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Marche Polytechnic University
Department of Agricultural, Food and Environmental Sciences

Scientific Field: AGR/07 - Plant Genetics

Ph.D. School of Agricultural, Food, and Environmental Sciences
XXXII cycle (2016-2019)

Genotypic variation of French bean yield and quality

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Acknowledgments

I'd like to thank all my colleagues that I worked with during my Ph.D. period, especially to the members of the Department D3a, section Plant Genetics. Above all, I'd like to express my gratitude to Prof. Roberto Papa for opening another door into my life and to Dr. Sergio Saia, for his support and supervision, for trying to do his best during the way…

I dedicate this thesis to my dearest, beautiful mother; Nuran Bozkır, and the man that no one can ever take his place on earth; my dad Süleyman Metin Bozkır… Without your support and love, neither I would be where I am nor who I am, I hope you're proud of me… Sizi çok seviyorum.

"La poesia non è fuori, è dentro: cos'è la poesia non chiedermelo più,
guardati allo specchio, la poesia sei tu!"
Roberto Benigni

Ancona, 15.02.2020

Dissertation Abstract and Structure

French beans are the fresh pods of *Phaseolus vulgaris*, which is an important crop both for agriculture, breeding, and nutritional studies. Aim of this research was to evaluate the genotypic differences among various green bean genotypes in terms of Brix, acidity, and pH. Three experiments were performed in the greenhouse and field conditions. In the literature, the protocols related to the sugar analysis in green beans are a few and not always applicable mainly due to the pod structure of green beans (less water, more fiber). Thus, the 1st experiment in the greenhouse was dedicated to find the most suitable method for green bean analysis. The 2nd small-scale greenhouse experiment served to evaluate the genotypic differences, and the yield traits of green beans. In the 3rd experiment, the genotypes were treated with/without the arbuscular mycorrhizal fungi (AMF) and the possible effects of AMF were evaluated. Finally, a systematic mapping of the resistant starch content of legumes, beans and green beans was conducted, to underline the health benefits and technological properties of RS.

Fresh green beans contained higher levels of malic acid than frozen ones. Sugar content was high and variable among the genotypes, and more in fresh samples than frozen ones. The results of the 1st greenhouse experiment showed that the yield and number of the pods were changing by the genotype x time interaction. However, the mean pod weight was strongly controlled by the genotype. In the field experiment, AMF didn't affect the sugar content and pod weight of the genotypes studied. In terms of pH and

malic acid content, there were significant differences among the genotypes; the string beans (Striga genotype) had the highest malic acid content and had lower pH than all others.

As a conclusion, for all the parameters studied in the greenhouse and field; especially the pod size and acidity seem to be strongly under genotypic control. The other parameters were highly variable among genotypes. The sugar contents are similar, but the acidity and pH levels seemed to shape the taste of green beans. Resistant starch content of green beans can additionally be included in future breeding studies to understand its effect on the taste and yield characteristics.

Riassunto e Struttura della Tesi

Il fagiolino è il baccello immaturo di molte specie di fagiolo e in particolare del Phaseolus vulgaris, e rappresenta un importante coltura orticola in diversi ordinamenti colturali. Tuttavia, le informazioni su determinanti genetici della qualità del fagiolino, e in particolare del contenuto zuccherino e dell'acidità, non sono disponibili in letteratura scientifica. Scopo della presente tesi è quindi stato studiare le differenze genotipiche in diverse accessioni di fagiolino in termini di contenuto zuccherino, contenuto in acidi e pH. In particolare, sono stati condotti tre esperimenti, in laboratorio, serra e campo, e varie metodologie per l'analisi del contenuto zuccherino sono state esplorate.

L'acido malico è stato sempre trovato in campioni concelati/surgelati. Il contenuto zuccherino è stato molto alto, ma variabile nei campioni freschi e basso e poco variabile in quelli congelati/surgelati. Nella prova in serra, sei genotipi sono stati studiati ed è emerso chiaramente che la dimensione del baccello aveva un forte controllo genotipico a parità di epoca di raccolta, mentre la resa e il numero di baccelli sono variati sia in funzione del genotipo, sia del momento di raccolta. Al contrario della serra, nella prova di campo il peso medio del baccello e il contenuto zuccherino non hanno mostrato differenze tra le tesi, mentre i genotipi hanno mostrato variazioni per il contenuto in acido malico e pH de baccello. Nella systematic map sull'amido resistente del fagiolo, è emerso che i lavori nel settore sono

pochi, sebbene sufficienti a delineare alcune caratteristiche relazionate a questo carattere. In particolare, sono stati trovati 1893 studi complessivi relazionati al carattere in esame nelle specie vegetali, dei quali solo 327 riguardavano i legumi. Ad ogni modo, i risultati hanno mostrato che l'amido resistente del fagiolo può conferire al prodotto interessanti caratteristiche nutrizionali e tecnologiche, le quali vanno esplorate adeguatamente.

In conclusione, il controllo genotipico sulla dimensione dei baccelli è apparso molto forte, analogamente al controllo sull'acidità. Questi caratteri, unitamente al contenuto in amido resistente, potrebbero essere presi in considerazione in futuri programmi di breeding.

Contents

List of Abbreviations

AMF	Arbuscular mycorrhizal fungi
AOM	Azoxymethane
BMI	Body mass index
CVD	Cardiovascular diseases
DAF	Days after flowering
DNA	Deoxyribonucleic acid
DAS	Days after sowing
DF	Dietary fiber
eGI	Expected glycemic index
FAO	Food and Agricultural Organisation
GIT	Gastrointestinal tract
GI	Glycemic index
HS-SPME	Solid-phase microextraction from headspace
HDL	High-density lipoprotein
IDL	Intermediate density lipoprotein
LDL	Low-density lipoprotein
MUFA	Mono-unsaturated fatty acids
NSP	Non-starch polysaccharides
NDF	Non-digestible fraction
NHANES	National Health and Examination Survey
RS	Resistant starch
RAPD	Random amplification of polymorphic DNA
RBC	Red blood cells
SCFA	Short-chain fatty acids
SCIE	Social Care Institute for Excellence
TIU	Trypsin inhibitor unit
UENF	Universidade Estadual do Norte Fluminense Darcy Ribeiro
WoS	Web of Science

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1 Introduction

1.1 Phylogeny of the beans

Common beans belong to the family of *Fabaceae*; class *Magnoliopsida* (dicots); division Magnoliophyta (flowering plants) (**Figure 1**). Various species can be called beans, most of which included in the genus *Phaseolus* (De Ron et al., 2016; Singh, 2016a). Beans are autogamous diploid species with 22 chromosomes (2n=2x=22) and its haploid genome size is estimated to be between 587–637 Mbp (Arumuganathan and Earle, 1991; Sicard et al., 2005; Rossi et al., 2009; Whitney et al., 2010; Singh, 2016a). There are five cultivated genera of beans, of which *Phaseolus* and especially the species *P. vulgaris*, is the most important one (Singh, 2016a). The other genus includes *Vigna* (cowpea), and *Glicine* (soya bean).

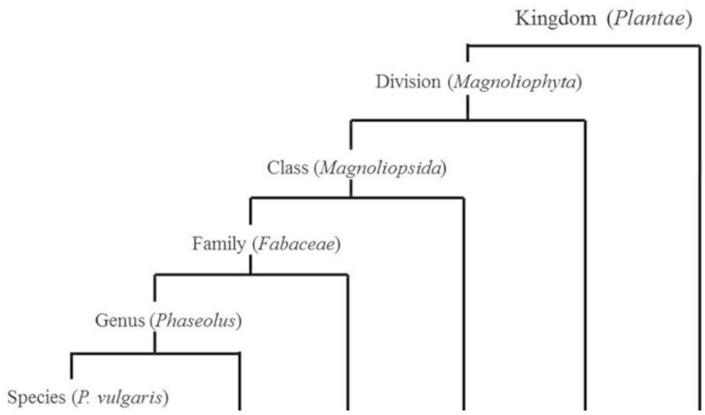

Figure 1. The phylogeny of *Phaseolus vulgaris*, adapted from (Duran-Flores and Heil, 2014)

The genus *Phaseolus* consists of about 70 species (Freytag and Debouck, 2002). The 5 most cultivated species (**Table 1**) are common bean (*P. vulgaris* L.), year bean (*P. dumosus* Macfad.), runner bean (*P. coccineus* L.), tepary bean (*P. dumosus* Macfad.) and lima bean (*P. lunatus* L.) (Paredes and Gepts, 1995; Delgrado-Salinas et al., 2006). From an economic point of view and impact on human consumption, the most important one is *Phaseolus vulgaris*, which also has one of the smallest genome among the legumes family – 625 Mbp per haploid genome (De Ron and Santalla, 2013; Schmutz et al., 2014; Bitocchi et al., 2017).

While *P. vulgaris*, *P. dumosus* and *P. coccineus* are closely related and are annual; lima bean is phylogenetically known to be more distant from the other domesticated species (De Ron and Santalla, 2013; Bitocchi et al., 2017).

Specifically, *P. vulgaris* is adapted to the warm temperatures and requires high amounts of water (usually growing in areas with ∼1,100 mm/year of rainfall). *P. coccineus* is found in more humid environments than *P. vulgaris*, at higher altitudes, and at cooler temperatures, it is predominantly allogamous and perennial. *P. dumosus* shows intermediate environmental needs and characteristics compared with the *P. coccineus* and *P. vulgaris*.

P. acutifolius is drought-tolerant and adapted more to warmer and arid environments. *P. lunatus* (lima beans) is much more adapted to the low-altitude and humid/sub-humid climates, as well as warmer environments (Bitocchi et al., 2017). These beans are also predominantly autogamous and include both annual determinate bush types and indeterminate climbers, which are often perennial (Baudoin, 1988; Salunkhe and Kadam, 1998).

Table 1. Phaseolus species and their main characteristics, based on the sources (Singh, 2016a; Bitocchi et al., 2017)

	Other names	**Geographic distribution of wild forms**	**Presumed domestication areas**	**Mating system**	**Life cycle**	**Genome size (Mbp)**	**Adaptation**	**Altitude (m)**	**Temperature (°C)**	**Precipitation (mm/year)**	**Growth cycle**
P. vulgaris	Common/dry/shell/snap/French bean	Mesoamerica and South America	Mesoamerica and Andes	Predominantly autogamous	Annual	587	Mesic and temperate	50-3000	14-26	400-1600	70-330
P. dumosus	Yearlong/year bean	Mesoamerica	Mesoamerica	Predominantly autogamous	Annual/Perrenial	709	Cool and humid	80-2600	14-24	1000-2600	110-365
P. coccineus	Runner/scarlet runner bean	Mesoamerica	Mesoamerica	Predominantly autogamous	Perrenial	660	Cool and humid	1400-2800	12-22	400-2600	90-365
P. acutifolius	Tepary bean	Southwestern USA to Central Mexico	Mesoamerica	Autogamous	Annual	734	Hot and dry	50-1900	20-32	200-400	60-110
P. lunatus	Lima bean (large seeded), sieve bean (small seeded), butter/Madagascar bean	Mesoamerica and South America	Mesoamerica and Andes	Predominantly autogamous	Annual/Perrenial	685	Warm and humid	50-2800	16-26	0-2800	90-365

Phaseolus crops are widely used for the study of evolution, plant genetics and breeding. The genetic diversity, collinearity, and synteny among their genomes; having different traits such as ranging between annual and perennial, autogamous and allogamous, and their adaptation to variable environmental conditions, even out of their center of origin make these crops important and preferable compared with many other crops (Bitocchi et al., 2017).

1.1.1 Phylogeny of Phaseolus

There are four natural gene pools of the genus *Phaseolus* and all the wild species grow under diverse environmental conditions. The primary gene pool of *Phaseolus* includes wild populations and cultivated varieties of the species; the plants in this gene pool can interact with each other and recombine without a genetic barrier. The secondary gene pool includes the runner bean and the year bean. The crossing between the common bean and the species of the secondary gene pool is possible without embryo rescue; however, using runner bean as a female parent usually requires *in vitro* embryo rescue techniques (Fouilloux and Bannerot, 2011). The tertiary gene pool consists of the tepary bean and the crosses with the common bean requiring *in vitro* techniques. Lima bean belongs to the quaternary gene pool, and no successful crosses between two species have been reported in this gene pool (De Ron et al., 2016). Specifically, even lima beans belong to the Andean gene pool; many varieties show intermediate characteristics that were previously demonstrated by RAPD markers (Nienhuis et al., 2019).

Phaseolus vulgaris has two main gene pools (from Mesoamerica and Andes) and these have been divided into six races (Mesoamerican gene pool: Mesoamerica, Durango and Jalisco; and Andean gene pool: Nueva Granada, Peru, and Chile). The members of each race have diverse morphological, agronomical, physiological and biochemical traits. And they differ from other races in allelic frequencies of the genes which are controlling these traits (Broughton et al.; Singh et al., 1991; Paredes and Gepts, 1995; Pedrosa-Harand et al., 2006). Among these, the races Nueva Granada, Peru, and Chile have a common origin and they seem to have diverged as a result of the human selection (Chacón S et al., 2005). Chile race has larger flat pods, while Nueva Granada has round-podded ones. Andean types also have larger flat pods that show similarity with some Spanish and Chinese accessions (Singh et al., 1991; Singh and Singh, 2016). Specifically, the Mesoamerican genotypes seem to have the best pod characteristics and a higher morphological variability, compared with the other genotypes (Nemeskéri et al., 2018b).

1.2 Domestication

During the domestication process, some traits of the plants were selected to create a superior crop in those characteristics compared with the non-domesticated one. With regard to the beans, ancestry had some effects on the plant characteristics during domestication such as the changes in the seed size, heliotropism, leaf size and phaseolin storage protein type (Wallace et al., 2018). The domesticated gene pools and wild ancestors of the common beans were successfully identified before (Bitocchi et al., 2013). In particular, *Phaseolus* differed from other genera because it had multiple parallels and independent domestication processes. It is thought that at least seven independent and isolated domestication events occurred (Bitocchi et al., 2017).

Some changes that arise under the domestication of *Phaseolus* beans have been the loss of the pod dehiscence (dispersal ability), seed dormancy, the transformation from perennial to annual life form and change in the seed size with a modified shoot architecture (Smartt, 1988).

There might also be an increase in the size of leaves, pods, and seeds; *e.g.* stems may become thicker and leaves larger. Other changes include trans-dehiscence, uniformity of the growth habit along with an increase in the number of pods and seeds per plant, fewer branches, longer to shorter growing period, changes from indeterminate to determinate growth habit (compact growth habit), reduced number of nodes, increase in the permeability of the seed coat (loss of dormancy) and decrease in the anti-nutritional factors.

In the common beans, pod dehiscence mainly depends on the presence of the fibers in the pods both in the walls and the sutures. The loss of these fibers may lead to the indehiscence of the pods and a lack of seed dispersal at maturity (Evans et al., 1976; Ladizinsky, 1987; Smartt, 1988; GIARDINI et al., 2007; Piperno, 2011; Singh and Singh, 2016).

Indeed, most of these characteristics seem to be correlated with each other. *E.g.* the number of pods was found to be positively correlated with the vegetative period (García et al., 1997). A close relationship was found between the number of nodes and the number of pods in the domesticated beans (Acosta Gallegos and Kohashi Shibata, 1989). Furthermore, the wild beans were found to be flowering earlier and having a longer duration of flowering compared with the domesticated ones; also the wild beans had more branches per main stem (Voysest and Dessert, 1991; García et al., 1997).

1.3 The diffusion of the species

Oaxaca valley is considered as the origin of the bean domestication in Mesoamerica, which later probably expanded through the Jalisco state, southern Peru, Southern Bolivia and northern Argentina in South America as the secondary domestication areas (Chacón S et al., 2005; Kwak and Gepts, 2009; Rau et al., 2012; Rodriguez et al., 2016; Singh, 2016a). However, phaseolin sequence studies suggested the origin of the domestication as Ecuador and northern Peru for *Phaseolus vulgaris* (Gepts and Bliss, 1985; Koenig et al., 1990; Koinange and Gepts, 1992; Gepts and Papa, 2003; Kami et al., 2006; Rossi et al., 2009; Angioi et al., 2010; McConnell et al., 2010; Rau et al., 2012; Bitocchi et al., 2012, 2013; Papa et al., 2016; De Ron et al., 2016; Nemeskéri et al., 2018b)

In these areas, the wild *Phaseolus* species are still observed at various altitudes from 1400 to 2100 m. Between the Andean and Mesoamerican gene pools, some differences were retrieved in terms of ability to accumulate lectin, Ca, P, S, phaseolin, and Fe (Islam et al., 2002); seed weight and type of phaseolin (Nienhuis and Singh, 1986; Koenig et al., 1990); diversity and population structures, and the degree of polymorphic markers (Cortés et al., 2011; Bitocchi et al., 2013; Goretti et al., 2014; Singh and Singh, 2016).

Phaseolin is the major storage protein in the *Phaseolus vulgaris* seeds and it can give information about the evolutionary process of the crop (Gepts, 1988). Based on the phaseolin type, the snap beans seem to have the Andean origin, specifically Peru (Koenig et al., 1990; Debouck, 1999). The common bean populations from northern Peru and Ecuador have a specific phaseolin type (Paredes and Gepts, 1995; Kami et al., 2006; Fouilloux and Bannerot, 2011; Rodriguez et al., 2016). In the European countries, the phaseolin types differ from those of the American countries (GIARDINI et al., 2007; Angioi et al., 2010).

1.3.1 Expansion of the crop

Presently, more than 260,000 accessions of different *Phaseolus* species are being conserved in more than 245 gene banks all around the world (Singh, 2016b).

Phaseolus species was thought to be introduced primarily to Spain and Portugal from Central America (Mesoamerica) around the sixteenth century (Berglund-Brücher and Brücher, 1976; Debouck and Smartt, 1995; De Ron et al., 2016). With regard to their use as green or snap beans, there have been two historical milestones: the first one was the invention of the "stringless trait" which was found by CN Keeney in the late nineteenth century. Romano snap beans which were being consumed in those periods had become the predecessors of the snap beans that we're consuming today. The other milestone for the snap bean production was the breeding of a new variety called Blue Lake in the 20th century in Willamette Valley/Oregon. This type had excellent canning and processing characteristics as well as the quality and yield traits (Singh and Singh, 2016).

The majority of the European common bean landraces (67%) were found to have an Andean origin. In a study dealing with the genetic diversity in Marche, 71% of the local *P. vulgaris* varieties in Marche were found to have the Andean origin (Sicard et al., 2005; Bellucci et al., 2014).

The origin of the bean accessions varies from Europe to Turkey, where white seed bean types were probably introduced through the Silk Route (De Ron et al., 2016). In particular, China is one of the most important exporters of snap beans in the world. However, it is thought that the snap beans were introduced to China through Japan. The majority of the Chinese snap bean varieties seem to have the Mesoamerican origin (Kumar et al., 2006; Zhang et al., 2008; Michael and Jackson, 2013; De Ron et al., 2016).

1.3.2 Types of beans and their growth habit

Common beans have 3 main uses (green or snap bean, shell bean, and dry bean) and the traits of the genotypes are usually evaluated depending on the traits needed for their usage.

Green or snap bean: The snap bean genotypes (**Figure 2.**) are selected for their succulent and tender pods with reduced fibers and strings (Nemeskéri et al., 2018b). The pods of the snap beans, generally contain the least inedible fiber as possible (no fiber at all is preferable) in the pod wall (Devi et al., 2015). The immature pods and seeds are consumed as the whole vegetable. Snap bean market classes are based on the pod characteristics and the plant type (Myers and Baggett, 1999). Briefly, the most important traits in snap beans include reduced pod wall fiber, the absence of the pod suture strings and thickened but succulent pod walls. Snap beans are generally selected for the round pod cross-section and long cylindrical shape. Specifically, dry bean genotypes have many interesting traits that could be transferred into the snap bean genotypes (Krause et al., 2012). This is because many snap bean genotypes have fibrous pods which make them less valuable for industrial processing (freezing, canning) (Cuesta-Marcos et al., 2016). For this reason improving the pod characteristics/traits have high importance in snap bean breeding both to satisfy the consumer preferences and to create superior crops which give higher yield (Dorna et al., 1993).

As an important factor, to obtain the optimum harvesting and rheological quality; all the green bean pods independently from their color, should be harvested before becoming mature and while still, they're growing. This means that they should have a bright color and fleshy, small seeds which usually coincide about 8-12 days after the flowering. If the harvesting date delays after the optimum maturity level is reached, the seeds get more mature, the pods become tougher and more fibrous, also the original greenish color fades away. All these changes lead to an undesirable reduction in quality (Singh, 2016b).

It is hypothesized that the snap beans were derived from dry beans through a series of mutations; first having a reduction in the pod fiber, then having changes in the pod shape and lastly, being bred into the stringless types (Singh and Singh, 2016). During these changes; it is thought that the genetic effects were higher than the environmental ones for the pod length, pod shape and the percentage of pod fiber (Mariguele et al., 2014).

Specifically, Romano beans are one of the most consumed snap beans. Romano beans have green pods, but a flat cross-sectional shape and comparing with other types of snap beans, these are almost fiber-free. In contrast to Romano types, other green beans have fleshy pods that are generally oval to round in cross-section. Wax beans are similar as shape to that of green beans but these have pale yellow to golden-colored pods as a consequence of the absence of chlorophyll in the pods, petioles and the young stems (Myers and Baggett, 1999).

Shell bean: Shell beans are harvested before the complete desiccation and the seeds are developed in the pods. Shell beans are more expensive in terms of manufacturing than dry beans because they must be harvested and shelled manually. Furthermore, they should be consumed shortly after the harvest or should be preserved by freezing because these contain high levels of moisture (del Castillo et al., 2010). Seed color is an important trait in both shell and dry beans, especially for the cultivars to be used for industrial processing. Anthocyanin pigments are responsible for the red and purple colors, including the parts of hypocotyl, seeds and the flowers (Leakey, 1988; Macz-Pop et al., 2006)

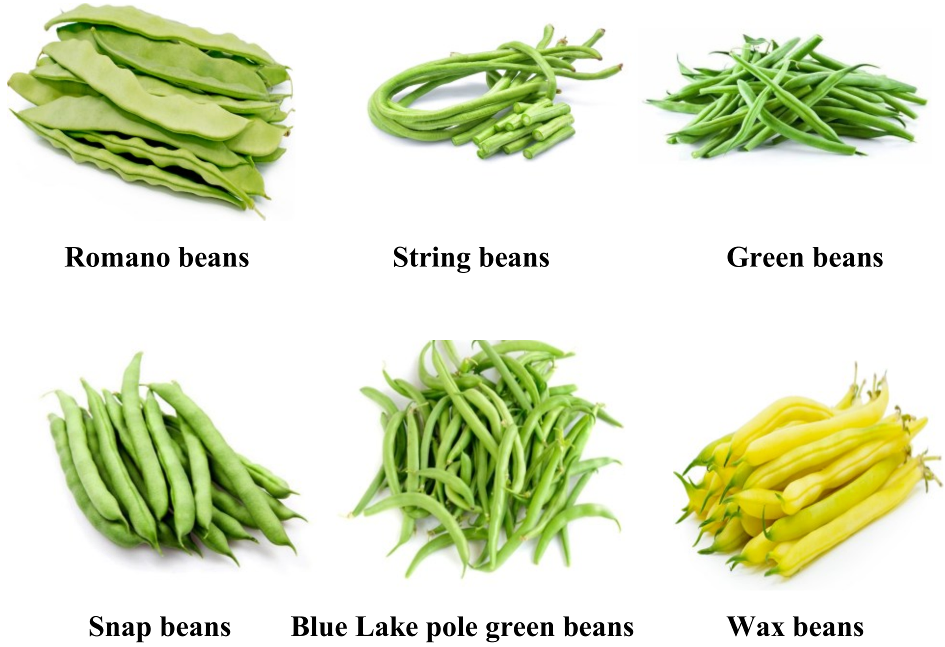

Figure 2. Green bean types

Dry bean: Dry beans are usually selected for their seed characteristics (color, shape, and size). Dry beans have a different pod shape and more pod fiber comparing with snap beans (Wallace et al., 2018). The seeds are harvested when completely become mature (del Castillo et al., 2010). The food industry prefers white-seeded cultivars because that the flavonoids in the colored seeds can change the color of the liquid in the can during and/or after the processing (Myers and Baggett, 1999). From an agronomical point of view, the advantage of the flavonoids is their positive effects on the germination and the emergence. Also due to their presence, the plant becomes more resistant to the pathogens. White seeded beans generally have reduced germination and emergence (Soltani et al., 2003; Martín-Cabrejas et al., 2014; Cuesta-Marcos et al., 2016).

Common beans have various growth habits (**Figure 3**) (García et al., 1997). Broadly, common bean genotypes can have two distinct growth forms of the stem: Type I bush types (determinate growth) and Type II pole types (indeterminate growth). Stem growth habit is controlled by three genes as L/l (long stem vs. short stem), A/a (indeterminate growth habit vs. determinate growth habit) and T/t (twining tendency vs. non-twining tendency). Both determinate and indeterminate types have green, wax and purple colored pods with or without the fiber (Myers and Baggett, 1999; Singh, 2016a). In general, the "bush" determinate types of *Phaseolus* beans are more common and they are mostly used for the shell and dry bean production. Because these also have shorter harvesting periods compared to the indeterminate types, more concentrated time of flowering and commercial maturity (Portal et al., 2018). Most of the tropical cultivars are indeterminate "climbing" types; while the determinate "bush" beans are more common in temperate zones (Blair et al., 2010). However, also in the temperate zones, indeterminate "climbing" types are mostly used for the green bean production for their capacity of giving higher yields as well as continuously flowering and producing pods (Paalhaar and Jansen, 2011). The bean varieties can also be classified as early or late, based on the time required for their physiological maturity. It has been observed that the bush cultivars are earlier than the climbing ones (Davis and Woolley, 1993).

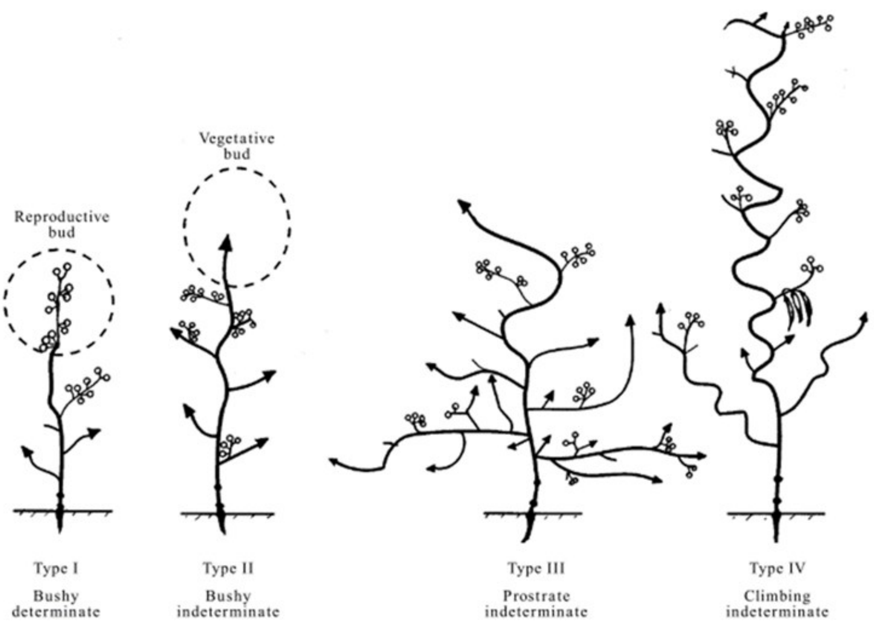

Figure 3. Common beans have different growing habits (García et al., 1997).

1.4 Yields and yield components of beans

There are many traits which can directly/indirectly affect the yield and these include growth habit, plant height, leaf number and size, number of primary branches, internode length, phenology (and especially days to flowering, and duration of flowering), number of flowers and pods, and pod fertility (usually measured as number of seed per pod) and harvest index. Some other pod traits are related more with the quality of the yield than quantity. These include the length of the pod, the sieve size (or pod width, measured through the ventral and dorsal sutures), the pod thickness (measuring through sidewall to sidewall), the pod cross-sectional shape, the pod straightness (curvature), the pod smoothness, the rate of the seed development, the stringiness on sutures, the fiber content in the pod wall, the presence of the interlocular cavitation, the point of detachment, the shape and the length of the spur, the flavor, the internal color of the pod and its texture. (Silbernagel and Drake, 1983; Myers and Baggett, 1999; Singh and Singh, 2016).

Indeed, the yields of snap beans, shell, and dry beans can dramatically vary both in terms of total dry matter of the pod or seed and, above all, when measured as the fresh matter. The differences for the pod, flavor compounds and agronomic practices needed for snap and dry beans also depend on the genotypes (Tofiño et al., 2004; Nemeskéri et al., 2018b).

1.4.1 Yield distribution in the world

Yield is a complex quantitative trait with low heritability, which can be modified with various environmental (including agronomical) conditions. The production of both dry and snap beans was increased significantly in the world during the last ten years (31,405,912 tonnes for dry beans and 24,221,252 tonnes for green beans in 2017; as it was 21,737,860 for dry beans and 18,017,731 tonnes for green beans in 2007). However, the harvested area in the world is still not as much due to the environmental stresses which have a negative effect on the yield. The production of the green beans and dry beans; respectively, 1,450,050 and 29,300,690 tonnes in 2007 haven't increased drastically during the last ten years; being 1,579,971 tonnes for green beans and 36,458,894 tonnes for dry beans in 2017. According to the Food and Agriculture Organization (FAO), the cultivation of *Phaseolus* crops is increasing each year; as it was 9 million in 2010, it became 21 million in 2014. The leading manufacturers are China, Indonesia, and India (Roberto dos Santos Trindade, 2012; Cruz et al., 2018; Oliveira et al., 2018b).

India, Myanmar, Brazil, USA, and China are the main producers with 6,4 Mt/yr, 5,5 Mt/yr, 3,0 Mt/yr, 1,6 Mt/yr, and 1,3 Mt/yr, respectively. Africa also contributes to a significant level of production, whereas in the European countries the dry bean production is lower.

China, Indonesia, India, Turkey, and Thailand are the most important producers of the green beans; with 19,2 Mt/yr, 0,9 Mt/yr, 0,6 Mt/yr, 0,6 Mt/yr, and 0,3 Mt/yr, respectively. In contrary to the dry beans; most of the green bean production is coming from Asian countries. Europe and America have a lesser contribution to green bean manufacturing.

1.4.2 Role of the management on yield and yield components

Plant density, planting pattern, quality and quantity of the seeds planted, time of sowing and fertilization are the factors affecting the yield components of the green, dry and shell beans (Sotomayor-Ramírez et al., 2008). P fertilization was found to have a positive effect on various yield components of the Red Wolaita variety of common beans (Turuko and Mohammed, 2014a). A related study was conducted at Arba Minch/Ethiopia in the rainy season of 2011. The experiment was designed as a randomized complete block design with three replications for each treatment. First, the field was treated with 60 kg/ha of N before the five different levels of P fertilizer were applied (0, 10, 20, 30 and 40 kg ha^{-1}). Results demonstrated that the P fertilization significantly increased the dry matter yield, yield components and some growth parameters like leaf area and number of branches per plant. However, no effect was observed for the plant height. 20 kg ha^{-1} P fertilization was recommended for the optimum production in the conditions studied (Turuko and Mohammed, 2014b). In another study, Puebla 152 and Carioca cultivars together with the breeding line UW 24-21 were either inoculated with rhizobia or treated with 150 ppm of N. And each genotype and nitrogen combination was grown at 8 levels of P. It was observed that the P level was directly correlated with the shoot dry weight, nodule number and nodule mass (Pereira and Bliss, 1987).

Cold stress is one of the major factors which affects the yield of *Phaseolus* beans. Green bean, *Phaseolus vulgaris* L. ssp. *volubilis* is one of the main greenhouse crops on the Spanish coast as well as it is in many other Mediterranean countries. It was shown that the growth of green beans was inhibited below the temperatures of 10–12 °C (López et al., 2008). The minimum temperature range for the seed germination was found for the cultivars IAC-Carioca-80 SH' RC, Rosinha G2, IAC-Carioca-. Akytã, Guarumbé, Iratim, Vermelho-2157, Campeão-1, Rudá and Aporé between 8≤t[°C]<13 °C. For IAPAR-57, it was found to be 13≤t[°C]<16 °C and for IAC-Carioca-80 SH' PP it was 16≤t[°C]<18°C. The maximum temperatures for the seed germination were found as 31≤t[°C]<35°C for IAPAR-57, Guarumbé and Iratim cultivars; and 35≤t[°C]<39°C for all the other cultivars (Neto et al., 2010). Konsens et al. (1991) found that under moderate temperatures (e.g. 27°C for day/17°C for the night) the onset of pod development was associated with cessation of flower bud production and with enhanced abscission of flower buds. Furthermore, under 32/27°C day/night temperature a large reduction in pod set was observed, which was thought to be related to the enhanced abscission of the flower buds, flowers and the young pods (≤ 3 cm) (Konsens et al., 1991). Especially during germination, the snap bean crops are very sensitive to the low temperatures.

The germination rate of the green beans decreases at 10°C, while temperatures between 18-20°C are considered as optimum (Kurtar, 2010). The time of sowing and plant spacing are also important factors that directly affect the pod yield. (Yoldas and Esiyok, 2009) found that when the sowing date of the various green bean genotypes studied (*Phaseolus vulgaris* vars. Amboto, Gina, Nassau, and Volare) in Turkey was delayed (August), the yield was decreasing (10926,7 kg) while those which were sown earlier in July had the highest yield (12783,7 kg ha^{-1}).

(Pérez-Barbeito et al., 2008) have studied with 35 local and 5 control cultivars (Maravilla de Venecia, Oro del Rhin, Felisa, Maravilla de Venecia var. Rapid, and Dorada de Aragón) in Spain (Tomiño and Salcedo) which were grown simultaneously for two years (2003 and 2004) and sown in three different planting seasons including: early (February-May), normal (May-August) and late (August-November). They found that the fresh pod yields were highest in the early planting season (February-May); considering that the longer pod maturation phase could be one of the main factors. Furthermore, earlier planting season seemed to contribute to a longer growing vegetative cycle and greater productivity than normal/summer and late autumn planting. Thus, the earliest maturing snap bean cultivars had the highest fresh pod yields in late planting seasons, while the latest maturing snap bean cultivars had the highest yields in early and normal planting seasons. Other studies also demonstrated that a higher number of pods were obtained in early sowing, and higher pod dry weight was obtained when the bush bean was planted at lower densities compared with wider spaces (Abubaker, 2008a; Getachew et al., 2014). However, there are other findings that show that as the planting density increases, the marketable pod yield of bush snap beans also increases (Mack et al.; Field and Nkumbula, 1986; WAHAB et al., 1986). Another study suggests that; higher plant populations, together with a more frequent irrigation and N fertilization, can give higher yields in French beans (*Phaseolus vulgaris* L.) (Sci and 1976) On the other side, some research show that wider row spacing seems to increase the number of pods, probably due to a reduced competition between the plants for the nutrients (Yoldas and Esiyok, 2009; Getachew et al., 2014). Furthermore, it was found that as the planting density decreases, the N, P, K and protein contents of the dry snap bean pods increase (Abubaker, 2008b).

1.5 Quality aspects of beans

1.5.1 Bromatological composition and sensory traits

Green beans are relatively fast-maturing crops in which the pods are being harvested immature especially for industrial processes. For measuring maturity, one of the most satisfactory methods is measuring the length of the developing seed within the pod. All the sensory characteristics (texture, aroma, flavor, appearance) are highly related to the physicochemical composition of the crop (Martínez et al., 1998a). It is known that the consumers prefer sweetness, juiciness, hardness, and fibrousness as the important sensory traits in the snap beans (MARTINEZ et al., 1995a; Mkanda et al., 2007).

One round (Strike) and one flat (Bina); in total two different varieties of green beans at different stages of maturity were studied for nine physiochemical and seven sensory variables which were grown in the greenhouse at Murcia in June 1992. Strike cultivar was identified by pod diameter following the seed company's recommendation as <6,5 mm, 6,6-8 mm., 8,1-9,0 mm and 8,1-9,0 mm. Bina variety was classified into two sizes including 10,0-15,0 mm. and 15,1-20,0 mm. Moisture, chlorophyll, soluble solids content and acidity were highly related to the growth and maturity of round green beans. And all of the parameters increased during the development of the plant; except chlorophyll. According to the panel tests performed; fibrousness, juiciness, and hardness were found to be the most important factors for the acceptance of the cooked green beans (Martínez et al., 1998b).

Three varieties of green beans: Cleo, Strike, and Sentry (Asgrow, Seed Co), were grown in a greenhouse at the Murcia, being harvested in June 1993. All the green bean pods were classified according to their pod diameter including extra-fine <7.0 mm, very fine 7.0-8.5 mm, fine 8.6-10.0 mm. and medium >10.0 mm. It was found that the ash and fiber content of Cleo and Strike varieties were increasing together with the pod size. It was concluded that the pod size had a significant effect on the chemical composition, protein digestibility, antinutritional factors and mineral availability of the green beans depending on each variety which are grown in the same conditions. Small pods were containing higher antinutritive factors which might affect the mineral availability of the snap beans, negatively (Martínez et al., 1998a).

In another study, the green bean cultivars Masai and Odessa were grown under standard greenhouse conditions at Wageningen/Netherlands in 1999. During the pod development, the cell wall material of the pods increased on a dry weight basis. The point where the white flowers of crops were turning into yellow, was indicated as the development of the bean pods and this stage was assigned as 0 daf (days after flowering). During the 0-7 daf, the seeds could not be separated from the pods. Between the 8-13 daf, the pods were observed to be extended approximately to 10 mm in length per day. After this stage, the seeds could be separated from the pods. Between the 14-23 daf stage, there was a cessation of pod elongation, in which the average pod length was measured to be 117 mm in length. Between 24-55 daf, the pods, as well as the seeds, became mature and seed cushion was started to degrade. This process is followed by the change of the color in the pods. After the 55 daf period, the dehydration and browning of the pods had begun and some mature seeds started spreading from the crop. The water content of the pods increased in the first stages of the pod development, while the high molecular mass components including proteins and starches decreased (Stolle-Smits et al., 1999).

There are three main compounds that contribute to the color of the snap beans: these are phenolic compounds, carotenoids, and chlorophylls. The major carotenoids in green beans are lutein and β-carotene. In a study, the potential differences of the chlorophyll and carotenoid content of Calisto cultivar and fresh green beans which were bought from a local supermarket were studied. All the pigments contributing to the color were found to increase after the green beans were frozen (Valverde and This, 2008). However, there are many other chemical processes that are responsible for the color degradation in green beans. One of the most important processes is the pheophytisation of the chlorophyll (Heaton et al., 1996; Martins and Silva, 2004).

The total chlorophyll content of the green beans is found to be associated with the pod length. Up to 11 mm diameter of pods, the chlorophyll synthesis seemed to increase in the field-grown green beans. However, after the maturation, it was found to be decreasing. In general, mature green bean pods were containing 300 ppm chlorophyll on a dry weight basis (Mayland, 1971). On the other side, the recent cultivars which are used for human consumption may contain higher levels of chlorophyll due to selective breeding, as color is one of the most important visual traits for consumer preferences.

Chemical compounds in the pods of the Berggold, Paulista, Re dei Burri, and Top Crop cultivars were examined in a research at Ljubljana/Slovenia, from April to July 2011. It was found that the pod size had a positive effect on the chemical compounds of the pod. Younger half-sized pods were containing higher fructose and glucose content as well as malic and citric acids comparing to the full-sized pods. However, the younger pods were containing significantly lower amounts of vitamin C and sucrose compared to the older pods (Selan et al., 2014).

1.5.2 Volatile compounds

HS-SPME analysis on cooked beans showed that most of the volatile compounds were getting lost during cooking. A total of 104 volatile compounds were identified in *Phaseolus vulgaris* L. which was studied. These were alcohols (23,62%-62,20%), terpenoids (2,45%-39,15%), heterocyclic compounds (1,21%-13,78%), hydrocarbons (2,22%-13,87%), esters (0,98%-12,98%), aldehydes (2,84%-4,45%), ketones (1,08%-1,53%) and sulphur compounds (0,52%-1.49%) (Barra et al., 2006). Aldehydes are known to be formed as a consequence of thermal Strecker oxidative degradation of amino acids and fatty acids (Williams and Allen, 1996; Tressl and Rewicki, 1999; Barra et al., 2006). Some other heterocyclic compounds were identified such as pyridine, furfural, and thiophenes, which were formed through the Maillard reaction between the sugars and the amino acids (Barra et al., 2006).

1.5.3 Acids

Dwarf bean is a rich source of folic acid, vitamin B6, minerals, carotenoids, carbohydrates, and various bioactive compounds. The most abundant organic acid in the dwarf beans is the malic acid (more than 90% of total organic acids), which is followed by citric acid. Tartaric and fumaric acids were found in trace amounts in the pods (Selan et al., 2014). Even though the snap beans contain a certain amount and combination of organic acids, in some cases these might decrease the quality of the vegetable. *E.g.* if large quantities of malic acid are being accumulated in the vacuoles of a vegetable, during cooking and processing some undesirable color changes might happen (MARTINEZ et al., 1995b). In addition to the malic, citric, tartaric and fumaric acids, some varieties may contain also pyruvic acid (Lake Shasta, Cascade, and Lake Geneva). Also in some varieties (Slimgreen and Cascade), lactic acid concentration was found to be higher (BIBEAU and CLYDESDALE, 2016).

1.5.4 Antinutrient compounds

Maturity of the seeds of dry beans is important as the maturity directly affects the protein and fiber levels. Common beans contain some anti-nutritive factors such as trypsin inhibitors (a protease inhibitor), which may influence the digestibility of the protein in legume seeds depending on the variety (8,8-26,8 TIU mg/g) (Janzen et al., 1976; Nemeskéri, 2002; De Mejía and Prisecaru, 2005). The colored seeded beans have a higher trypsin inhibitor activity; particularly red kidney beans (Nemeskéri, 2012). Other anti-nutritive factors, which lower the digestibility of common beans are amylase inhibitors; lectins; phytates and tannins. However, the activity of these can be decreased by thermal treatment (Khattab et al., 2009).

1.5.5 Proteins and amino acids

Leucine and glutamic acid are found in high levels in the beans (ORUNA-CONCHA et al., 1996; Tessari et al., 2016). Specifically, snap beans contain high levels of aspartic acid and leucine; almost 13,8-14,5/100 g and 8,85-9,94 g/100 g, respectively (Oseguera Toledo et al., 2016; Tessari et al., 2016). In the snap beans, the cysteine is generally oxidized into the cysteic acid. For this reason, snap beans do not contain significant amounts of cysteine (Fukuji et al., 2019). However, colored pods with larger seeds do contain higher amounts of methionine, cysteine, phenylalanine, and leucine comparing with white-seeded genotypes (Oseguera Toledo et al., 2016). Dry beans contain high levels of serine, leucine, phenylalanine and histidine contents compared with the other legumes (Gyõri et al., 1998). Irrigation may increase the levels of some amino acids such as leucine, valine, glycine, and proline (Nemeskéri, 2012). Genotypes that have higher yields have also higher protein content (Oseguera Toledo et al., 2016). Depending on the variety, the common bean seeds may contain 24-31% protein (Nemeskéri and Nagy, 2003; Nemeskéri, 2012; Nemeskéri et al., 2018a). According to data, the protein contents of the landraces range between 16,54% to 25,23%, while the protein contents of the modern varieties range between 19,70% to 24,30%. Furthermore, the protein content of the cultivated beans is less (16,6–24,6 g/kg) than those of wild beans (18,8–33,3 g/kg) (Guzmán-Maldonado et al., 2000).

The main proteins of the common beans include 12-30% albumin, 54-79% globulin of the total protein, and high amounts of glutelin (20-30%) (Campos-Vega et al., 2010). Phaseolin is the main globulin fraction and it forms 40-50% of the total seed proteins (Derbyshire et al. 1976). Even though it is resistant to *in vivo* digestion; digestion can be improved up to 90% through cooking (Genovese and Lajolo, 1998; Montoya et al., 2006; Campos-Vega et al., 2010; Nemeskéri, 2012). Lectins are undesired proteins that are found in the common beans. These are glycoproteins which agglutinate erythrocytes in blood *in vitro* (Kim et al., 2010). Even though they're known to be toxic; they can be highly deactivated through cooking. In kidney beans, lectins and moderate trypsin inhibitors are found between 2,4-5% of the total protein content (17-23%) (Genovese and Lajolo, 1996; Campos-Vega et al., 2010).

1.5.6 Lipids

According to the United States Department of Agriculture Food Composition Database, raw green beans contain 0,22 g of lipids per 100 gr which is quite insignificant compared with other major chemical compounds (https://ndb.nal.usda.gov/ndb/foods/show/11052). The common bean seeds contain between 0,98-2,53% crude fat (Nemeskéri et al., 2018b). Mono-unsaturated fatty acids (MUFA) content varies between 19,42% to 24,26% in the landraces and between 21,90% to 25,86% in the modern varieties. And the modern varieties contain the fat between 0,33% and 1,33%, while the landraces contain between 0,33% and 1,00% (Guzmán-Maldonado et al., 2000).

The lipid contents of different genotypes aren't affected by the growing conditions with the exception of being decreased by irrigation (Florez et al., 2009). The dried edible seeds of two *Phaseolus vulgaris* cultivars which were grown in Northern Nigeria were analyzed and found to contain 1,14 - 3,03% of fat (Onwuliri and Obu, 2002). Specifically, lipids in green beans and common bean seeds not only contribute to the chemical content but also to the taste of green beans via the formation of some volatile compounds. In the green beans *trans*- 2- hexanal, 1- penten- 3- ol, 3- penten- 1- ol, *trans*- 2- hexenol and *cis*- 3- hexenol; in common beans, 1- penten- 3- ol and 3- penten- 3- ol with a small amount of *trans*- 2- hexenal were formed due to the formation of linoleic acid (de Lumen et al., 1978).

1.5.7 Secondary Compounds

Phenolic compounds are secondary metabolites that protect the plant cells against lipid peroxidation, protein denaturation, DNA damage, etc. Phenolics also support the plant through pigmentation, growth, reproduction and give resistance to various environmental stress factors (Nascimento & Fett-Neto, 2010; Weidner et al., 2018). The primary phenolic acids found in the snap bean pods are caffeic acid, p-coumaric acid, ferulic acid, and sinapic acid. These comprise 2,79-5,34 mg/g extract of the white bean varieties (Weidner et al., 2018). The green-podded cultivars seem to have a higher content than yellow-podded varieties. Nyau et al. (2016) demonstrated that the red beans had higher amounts of total polyphenols than the grey mottled, brown and white beans (Reynoso-Camacho et al., 2006; Nyau et al., 2016). Other legumes together with white beans such as chickpea, pea, and lentil are also known to contain lower amounts of phenolic acids (Reynoso-Camacho et al., 2006; Weidner et al., 2018).

1.5.8 Minerals

The potassium content of the fresh pods is found to vary between 24,3 g kg^{-1}-34,6 g kg^{-1} (Carović-Stanko et al., 2018). In addition, a positive correlation was found between the P and K content (Carović-Stanko et al., 2018).

(Quintana et al., 1999) studied the Ca contents of thirty-nine crops which were widely consumed and found that the green beans were the third crop highest in Ca among all other vegetables. Normally, different cultivars are expected to accumulate different amounts of Ca in their pods. Two studies found the pod Ca content of snap bean cultivars between 3,82-6,80 mg/g (Miglioranza et al., 1998; Quintana et al., 1999).

(Celmeli et al., 2018) found the Zn content of the common bean landraces between 17,81 and 37,90 mg/kg, and the modern varieties between 25,03 and 35,1 mg/kg. Additionally, a correlation was found between the Zn and the Se contents (Celmeli et al., 2018).

1.6 Aim of the work

There are two principal aims of this thesis which can be described as below:

- To understand and detect the differences of certain chemical traits between various snap bean genotypes; mainly sugar, acidity, and pH;
- To determine the differences between selected genotypes in terms of key plant traits, including seed shape, seed color, yield components, duration of flowering and fruiting.

2 Materials and methods

The main aim of the thesis was to study the genotypic differences of the green beans (*Phaseolus vulgaris*) in terms of sugar, acidity, and pH.

The experimental setup included:

- **Experiment 1:** A preliminary experiment aimed to choose a proper method to analyze the sugar and acidity contents of the green bean genotypes chosen,
- **Experiment 2:** A pot experiment aimed to evaluate the yield potential and quality of various bean genotypes which were collected various times,
- **Experiment 3:** A field experiment aimed to evaluate the yield of the green beans and the sugar contents of variable genotypes of *P. vulgaris* which were either inoculated or not inoculated with the arbuscular mycorrhizal fungi (AMF),
- **Experiment 4:** A systematic mapping about the resistant starch (RS) which is found in mainly common beans, as it is an important but understudied trait in this group of species.

2.1 Experiment 1. Methodologies for the determination of the sugar and acidity

Various green beans with variable morphology were purchased from different supermarkets at Ancona in December 2017; and February, April 2018.

The green beans were prepared and analyzed according to the protocol described in (Proulx et al., 2010). The procedure of the sample preparation, sugar, and acidity analysis both for the preliminary and the main experiments are described below:

Sugar content:

- ≈20 g of sample for each replicate was weighed and placed in the plastic plates (for the frozen samples, the plates were exposed to the room temperature for a certain amount of time depending on the dissolution of the green bean tissue),
- All the samples in the plates were chopped into the small parts by knife,
- Chopped samples were broken into smaller tissues (homogenization) with the diverse methodology to understand which was the most accurate and compatible method for the

current study; which will be described later in detail. Homogenizing was made either through a home blender, a home-type juicer, Ultra Turrax T25 homogenizer or liquid N as will be described later. Then, the homogenized green beans were put into the Falcon tubes separately,

- After homogenizing, ≈10 g of distilled water was added to each sample,
- Samples were put into the centrifuge at the speed of 800 gN for 30 min,
- "Filtering through the cheesecloth" as it was mentioned in the original protocol was not applied due to the laboratory facilities and the organizational issues,
- After centrifuge, the clear green bean juice was transferred into new Falcon tubes;
- For the determination of Brix quantity: 3 drops of clear crop juice were taken from each Falcon and they were inserted into the related part of the hand refractometer (Atago N-1E hand refractometer and Atago PR-101α Palette automatical refractometer);
- The results were expressed as the percentage of sucrose in all the green bean samples, separately.

Acidity:

- ≈6.0 g of aliquots (clear green bean juices) from each sample which was taken from the Falcon tubes after the centrifuge was diluted with 50 ml of distilled water and put into the beaker;
- Each sample was titrated with 0,1 *N* NaOH (Sodium hydroxide) to the endpoint of 8.2 after adding 3-5 drops of Bromothymol Blue on a magnetic stirrer (Hanna HI 190M) with a bar inside, which helps to homogenize the solution during the analysis,
- The milliliters (*ml*) of NaOH used were recorded and obtained results were expressed as the percentage of malic acid,
- For the conversion of the NaOH into the malic acid this formula was used: [(mL NaOH x 0.1 N x 0.064 meq/6.00 g of juice) x 100] (Proulx et al., 2010).

As mentioned before, some different techniques were used to break and/or to homogenize the tissues of the green beans. These were:

- ✓ A home-type blender (Moulinex Turbomix R30 R1),
- ✓ A home-type juicer (Bosch CNC J03),
- ✓ Ultra Turrax T25 homogenizer (Tanke&Kunkel IKA-Labortechnik),
- ✓ Liquid Nitrogen.

All of the methods used had some advantages and disadvantages. *E.g.* the home blender and fruit juice extractor could not break the tissue of the green beans efficiently. To break the tissue of the green beans or to extract enough clear juice, a mass amount of green beans was needed. It was also time-consuming compared with the other techniques which require the cleaning and re-preparing the instrument. Another instrument used (Ultra Turrax T25 homogenizer) didn't work efficiently to break the tissues of the green beans. At the end of the process, most of the green bean tissue has remained intact. Instead, the utilization of liquid N was fast, accurate and easy to apply. The only disadvantage was the higher costs compared to the other methods.

The protocols were repeated with the pods from the plants which were obtained from the greenhouse experiment. In particular, the genotypes Midas and MG38 were sown in a 98x27x37 cm pot in February 2018 (10 replicated seeds per genotype, 1 plant per pot) or March 2018 (20 replicated seeds per genotype, 1 plant per pot). Plants were grown until the pods were ready to be collected. The pots were filled with a commercial substrate (1:4 ratio of perlite to soil).

2.2 Experiment 2. Comparisons of 6 genotypes grown in pots in the greenhouse

The last greenhouse experiment was established in July 2018.

The aim of the experiment was studying the yield of six green bean genotypes which were grown in the pots in the greenhouse of the Department of Agricultural, Food and Environmental Sciences (D3A) of Marche Polytechnic University. The genotypes used were two parental lines of the RIL (Midas and MG38), two genotypes among the best crosses of the parental lines (FG21 and FG26) and two elite cultivars (Moonstone and Kysia). The RILs are created through the crossing of two inbred strains, followed by the repeated generations of selfing or sibling mating to produce an inbred line. In the end, a new line with a genome which is a mosaic of its parental lines is formed. The experiment was a randomized block design with six replicates.

The following procedures were followed in the experiment:

1. The first stage was the germination of the seeds. The Petri dishes were prepared for the germination. Sticky labels were put on the outside of the Petri dishes in which the genotype, replicate number and the dates were written.

2. A filter paper was put into each Petri dish and 10 ml of distilled water was added to give some moisture for the seeds to germinate.
3. In total 72 seeds were placed onto the filter paper which was already placed into the adequate number of the Petri dishes before.
4. After adding the seeds into the Petri dish, a few drops of more water were given on the seeds to provide the prerequired humidity for germination.
5. Each Petri dish was covered with Aluminum foil to avoid light exposure.
6. First, the Petri dishes were put into the fridge to facilitate the germination process for one night; the next day they were transferred into the room temperature, being left in a non-humid and dark ambient for a few days for the germination to proceed.
7. Periodically the seeds were controlled to see if they were germinated or not. The same process was repeated for other seeds that were not germinated.
8. After the germination of all seeds was completed, the seeds were weighed and the total weight of each 12 seeds per genotype was measured and recorded.
9. Then, the germinated seeds were transferred to the greenhouse to be sown in the pots prepared before.
10. During the germination of the seeds, all the pots were washed and dried before the sowing process in the greenhouse was initiated.
11. After the germination of the seeds, each pot which was already filled with 5 kg of soil + 0.5 kg of perlite (density 0.22 t/m^3) and sown

with two pre-germinated seeds for each replicate. Pots were given water gently until the drainage water was appeared and then, all of the pots were weighted. This measured weight was considered as the total weight of the pot with the 100% moisturized soil.

12. Before putting water, weights of each pot were measured and recorded and an amount of water corresponding to the loss of weight was given. Such procedure was repeated 2 to 3 times per week depending on the air temperatures.
13. The day of the emission of flowers and their position was recorded 3 times per week, the height of the plants was also recorded.

All the data about the yield, mean pod weight and the number of pods were analyzed with a general linear mixed model in SAS/STAT 9.2 and differences among means separated with a Tukey Kramer test.

2.3 Experiment 3. Role of the inoculation with arbuscular mycorrhizal fungi on bean quality under field conditions

The experiment was performed in the experimental station of Agugliano (AN, 43.545N, 13.363E, 80 m asl), located around 20 km in the inland of Ancona, Marche. The site has a 780 mm mean yearly rainfall and a mean annual air temperature of 13,3 °C. The soil is Vertic Haplustept. The experiment was established on the 3rd of June 2019. The experimental unit was a pot with 16 plants in a bine: interline distance was 1,6 m, intra-bine row distance was 0,2 m, the distance between 2 adjacent plants in a row was 10 cm. The experiment was hand sewn.

The genotypes used were as in **Table 2**.

Table 2. List of the genotypes used in the field experiment

Group	Genotype	Code	Group	Genotype	Code
Cultivar	Rocquencourt	1	**Introgression Line**	IL_250/309	17
	Marconi nano	5		IL_320/962	19
	Metro o Stringa	6		IL_321/967	20
	Purple king	8		IL_398/1603	23
	Contender	11		IL_471/1870	27
				IL_77/2259	31

Two different treatments were applied, including the genotype alone and inoculation with the arbuscular mycorrhizal fungi (AMF). AMF is a soil-borne microorganism which enhances a mutualistic symbiosis with the cultivated species and helps them exchange the nutrients from the soil through the contribution of fixed Carbon.

The AMF used was *Glomus iranicum var. tenuihypharum* (120 propagules/gram of inoculum, referred to as MYC) applied at a rate of 2 g inoculum/plot as a seed coating, compared to a non-inoculated treatment (referred as CONTROL).

The experiment received 20 mm irrigation twice per week by means of low pressure (<1 atm) drip irrigation system. The irrigation was avoided for four days (from 9th to 12th July) due to two rainfall events (28 mm on the 9th of July and 38 mm on the 10th of July).

The plants were not given any fertilizer and were hand weeded.

During the growth, flower emissions were counted. Pods were harvested when they were at 7 cm length. Then they were counted, weighed and analyzed for their sugar content and other characteristics mentioned before, according to the protocol described in Experiment 1.

The mean pod weight, data of the yield and the number of the pods were analyzed with a general linear mixed model in SAS/STAT 9.2 and the differences among means were evaluated with a Tukey Kramer test.

2.4 Experiment 4. A systematic mapping on the resistant starch content in beans

Resistant starch is an important component of many foods. RS can be found in plenty of the plant species, but most of the studies indicate the starchy foods as the primary nutritional resources; especially the cereals. The reports available for the legumes, especially the beans are fewer.

Detailed research and collection of the relevant reports on the common bean (and especially the genus *Phaseolus*) were made through a systematic mapping on the Web of Science (WoS v. 5.31) and Scopus (dates of collection: 12th to 17th October 2018, see supplementary material for the raw files). Systematic mapping is a detailed research which aims to synthesize the whole scientific evidence that exists on a particular subject [See (Schillaci et al., 2018; Saia et al., 2019) for further details and the guideline by the Social Care Institute for Excellence (SCIE, at http://www.scie.org.uk/research/maps.asp)].

This systematic mapping aimed to focus on the studies of the resistant starch in the common beans. The search was carried out, using “title+abstract+keywords”. In the WoS, “all databases” were selected and analyzed.

To achieve this, we firstly expanded the research into a wider aspect in both databases (WoS and Scopus) as “resistant starch” with no further specification. Additional researches included “resistant starch” (hereafter will be referred to as RS) AND “legume*” OR “*Phaseolus*” OR “plant*” OR “bean*” OR “grain*”. The detailed screening was made, only for the “legume*”, “*Phaseolus*” and “bean*” searches.

The obtained results were used to compile and underline the importance of the RS in beans for human health and the potential improvement of the RS content in beans.

3 Results and discussion

3.1 Experiment 1. Methodologies for the determination of the sugar and acidity

Preliminary results were used to evaluate if the chosen laboratory analysis methods were enough compatible and applicable to the current laboratory conditions and samples collected. Results indicated that the acidity levels of fresh and frozen samples were quite different (**Figure 4**). It is known that the acidity levels of green beans decrease by cold storage. Thus, it was an expected result for the frozen beans to have lower acidity. Mean BRIX levels of the fresh and frozen samples were also found to be different for each sample group (**Figure 5**). After choosing the right method for analysis, the next greenhouse experiment was performed and the below-mentioned results were obtained.

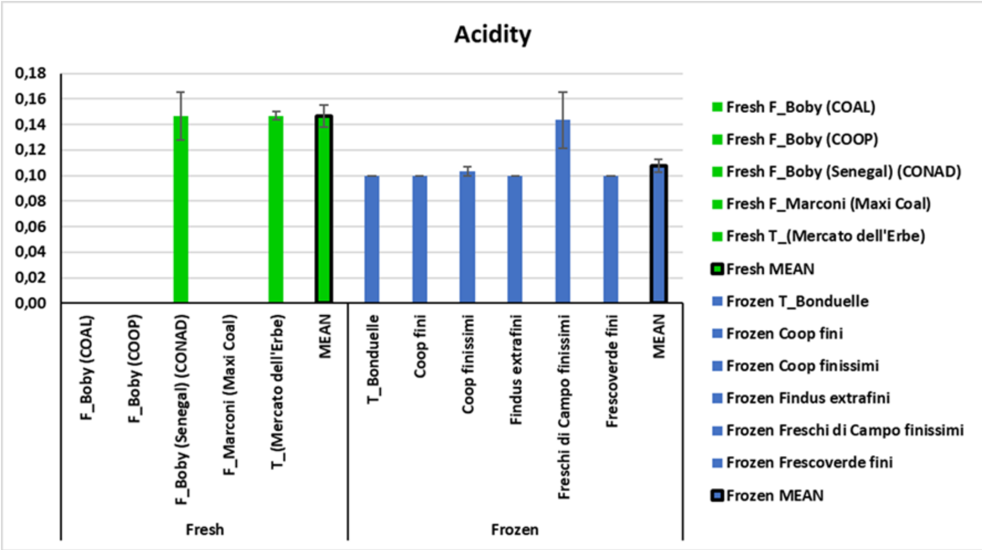

Figure 4. Acidity of the samples (content in malic acid). The mean and standard error (n=3)

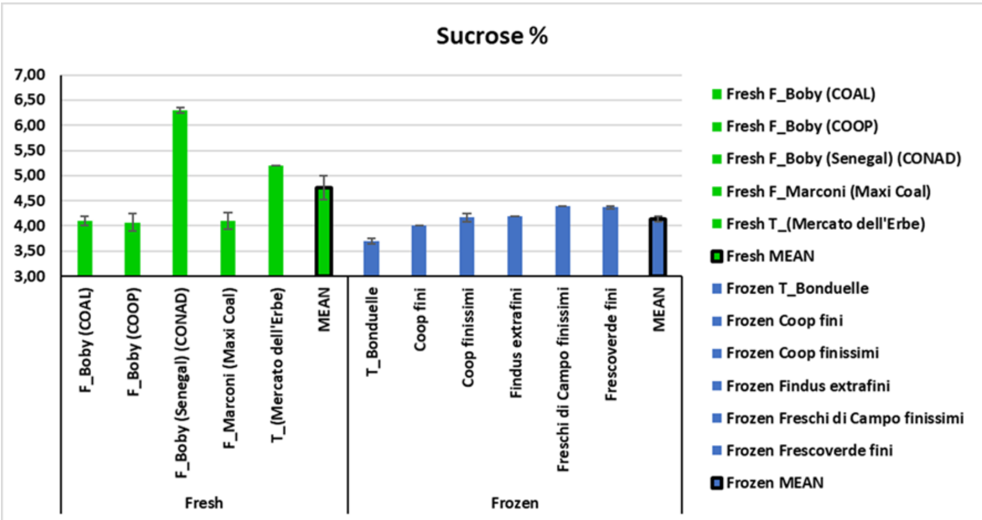

Figure 5. Brix level (%) of the samples. The mean and standard error (n=3)

3.1.1 Discussion

The results of the first experiment were only used as a confirmation of the selected protocol's applicability. It is known that as the storage time increases, the starches convert into the reducing sugars (mainly glucose) for preventing the green beans from the chilling injury. This leads to a sweeter taste as the storage time of the green beans increases. On the other hand, the total soluble solids (Brix) and the titrable acidity tend to decrease during the early storage (Guo et al., 2008). Especially if the vegetable contains lower quantities of protein, there is a higher tendency for pH to be decreased. This decline in pH levels increases the acid-catalyzed reactions rate, as well as starch hydrolysis. In addition to these changes, chlorophyll's pheophytization by time changes the bright green color of the green beans into an olive-brown color (Martins et al., 2005).

In the first experiment, 11 green bean samples (5 fresh green beans, 6 frozen green beans) were analyzed. Some of the fresh samples were bought from local producers and all other fresh and frozen green beans were bought from different supermarkets at Ancona. The results showed that there was variability within the cultivars both for the Brix and acidity contents as it was expected. Like any other chemical compound existent in green beans, these also depend on many factors; primarily due to the genotypic differences as well as the growing conditions of the plant (irrigation, fertilization, inoculation, weather conditions, the soil characteristics, etc.). Specifically, the Brix and acidity levels are good indicators for the maturity of the green beans. Because during the development of the plant, both of them tend to increase (MARTINEZ et al., 1995a).

In this first experiment, fresh green beans seemed to have higher levels of Brix and acidity levels compared to the frozen samples. Storing conditions have a marked influence on the Brix levels as well as on the color of the snap beans (Kinyuru et al., 2011). Accordingly, (Hunter and Fletcher, 2002) found that the average ascorbic acid content of the fresh green beans (0.057 g kg^{-1} wet weight) was decreasing (0.020 g kg^{-1} wet weight) after being frozen (Hunter and Fletcher, 2002). In another research, Opus and Leon cultivars were harvested, hydro cooled and held from 2 to 7 days in different temperatures. Snap beans stored at the temperatures higher than 10°C were observed to have lower acidity and soluble solids content than those stored in cooler temperatures (1, 5 and 10°C) (Proulx et al., 2010). Even though there are many other components that contribute to the taste of the green beans, the major factor that affects the taste of the green beans is sugar to acidity ratio. When the acidity content decreases during the storage conditions or as a result of any external influences, this contributes to a sour taste depending on the brix content; especially free sugars level, which makes the green beans less desirable for the consumers.

The literature on the Brix and acidity levels of green beans are whether focused on different industrial and home processing methods' (cooking, bleaching, canning, extrusion, soaking, etc.) or different post-harvest treatments' effects on both parameters. However, the studies which solely address the differences between diverse genotypes are few and not applicable to a wider range of genotypes. Expanding the future studies of sugar to acidity ratio into a wider number of genotypes may help the breeders evaluate the most suitable genotypes which have a better taste.

This thesis contributes to the new future studies by underlining the differences of snap beans in terms of Brix, acidity and pH levels and how they can be different in each genotype; also, how these might affect the taste of the green beans. It is also important and valuable for choosing some genotypes which weren't studied before in the literature. However, to have a wider knowledge about these parameters' relationship with the taste, other components that contribute to the taste of green beans should be considered. This would give more detailed and complete information for future breeding studies. In addition, a panel test can be very useful to have a realistic reflection for comparing the prementioned factors' relationship with the real human experience.

3.2 Experiment 2. Comparison of the 6 genotypes grown in the pots, in greenhouse

The fresh weight of the pods for each genotype showed a variation depending on the collection times (**Table 3**), with no mean difference among the genotypes irrespective of the sampling times. Similar results were obtained for the number of the pods. On the contrary, the pod weight was dependent on the genotype.

When the data was evaluated considering the sampling times, differences among the genotypes only seemed to occur during the first and the second sampling times for the mean pod weight. This can be a result of the genotypic differences as each genotype accumulates different amounts and types of chemical compounds; thus, the mean pod weight of each changes differently by time. Most of the genotypes studied reached a peak in the production in the first and second harvesting dates. After that, the pod numbers, as well as the pod weights, started decreasing. Considering together with the lack of differences in the mean pod weight by time may suggest that this trait can be substantially under genotypic control.

Only a few differences were found among the genotypes in terms of the production quantity between the sampling times. It was observed that the lower total yielding genotypes, gave a higher yield in the first sampling (**Figure 7**). It is likely that these low yielding genotypes have a lower ability to continuously produce flowers. However, after the third sampling, the differences among the samples, in terms of the cumulated weight of the pods were similar for each genotype (**Figure 8**).

Some results of the statistical analysis of the greenhouse experiment were shown (**Table 3.**). F values at $p<0.05$ were indicated in red and bold. Differences by sampling times for fresh weight, mean pod weight and the number of the pods were shown.

Table 3. Results of the statistical analysis of the greenhouse experiment. F values at $p<0.05$ were indicated in red and bold. Sliced differences by collection time(s) were shown.

fresh weight				
Effect	Num DF	Den DF	F Value	Pr > F
gen	5	28.27	2.27	0.074
Time	2	37.27	11.32	**1E-04**
gen*Time	3	28.81	5.08	**0.006**
Tests of Effect Slices for gen*Time Sliced By Time				
Time	Num DF	Den DF	F Value	Pr > F
54	5	54.98	13.44	**<.0001**
64	5	55.47	0.89	0.497
71	4	53.35	0.79	0.535
96	2	55.2	0.12	0.889

mean pod weight				
Effect	Num DF	Den DF	F Value	Pr > F
gen	5	50.34	6.33	**1E-04**
Time	3	48.28	0.6	0.62
gen*Time	11	47.63	0.31	0.98
Tests of Effect Slices for gen*Time Sliced By Time				
Time	Num DF	Den DF	F Value	Pr > F
54	5	55.73	3.64	**0.006**
64	5	55.71	3.22	**0.013**
71	4	55.37	1.95	0.114
96	2	55.69	0.76	0.474

number of pods				
Effect	Num DF	Den DF	F Value	Pr > F
gen	5	29.28	1.43	0.241
Time	3	40.43	8.87	**1E-04**
gen*Time	11	34.57	2.18	**0.04**
Tests of Effect Slices for gen*Time Sliced By Time				
Time	Num DF	Den DF	F Value	Pr > F
54	5	55.77	7.7	**<.0001**
64	5	55.88	0.16	0.976
71	4	55.69	0.14	0.967
96	2	55.72	0.03	0.967

For the total weight of the pods (**Figure 6**) FG21, FG25 and Midas had similar yields. Moonstone genotype was quite close to FG21, FG25, and Midas; however, not significantly in terms of the total weight of the pods. Expectedly, MG38 was shown to have a lower yield compared with the others.

Differences among the genotypes in terms of yield were expressed through the number of pods for each genotype (**Figure 9**). It was scarcely depended on the pod size. Also, these differences among the genotypes for the number of the pods were compatible with each genotype's total yield. Similar results were found for the distribution of the number of pods by sampling times (**Figure 10**).

The number of pods obtained from the 1^{st} harvest compared to the number of the pods in the whole harvest, for each genotype was similar. For the 2^{nd} harvest; MG38, Kysia and Moonstone genotypes produced many more pods than the others. For the 3^{rd} harvest, Midas was the most productive genotype. For the last harvest only FG21, FG25 and Moonstone gave pods, suggesting that these genotypes may need a higher thermal sum degree to produce flowers.

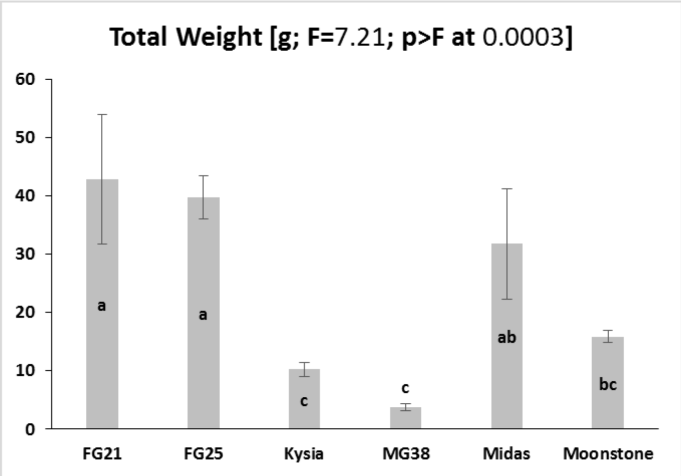

Figure 6. Total weight of the pods from various collection times in the greenhouse experiment. The mean and standard error (n=3). Bars with a letter in common are not different statistically according to the Tukey test.

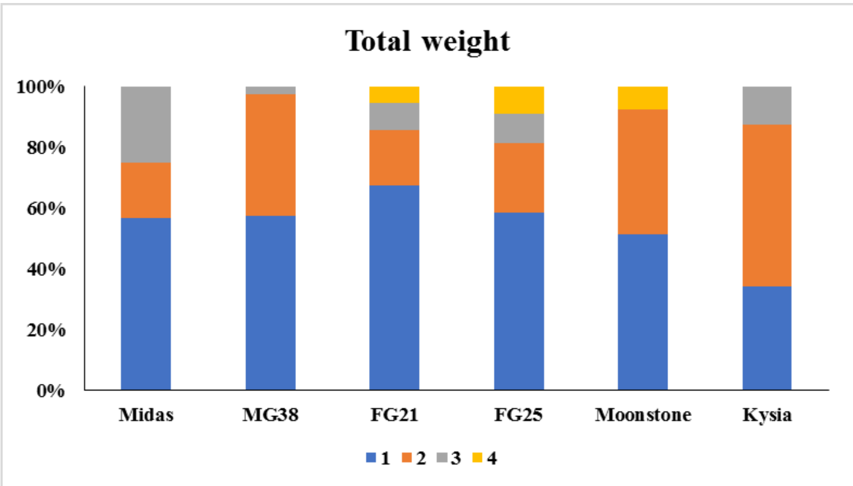

Figure 7. The fraction of the total weight of pods by each collection in the greenhouse experiment.

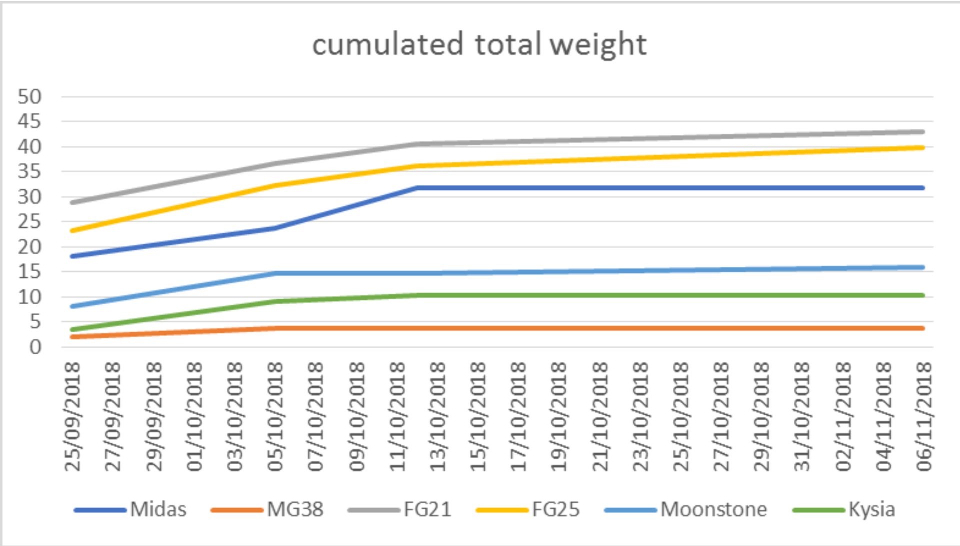

Figure 8. Cumulated weight of the pods by time in the greenhouse experiment.

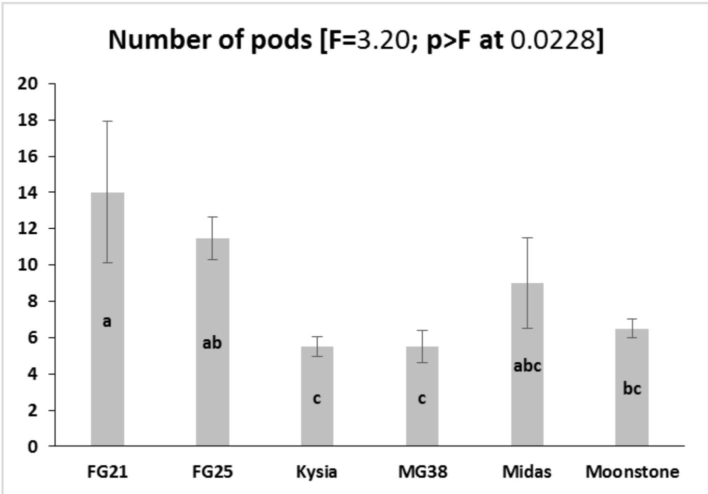

Figure 9. The number of pods from the greenhouse experiment. The mean and standard error (n=3). Bars with a letter in common bean are not different statistically according to the Tukey test.

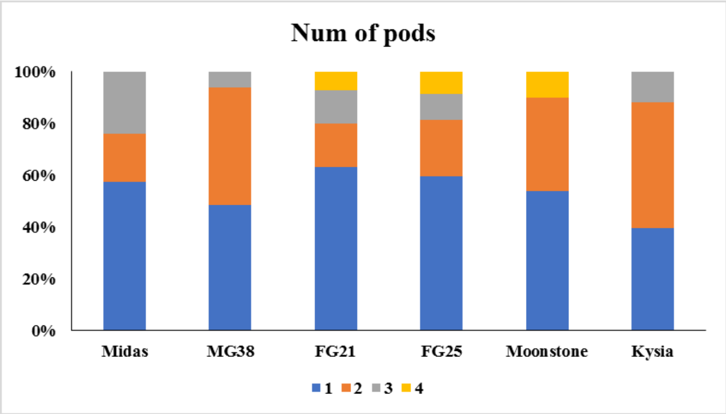

Figure 10. The fraction of the number of pods by each collection time in the greenhouse experiment.

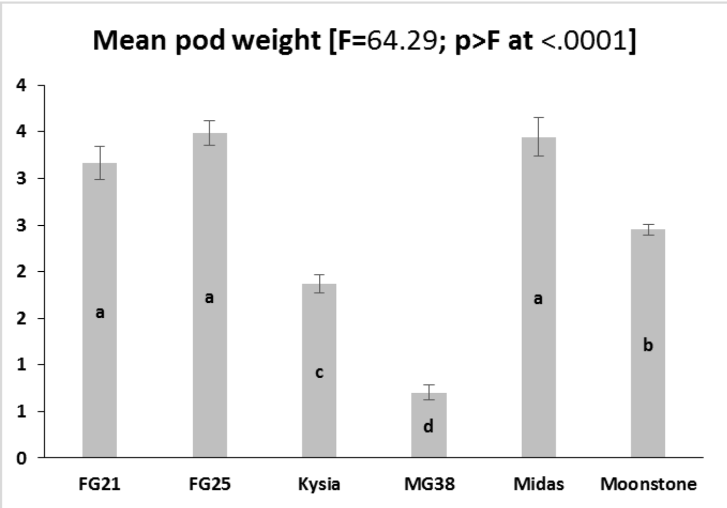

Figure 11. Mean pod weights in the greenhouse experiment. The mean and standard error (n=3). Bars with a letter in common are not different statistically according to the Tukey test.

Midas and FG25 genotypes had the highest mean pod weights; following FG21, Moonstone, Kysia, and MG38 genotypes, respectively. On the other side, mean pod weight patterns were similar for FG21, FG25 and Midas genotypes.

Mean pod weight for each harvest date among the genotypes was highly variable (**Figure 12**). Especially FG25, Moonstone, and FG21 produced significantly heavier pods than Midas and MG38 in the first and second harvest. FG25, Moonstone, and Kysia showed a decreased pattern for the pod size after the second sampling. However, the pod size for FG21 and Midas genotypes weren't decreased. The differences of the yield, during whole harvests between the introgression lines or among other genotypes, were observable through the pod sizes and the number of the pods for each genotype.

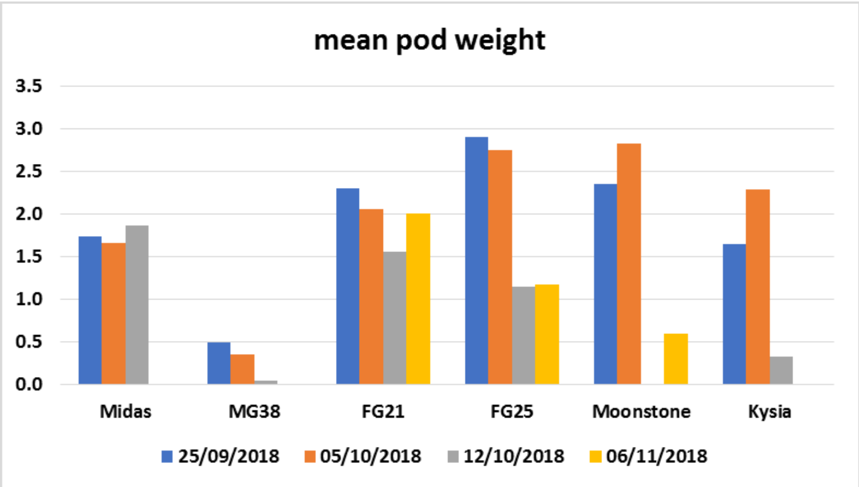

Figure 12. Mean pod weight for each sampling in the greenhouse experiment.

The average mean pod weight tended to increase with the increasing yield in each harvest date (**Figure 13**) and then, it reached a peak (more than 10 g f.w.). However, this variation of the relationship between the pod size and the yield for each harvest date strongly depended on the genotype (**Figure 14**) and the highest (and earlier) yielding genotypes also seemed to have heavier pods.

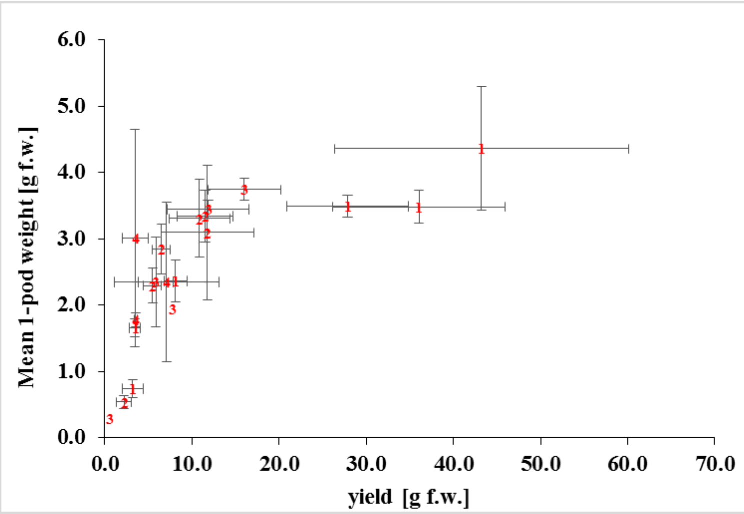

Figure 13. Relationship between yield and mean pod weight for each sampling (the numbers in the centroids) in the greenhouse experiment. Bars are S.E. around the mean.

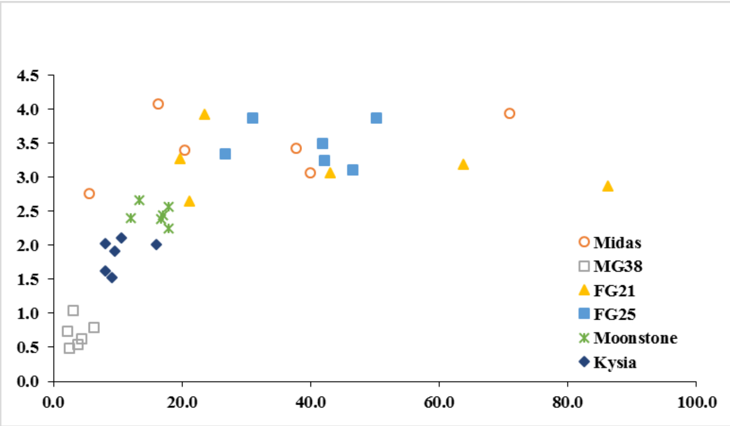

Figure 14. Relationship between the yield and mean pod weight by genotype in the greenhouse experiment. Bars are S.E. around the mean.

3.2.1 Discussion

The genotypes chosen for this study are interesting because of being introgressed with wild genetic diversity. This is an important aspect because the elite germplasms have reduced genetic diversity due to the processes of domestication and modern breeding.

The results of the second experiment demonstrated that for the number of pods and the fresh weight, the genotypes were found statistically different from each other ($p<0.05$). This was more apparent for the 1^{st} harvest (54^{th} day after sowing) than the others ($p<0.05$). The most yielding genotypes were respectively FG21 and FG25, and the least yielding one was MG38 (**Figure 6**) ($p<0.05$). The cumulative weight of the genotypes during the whole harvests also supports this finding (**Figure 8).** The yield in the snap beans is highly correlated with the seeds per pod. It is known that most yielding genotypes also give the highest number of seeds per pod (Singh et al., 2019). For this reason, FG21 and FG25 genotypes can be included in the future breeding studies to create high yielding new genotypes.

Another finding from the second experiment was that FG25, FG21, and Midas genotypes were similar to each other in terms of mean pod weight (**Figure 11**), which might indicate some morphological similarities in between these genotypes together with other findings (**Figure 6-11**). Even though, FG21 was the highest yielding genotype; FG25 had the highest mean pod weight (**Figure 11**). Significant differences in the pod weight of different genotypes are normally expected. In one study, which 6 different green bean genotypes (Burgundy, California #5, Crocket. Gold Dust, Queen Ann, Supremo) were evaluated, the pod weights of the genotypes were between 12,5-38,7 g, which shows a great variability (Richardson, 2011). For all the genotypes except the Midas, the 1st harvest was the most yielding compared to the others. And Midas, gave more yield than all the other genotypes in the 3rd harvest, even compared to the initial yield of itself. This might be due to some environmental factors' effect on the related trait, but it might also indicate the higher stability of this genotype. However, to understand the exact cause of this variability, further researches are needed (**Figure 12**). In any case, the mean yield of the pulses and French beans are quite lower compared to the yield of the cereals. Because most of the cultivars are instable to the environmental conditions and are susceptible to plant diseases (Singh et al., 2019).

A genotype that gives a higher yield is not necessarily a stable genotype. For this reason, it is essential to consider the genotypes not only with the higher yields but also with higher stability during breeding studies (Hassan et al., 2019). In this study, the variability of the parameters studied was highly dependent on the productive traits of each genotype. However, this again does not mean that the genotype which yields the most in the first harvest is the most productive one. Some genotypes have a longer harvesting period. Thus, in the end, they produce more edible pods than those which yield more during the first two or three harvests. Considering all these factors, in addition to higher stability; the length of the harvesting period for each genotype should also be considered for choosing higher yielding genotypes. Even all these conditions are met, a productive genotype may not always give high yield as this depends on many extrinsic factors as well. Arunga et al. (2010) evaluated seven varieties of snap beans (*Phaseolus vulgaris L.*) (Wade, Earligreen, Earliwax, Streamliner, Harvest King, Slendergreen, and Tendercrop) and all possible F_2's among them for some productive traits including the pod number per plant. Pod number trait was found to show some dominance, with recessive genes contributing to the high pod number. Furthermore, for the studied characters Earligreen and Slendergreen genotypes were superior compared with the others (Arunga et al., 2010). In another study, fourteen snap bean accessions taken from the Germplasm Bank of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) in Brazil were evaluated for their morpho-agronomical diversity based on the genotype X environment (sowing season) interaction. During two sowing seasons in 2011 (April-August, October-December) a randomized block design with 14 treatments and four replications were applied. For all the traits studied, including the total pod weight; the total number of pods; mean pod weight per plant and the mean number of pods per plant, a significant diversity between the genotypes was shown ($p<0.05$). Furthermore, this variance between the genotypes was seemed to be higher in the summer period than the winter period (Teixeira et al., 2004).

In the literature, information about the yield and yield components are mostly examined based on the effects of different agricultural methods (row spacing, irrigation, and fertilization techniques). Furthermore, most of the researches are focused on common beans instead of snap beans. The genotypes which are studied are also limited. To understand different genotypes' basic differences in terms of the chemical component(s) chosen, more future studies are needed without implicating complicated factors and agricultural treatments. Thus, the breeders might have a chance of evaluating the high-quality cultivars based on solely genotypic differences without the effect of another treatment.

In the 2nd experiment, no fertilization neither inoculation was applied to the plants. The irrigation was given every day during the first week, then 2-3 times a week depending on the temperature. It is known that the irrigation method and frequency have a direct effect on the chemical content and physiological quality of the plant. *E.g.* a study revealed that the snap beans which were furrow-irrigated had a higher level of vitamin C, free phenolics, protein, and proline, while the drip-irrigated ones were higher in amino acids content. However, some components such as chlorophylls, carotenoids, and reducing sugars were the same for both samples. Interestingly, the irrigation not only before but also after the harvest continued to influence the quality of snap beans (El-tahan et al., 2016).

All the genotypes, after reaching the maximum productivity, normally are expected to have a decreased yield by time. Accordingly in this study, Midas and Kysia seemed to have less yield in the beginning. However, after the 2nd harvest, they produced more pods compared with the other higher-yielding genotypes (**Figure 7**). Oliveira et al. (2018a) studied different green bean cultivars to understand the relationships between certain yield characteristics in Brazil during the years 2011-2013. The number of seeds per pod showed a negative correlation with the pod weight, grain weight and the seed weight per plant. Furthermore, the lines which had a higher number of the seeds per pod were less productive; specifying that the genotypic selection shouldn't be based on obtaining more seeds or pod yield for these genotypes (Oliveira et al., 2018a). Analogically, in this study, the Kysia and MG38 genotypes gave fewer seeds per pod comparing with other genotypes and they were found to be less productive as well.

Specifically, the number of the edible pods for each and/or the whole harvest is an important indication of the yield quality for the green bean cultivars. And different genotypes are expected to show high variability for the number of the edible pods. In this study, the total yield for each genotype and the differences in yield between the genotypes were significant and consistent with other findings for the total weight, cumulative total weight and the number of pods analyzed (**Figure 6-9**). The chemical content of the pod is affected by the number of pods, interestingly. According to research conducted in China in 2016; two green bean cultivars (Bronco and Paulista) were examined to understand the relationship between the pod parameters and the chemical content of the pods. It was found that the cultivar Paulista, which had the highest number of pods, also contained a higher soluble sugar level compared with the others (Saleh et al., 2018).

In the 2nd study, the number of pods for the 1st harvest: total number of the pods in the whole harvest for each genotype were similar. During the development of a plant, all the phases, including the harvesting phase requires a minimum accumulation of temperature before that stage can be completed, so that the plant can move to the next stage. A plant senses the temperature each day of the development and adds the average for each day to reach a total temperature which is required for the next stage. This temperature for each specific phase of the development is called "thermal time or heat sum" and is expressed as degree days (°Cd) (Howard and Macpherson, 2000). (Yoldas and Esiyok, 2009) studied the thermal time and yield characteristics for the Amboto, Gina, Nassau and Volare cultivars of *Phaseolus vulgaris L.* in Turkey in 2004 and 2005. The thermal time values were evaluated from emergence to harvest; from emergence to the beginning of the flowering and from the beginning of the flowering to the harvest. The highest yield (12783.7kg ha^{-1}) was obtained from the early sowing in July. The delay in the sowing date seemed to decrease the yield (10926.7 kg) in 2005. The later sowing dates led to the decreasing yields. 1552.6°C day in Gina and Nassau, 795.3°C day in Gina and 958.7°C day in Volare were calculated as the highest thermal times (Yoldas and Esiyok, 2009).

3.3 Experiment 3. Role of the inoculation with arbuscular mycorrhizal fungi on bean quality under field conditions

The results of the statistical analysis are expressed in **Table 4**. The pod weight and sugar content did not differ by the treatments. However, significant differences were found for the malic acid and pH content among the genotypes.

Table 4. Results of the statistical analysis of the field experiment. Variables were genotypes (Gen) and inoculation with arbuscular mycorrhizal fungi (INOC).

			Pod weight		Brix		Malic acid		pH	
Effect	Num DF	Den DF	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
GEN	10	17	1.6	0.188	1.12	0.412	3.17	0.024	16.21	<.0001
INOC	1	17	2.23	0.154	0.55	0.472	1.98	0.181	0.08	0.784
GEN*INOC	10	17	1.22	0.343	2.07	0.104	0.85	0.595	1.14	0.398

The mean yield was found as 181±66 g $plot^{-1}$ and the sugar content was 3.61±0.12.

For the malic acid content (**Figure 15**) of the green bean genotypes, only “Metro o Stringa” (genotype 6) had higher levels compared with the rest of the genotypes, which were similar to each other.

Results found for pH levels only partly matched those of the Malic acid content. Metro o Stringa genotype (the 6th genotype) had the lowest pH and the 20th genotype (IL_321/967) was also different from the others in terms of pH.

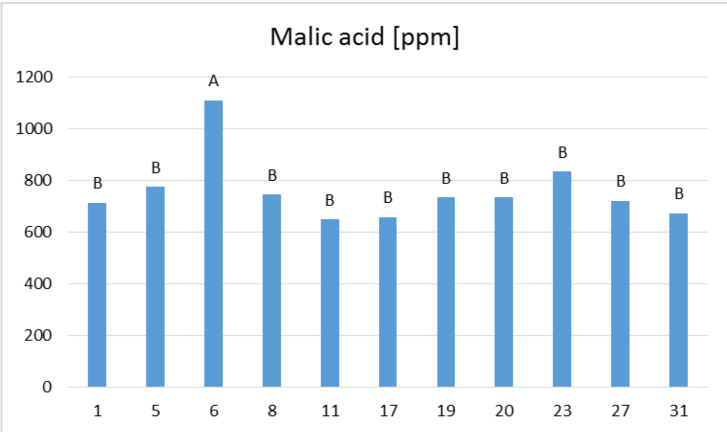

Figure 15. Malic acid contents (ppm) of the genotypes. Bars with a letter in common are not different statistically at $p<0.05$ according to the Tukey test.

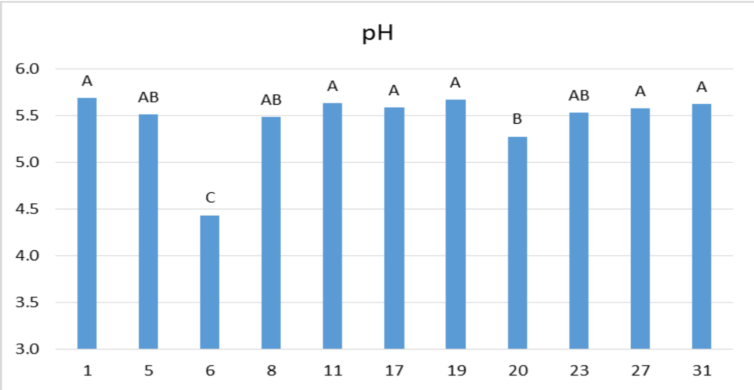

Figure 16. pH levels of the genotypes. Bars with a letter in common are not different statistically at $p<0.05$ according to the Tukey test.

3.3.1 Discussion

There are many other components that contribute to the taste of the green beans. However, the ratio of sugar to acidity is the main factor that determines the taste and consumer acceptability. Malic acid is the main organic acid in the snap beans (Proulx et al., 2010). The results of the 3rd study showed that only for the malic acid and pH contents, there was a statistically significant difference ($p<0.05$) among the genotypes **(Table 4.)**. This means if the sugar levels of the genotypes are quite similar; the only factors which could make the snap beans much or less acidic (much or less sweet) are the levels of malic acid and pH. In this study, the pH levels of the genotypes were between 5,70-6,00 and the malic acid levels were between 0,043-0,128. (Proulx et al., 2010) studied with the Opus and Leon cultivars and found that the malic acid contents of the genotypes were respectively 0,09 and 0,08; the pH levels were 6,3 and 6,4. Just like any other chemical component; soluble sugars, acidity, and pH levels vary depending on the traits of the genotype. However, abiotic and biotic factors (climate, soil characterization, etc.) may have a significant effect on the expression of these traits. In this study, the 6th genotype contained the highest level of malic acid, while the 11th genotype had the least (**Figure 15.**). MARTINEZ et al., (1995) found the malic acid content of green bean cultivars Strike and Bina 1410 and 1620 ppm; respectively. In another research, the malic acid content of the fresh green beans bought from the local supermarkets in Spain was found to be 960-1950 ppm (De La Cruz-García et al., 1997). This variability among genotypes is hugely affected by the traits of the genotypes but all the extrinsic factors as well, shape the quantity of the chemical components which are found in green beans.

In contrary to the previous results, there was no difference among the genotypes for the Brix levels. It is interesting because normally different genotypes were expected to have differing amounts of total soluble solids (Brix). For this reason, the malic acid and pH contents can be counted as the primary dynamic parameters which have an influence on the taste of the genotypes studied.

Microorganisms are the living part of the soil and they actively participate in its formation. For all the microbiological processes in soil, a certain amount of diverse organic matters needed. Depending on the microflora activity, soil fertility either become enriched or weakened. Diverse microorganisms which are the parts of the microbiological fertilizers, increase the efficiency of the soil and help the crops yield more than they could in normal conditions (Milic et al., 2006). In this study, the inoculation and genotype - inoculation interaction parameters weren't found to be effective on the Brix level and pod weight ($p>0.05$). In a randomized experiment with three replications, the effect of the arbuscular mycorrhizal inoculation on the growth and the productivity of snap bean cultivar Paolista was studied in Egypt, during the years 2014 and 2015. The results demonstrated that the vegetative growth characteristics, the yield, total carbohydrates of the pods and chlorophyll, the nutrition content of the leaves were increasing significantly after arbuscular mycorrhizal fungi

(AMF) treatment (Youssef et al., 2017). The influence of Rhizobium inoculation on nodules, growth, and yield of French bean (*Phaseolus vulgaris L.*) cultivars, were studied in Pakistan, during 2016. Evergreen, Komal green, and Winner cultivars were inoculated with Rhizobium. Inoculation with Rhizobium significantly enhanced the growth and yield parameters of French bean cultivars (Abbas Khan et al., 2016). On the contrary, AMF treatment did not have a significant effect on the pod weight, Brix, malic acid and pH levels of the genotypes. Such lack of differences may be a result of the various conditions; including the P level of the soil which may have impaired the mycorrhizal growth, or the unresponsiveness of the mycorrhizal species used (Lambais et al., 2003; Aguirre-Medina et al., 2017; Mohamed et al., 2019). In addition, early results on the relationship between AMF and *Phaseolus* showed that the fungus can stimulate plant growth depending on the plant stage, with stronger stimulations in the juvenile phase and lesser in the reproductive stage (Daft & Elgiahmi, 1974), such as in the present study. Particularly, these authors showed that the mycorrhizal stimulation of beans mostly occurs in the root, which the growth can be detrimental for the plant yield if the nutrient availability is high.

3.4 Experiment 4. A systematic mapping on the resistant starch content in beans

The systematic mapping mainly based on searches on WoS and Scopus. In WoS, the keyword "RS only" led to the 4504 results. In WoS, "title+topic" search led to 4120 results; in Scopus, only "topic" search led to 3039 records. All the files found were organized and unified, then the duplicated results were eliminated. Expectedly, broader searches (*e.g.* "legume*" in topic and field) did not include all the records which were found during the specific keyword searches (e.g. *Phaseolus*). After merging the original files and selecting the "bean*" + "*Phaseolus*" + "legume*" keywords, we obtained a total of 1891 records (from 1962 to 2018). We also reached the 6 articles which were published in 2019. A total of 171 records were found in 2018, which could increase with the articles which are published from the 12^{th} of October to 31^{st} of December 2018 (**Figure 17**).

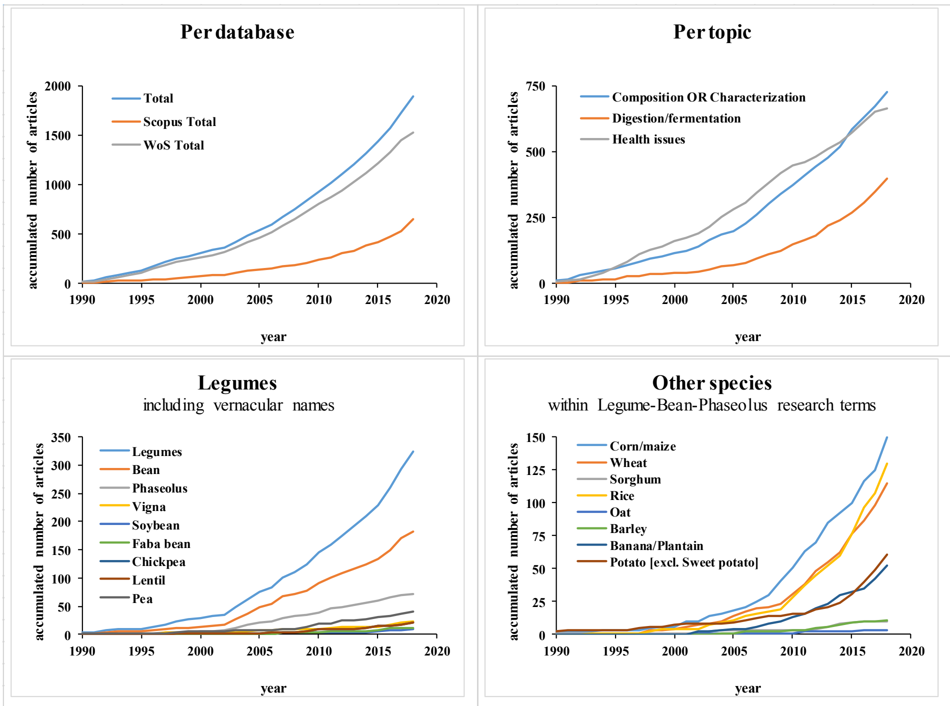

Figure 17. Main results of the systematic map performed

The titles of the articles found were scanned for the pre-determined species' names (either the binomial or vernacular name) in the topic. When the matches were not compatible with the species which were being studied, either abstract or the whole paper were screened again to obtain any information related to the species studied. The species and/or the topics which were screened are: "Composition OR Characterization", "Digestion/fermentation", "Health issues", "Legumes", "bean", "*Phaseolus*", *Vigna*", "Faba bean", "*Pisum sativum* or Pea", "*Lens culinaris* or lentil", "*Cicer arietinum* or chickpea", "*Glycine max* or soybean", "*Zea* *mays* or corn or maize", "*Triticum spp*. or wheat", "*Hordeum vulgare* or barley", "*Avena sativa* or oat", "*Oryza sativa* or rice", "*Sorghum bicolor*", "*Musa spp.* or banana or plantain", "*Solanum tuberosum* or potato (excluding sweet potato)". In both the Scopus and WoS a total of 340 articles were found which were directly related to the parameters we searched (**Table 4**). Unfortunately, the reports which were dealing with the RS of *Phaseolus* and other species were scarce in terms of the keywords we used (*i.e.* considering the legumes and other species with "Title" and "Topic" search). Furthermore, 20 articles were comparing the *Phaseolus* with other legumes, and 12 were comparing *Phaseolus* with the non-legume species.

Table 4. Total studies retrieved in the systematic map and dealing with *Phaseolus*

		Total records	dealing with *Phaseolus spp.*
Total		1893	75
Scopus	RS *Phaseolus*	474	14
	RS bean	235	58
	Scopus Total	653	58
WoS	RS *Phaseolus* TT*	1398	64
	RS bean TT	1513	65
	RS *Phaseolus* TO	162	61
	RS bean TO	219	50
	WoS Total	1532	66
Research aspect	Composition OR Characterization	736	50
	Digestion/fermentation	396	28
	Health issues	665	17
Legumes	Legumes	327	-
	bean	200	-
	Phaseolus	75	75
	Vigna	26	5
	Vicia Faba or fava bean	14	2
	Pisum sativum or Pea	44	4
	Lens culinaris or lentil	26	5
	Cicer arietinum or chickpea	27	5
	Glycine max or soybean	9	0
Other species	Zea mays or corn or maize	150	2
	Triticum spp. or wheat	117	4
	Hordeum vulgare or barley	13	2
	Avena sativa or oat	3	0
	Oryza sativa or rice	132	3
	Sorghum bicolor	10	0
	Musa spp. Or banana or plantain	52	0
	Solanum tuberosum or potato [excluding sweet potato]	62	1

3.4.1 Sources and Utilization of RS in the Body

RS is a part of dietary fibers (DF) and is a non-nutrient dietary component, which can have some beneficial effects on health such as laxation, regulation of serum cholesterol and glucose levels (Larrauri, 1999; DeVries, 2003). RS (especially RS3) can contribute to bowel health by preventing bowel inflammatory diseases and colorectal cancer, supporting colonic fermentation and beneficial bacterial growth and increasing fecal bulk and fecal transition time (Topping et al., 2003). For example, supplementation of 25 g per day to healthy adults led to an increased daily fecal output (above the normal levels) with minimal gastrointestinal discomfort (Maki et al., 2009; Rabbani et al., 2009). It was shown that RS can be used respectively to control Shigellosis in children by decreasing the stool volume, numbers of stool per day and red blood cells (RBC) in stool; increase short-chain fatty acid (SCFA) concentrations in feces; increase probiotics population in the gut; and suppress the development of pathogenic *Enterobacter* species (Silvi et al., 1999; Rabbani et al., 2009). However, it should be considered that the overconsumption of RS may also have some negative effects. *E.g.* consumption above 60 g of RS3 was associated with a mild laxative effect, including other symptoms like bloating, borborygmi (audible abdominal noises), flatulence and colic-watery feces (Storey et al., 2007).

The colonic bacteria are more likely to use RS than other DF for the fermentation (Torres-pacheco, 2006). After reaching the large bowel, RS acts as a substrate for microbial fermentation resulting in various end-products. Among these, SCFA has specific importance as it's being used by the beneficial colonic bacteria as an energy source (Cummings et al., 1996). When it's digested, RS has also lower calories (8 kJ/g) compared with fully digestible starches (15 kJ/g) (Rochfort and Panozzo, 2007).

3.4.2 Nutritional, health and sensory aspects

Foods rich in RS should be consumed in adequate, recommended amounts to get the health benefits related to their RS (Niba, 2002). While other types of DF may cause some gastrointestinal symptoms such as floating etc. even in lower doses (≈10 g), RS can be tolerated in higher amounts (up to 50 g/d and for RS3> 60 g/d) without causing any symptoms (Storey et al., 2007; Klosterbuer et al., 2013). RS may also be used in the prevention and treatment of postprandial glycemia, mineral absorption and gallstone formation with or without medication therapy depending on the needs of the individual (Annison and Topping, 1994; Fuentes-Zaragoza et al., 2010).

However, despite the market availability and variety of RS-enriched products in many countries, RS consumption in the diet is still below the recommended values (Murphy et al., 2008; Sanz et al., 2008). Consuming a diet poor in RS and other DF can consequently lead to chronic health problems which are primarily obesity, colon cancer, diabetes and other obesity-related diseases (Niba, 2002; Marlett et al., 2002; Hayat et al., 2014).

The consumption of the bean and bean-based products in the diet can cover the fiber requirements of the body as well as help preventing chronical diseases. However, the excess consumption of the beans and other legumes may cause some gastrointestinal discomfort such as flatulence. This is due to some highly fermentable compounds existing in beans such as the α-galactosides, soluble DF and RS. The natural fermentation may reduce the quantity of these compounds up to 90% (Granito et al., 2005). *E.g.* unfermented and fermented beans were studied with the participation of 10 female volunteers between the age of 25-40 for 28 days. The subjects consumed either 45 g of fermented and cooked beans or only cooked beans for 7 days, with a 2-week break between the experimental periods. Also, a sensorial evaluation was performed by 51 panelists, concluding that 56% of them "slightly liked" and "really liked" the fermented and cooked beans. The results showed that the fermented bean consumption could significantly decrease the flatulence problem up to 56,1%, the intestinal noises (borborygmi) up to 48%, nausea up to 80% and abdominal bloating up to 11% (Granito et al., 2005). In another study, a mixture of the cereals (corns and oats) and some snack bars which were containing 20-30% beans were given to individuals and their preference and feedbacks about the sensory qualities of both products were asked. Most of the panelists chose the snack bar with 30% bean content (Zambrano et al., 2013). These products are healthier than most of the snacks in the market, as they contain higher levels of protein and fiber.

3.4.3 Prebiotics and the prevention of colon cancer

Prebiotics are non-digestible food components that can positively affect health, mainly by selectively stimulating the activity of beneficial bacteria in the GIT (Roberfroid, 2000). RS was claimed to be the most important substrate for bacterial carbohydrate fermentation in the colon (Hylla et al., 1998) and can support the growth of beneficial microorganisms in the bowel (Brown, 2009). Immediately after RS fermentation, colonic pH also decreases (Haralampu, 2000). Indeed, legume-based diets, which are also rich in RS, were found to diminish the quantity of pathogenic intestinal bacteria while supporting the growth of the beneficial species (Queiroz-Monici et al., 2005). It was reported that the consumption of RS in freeze-dried haricot beans decreased the cecal pH of rats, which is related to colon cancer parameters (Key and Matherst, 1993).

Among all RS fermentation by-products, SCFA, and especially butyric acid, has huge importance as an energy source for the large intestinal epithelial cells (Liljeberg Elmståhl, 2002). Butyrate inhibited the growth and proliferation of tumor cells *in vitro* by blocking a phase of the cell cycle (Feregrino-Pérez et al., 2008). According to some animal studies, butyrate was also associated with a lower risk of bowel cancer due to its apoptotic response (Govers et al., 1999; Bauer-Marinovic et al., 2006; Obiro et al., 2008; Le Leu et al., 2010).

The information about the other legumes' effects on butyrate production is relatively fewer. Particularly, it was seen that the indigestible fraction of the black beans and lentils have the ability to induce butyric acid production compared to the chickpea (Hernández-Salazar et al., 2010).

In another study, processed red kidney beans with different types and quantity of indigestible carbohydrates were analyzed to understand the hindgut fermentability and SCFA production (Henningsson et al., 2001). Low and high RS bean flours, mainly containing raw and physically inaccessible starch, were obtained by milling raw or boiled beans and various diets prepared from these flours. These diets contained various amounts of indigestible carbohydrates. The authors found that the raw and physically inaccessible starch was more readily fermented than retrograded starch (97-99% vs. 86-95%, respectively) and RS was more fermented than the non-starch glucans. However, the fermentability of the autoclaved non-starch glucans (50-54%) was higher than the boiled beans (37-41 %). Also, the butyric acid level was higher in the high RS than low RS diets (Henningsson et al., 2001).

According to another research investigating the short (1-3 d) and medium (14 d) term adaptations of rat large bowel to the cooked haricot beans in diets based on white bread, it was observed that among all other cecal SCFAs, butyrate was the most abundant one. In addition, all the RS in the diet seemed to be completely fermented (Key and Mathers, 1995).

An experiment was conducted to understand the rats' digestion of the non-starch polysaccharides (NSP) and RS. A whole-meal, bread-based diet containing 0-450 g of freeze-dried and cooked haricot beans per kg of the body weight was given to two groups of rats for 14 days and 21 days. After the consumption of the diet, RS was not detected in feces and it was assumed to be completely fermented in the large bowel. Furthermore, a greater amount of volatile fatty acids (VFA), including butyrate, acetate, and propionate were absorbed from the large bowel which also affected the VFA pattern of the portal and heart blood (Key and Matherst, 1993).

Non-digestible fraction (NDF) from the cooked common beans inhibited azoxymethane (AOM) induced colon cancer, by altering the expression of the genes involved in the induction of the apoptosis and cell cycle arrest through the action of butyrate (Vergara-Castañeda et al., 2010). The same authors confirmed that the NDF of cooked common beans may inhibit colon carcinogenesis at an early stage by inducing the cell cycle arrest of the colon cells and morphological changes linked to apoptosis (Feregrino-Perez et al., 2014). This non-digestible fraction (NDF) of common beans were also shown to be able to modulate the protein expression in HT-29 cells, which is associated with the parameters of the colon cancer (Campos-Vega et al., 2012). As a disadvantage, some anti-nutritive factors in the common beans; such as protease inhibitors, tannins and lectins may reduce protein digestibility as well as some other minerals (Key and Matherst, 1993). However, these factors can be inactivated through thermal processing.

3.4.4 Glucose metabolism

RS can help to regulate glucose and lipid metabolisms after starchy food ingestion through some direct and indirect effects. For example, RS containing products can give satiety by giving a lesser glycaemic response, which is important in the management of obesity and obesity-linked diseases (Carter and Drewnowski, 2012).

Compared with some potato or rice products, legume starches have dramatically lower levels of the glycaemic index: with 20%, 85%, 77% and 50% (compared to glucose as 100%) for beans, baked potatoes, wholemeal bread, and rice, respectively (Foster-Powell et al., 2002). For these reasons, consumption of the common beans and other legumes is adversely associated with the risk of type-2 diabetes mellitus (Villegas et al., 2008) and people with diabetes are highly recommended to cover their carbohydrate needs from low GI foods such as common beans (Wolever et al., 1992). These effects might be indirectly related to the natural starch blocker of beans "α-amylase inhibitor isoform 1 (alpha-AI1)" as well (Obiro et al., 2008).

All types of DF, including the RS, affect the response and the action of gut hormones; especially cholecystokinin which mediates the postprandial glycaemic and insulinemic response to dietary carbohydrates. In male subjects, bean intake has been associated with increased cholecystokinin levels (Bourdon et al., 1999; Brewer et al., 2015). However, to achieve this, the consumption of RS should be higher than at least 14% of the total starch intake (Wu et al., 2009). The physicochemical properties, including *in vitro* starch digestibility and expected glycemic index (eGI) of bean flour and isolated bean starch from different bean cultivars were investigated by (Chung et al., 2008). The bean flour had a significant amount of RS (32.4% and 36.0%) and the bean starch had slightly more slowly digestible starch than the flour. However, consuming foods with high levels of RS cannot help alone an individual to be healthy, as there are many other factors depending on the health status of an individual, including their lifestyle (Yamada et al., 2005).

3.4.5 Lipid metabolism

RS can strongly affect the lipid metabolism by reducing the associated parameters for the CVD (total cholesterol, triglycerides, LDL, HDL, VLDL, and IDL) (Morand et al., 1994; Han et al., 2003). The common beans' effect on hypercholesterolemia is highly linked with their RS and DF content which affects the cecal SCFA concentrations in the bowel. (Han et al., 2003) found that feeding rats with a cholesterol-free diet containing *V. angularis* and 2 different genotypes of *P. vulgaris* improved most of the lipid parameters compared to a cornstarch-based diet.

After feeding 13 normocholesterolemic male students, separately with 450 g/day baked beans and 440 g/day spaghetti for 14 days, it was observed that there was a significant decrease in the total plasma cholesterol and HDL levels of the participants (Shutler et al., 1989). Similar to these findings, LDL and serum cholesterol levels of hypercholesterolemic patients who consumed 275 g of navy beans for three weeks were both decreased up to 19 and 24%, respectively (Anderson et al., 1994).

Similarly, the substitution of white wheat bread with the brown beans during the dinner of 16 healthy young adults, significantly lowered some parameters related to both sugar and lipid metabolisms; including blood glucose and insulin responses. It also improved the hormonal status related to satiety and hunger, as well as other biomarkers for the CVD risk. In addition, an increased SCFA concentration in the plasma and an improvement in the colonic fermentation were observed (Nilsson et al., 2013).

Resistant starch in the common beans is thought to act together with the proteins to affect the serum cholesterol levels by limiting the digestion of the high-calorie macronutrients and binding the steroids in the gut (Phillips, 1993). The existing researches about bean consumption's effect on cardiovascular health are promising. However, there are many other dimensions and complicated relationships to be understood in the future studies.

3.4.6 Weight control and the activity of "starch blockers"

According to the National Health and Examination Survey (NHANES) (Papanikolaou and Fulgoni, 2008), bean consumers had better overall fiber and mineral intakes, which lead to lower body weight and smaller waist size comparing with the non-consumers. These effects are thought to directly/indirectly related to the RS content of the beans. The rats which were fed with the dry bean extract were found to have a dose-dependent reduction of daily food intake, which consequently resulted in a reduction of the body weight and a decrease in postprandial glycemia (Fantini et al., 2009). It was a confirmation of a past study conducted about bean consumption's effect on body weight (Pusztai et al., 1998).

Starch blockers are known to be natural weight loss supplements. These compounds interfere with the breakdown of complex carbohydrates, leading to a reduced digestibility or prolonged digestion of the carbohydrates. As a result, generally, much less energy is derived from the carbohydrate source (Celleno et al., 2007). Beans contain "natural starch blockers" such as amylase inhibitors. These starch blockers which are derived from the beans are widely used as an alternative source to the synthetic obesity remedies (Lockwood, 2004), because of having less undesirable effects compared to the industrial products for bodyweight management and helping to reduce the fat tissue without any reduction in the muscle quantity (Obiro et al., 2008).

In a randomized, double-blinded and placebo-controlled study, a dietary supplement containing 445 mg of an extract derived from the white kidney beans was given to 60 slightly overweight individuals. The participants were divided into two homogeneous groups considering their age, gender, and body weight. The test product containing *P. vulgaris* extract and the placebo were taken one tablet per day for 30 consecutive days before the main meal which was rich in carbohydrates. After 30 days of receiving the bean extract with a carbohydrate-rich 2000-2200 calorie diet, the group which took the *P. vulgaris* extract before the meal seemed to have a greater reduction of the body weight, BMI, fat mass, adipose tissue thickness, and waist/hip/thigh circumferences while maintaining lean body mass compared to the subjects receiving placebo (Celleno et al., 2007).

4 Conclusions

The initial aim of this study was to understand the genotypic variation in terms of Brix, malic acid content and pH of the French beans, also considering the yield characteristics and other quality aspects of the genotypes. The genotypes were characterized with the expected contrasts in both the yield characteristics and the contents of Brix, acidity, and pH as mentioned in the discussion sections.

The Brix, acidity and pH levels are important to obtain pods with high quality, which also have a desired color by the consumers. A high-quality green bean pod should have a bright green color, have a higher sugar content and less acidity, should be succulent and least fibrous as possible.

The main results of the study indicated a high variability for the characteristics studied, which are mainly pod weight and pod number. On the contrary, variability among the genotypes for the Brix content was observed under the controlled but not field conditions. However, this may be a consequence of the stressful conditions in the field, such as the extremely high temperatures in which the plants were exposed. This is known to increase sugar oxidation.

As a conclusion, the genotypes which had higher sugar to acidity ratio can be considered to develop new lines with a superior quality which have a higher resistance to diverse abiotic and biotic stresses.

Finally, the whole bromatological composition should be considered for the breeding studies, especially for the quality aspects which are important for the consumers. Also, RS content of the snap beans can be studied in the future to understand and reveal its relationship with the plant’s resistance to high temperatures as it is observed in some legumes.

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