Landry et al., 2015) as well as in intercropping systems (Zhang et al., 2004; Song et al. 2007; Li et al., 2009). Arguably, the most important contribution of faba bean to agricultural ecosystems is the substantial amount of atmospheric N that can be fixed by the crop and its associated rhizobia. Depending on the growing conditions, faba bean is reported to fix up to 160 kg N ha^{-1} (Cline and Silvernail, 2002; Hoffmann et al., 2007; Horst et al., 2007). For this reason, faba bean can be considered as one of the most efficient nitrogen-fixing cool season legumes (Herridge et al., 1994). Interestingly, faba bean maintains its nitrogen-fixing capabilities even in soils that are rich in N (Peoples et al., 2009; Ko¨pke and Nemecek, 2010). Other benefits and potential uses for faba bean include the following: i. break crop in a broader rotational program (Ko¨pke and Nemecek, 2010; Abera et al., 2015; Landry, et al., 2015), ii. feed source for pollinators and beneficial insects (Somerville, 2002; Etemadi et al., 2015), iii. enhanced soil microbial activity (Wang et al., 2007; Van der Putten et al., 2013; Wahbi et al., 2016), iv. control of soil-borne diseases (Jensen et al., 2010), and v. a rich source of L-Dopa for medicinal use (Etemadi et al, 2017). Therefore, integrating faba bean into cropping systems as either a cover crop or a dual-purpose cash/cover crop can enhance the sustainability and resiliency of agricultural production systems.

In the northeast U.S., unlike many common legumes such as peas, lentils, and beans, faba bean continues its growth and fixation of atmospheric N until winterkill in early to mid-December (Etemadi and Hashemi, 2014). Griffin et al. (2000) stated that in northern climates, legume cover crops seeded after the main crop harvest is limited opportunity for the establishment and significant biomass accumulation. (Griffin et al. (2000) stated that in northern climates, limited opportunity provided for the establishment and significant biomass accumulation if legume cover crops seeded after harvesting the main crop.) When grown solely for a cover crop, faba bean can be sown as late as early September; however, pod harvest is very unlikely. As expected, earlier plantings of faba bean result in greater biomass production and root activity (Song et al., 2007; Ko¨pke and Nemecek, 2010) that provide more ecological services – including increased N contributions to the subsequent cash crop. Growing dual-purpose faba bean as a cash and cover crop requires an earlier sowing in July (unpublished data).

In New England, sweet corn is cultivated on 5,540 hectares (Nass, 2014), and is the most widely grown vegetable crop. Like other types of corn, sweet corn is considered to be a high N demanding crop, requiring 130-160 kg N ha^{-1} on average (New England Vegetable Guide, 2016). Nitrogen utilization efficiency can be substantially improved by including legume cover crops in cropping systems, and also by improving the synchronization of N inputs and uptake sinks (Essah and Delgado, 2009). A previous report suggested that faba bean as green manure can significantly lower the cost of N fertilizer and the crop may also offer additional soil health benefits (Ko¨pke and Nemecek,2010).

In southern New England, active decomposition of winterkilled cover crops, including fall grown faba bean, generally begins as early as mid, March. However, sweet corn is commonly planted in mid, May. In contrast to winterkilled faba bean, spring grown faba bean residues generally have a significantly lower C:N ratio that causes the N mineralization of the residues to occur much faster (Shi, 2013). This increased rate of decomposition may not be well synchronized with the N uptake pattern of the ensuing crop, and poor synchrony results in a higher risk of N loss to the environment (Jensen et al., 2010), especially in CT systems. Improving the synchrony of N release from faba bean residues can enhance the N uptake/utilization efficiency of the succeeding crop, thus reducing potential environmental hazards (Thompson et al., 2015). The C:N ratio of cover crop residues is an important functional trait that influences subsequent crop yield (Kuo and Sainju, 1998; Starovoytov et al., 2010), and the C:N ratio can be manipulated by mixing legume and non-legume cover crop species (Teasdale and Abdul-Baki, 1998; Finney et al., 2016). For example, a mixed vetch and winter rye cover crop may supply sufficient, timely N to NT field corn (Griffin, et al., 2000; Cline and Silvernail, 2002). Mixing faba bean with a grass companion cover crop has been suggested in order to balance its C:N ratio (Ranells and Wagger, 1997; Villamil et al., 2006). However, when faba bean is grown as a dual- purpose cash and cover crop, the use of a companion grass with faba bean is not practical. Implementing a NT system in which winter killed faba bean residues remain on the soil surface may be a viable option to slow down the N mineralization rate in the spring, thus providing improved synchrony with N uptake of sweet corn. Moreover, an integrated cover crops and NT system is an effective strategy to build soil organic matter (Sainju et al., 2002).

There is a need for a better understanding of the trend of N release from winterkilled faba bean residues. We hypothesized that leaving fall sown faba bean cover crop residues on the soil surface would provide a better N release/uptake synchrony with spring sown sweet corn as opposed to incorporating the faba bean residues into the soil. Therefore, the main goals of the current study were to:

1) Evaluate the decomposition trend of winterkilled faba bean residues in CT and NT systems.
2) Assess the contribution of faba bean residues to the N demand of succeeding sweet corn.

Materials and methods

Experimental site and weather conditions

A 2-year experiment was conducted in 2013-2015 at the University of Massachusetts Amherst Agricultural Experiment Station Crops and Animal Research and Education Farm in South Deerfield (42° 28′ 37″N, 72° 36′ 2″ W). The soil type at the experimental site was a Hadley fine sandy loam (nonacid, mesic Typic Udifluvent). Composite soil sample (0-0.2 m depth) taken prior to planting indicated soil pH (1:1, soil/H_2O) was 6.6, cation exchange capacity (CEC) was 8.1 meq/100g, and available P, K and Mg were all in the optimum range thus no fertilizer was applied to the experimental plots in either year. Due to the humid northeastern climate, N recommendations are not based on specified soil nitrate test results (Lawrence et al., 2008). Weather conditions including growing degree days (GDD), precipitation throughout the experiment period (2013-2015), and the norm (average of 20 years), for the region are presented in Table 3.1.

Experimental setup, treatments and field operations

The experiment was laid out as split-plot arrangement within a randomized complete block design with four replications at the same experimental units as the previous year. Main plots consisted of NT and CT treatments. Sub-plots were allocated to a factorial combination of three faba bean planting dates (1, 7, and 14 August) and no cover crop (control) with supplemental N rates (0, 25, 50, 75, and 100 kg N ha^{-1}) applied to sweet corn the following spring. Individual sub-plots consisted of three rows, 4.2 m long and 0.76 m wide. Faba bean

‘Windsor’ was planted with a plot grain drill at a density of 8.8 plant m^{-2}. Seeds were inoculated with peat base *Rhizobium leguminosarum* (N-Dure, Verdesian, Cary, NC) before sowing in both years. No irrigation was used in this experiment as irrigation is not a common practice for agronomic field crops in Massachusetts. Faba bean biomass was determined prior to winter-kill (roughly mid-December in both years) by harvesting 1 m^2 from all plots. In the spring, faba bean residues were disked into the soil in the CT plots. Residues remained on the soil surface in the NT treatment. An early maturity sweet corn (Spring Treat F1 (se) Nat II, 66 d) was planted on faba bean stubble rows on 15 May in both years at a population density of 65,000 plants ha^{-1}. Nitrogen fertilizer in the form of calcium ammonium nitrate (27% N) was side dressed to sweet corn at 0, 25, 50, 75, and 100 kg N ha^{-1} within each faba bean cover crop date of planting. In faba bean plots, weeds were controlled mechanically (by hand and rototiller) three times during its growing period. Weed control in sweet corn was similar in both years and consisted of 2.2 kg a.i. ha^{-1} dual magnum (2-chloro-2',6'-diethyl- *N*-(methoxymethy1)-acetanilide, and 1.8 kg a.i. ha^{-1} treflan (*a*, *a*, *a*-trifluoro-2,6-dinitro-*N*, *N*-dipropyl-*p*-toluidine) preemergence herbicide. Sweet corn ears from center rows were hand harvested on 6 Aug. 2014 and 10 Aug. 2015 at peak marketable stage. The marketable ear number (minimum of 17 cm in length), ear fresh yield, and percentage of unfilled ear tip were determined.

Faba bean residues decomposition

A mesh bag technique was used based on the procedure fully described by Jahanzad et al. (2016). Mesh bags (60 µm) were made of polyamide nylon with a finished size of 20 × 10 cm. Prior to winter-kill, 10 faba bean plants were dug out carefully, washed, and air dried. Total fresh biomass including roots and shoots were determined. The tissue samples were dried in a forced air oven at 80°C for 36 h. Dried samples were weighed, then ground fine to pass through a 0.42-mm screen. A 200-mg subsample was used for N analysis. A Kjeldahl method of digestion (potassium sulfate, cupric sulfate, sulfuric acid) was used, followed by N measurement with a Lachat 8500 FIA spectrophotometer, Lachat Method no. 13-107-06-2-D (Zellweger Analytical, Milwaukee, WI).

Eighty mesh bags were filled with 200 g of uniformly mixed fresh chopped plant tissues. Forty mesh bags were left on the soil surface (simulating NT system) and 40 bags were buried 20 cm deep in the soil (simulating CT system). Three mesh bags, representing three replications, were recovered from both tillage systems on a weekly basis beginning 1 April. At each retrieval time, the content of each bag was dried in a forced-air oven set at 70°C, ground, weighed, and analyzed for dry matter and N content.

Statistical analysis

Analysis of variance (ANOVA) was conducted with the mixed model procedures in SAS (SAS Institute, 2003). Data shown in figures are the arithmetic means of four replicates of each treatment. Mesh bag retrieval dates were a continuous array of treatments, so trends in cover crop residue dry matter and N release during decomposition were assessed by regression analysis. Effects were considered significant at $P \leq 0.05$ by the F test, and when the F test was significant, Fisher's Least Significant Difference Test (LSD) was used for mean separation.

Results and discussion

Weather

Cumulative growing degree days (GDD4°C) at Orange Airport, MA (roughly 27 km away from the research site), during the growth period of faba bean cover crop (August–December), were 1843 and 1889 in 2013 and 2014, respectively, which were lower than the norm for this location (Table 3.1). Cumulative GDD during the months of May through August when sweet corn normally grows in New England were similar to the norm for the location in both years of the study. From August to December, months that spanned the faba bean growth period, precipitation totaled 418 mm in 2013 and 499 mm in 2014, which were comparable to the norm for the area. However, in spring 2014, the sweet corn crop experienced an unusual drought condition throughout the entire growing season (Table 3.1). Therefore, when averaged across all faba bean planting dates, the sweet corn yielded roughly 15% less overall in 2014 than in 2015. The influence of year and the interaction of year by trait were significant; accordingly, the results of the 2 yr are presented separately.

Cover crop biomass and decomposition trend

Faba bean biomass just prior to winter kill was dramatically influenced by date of planting (Table 3.2). The key benefit of legume cover crops is their role in supplying significant amounts of atmospheric N to a successive crop. However, it is well documented that the magnitude of agronomic services from cover crops in general, and N contribution from legumes in particular, are largely dependent on the amount of biomass they can produce by the termination time (Snapp et al., 2005; Hashemi et al., 2013; Finney et al., 2016; Mirsky et al., 2017). Early planting has been recognized as one major factor that influences cover crop biomass yield (Hashemi et al., 2013; Lounsbury and Weil, 2014; Komainda et al., 2016). In the current study, faba bean biomass decreased linearly as planting date was delayed. The amount of biomass loss for each day of delay was significant and estimated around 180 and 210 kg ha^{-1} in 2013 and 2014, respectively. However, the first week of planting delay (8 August vs. 1 August) was responsible for only a 19 and 7% biomass reduction in 2013 and 2014, respectively. Conversely, the second week of planting delay (14 August vs. 1 August) resulted in a reduction of faba bean biomass of up to 62 and 55% in 2013 and 2014, respectively. Drier conditions in 2013, especially during the month of October when faba bean is actively growing, could in part explain the difference in total biomass production in the 2 yr of this experiment. We presumed that a higher *Rhizobium* population in the soil in the second year of the experiment could also have played a role in the production of more faba bean biomass in 2014. The presence of higher N concentrations in faba bean tissues in 2014 support this assumption (Table 3.2). Nitrogen yield of a cover crop is a product of tissue N concentration and biomass accumulation, which indicates the potential amount of N that can be released into the soil during the decomposition process. Nitrogen accumulation of faba bean in 2013 and 2014 is presented in Table 3.2. As mentioned, above, the N concentration in faba bean plants was significantly higher in 2014 compared with 2013. Although the *Rhizobium* population was not analyzed in this experiment, it is logical that the *Rhizobium* population would naturally be higher in the second year of the experiment conducted in the same location, compared with the first year of the study. The difference in the N concentration of faba bean in each year of the experiment could be at least partly attributed to the hypothesized higher bacterial population and resultant activity in 2014. The results indicate that faba bean sown on 1 Aug. 2014 accumulated as much as 192 kg ha^{-1} (Table 3.2), which is greater than earlier reports (Bremer et al., 1988; Unkovich and Pate, 2000; Cline and Silvernail, 2002), which reported that faba bean can fix up to 160 kg N ha^{-1}. Also, Duc et al. (1988) and Schwenke et al. (1998) reported that faba bean can fix up to 330 kg N ha^{-1}. Additionally, in the dryer condition of 2013, faba bean fixed 50% less N than in 2014, averaged over the three planting dates. The results obtained in the current study, as well as in earlier reports (Oplinger et al., 1990; Baddeley et al., 2014), revealed that in general, faba bean and its associated *Rhizobium* are more efficient in fixing atmospheric N than vetch (Spargo et al., 2016; Mirsky et al., 2017) and many other grain legumes (Peoples et al., 2009).

The trends of N release for each of the two tillage systems are presented in Fig. 3.1. When the first decomposition sample was retrieved on 1 April for analysis, almost 30% of the N content of the faba bean residues in CT, and 20% in NT, had already been mineralized. The difference in the N release rate between the two tillage systems widened as the growing season progressed. Rapid decomposition of cover crop residues is not necessarily desirable, because the mineralized N is subject to various avenues of environmental loss if it is not captured by plants (Lupwayi et al., 2004; Tonitto et al., 2006; Cook et al., 2010). The results confirmed our hypothesis and also the earlier report by Drinkwater et al. (2000) that the use of a NT method would slow down decomposition of faba bean residues, providing a better timing between N mineralization and N uptake by sweet corn plants. In a CT system, almost 50% of N was released by the end of May, whereas in NT, release of 50% of N was delayed for approximately one month and occurred at the end of June. In New England, sweet corn traditionally is planted in early-mid May and commences its rapid growth stage in mid-June (4R Plant Nutrition, International Plant Nutrition Institute, 2015). Therefore, the delay in 50% N release from faba bean residues can significantly improve the synchrony between N release from faba bean residues and N uptake by sweet corn.

Sweet corn yield

<u>Influence of faba bean residues and tillage systems</u>

Sweet corn marketable ear number and fresh ear yield, as well as percentage of unfilled ear tip, were significantly influenced by the amount of faba bean biomass accumulated at each planting date (Table 3.3). Averaged over 2 yr, sweet corn planted into residues of the earliest sown faba bean produced roughly 19% more marketable ear, 23% higher fresh ear weight, and 39% less unfilled ear tip compared with those grown in no faba bean plots (Table 3.3). The positive influence of faba bean residues on aforementioned traits dropped to 11, 7, and 6%, respectively, when planting of faba bean was delayed for only 2 wk. The difference in influence of faba bean residues on yield performance of sweet corn is mainly attributed to the total N contribution of their residues (Table 3.2). As previously stated, more ecological benefits can generally be expected from higher cover crop biomass production. In addition to the higher accumulated N in early sown faba bean residues, other factors, such as type of tillage system, might have played a role in this study. Marketable ear yield of sweet corn was significantly higher in NT than in CT systems (Fig. 3.2a).

Averaged over faba bean residue, sweet corn plants produced roughly 22% higher marketable ear and 31% less unfilled ear tip compared with a conventional system (Fig. 3.2a, b). This could be primarily due to better synchrony existed between faba bean decomposition with sweet corn growth in a no-till system (Fig. 3.1). The interaction between a tillage system and presence of faba bean residue was significant. For example, the ear yield difference between corn grown into faba bean residues was higher in no-till plots compared with a conventional tillage system (Fig. 3.4). Averaged over 2 yr, the maximum marketable ear (12.8 Mg ha^{-1}) was obtained from corn planted in no-till plots covered with faba bean residues (Fig. 3.4). Reports on the influence of tillage systems on sweet corn yield planted following various types of cover crops are contradictory and seem greatly influenced by the amount of residues produced at cover crop termination time (Teasdale et al., 2008) and the C/N ratio of the cover crops (Cline and Silvernail, 2002; Kuo and Jellum, 2002). Cline and Silvernail (2002) concluded that NT sweet corn yielded similar to CT when planted after vetch, whereas sweet corn grown after rye or rye–vetch mixtures experienced a yield penalty. In addition to C/N, time and method of termination of cover crops may also interact with the influence of a tillage system. Winter legumes grown as green manure in spring and/or summer generally have a lower C/N, and they thus release substantial amounts of N in CT systems. In NT systems, the release of N is slower and therefore may not meet the N requirement of a high N demanding crop such as sweet corn.
Recently, Lowry and Brainard (2017) reported that the influence of the tillage system on organic sweet corn biomass is pronounced when soil moisture was non-limiting. Also, the lack or negative response of NT systems on integrated organic cover crop/ sweet corn production might be related to a higher weed population that is usually higher in NT compared with CT systems.

<u>Influence of supplement N application</u>

In both years, averaged over faba bean planting dates and tillage systems, the response of sweet corn marketable ear number and fresh ear yield to application of supplemental N was quadratic (Table 3.3) and reached its peak at approximately 80 kg N ha^{-1} (Fig. 3.3). Unfilled ear tip decreased linearly as N application rate increased (Table 3.3). As expected, the response of sweet corn to supplemental N was more pronounced when sweet corn was planted in plots without a prior faba bean cover crop (Fig. 3.3). In plots with no faba bean cover crop, a linear increase in sweet corn marketable ear yield was detected with increased supplement N fertilizer rate (Fig. 3.3). Although the faba bean cover crop was effective in fixing and conserving N, the faba bean residues did not provide sufficient N to the following sweet corn crop. As a result, when corn was planted into faba bean residues, an asymptotic response to increased N application rate with a peak at approximately 60 kg ha^{-1} was observed (Fig. 3.3). The impact of faba bean residues on sweet corn response to supplemental N was more noticeable in 2014 than 2015, presumably due to differences in the amount of percipiation (Table 3.1). The results obtained from this study do not confirm some of the earlier reports (Griffin et al., 2000; Cline and Silvernail, 2002) that indicated sweet corn planted after a legume cover crop usually does not respond to supplemental N.

Based on results obtained in the current study, we concluded that averaged over 2 yr, the yield of sweet corn grown after faba bean was responsive to the first three increments of N up to 60 kg ha^{-1}, as opposed to the linear response in no faba bean plots. We found no significant interaction between faba bean date of planting and tillage system or between supplemental N application rate and tillage system. Further investigation on faba bean as the major N source for succeeding vegetables under different sets of environmental conditions and management practices would be valuable for developing strategies to increase economic returns and to limit adverse environmental impacts of commercial fertilizers.

Conclusion

The results of this experiment provide a better understanding of the efficiency of N contribution of faba bean grown as a cover crop in rotation with sweet corn in the northeastern United States. Nitrogen yield of faba bean is a function of both its biomass production and its plant tissue N concentration. Therefore, earlier planting of faba bean dramatically influences the potential N that could be fixed and subsequently contributed to the following crop. In the current study, our measurements revealed that potential N was as much as 192 kg N ha^{-1} when faba bean was planted as early as 1 August and weather conditions were favorable (2014). The tillage system can significantly influence the trend of residue decomposition. No-till system delayed 50% N release from faba bean residues by approximately one month, which can significantly improve the synchrony between mineralization of faba bean residues and N uptake by sweet corn. Sweet corn planted into faba bean residues produced greater marketable ear number, higher fresh ear weight, and less unfilled ear tip compared with those grown in no faba bean plots. Since fall-grown faba bean is not considered to be a high-residue cover crop, spring-sown sweet corn can benefit from NT compared with CT system. Averaged over 2 yr and faba bean planting dates, sweet corn yielded 26% higher and unfilled ear tip 30% lower in NT compared with CT, respectively. Although the faba bean cover crop was effective in fixing and conserving N, its residues did not provide sufficient N to the following sweet corn crop. On average, sweet corn responded positively to applications of supplemental N up to 60 kg ha^{-1}. Averaged over 2 yr, sweet corn following faba bean, plus approximately 50 kg N ha^{-1}, yielded similarly to those that received 100 kg N ha^{-1} without a prior faba bean cover crop.

Table 3. 1 Precipitation and accumulated GDD during growing seasons of faba bean (2013 and 2014), sweet corn (2014 and 2015), and faba bean residues decomposition (2014 and 2015) compared with 20 years average of corresponding months for the experimental location.

Month	**GDD †**			**Precipitation (mm)**		
(Faba bean)	**2013**	**2014**	**Norm**	**2013**	**2014**	**Norm**
Aug	858	835	907	99	92	91
Sep	591	630	643	77	41	109
Oct	316	368	286	67	160	110
Nov	68	45	89	94	90	77
Dec	10	11	16	81	116	79
Total	**1843**	**1889**	**1941**	**418**	**499**	**466**
(No crop)	**2014**	**2015**	**Norm**	**2014**	**2015**	**Norm**
Jan	9	0	3	82	83	67
Feb	0	0	2	59	37	69
Mar	3	2	47	82	43	95
Apr	167	157	204	112	51	80
Total	**179**	**159**	**256**	**335**	**214**	**311**
(Sweet corn)	**2014**	**2015**	**Norm**	**2014**	**2015**	**Norm**
May	516	674	515	29	26	88
Jun	785	715	767	1	192	116
Jul	947	938	942	55	85	98
Total	**2248**	**2327**	**2224**	**84**	**303**	**302**

†GDD calculated as GDD = Σ (T_{max} - T_{min})/2 –T_b where T_{max} and T_{min} are daily maximum and minimum temperatures, respectively. Base temperature (T_b) was set as 4^o C and 10^o C for faba bean and sweet corn, respectively.

Table 3. 2 Faba bean biomass and N concentration in faba bean aerial biomass prior to winter kill as influenced by its date of planting.

Faba bean planting date	**Biomass (Mg ha^{-1})** 2013	2014	**N in residues (g kg^{-1})** 2013	2014	**N yield (kg ha^{-1})** 2013	2014
Aug 01	4.0±0.1	5.3±0.1	25±0.2	36±0.5	101±0.1	192±0.5
Aug 07	3.3±0.1	4.9±0.1	23±0.1	31±0.2	75±0.3	154±0.2
Aug 14	1.5±0.1	2.4±0.2	22±0.1	28±0.2	33±0.3	67±0.2
Trend	Q*	Q*	Q*	Q*	Q*	Q*

L = linear.

* significantly at *P*= 0.05

Table 3. 3 Sweet corn marketable ear number and fresh ear yield and unfilled tip percentage affected by faba bean (FB) residues planted at three dates of planting (DOP) and supplement N application rate to sweet corn.

Treatments	**Ear # (ha^{-1})** 2014	2015	**Ear wt. (Mg ha^{-1})** 2014	2015	**Unfilled ear tip (%)** 2014	2015
FB DOP						
Aug01	51793	55380	11.94	13.53	11.1	5.8
Aug07	49800	52591	10.34	12.73	16.4	7.2
Aug14	45760	51630	9.85	11.14	17.4	8.5
No FB	38547	48607	9.15	10.43	18.3	9.2
Trend	L*	L*	Q*	L*	Q*	L*
Supp. N rate (kg ha^{-1})						
0	37050	45816	6.37	9.95	17.1	10.0
25	49005	54582	11.14	12.73	16.4	8.2
50	52629	57568	11.65	14.32	14.3	6.6
75	52721	57680	11.66	14.33	14.2	6.1
100	53754	58621	11.78	14.43	13.9	5.6
Trend	Q*	Q*	Q*	Q*	L*	L*

*, ** significantly at *P*= 0.05 and *P*=0.01, respectively, ns= not significant

L and Q = linear and quadratic, respectively.

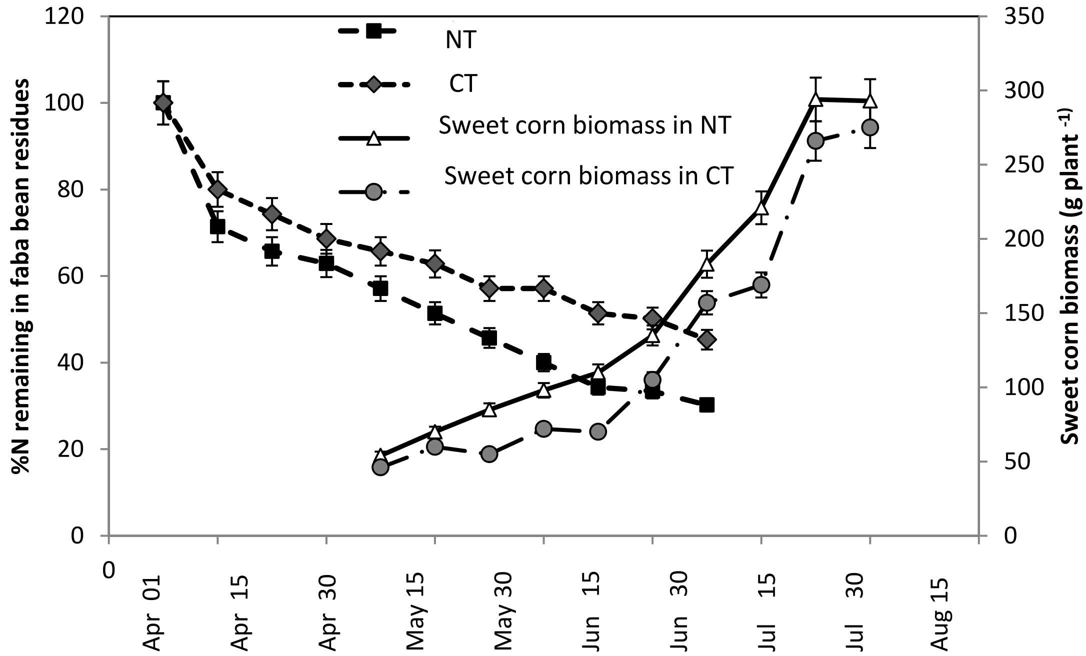

Figure 3.1 Nitrogen release trend from decomposing faba bean residues in conventional tillage (CT) and no-till (NT) systems and sweet corn growth pattern. Means are averaged over two growing seasons.

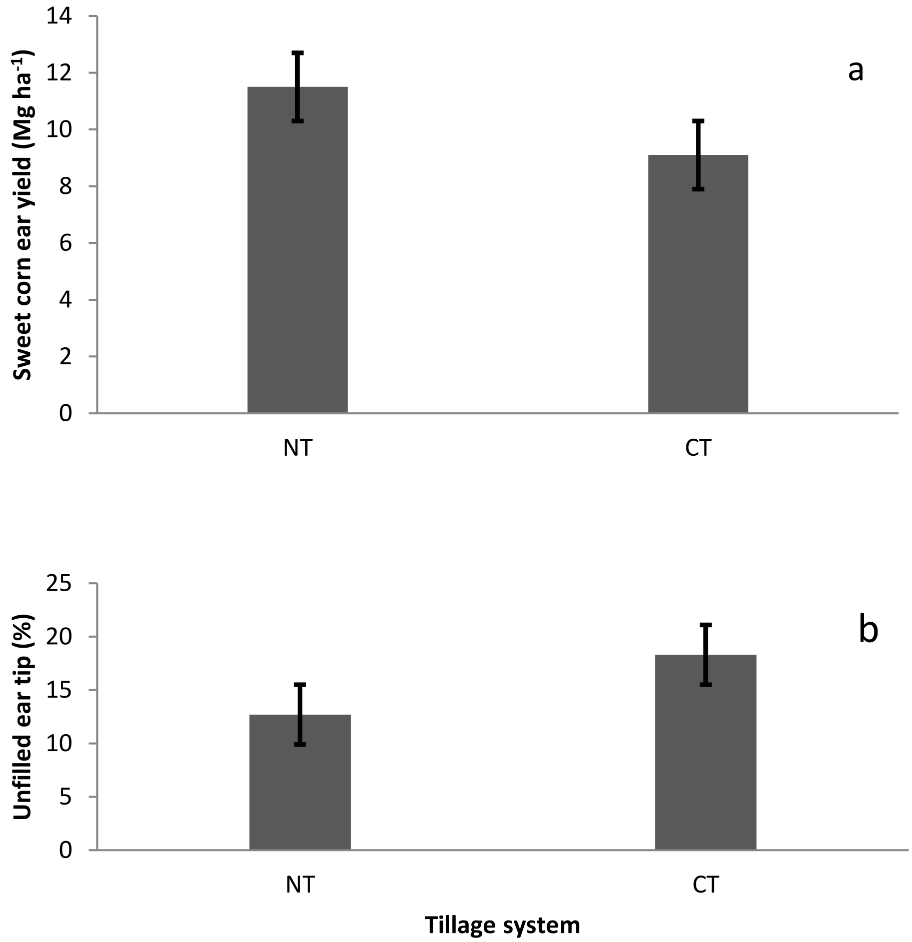

Figure 3. 2 Effect of tillage system on sweet corn ear yield (a) and unfilled ear tip percentage (b). Means are averaged over three faba bean dates of planting and two growing seasons.

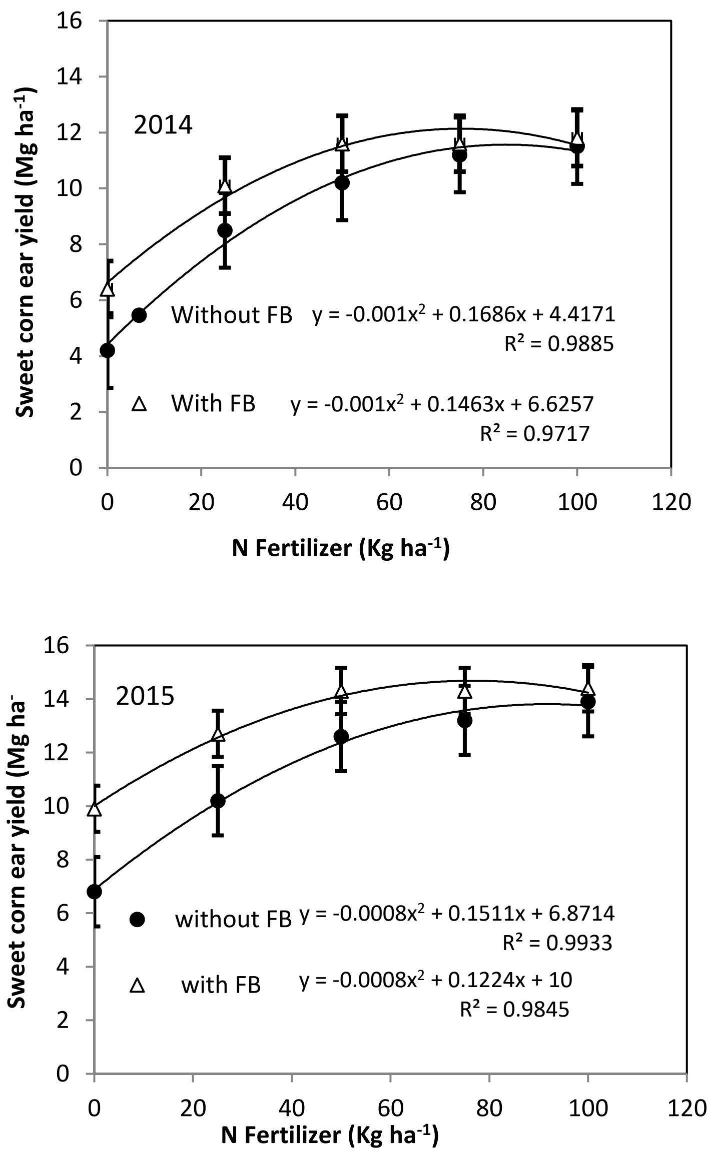

Figure 3.3 Sweet corn ear yield influenced by presence or absence of faba bean residues and supplement N fertilizer rates in 2014 and 2015. Values are averaged over two tillage systems.

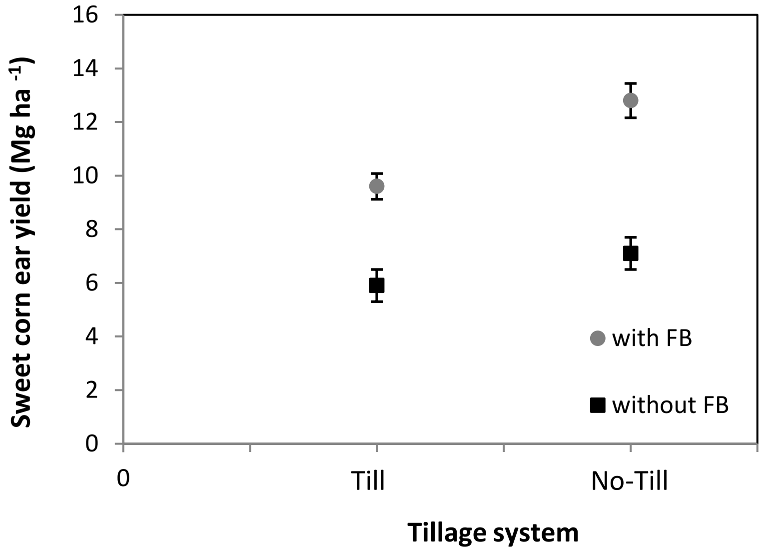

Figure 3.4 Interactive effect of tillage system and faba bean residues on sweet corn ear yield. Values are averaged over 2014 and 2015.

CHAPTER IV

APPLICATION OF DATA ENVELOPMENT ANALYSIS TO ASSESS PERFORMANCE EFFICIENCY OF EIGHT FABA BEAN VARIETIES

Abstract

Faba bean (*Vicia faba* L.) seeds are generally large which limits its adoption as cover crop and/or dual-purpose cover crop/cash crop due to the high seed cost. The purpose of this study was to apply data envelopment analysis (DEA) by using seed size as input and fresh pod, fresh seed, and L-Dopa yield as output to evaluate efficiency of eight faba bean varieties. Eight faba bean varieties were evaluated in a 2-yr study. Four common methods of DEA were used for ranking faba bean varieties. Aquadulce and Delle Cascine out-yielded other varieties in both years. Averaged over two yrs Aquadulce and Delle Cascine produced 16.15 and 16.27 Mg ha^{-1} fresh pod, respectively. However, Aquadulce had 21% lower seed size than Delle Cascine which significantly reduces the cost of production. L-Dopa yield ranged from 4 kg ha^{-1} in Sweet Lorane to 46 kg ha^{-1} in Aquadulce. Although no significant difference was found in fresh pod yield and fresh seed yield of Aquadulce and Delle Cascine, Aquadulce ranked first in both years while Delle Cascine ranked fourth in 2015 and third in 2016 due to its larger seed size and lower L-Dopa. Bell bean and Sweet Lorane had the smallest seed size yet their efficiency ranked last due to their low fresh pod yield, fresh seed yield, and L-Dopa yield. Results revealed that DEA could successfully use multiple traits in a single mathematical model without the need for the specification of tradeoffs among multiple measurements.

Introduction

Faba bean is a cool season legume crop, which originated in the Middle East in the pre-historic period where it has been used as a main source of protein for human and animal nutrition. Faba bean is the fourth most important pulse crop in the world due to its richness in protein, carbohydrate, B-group vitamins, and minerals (Crépon et al., 2010). Faba bean is considered an important dietary component used by many ethnic groups, especially Portuguese-speaking citizens. Massachusetts has the largest population of Portuguese-speaking people in the United States. The community comprises people from Portugal, the Azores, Cape Verde, and a growing Brazilian population, estimated by the Brazilian Consulate in Boston at approximately 250,000 in 2013 (Etemadi et. al., 2015). However, faba bean is not currently grown in New England, partly due to its large seed size and relatively low yield which makes it non-competitive compared with other legumes. Currently only two varieties of faba bean are available to the Northeast growers where their yields are relatively low, and the seed cost is high due to their large seed size. Projected population density of grain crops is traditionally determined by seeding rate per unit area and is related to the number of seeds per unit weight (kg). Therefore, seeding rate of cultivars with smaller seed size is significantly lower than those with larger seeds which in turn influence the cost of production.

Due to its diverse and significant ecological services (Köpke and Nemecek, 2010) faba bean has increasingly received attention and is now widely used in many regions of the world as a source of food and also as a break crop in cereal crop production to minimize the occurrence of cereal cyst nematode (*Heterderaavenae*) (Sattell et. al., 1998) and some soil-borne pathogens (Landry et. al., 2016). Faba bean has been identified for its efficient N_2 fixation capacities among the cool season pulses (Herridge et. al., 1994; Mekkei, 2014). Reports indicated that faba bean can fix between 53 and 330 kg N ha^{-1} (Galloway et. al., 2004; Visalli, 2015) depending on management and environmental conditions. In New England, faba bean can be seeded as early as mid-March if soil is dry enough for cultivation practices and be harvested in time for planting an additional crop due to its relatively short growing season. When planted early, some marketable pods can be harvested in late June and N- rich residues be incorporated into soil as green manure. Alternatively, faba bean can be sown after harvesting winter grains or a spring crop as a dual purpose, that is, cash crop/cover crop until it is winter killed (Etemadi et. al., 2015).

Faba bean also has been identified as a rich source of natural L-Dopa which is the precursor to the neurotransmitter's dopamine, norepinephrine, and epinephrine collectively known as catecholamines (Randhir and Shetty, 2004; Singh et al., 2013; Mohseni and Golshani, 2013). L-Dopa elevates dopamine concentrations in the brain thus traditionally it has been used for curing Parkinson's disease (Hiroshima et. al., 2014). Faba bean varieties vary considerably in L-Dopa content. For example, Etemadi et al. (2014) reported that L-Dopa concentrations in different varieties varied between 7.6 to 10.9 g kg^{-1}.

Data envelopment analysis is a non-parametric method that uses linear programming to evaluate the performance of decision-making units (DMUs) with multiple inputs and outputs. It has opened up possibilities for use in cases which have been resistant to other approaches because of the complex and often unknown nature of the relations between the multiple inputs and outputs involved in many of their activities Data envelopment analysis was first introduced by Charnes et al. (1978) and since then a continuous growth in its implication has been observed. The DEA has the ability to model multiple-input and multiple-output relationships without a priori underlying functional form assumption. This ability provides wide application areas such as in agriculture, banking, education, environment, healthcare, energy, manufacturing, transportation, and supply chain management (Emrouznejad et al., 2008). Researchers have successfully used DEA to analyze the production efficiency (Huang and Hu, 2006), productivity (Aldaz and Millán, 2003), land use (Toma et. al., 2015), and irrigation (Díaz et al., 2004). For a comprehensive survey on DEA applications from 1978 to 2010, see Liu et al. (2013). In general, DEA models minimize “inputs” and maximize “outputs”; where smaller levels of the former and larger levels of the latter represent better performance or efficiency (Cook et. al., 2014). The most popular DEA models were introduced by Charnes et al. (1978) (CCR model), Banker et al. (1984) (BCC model), Färe and Grosskopf (1985) (FG model), and Seiford and Thrall (1990) (ST model). The main difference between above DEA models can be summerized as the CCR model is constant returns to scale, the BCC model is variable returns to scale, the FG model is non-increasing returns to scale, and the ST is non-decreasing returns to scale (Foroughi and Shureshjani, 2016). For more details about qualitative and quantitative aspects of returns to scale in DEA models, readers are referred to Banker et al. (2011).

Yu et al. (1996 a, 1996b) proposed a generalized model that included the above-mentioned popular DEA models. In agriculture, selection process of inputs is important because the outputs such as productivity depend on the input consumption. However, when additional information or assumptions are available, some weights need to be restricted. Therefore, in this regard a DEA problem with an assurance region should be considered. A DEA problem with an assurance region (DEA/AR) first was introduced by Thompson et al. (1990) and made DEA more applicable. In crop production, yield is acknowledged as having a positive correlation with economic efficiency.

The main objective of this research was to evaluate the performance (economic yield) of eight faba bean varieties based on their seed size, pod fresh weight (PFW) and seed fresh weight

(SFW) and L-Dopa yield. Four different DEA models were used to assess the performance efficiency of these spring-sown faba bean varieties in Massachusetts.

Materials and methods

Farm Experiment

Eight varieties of faba bean including Bell Bean, Early Violletto, Aquadulce, Delle Cascine, Windsor, Sweet Lorane, Early White, and D'Aquadulce were selected for this study based on their past performance when sown in early spring at the research site (Etemadi et al., 2015). The seed size of the varieties was determined by taking the average of five sets of 100-seeds and were ranged from 51 g per 100 seeds (Bell Bean) to 392 g per 100 seeds (D'Aquadulce). Seeds were hand-planted in 15 Apr. 2015 and 17 Apr. 2016 at the University of Massachusetts Crops and Animal Research and Education Center in South Deerfield (42°28'37" N, 720°36'2" W). The soil type was a Hadley fine sandy loam (nonacid, mesic Typic Udifluvent) with pH of 6.7, organic matter content of 12 g kg^{-1}, N, P, K, and Ca content of 3, 9, 73, and 868 mg kg^{-1} (Morgan, 1941), respectively. These nutrients content was considered adequate (New England vegetable management guide, 2016). Therefore, no additional fertilizer was applied to the experimental plots. A complete randomized block design with four replications was used. Research plots consisted of three rows, 5 m long and 0.76 m apart. Space between the plants within the rows was 15 cm. Three meters from the middle rows (2.28 m^2) from each plot was randomly selected and marked as final harvest area. Pods of plants in final harvest areas were harvested manually three times beginning on 7 July 2015 and 10 July 2016 for determination of pod and seed yield. Weeds were controlled three times prior to canopy closure using a hand rotivator.

Measurements

Faba bean yield including PFW and SFW were determined. Seed cost was calculated based on 60,000 seeds ha^{-1}, number of seeds kg^{-1}, and the cost of purchased seeds. L-Dopa concentration in seeds was measured at physiological maturity and presented as L-Dopa yield (SFW × L-Dopa concentration). Seed samples were oven dried for 36h at 55°C. Dried samples were ground fine to pass through a 0.42 mm screen before extraction.

High Performance Liquid Chromatography Procedure

We immersed 200 mg of ground samples in 95% ethanol and then kept in a freezer for 72 h. Samples were homogenized using a tissue tearer. Samples were centrifuged at 13,000 rpm for 10 min. The liquid portion was left under hood until the ethanol evaporated. The residue was dissolved in buffer solution and left in fridge until particles settled. The supernatant was passed through a 0.45 µm syringe filter and analyzed using an HPLC (Shimadzu Prominence HPLC with DAD detector, Phenomenex Gemini C18 column). Four concentrations of stock solution (50, 100, 200, 400 mg L^{-1}) were used and diluted and injected with the mobile phase. The calibration curve was obtained by plotting the absorbance area vs. the concentrations of the standard solution.

Data Envelopment Analysis

Generalized Data Envelopment Analysis with Assurance Region

Data Envelopment Analysis (Eq. [1]) was used as a tool in ranking varieties based on three traits that had highest priority for our assessment.

Assume x_{ij} and y_{rj} are the amount of the *i*th input and *r*th output of *j*th DMU_j, respectively.., Then the generalized DEA model was formulated as following (Yu et al., 1996a, 1996b):

$$\begin{aligned}
\max z &= \sum_{r=1}^{s} u_r y_{ro} - \mathrm{d}_1 u_0 \\
\text{s.t.} \quad & \sum_{i=1}^{m} v_i x_{io} = 1 \\
& \sum_{r=1}^{s} u_r y_{rj} - \sum_{i=1}^{m} v_i x_{ij} - \mathrm{d}_1 u_0 \leq 0, \; j = 1, ..., n \\
& \mathrm{d}_1 \mathrm{d}_2 (-1)^{\mathrm{d}_3} u_0 \geq 0, v_i, u_r \geq 0, i = 1, ..., m, r = 1, ..., s
\end{aligned} \qquad [1]$$

whereδ_1, δ_2, and δ_3 are binary parameters:

(i) If $\delta_1 = 0$, then the generalized DEA model is reduced to the CCR model.
(ii) If $\delta_1 = 1$ and $\delta_2 = 0$, then the generalized DEA model is reduced to the BCC model.
(iii) If $\delta_1 = \delta_2 = 1$ and $\delta_3 = 0$, then the generalized DEA model is reduced to the FG model.
(iv) If $\delta_1 = \delta_2 = \delta_3 = 1$, then the generalized DEA model is reduced to the ST model.

In theory, DEA models allow each decision-making units (DMU) to select the weights that are most favorable to themselves in calculating the ratio of the virtual output to the corresponding virtual input. However, the weights of inputs and outputs must be kept in some ranges to be applicable to agricultural usage. Therefore, the concept of the assurance region (AR) was developed to restrict some weights to reasonable ranges (Thompson et. al., 1990, 1992). These restrictions were applied to DEA models to prevent DMUs from ignoring and/or relying too much on any input or output. Zhou et al. (2012) developed the following model for AR (Eq. [2]).

$$
\begin{aligned}
&\max z = \sum_{r=1}^{s} u_r y_{ro} - \mathrm{d}_1 u_0 \\
&\text{s.t.} \quad \sum_{i=1}^{m} v_i x_{io} = 1 \\
&\sum_{r=1}^{s} u_r y_{rj} - \sum_{i=1}^{m} v_i x_{ij} - \mathrm{d}_1 u_0 \le 0,\ j = 1,...,n \\
&C_{pq}^{L} \le \frac{v_q}{v_p} \le C_{pq}^{U},\ 1 \le p \prec q = 2,...,m \\
&D_{pq}^{L} \le \frac{u_q}{u_p} \le D_{pq}^{U},\ 1 \le p \prec q = 2,...,s \\
&\mathrm{d}_1 \mathrm{d}_2 (-1)^{\mathrm{d}_3} u_0 \ge 0,\ v_i, u_r \ge 0,\ i = 1,...,m,\ r = 1,...,s
\end{aligned} \qquad [2]
$$

In this study, we assumed that $0 \le C_{pq}^{L} \prec C_{pq}^{U}$ and $0 \le D_{pq}^{L} \prec D_{pq}^{U}$.

From the generalized DEA model (model 1), parameter u_0 in the CCR model is zero; in the BCC model is a free in sign variable; in the FG model is a non-negative variable; and in the ST model is a non-positive variable. Thus, the following relations were considered to obtain the efficiency when using these four models:

The efficiency of a DMU from the CCR model is less than or equal to its efficiency from the FG or the ST model.

The efficiency of a DMU from the FG or the ST model is less than or equal to its efficiency from the BCC model.

The efficiency of a DMU from the BCC model with a positive u0 will be the same as its efficiency from the FG model.

The efficiency of a DMU from the BCC model with a negative u0 will be the same as its efficiency from the ST model.

The efficiency of a DMU from the FG or ST model with $\boldsymbol{u_0 = 0}$ will be the same as its efficiency from the CCR model

Statistical Analysis

Proc GLM (SAS Institute, 2003) was used for analysis of variance. Effects were considered significant at *P*□ 0.05 by the *F* test, and when the *F* test was significant, Fisher's Least Significant Difference Test (LSD) was used for mean separations.

Results

Fresh Pods, Fresh Seeds, and L-Dopa Yield

A significant year by dependent variables including PFW, SFW, and L-Dopa was detected; therefore, data was analyzed by year. Bell bean performed poorly in 2016 thus it was eliminated from variety evaluation in the second year of the experiment. Pod fresh weight, seed fresh weight, and L-Dopa of faba bean varieties harvested in 2015 and 2016 are presented in Tables 4.1 and 4.2, respectively. On average, PFW, SFW, and seed/pod ratio were significantly higher in 2015 compared with 2016. This was mainly attributed to the wetter and warm but slightly cooler conditions in 2015 (Table 4.3), particularly during the months of June and July when pod formation and pod filling of faba bean normally takes place in northeastern United States. The response of the tested varieties to growing conditions varied significantly, but Aquadulce and Delle Cascine out-yielded other varieties in both years (Tables 4.1 and 4.2). Windsor is the common variety available to the growers in the Northeast. However, our results revealed that Aquadulce yielded roughly threefold higher than Windsor while its seed size was 15% smaller thus its seed cost is also lower. Moreover, L-Dopa yield among the tested varieties varied dramatically and ranged from 4.0 to 46.0 kg ha^{-1} in 2015 and 2.8 to 30 kg ha^{-1} in 2016. Higher L-Dopa in 2015 compared with 2016 can be attributed to higher fresh pod yield which was 12% higher averaged over all varieties (Tables 4.1 and 4.2).

Varieties Efficiency

In this study we used four major traits including seed size, PFW, SFW, and L-Dopa yield for ranking the varieties using the DEA/AR model. To analyze the data by the model, the inputs and outputs of the DEA model were defined. The PFW, SFW, and L-Dopa values were used directly as the outputs in all four models with no transformation required where the larger numbers considered more favorable. Seed size however, is a quantity where too large and/or too small values cannot be considered desirable. Hence, seed size values were converted into a set of data before being used. An average of the smallest (Bell Bean) and the largest seed size (D'Aquadulce) was calculated and seed size deviation of all varieties from the average was determined and used as input. The smaller the deviation from the average value was considered optimum. The adjusted data for seed size as input to the DEA model is included in Tables 4.1

and 4.2. In summary, there were one input (deviation from the average seed size) and three outputs including PFW, SFW, and L-Dopa (kg ha^{-1}) in the DEA models.

The ARs should be determined based on experts' opinions or other logic explanations. In the current study the ARs were defined based on authors'experience and considered as Eq. [3].

$$\frac{1}{3} \le \frac{u_2}{u_1} \le 3 \qquad \text{and} \qquad \frac{1}{3} \le \frac{u_3}{u_2} \le 3 \quad [3]$$

We utilized the GAMS software for running the model. In this study four models, that is, CCR, BCC, FG, and ST were used for evaluation of the efficiency of the faba bean varieties in 2015 and 2016 (Tables 4.4–4.7).The results indicated that Aquadulce had the highest performance efficiencies in all four models in 2015 (Tables 4.4 and 4.5) and 2016 (Tables 4.6 and 4.7) followed by Early White. Interestingly, Delle Cascine yielded similar or better than Aquadulce and Early White varieties however due to its larger seed size, lower seed/pod ratio, and lower L-Dopa content it was ranked fourth in the efficiency evaluation list. On the other hand, although Early White ranked second in almost all four models, its PFW, SFW, and L-Dopa yields were dramatically lower than Aquadulce and therefore the ranking might be misleading. This clearly indicates that DEA can be used efficiently when the number of testing faba bean varieties is fairly high.

Discussion

Although numerous ecological services from faba bean have been documented (Köpke and Nemecek, 2010; Jensen et. al., 2010) and despite an increasing market for fresh faba bean in southern New England, its cultivation has been neglected thus markets in this region are relying on imported fresh faba bean from Mexico and other states. Large seeds of many faba bean varieties, at least those that are currently available to the growers in the Northeast, should be considered as one of the major factors that limits faba bean production. The authors agree with Landry et al. (2016) that potential agronomic benefits, including the economics as well as various ecological services of faba bean should be explored further.

Despite no statistical differences between Aquadulce and Delle Cascine in regard to PFW, SFW, and L-Dopa content, Delle Cascine ranked fourth in 2015 and third in 2016 compared with Aquadulce which came first in terms of variety efficiency in both years. This was primarily due to the smaller seed size of Aquadulce which was 21% lower than Delle Cascine. In other words, while having similar yield, Aquadulce was selected as a better choice than Delle Cascine especially when faba bean is grown primarily as cover crop and/or dual-purpose cash/cover crop.

L-Dopa concentration in fresh seeds of the tested varieties were higher in 2016 because growing conditions was drier and warmer than 2015. L-Dopa is a secondary metabolite and it is well documented that accumulation of secondary metabolites in plants strongly interacts with growing conditions and environmental stresses (Keshavarz Afshar et al., 2015). Nevertheless, despite higher L-Dopa concentrations in 2016 (averaged across all varieties) since SFW in 2015 was roughly 30% more than in 2016, L-Dopa yield was higher in 2015 compared with 2016.

There is a dramatic variation in seed size of faba bean varieties (Duc, 1997; Landry et al., 2016). In the current study with a limited number of varieties, the seed of D'Aquadulce was almost 7.7 and 5.8-fold larger than bell bean and Sweet Lorane, respectively. Such significant variation makes faba bean a unique legume crop when it comes to variety evaluation. For example, in common bean (*Phaseolus vulgaris* L.) (Peksen and Gulumser, 2005; Sabokdast and Khyalparast, 2008), or chickpea (*Cicer arietinum* L.) (Atta et. al., 2008), variety selection was simply based on seed yield which is the highest contributor to the overall yield. However, in faba bean, due to the large variation in seed size, economic yield which includes seed cost must also be considered. In the current study replacing Windsor (the current available variety in the Northeast) with Aquadulce seems an easy decision. Aquadulce has a smaller seed (265 g 100 seed^{-1} vs. 311 g 100 seed^{-1}) therefore it requires a lower seeding rate (84 vs. 102 kg ha^{-1}) and lower seed cost (US$538 vs. $727 ha^{-1}) and had a higher seed yield (1925 vs. 695 g dry seed). However, in other examples the selection of a variety could be more complicated where the seed cost of a variety might be less, but its yield is also lower. The selection becomes even more complicated when L-Dopa yield is among the goals of faba bean cultivation.

The results of the current study indicated that DEA can be used efficiently especially when the number of testing faba bean varieties is fairly high. Varieties ranked high by DEA models can be significantly used to promote faba bean production in the Northeast.

Table 4. 1 Seed size, pod fresh weight (PFW), seed fresh weight (SFW), and L-Dopa yield of faba bean in 2015.

Variety	**Seed size**	**Deviation from the avg. seed size (input)**	**PFW (output 1)**	**SFW (output 2)**	**L-Dopa (output 3)**
	g 100 seed^{-1}		–Mg ha^{-1}–		**kg ha^{-1}**
Bell Bean	51	□170.5	3.73cd†	1.46c	13.6e
Sweet Lorane	68	□153.5	2.05d	0.52d	4.0f
Early White	253	31.5	11.86b	3.82b	28.6c
Aquadulce	265	43.5	16.94a	6.67a	46.0a
Early Violletto	280	58.5	13.52b	4.98ab	21.9cd
Windsor	311	89.5	5.77c	2.13bc	15.3de
Delle Cascine	335	113.5	17.04a	5.28a	37.5b
D’Aquadulce	392	170.5	6.22c	1.81c	13.5e

†Values within a column followed by the same letter do not differ significantly (*p*< 0.05).

Table 4. 2 Seed size, pod fresh weight (PFW), seed fresh weight (SFW), and L-Dopa yield of faba bean in 2016.

Variety	**Seed size**	**Deviation from the avg. seed size (input)**	**PFW (output 1)**	**SFW (output 2)**	**L-Dopa (output 3)**
	g 100 seed^{-1}		–Mg ha^{-1}–		kg ha^{-1}
Sweet Lorane	68	□162	1.34e†	0.36d	2.8c
Early White	253	23	10.59c	2.08c	15.6b
Aquadulce	265	35	15.37a	4.35a	30.0a
Early Violletto	280	50	11.25b	3.45b	15.2b
Windsor	311	81	5.02d	1.84c	13.3b
Delle Cascine	335	105	15.50a	4.12a	29.3a
D’Aquadulce	392	162	5.44d	1.79c	13.4b

† Values within a column followed by the same letter do not differ significantly (*p*< 0.05).

Table 4. 3 Precipitation and growing degree days (GDD) during faba bean growing season in 2015, 2016, and norm (50 yr average) for the experimental site.

Month	**Precipitation**		**GDD†**		**50-yr mean**
	2015	**2016**	**2015**	**2016**	**Precipitation**
	mm				mm
April	55	50	206.7	234.1	77
May	49	74	751.9	584.2	84
June	183	50	773.5	823.1	112
July	14	82	994.0	1040.7	90

† GDD = (T_{max}+ T_{min})/2– T_{base} where $T_{base}$is 4.4°C (Etemadi et al., 2015).

Table 4. 4 Efficiency ranking of faba bean varieties using Charnes, Cooper, Rhodes (CCR) and Färe and Grosskopf (FG) models (2015).

DMU† (Variety)	**Efficiency CCR and FG models**	**Rank**	**Input weight**	**Output weights**		
			v_1	u_1	u_2	u_3
Bell Bean	0.056259	7	0.005865	0.000007	0.000020	0.000061
Sweet Lorane	0.032948	8	0.006515	0.000015	0.000005	0.000002
Early White	0.946632	2	0.031746	0.000072	0.000024	0.000008
Aquadulce	1.000000	1	0.022989	0.000052	0.000017	0.000006
Early Violletto	0.589044	3	0.017094	0.000039	0.000013	0.000004
Windsor	0.164294	5	0.011173	0.000025	0.000008	0.000003
Delle Cascine	0.376081	4	0.008811	0.000020	0.000007	0.000002
D'Aquadulce	0.090841	6	0.005865	0.000013	0.000004	0.000001

† DMU, decision-making units.

Table 4. 5 Efficiency ranking of faba bean varieties using Banker, Charnes, and Cooper (BCC) and Seiford and Thrall (ST) models (2015).

DMU† (Variety)	**Efficiency BCC and ST models**	**Rank**	**Input weight**	**Output weights**			**Free in sign variable**
			v_1	u_1	u_2	u_3	u_0
Bell Bean	0.184751	6	0.005865	0.000000	0.000000	0.000000	–0.184751
Sweet Lorane	0.205212	5	0.006515	0.000000	0.000000	0.000000	–0.205212
Early White	1.000000	1	0.031746	0.000000	0.000000	0.000000	–1.000000
Aquadulce	1.000000	1	0.022989	0.000020	0.000061	0.000020	–0.252927
Early Violletto	0.615794	2	0.017094	0.000015	0.000045	0.000015	–0.188074
Windsor	0.351955	4	0.011173	0.000000	0.000000	0.000000	–0.351955
Delle Cascine	0.376964	3	0.008811	0.000018	0.000006	0.000002	–0.047094
D'Aquadulce	0.184751	6	0.005865	0.000000	0.000000	0.000000	–0.184751

† DMU, decision-making units.

Table 4. 6 Efficiency ranking of faba bean varieties using Charnes, Cooper, Rhodes (CCR) and Färe and Grosskopf (FG) models (2016).

DMU† (Variety)	**Efficiency CCR and FG model s**	**Rank**	**Inputweight**	**Output weights**		
			v_1	u_1	u_2	u_3
Sweet Lorane	0.018692	6	0.006173	0.000004	0.000011	0.000022
Early White	1.000000	1	0.043478	0.000043	0.000014	0.000089
Aquadulce	1.000000	1	0.028571	0.000016	0.000049	0.000102
Early Violletto	0.532079	2	0.020000	0.000012	0.000035	0.000025
Windsor	0.160575	4	0.012346	0.000007	0.000021	0.000136
Delle Cascine	0.333162	3	0.009524	0.000009	0.000003	0.000034
D'Aquadulce	0.082388	5	0.006173	0.000006	0.000002	0.000068

† DMU, decision-making units.

Table 4. 7 Efficiency ranking of faba bean varieties using Banker, Charnes, and Cooper (BCC) and Seiford and Thrall (ST) models (2016).

DMU† (Variety)	**Efficiency BCC and ST models**	**Rank**	**Input weight**	**Output weights**			**Free in sign variable**
			v_1	u_1	u_2	u_3	u_0
Sweet Lorane	0.141975	5	0.006173	0.000000	0.000000	0.000000	–0.141975
Early White	1.000000	1	0.043478	0.000000	0.000000	0.000000	–1.000000
Aquadulce	1.000000	1	0.028571	0.000000	0.000000	0.000030	–0.657143
Early Violletto	0.558948	2	0.020000	0.000201	0.000602	0.000021	7.553890
Windsor	0.283951	4	0.012346	0.000000	0.000000	0.000000	–0.283951
Delle Cascine	0.333162	3	0.009524	0.000000	0.000000	0.000034	–0.219048
D'Aquadulce	0.141975	5	0.006173	0.000000	0.000000	0.000000	–0.141975

† DMU, decision-making units.

CHAPTER V
NUTRIENT ACCUMULATION IN FABA BEAN VARIETIES

Abstract

Increasing the amount of nutrients in plant-based foods will help to improve the nutritional status of people in the World. Faba bean (*Vicia faba* L.) as a staple food in developing countries has the potential to provide many nutrients in human diets. The objective of this study was to characterize the nutrient concentrations in leaf, pod, and seed of faba bean varieties. A field study was conducted with six faba bean varieties. Variation in the elemental concentrations of nutrients and protein occurred among varieties and different parts of the plants. The highest concentrations nitrogen, phosphorus, potassium, zinc, or copper and protein were in the seeds. However, the highest accumulation of calcium, magnesium, iron, or manganese was in the leaves. We recommend that faba bean be considered as a valuable crop in the diet of nutrient-deficient consumers due to high concentration nutrients in edible parts of the plant.

Introduction

Faba bean as a cool-season legume is an ancient crop of Mediterranean region and meets its nitrogen need by biological fixation (Etemadi, Hashemi, and Autio 2016; Etemadi et al. 2017c). It plays an important role in global human diet and agriculture (Jensen, Peoples, and Hauggaard-Nielsen 2010; Etemadi et al. 2016; Etemadi et al. 2017b; Etemadi et al. 2018). Faba bean is the most common legume in the diets of inhabitants of the Middle East, Mediterranean region, China, and Ethiopia and can be used as a vegetable, green or dried, fresh or canned (Bond et al. 1985). The nutritional value of faba bean is high, and in some areas of the World is superior to peas (*Pisum sativum* L.) or other grain legumes (Cre´pon et al. 2010). Globally, faba bean has been ranked as the third most important grain legume after soybean (*Glycine max* Merr.) and pea in terms of cultivated area and production (Mihailovic, Cupina, and Eric 2005; Singh et al. 2013). Faba bean is rich in proteins, complex carbohydrates, dietary fiber, choline, lecithin, minerals, and secondary metabolites (Etemadi et al. 2015; Mohseni and Golshani 2013). So, it is a rich source of nutrients in human diet, while being low in fats and sodium and cholesterol-free (Adamu et al. 2015; Gepts et al. 2008). On a global scale, common bean

(*Phaseolus vulgaris* L.) is the most important legume staple for human consumption (López-Pedrouso et al. 2012). Its high protein content makes common bean a cheap alternative protein source compared to the highly priced animal or meat-protein sources for households in developing countries (Adamu et al. 2015).

Diversification in the use of various beans, including faba and common beans, can stimulate an increased cultivation and supply of this legume. Furthermore, the increased utilization of beans in human nutrition may contribute to achieving the targeted daily dietary protein intake recommended by the World Health Organization (WHO 2007), especially in poor, developing nations. This use is essential to attaining sustainable food security in terms of nutritional quality (Jahanzad et al. 2014). Legume seeds are typically rich in micronutrients such as Fe and Zn (Khazaei et al. 2017). Micronutrient deficiency is recognized as a "hidden hunger" in the world. Iron and Zn are essential for sustenance and optimal physiological function of all life forms on the planet (Bailey et al. 2015). Nearly one-third of the population of the world is Fe-deficient, and one-fifth is Zn-deficient (de Benoist et al. 2008). Two billion people around the world suffer from micronutrient malnutrition (Gupta et al. 2016). Different methods are available today to prevent micronutrient malnutrition, including food fortification, dietary supplementation, diet diversification, and biofortification (Gupta et al. 2016). The development of biofortified crop varieties, particularly nutrient-rich food legumes, will have a positive impact in alleviating mineral malnutrition in Asian and African nations according to Gupta et al. (2016). Mineral elements play important physiological roles in plants and in the human body. The human body requires more than 22 chemical elements that can be supplied by an appropriate diet (White and Broadley 2005). Dietary deficiencies in elemental nutrients can have significant negative impacts, such as imparting learning disabilities in children, increasing morbidity and mortality, causing low worker productivity, and imparting high healthcare costs (Karaköy et al. 2012). The most common micronutrient deficiencies occur with Fe and Zn, but certain populations may suffer from deficiencies in Mg and Ca. It has been estimated that nearly 3.7 billion people worldwide are Fe deficient (60%) and that 54% of these billion people are severely deficient (Yang, Chen, and Feng 2007). Zinc deficiency ranks 11th among the 20 most important nutritional deficiencies worldwide and the 5th among the 10 most important deficiencies in developing countries (Cakmak 2008). Hotz et al. (2004) reported that Zn deficiency affects about one-third of the world population and that its incidence ranges from 4%

to 73% depending on the country (Karaköy et al. 2012). Micronutrient deficiencies mainly result from low inputs from the daily diet. The concentrations of mineral nutrients in most plant foods are not sufficient to meet daily dietary requirements when these foods are consumed in typical amounts.

Regions with Zn-deficient soils, such as India, Pakistan, China, Iran, and Turkey, are regions where human Zn deficiency is most widespread (Karaköy et al. 2012; Khan, Fuller, and Baloch 2008). Enrichment of food crops with mineral nutrients is currently a high-priority research area. Producing micronutrient-enriched cultivars (biofortification), particularly those with increased Zn and Fe either agronomically or genetically and improving the bioavailability of these minerals are considered a promising and cost-effective method to manage micronutrient deficiencies (Karaköy et al. 2012). One approach that can be used to increase the level of mineral nutrients in food crops is to identify natural variants that have favorable traits and to use these variants to develop new cultivars.

Protein malnutrition is prevalent in developing and underdeveloped countries due to the limited availability of animal protein (Raikos et al. 2014). Sourcing sufficient amounts of protein to meet future dietary requirements is a critical issue, which scientists and policymakers are currently addressing worldwide. Indeed, a recent report by the European Innovation Partnership, entitled "Agricultural Productivity and Sustainability" (Schreuder and Visser 2014) identified the need for greater plant-based protein production for animal feed and increasingly for direct human consumption. An accruing body of evidence identifies the urgent need to shift toward a more plant-based diet for environmental and physiological reasons (Clonan et al. 2015; Machovina, Feeley, and Ripple 2015). At the moment, the global population is growing rapidly, and with it, the demand for dietary protein, mainly from animal origin, is projected to increase by more than 50% by 2030 compared to that of 2000 (Westhoek et al. 2011).

The production and consumption of local food, including high-protein crops, may contribute toward achieving a sustainable diet. However, there is limited information regarding the variation in mineral concentrations among faba bean varieties. Karaköy et al. (2012) evaluated mineral concentration of a set of Turkish landraces and cultivated genotypes of lentil (*Lens culinaris* Medikus) and reported considerable variability for Fe, Zn, Cu, Ca, and Mg concentrations. Alghamdi et al. (2014) evaluated 35 advanced breeding lines in Saudi Arabia at one field location over two seasons and reported significant variation for Fe, Zn, Cu, Ca, Mg, P, K, and Mn concentrations (Gupta et al. 2016). However, these previous studies did not investigate the mineral compositions of faba bean varieties. In the present study, we examined the genetic variation in macronutrients (N, P, K, Mg, and Ca), micronutrients (Zn, Fe, Cu, and Mn), and protein to identify germplasm that could be used to improve the nutritional quality of faba bean. The objective of this study was to determine different mineral concentrations of six varieties of faba bean grown under field conditions.

Material and methods

A two-year experiment was conducted in 2015-2016 at the University of Massachusetts Amherst Agricultural Experiment Station Crops and Animal Research and Education Farm in South Deerfield (42° 28′ 37″N, 72° 36′ 2″ W). The soil type at the experimental site was a Hadley fine sandy loam (nonacid, mesic Typic Udifluvent). A soil test was taken prior to planting. Soil pH (1:1, soil/H_2O) was 6.6; cation exchange capacity (CEC) was 8.1 meq/100g, and available P, K, Ca, and Mg were in the optimum range; thus, no fertilizer was applied to the plots in either year. Seeds were inoculated with peat-based *Rhizobium leguminosarum* (Verdesian Life Sciences, Cary, NC) prior to sowing. Weather condition including growing degree days and precipitation throughout the experiment period (2015-2016) and the norm (average of 20 years) for the region are presented (Table 5.1).

Farm Experiment

Six varieties of faba bean including Early Violletto, Aquadulce, Delle Cascine, Windsor, Early White, and D'Aquadulce were selected based on the previous experiments (Etemadi et al. 2017a). Seeds were hand-planted on 15 April 2015 and 17 April 2016. The experimental design was a randomized complete block design with four replications. Research plots consisted of three rows, five-meters long and 0.76-m apart. Space between the plants within the rows was 15 cm. Three meters of the middle rows (2.28 m^2) from each plot were cut at ground level at faba bean marketable size when the leaves were still green, and pods and seeds were not dried out.

Tissue analysis for nutrients

Faba bean harvests were divided to different parts including leaf, seed, and pod walls. Parts were ground through a 30-mesh screen after drying in a forced-draft at 70º C for 48 hr. A 0.5-g portion of ground sample was put in a porcelain crucible and incinerated in a furnace at

500^{o} C for 8 hours. After incineration and cooling, 15 mL of 1 M HCl (Zandvakili et al. 2017a, b) were added to each crucible to dissolve the ash. The sample was filtered through filter paper (Whatman #1) into glass scintillation vials. Then, 1 mL of solution was transferred into a 25-mL volumetric flask, which was filled to volume with 1 M HCl. All measurements were performed by nitrogen gas plasma spectrometry (Agilent 4200 MP-AES, Agilent Technologies, Santa Clara, CA; Zandvakili et al. 2017b). Total nitrogen was measured by Kjeldahl procedures (Bremner and Mulvaney 1982).

Crude protein

About 16 % of the protein molecule is N. The protein content in this study was estimated by multiplying the determined total N concentration by 6.25 (Mirshekari et al. 2013), a universal conversion factor (Merriam-Webster Medical Dictionary, https://www.merriam-webster.com/medical).

Statistical analysis

The experimental design was a randomized complete block with three replicates of six faba bean varieties. Analysis of variance was performed using the General Linear Model (PROC GLM) of SAS version 9.3 (SAS Institute 2009). Means were separated by Fisher's protected least significant difference (LSD) or by Duncan's New Multiple Range Test (Steel and Torrie 1980).

Results and Discussion

Dry matter accumulation

Aquadulce produced the highest yield of seed with 2618 kg ha^{-1} followed by Early Violletto and Delle Cascine with 1962 and 1920 kg seed ha^{-1}, respectively (Table 5.2). Varieties with high seed production had dry pod wall weights in the same order, whereas Delle Cascine with 1020 kg leaf ha^{-1} ranked first for producing leaf mass.

The nutritive value of faba bean varieties is a function of the composition of components of the plant individually (leaf, pod wall, seed). As faba bean leaves are edible as a green vegetable in salad (Gamble 2018), it is valuable to have information about the nutrients in this plant component. Faba bean seeds are used as a cooked vegetable (Stephens 2005), and pods could be roasted, and smaller ones are edible (Brickman 2011).

Faba bean partitioning of dry matter is different among each component in different varieties. Most of the studies have focused on biomass production and the effect of different agronomic management on production (Agegnehu 2018; Chaichi et al. 2015; Gómez et al. 2017; Kubure, Raghavaiah, and Hamza 2016; Kusvuran, Parlak, and Saglamtimur 2015; Landry, Coyne, and Hu 2015; Loss and Siddique 1997; Singh et al. 2012). Very few studies considered nutritive value of various components of faba bean varieties. Our results are based on the sampling at physiological maturity, the time point that allocation to vegetative tissue ceases and accumulation of dry matter is directed toward the seeds and pods with remobilization of carbohydrates from vegetative component. This pattern has been reported for faba bean (Kumar et al. 2015) and soybean varieties (Bender, Haegele, and Below 2015).

Nitrogen and protein content

Nitrogen concentration of faba bean varieties differed among the components at maturity with the order of seed>pod wall>leaf. Overall, N concentration was the highest for seed with 42% and 48% more N than leaf and pod wall, respectively (Table 5.3). However, no significant difference occurred between leaf and pod wall. Nitrogen concentration was highest in seeds regardless of type of faba bean varieties, but the highest concentration of N was obtained with Windsor with 3.47% followed by Delle Cascine with 3.43%, and the lowest N concentration in seed was with Early Violletto with 2.71% (Table 5.3). Higher accumulation of N in seeds than in other parts is expected since N is mobilized from the leaf, pod wall, and other vegetative tissues to the seeds during the seed-filling period. Our results support the nutrient-drain hypothesis of Sesay and Shibles (1980) that states that the developing reproductive structures drain sources from vegetative parts (Nooden 1980). In fact, the high demand for N during seed growth exceeds the estimated maximum rate of N uptake. Aquadulce had the highest leaf concentration of N with 2.01%, and Delle Casine had the lowest N concertation with 1.53% (Table 5.3). High N demand of seeds has been reported previously for soybean with between 68%-86% of total N accumulation partitioned to the seed depending on the cultivar and yield level (Hanway and Weber 1971; Pazdernik, Graham, and Orf 1997; Bender, Haegele, and Below 2015). Protein concentration was calculated from total N concentration. In general, concentration of protein was the highest to lowest for seed, leaf, and pod wall (Table 5.3). Protein concertation was highest in seeds regardless of type of faba bean varieties. The highest concentration of protein obtained was with Windsor with 21.69% and the lowest protein concentration was obtained with Early Violletto with 16.94% (Table 5.3).

Phosphorus and potassium contents

On the whole, the P among plant parts differed in the order of seed> pod wall> leaf (Table 5.4). Our results showed that the highest P concentration obtained in seed was 0.76% with 40% and 52% higher value than with pod wall and leaf, respectively. Similar to N mobilization, P stored in leaves and pods is mobilized (Bender, Haegele, and Below 2015; Hanway and Weber 1971). The highest P concentration was 0.79% in seeds of D'Aquadulce, and the lowest was 0.30% in leaves of Delle Cascine (Table 5.4). The difference in P accumulation among plant organs was much more pronounced that the difference among varieties. In general, there was a significant difference in P concentrations among the plant components with the order of pod wall > seed=leaf (Table 5.4). Unlike N and P, which had the highest concentration in the seed part of all varieties, the highest K concertation was in pod wall with 38% and 44% higher values than seed and leaves, respectively (Table 5.4). The highest accumulation of K was with Aquadulce with 1.89% in the seed component, and the lowest accumulation was with Delle Cascine at 1.22% in the leaf component. In our study unlike N and P, K was partitioned equally distributed between the vegetative organs leaf and the seed. This partitioning is indicative of the lack of mobilization of K into the reproductive seed component. In Mediterranean diets, pod is an edible part and is considered as nutrient source when eaten with immature seeds (Sing et al. 2013).

Calcium and Magnesium Contents

Unlike N, P, and K, for which the lowest accumulation was obtained in the leaf parts of faba bean varieties the Ca concentration in the leaf was more than three-fold and eight-fold greater than the Ca concertation in the pod wall and seed, respectively (Table 5.4). The highest accumulation of Ca was with Delle Cascine with 2.15% in the leaf, and the lowest accumulation was with the varieties Aquadulce and D'Aquadulce with 0.19% in the seed component. Similar to Ca the highest concentration of Mg was in the order of leaf > pod wall > seed (Table 5.4). The difference between plant organs on Mg accumulation was more pronounced that the difference among the varieties. The Mg concentration in the leaf component was more than two-fold the seed components (Table 5.4). Calcium in an immobile element and Mg is mobile; however, the similar pattern of partitioning of these nutrients in plants organs may indicate that mobilization of Ca and Mg from vegetative plant parts is minimal. The higher Ca and Mg in the leaf tissue can be important from the nutritional point of view for human diet if the leaves are eaten. The low Ca concentration of the seed has been associated with poor germination and weak seedlings in legumes (Keiser and Mullen 1993).

Iron, Copper, Zinc, and Manganese Contents

The concertation of Fe in different organs of faba bean was significantly different and in the order of leaf > pod > seed (Table 5.5). The accumulation of Fe in the leaf component of the plants was 8.7-fold and 3.2-fold of the accumulation in seed and pod wall, respectively. Iron deficiency is one of the biggest problems with regard to nutrition in developing countries. Generally, legumes are considered as a major source for essential elements, such as Fe and Zn. However, the high amount of Fe concentration in leaves of all faba bean varieties may be important in human diets. This plan, however, should be practiced cautiously since it is reported that some legumes can contain high amounts of Fe binding polyphenols that inhibit Fe absorption (Sandberg, 2002). The highest and lowest Fe concentration was with Delle Cascine at 804 mg kg^{-1} in the leaf component, and the lowest concentration was in seeds of Early Violletto with 59 mg kg^{-1}. Mahmoud et al. (2006) reported a similar result on the accumulation of Fe in the leaf component of faba bean. There is a paucity of reliable data on the mineral composition of different in different organs of faba bean. However, our results reveal that faba bean could be considered as a potential source to alleviate Fe deficiency. Our results revealed a significant difference among plant organs on Zn accumulation in the order of seed > leaf > pod wall. The concentration of Zn in the seed was 3 and 2.5-fold of pod wall and leaf, respectively. Faba bean can be a good source of Zn and Fe in human diets. Concentration of Cu in our study was significantly different among plant organs and in the order of seed=leaf > pod. The highest accumulation was with D'Aquadulce at 24 mg kg^{-1} in the leaf, and the lowest was with Early Violletto at 12 mg kg^{-1} ppm in the pod wall. We conclude that faba bean leaves can be a good source for Cu in human diets. However, unlike Cu and similar to Mg, Ca, and Fe the accumulation of Mn was in order of leaf > pod > seed. Manganese accumulation in the leaf was five-fold and two-fold of seed and pod wall, respectively.

Conclusion

In summary, considerable variation in the macro- and micronutrient contents of faba bean varieties and in plant organs was identified. Our results provide a useful foundation for the development of new cultivars of faba bean that have high mineral content. Identification of genetic variation is essential for achieving improvements in the mineral content of crops. Faba bean is an integral part of people's diets in many countries in Asia, including Bangladesh, Nepal, India, and Pakistan. People living in these areas are affected with mineral deficiencies, particularly iron deficiency anemia. Biofortification of minerals in faba bean will have a positive impact on people's health in these mineral-deficient areas. This study reports on the mineral status of different parts of faba bean varieties of micro- and macro-nutrients. This information suggests that the high concentration in leaves of all faba bean varieties may be further considered for improving the nutritional status. Faba bean can be a good source of mineral nutrients, especially Zn and Fe, in human diets.

Table 5. 1 Precipitation and temperature during faba bean growing seasons of 2015 and 2016 and norm (20-year mean) for the experimental site

Month	Precipitation, mm			Temperature, ^{o}C		
	2015	2016	20-year mean	2015	2016	20-year mean
April	51	53	80	7.6	7.4	7.7
May	26	65	88	15.5	14.2	13.9
June	192	34	116	17.9	19.0	18.6
July	84	43	98	21.0	22.3	21.4

Table 5. 2 Leaf, pod wall, and seed dry matter accumulation of faba bean varieties (average of two years)

Variety	Dry matter production, kg ha^{-1}		
	Leaf	Pod wall	Seed
Windsor	519d†	817e	876d
Early White	890b	956d	1085c
Early Violletto	755c	1839b	1963b
Delle Cascine	1020a	1668c	1920b
Aquadulce	873b	2315a	2618a
D'Aquadulce	486d	700e	803d

†Means followed by the same letter within a column do not differ significantly by Duncan's New Multiple Range Test (*P*= 0.05).

Table 5. 3 Total nitrogen and crude protein concentrations of leaves, pod walls, and seeds of faba bean varieties (average of two years)

Plant part	Variety of faba bean						
	Windsor	Aquadulce	D'Aquadulce	Early White	Early Violletto	Delle Cascine	Mean†
			Total N, % dry wt				
Leaf	1.76	2.01	1.81	1.99	1.72	1.53	1.80b
Podwall	1.79	1.62	1.73	1.38	1.47	1.49	1.60b
Seed	3.47	3.11	3.06	2.81	2.71	3.43	3.10a
			Crude protein, % dry wt‡				
Leaf	11.00	12.56	11.31	12.44	10.75	9.56	11.27b
Podwall	11.19	10.13	10.81	8.63	9.19	9.31	9.88c
Seed	21.69	19.44	19.13	17.56	16.94	21.44	19.36a

†Means followed by the same letters within columns of Total N or Crude Protein do not differ significantly by Duncan's New Multiple Range test (*P*=0.05). LSD (*P*=0.05) for interaction is 0.70 for Total N and 2.89 for Crude protein.
‡Crude protein is 6.25 x % total N.

Table 5. 4 Leaf, pod wall, and seed concentrations of P, K, Ca, and Mg of faba bean varieties (average of two years)

Plant part	Windsor	Aquadulce	D'Aquadulce	Early White	Early Violletto	Delle Cascine	Mean
			P, % dry wt				
Leaf	0.34	0.44	0.39	0.37	0.31	0.30	0.36c
Pod wall	0.40	0.51	0.45	0.49	0.30	0.54	0.45b
Seed	0.68	0.75	0.79	0.73	0.71	0.79	0.76a
			K, % dry wt				
Leaf	2.09	1.73	1.59	1.62	1.46	1.22	1.62b
Pod wall	3.05	3.67	2.44	2.51	3.12	2.54	2.89a
Seed	1.78	1.88	1.65	1.86	1.67	1.85	1.78b
			Ca, % dry wt				
Leaf	0.64	1.53	1.91	1.49	1.68	2.15	1.56a
Pod wall	0.44	0.53	0.45	0.59	0.50	0.51	0.50b
Seed	0.21	0.19	0.19	0.21	0.16	0.24	0.19c
			Mg, % dry wt				
Leaf	0.51	0.40	0.56	0.47	0.42	0.47	0.47a
Pod wall	0.51	0.40	0.56	0.47	0.42	0.47	0.47a
Seed	0.23	0.22	0.19	0.21	0.18	0.20	0.20c

*Means of elements followed by the same letter within a column do not differ significantly by Duncan's New Multiple Range Test (*P*=0.05). LSD (*P*=0.05) are for K interaction, 0.92; Mg interaction, 0.14; P interaction, 0.15; Ca interaction, 0.92

Table 5. 5 Leaf, pod wall, and seed Fe, Cu, B, Mn, and Zn dry weight concentrations of faba bean varieties (average of two years)

Plant part	Windsor	Aquadulce	D'Aquadulce	Early White	Early Violletto	Delle Cascine	Mean
			Fe, mg kg^{-1}				
Leaf	642	478	800	508	492	804	621a
Pod wall	221	190	156	153	230	219	195b
Seed	63	70	80	72	59	83	71c
			Cu, mg kg^{-1}				
Leaf	20	14	24	19	19	26	20a
Pod wall	13	14	13	14	12	18	14b
Seed	21	20	18	20	17	23	20a
			B, mg kg^{-1}				
Leaf	22	26	26	24	22	25	24b
Pod wall	31	32	34	32	30	34	32a
Seed	23	26	26	23	23	25	25b
			Mn, mg kg^{-1}				
Leaf	62	54	81	72	67	76	68a
Pod wall	30	31	25	36	39	33	32b
Seed	18	15	14	17	15	18	16c
			Zn, mg kg^{-1}				
Leaf	15	19	25	15	13	11	16b
Pod wall	10	14	20	15	18	14	13c
Seed	36	45	41	43	29	45	40a

*Means followed by the same letter within a column do not differ significantly by Duncan's New Multiple Range Test (P= 0.05). LSD (P=0.05) Fe interaction, 276; for Cu interaction, 7; for B interaction, 2; for Mn interaction, 21; for Zn interaction, 11

CHAPTER VI

YIELD AND ACCUMULATION TREND OF BIOMASS AND L-DOPA IN DIFFERENT PARTS OF EIGHT FABA BEAN VARIETIES

Abstract

Faba bean (*Vicia faba* L.) is a rich source of 3,4-dihydroxyphenyl-L-alanine (L-Dopa) and thus can be used as an alternative to synthetic compounds for curing Parkinson's disease. Accumulation of biomass and L-Dopa in different organs of eight cultivars of faba bean was investigated in a field-grown study in 2015 and 2016 at the Research and Education Center, University of Massachusetts, Amherst. Plants were harvested at five stages of growth and partitioned into various organs including roots, leaves, stems, pod walls, and fresh seeds. 'Aquadulce' and 'Delle Cascine' outyielded other cultivars in both years. Averaged over 2 yr, Aquadulce and Delle Cascine produced 16,152 and 16,270 kg fresh pods ha^{-1}. Thus, the highest L-Dopa seed yield was obtained from these two cultivars. Accumulation of L-Dopa during faba bean growing period exhibited a quadratic trend and reached its peak values at 65 to 75 d after transplanting, whereas accumulation of dry matter exhibited a linear trend. The results revealed that distribution of dry matter among various organs of faba bean was significantly different among the cultivars. The L-Dopa yield (concentration × dry matter) varied dramatically among the cultivars where Aquadulce and 'Sweet Lorane' yielded 55.2 and 6.4 kg L-Dopa ha^{-1}, respectively. The superiority of Aquadulce in regard to the L-Dopa yield was primarily due to its photosynthesis capacity, which produced 6871 kg dry matter ha^{-1}, rather than its L-Dopa concentration. We concluded that if the goal of producing faba bean is dual purpose, the leaves can be used as the richest L-Dopa organ, whereas the green pods can be harvested as a cash crop.

Introduction

Faba bean (*Vicia faba* L.) is a cool-season legume crop and has the potential to be grown as a new multi-purpose vegetable crop in the Northeastern U.S. (Etemadi et al. 2015a; Hu et al. 2015, Etemadi et al. 2017c). Globally, faba bean has been ranked as third important grain legumes after soybean (*Glycine max* L.) and pea (*Pisum sativum* L.) in terms of cultivated area and production (Mihailovic et al. 2005; Singh et al. 2013). Faba bean is rich in various nutrients and an excellent source of protein, complex carbohydrates, dietary fiber, choline, lecithin, minerals and secondary metabolites including phenolic compounds (Mohseni and Golshani 2013). Additionally, faba bean has been identified as a medicinal plant, given that it accumulates a large amount of L-Dopa in its various parts. (Etemadi et al. 2015c; Holden, 2016, Etemadi et al. 2017 b). Therefore, faba bean has the potential to be grown as a functional food crop.

Although faba bean is a cool-season crop and can be sown as early as mid-March in Northeast, in many years the soil in early spring is often wet and sometimes still covered with snow which delays direct seeding method. Transplanting faba bean as an alternative to direct seeding was explored successfully and offered some potential benefits (Etemadi et al. 2018):

1- Early planting would not be limited by soil condition.
2- Drip irrigation and/or fertigation can be employed which minimizes the negative impact from prolonged drought incidents.
3- It provides an opportunity for earlier harvesting for more successful double cropping.
4- Flowering which is the most sensitive growth stage to the incident of chocolate spot disease takes place earlier.
5- It avoids planter issues related to significant seed size variability.

L-Dopa (3,4-dihydroxyphenyl-L-alanine) is a precursor of dopamine currently used as a major ingredient in treating Parkinson's disease (PD) and hormonal imbalance (Ali et al., 2005; Rani et al., 2007; Surwase et al., 2012; Inamdar et al., 2013; Hu et al., 2015). In the 1960s, major improvement in PD was made when researchers identified the low level of dopamine in the brain of diseased patients, which led to the development of L-Dopa (Davie, 2008). L-Dopa is an amino acid naturally isolated from various legumes (Chattopadhyay et al., 1994; Inamdar et al., 2013) and other plants such as banana (*Musa* spp.; Bapat et al., 2000; Rani et al., 2007) in various forms (Gautam et al., 2012). It is synthesized from the amino acid L-tyrosine in the mammalian body and brain (Randhir and Shetty, 2004; Haq and Ali, 2006; Miller et al., 2009). Earlier reports (Nagatsu et al., 1964; Peaston and Weinkove, 2004; Miller et al., 2009) indicated that L-Dopa is an important precursor for the various neurotransmitters like dopamine (Upadhyay et al. 2012), noradrenaline and adrenaline collectively known as catecholamines (Gautam et al. 2012). Commonly, PD patients are treated with synthesized L-Dopa. Synthesized L-Dopa is expensive and often causes a variety of side effects including nausea, vomiting, low blood pressure, drowsiness, and restlessness (Lee et al., 1996; Knowles, 2003; Patil et al., 2013). Therefore, consuming natural sources of L-Dopa to avert potential side effects has been recommended (Singh et al., 2012; Patil et al., 2013). It is estimated that the annual world demand for L-Dopa is ~250 metric tons (Katayama and Kumagai 2010), with a market value of ~ US$101 billion (Koyanagari et al., 2005; Inamdar et al., 2013; Patil et al., 2013). Cultivation of crops that are rich in natural L-Dopa to overcome side effects and the high cost of production of synthetic L-Dopa thus seems justifiable (Singh et al., 2012; Patil et al., 2013). Faba bean is ranked after velvet bean [*Mucuna pruriens* (L.) DC.] in terms of L-Dopa content (Soares et al. 2014). The amount of L-Dopa in faba bean, however, is dependent on genotypes (Lorenzetti et al., 1998; St. Laurent et al., 2002; Etemadi et al., 2017a), environmental conditions (Goyoaga et al., 2008; Multari et al., 2015), plant growth stage (Geng, 2012), and organs (Etemadi et al., 2018a). Cao (2010) reported a large variation of L-Dopa content among faba bean genotypes, including flowers across 197 cultivars, seedlings across 32 cultivars, and seeds across 52 cultivars with the green seed coat. Similarly, Hu et al. (2015) reported a significant variation in L-Dopa concentration among six faba bean lines differing in flower color. Distribution of L-Dopa in various parts of faba bean is not uniform, and different organs accumulate L-Dopa at different rates (Geng, 2012; Etemadi et al., 2015d). However, many earlier reports have been focused on L-Dopa content in seeds, whether as fresh or dried, and less attention has been given to the amount of L-Dopa in other parts of the plant. In general, L-Dopa concentration in various faba bean organs reaches its peak concentration at early stages of growth (Etemadi et al., 2014). However, L-Dopa yield is a product of concentration and plant biomass (Etemadi et al., 2018a), and thus the highest L-Dopa yield can be obtained at later stages of growth. The main objective of this study was to determine the L-Dopa concentration and distribution among various parts of eight cultivars of faba bean at different developmental stages. This information is helpful to researchers and healthcare providers who study the medicinal values of faba bean.

Materials and Methods

Experimental site and weather conditions

A 2-year field study was conducted at the University of Massachusetts Amherst Agricultural Experiment Station, Crops and Animal Research and Education Farm in South Deerfield (42° 28′ 37″N, 72° 36′ 2″ W) in 2015 and 2016. The soil type at the experimental site was a Hadley fine sandy loam (nonacid, mesic Typic Udifluvent).

Treatments and Measurements

Seeds of eight faba bean varieties including Windsor, Aquadulce, D'Aquadulce, Delle Cascine, Sweet Lorane, Early White, Early Violletto, and Bell bean were inoculated with peat base *Rhizobium Leguminosarum* (*VERDESIAN, N. DURE, Carry, NC*) and grown in greenhouse condition on April 1st in 2015 and April 5th in 2016, where temperature was set at 15^{0}C. The seeding trays were 25 × 50 cm, filled with potting soils and kept for eight days in the greenhouse condition prior to being transplanted to the main research plots. Some morphological characteristics and susceptibility to chocolate spot disease of varieties used in this experiment are presented in Table 6.1.

Chocolate spot disease is the major disease of faba bean in New England when flowering stage coincides with high temperatures in summer. Faba bean seedlings were transplanted on April 8th and April 12th in 2015 and 2016, respectively. In both years, composite soil samples (0-0.2 m depth) taken prior to planting indicated soil pH (1:1, soil/H_2O) was 6.6, cation exchange capacity (CEC) was 8.1 meq/100g, and available P and K were all in the optimum range for faba bean thus no fertilizer was applied to the experimental plots. Weather conditions including precipitation throughout the experiment period (2015 and 2016) and the norm (average of 20 years) for the experimental site are presented in Table 6.2. No irrigation was used in this experiment as irrigation is not a common practice for the field crops in Massachusetts. The experiment was laid out as complete randomized block design with three replications. Weeds were controlled mechanically three times prior to the canopy closure. Research plots consisted of six rows, eight meters long and 0.76 m apart. Space between the plants within the rows was 15 cm. Two meters from rows 2 and 5 (1.5 m^2) from each plot were harvested at five growth stages including; seedling (three and six leaf stages), flowering and pod emerging, pod ripening and physiological maturity. At each harvest, crops were dogged out from the soil with extra care, washed and divided into various parts including roots, stems, leaves, flowers, pod walls, and seeds (when existed). Plant parts were dried separately in an air-forced oven at 50^{0}C for 36h. Dried samples were ground fine to pass through a 0.42 mm screen and prepared for extraction. At physiological maturity, pods were harvested from 3 m^2 using rows 3 and 4 to determine pods and seeds fresh yield.

Extraction Method

The procedure for the extraction of L-Dopa has been described fully in our recent publication (Etemadi *et al.* 2017*b*). 200 mg of dried samples immersed in 95% ethanol and kept in the freezer for 72 hrs. Samples then homogenized using a BioHomogenizer (BIOSPEC PRODUCTS, Bartlesville, OK). Samples centrifuged at 13,000 rpm for 10 min. The liquid portion left under the hood until ethanol was evaporated. The residue dissolved in buffer solution (32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM EDTA, 0.215 mM octyl sulfate pH 4) and left in the fridge until particles settled. The supernatant was passed through a 0.45 µm syringe filter for analysis by HPLC (LC 2010A, Shimadzu Prominence HPLC with DAD detector (SPD-M20A Photodiode Array Detector, Shimadzu, Japan, Tokyo), Phenomenex Gemini C18 column).

Standard solution

A stock solution of L-Dopa was prepared by dissolving 25 mg of L-Dopa (SIGMA-ALDRICH, INC., WI) in 25 ml of 0.1N HCl in a volumetric flask. Standard solutions (50 to 400 mg L^{-1}) were used for diluting the stock solution with 0.1mol HCl L^{-1}.

HPLC Procedure

L-Dopa was analyzed by HPLC equipped with an auto-sampler and diode array detector. Data were acquired and processed by an LC Solution Data System (Shimadzu, Japan). Chromatographic separation was performed on a Phenomenex C18 column (250 mm × 4.6 mm, 5 µm) (Phenomenex, CA, USA) with an isocratic flow of 1 mL min^{-1}. The mobile phase was 0.1% acetic acid (98%) and methanol (2%). The mobile phase was filtered through 0.2 µm filters and degassed by sonication for 30 min. Injection volume was 20 µL and L-Dopa detection was at 283 nm.

Calibration Curve

Four concentrations of the stock solution after dilution (50, 100, 200, 400 ppm) with mobile phase were injected in triplicates. The calibration curve was obtained by plotting the absorbance area versus the concentrations of the standard solution. A linearity graph of control solution of L-Dopa is shown in Fig. 6.1.

Statistical analysis

Data were processed by ANOVA (Steel and Torrie, 1980; SAS Institute, 2003). When the *F* test was significant, Fisher's LSD was used for mean separations. Regression analysis method was performed to determine the dates after planting for reaching the peak values of L-Dopa. To determine confidence interval, the vertices of the quadratic functions from the regression fit

of the generated dataset were calculated through bootstrapping (DeSousa, 2006). Since the residual followed a normal distribution, prediction interval was calculated with a significance of 95% for the desired statistics.

Results

Weather

Cumulative growing degree days at Orange Airport, MA (roughly 27 km away from the research site), during the growing period of faba bean (April–July) were 2483 and 2457°C d in 2015 and 2016, respectively, which were comparable with the norm for this location (Table 6.2). However, in both years, faba bean suffered from some level of drought stress, especially during the first 8 wk after transplanting. The amount of precipitation in these 8 wk was 54 and 30% lower than the norm for this location in 2015 and 2016, respectively. As a result, in both years, total dry matter produced at the time of harvest was lower than for other experiments conducted at the same location (e.g., Etemadi et al., 2018a). We found no statistical significance for the interaction of year by dry matter and L-Dopa concentration; therefore, an average of the 2 yr is presented.

Dry matter accumulation

Dry matter accumulation trends of the faba bean varieties used in this study are shown in Table 6.3. Overall, the whole plant dry matter accumulation during faba bean growing period followed a quadratic trend. The highest dry matter (6871 kg ha^{-1}) was accumulated by Aquadulce, 95 days after planting when the pods were ready to harvest. The results were consistent with our earlier report (Etemadi et. al, 2017a) where Aquadulce produced the highest seed dry yield (2755 kg ha^{-1}) among the tested varieties. To our surprise, Sweet Lorane which is a late maturity cultivar produced the lowest dry matter yield (829 kg ha^{-1}) despite being harvested 135 days after transplanting. Photosynthesis and biomass production of Sweet Lorane thus requires further investigation. Distribution of dry matter among various organs of faba bean was significantly different amongst the varieties. For example, Sweet Lorane followed by Early White were the leafiest varieties and accumulated 27.9 and 22.9 percent of their final dry matter in leaves (Table 6.4). On the other hand, 38.1 and 37.2 percent of dry matter were accumulated in seeds of Aquadulce and Bell bean, respectively. Excluding Sweet Lorane which performed poorly in terms of pod production, the contribution of pods (pod wall plus seeds) to the total dry matter ranged from 53-72% in tested varieties at harvest time (Table 6.4).

L-Dopa accumulation

Accumulation of L-Dopa during the growing period exhibited a quadratic trend in all varieties (Table 6.5) with the peak values about 65-75 days after transplanting (excluding Sweet Lorane) (Figure 6.2).

There was a significant difference in the maximum accumulated L-Dopa concentrations among the tested varieties. Using regression model (Mirshekari *et al.* 2013), the highest and lowest L-Dopa were estimated as high as 62.0 mg g^{-1} and 43.0 mg g^{-1} in Bell bean and Early Violletto, respectively (Table 6.5).

Partitioning of L-Dopa in various organs of faba bean varieties over time is shown in Fig. 6.3. The highest L-Dopa concentration in all varieties was extracted from leaves followed by pod walls, seeds, roots, and stems, respectively. Accumulation of L-Dopa in various parts of faba bean was not exactly similar to the accumulation of dry matter throughout the entire growing season. Dry matter production at the onset of first pod formation exceeded the L-Dopa accumulation, thus L-Dopa content (mg) per g dry matter in all plant parts exhibited a declining trend (Fig. 6.3, a-h).

Biomass and fresh seed L-Dopa yield

The L-Dopa yield of faba bean cultivars varied dramatically. For example, Aquadulce produced over eightfold more L-Dopa than Sweet Lorane. The L-Dopa yield of each plant organ is the product of its dry matter by L-Dopa concentration. The L-Dopa yield of the faba bean cultivars at each sampling time is presented in Table 6.6. Although dry matter yield in all cultivars increased as growing season proceeded, the highest L-Dopa yield was obtained 65 to 75 d after planting, excluding Sweet Lorane due to its late maturation. This response was primarily due to the declining trend in L-Dopa concentration in all plant parts in later stages of growth. The results obtained in this study revealed that greater accumulation of biomass in late stages of growth can be compromised by the lower L-Dopa concentration. The highest L-Dopa yield (55.2 kg ha^{-1}) was obtained from Aquadulce. Pod fresh weight and seed fresh weight of faba bean cultivars are presented in Table 6.7. Aquadulce and Delle Cascine outyielded other cultivars. Windsor is currently the common cultivar available to the growers in US Northeast. Results from the current study revealed that L-Dopa seed yield ranged from 3.4 to 38 kg ha^{-1}, and Windsor performed relatively poorly in terms of L-Dopa yield (Table 6.7). The highest L-Dopa seed yield (38.0 kg ha^{-1}) was obtained from Aquadulce, followed by Delle Cascine, which yielded 33.4 kg ha^{-1} (Table 6.7).

Discussion

L-Dopa yield of various faba bean organs is the product of their dry matter by L-Dopa concentration (Etemadi et al. 2017 a). Faba bean varieties evaluated in the current study not only exhibited dramatic differences in biomass but also differed significantly in distributing the assimilates among their organs. Although fresh pods of faba bean are not the richest part of the plant in terms of L-Dopa concentration, averaged over all varieties, 60% of dry matter was accumulated in pod walls and fresh seeds (Fig. 6.4). Therefore, varieties with highest seed weight more likely produce higher L-Dopa yield.

In this study, Bell bean accumulated the highest L-Dopa concentration but Aquadulce produced the highest L-Dopa seed yield amongst tested varieties. Results obtained in the current study revealed that pod walls are even richer source of L-Dopa compared to the fresh seeds (Fig. 6.3). This information is especially important if faba bean is grown as cash/medicinal dual-purpose crop. Although mature faba bean pods are considered as nutritious fruit, if the main goal of growing faba bean is to obtain L-Dopa, it is recommended to harvest plants around early stages of pod formation when L-Dopa yield is maximized. On the other hand, if the goal is dual purpose use of faba bean, leaves can be used as the richest L-Dopa organ while the green pods can be harvested as a cash crop.

Accumulation of L-Dopa in faba bean plants was not quite similar to accumulation of dry matter. Excluding Aquadulce and Sweet Lorane, L-Dopa of faba bean varieties reached their peak values about 75 days after planting (Fig. 6.2 and 6.3). Unlike L-Dopa, dry matter accumulation followed a linear trend in all varieties (Fig. 6.5).

The L-Dopa content of faba bean in each individual organ as well as whole plants at final harvest was clearly lower than their peak concentrations. Similarly, Wang et al. (2017) indicated that the pattern of dry matter accumulation is different than the accumulation of active components. The maximum concentration of L-Dopa (62 mg g^{-1}) was extracted from Bell bean which was comparable to the values reported by Hu et al. (2015) who measured 57 mg L-Dopa per gram dry matter of faba bean. The reduction of L-Dopa between its peak concentration and harvest time indicates that during this period a significant amount of the L-Dopa had been transformed into other compounds, possibly in addition to the slower or halt of its biosynthesis. Results confirmed the earlier conclusion made by Teramoto and Komamine (1988) that L-Dopa is at its peak level in young parts of plants compared with mature organs.

Exogenous doses of synthetic L-Dopa are often associated with a variety of chronic side effects (Shetty et al. 2001). Therefore, search for a naturally rich source is becoming more imminent. Currently published studies that have evaluated L-Dopa content of faba bean were mainly focused on fresh and dry seeds (Longo et al. 1974; Shetty et al. 2001; Randhir and Shetty 2004). However, Mohseni and Golshani (2013) indicated that in the management of PD, consumption of large quantities of un-sprouted faba bean seeds may cause flatulence in the patients. Recently we reported that leaves were the richest L-Dopa organ of faba bean followed by flowers, young pods, mature seeds, roots, and stems, respectively (Etemadi et al. 2017b). Synthetic compounds contain between 100-250 mg L-Dopa (rxlist, SINEMET Drug Info.). Results from the current study indicated that on the average 2-4.5 g dry matter of Aquadulce which produced the highest biomass and L-Dopa yield contained the corresponding amount of L-Dopa compared with the synthetic product.

Conclusion

The results of the current experiment suggest that although faba bean is a rich source of natural L-Dopa but to maximize the optimum L-Dopa yield the crop should be harvested prior to the physiological maturity, roughly 70 days after transplanting. If the goal is dual purpose use of faba bean, leaves can be harvested as a rich source of L-Dopa whereas green pods can be harvested as a cash crop. We concluded that the amount of dry matter produced by faba bean is considerable enough to be grown as a viable source of natural L-dopa compared to the synthetic sources.

Table 6. 1 Select morphological characteristics and susceptibility to chocolate spot disease of faba bean varieties used in this experiment **(**Etemadi et. al., 2015b).

Variety	**Seed size**	**Seed color**	**Flowering time**	**Maturity**	**Chocolate spot**
Aquadulce	Large	Light green	early	early	MS
Bell bean	small	Light brown	mid	mid	MRMS
D'Aquadulce	Large	Dark green	mid	early	MS
Delle Cascine	Large	Light green	early	early	MS
Early Violletto	Med-large	purple	early	early	S
Early White	Med-large	green	late	early	MS
Sweet Lorane	Small	Light brown	late	late	MRMS
Windsor	Large	Light green	Early	early	MRMS

R = Resistant
MR = moderately resistant
MRMS = moderately resistant to moderately susceptible MS = moderately susceptible
MSS = moderately susceptible to susceptible S = Susceptible

Table 6. 2 Precipitation and GDD during faba bean growing season in 2015, 2016 and the norm (20 years average) for the experimental site

Month	**Precipitation (mm)**			**GDD†**		
	2015	**2016**	**20-yr mean**	**2015**	**2016**	**20-yr mean**
April	51	53	80	157	184	204
May	26	65	88	674	529	515
June	192	34	116	714	764	767
July	84	43	98	938	980	942
Total	**353**	**195**	**382**	**2483**	**2457**	**2428**

†GDD = (Tmax+ Tmin)/2 – Tbase where Tbase is 4.4^{o}C

Table 6. 3 Dry matter (kg ha^{-1}) accumulation trend of faba bean varieties during growth period average over two years

Varieties	**Dry matter accumulation in days after planting**								
	45	**55**	**65**	**75**	**95**	**115**	**135**	**Trend/Equation**	**R^2**
Aquadulce	643.0±17.5	1354.8±59.3	3669.5±62.2	5002.5±69.7	6870.9±64.0			**Q=-0.7899t^2+242.76t-8977.0	0.98
Bell bean	354.8±12.3	405.0±14.2	654.0±17.8	1240.0±47.7	2217.8±38.2			**Q=0.5816t^2-42.675t+1050.6	0.99
D'Aquadulce	610.7±24.1	762.4±23.2	1284.8±59.8	1930.0±59.6	2573.3±30.8			**L=42.121t-1389.9	0.97
Delle Cascine	637.0±28.5	1263.0±59.7	3381.0±61.0	4140.0±42.5	5730.8±55.7			**Q=-0.8093t^2+220.49t-7869.5	0.97
Early Violletto	636.0±15.8	1121.0±26.5	2378.5±52.6	3208.3±51.4	5392.7±42.1			**L=97.608t-3992.5	0.99
Early White	614.0±17.6	820.0±57.4	2166.0±52.0	2773.0±61.5	3887.3±51.9			**Q=-0.2504t^2+105.09t-3790.7	0.97
Sweet Lorane	86.7±1.70	223.2±13.3	296.6±13.1	397.1±14.6	493.3±15.3	710.0±18.4	829.0±19.6	**Q=-0.0149t^2+10.686t-341.5	0.99
Windsor	403.0±14.7	680.0±27.0	1445.0±58.1	1865.0±29.0	2808.0±25.8			**L=49.749t-1893.0	0.99
V*ST†								**	

†V, ST = variety and sampling time, respectively.

*Q and **Q are significant at P ≤0.05 and P ≤0.01, respectively. Values are mean ± standard error.

Table 6. 4 Distribution of dry matter in various organs of faba bean varieties as % of the total dry matter at physiological maturity harvest. Values are average of two years

Variety	Dry matter (kg ha^{-1})	Root	Stem	Leaf	Pod wall	Seed
Aquadulce	*6870.9±64.0	4.5±0.3	11.0±1.3	12.7±1.1	33.7±2.5	38.1±2.9
Bell bean	2217.8±38.2	6.1±0.2	10.4±1.1	15.3±1.4	31.0±2.0	37.2±2.8
D'Aquadulce	2573.3±30.8	7.6±1.2	15.1±1.3	18.9±1.6	27.2±2.1	31.2±2.1
Delle Cascine	5730.8±55.7	3.9±0.5	15.7±1.4	17.8±1.5	29.1±1.8	33.5±2.4
Early Violletto	5392.7±42.1	3.5±0.5	12.0±1.0	14.0±1.3	34.1±2.1	36.4±2.8
Early White	3887.3±51.9	3.4±0.4	21.2±1.8	22.9±2.0	24.6±2.0	27.9±2.1
Sweet Lorane	829.0±19.6	9.9±1.3	24.5±2.2	27.1±2.3	18.4±1.1	20.1±1.7
Windsor	2808.0±25.8	4.1±0.2	17.1±1.2	18.5±1.7	29.1±2.3	31.2±2.4
Average	**3774.2±61.7**	**5.4±1.2**	**15.9±1.8**	**18.4±1.9**	**28.5±1.9**	**31.8±2.3**

Values are mean ± standard error.

Table 6. 5 L-Dopa concentration (mg g^{-1}) of faba bean varieties during growth period. Values are average of two years

Varieties	**L-Dopa Conc. measured in days after planting**								
	45	55	65	75	95	115	135	Equation	R^2
Aquadulce	8.3±1.2	31.4±2.5	55.1±3.2	52.9±3.3	27.3±1.7			*Q=-0.0593t^2+8.7059t-264.35	0.98
Bell bean	13.3±1.2	38.1±2.6	51.1±3.0	62.0±3.2	30.1±1.2			**Q=-0.0578t^2+8.4711t-251.96	0.98
D'Aquadulce	13.0±1.6	36.5±2.7	54.5±3.3	52.3±3.3	28.7±1.3			**Q=-0.0539t^2+7.8574t-231.37	0.99
Delle Cascine	8.5±1.5	32.4±2.5	50.5±3.0	47.6±2.3	27.4±1.7			**Q=-0.0516t^2+7.5901t-228.15	0.98
Early Violletto	5.2±1.6	27.3±1.6	43.1±3.1	39.1±3.4	22.0±1.7			*Q=-0.0455t^2+6.686t-202.79	0.97
Early White	11.0±1.3	33.4±2.5	53.8±3.2	52.0±3.4	27.7±1.1			*Q=-0.0553t^2+8.0842t-241.43	0.98
Sweet Lorane	14.1±1.6	31.6±2.7	39.1±2.6	43.5±2.5	43.4±2.1	41.0±2.2	26.0±1.0	**Q=-0.0126t^2+2.3452t-62.506	0.94
Windsor	13.1±1.2	36.9±2.7	54.4±2.7	49.0±2.7	27.1±2.0			**Q=-0526t^2+7.6116t-222.36	0.97
V*ST†								**	

†V and ST = Variety and sampling time, respectively.
*Q and **Q are significant at P ≤0.05 and P ≤0.01, respectively. Values are mean ± standard error.

Table 6. 6 L-Dopa yield (kg ha^{-1}) of faba bean varieties during growth period. Values are average of two years

Varieties	L-Dopa yield calculated in days after planting								
	45	**55**	**65**	**75**	**95**	**115**	**135**	**Equation**	**R^2**
Aquadulce	2.0±0.1	14.5±0.1	42.7±3.9	55.2±2.9	37.1±2.6			*Q=-0.0463t²+7.3175t-238.37	0.92
Bell bean	1.7±0.1	5.5±0.1	8.8±4.2	16.6±1.3	14.7±1.0			*Q=-0.0072t²+1.2947t-42.991	0.90
D'Aquadulce	2.9±0.1	10.1±0.3	14.2±0.0	21.0±1.1	15.4±1.5			**Q=-0.014t²+2.2252t-69.604	0.95
Delle Cascine	2.3±0.4	15.1±1.6	35.6±2.1	38.3±2.9	34.6±2.8			*Q=-0.0296t²+4.8301t-156.64	0.97
Early Violletto	1.5±0.6	11.4±0.2	19.8±1.4	25.3±2.8	25.2±1.9			*Q=-0.015t²+2.5891t-84.885	0.99
Early White	2.9±0.4	9.7±1.5	29.1±1.7	32.7±2.5	22.4±1.7			**Q=-0.0286t²+4.4674t-143.09	0.91
Sweet Lorane	0.4±0.1	2.7±0.1	2.6±0.2	4.3±0.8	4.4±0.8	6.4±1.0	4.1±0.9	**Q=-0.0012t²+0.2559t-8.5008	0.84
Windsor	2.3±0.1	9.1±0.1	16.3±2.2	18.9±1.6	15.2±1.2			**Q=-0.0144t²+2.2838t-71.931	0.99
V*ST†								**	

†V and ST = Variety and sampling time, respectively.
*Q and **Q are significant at P ≤0.05 and P ≤0.01, respectively. Values are mean ± standard error.

Table 6. 7 Pod fresh weight (PFW), seed fresh weight (SFW), and L-Dopa seed yield of faba bean varieties. (values are averaged over two years)

Variety	**PFW ($kg\ ha^{-1}$)**	**SFW ($kg\ ha^{-1}$)**	**L-Dopa Seed yield ($kg\ ha^{-1}$)**
Bell bean	3731cd†	1462c	13.6d
Sweet Lorane	1695d	444d	3.4e
Early white	11225b	2946b	22.1c
Aquadulce	16152a	5511a	38.0a
Early violletto	12383b	3454ab	18.5c
Windsor	5391c	1842bc	14.3d
DelleCascine	16270a	4703a	33.4b
D'Aquadulce	5830c	1799c	13.4d

†Values within a column followed by the same letter do not differ significantly ($p < 0.05$).

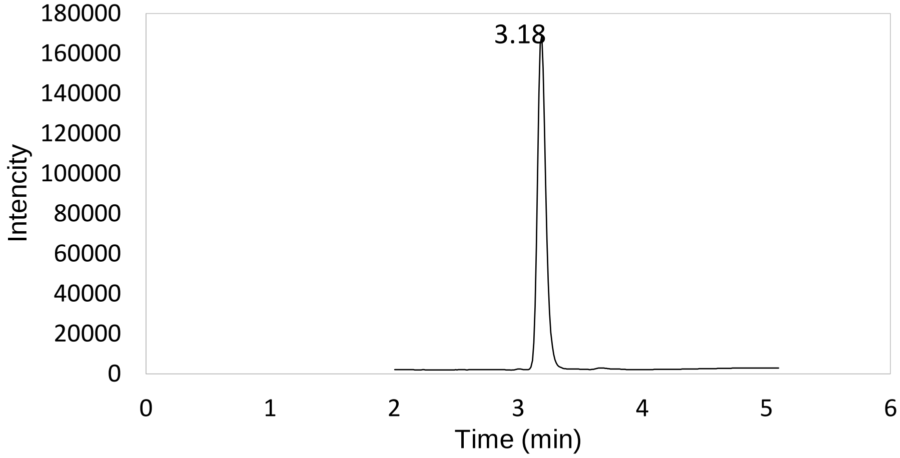

Figure 6.1 HPLC chromatogram of L-Dopa standard solution

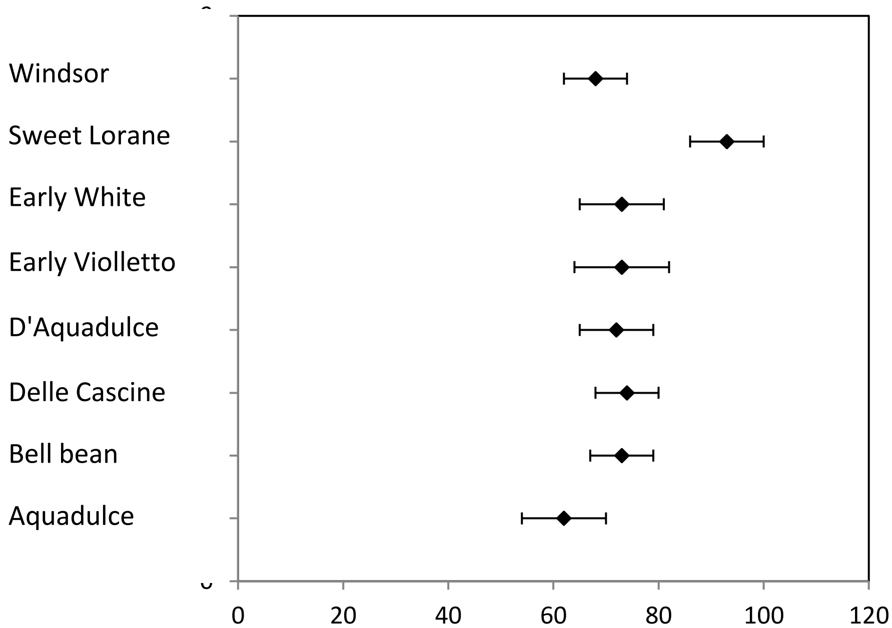

Figure 6.2 Confidence interval of days after planting to reach maximum L-Dopa conc. (mg g^{-1}) in eight faba bean varieties used in this study. Presented values are average of two years.

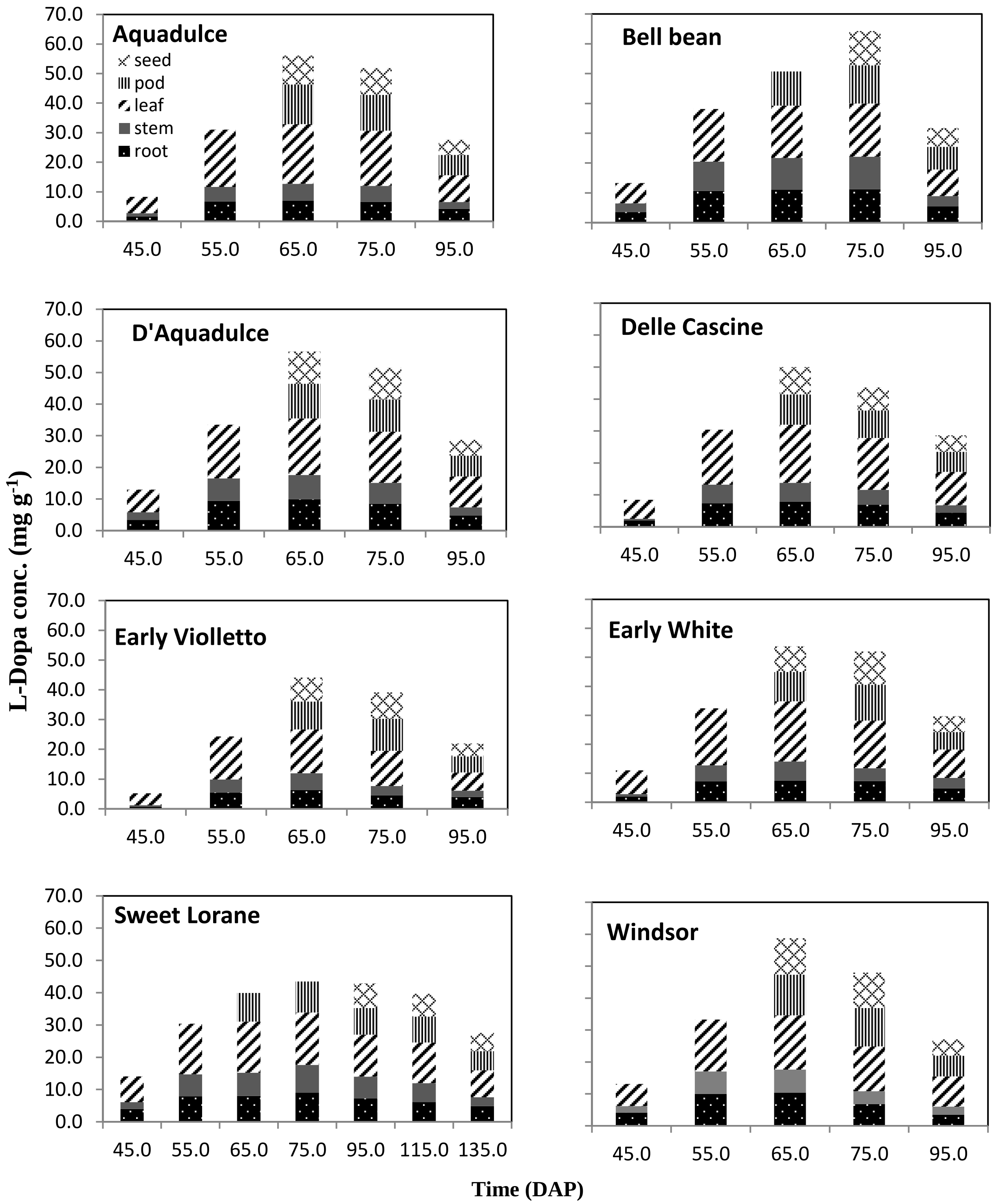

Figure 6.3 L-Dopa accumulation in various organs of faba bean varieties during the growth period. Measurement was continued for sweet Lorane (2-g) until 135 days after planting. Presented values are average of two years.

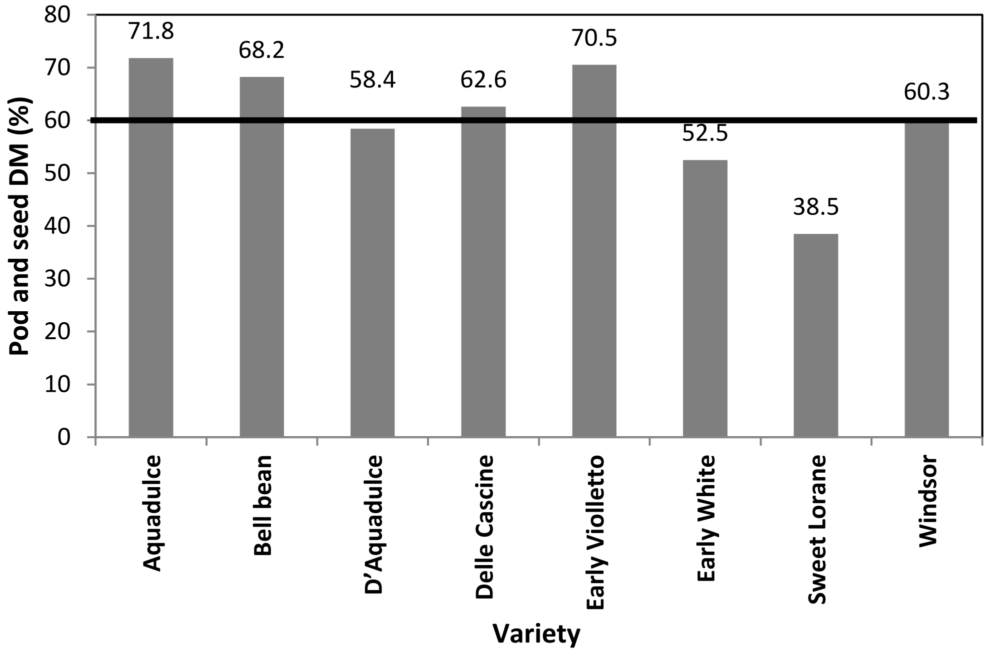

Figure 6.4 Pod and seed dry weight percentage of total dry matter in eight faba bean varieties. Presented values are average of two years.

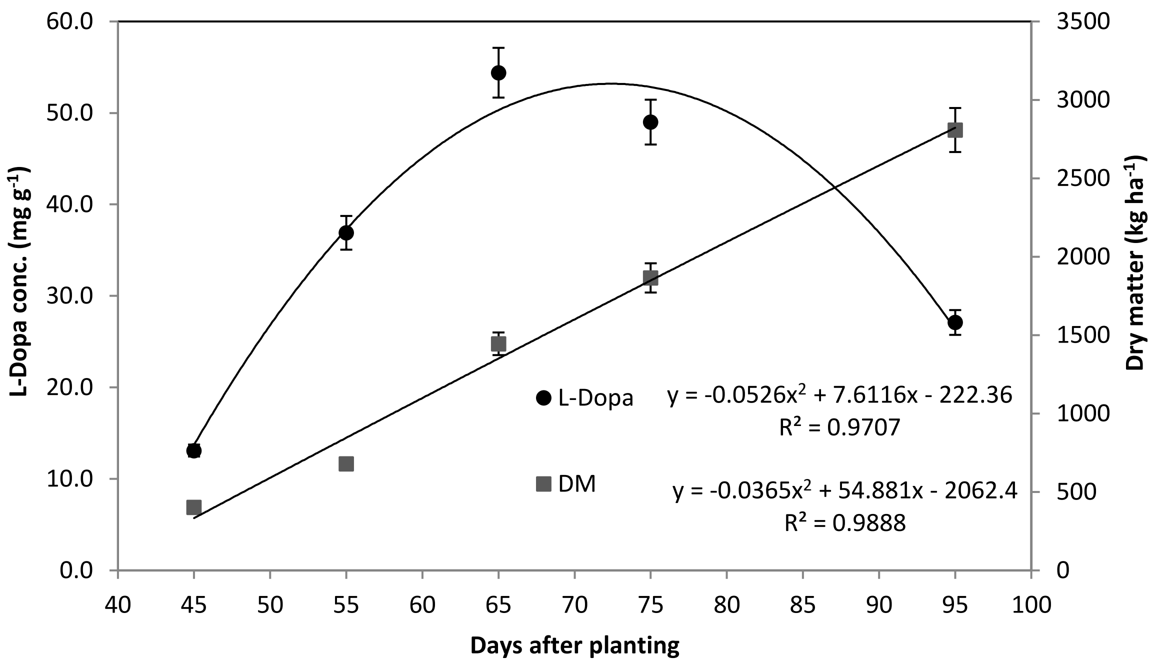

Figure 6.5 Dry matter and L-Dopa accumulation trend of Windsor (the most common variety in New England) during its growth period. Presented values are average of two years.

CHAPTER VII

ACCUMULATION OF L-DOPA IN VARIOUS ORGANS OF FABA BEAN AND INFLUENCE OF DROUGHT, NITROGEN STRESS, AND PROCESSING METHODS ON L-DOPA YIELD

Abstract

Faba bean (*Vicia faba* L.) has been identified as a rich source of L-Dopa, which is used in treating Parkinson's disease. Biosynthesis and accumulation of active substances such as L-Dopa in plant tissues may interact with growing conditions and processing methods. Accumulation trends of L-Dopa in various faba bean organs and the effect of drought stress and N fertilization on L-Dopa content were studied in a field and two greenhouse experiments. The influence of various processing methods on L-Dopa content of faba bean tissues was evaluated. The highest L-Dopa content was detected in fresh leaves (22.4 mg g^{-1}) followed by flowers, young pods, mature seeds, and roots. Regardless of processing method, L-Dopa concentration in faba bean tissues was significantly reduced when tissues were boiled or dried. Among various methods of processing, freezing had the lowest detrimental effect, reducing L-Dopa concentrations by 24.1% and 21.1% in leaves and seeds, respectively. Drought stress elevated L-Dopa concentration, and maximum L-Dopa (23.3 mg g^{-1} of biomass) was extracted from plants grown under severe drought stress. However, L-Dopa yield (L-Dopa concentration × biomass) was compromised, owing to the adverse influence of drought stress on dry matter production. No significant difference in L-Dopa concentration was detected among various N application rates.

Introduction

Faba bean is a cool-season legume crop with high nutritional value (Etemadi, et. al., 2015) and has also been identified as a medicinal plant, given that it accumulates a large amount of L-Dopa (3,4-dihydroxyphenyl-L-alanine), a precursor of dopamine currently used in treating Parkinson's disease (PD). L-Dopa is synthesized from the amino acid L-tyrosine in the mammalian body and brain (Randhir and Shetty, 2004; Haq and Ali, 2006; Miller, et. al., 2009). Synthesized L-Dopa is expensive and often causes a variety of side effects including nausea, vomiting, low blood pressure, drowsiness, and restlessness (Lee, et. al., 1996; Knowles, 2003; Patil, et. al., 2013). For this reason, use of natural sources of L-Dopa to avert potential side effects has been recommended (Lee, et. al., 1996; Knowles, 2003; Singh, et. al., 2012; Patil, et. al., 2013). The world demand for L-Dopa is estimated to be as high as 250 t year^{-1} (Katayama and Kumagai, 2010) with an annual market value of about $100 billion (Koyanagari, et. al., 2005; Inamdar, et. al., 2013; Patil, et. al., 2013). Different parts of faba bean accumulate L-Dopa with different rates and trends (Etemadi, et. al., 2014).

Environmental conditions influence the accumulation of secondary metabolites in plants (Falk, et. al., 2007; Selmar, M. Kleinwächter, 2013; Afshar, et. al., 2015). Environmental stresses may influence metabolic pathways involved in the synthesis of secondary metabolites, thus changing the synthesis and accumulation of natural compounds in plants (Bohnert, et. al., 1995). Adaptation of plants to biotic and abiotic stresses is due primarily to the stimulation of protective biochemical systems and synthesis of secondary metabolites such as phenolics derived from the phenylpropanoid pathway (Randhir and Shetty, 2004; Jensen, et. al., 2010). Plants grown under high temperature (López-Bellido, et. al., 2005; Köpke and Nemecek, 2010) and nutritional disorders (Randhir and Shetty, 2004; Agegnehu, et. al., 2006) produced phenylpropanoid metabolites such as flavonoids, isoflavonoids, anthocyanins, and polyphenols. Both environmental and genetic factors play roles in L-Dopa production (Lorenzetti, et. al., 1998; St-Laurent, et. al., 2002). An earlier report indicated that the nature of the N source and the presence/absence of illumination affected the production of L-Dopa by cell suspension cultures of velvet bean (Wichers, et. al., 1985). Randhir and Shetty (Randhir and Shetty, 2004) reported that under microwave-induced stimulation, L-Dopa content of germinated sprouts increased by 59%.

Plants exposed to some degree of drought stress accumulate higher concentrations of secondary metabolites (Selmar and Kleinwächter, 2013; Bistgani, et. al., 2017a). Such enhancement is reported to occur in nearly all classes of natural products including simple and complex phenols, numerous terpenes, and also N-containing substances such as alkaloids, cyanogenic glucosides, and glucosinolates. Owing to the beneficial effects of drought stress on accumulation of secondary metabolites, especially phenolic compounds, exposing plants to some degree of water stress has been suggested as a potential approach to increasing the production of these active substances (Bettaieb, et. al., 2009). There is limited information available on the response of L-Dopa content of faba bean to drought stress.

Although faba bean is an N_2-fixing plant, there is a lack of information about the effect of supplemental N fertilization on its L-Dopa concentration. Deficiencies in N and P directly influenced the accumulation of phenylpropanoids (Ramakrishna and Ravishankar, 2011) and lignification (Chalker-Scott and Fnchigami, 1989).

Because fresh faba beans are not available at all times in all locations, Parkinson patients may store relatively large numbers of plants for consumption. Plants may be processed by various methods, including chopping frozen tissues and powdering dried plants. However, it is not known to what extent processing influences the L-Dopa content of faba bean plants. Earlier reports (Echeverria and Bressani, 2006; Vadivel and Pugalenthi, 2010) indicated that L-Dopa in legumes can be destroyed by cooking and soaking in alkaline solutions. Dahouda et al. (2009) concluded that the L-Dopa in velvet bean was degraded by cooking but not by roasting. More information about the potential negative impact of processing methods on L-Dopa concentration is needed.

The main objectives of this study were to investigate:

1)the accumulation trend and concentration of L-Dopa in various organs of faba bean plant
2)the influence of drought stress on L-Dopa concentration
3)whether supplemental N fertilization can improve L-Dopa content
4)the influence of processing methods on L-Dopa content of faba bean

Materials and methods

Measuring L-Dopa concentration in plant parts

A two-year field study was conducted at the University of Massachusetts Amherst Agricultural Experiment Station, Crops and Animal Research and Education Farm in South Deerfield (42°28′37″N, 72°36′2″W) in 2013 and 2014. The soil type at the experimental site was a Hadley fine sandy loam (nonacid, mesic Typic Udifluvent). Seeds were inoculated with peat-based Rhizobium leguminosarum (VERDESIAN, N.DURE, Carry, NC) prior to sowing in both experiments. Seeds were planted on March 5 in 2013 and March 10 in 2014 in three replications. Faba bean plants were harvested at two stages of growth. The first harvest was at the six-leaf stage (15 days after germination) and the second harvest was at 95 days after germination when pods were fully grown. In the first harvest, whole seedlings were used for L-Dopa determination. However, in the second harvest, plants were divided into various organs including roots, stems, leaves, terminal buds, flowers, young pods, and mature seeds. All organs weredigested separately and analyzed for L-Dopa using highperformance liquid chromatography (HPLC), a reliable method to measure L-Dopa (Shetty, et. al., 2001).

Processing method influence on L-Dopa concentration

Five plants were harvested randomly from each experimental plot replication when the lower pods were physiologically mature. Pods and leaves were separated and processed by various methods including air drying, oven drying, boiling, and freezing. Air drying: samples were placed on paper bags and left to dry at room temperature for two, four, and seven days. Oven drying: samples were placed on metal trays and dried in a forced-air oven at 50 °C for 24 h. Freezing: samples were chopped and stored in a freezer immediately after harvest at −23 °C for 48 h prior to analysis. Boiling: samples of leaves and seeds were cooked in boiling water (five times the sample weight) for 40 min. After each processing method, 200-mg samples from each treatment were taken. For the drying method, samples were ground to pass a 0.42 mm screen and prepared for extraction. For the other processing methods, samples were ground with mortar and pestle prior to analysis.

Drought-stress influence on L-Dopa concentration

A greenhouse experiment was conducted at the College of Natural Sciences, University of Massachusetts Amherst. Seeds of faba bean (cv. Windsor) were inoculated and planted two times, on October 14 and December 15, 2014, in 25 cm × 50 cm trays with 3.2-cm cells filled with a pro-mix soil. Seedlings were transferred to individual pots filled with sterilized play sand after nine days. Greenhouse temperature was set at 15 °C with 16 h light during daytimeand 12 °C at night with a relative humidity of 50% ± 5%. The experiment was laid out in a randomized complete block design with three soil moisture treatments and replicated three times. Soilmoisture treatments were planned to expose plants to moderate and severe drought stress, based on soil moisture depletion. Soil moisture holding characteristics, including the volumetric soil moisture content at −0.03 MPa (field capacity, FC) is the amount of soil moisture or water content held in the soil after excess water has drained away, and −1.5 MPa (permanent wilting point, PWP) is defined as the minimal value of soil moisture the plant requires not to wilt and was determined with a pressure-plate apparatus. The difference between FC and PWP was taken as the available soil water content. Soil moisture content in all pots was monitored twice a day using time-domain reflectometry (TDR) (MiniTrase System, Soil Moisture Equipment Corp., Santa Barbara, CA) fitted with 20-cm probe rods. Watering of control pots (nonstress) was based onmaintaining soilwater content at 80% FC to avoid drought stress. Pots under moderate and severe levels of water deficit received 60% (moderate stress) and 40% (severe stress) of the amount of irrigation water used in the control pots. Plants were harvested at four vegetative growth stages: two, four, six, and eight leaves. At each stage, three plants were cut at the soil surface and oven-dried at 50 °C. Dried plant samples were ground to pass a 0.42 mm screen and prepared for extraction.

Nitrogen stress influence on L-Dopa concentration

Seeds of faba bean (cv. Windsor) were inoculated and planted twice: October 14 and December 15, 2014. Growing conditions and layout of this experiment were similar to those of the drought stress experiment. After seedlings were transplanted to the main pots, N fertilizer (calcium ammonium nitrate with 27% N) was applied at five rates: 0, 0.250, 0.050, 0.075, and 0.100 g N pot^{-1} (equivalent to 0, 25, 50, 75, and 100 kg ha^{-1}). Faba bean seedlings were harvested at four vegetative growth stages: 2, 4, 6, and 8 leaves. At each growth stage, three plants were harvested at the soil level and oven-dried at 50 °C. Dried plant samples were ground to pass a 0.42 mm screen and prepared for extraction.

Plant preparation procedure

The procedure described by Shetty et al. (2001) for the extraction of L-Dopa was used with some modifications. Dry samples of 200 mg were immersed in 95% ethanol and kept in the freezer for 72 h. Samples were then homogenized using a BioHomogenizer (BIOSPEC PRODUCTS, Bartlesville, OK) and centrifuged at 13,000 r min^{-1} for 10 min. The liquid portion was left under a fume hood until the ethanol evaporated. The residue was dissolved in a buffer solution (32 mmol L^{-1} citric acid, 54.3 mmol L^{-1} sodium acetate, 0.074 mmol L^{-1} EDTA, 0.215 mmol L^{-1} octyl sulfate pH 4) and refrigerated until particles settled. The supernatant was passed through a 0.45 μm syringe filter for analysis by HPLC (LC 2010A, Shimadzu Prominence HPLC with DAD detector (SPD-M20A Photodiode Array Detector, Shimadzu, Japan, Tokyo), Phenomenex Gemini C18 column).

HPLC

L-Dopa was analyzed by HPLC equipped with an autosampler and diode array detector. Data were acquired and processed by an LC Solution Data System (Shimadzu, Japan). Chromatographic separation was performed on a Phenomenex C18 column (250.0 mm × 4.6 mm, 5 μm) (Phenomenex, CA, USA) with an isocratic flow of 1 mL min^{-1}. The mobile phase was 0.1% acetic acid (98%) and methanol (2%). The mobile phase was filtered through 0.2 μm filters and degassed by sonication for 30 min. Injection volume was 20 μL and L-Dopa detection was at 283 nm. 2.7. Calibration curve Four concentrations of stock solution (50, 100, 200, and 400 mg L^{-1}) were prepared and injected with mobile phase. The calibration curve was obtained by plotting the absorbance peak area against the concentrations of the standard solution. A linearity graph of control solution of L-Dopa is shownin Fig. 7. 1. 2.8. Statistical analysis

Collected data were subjected to ANOVA using PROC GLM of SAS (SAS, 2003; Zandvakili, et. al., 2017). Effects were considered significant at $P \leq .05$ by the F-test, and when the F-test was significant, Fisher's least significant difference (LSD) test was used for mean separation. The variability of the data in all of the figures is indicated by corresponding error bars. Regression equations for faba bean biomass and L-Dopa concentration under drought stress and N application rates were determined by fitting the data to linear or quadratic trend using PROC REG.

Results and discussion

L-Dopa concentration in various plant parts

L-Dopa concentration differed significantlyamong organs of faba bean (Fig. 7. 2-a). The highest L-Dopa concentration (13.3 mg g^{-1}) was extracted from young seedlings harvested 15 days after germination. L-Dopa content decreased as plants matured. When plants were harvested at physiological maturity, the highest L-Dopa content was detected in leaves (10.5 mg g^{-1}) followed by flowers, young pods, mature seeds, and roots. Earlier reports focused mainly on L-Dopa content of seeds (Randhir and Shetty, 2004; Mohseni and Golshani, 2013). Results from the present study indicated that mature seeds were not the richest part of faba bean and contained on average 7.2 mg g^{-1}, a concentration lower than reported previously (Randhir and Shetty, 2004).

The L-Dopa yield of each plant part is a product of its concentration and the dry weight of the organ. Accordingly, the highest L-Dopa yield was obtained from leaves (Fig. 7.2-b). Recently (Etemadi, et. al., 2017) we reported that L-Dopa yields of seeds differed markedly among cultivars, with Aquadulce producing the highest L-Dopa yield with 38 kg ha^{-1}.

Influence of processing method on L-Dopa concentration

Processing methods influenced the L-Dopa content of seeds and leaves (Fig. 7.3). As expected, the highest L-Dopa was extracted from freshly harvested plants. The highest L-Dopa concentrations extracted from fresh leaves and seeds were 22.4 mg g^{-1} and 16.1 mg g^{-1}, respectively. When leaves and seeds were chopped and kept frozen for 48 h, their L-Dopa content decreased by 24.1% and 21.1%, respectively. The reduction in L-Dopa due to processing was much higher when plant parts were processed by methods other than freezing. When leaves or seeds were oven- or air-dried for two, four, or seven days, the L-Dopa content decreased similarly, and the extracted concentrations were as low as 50% of those of fresh-harvested leaves and seeds. Among various processing methods, boiling caused the greatest reduction in L-Dopa concentration in leaves and seeds. L-Dopa is a simple phenolic secondary metabolite that is synthesized in the shikimic acid pathway. It is derived from Ltyrosine by the action of tyrosinase (Fig. 7.4). In general, activity of enzymes is negatively impaired by extreme changes in their normal conditions. Storing plant materials for relatively short period of time in room temperatures may result in significant decrease in L-Dopa concentration. We found a

decrease of up to 50% in L-Dopa concentration when leaves and seeds of faba bean were kept at room temperature for 48 h or oven-dried. However, we found no further
reductions when plant samples were kept for four and seven days. Drying and heating of plant parts may result in accelerating the degradation and/or reduction of enzyme activities, owingmainly to protein denaturation (Shetty, et. al., 2006). Nyirenda et al. (Nyirenda, et. al., 2003) reported that boiling, which is a combination of soaking and high temperature, severely reduced the L-Dopa content of velvet bean. In the present study we extracted less L- Dopa by boiling plant materials than by applying other processing methods (Fig. 7.3). The result was in accord with earlier reports indicating that L-Dopa in legumes is destroyed by cooking or roasting (Laurena, et. al., 1991; Echeverria and Bressani, 2006; Vadivel and Pugalenthi, 2010).

Influence of drought stress on faba bean biomass and L-Dopa concentration

Under all irrigation treatments, change in biomass of faba bean showed a quadratic response with a typical sigmoid trend (Table 7.1). The amount of biomass production decreased significantly as drought stress intensified. At eight-leaf stage, faba bean plants grown under moderate and severe drought stress produced 27% and 42% less biomass than well-watered plants. Reduction in biomass of plants under drought stress condition is not surprising, given that growth of plant organs requires maximum turgor pressure. Such pressure is not available when irrigation water is limited, resulting in reduced plant height, leaf size, and stem diameter (Mirshekari, et. al., 2012; Mirshekari, et. al., 2013). Other than cell expansion, photosynthesis is also considered to be one of the earliest physiological functions impaired when plants experience drought stress, owing mainly to stomatal closure (Afshar, et. a., 2015; Bistgani, et. al., 2017a). L-Dopa contents of well-watered faba bean seedlings and those grown under stress conditions increased until the fourleaf stage followed by a declining trend (Table 7.2). Imposing drought stress elevated the L-Dopa concentration to its peak value of 23.3 mg g^{-1} under the severe-stress condition, almost twofold that of well-watered seedlings. A similar result reported by Khang et al. (2016) was an increased accumulation of phenolic compounds in six different legumes subjected to drought stress. Production of secondary metabolites in plants interacts strongly with growing condition and environmental stress (Falk, et. al., 2007; Bistgani, et. al., 2017b). It appears that enhancement of L-Dopa accumulation in faba bean seedlings is part of the defense mechanism in plants experiencing drought stress. The defense mechanism helps plants to tolerate the stress condition for a longer period of time (Shetty, et. al., 2006; Ramakrishna and Ravishankar, 2011). Reactive oxygen species (ROS), also called active oxygen species (AOS) or reactive oxygen intermediates (ROI) are the result of the partial reduction of atmospheric O2, which is increased by lipid peroxidation, chlorophyll destruction, protein oxidation, and nucleic acid damage under stress situations during plant growth (Kasote, et. al., 2015; Bistgani, et. al., 2017a). Plants have an innate ability to biosynthesize a wide range of non-enzymatic antioxidants capable of attenuating ROS-induced oxidative damage (Cruz de Carvalho, 2008; Kasote, et. al., 2015). L-Dopa yield is a product of its concentration and plant biomass. Although drought stress elevated L-Dopa concentrations in faba bean seedlings, L-Dopa yield was compromised by a large adverse effect of drought stress on biomass production (Table 7.1), so that well-watered plants produced the highest L-Dopa yield (Fig. 7.5). Thus, imposing drought stress should not be considered a viable option for increasing L-Dopa yield in faba bean.

Influence of N application rate on faba bean biomass and L-Dopa concentration

Faba bean biomass increased with N application rate up to the equivalent of 75 kg ha^{-1} (Table 7.3). As a legume, faba bean meets its N need by biological fixation. However, the results of the present study revealed that due to the conditions of the experiment, the amount of biological fixation was not enough to satisfy faba bean N requirements. Even under field conditions, many legumes often respond positively to supplemental N (Lou, 1994; Hungria, et. al., 2006; Salvagiotti, et. al., 2008; Zandvakili, et. al., 2012). Given that L-Dopa is an N-containing compound, we assumed that higher availability of N in the soil stimulates L-Dopa synthesis, so that more L-Dopa can be extracted. The results of our study, however, did not fully confirm this hypothesis. At all growth stages, the lowest L-Dopa concentrations were found in plants grown with no supplementary N. At the four-leaf stage, maximum L-Dopa content was extracted from plants receiving the equivalent of 100 kg N ha^{-1}, but in later stages of growth, no significant differences in L-Dopa concentration were detected among the N application rates (Table 7.4).

Conclusions

The highest L-Dopa concentration was accumulated in seedlings. However, given that total extractable L-Dopa is a function of both concentration and biomass, the highest L-Dopa yield was extracted from mature plants. The highest L-Dopa content was found in fresh leaves. Immediate freezing of plant tissues preserved more L-Dopa than other processing methods. Although drought stress stimulated L-Dopa synthesis, especially at the early stages of growth, plants grown under well-watered conditions yielded higher L-Dopa than stressed plants. We conclude that application of exogenous N cannot be used as a strategy to stimulate L-Dopa synthesis, but that the use of higher N application rates may improve L-Dopa yield by promoting biomass production.

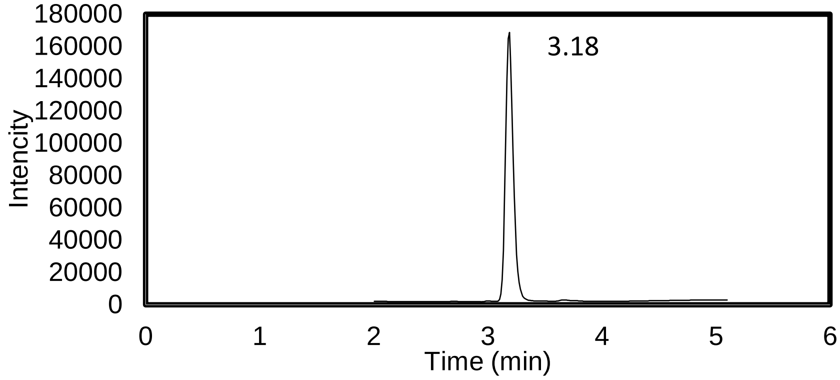

Figure 7. 1 HPLC chromatogram of L-Dopa standard solution

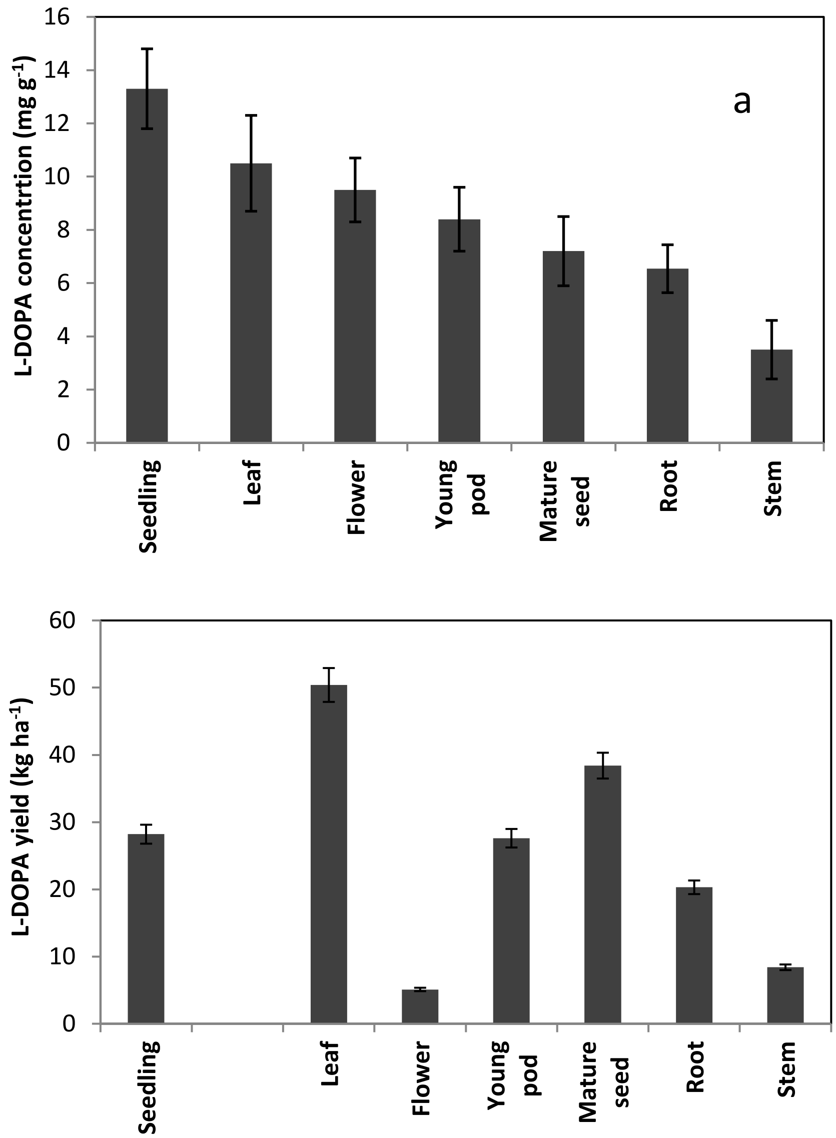

Figure 7. 2 L-Dopa concentration (a) and yield (b) of various organs of faba bean. Values are mean of two experiments and three replications.

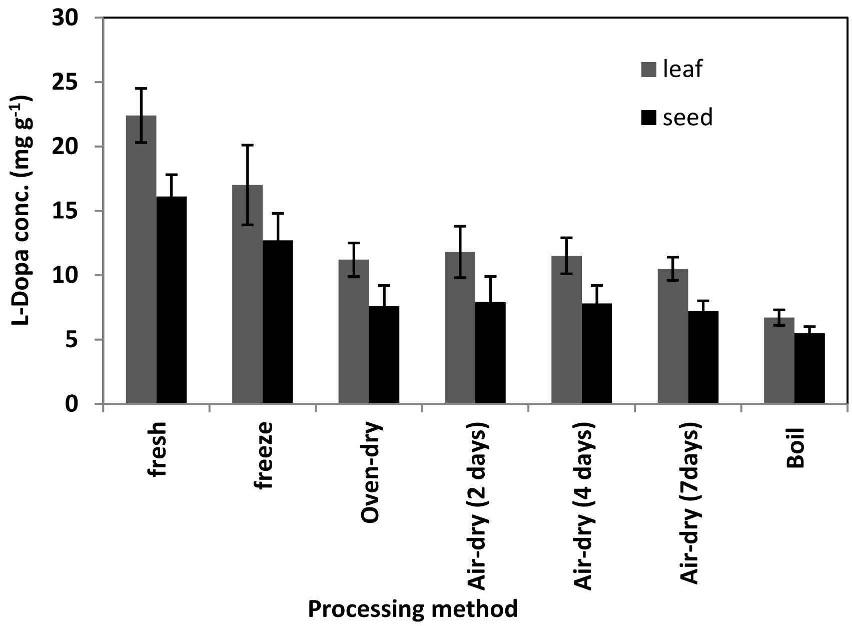

Figure 7. 3 The influence of various processing methods on L-Dopa content of faba bean leaves and seeds. Values are average of two experiments and three replications.

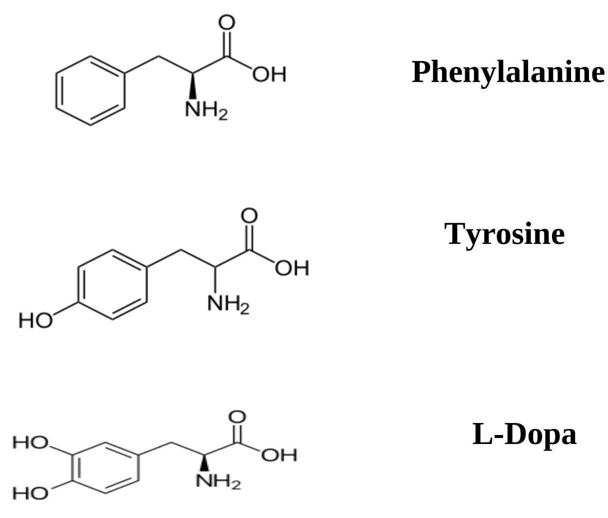

Figure 7. 4 Two initial steps of L-Dopa biosynthesis

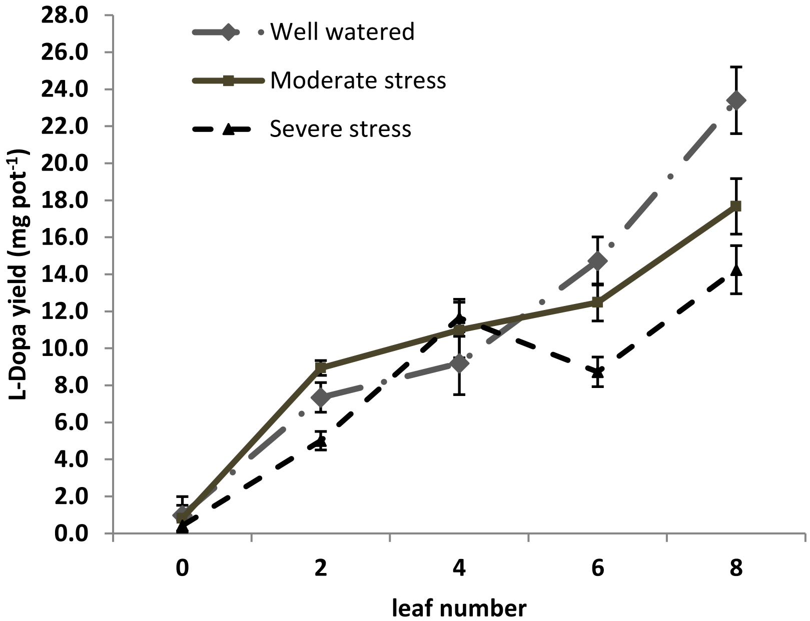

Figure 7. 5 Influence of drought stress on L-Dopa yield of faba bean in early stages of growth. Values are average of three replications and two experiments.

Table 7. 1 Influence of drought stress on faba bean biomass (g pot^{-1}) harvested in early growth stages.

				Growth Stage (Leaf numbers)			
Stress Condition	**Protrude**	**2**	**4**	**6**	**8**	**Trend/Equation**	R^2
Well- watered	0.3	0.7	0.8	1.6	2.6	Q** biomass=0.04GS^2-0.02GS+0.40	0.98
Moderate stress	0.2	0.6	0.7	1.3	1.9	Q** biomass=0.01GS^2+0.09GS+0.18	0.99
Severe stress	0.1	0.3	0.5	0.9	1.5	Q** biomass=0.02GS^2+0.03GS+0.09	0.96
Trend	*Q	*Q	**Q	**Q	**Q		
CV % (0.05)	18.8	15.8	4.9	6.4	4.9		

*, and ** significant at 0.05 and 0.01, respectively.
L and Q = linear and quadratic, respectively.

Table 7. 2 Influence of drought stress on faba bean L-Dopa concentration (mg g^{-1}) in early growth stages.

				Growth Stage (leaf numbers)			
Stress Condition	**Protrude**	**2**	**4**	**6**	**8**	**Trend/Equation**	R^2
Well- watered	3.3	10.5	11.5	9.2	9.0	Q**L-Dopa=-0.39GS^2+3.95GS+1.58	0.68
Moderate stress	4.1	14.9	15.7	9.6	9.3	Q**L-Dopa=-0.57GS^2+5.43GS+2.33	0.57
Severe stress	4.2	16.7	23.3	9.7	9.5	Q**L-Dopa=-0.99GS^2+8.80GS-0.32	0.59
Trend	Q*	Q**	Q**	ns	ns		
CV % (0.05)	6.7	4.2	1.8	8.4	7.6		

*, and ** significant at 0.05 and 0.01, respectively.
L and Q = linear and quadratic, respectively.

Table 7. 3 Influence of N application rate on faba bean biomass (g pot^{-1}) in early growth stages.

	Growth Stage (leaf numbers)					
N Rate ($Kg\ ha^{-1}$)	**2**	**4**	**6**	**8**	**Trend/Equation**	**R^2**
0	0.5	0.7	0.9	1.3	Q*biomass=0.012GS^2+0.005GS+0.45	0.99
25	0.7	1.1	1.4	1.7	L**biomass=0.16GS+0.4	0.99
50	1.2	1.5	1.7	2.2	Q*biomass=0.012GS^2+0.035GS+1.1	0.98
75	1.4	1.9	2.2	2.5	L**biomass=0.18GS+1.1	0.98
100	1.7	1.9	2.3	2.5	Q**biomass=0.14GS+1.4	0.98
Trend	Q*	Q*	Q**	Q*		
CV %(0.05)	5.3	7.0	6.9	9.1		

*, and ** significant at 0.05 and 0.01, respectively.
L and Q = linear and quadratic, respectively.

Table 7. 4 Influence of N application rate on faba bean L-Dopa concentration (mg g^{-1}) in early growth stages.

	Growth Stage (leaf numbers)					
N Rate ($Kg\ ha^{-1}$)	**2**	**4**	**6**	**8**	**Trend/Equation**	**R^2**
0	10.1	12.2	10.8	10.0	Q** L-Dopa=-0.18GS^2+1.73GS+7.6	0.75
25	12.2	14.3	13.2	11.2	Q** L-Dopa=-0.26GS^2+2.36GS+8.62	0.95
50	13.2	16.7	13.8	11.5	Q** L-Dopa=-0.36GS^2+3.22GS+8.5	0.83
75	15.3	17.4	14.2	12.1	Q* L-Dopa=-0.26GS^2+1.98GS+12.7	0.86
100	14.5	22.3	13.4	12.5	Q* L-Dopa=-0.36GS^2+2.87GS+12.78	0.72
Trend	Q**	Q**	Q*	ns		
CV %(0.05)	2.6	3.6	1.0	5.1		

*, and ** significant at 0.05 and 0.01, respectively.
L and Q = linear and quadratic, respectively.

CHAPTER VIII

OVERALL SUMMARY AND CONCLUSION

The current comprehensive research study revealed that faba bean as a cool season grain legume can be successfully integrated in various cropping systems. Multiple ecological benefits are associated with faba bean and argubly the major benefit of faba bean cultivation is related to its high capacity biologically nitrogen fixation.

The result of this study showed that under optimum management such as early planting, faba bean can fix roughly 200 kg N ha^{-1} (Chapter 3). Other notable ecological benefit associated with faba bean is that the crop can be integrated into cropping systems as a break crop to control various pests and diseases in cereal crops such as corn and barley.

Although the influence of faba bean on bacterial community was not the focus of this research, it has been well documented that integrated faba bean into cropping systems enhance bacterial diversity and activity (Köpke, and Nemecek, 2010; Zhang, et. al., 2010; Orr, et. al., 2011; Siczek, and Lipiec, 2016).

One of the economic benefits of faba bean cultivation is the fact that faba bean as a cool-season grain legume can be planted as early as mid-March, if soil is workable, thus it significantly extends the growing season which is highly important in shorter-growing season area such as Northeast U.S.A. Additionaly faba bean can also be planted in July as dual purpose cash/cover crop and continues growing till early December before it winter kills. Results from this study showed that there are varieties available that require fewer GDD yet producing higher seed yield. For example, Aquadulce with 21.7% fewer GDD than Windsor (the current variety available to the growers in New England) while produced 26.5% higher yield (Chapter 2). Varieties that require fewer GDD for ripening are especially important in shorter-season regions.

One of the innovative planting method introduced in the current study is transplanting faba bean seedlings as opposed to traditional direct seeding to ensure its early planting in spring for maximum benefit. Early planted faba bean provides the opportunity for successful double cropping hence higher income for growers. When proper faba bean variety (in current study Aquadulce) was plnated in early spring, the net income from one-hectare transplanted faba bean estimated about $10,960 and direct seeded as much as $11,733 (Table 8.1 and 8.2).

When faba bean is used in a double cropping system, the nitrogen contribution from its roots and aerial residues to the N need of succeeding crop should be also accounted in faba bean economic benefits.

We concluded that seeds, pods, and leaves of faba bean are rich in various nutrients required in human diet (Chapter 5). For example, the protein content of seed, pods, and leaves were 19, 10, 11% respectively. Therefore, the results obtained in current study revealed that pod wall and leaves should be taken seriously in dietary consumption. For example, faba bean leaves can be easily mixed with other greens in garden salads.

One of the important and unique outcome in this research project was related to the findings of L-Dopa accumulation trend in various parts of the plant, and the influence of various processing methods on its availability. We found in this study that in general faba bean is a rich natural source of L-Dopa and its leaves and fresh seeds contain the highest amount of L-Dopa (Chapter 6). Therefore, Parkinson disease patients can use these two plant parts as natural medicine. For example, it was estimated that on average, the 2 to 4.5 g dry matter of Aquadulce that produced the highest biomass and L-Dopa yield contained a similar amount of L-Dopa to the synthetic product which contain between 100 and 250 mg L-Dopa (rxlist, Sinemet drug information).

Processing of faba bean for preserving L-Dopa for year around consumption may negatively impact its concentration in various parts. The results obtained in this study revealed that freezing chopped fresh leaves and seeds had the lowest negative influence where L-Dopa content reduced by 24% and 21%, respectively. Other processing methods including drying and boiling considerably reduced L-Dopa content of plant parts (Chapter 7).

The results of the experiments indicated that faba bean is an economically and ecologically valuable cash crop. However, it was also concluded that due to its relatively large seed size and thus the cost of seeds, using faba bean as sole cover crop is not economic and cannot be recommended.

The general perception is that larger seeds produce plants with better vigor and higher yield. The results from studying the influence of faba bean seed size on yield and L-Dopa content revealed that the high yielding varieties do not necessarily have larger seeds. We performed analysis using DEA models to evaluate the efficiency of eight faba bean varieties and concluded that Aquadulce with a seed size about 15% smaller than Windsor (the common available variety in New England), produced 26% more seed yield and as much as two-fold L-Dopa yield (Chapter 4).

The overall conclusion obtained from this comprehensive study revealed that faba bean can be grown successfully as a multi-porpuse (cash/cover/ medicinal) in the Northeast U.S.A.

Table 8. 1 Enterprise budget for transplanted faba bean (variety Aquadulce)[1] (Only variable Costs based on 1 hectare)

	Labor hrs ($14.00/hr)	**Machinery hrs ($20.00/hr)**
Baseline soil sampling	2	-
Plow and disk	-	4
Transplanting	10	10
Weed control (3 times rototilling)	30	-
Scouting for pests	10	-
Harvesting and packing[2]	133	-
Total hours	185	14
Total costs	**$2,590**	**$280**
MATERIALS		
Soil testing lab charge/sample	$20	
Seed[3]	$538	
700 tray (Transplant)[4]	$553	
Media[5]	$60	
Fertilizer[6] (if needed)	$350	
Boxes[7]	$849	
Total transplanting	**$2,370**	
SUMMARY OF VARIABLE COSTS & RETURNS		
Labor	$2,590	
Machinery	$280	
Material	$2,370	
Delivery	$300	
Total Costs	$5,540	
Total returns[8]	$16,500	
Net Income	**$10,960**	

[1] Updated February 2017
[2] Based on 7,500 kg/ha; 14 kg/ boxe; 535 boxes, 4 boxes/person/hour = 133 hours
[3] Based on 60,000 seeds/ha which is 84 kg = $538 (Johnny's Selected Seeds)
[4] 700 trays @ $79/100 trays
[5] 3 boxes @ $ 20/box
[6] Based on soil test results
[7] 535 boxes @ $1.58/box = $849
[8] 7,500 kg ha^{-1} yield @ $2.20/kg; Based on preliminary price provided by Whole Foods

Table 8. 2 Enterprise budget for faba bean (variety Aquadulce)[9]
(Only variable costs based on 1 hectare)

	Labor hrs ($14.00/hr)	**Machinery hrs ($20.00/hr)**
Baseline soil sampling	2	-
Plow and disk		4
Direct seeding		6
Weed control (3 times rototilling)	30	
Scouting for pests	10	-
Harvesting and packing[10]	133	
Total hours	175	10
Total costs	**$2,450**	**$200**

MATERIALS

Soil testing lab charge/sample	$20
Seed[11]	$538
Media	$60
Fertilizer[12] (if needed)	$350
Boxes[13]	$849
Total direct seeding	**$1,817**

SUMMARY OF VARIABLE COSTS AND RETURNS

Labor	$2,450
Machinery	$200
Material	$1,817
Delivery	$300
Total Costs	$4,767
Total returns[14]	$16,500
Net Income	**$11,733**

[9] Updated February 2017
[10] Based on 7,500 kg/ha; 14 kg/ boxes; 535 boxes, 4 boxes/person/hour = 133 hours
[11] Based on 60,000 seeds/ha which is 84 kg = $538 (Johnny's Selected Seeds)
[12] Based on soil test results
[13] 535 boxes @ $1.58/box = $849
[14] 7,500 kg/ha yield @$2.20/kg; Based on preliminary price provided by Whole Foods

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