AGRONOMIC, GENETIC AND NUTRACEUTICAL CHARACTERIZATION OF BEAN (*Phaseolus spp.*) LANDRACES FROM VENETO, ITALY

by

PIETRO M. S. SICA

(Under the Direction of Miguel Cabrera and Dorcas H. Franklin)

ABSTRACT

Conserving bean genetic materials plays an important role in achieving global food security through sustainable agriculture. This study aimed to collect and perform agronomic, morphologic, genetic, and nutraceutical characterization of Italian accessions and Venetian landraces. Italian beans showed different nutraceutical compositions, plants and seeds morphological characteristics, and agronomical performance in different Venetian environments. These accessions were clustered and linked to their domestication centers, Andean and Mesoamerican. Six landraces with high-performance, good nutraceutical qualities, and seeds with characteristics of commercial interests were selected and evaluated under drought conditions. Therefore, this study has economic, cultural, and social importance through the maintenance of traditional beans from Italy.

INDEX WORDS:Genetic diversity; bean germplasm; conservation genetics;Venetian bean landraces; organic bean production.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

In the last decades, the world population has grown steadily and increasingly urbanized; consequently, the way food is produced, distributed, and consumed worldwide also changed dramatically (FAO, 2019b). Today, it is estimated that 821.6 million people in the world are going hungry, that is, they do not consume the sufficient amount of calories regularly to lead a normal, active and healthy life (FAO, IFAD, UNICEF, WFP, and WHO, 2019). It is expected that by 2050 the world population will grow 34% achieving 9.1 billion people - and the percentage of people living in urban areas will increase from 49% to 70%. Urbanization, combined with income growth, will bring with it changes in lifestyles and consumption patterns, requiring an increase of 70% of the world food production by 2050 (FAO, 2019a). A significant challenge faces the world: supply enough food for a larger and more affluent population in an environmentally and socially sustainable way, and also ensuring that the world's poorest people will have access to enough food to supply their daily calories demand. Increasing food production is essential but finite land, ocean, and atmosphere resources will make it more difficult (Conway, 1998), raising concern on environment and food security, with the need for a more sustainable agricultural production (Rubiales and Mikic, 2015).

Grain legumes present outstanding nutritional and nutraceutical properties while being an affordable food that contributes to achieving future global food and feed security in the context of an increasing world population (Frontiers Research Topic, n.d.). According to Calles (2016), Peix et al. (2015) and Courty et al. (2015), legumes can play an essential role in addressing issues related to the environment, health, and food security by:

- i) fixing atmospheric nitrogen biologically, which is a cornerstone of sustainable agriculture,
- ii) providing different services in multiple crop systems (crop rotation, intercropping, agroforestry),
- iii) improving nutrient cycling,
- iv) reducing energy costs and greenhouse gases emissions of the production of synthetic nitrogen,
- v) improving soil physical conditions and its biodiversity, consequently leading to a sustainable improvement of the agricultural system, also increasing its resilience.

Currently, in Latin America and eastern and southern Africa beans (*Phaseolus spp.*) are the most important legume cultivated by smallholders (Butare et al., 2011; Polania et al., 2016a) and are the principle food legume for 250 million people in South and Central America and 400 million people in eastern Africa (McClean and Raatz, 2017), providing essential food components to combat malnutrition in these regions (Katungi et al., 2009).

THE BEANS

Phaseolus spp.

The term beans refer mostly to the genus *Phaseolus*' food legumes, belonging to the Fabaceae family (formerly known as Leguminosae), subfamily Papilionoidae, tribe Phaseoleae, subtribe Phaseolinae. All the genus species are diploid, and most have 22 chromosomes (2n = 2x = 22) (Gepts, 2001). The genus *Phaseolus* contains 150 species in warm and tropical countries, annuals and perennials, mostly grown for edible seeds, but can also be ornamentals.

Beans are usually herbs but woody at the base. Mostly twinning, some forms erect. Leaves pinnately 3-foliate but sometimes reduced to 1 leaflet, stipulate and stipellate, the stipulate persistent, leaflets entire. The number of flowers ranges from a few to many, racemose or fascicled on axillary peduncles and with a diversity of colors like white, yellow, red, purple, with Papilionaceous corolla, usually with five petals, including a large upper petal enclosing two lateral wings and a lower carina of two united petals. Pods are compressed or with convex sides, containing several to many seeds, and dehiscent. The germination can be hypogeal or epigeal (MC – these sentences are not complete). (Bailey, 1924; Moore, 1936; Nassar et al., 2010).

Five species of the genus *Phaseolus* are domesticated: in decreasing order of importance, common bean (*P. vulgaris* L.), lima bean (*P. lunatus* L.), runner bean (*P. coccineus* L.), teary bean (*P. acutifolius* A. Gray), and year bean (*P. polyanthus* Greenman). Among those, common bean is the most important specie economically and

scientifically (Gepts, 2001), being the third legume crop in importance, after soybean and peanut, but first in direct human consumption (Broughton et al., 2003).

P. vulgaris stem can vary from 0.3 to 7 m, so that the plants can be dwarf or have climbing growth habit. The color of flowers can be from light pink, white to violet, with size from 1.0 to 1.8 cm. The fruit shape is linear or oblong, with its dimensions ranging from 11-15 mm length, 5-8 width, and 5 mm thickness. The weight of 1,000 grains is from 200 to 500 g (Giurcă, 2009), and the main components of mature grains are carbohydrates (54.3 - 59.9%), proteins (20.9 - 22.1%), lipids (2.49 - 2.52%), crude fiber (6.26 - 8.55%), ashes (3.8 - 4.0%), and moisture (5.29 - 9.93%) (Costa et al., 2006).

P. coccineus, also known as Scarlet runner beans or bean from Spain, differs from the *P. vulgaris* because its cotyledon emergence is hypogeal and has tuberous roots, rich in starch. Stem varies from 2 to 7 m, being a climbing specie. The length of the seeds can range from 18 to 25 mm, the width 12-14 mm, and the thickness of 7-12 mm. Flowers can be white or red and larger than *P. vulgaris*. Seeds can be white or violet with black spots. The weight of 1,000 grains is from 1,000 to 1,400 g. Mature grains are composed of 20% protein, 63% carbohydrates, 1.5% fibers, and 3.5% ash (Kay, 1979; Giurcă, 2009). Compared to *P. vulgaris* species, *P. coccineus* has a high allogamy degree (Giurcă, 2009), attracting more pollinators (Fig. 1.1).

Vigna unguiculata, also known as cowpea, is one of the most widely adapted, versatile, and nutritious grain legumes (Rachie and Singh, 1985). Cowpea flowers are large, about 2 cm, and breadth (when open), has cleistogamous structure, and simultaneous pollen shed and stigma receptivity. These factors lead to a high degree of self-pollination (Ehlers and Hall, 1997). Flowers are yellow, white, red, or purple

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(IBPGR, 1983). Length, width, and thickness are, on average, 9.28 mm, 6.55 mm, and 6.08 mm, respectively. Seeds are composed of 24.8% protein, 1.9% fat, 6.3% fibre, and 63.6% carbohydrates (Kabas et al., 2006). Some morphological, physical, and nutraceutical characteristics of common bean, runner bean and cowpea are summarized in table 1.1.

COMMON BEAN PHENOLOGY

Common beans are cultivated in different crop systems and a wide range of environments such as the Americas, Europe, the Middle East, China, and Eastern Africa. It is one of the major crops with highest levels of variation in growth habit, seed characteristics (size, shape, color), maturity, and adaptation. It also has high variability, with more than 40,000 varieties worldwide (Jones, 1999).

Common bean plants can be classified as determinate bush, indeterminate bush with erect branches, indeterminate bush with prostrate branches, indeterminate with semiclimbing main stem and branches, indeterminate with moderate climbing ability and pods distributed evenly up the plant and indeterminate with aggressive climbing ability and pods mainly on the upper nodes of the plant (IBPGR, 1982).

Phaseolus vulgaris plants have three main phenological growth phases: vegetative, reproductive, and senescence. In the vegetative growth, the plant increases the number and area of leaves and improves its distribution by forming side shoots. Reproductive phase is when the plant blooms and produces pods, that later have grain filled. Senescence is when the plant reaches its maturity, dying, and having the pods dry and

ready for harvesting. The BBCH-identification keys of beans with the phenological growth stages is on the table 1.2 (Feller et al., 1995).

Heat units expressed in Growing degree days (GDD) are frequently used to describe the timing of biological processes. It uses the average air temperature and the crop base temperature to predict crop development. It intends to describe the heat energy received by the crop in a given period of time (McMaster and Wilhelm, 1997). Then, air temperature is a dominant factor controlling crop development (Yang et al., 1995) and the average temperature should increase in the next decades in different parts of the world, bringing the necessity of changes in the cultivation systems of various crops (Schlenker et al., 2007; Tait, 2008).

The selection of an appropriate base temperature is essential to calculate the growing degree days. However, there are different methods to define it and each method provides a different value (Yang et al., 1995). For common beans, 10°C is commonly used as the base temperature (Yang et al., 1995; Jenni et al., 2000). However, different base temperatures can be found for different varieties, at different growth stages and different locations (Jenni et al., 2000). Assessing different accessions, Scully and Waines (1988) found base temperature ranging from even -4.5°C to 6.4°C, while Jenni et al. (2000) found from 0°C from sowing to maturity for the cultivars labrador, goldrush, and teseo, to 15°C to the cultivar flevoro in the period from flowering to maturity.

DRY BEAN PRODUCTION IN THE WORLD AND IN ITALY

In 1987, 15 million Mg of dry beans were produced in the world, in 25.6 million hectares, with yields of about 588 kg per hectare. After 30 years, the production more than doubled to over 31 million Mg in 36.6 million hectares, with yields of 861 kg per hectare, with almost 50% (15.4 million) produced in Asia. The yield increase is due to new technologies, mainly breeding programs around the world. Brazil, Myanmar, India, and China together (16.19 million) account for more than 50% of the total world production (FAOSTAT, 2017).

In China, the production slightly changed from 1987 to 2017, from 1.48 million Mg to 1.33 million Mg; however, the yield increased from 1,044 kg per hectare to 1,651 kg per hectare. Brazilian production increased by almost 50% in the same period, from 2 to 3 million Mg, and the yield more than doubled from 384 kg per hectare to 1,085 kg per hectare. In India, the total production increased from 3.2 million to 6.39 million Mg, and the yields remained at about 400 kg per hectare. In Myanmar, the dry bean production increased about fifteen times, from 362,000 Mg to 5.47 million Mg in the same period, and the yields increased from 933 kg per hectare to 1,719 kg per hectare, one of the highest yields in the world currently (FAOSTAT, 2017). In Italy, however, bean production is decreasing.

In 1967, the Italian production was 174,000 Mg, which decreased to 51,200 Mg in 1997, achieving the lowest level in 2017, 11,200 Mg. In this same period, Italy increased its dry bean importation from 22,000 Mg in 1967 to 118,000 Mg in 2017, about ten times more than the current production (FAOSTAT, 2017). According to Ranalli and Parisi

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(2018), the drastic reduction of bean production in Italy in the last five decades can be explained by:

- i) the abandonment of promiscuous cultivation,
- ii) the low economic competitiveness towards other crops (straw cereals, corn, chard, etc.),
- iii) the worsening of diseases caused by viruses, bacteria, and fungi that are not easily controlled,
- iv) the scarce availability of varieties endowed with genetic resistance to plant diseases and suitable for the mechanization of the various cultivation operations, in particular the harvest.

However, the Italian dry bean production is expected to increase in the following years due to the following reasons (Nelson et al., 2016; Ranalli and Parisi, 2018):

- the availability of the first varieties constituted, or being created, resistant to diseases, in particular to some virosis and bacteriosis,
- the significant importation of dry grain from abroad for the relatively large consumption still being made of it, thanks to consolidated traditional food habits and nutritional and dietetic value,
- iii) the need for alternative cultivation in soils subjected to very tight alternations and for problems of a surplus of other productions, especially cereals,
- iv) the growing interest in vegetable source of protein,

v) the increasing demand for Made in Italy products and typical local products.

This same downward trend in production in the last decades, followed by a sharp rise is being observed in Europe. In 1961 Europe produced 1.1 million Mg of dry beans, almost five times more than in 2007, 237,000 Mg. However, in the last decade, European production increased to 1.1 million Mg again (FAOSTAT, 2017). Thus, the bean production increase in Italy, as predicted by Ranalli and Parisi's (2018) is already happening in Europe in the last few years.

IMPORTANCE OF BEANS ON HUMAN DIET

NUTRITIONAL AND NUTRACEUTICAL PROPERTIES

Bean is a grain legume, also known as pulse, and represents the primary source of essential amino acids for people in many developing regions in the world. It is recognized as an essential source of protein (Duranti and Gius, 1997). Legumes and beans are also gaining importance because of their health benefits preventing and helping to manage hypercholesterolemia, hypertension (Arnoldi et al., 2015), obesity, diabetes, and coronary conditions (Calles, 2016). Indeed, legumes are a critical and affordable source of plantbased proteins, vitamins, and essential minerals like calcium, magnesium, and zinc, contributing to the food security and nutrition of people around the world, especially subsistence smallholder farmers in developing countries (Calles, 2016). In developed countries, vegetarians, vegans, and flexitarian diets tend to increase and the legumes are recommended as the main plant-based protein source (Nelson et al., 2016).

The legumes are rich in protein and water-soluble fibers. In the United States, legumes consumption four times or more a week was associated with 11% lower risk of cardiovascular diseases and 22% lower risk of coronary heart diseases, when compared with the consumption of less than once a week (Bazzano et al., 2001). Pulses have low glycemic index values, which increases satiety, helping obese and overweight individuals to control their weight. The low glycemic index values of legumes also bring benefits for healthy individuals in terms of post-prandial glucose and lipids metabolism (Rizkalla et al., 2002). Also, pulses have a variety of compounds that, depending on the consumed quantities, may help to prevent colon, breast, and other cancers (Mathers, 2002).

In 1990, the global prevalence of anemia was about 40.2%, and although it decreased to 32.9% in 2010, it still affects more than 2.2 billion people in the world, with iron deficiency being the most common cause. The lowest burden by iron deficiency is in North America's high income (2.9% of the envelope), while in Central Asia and Andean Latin America, this percentage is over 60%. Children aged 0-5 years, women of childbearing age, and pregnant woman are the categories with higher risks. Anemia and iron deficiency are associated with several chronic diseases (Kassebaum et al., 2014; Lopez et al., 2016). Iron and zinc are found in similar foods and adsorption of both may be affected by other food compounds. Dietary iron and zinc intakes and status can be positively associated (Lim et al., 2015).

Human and soil zinc deficiency can be correlated in African, Asian, Andean South America, and Mexico (Alloway, 2008; Wessells and Brown, 2012; Cakmak et al., 2017). In these same regions where beans are common sources of protein, beans can also be an excellent source of zinc for human diets (Jones, 1999; USDA, 2015). Its consumption in sufficient quantities is considered a strategic remedy for hidden hunger and healthy diets (Larochelle et al., 2015). Although beans are rich in iron, isotopes studies indicate that iron from beans is lowly absorbed by humans since the phytic acid content is also high and inhibits it (Petry et al., 2015).

BEANS' CARBOHYDRATES

Bean's carbohydrates are majorly composed of starch and non-starch polysaccharides, together with a considerable amount of their derivatives, such as oligosaccharides (Bravo et al., 1998; Reynoso-Camacho et al., 2006). Starch can be classified in three different types based on its susceptibility to amylases and the release rate of glucose and its absorption in the gastrointestinal tract: slowly digestible starch (SDS), rapidly digestible starch (RDS) and resistant starch (RS) or non-digestible starch (NDS) (Englyst et al., 1992).

Compared to the cereals and tuberous crops, the digestibility of the bean starch is considered much lower since it has a high content of resistant starch and other components (Tovar and Melito, 1996). It contains a greater amount of amylose, which confers to those starches a lower bioavailability than of most starches, when raw or retrograded (Guillon and Champ, 2002), and interactions of the starch with proteins can reduce its digestibility (Ovando-Martínez et al., 2011). These characteristics of the starch contained in beans can have important physiological effects, promoting a slow and moderate postprandial response to the level of blood glucose and insulin (Guillon and Champ, 2002; Sievenpiper et al., 2009). The most common oligosaccharides present in legumes are α -galactosides, which are sugars of low molecular weight, mainly

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represented by oligosaccharides of the raffinose family. Their total and relative abundance varies between different species and cultivars (Díaz-Batalla et al., 2006b). The α -galactosidase are not produced by the mammals, so it is not digested in the upper part of the gastrointestinal tract, becoming available to the bacterial fermentation in the colon, which produces carbon dioxide, causing flatulence in humans and other animals (Rackis, 1975). For this reason, α -galactosides are considered an antinutrient. However, it apparently has prebiotic effects, that can be an opportunity to valorize these oligosaccharides (DF – divide sentence in two) (Guillon and Champ, 2002; Martínez-Villaluenga et al., 2008).

BEANS' AMINO ACIDS AND PROTEINS

Seeds contain different types of proteins that are accumulated during plant development. Some of these are present in high amounts and serve to provide a store of amino acids for use during the germination and seedling growth, such as water-soluble albumins and salt-soluble globulins (Shewry et al., 1995). Those, respectively, account for 10 to 30% and 45 to 70% of common bean total protein content on a dry weight basis (Sathe, 2002). Dry bean is known as a major source of protein. Half cup of beans (equivalent to 90 g) provides from 7 to 8 g of protein, about 15% of the daily requirement of a 70 kg adult (Messina, 1999). Dry bean is considered to be not only in the vegetable group, but also in the protein food groups as dry beans have similar protein profiles as foods in both groups and also provide other nutrients that are found in seafood, meats, and poultry, such as iron and zinc (USDA, 2015). Animal and plant proteins are composed of about 20 amino acids that are present in all kinds of food proteins, varying according to the type of protein (NCBI, 1989). Although other elements can be found in the side chains of amino acids, they are mainly composed of carbon (C), hydrogen (H), oxygen (O), and nitrogen (N), known as its key elements (ChEBI, 2018). In general, the requirement of the human body is represented in terms of protein. However, the biological requirement is, in fact, in terms of amino acids. In the human body, amino acids are required for the synthesis of body proteins and other nutrient-containing compounds (creatine, peptide hormones, neurotransmitters). The protein and amino acids consumed in excess are not stored – as carbohydrates and lipids are - and are degraded. The nitrogen is excreted as urea in the urine. Nitrogen can also be lost in feces, sweat, and other body secretions or losses such as hair, and skin exfoliation, and cut nails (NCBI, 1989).

There are nine amino acids that, although required, are not synthesized by the human body. These amino acids need to be obtained through the food and are known as essential amino acids. The essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (NCBI, 1989).

BEAN'S TOTAL PHENOLS AND ANTIOXIDANT CAPACITY

Beans also have considerable contents of phenols and flavonoids. Polyphenols usually are present in the seed coat, rarely in the cotyledons. Varieties with red, black, blue-violet colors are also rich in anthocyanin and, consequently, have a high antioxidant and antiradical activities (Díaz-Batalla et al., 2006b; Ombra et al., 2016). Colored varieties also tend to have a higher content of total phenols and greater antioxidant activities compared to non-pigmented ones (Oroian and Escriche, 2015; Ombra et al., 2016). Phenols can bring benefits such as reducing the risk of cancer, aging, and apoptosis, and oxidative stress (Cardador-Martínez et al., 2006, Kalogeropoulos et al., 2010, Ranilla et al., 2010). The antioxidants can be found in vegetable and fruits and prevent or delay some types of cell damages (NIH - U.S., 2013). Previous studies have shown that different bean accessions have a great range of total phenols and antioxidant contents (Lee et al., 2000, Madhujith et al., 2004, Oomah et al., 2005). However, dry beans need to be cooked in order to be consumed by humans. Different accessions are also affected by different cooking processes and, most of the times, the nutritional value of the consumed bean is reduced after it is processed (Rocha-Guzmán et al., 2007; Boateng et al., 2008; Ranilla et al., 2009; Akillioglu and Karakaya, 2010).

BEANS DOMESTICATION AND GENETIC DIVERSITY

Beans (*Phaseolus spp.*) are used as a model for understanding crop evolution. One almost unique situation that characterizes the *Phaseolus* genus is that for *P. vulgaris* and *P. lunatus*, the wild forms are distributed in both Mesoamerican and South America (Andean mountains), where at least two independent and isolated episodes of domestication occurred (Bitocchi et al., 2017). A strong pre-domestication bottleneck is responsible for the formation of the Andean population. Despite having undergone different domestication processes, both gene pools are partially sexually compatible and morphologically similar. The differences between these populations can be detected through molecular tools and were revealed using different molecular markers: random

amplified polymorphic DNA (RAPD) (Johns et al., 1997; Beebe et al., 2000), amplified fragment length polymorphism (AFLP) (Tohme et al., 1996; Beebe et al., 2001), microsatellites (SSRs) (Blair et al., 2006, 2007; Díaz and Blair, 2006), and more recently single nucleotide polymorphism (SNP) markers (Cortés et al., 2011; Bitocchi et al., 2013).

The independent domestication processes started about 8,000 years ago. Beebe et al. (2001) and Bitocchi et al. (2013) results suggested that, in South America, the origin of bean domestication is between Northern Argentina and Southern Bolivia. Bitocchi et al. (2013) proposed a single domestication event within each gene pool. The origin of bean domestication in Mesoamerica is likely to have occurred in the Oaxaca valley (Bitocchi et al., 2013) in central Mexico, located south of Mexico City. However, Kwak et al. (2009) suggested that the origin of the Mesoamerican bean domestication was in Rio Lerma-Rio Santiago basin, north of Mexico City.

Angioi et al. (2010) analyzed six chloroplast microsatellite loci and two unliked nuclear loci of 307 *P. vulgaris* European landraces and compared with 94 accessions representing Mesoamerican and the Andean gene pools. They found that 67% of the European landraces are from the Andes. In addition to that, 44% of the European landraces have undergone hybridization between Andean and Mesoamerican accessions. These hybrids are present everywhere in Europe, with uneven distribution and high frequencies in Central Europe and lower frequencies in Spain and Italy. In Italy, onequarter of the assessed accessions were from Mesoamerica.

Plant genetic resources for food and agriculture conservation and sustainable use are essential to ensure that the world will produce enough food to feed its growing population. For this reason, the International Treaty on Plant Genetic Resources for Food and Agriculture was adopted by FAO in November 2001 (FAO, 2020). Genetic resources can be conserved *ex-situ* and *in-situ*. *Ex-situ* means that conservation is held outside the natural habitat, sampling, transferring, and storing the genetic materials that can be maintained via seed/ovule/semen, in-vitro, or in botanical gardens or zoos (Maxted, 2013). There are almost 117,000 conserved ex situ accessions of Phaseolus spp., of which 86% is of *P. vulgaris*. About two-thirds of these accessions are traditional cultivars and landraces, 15% are improved cultivars, and only 3% are wild relatives (CGIAR (Genebank Platform), 2020). Currently, the International Center for Tropical Agriculture holds the largest collection, preserving almost 40,000 accessions from 112 countries. Followed by the USDA (National Plant Germplasm System) Western Regional Plant Introduction Station at Pullman, WA, USA, which have more than 17,000 accessions, of which 14,075 are *P. vulgaris*. *In-situ* conservation is the maintenance of viable populations of species in the surroundings where they have developed their distinctive properties. Its steps are designation, management and monitoring in a reserve or on-farm (Maxted, 2013). Thus, *in-situ* conservation can have a complementary role in the *ex-situ* conservation. It is important to develop the plant genetic resources for food and agriculture simultaneously with the needs of farmers and social-cultural, economic and environmental changes (Negri and Tiranti, 2010).

BEANS IN ITALY

INTRODUCTION OF COMMON BEAN IN ITALY

The first introduction of common bean from Central/South America into Western Europe most likely took place around 1500, in the sixteenth century (Zeven, 1997). In Italy, beans are present in a 1515 Giovanni di Udine painting (Albala, 2007), and mentioned in historical documents fixing 1532 as the year of common bean introduction in Italy. It created a long tradition that allowed the evolution of many landraces that are adapted to microclimates in restricted areas. It represents a pastiche of cultures and traditions that are an irremissible good for Italy, and they are used by low impact environmental agriculture (Venora et al., 2009). In Veneto, the diffusion of common bean occurred quickly, and today, the cultivation of this pulse is of great economic relevance in Belluno region (Piergiovanni and Lioi, 2010). However, although those landraces have original morpho-agronomic and nutritional characteristics, with high organoleptic qualities, they have been gradually substituted by genetic uniform commercial varieties (Spagnoletti Zeuli et al., 2004; Venora et al., 2009). After the 1950's the large scale of breeding programs and fast disappearance of landraces, increased the importance of collecting and preserving those landraces in germplasm, *in-situ* or *ex-situ*, contributing to the improvement of food crops and preserve its genetic diversity (Piergiovanni and Laghetti, 1999). Conserve and preserve landraces from the Veneto region are one of the objectives of the Valebio project.

PRODUCT DESIGNATION OF ORIGIN (PDO) AND PROTECTED GEOGRAPHIC INDICATION (PGI) OF ITALIAN BEANS

In Italy, centuries of traditions and dedication to quality and excellence have built a reputation for high-quality food. The protected designation of origin (PDO) is a label - certified by the European Union – that is awarded to products that have a close tie to its territory of origin. The product must be produced, processed, and prepared in a given geographical area, recognizing production methods. Protected Geographic Indication (PGI) is awarded to agricultural products and foodstuff closely linked to the geographical area (Italian Made, n.d.). Most of the consumers are willing to pay a premium price for products with PDO labels since they see it as a positive aspect (Fotopoulos and Krystallis, 2003) and a reliable indicator of product quality (Menapace et al., 2011).

Of the more than two hundred products with PDO and PGI labels from Italy, six are beans. The fagioli bianchi di Rotonda are a PDO from Potenza, in the region of Basilicata. Potenza (in Basilicata) also has a PGI bean, the fagiolo di Sarconi. Frosinone, in the region Lazio, has the other PDO bean from Italy, the fagiolo canellino di Atina. The other three PGI beans are from Piemonte (fagiolo di Cuneo, from Cuneo), Toscana (fagiolo di Sorana, from Pistoia), and Veneto (fagiolo di Lamon della Vallata Bellunese, from Belluno) (Fig. 1.2).

However, the fagiolo di Lamon is not the only type of bean traditionally cultivated in Veneto. The dry beans Blu, Borlotto, Diavolóni, Fasóla, Gialèt , Gnòchi, Madrìna, Mama, da l'òjo, da l'òro, del Papa, de Pósina, Scalda o Scaldafèro, Scarpari and the green beans Cornéte o téghe Cornéte, Slandróne, Stringhe are other examples of typical beans cultivated for centuries in Veneto. The fagiolo Scalda e la Fasóla posenàta di Posina (VI), Borlotto nano di Levada e fagiolino "Meraviglia di Venezia" have the cerficate of typicality (Giallo Zafferano, n.d.).

DESCRIPTION OF DAFNAES GERMPLAM

The Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) from the University of Padova has a germplasm to conserve the genetic material of Italian and Venetian landraces. Currently, this germplasm contains 48 accessions of which three are cowpeas (*Vigna unguiculata*), two are runner beans (*P. coccineus*) and 43 common beans (*P. vulgaris*). The cowpeas and 18 of the *P. vulgaris* are Italian accessions, provided by Blumen S.A. and are elite lines or pre-commercial varieties. Of these, eight are dwarf and 10 are climbing. The other 27 are climbing landraces from the mountainous area of the Veneto. The accessions' id number, name and growth habits are presented on Table 1.3. Table 1.4 shows some morphological characteristics of the pods and seeds of 26 of the accessions used on Chapter 2. Pictures of the pods, leaves and seeds of these accessions are presented in the Annexes.

The genetic characterization of these accessions was performed by using microsatellites and single nucleotide polymorphisms (SNPs). The genetic similarity within and among the populations is in figure 1.3. Of the 48 accessions, the three cowpeas were detected, thus, are not included into the genetic characterization. Other five accessions were not included into the genetic characterization because the seeds did not germinate, and it was not possible to collect genetic material from the leaves. The accessions were divided into three main clusters: -Cluster A, 22 accessions: 1, 2, 3, 6, 8, 10, 11, 15, <u>19</u>, 20, **26**, **27**, **30**, **32**, **33**, **34**, **36**, **38**, **39**, **41**, **42**, **47** (landraces in bold)

-Cluster B, 16 accessions: 5, 7, 9, 12, 14, 17, 18, <u>19</u>, **23**, **24**, **25**, **28**, **29**, **31**, **44**, **46** -Cluster C, 2 accessions: 4 and 22.

The cluster C is composed by two *P. coccineus* accessions and is 32.06% genetically similar to the cluster A and 30.08% similar to the cluster B. Cluster A has 97.48% genetic similarity within it, and 61.12% when compared to the cluster B, that has 90.7% genetic similarity within it. Based on the National Center for Biotechnology Information (NCBI) dataset it was possible to relate clusters A and B ancestry to the domestication centers. Cluster A was related to the Andean domestication center and B to the Mesoamerican.

EFFECTS OF DROUGHT CONDITIONS ON BEAN PRODUCTION

During the production cycle, crops are subjected to stresses that are external conditions that adversely affect the growth and development of the plant and, consequently, the yield. Stresses are classified into biotic and abiotic stresses. Biotic stress is when the plant is attacked by pathogens and/or other living organisms. Abiotic stress is caused by the physical environment, leading to the loss of major crops worldwide (Gull et al., 2019).

Less than 1% of the water absorbed by the plant is retained in the biomass and the remainder is lost by evapotranspiration (Beebe et al., 2013a). The evapotranspiration is essential and responsible for high water losses by the plant because (Beebe et al., 2013a):

- i) it is part of the stomatal opening process, that allows the leaves to get carbon dioxide from the atmosphere, which will be a substrate for the photosynthesis (Medrano et al., 2002),
- ii) leaves and stems can lose water to the atmosphere even when the stomata are closed (Radin et al., 1994),
- when the plant is exposed to high temperatures, heating effect of light and/or low atmospheric relative humidity transpiration rate increases to cool leaves (Radin et al., 1994),
- iv) the transpiration rate is essential for the transport of nutrients from the soil and of compounds chemically synthesized by the roots, that end in the leaves (Peuke et al., 2002).

Most of the bean cultivated area is found in Latin America and Africa (FAOSTAT, 2017), in regions with climate susceptible to drought during the growing period (NIDIS, 2019). For beans with 60 to 120 days cycle, depending on environmental conditions, a range from 300 to 500 mm of water is required to achieve the maximum production potential (Beebe et al., 2013a). It is estimated that 73% of the bean cultivated area in Latin America and 40% in Africa is under micro-climate conditions that will have moderate to severe water-deficits at some time during the cropping season (Broughton et al., 2003). In countries and regions with intensive agriculture and with more technology, these effects can be relatively reduced. In the USA, average yields are above 1.6 Mg ha⁻¹, and in Brazil under intensive management and irrigation yields average 1.8 Mg ha⁻¹.

However, in over a million hectares cultivated in highland Mexico yields are below 0.4 Mg ha⁻¹. In Northeastern Brazil, where at least another million hectares of beans are cultivated, yields are about 0.45 Mg ha⁻¹ (Broughton et al., 2003; Beebe et al., 2013a). In Africa, hundreds of thousands of hectares are also affected by drought, leading to the loss of almost one million tons of production each year due to the water-deficit (Wortmann et al., 2004). Thus, common bean low yields are clearly related to drought stress and, unfortunately, to the fact that dry areas where beans are cultivated are also poverty hot spots where there is less capital of investment (Beebe et al., 2013a). To screen and identify drought stress tolerant beans is important tools to help reduce the yield loss by water-deficit in these regions.

SCOPE AND OBJECTIVES OF THE STUDY

The research project Valebio is a partnership between the University of Padova and Blumen Group SpA and will assess different landraces from the Veneto region and precommercial Italian varieties. The accessions used in the project were divided into three main groups: pre-commercial varieties, elite lines, and Venetian landraces. The Italian pre-commercial accessions are climbing pre-commercial cultivars provided by the Blumen S.A. seed company and the elite lines are pre-commercial Italian dwarf accessions. The Venetian landraces used in this project were collected from Venetian lowlands and high hill or mountain environments. Although the Veneto is not one of the primary domestication centers of the common beans, the collected material can be considered autochthonous because these beans have been cultivated in isolation for centuries. This material essentially underwent an ecotypization process, which determined the selection of accessions and gene combinations, able to achieve the maximum adaptation to the new pedo-climatic and anthropic conditions. The long-term germplasm utilization and conservation operated yearly by farmers, along with the natural evolutionary processes through climate changes, have therefore brought to the formation of Veneto germplasm itself for many species, including common beans. This project also aims to perform the agronomic, morphological, genetic, and nutraceutical characterization. It will select the ones suitable for cultivation in organic agricultural systems of this region, improving the value of biodiversity of culturally and historically typical leguminous species for Veneto. The seeds will be stored at the Veneto Germplasm Bank at the University of Padova. In addition, the project aims to develop commercial varieties based on single pure lines of some accessions, including common beans with colored seeds, improved nutraceutical traits in order to use their seeds as a source of proteins, fibers, and polyphenols, especially for aged and medically fragile populations. These newly bred varieties are expected to be important at both the regional and national levels.

This thesis is part of the Valebio Project and aimed to perform the agronomic, genetic, nutraceutical and morphological characterization of these accessions cultivated in two typical Venetian environments: mountains (montagna) and sea level (pianura). Landraces with agronomic high-performance and interesting nutraceutical characteristics were selected and evaluated under drought conditions in the state of Georgia, USA.

The Valebio Project consists of two-year field trials. This work presents the data and results from the first-year trials and a trial assessing the effect of drought conditions on the agronomic performance of six highly productive Venetian landraces. It is
important to highlight that in the first year (2019) the number of seeds, mainly from landraces, was limited. The main reasons are that those seeds were collected from farmers or collectors that did not provide a large number and some seeds were not in ideal storage conditions, thus, some landrace bags came infested with woodworm, which reduced the number of viable seeds. For this reason, in the first-year one of the focus was to multiply these seeds and store properly to next year. Thus, it was not possible to perform the nutraceutical characterization of these accessions after cooking, nor sensorial testing.

Specific objectives:

-Genetic characterization: to determine the degree of homozygosity of each individual cultivated. In addition, determining the genetic similarity within and among the populations, relating them to the Andean and Mesoamerican domestication centers,

-Agronomic characterization: to evaluate the responses of different accessions in different cultivation environments and how that affects the phenological development of the plant and its productivity

-Nutraceutical characterization: to assess the effect of the accession and of the environment in nutraceutical properties of the seeds

-Morphological characterization: to assess the effect of the accession and of the environment in the plants' and seeds' morphological characteristics

-Screening for drought tolerance: to assess the effect of drought conditions on the agronomic performance of six Venetian landraces.

LITERATURE CITED

Akillioglu HG, Karakaya S (2010) Changes in total phenols, total flavonoids, and antioxidant activities of common beans and pinto beans after soaking, cooking, and in vitro digestion process. Food Science and Biotechnology 19: 633–639.

Albala K (2007) Phaseolus vulgaris: Mexico and the World. Beans: a History London: Bloomsbury Academic. p. 240.

Alloway BJ (2008) Zinc in soils and crop nutrition. Paris: IZA and IFA.

Angioi SA, Rau D, Attene G, Nanni L, Bellucci E, Logozzo G, Negri V, Zeuli

PLS, Papa R (2010) Beans in Europe: Origin and structure of the European landraces of Phaseolus vulgaris L. Theoretical and Applied Genetics 121: 829–843.

Arnoldi A, Zanoni C, Lammi C, Boschin G (2015) The Role of Grain Legumes in the Prevention of Hypercholesterolemia and Hypertension. Critical Reviews in Plant Sciences 34: 144–168.

Bailey LH (1924) Manual of Cultivated Plants. London: Macmillan & Co.

Bazzano LA, He J, Ogden LG, Loria C, Vupputuri S, Myers L, Whelton PK

(2001) Legume consumption and risk of coronary heart disease in US men and women:

NHANES I Epidemiologic Follow-up Study. Archives of internal medicine 161: 2573–2578.

Beebe S, Rengifo J, Gaitan E, Duque MC, Tohme J (2001) Diversity and Origin of Andean Landraces of Common Bean. Crop Science 41: 854–862.

Beebe S, Skroch PW, Tohme J, Duque MC, Pedraza F, Nienhuis J (2000) Structure of Genetic Diversity among Common Bean Landraces of Middle American Origin Based on Correspondence Analysis of RAPD. Crop Science 40: 264–273. Beebe SE, Rao IM, Blair MW, Acosta-Gallegos JA (2013) Phenotyping common beans for adaptation to drought. Frontiers in Physiology 4: 35.

Bitocchi E, Bellucci E, Giardini A, Rau D, Rodriguez M, Biagetti E, Santilocchi R, Spagnoletti Zeuli P, Gioia T, Logozzo G, Attene G, Nanni L, Papa R (2013) Molecular analysis of the parallel domestication of the common bean (Phaseolus vulgaris) in Mesoamerica and the Andes. New Phytologist 197: 300–313.

Bitocchi E, Rau D, Bellucci E, Rodriguez M, Murgia ML, Gioia T, Santo D, Nanni L, Attene G, Papa R (2017) Beans (Phaseolus ssp.) as a model for understanding crop evolution. Frontiers in Plant Science 8: .

Blair MW, Díaz JM, Hidalgo R, Díaz LM, Duque MC (2007) Microsatellite characterization of Andean races of common bean (Phaseolus vulgaris L.). Theoretical and Applied Genetics 116: 29–43.

Blair MW, Giraldo MC, Buendía HF, Tovar E, Duque MC, Beebe SE (2006) Microsatellite marker diversity in common bean (Phaseolus vulgaris L.). Theoretical and Applied Genetics 113: 100–109.

Boateng J, Verghese M, Walker LT, Ogutu S (2008) Effect of processing on antioxidant contents in selected dry beans (Phaseolus spp. L.). LWT - Food Science and Technology 41: 1541–1547.

Bravo L, Siddhuraju P, Saura-Calixto F (1998) Effect of Various Processing Methods on the in Vitro Starch Digestibility and Resistant Starch Content of Indian Pulses. Journal of Agricultural and Food Chemistry 46: 4667–4674.

Broughton WJ, Hernández G, Blair M, Beebe S, Gepts P, Vanderleyden J (2003) Beans (Phaseolus spp.) - Model Food Legumes. p. 55–128. Butare L, Rao I, Lepoivre P, Polania J, Cajiao C, Cuasquer J, Beebe S (2011) New genetic sources of resistance in the genus Phaseolus to individual and combined aluminium toxicity and progressive soil drying stresses. Euphytica 385–404.

Cakmak I, McLaughlin MJ, White P (2017) Zinc for better crop production and human health. Plant and Soil 411: 1–4.

Calles T (2016) Preface to special issue on leguminous pulses. Plant Cell, Tissue and Organ Culture (PCTOC) 127: 541–542.

Cardador-Martínez A, Albores A, Bah M, Calderón-Salinas V, Castaño-Tostado E, Guevara-González R, Shimada-Miyasaka A, Loarca-Piña G (2006) Relationship among antimutagenic, antioxidant and enzymatic activities of methanolic extract from common beans (Phaseolus vulgaris L). Plant Foods for Human Nutrition 61: 161–168.

CGIAR (Genebank Platform) (2020) [Internet]. Available from:

https://www.genebanks.org/resources/crops/beans/

ChEBI (2018) [Internet]. Available from:

https://www.ebi.ac.uk/chebi/searchId.do?chebiId=33709

CIAT (International Center for Tropical Agriculture) [Internet]. Available from: https://ciat.cgiar.org/what-we-do/crop-conservation-and-use/bean-diversity/

Conway G (1998) The doubly green revolution : food for all in the twenty-first century. Itacah, USA: Comstock Pub. Associates.

Cortés AJ, Chavarro MC, Blair MW (2011) SNP marker diversity in common bean (Phaseolus vulgaris L.). Theoretical and Applied Genetics 123: 827–845. Costa GEA, Queiroz-Monici KS, Reis SMPM, Oliveira AC (2006) Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. Food Chemistry 94: 327–330.

Courty PE, Smith P, Koegel S, Redecker D, Wipf D (2015) Inorganic Nitrogen Uptake and Transport in Beneficial Plant Root-Microbe Interactions. Critical Reviews in Plant Sciences 34: 4–16.

Díaz-Batalla L, Widholm JM, Fahey GC, Castaño-Tostado E, Paredes-López O (2006) Chemical Components with Health Implications in Wild and Cultivated Mexican Common Bean Seeds (Phaseolus vulgaris L.). Journal of Agricultural and Food Chemistry 54: 2045–2052.

Díaz LM, Blair MW (2006) Race structure within the Mesoamerican gene pool of common bean (Phaseolus vulgaris L.) as determined by microsatellite markers.

Theoretical and Applied Genetics 114: 143–154.

Duranti M, Gius C (1997) Legume seeds: protein content and nutritional value. Field Crops Research 53: 31–45.

s JD, Hall AE (1997) Cowpea (Vigna unguiculata L. Walp.). Field Crops Research 53: 187–204.

Englyst HN, Kingman SM, Cummings JH (1992) Classification and measurement of nutritionally important starch fractions. European Journal of Clinical Nutrition 46: 33– 50.

FAO (2019a) [Internet]. Available from: http://www.fao.org/hunger/en/ FAO (2019b) How to Feed the World in 2050. . FAO (2020) [Internet]. Available from: http://www.fao.org/planttreaty/overview/en/

FAO, IFAD, UNICEF, WFP, WHO (2019) The State of Food Security and Nutrition in the World 2019. Safeguarding against economic slowdowns and downturns. Rome:

FAOSTAT (2017) [Internet]. Available from:

http://www.fao.org/faostat/en/#data/QC

FELLER C. H, BLEIHOLDER L, BUHR H, HACK M, HESS R, KLOSE U, MEIER R, STAUSS T, VAN DEN BOOM E. (1995) Phenological stages of development of vegetables: II. Fruit vegetables and legumes, cited by: In: U Meier, editor. Growth stages of mono-and dicotyledonous plants BBCH Monograph Federal Biological Research Centre for Agriculture and Forestry (2001) BBCH working group. p. 141–144.

Fotopoulos C, Krystallis A (2003) Quality labels as a marketing advantage. European Journal of Marketing 37: 1350–1374.

Frontiers Research Topic [Internet]. Available from:

https://www.frontiersin.org/research-topics/9002/legumes-for-global-food-security

Gepts P (2001) Phaseolus vulgaris (Beans). In: S Brenner and JH Miller, editor.

Encyclopedia of genetics New York: Academic Press. p. 1444–1445.

Giallo Zafferano [Internet]. Available from:

https://blog.giallozafferano.it/timoelenticchie/il-veneto-e-i-suoi-fagioli/

Giurcă DM (2009) Morphological and phenological differences between the two species of the Phaselous genus (Phaseolus vulgaris and Phaseolus Coccineus). Cercetări Agronomice în Moldova XLII: .

Guillon F, Champ MJ (2002) Carbohydrate fractions of legumes: uses in human nutrition and potential for health. British Journal of Nutrition 88: 293–306.

Gull A, Ahmad Lone A, Ul Islam Wani N (2019) Biotic and Abiotic Stresses in Plants. In: A Oliveira, editor. Abiotic and Biotic Stress in Plants IntechOpen.

IBPGR (1982) [Internet]. Available from:

https://www.bioversityinternational.org/fileadmin/_migrated/uploads/tx_news/Phaseolus _vulgaris_descriptors_160.pdf

IBPGR (1983) [Internet]. Available from:

https://www.bioversityinternational.org/fileadmin/_migrated/uploads/tx_news/Descriptor s_for_cowpea_377.pdf

Italian Made [Internet]. Available from: http://www.italianmade.com/usa/pdo-pgi/

Jenni S, Bourgeois G, Laurence H, Roy G, Tremblay N (2000) Improving the

Prediction of Processing Bean Maturity Based on the Growing-degree Day Approach.

American Society of Horticulture Science 35: 1234–1237.

Johns MA, Skroch PW, Nienhuis J, Hinrichsen P, Bascur G, Muñoz-Schick C

(1997) Gene Pool Classification of Common Bean Landraces from Chile Based on

RAPD and Morphological Data. Crop Science 37: 605–613.

Jones A (1999) [Internet]. Available from: http://www.fao.org/3/a-av015e.pdf

Kabas O, Yilmaz E, Ozmerzi A, Akinci I (2006) Some physical and nutritional properties of cowpea seed (Vigna sinensis L.). Journal of Food Engineering 79: 1405–1409.

Kalogeropoulos N, Chiou A, Ioannou M, Karathanos VT, Hassapidou M, Andrikopoulos NK (2010) Nutritional evaluation and bioactive microconstituents (phytosterols, tocopherols, polyphenols, triterpenic acids) in cooked dry legumes usually consumed in the Mediterranean countries. Food Chemistry 121: 682–690.

Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, Regan M, Weatherall D, Chou DP, Eisele TP, Flaxman SR, Pullan RL, Brooker SJ, Murray CJL (2014) A systematic analysis of global anemia burden from 1990 to 2010. Blood 123: 615–624.

Katungi E, Farrow A, Chianu J, Sperling L, Beebe S (2009) Common bean in Eastern and Southern Africa: a situation and outlook analysis. International Center for Tropical Agriculture 1–61.

Kay D (1979) Food legumes. Tropical Products Institute, London. Crop and Product Digest 435.

Kwak M, Kami JA, Gepts P (2009) The Putative Mesoamerican Domestication Center of Phaseolus vulgaris Is Located in the Lerma-Santiago Basin of Mexico. Crop Science 49: 554–563.

Lee KG, Mitchell AE, Shibamoto T (2000) Determination of antioxidant properties of aroma extracts from various beans. Journal of Agricultural and Food Chemistry 48: 4817–4820. Lim K, Booth A, Szymlek-Gay EA, Gibson RS, Bailey KB, Irving D, Nowson C, Riddell L (2015) Associations between Dietary Iron and Zinc Intakes, and between Biochemical Iron and Zinc Status in Women. Nutrients 7: 2983–2999.

Lopez A, Cacoub P, Macdougall I, Peyrin-Biroulet L (2016) Iron deficiency anaemia. The Lancet 387: 907–916.

Madhujith T, Naczk M, Shahidi F (2004) Antioxidant activity of common beans (*Phaseolus vulgaris* L.). Journal of Food Lipids 11: 220–233.

Martínez-Villaluenga C, Frias J, Vidal-Valverde C (2008) Alpha-galactosides: Antinutritional factors or functional ingredients? Critical Reviews in Food Science and Nutrition 48: 301–316.

Mathers JC (2002) Pulses and carcinogenesis: potential for the prevention of colon, breast and other cancers. British Journal of Nutrition 88: 273–279.

Maxted N (2013) In Situ, Ex Situ Conservation. In: S Levin, editor. Encyclopedia of Biodiversity Elsevier Inc. p. 313–323.

McClean PE, Raatz B (2017) Common Bean Genomes: Mining New Knowledge of a Major Societal Crop. In: MP de la Vega, F Marsolais, and M Santalla, editor. The Common Bean Genome Gewebestrasse, Switzerland: Springer International Printer. p. 295.

McMaster GS, Wilhelm WW (1997) Growing degree-days: One equation, two interpretations. Agricultural and Forest Meteorology 87: 291–300.

Medrano H, Escalona JM, Bota J, Guli As J, F L Ex J (2002) Regulation of Photosynthesis of C 3 Plants in Response to Progressive Drought: Stomatal Conductance as a Reference Parameter. Annals of Botany 89: 895–905. Menapace L, Colson G, Grebitus C, Facendola M (2011) Consumers' preferences for geographical origin labels: evidence from the Canadian olive oil market. European Review of Agricultural Economics 38: 193–212.

Messina MJ (1999) Legumes and soybeans: Overview of their nutritional profiles and health effects.

Moore JA (1936) The Vascular Anatomy of the Flower in the Papilionaceous Leguminosae. II. American Journal of Botany 23: 355.

Nassar RMA, Ahmed YM, Boghdady MS (2010) Botanical studies on Phaseolus vulgaris L. I-morphology of vegetative and reproductive growth. International Journal of Botany 6: 323–333.

NCBI (1989) [Internet]. Available from:

https://www.ncbi.nlm.nih.gov/books/NBK234922/

Negri V, Tiranti B (2010) Effectiveness of in situ and ex situ conservation of crop diversity. What a Phaseolus vulgaris L. landrace case study can tell us. Genetica 138: 985–998.

Nelson ME, Hamm MW, Hu FB, Abrams SA, Griffin TS (2016) Alignment of Healthy Dietary Patterns and Environmental Sustainability: A Systematic Review. Advances in Nutrition 7: 1005–1025.

NIDIS (2019) [Internet]. Available from: https://www.drought.gov/gdm/currentconditions

NIH - U.S. (National Center for Complementary and Integrative Health) (2013) [Internet]. Available from: https://www.nccih.nih.gov/health/antioxidants-in-depth Ombra MN, D'acierno A, Nazzaro F, Riccardi R, Spigno P, Zaccardelli M, Pane C, Maione M, Fratianni F (2016) Phenolic Composition and Antioxidant and Antiproliferative Activities of the Extracts of Twelve Common Bean (Phaseolus vulgaris L.) Endemic Ecotypes of Southern Italy before and after Cooking. Oxidative Medicine and Cellular Longevity 2016: 1–12.

Oomah BD, Cardador-Martínez A, Loarca-Piña G (2005) Phenolics and antioxidative activities in common beans (Phaseolus vulgaris L). Journal of the Science of Food and Agriculture 85: 935–942.

Oroian M, Escriche I (2015) Antioxidants: Characterization, natural sources, extraction and analysis. Food Research International 74: 10–36.

Ovando-Martínez M, Bello-Pérez LA, Whitney K, Osorio-Díaz P, Simsek S (2011) Starch characteristics of bean (Phaseolus vulgaris L.) grown in different localities. Carbohydrate Polymers 85: 54–64.

Peix A, Ramírez-Bahena MH, Velázquez E, Bedmar EJ (2015) Bacterial Associations with Legumes. Critical Reviews in Plant Sciences 34: 17–42.

Petry N, Boy E, Wirth J, Hurrell R (2015) Review: The Potential of the Common Bean (Phaseolus vulgaris) as a Vehicle for Iron Biofortification. Nutrients 7: 1144–1173.

Peuke AD, Jeschke WD, Hartung W (2002) Flows of Elements, Ions and Abscisic Acid in Ricinus Communis and Site of Nitrate Reduction Under Potassium Limitation. Journal of experimental botany 53: .

Piergiovanni AR, Laghetti G (1999) The common bean landraces from Basilicata (Southern Italy): an example of integrated approach applied to genetic resources management. Genetic Resources and Crop Evolution 46: 47–52.

Piergiovanni AR, Lioi L (2010) Italian Common Bean Landraces: History, Genetic Diversity and Seed Quality. Diversity 2: 837–862.

Polania J, Poschenrieder C, Rao I, Beebe S (2016) Estimation of phenotypic variability in symbiotic nitrogen fixation ability of common bean under drought stress using 15 N natural abundance in grain. Europ. J. Agronomy 79: 66–73.

Rachie KO, Singh SR (1985) Cowpea research, production and utilization. New York: Wiley.

Rackis JJ (1975) Oligosaccharides of Food Legumes: Alpha-Galactosidase Activity and the Flatus Problem. In: A Jeanes and J Hodge, editor. Physiological Effects of Food Carbohydrates Washington D. C.: American Chemical Society. p. 207–222.

Radin JW, Lu Z, Percy RG, Zeiger E (1994) Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. Proceedings of the National Academy of Sciences of the United States of America 91: 7217–7221.

Ranalli P, Parisi B (2018) Bean and String Bean. Cultivation, Choice of Cultivars and Post-harvest (in Italian). Milan: Edagricole - New Business Media.

Ranilla LG, Genovese MI, Lajolo FM (2009) Effect of different cooking conditions on phenolic compounds and antioxidant capacity of some selected Brazilian bean (Phaseolus vulgaris L.) cultivars. Journal of Agricultural and Food Chemistry 57: 5734–5742.

Ranilla LG, Kwon YI, Genovese MI, Lajolo FM, Shetty K (2010) Effect of thermal treatment on phenolic compounds and functionality linked to type 2 diabetes and hypertension managements of Peruvian and Brazilian Bean cultivars (*Phaseolus vulgaris*L.) using in vitro methods. Journal of Food Biochemistry 34: 329–355.

Reynoso-Camacho R, Guevara-González RG, Torres-Pacheco I (2006) Bioactive components in common beans (Phaseolus vulgaris L.). Advances in agricultural and food biotechnology 666: 217–236.

Rizkalla SW, Bellisle F, Slama G (2002) Health benefits of low glycaemic index foods, such as pulses, in diabetic patients and healthy individuals. British Journal of Nutrition 88: 255–262.

Rocha-Guzmán NE, González-Laredo RF, Ibarra-Pérez FJ, Nava-Berúmen CA, Gallegos-Infante JA (2007) Effect of pressure cooking on the antioxidant activity of extracts from three common bean (Phaseolus vulgaris L.) cultivars. Food Chemistry 100: 31–35.

Rubiales D, Mikic A (2015) Introduction: Legumes in Sustainable Agriculture. Critical Reviews in Plant Sciences 34: 2–3.

Sathe SK (2002) Dry bean protein functionality. Critical Reviews in Biotechnology 22: 175–223.

Schlenker W, Hanemann WM, Fisher AC (2007) Water availability, degree days, and the potential impact of climate change on irrigated agriculture in California. Climatic Change 81: 19–38.

Scully B, Waines JG (1988) Ontogeny and Yield Response of Common and Tepary Beans to Temperature. Agronomy Journal 80: 921–925.

Shewry PR, Napier JA, Tatham AS (1995) Seed Storage Proteins: Structures and Biosynthesis. The Plant Cell 7: 945–956.

Sievenpiper JL, Kendall CWC, Esfahani A, Wong JMW, Carleton AJ, Jiang HY, Bazinet RP, Vidgen E, Jenkins DJA (2009) Effect of non-oil-seed pulses on glycaemic control: A systematic review and meta-analysis of randomised controlled experimental trials in people with and without diabetes. Diabetologia 52: 1479–1495.

Spagnoletti Zeuli PL, Baser N, Riluca M, Laghetti G, Logozzo G, Masi P,

Molinari S, Negri V, Olita G, Tiranti B, Veronesi F (2004) Valorisation and certification of Italian bean agro-ecotypes (Phaseolus vulgaris) (in Italian). p. 19–19.

Tait (2008) Future projections of growing degree days and frost in New Zealand and some implications for grape growing. Weather and Climate 28: 17.

Tohme J, Gonzalez DO, Beebe S, Duque MC (1996) AFLP Analysis of Gene Pools of a Wild Bean Core Collection. Crop Science 36: 1375–1384.

Tovar J, Melito C (1996) Steam-Cooking and Dry Heating Produce Resistant Starch in Legumes. Journal of Agricultural and Food Chemistry 44: 2642–2645.

USDA (National Plant Germplasm System) [Internet]. Available from: https://npgsweb.ars-grin.gov/gringlobal/search.aspx

USDA (2015) 2015-2020 Dietary Guidelines for Americans. .

Venora G, Grillo O, Ravalli C, Cremonini R (2009) Identification of Italian landraces of bean (Phaseolus vulgaris L.) using an image analysis system. Scientia Horticulturae 121: 410–418.

Wessells KR, Brown KH (2012) Estimating the Global Prevalence of Zinc Deficiency: Results Based on Zinc Availability in National Food Supplies and the Prevalence of Stunting. PLoS ONE 7: e50568. Wortmann CS, Kirkby RA, Eledu CA, Allen DJ (2004) Atlas of common bean (Phaseolus vulgaris L.) production in Africa.

Yang S, Logan J, Coffey DL (1995) Mathematical formulae for calculating the base temperature for growing degree days. Agricultural and Forest Meteorology 74: 61–74.

Zeven AC (1997) The introduction of the common bean (Phaseolus vulgaris L.) into Western Europe and the phenotypic variation of dry beans collected in the Netherlands in 1946. Euphytica 94: 319–328.

TABLES

Scientific name	Phaseolus vulgaris	Phaseolus coccineus	Vigna unguiculata
Common name	Common bean	Scarlet runner bean	Cowpea
Stem height (m)	0.3 - 7	2 - 7	-
Flower colours	Pink, white, violet	White, red	Yellow, white, purple
Seed length (mm)	11-15	18-25	9.28
Seed width (mm)	5-8	12-14	6.55
Seed thickness (mm)	5	7-12	6.08
Flower size (mm)	10-18	>18	20
Seed protein (%)	23.27	20	24.8
Seed fat (%)	2.70	12	1.9
Seed fiber (%)	8.01	1.5	6.3
Seed ashes (%)	4.22	3.5	3.4
Seed carbohydrates (%)	61.80	63	63.6

Table 1.1. Morphological and nutraceutical characteristics of common bean, runner bean and cowpea

Sources: adapted from (Kay, 1979, IBPGR, 1982, Rachie and Singh, 1985, Ehlers and Hall, 1997,

Costa et al., 2006, Kabas et al., 2006, Giurcă, 2009).

Code	Description
	Principal growth stage 0: germination
00	Dry seed
01	Beginning of seed imbibition
03	Seed imbibition complete
05	Radicle emerged from the seed
07	Hypocotyl with cotyledons breaking through seed coat
08	Hypocotyl reaches the soil surface; hypocotyl arch visible
09	Emergence ("cracking stage")
	Principal growth stage 1: leaf development
10	Cotyledons completely unfolded
12	2 full leaves
1	Stages continuous till
19	9 or more visible full leaves
	Principal growth stage 2: formation of side shoots
21	First side shoot visible
29	9 or more side shoots visible
	Principal growth stage 5: inflorescence emergence
51	First flower bud visible
55	First flower bud enlarged
59	First petals visible, flowers still closed
	Principal growth stage 6: flowering
60	First flowers open
61	Beginning of flowering
62	20% of flowers open
67	Flowering finishing: majority of petals fallen or dry
69	End of flowering: first pods visible
	Principal growth stage 7: development of fruit
71	10% of the pods have reached the typical length
78	80% of the pods have reached the typical length
79	Pods: individual beans easily visible
	Principal growth stage 8: ripening fruit and seed
81	10% of pods ripe
88	80% of pods ripe
89	Fully ripe
	Principal growth stage 9: senescence
97	Plant dead
99	Harvested product

Table 1.2. Phenological growth stages and BBCH-identification keys of Beans. Adapted from Feller et al. (1995).

Genotype			
Id	Name	Туре	Growth
1	Fagiolino mangiatutto rampicante	Pre-commercial	Indeterminate (climbing)
2	Borlotto nano	Elite line	Determined (Dwarf)
3	Borlotto nano	Elite line	Determined (Dwarf)
4	Fagiolo bianco nano	Elite line	Determined (Dwarf)
5	Fagiolo rampicante	Pre-commercial	Indeterminate (climbing)
6	Fagiolo nano creso	Elite line	Determined (Dwarf)
7	sel. Gia)	Pre-commercial	Indeterminate (climbing)
8	Fagiolo nano mangiatutto anellino di trento	Elite line	Determined (Dwarf)
9	giallo	Pre-commercial	Indeterminate (climbing)
10	Fagiolo rampicante stortino di trento	Pre-commercial	Indeterminate (climbing)
11	Fagiolo borlotto rampicante (bortollo lingua di fuoco 3)	Pre-commercial	Indeterminate (climbing)
12	Fagiolo rampicante (blue lake a grano nero sel. Tom)	Pre-commercial	Indeterminate (climbing)
13	Fagiolo dolico (nano dall'occhio)	Elite line	Determined (Dwarf)
14	Fagiolo nano mangiatutto (OR arno)	Elite line	Determined (Dwarf)
15	Fagiolo nano (montalbano)	Elite line	Determined (Dwarf)
16	Fagiolo rampicante (dolico del metro)	Pre-commercial	Indeterminate (climbing)
17	Fagiolo nano valdarno	Elite line	Determined (Dwarf)
18	Fabiolo nano coco nain blanc precoce (lotto verdone)	Elite line	Determined (Dwarf)
19	Fagiolo rampicante tondino abruzzese	Pre-commercial	Indeterminate (climbing)
20	Fagiolo verdone del piave (professional seed)	Elite line	Determined (Dwarf)
21	Fagiolo dolico rampicante mangiatutto o stringa	Pre-commercial	Indeterminate (climbing)
22	Fasole del diavolo	Landrace	Indeterminate (climbing)
23	Gialet	Landrace	Indeterminate (climbing)
24	Posenati	Landrace	Indeterminate (climbing)
25	Semi-rampicante abruzzese	Landrace	Indeterminate (climbing)
26	Fasol dela nonna	Landrace	Indeterminate (climbing)
27	Maseleta rossa	Landrace	Indeterminate (climbing)
28	Zia Orsolina	Landrace	Indeterminate (climbing)
29	Meraviglia di Venezia	Landrace	Indeterminate (climbing)
30	Secle	Landrace	Indeterminate (climbing)
31	Della Clorinda	Landrace	Indeterminate (climbing)
32	Pegaso	Landrace	Indeterminate (climbing)
33	SC-iosela	Landrace	Indeterminate (climbing)
34	D'oro (val di fiemme)	Landrace	Indeterminate (climbing)
35	Meso e Meso	Landrace	Indeterminate (climbing)

Table 1.3. Genotypes Id number, names, classifications, and growth habits

36	Maron	Landrace	Indeterminate (climbing)
37	Righetti 1	Landrace	Indeterminate (climbing)
38	Verdine	Landrace	Indeterminate (climbing)
39	Cuna	Landrace	Indeterminate (climbing)
40	Oci Della Madona	Landrace	Indeterminate (climbing)
41	Sciosele	Landrace	Indeterminate (climbing)
42	Monachelle	Landrace	Indeterminate (climbing)
43	Righetti 2	Landrace	Indeterminate (climbing)
44	Mamme bianche di Bassano - Prod 2018 Azienda	Landrace	Indeterminate (climbing)
45	Mame Bianche B1 - Prod Azienda 2018	Landrace	Indeterminate (climbing)
46	Banel fonzaso - Prod Azienda 2018	Landrace	Indeterminate (climbing)
47	Zolferini Rovizetti - Prod Azienda 2018	Landrace	Indeterminate (climbing)
48	Bala rossa	Landrace	Indeterminate (climbing)

Genotype ld	Pod cross- section shape	Pod curvature	Seed color pattern	Seed color	Seed shape
1	Very flat	Curved	Absent	Pinkish white	Cuboid
2	Round	Slightly curved	Striped	Light Brown and purple	Oval
3	Round	Curved	Striped	Light Brown and red	Cuboid
6	Very flat	Straight	Absent	White	Oval
7	Very flat	Straight	Absent	White	Oval
8	Very flat	Curved	Striped	Light Brown and purple	Cuboid
9	Round	Curved	Absent	Black	Oval
11	Round	Slightly curved	Striped	Light Brown and purple	Round
12	Very flat	Straight	Absent	Black	Cuboid
17	Pear shape	Curved	Absent	White	Oval
18	Very flat	Straight	Absent	Yellowish white	Oval
19	Round	Straight	Absent	Yellowish white	Round
20	Round	Slightly curved	Absent	Yellowish white	Cuboid
23	Pear shape	Recurving	Absent	Yellow	Truncate fastigiate
24	Round	Curved	Striped	Light and dark brown	Round
25	Round	Slightly curved	Speckled	Light and dark brown	Truncate fastigiate
26	Round	Straight	Striped	Light brown and black	Round
27	Very flat	Slightly curved	Bicolor	Red and white	Oval
28	Round	Curved	Striped	Light brown and black	Oval
29	Very flat	Slightly curved	Absent	White	Cuboid
30	Round	Curved	Absent	Dark purple	Round
31	Round	Straight	Absent	Black	Oval
32	Very flat	Straight	Striped	Light and dark brown	Oval
33	Round	Straight	Striped	Light and dark brown	Oval
34	Round	Slightly curved	Absent	Golden brown	Round
36	Round	Curved	Absent	Brown	Round

Table 1.4. Pod and seed morphological description of each genotype

FIGURES



Fig. 1.1 Flowers of a variety of P. vulgaris with the absence of pollinators (left). Flowers of a variety of P. coccineus cultivated in the same environment and at the same period, with the presence of bees (right). Source: Author.



Fig. 1.2 Distribution of the six Italian beans with PDO and PGI. Source: adapted by the author.



CHAPTER 2: AGRONOMIC, GENETIC AND NUTRACEUTICAL CHARACTERIZATION OF VENETIAN BEAN (*Phaseolus vulgaris*) LANDRACES CULTIVATED IN DIFFERENT ENVIRONMENTS

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ABSTRACT

Beans are an essential source of protein around the world and are also essential in sustainable agricultural systems. Thus, conserving bean genetic materials plays an important role in achieving global food safety and security with the preservation of functional and serependic opportunities afforded by plant species diversity for a more sustainable agriculture future. The Valebio project, from the University of Padova, aims to collect and perform agronomic, morphologic, genetic, and nutraceutical characterization of 48 Italian accessions and mountainous Venetian landraces that will be conserved in germplasm. A genetic characterization grouped these 48 accessions in three clusters that were linked to Andean and Mesoamerican centers of origins and to the P. *coccineus* species. In 2019, 26 of these accessions were cultivated in two Venetian environments: sea level and mountains. The environment did not affect these accessions nutraceutical properties. However, plants cultivated at the sea level had its weight and density reduced, which led to yield reductions of more than 50%. Thus, the agronomic performance of these landraces showed their strong connection and adaptation to mountainous environment.

1. INTRODUCTION

Legumes play an important role in addressing issues related to the environment, health, and food security and are also important due to their health benefits, preventing and helping to manage hypercholesterolemia, hypertension (Arnoldi et al., 2015), obesity, diabetes and coronary conditions (Calles, 2016). They are also a critical and affordable source of plant-based proteins, vitamins and essential minerals like calcium, magnesium, and zinc, contributing for the food security and nutrition of people around the world, especially subsistence smallholder farmers in developing countries (Calles, 2016). In developed countries, vegetarians, vegans, and flexitarian diets tends to increase and the legumes are recommended as the main plant-based protein source (Nelson et al., 2016).

Common bean (Phaseolus vulgaris L.) is a diploid (2n = 2x = 22) annual species belonging to the Fabaceae family are grown worldwide for their edible green pods and dry seeds. Given the relative simplicity and the small dimension (650 Mb) of its genome, *P. vulgaris* provides a useful model for studying closely related species of agronomic interest. It is a predominantly self-pollinating plant, with occasional occurrence of insectmediated cross-pollination (Rendón-Anaya et al., 2017). Breeding strategies for common bean rely on the selection of homozygous individuals for the development of pure lines of high agronomic value.

The domestication process of the common bean was a unique process. It occurred in two geographically distinct regions, simultaneously, and partially isolated gene pools: Mesoamerican and Andean. Genetic evidence suggests the Mesoamerica as the center of origin of common bean. The Andean population derived as a consequence of a strong pre-domestication bottleneck. Despite having undergone through independent domestication processes, both gene pools are partially sexually compatible and morphologically similar. Differences between the two gene pools have been revealed using different molecular markers such as random amplified polymorphic DNA (RAPD) (Johns et al., 1997; Beebe et al., 2000), amplified fragment length polymorphisms (AFLP) (Tohme et al., 1996; Beebe et al., 2001), or microsatellites (Díaz and Blair, 2006).

P. vulgaris varieties are distributed worldwide and are cultivated in the tropics, subtropics, and temperate zones (Hidalgo, 1988), showing great variability in terms of agronomic performance, seed size, shape and color, the relative duration of the reproductive cycle and many other qualitative and quantitative traits (Rodiño et al., 2003). This diversity enabled its cultivation in a wide range of cropping systems and environments such as China, Eastern Africa, the Americas, the Middle East and Europe, with more than 40,000 varieties (Jones, 1999).

The first introduction of common bean from Central/South America into Western Europe most likely took place around 1500, in the sixteenth century (Zeven, 1997). A peculiarity of the European population of *P. vulgaris* is the high proportion of Mesoamerican and Andean hybrids, estimated to be about 44%. This could be explained by the presence of different landraces, which are traditionally cultivated in proximity to each other, facilitating occasional outcrossing and gene flow (Angioi et al., 2010). In Italy, beans were first presented in a 1515 Giovanni di Udine painting (Albala, 2007) and mentioned in historical documents fixing 1532 as the year of common bean introduction in Italy. Bean cultivation created a long tradition that allowed the evolution of many landraces adapted to microclimates in restricted areas, representing a pastiche of cultures

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and traditions that are an irremissible good for Italy and that are being used by low environmental impact agriculture (Venora et al., 2009). In the region of Veneto, the diffusion of common bean occurred quickly, and nowadays, the cultivation of this pulse still has great economic relevance in the Belluno region (Piergiovanni and Lioi, 2010). However, although those accessions have original morpho-agronomical and nutritional characteristics, with high organoleptic qualities, they have been gradually substituted by genetic uniform commercial varieties (Spagnoletti Zeuli et al., 2004; Venora et al., 2009).

After the 1950's the large scale of breeding programs and fast disappearance of landraces, caused the disappearance of an unknown number of populations and the marginalization of others in private gardens. The commercial value of these landraces is generally limited and is often sold only in local markets, appreciated and used in the preparation of local dishes (Piergiovanni et al., 2000). This fact increases the importance of collecting and preserving those local accessions in germplasm, in situ, or ex-situ, contributing to the improvement of food crops and preserve its genetic diversity (Piergiovanni and Laghetti, 1999). Collect, conserve, and preserve landraces from the Veneto region are some of the objectives of this study.

Although the Veneto is not one of the primary domestication centers of the common beans, the collected material can be considered an example of serependic value of conserving landraces in a variety of places. Through their conservation in the Veneto region these landraces have undergone an autochthony with minor adaptations as they have been cultivated in isolation for centuries, undergoing to a sort of ecotypization process. This process selected accessions and gene combinations different from the original ones that are able to achieve the maximum adaptation to the new pedo-climatic

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and anthropic conditions which could be critically important as we adapt to changing climates. The long-term germplasm utilization and conservation operated yearly by farmers, along with the natural evolutionary processes through climate changes, have therefore brought to the formation of Veneto germplasm itself for many species, including common beans (.

In 2019, 26 accessions were assessed in two different environments of the Veneto: Legnaro, at sea level, and Asiago, in the mountains. In agronomic terms, the yield and phenological development were assessed. The seeds' nutritional values were characterized based on total phenols, antioxidants activity, protein and starch content, and amino acid composition. The seeds were also characterized based on its physical properties: the weight of 100 seeds, seed volume, density, length, width, and thickness. At the early stage, leaf samples were collected, and the accessions were characterized genetically within and among populations. This study aimed to assess the agronomic performance, morphological and nutraceutical characterization, molecular markerassisted selection and the socio-economic valorization of ancient local varieties of common suitable for cultivation in organic agricultural systems.

2. MATERIALS AND METHODS

2.1. Experimental sites and environmental conditions

Field trials were conducted between the months of June and November of 2019, in two different environments of the Veneto region: at the sea level (SL), (45°20'43.52"N, 11°57'11.56"E) and Asiago, in the mountain (MT) (45°53'30.58"N, 11°33'43.58"E). At both locations, planting layout used was 50 cm between rows, 50 cm on row and about 150 cm between accessions. At the sea level, 20 plants of each accession were transplanted in plots with the dimensions of 5 m x 1 m. Climbing accessions, 19 in total, were planted in five rows each 44 m long. Seven dwarf accessions were transplanted in three rows. In the mountain, 10 plants of each accession were transplanted in plots of 2.5 m x 1 m. For climbing accessions, a structure to direct the plant vertical growth was constructed, consisting of four 2.1 m bamboo canes, tied at the top forming "hut" structures. Each plant was attached to one bamboo cane. Thus, in each parcel, in SL the 20 individuals were grouped into five groups of four plants and in MT the 10 plants were in two groups of four plants and in a pair of plants.

In SL, trials were conducted in the Experimental Farm 'Lucio Toniolo', from the University of Padova, in an altitude of 7 m above sea level. The soil in SL is a eutric fluvisol (World Reference Base) and was composed of 65% silt, 15% sand, and 25% clay, with pH 8.15 (1:1 H₂O). The organic matter was 1.77%, the total nitrogen 0.11%, and the C/N ratio 9.72. Total Olsen phosphorus by 810 mg P₂O₅ kg⁻¹, potassium 59.9 mg K₂O kg⁻¹, magnesium 247 mg kg⁻¹, calcium 2,619 mg kg⁻¹, sodium 26.1 mg kg⁻¹ and total sulfur 408 mg kg⁻¹. The cycle in SL was from June 10th until October 25th (137 days), with an average temperature of 20.4°C, maximum and minimum average temperature were 28.4°C in the 37.6°C in the 26th day and 7.7 °C in the 135th day (Fig. 2.1), and accumulated precipitation 236mm. An irrigation system was installed, and water was supplied according to the need of the plants.

In MT, the trials were conducted on a local farm, at an altitude of 994m. The soil in MT is a eutric cambisol (World Reference Base) and was composed of 45% silt, 15% sand, and 40% clay, with pH 6.77. The organic matter was 2.6%, the total nitrogen 0.10%, and the C/N ratio 15.11. Total phosphorus 750 mg P_2O_5 kg⁻¹, potassium 67.3 mg K₂O kg⁻¹, magnesium 201 mg kg⁻¹, calcium 1,932 mg kg⁻¹, sodium 18.1 mg kg⁻¹ and total sulfur 550 mg kg⁻¹. The cycle in MT was from June 24th until November 7th (136 days), with an average temperature of 14.1°C, maximum and minimum average temperature were 28.4°C in the 34th day and -1.4 °C in the 117th day (Fig. 2.1), and accumulated precipitation 512mm. An irrigation system was installed, and water was supplied according to the need of the plants.

2.2. Cultivation operations

Cultivation operations were carried out primarly in the same way in both locations. The soil was prepared by performing a harrowing (milling in the mountain plot) and, subsequently, the organic fertilizer Biorex (pelleted manure) of Italpollina (derived from poultry litter) was distributed, at a rate of 0.8 Mg ha⁻¹. The fertilizer has the following composition: N 2.8%, P₂O₅ 2.5%, and K₂O 3.0%, organic carbon of biological origin 38%, organic matter 65%, C / N 13, water content 16%, pH 7.

Accessions seeds were sown in seedling starter trays (40 holes), arranging one seed in each hole, and using fine peat-based Geo Substrat (PBE Substrates), containing 2-6mm perlite, and with pH 5-6 and EC 0.4 mS cm⁻¹. The sowing was carried out on May 30 for the material destined for SL and on June 10 for that to be used in MT. Although the traditional agronomic practice provides for direct sowing for the bean, it was decided to sow on the substrate and then proceed with the transplant due to the scarce availability of seed of some accessions. This was done to ensure the emergence of enough seedlings to be transplanted in each location, avoiding the possible drawbacks caused by direct sowing in the field, i.e. the formation of surface crust and attack by pathogens and parasites during germination.

After sowing, seedling starter trays were kept in a protected environment and watered regularly to ensure optimal substrate water content. After about ten days from sowing the seedlings had already developed the first two true leaves and had adequately colonized the media with the root system. Transplants were carried out on June 10 in SL and on June 21 in MT. Weeding in the inter-row, where the soil was free of mulching, was carried out mechanically in MT, with localized spraying of glyphosate in SL.

The harvesting of pods was carried out gradually when they appeared completely dry on the plant. In SL, this phase started in the second half of August and lasted until the end of October, while in MT, it started in the second half of September and ended in early November. The maturation of the pods was therefore scaled in a more accentuated way for climbing accessions that tend to have indeterminate growth.

2.3. Plant materials

Accessions assessed in this study were selected from the germplasm bank of the Department of Agronomy, Food, Natural Resources, Animal and Environment (DAFNAE), of the University of Padova, in Legnaro, Italy. DAFNAE's germplasm is mostly by composed *Phaseolus vulgaris*, with 48 accessions, of which 26 were selected and cultivated in both locations, SL and MT, all *P. vulgaris*. A list with the names, identification number, and some characteristics of these accessions is shown in Table 2.1.

Accessions used in this study are highlighted in light grey. Pictures with its leaves, pods, flowers, and seeds are in the Annexes. Due to differences in the cultivation process, accessions were divided between dwarf and climbing.

2.4. Agronomic and morphological characterizations

2.4.1. Phenological assessment

In SL, for each accession, ten plants were identified, positioned more internally in the parcel (considering the pairs, five in one row and five in the other), which were then kept as samples for phenological observations up to collection. In MT, on the other hand, having only ten plants per accession, six plants were used as models for each parcel, following the same criterion adopted in SL. Phenological surveys were carried out weekly in SL and every two weeks in MT. The identification codes obtained from the observations were subsequently averaged to obtain a single value referred to the individual parcel.

The BBCH-scale uses a decimal scale for the description of phenological growth stages of plants and weeds. In this study, it was used the scale proposed by Feller et al. (1995) divided in 8 principal growth stages: 0) Germination (0-09); 1) Leaf development (10-19); 2) Formation of side shoots (21-29); 5) Inflorescence emergence (51-59); 6) Flowering (60-69); 7) Development of fruit (71-79); 8) Ripening of fruit and seed (81-89); 9) Senescence (97-99). For practical reasons, this scale can also be divided into three main groups: vegetative growth (0-2), reproductive growth (5-7) and harvesting (8-9).

2.4.2. Growing degree days

Cumulative GDD (growing degree days) was calculated by summing the positive values of the Eq. 1 for each day and considering a base temperature of 10°C (Jenni et al., 2000).

$$GDDx = \left(\frac{Tmax+Tmin}{2}\right) - Tbase$$
 (Eq. 1)

Where: GDDx is the growing degree day of the day x, Tmax is the maximum average temperature of the day x, Tmin is the minimum average temperature of the day x, and Tbase is the base temperature.

Temperature at both locations were monitored by weather stations from the Regional Agency for Environmental Prevention and Protection of Veneto (ARPAV).

2.4.3. Seed production

After maturing and drying in the plant, pods were harvested and completely dried at room temperature (until about 10% moisture). The production was considered as grams of dry grain per plant.

2.4.4. Seeds' physical properties

Seeds of each accession and location were assessed for seed weight, bulk density, volume, and imbibition capacity, according to Bishnoi and Khetarpaul (1993) and Shimelis and Rakshit (2005). For each accession, three replicates of 100 seeds from MT and SL were collected and weighed, giving the weight of 100 seeds in grams (Nicoletto et al., 2019). Of these, ten were collected and inserted in a 100 mL graduated cylinder containing 50 mL of distilled water. After the seeds were added to the cylinder, the

variation in the volume was considered as the volume of 10 seeds. Density, in g mL⁻¹, was calculated according to Eq. 2.

Seed density =
$$\frac{100 \text{ seeds weight } (g)}{10 \text{ x 10 seeds volume } (mL)}$$
 (Eq. 2)

2.5. Genetic characterization

The genetic characterization was performed in all 48 accessions from DAFNAE's germplasm. A total number of genomic DNA samples were extracted from young leaves using the DNeasy 96 Plant kit (Qiagen, Hilden, Germany) following the instruction provided by the supplier. After extraction, the DNA quality and quantity were evaluated using the NanoDrop 2000c UV-Vis spectrophotometer (Thermo Fisher, Pittsburgh, PA). DNA samples integrity was checked by electrophoresis on a 2% agarose/1× TAE gel containing 1× Sybr® Safe DNA gel stain (Life Technology, Carlsbad, CA).

The SSR raw data were analysed with POPGENE 32 software package v. 1.32 (Yeh et al., 1997) and the following statistics were calculated: observed and expected homozygosis (Obs_Ho and Exp_Ho, respectively), the average number of alleles per locus (na), the effective number of observed alleles per locus (ne) and the SSR allele frequencies. All the statistics were calculated both for the used SSR loci and the 48 accessions analysed. To determine the alleles variability of the assessed marker loci, Nei's index was calculated and assumed as Polymorphic index content (PIC) (Palumbo and Barcaccia, 2018). Otherwise, considering the sub-population later identified, the same index was used to express their heterozygosis grade. Finally, to investigate the presence of any possible private allele in the single populations identified, GenAlEx

software (Peakall and Smouse, 2006, 2012) was used. Only those alleles showing a frequency higher than 10% in a specific subgroup and lower than 2% in the remaining sub-groups were considered.

Genetic similarity (GS) estimates were also calculated between individuals in all possible pair-wise comparisons by applying the simple matching (SM) coefficient. The resulting similarity matrix was later used for the construction of an UPGMA dendrogram. The average similarity was also calculated within and among both the landraces and the pre-commercial varieties and a Principal Coordinates Analysis (PCoA) graph was developed starting from the similarity matrix, and samples were than labelled on the base of the results obtained both by STRUCTURE software and the UPGMA dendrogram.

Population structure analysis of the core collection has been performed using the STRUCTURE software, which exploits a systematic Bayesian clustering approach applying Markov Chain Monte Carlo (MCMC) estimation (Pritchard et al., 2000), which compares the molecular marker data belonging to each accession among themselves to infer their membership to a series of putative clusters. The simulation was performed assuming the admixture model, with no a priori population information. The SNPs data were analyzed with 106 iterations and a burning period of 2·105, ten replicates runs were executed with the value of K ranging between 1 and 16. The most likely K value was estimated using ΔK (Evanno et al., 2005) and was considered for the assignment to an ancestral group.

2.6. Nutraceutical characterization
2.6.1. Total phenols and antioxidant capacity

Bean freeze-dried samples (0.2 g) were homogenized in methanol (20 mL) with an Ultra Turrax T25 until uniform consistency at 13.500 rpm. Samples were filtered (filter paper, 589 Schleicher), and appropriate aliquots of extracts were assayed by Folin Ciocalteau (FC) assay for total phenol (TP) content and by Ferric Reducing Antioxidant Power (FRAP) assay for total antioxidant activity.

The content of TP was determined using the FC assay with gallic acid as calibration standard by a Shimadzu UV-1800 spectrophotometer (Columbia, MD, USA). The FC assay was carried out by pipetting 200 μ L of bean extract into a 10 mL polypropylene tube. This was followed by the addition of 1 mL of FC's reagent. The mixture was vortexed for 30 s, and 800 μ L of filtered 20% sodium carbonate solution was added after 1 min and before 8 min from the addition of the FC reagent. This was recorded as time zero; the mixture was then vortexed for 30 s after the addition of sodium carbonate. After 2 h at room temperature, the absorbance of the colored reaction product was measured at 765 nm. The content of TP in the extracts was calculated from a standard calibration curve, built with different concentrations of Gallic acid, ranging from 0 to 600 μ g mL⁻¹ (Correlation coefficient: R²: 0.9982). Results were expressed on the basis of mg of Gallic Acid Equivalent per kg (mg GAE kg⁻¹) of dry bean powder (Singleton et al., 1999).

FRAP reagent was prepared fresh so that it contained 1mM 2,4,6 tripyridyl-2triazine and 2mM ferric chloride in 0.25M sodium acetate at pH 3.6 (Benzie and Strain, 1996). A 100 μ L aliquot of the methanol extract prepared as above was added to 1900 μ L of FRAP reagent and accurately mixed. After leaving the mixture at 20 °C for 4 min, the absorbance at 593 nm was determined. Calibration was against a standard curve (0–1200 μ g mL⁻¹ ferrous ion) produced by the addition of freshly prepared ammonium ferrous sulfate. FRAP values were calculated as mg mL⁻¹ ferrous ion (ferric reducing power) from three determinations and are presented as mg of Fe²⁺ (ferrous ion equivalent) kg⁻¹ dw.

2.6.2. Amino acids composition

The determination of the amino acid content was carried out in accordance with the provisions of the European Pharmacopoeia 5.0 - 2.2.56. Amino acid analysis - Protein hydrolysis - Method 1 for hydrolysis and from the European pharmacopoeia 5.0 - 2.2.56. amino acid analysis - Methodologies of amino acid analysis: general principles - Method 5 and Method 7 for derivatization. The HPLC identification and quantification was performed following Agilent ZORBAX Eclipse AAA - Technical Note (publication number 5980-1193).

2.6.3. Starch content

Starch content was determined using the OAC Official Method 996.11 Starch (total) in cereal products and the AOAC Official Method 979.10 Starch in Cereals. Following the University of Florida, IFAS, Bulletin 339-2000 "Starch Gelatinization & Hydrolysis Method" Boehringer Mannheim, Starch determination, cat. N ° 207748. A method adapted for chromatographic analysis. 2.6.4. Protein content

Crude protein was determined by the analysis of the nitrogen content according to the semi-micro Kjeldhal technique (Kjeltec-System Foss Tecator) (Hjalmarsson and Akesson, 1983); the protein content was calculated by multiplying the N content by a factor of 6.25 (Adler-Nissen, 1986).

2.7. Statistics

Data obtained from the various findings were separated between climbing and dwarf accessions due to differences in growth habit and production systems. To assess the effect of the cultivation environment, mountain and sea level, (E), the accession (A), and their interaction (E x G), analysis of variance was performed. The differences between means were assessed by Tukey's HSD test for P <0.05 in JMP Pro 15. Excel and SigmaPlot 11.0 were used to prepare the graphs.

3. RESULTS

3.1. Genetic characterization

On fig 2.2., it is possible to see that all the accessions were separated into three main clusters (A, B, and C). The cowpeas (accessions 13, 16, and 21) DNAs were not detected due to incompatibility with the primers used since it is from a different genus. Accessions 4 and 22 were in cluster C, being separated at 0.3 genetic similarity. Both 4

and 22 are *P. coccineus*; thus, it was expected to have them as part of a separated cluster. Most of the accessions (all the *P. vulgaris*) were grouped into two different clusters:

-Cluster A, 22 accessions: 1, 2, 3, 6, 8, 10, 11, 15, <u>19</u>, 20, **26**, **27**, **30**, **32**, **33**, **34**,

36, 38, 39, 41, 42, 47 (landraces in bold)

-Cluster B, 16 accessions: 5, 7, 9, 12, 14, 17, 18, <u>19</u>, **23**, **24**, **25**, **28**, **29**, **31**, **44**, **46** -Cluster C, 2 accessions: 4 and 22.

Accession 19 was the only one in common between clusters A and B. All the DNA samples were collected in Legnaro and as all the plants of the accession 35 died in that location before sampling, it was not assessed. Accessions 37, 40, 43, 45, 48 seeds did not germinate.

When comparing the accessions genetic similarity within their clusters and between the clusters, it is possible to observe that besides having more accessions, cluster A is more homogeneous (97.48%), and cluster B accessions had a 90.70% genetic similarity. A difference is visible in the number of sub-clusters that each cluster has. Cluster A has only three sub-clusters, while cluster B has at least six sub-clusters. When comparing between the clusters, A and B are 61.12% genetically similar, and cluster C is 32.06% and 30.08% genetically identical to the clusters A and B, respectively.

Fig. 2.2. also presents the percentage of ancestry membership in each cluster. The ancestral membership of *P. vulgaris* suggests that the accessions assessed are likely to have been originated from two genetically distinct subgroups. Of 92 entries assessed, 90 had a membership of almost 100%, all from the *P. vulgaris* clusters. In contrast, *P. coccineus's* two entries, from populations 4 and 22, had a greater rate of mixing, with about 80% from cluster A and 20% from cluster B. Thus, most of the populations had not

undergone trough hybridization between Andean and Mesoamerican accessions. These results were interpreted and aligned with known accessions from both geographic domestication centers, Andean and Mesoamerican, available in the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA). Cluster A was closely linked to the Andean accessions, and the cluster B was linked to the Mesoamerican accessions.

Assessing the presence of the six molecular markers, mostly, all the accessions of the cluster A had the allele A. The HSF had allele B and characterized a subgroup formed by the accessions 1, 2, 3, 6, 8, 10, 15, 19, 20, 26, 27, 30, 38, 39, 41, and 42. Accessions 2 and 8 were part of individual subgroups within this major subgroup, and their β -GDP were from the alleles B and C, respectively. In the other subgroup within the cluster A, accessions 6, 32, 33, 36, and 47 were grouped together. Entries 33a and 36a, b, d, and e, had their PEPC with allele B. In cluster B, the accessions were more mixed and heterogeneous (Fig. 2.2).

3.2. Phenological assessment

On table 2.2. is shown that the interaction between accession and environment did not have a significant effect on the duration of vegetative phase, flowering phase, fresh pods period, dry pods period, and total cycle length. All these variables were significantly affected by the accession (Fig. 2.3).

The length of flowering phase and fresh pods period of climbing accessions were significantly affected by the environment. Dwarf accessions had their fresh pods and dry pods period length significantly affected by the environment (Table 2.2). The average flowering phase of climbing accessions at the sea level was 31 days, whereas in the mountains it was significantly shorter, 18 days. Dwarf accessions had a significantly longer dry pods period at the sea level (31 days) when compared to the mountain (18 days). Both, dwarf and climbing, had significantly longer fresh pods periods in the mountain (45 and 49 days, respectively) when compared to the sea level (29 days for both) (Table 2.3.).

3.3. Seed production

Accession had a significant effect on seed production of climbing and dwarf accessions. The environment and the interaction between accession and environment significantly affected the seed production of climbing accessions but did not affect it dwarf accessions (Table 2.2).

Climbing accessions seed production ranged from 12.4 (36 at the sea level) to 326.4 g of seeds per plant (24 in the mountains). On average, climbing accessions produced about two times more seeds per plant in the mountain than at the sea level. Of the 19 accessions, only two produced more at the sea level than in the mountain, however, with no significant difference. Accession 36 produced about 20 times more seeds in the mountains when compared to the seal level (Fig. 2.5.). On average, the production of seeds per plant of dwarf accessions ranged from 20.3 (accession 20) to 82.7 g of seeds per plant (accession 6). Accession 6 dry seeds production was significantly greater than accessions 17 (27.7 g) and 20 (20.3 g) (Table 2.4).

3.4. Seeds' physical characterization

The accession and the environment had a significant effect on the weight of 100 seeds, volume of 10 seed, and seed's length, width, and thickness of climbing and dwarf accessions. These variables of both, the climbing and the dwarf accessions, were also significantly affected by the interaction of accession and environment. The environment significantly affected the seed density of climbing and dwarf accessions and the accession had a significant effect on the density of climbing accessions (Table 2.2).

The weight of 100 seeds ranged from 25 g of the accession 7 at the sea level to 94 g of the accession 33 in the mountain. Most of the 19 accessions had greater weight of 100 seeds in the mountain when compared to the sea level. The volume of 10 seeds ranged from 2 (accession 7 in the mountain) to 7.7 mL (accession 32 at the sea level). Seeds' length ranged from 10.5 (19 in the mountain) to 17.6 mm (32 in the mountain), width from 5.8 (12 in the mountain) to 12.2 mm (36 in the mountain), and thickness from 4.7 (12 in the mountain) to 9.4 mm (36 in the mountain) (Table 2.5).

3.5. Nutraceutical characterization

The environment and accession interaction did not have a significant effect on the total phenols, antioxidant capacity, protein and starch content. The environment significantly affected the protein content of climbing accessions and the starch content of dwarf accessions, and the antioxidant capacity of both climbing and dwarf accessions (Table 2.2).

Total phenols content was significantly affected by the accession in both climbing and dwarf accessions. All the accessions had total phenols content greater than 3,000 mg GAE kg⁻¹ of dry matter. Among the climbing accessions, accession 1 was the one with the highest total phenol content, with a significant difference when compared to most of the other accessions. However, accession 1 did not significantly differ from the accessions 12, 19, 24, 25, 30, 31, 32, 33, and 36. Accessions 7, 23, 29, and 34 total phenols content were lower than 4,000 mg GAE kg⁻¹ of dry matter. Among the dwarf accessions, the total phenols content of the accession 8 seeds was significantly greater than the accessions 6, 7, and 18 seeds. Besides accession 8, accessions 2 and 3 seeds also had a total phenols content greater than 4,000 mg GAE kg⁻¹ of dry matter (Fig. 2.6).

Genotypic effects on antioxidant capacity was significant. Most of the accessions' antioxidant capacity was over 6,000 mg Fe⁺² kg⁻¹. Among the climbing, accessions 25, 33, and 36 were over 10,000 mg Fe⁺² kg⁻¹, and had a significantly higher content than the accessions 7, 9, 19, 23, 27, and 29, which had the content lower than 6,000 mg Fe⁺² kg⁻¹. Among the dwarf accessions, the 8 was significantly greater than accessions 3, 6, 17, and 18. Besides the accession 8, that the content was over 13,000 mg Fe⁺² kg⁻¹, accession 2 also had the content greater than 10,000 mg Fe⁺² kg⁻¹. Accessions 6, 17, and 18 were lower than 4,000 mg Fe⁺² kg⁻¹ (Fig. 2.7).

Considering the average content of the starch from the mountains and from the sea level, accessions 24, 27, 28, and 30 contents were higher than 30% of the dry matter, and accessions 7 and 29 were lower than 20%. Some accessions had considerable variation between the two locations. Among the climbing accessions, there were no

significant effects of the environment on the starch content. Regarding the dwarf accessions, seeds from the cultivation in the mountains had a significantly higher starch content than the ones cultivated at the sea level. Considering the protein content from both locations average, the accessions 23, 29, and 32 were greater than 30%, while the protein content of the accessions 19 and 26 were lower than 26%. When comparing the effects of the environments, seeds of climbing accessions from the sea level had a significant greater protein content, 9.75% more than the seeds from the mountains. Among the dwarf accessions, there no significant difference between both cultivation environments. All dwarf accessions had a protein content lower than 30%, but only accessions 17 and 18 were below 26% (Table 2.5).

The amino acid profile was not affected by the environment and by the interaction of environment and accession. Considering the essential amino acids, the accession significantly affected the content of methionine and threonine of climbing accessions and the threonine content of dwarf accessions. Among climbing, accessions 1 (321 mg 100 g⁻¹) and 36 (310 mg 100 g⁻¹) methionine contents were significantly greater than accessions 12, 19, 26 (all 256 mg 100 g⁻¹), and 7 (253 mg 100 g⁻¹), and accession 1 threonine content (1,261 mg 100 g⁻¹) was significantly greater than accessions 25 (899 mg 100 g⁻¹) and 30 (911 mg 100 g⁻¹). Among dwarf accessions, accession 2 threonine content (1179 mg 100 g⁻¹) was significantly greater than accession 20 (1100 mg 100 g⁻¹) that was significantly greater than accessions 3 (1003 mg 100 g⁻¹), 6 (1013 mg 100 g⁻¹), 17 (1023 mg 100 g⁻¹), and 18 (1045 mg 100 g⁻¹) (Table 2.8). Of the non-essential amino acids, tyrosine of climbing, and serin and glycine of the dwarf were significantly affected by the accession. Considering the climbing accessions, accession 1 tyrosine content (811 mg 100 g⁻¹) was

significantly greater than accession 26 (596 mg 100 g⁻¹). Considering the dwarf accessions, accession 6 serin content (1334 mg 100 g⁻¹) was significantly lower than accessions 2 (1611 mg 100 g⁻¹), 8 (1549 mg 100 g⁻¹), 17 (1433 mg 100 g⁻¹), 20 (1567 mg 100 g⁻¹), and the glycine content of accessions 2 (1004 mg 100 g⁻¹) and 20 (986 mg 100 g⁻¹) were significantly greater than accession 3 (852 mg 100 g⁻¹) (Table 2.9).

4. DISCUSSION

The region of Veneto has about 18,364 km², of which 57% is a vast plain and 29% is a mountainous area, composed by the Carnic Alps, Eastern Dolomites, and the Venetian pre-alps (Veneto Inside). This specific geographic formation allowed, after the introduction of beans from the Americas, farmers or small farms and rural communities to grow beans in isolation for centuries. This study shows that over the years, new introductions and exchanges of different accessions have occurred, from different domestication centers and origins. However, currently, just based on visual characteristics, it is possible to identify dozens of accessions typical from that region.

In this study, seeds were collected from different regions of Veneto, but most came from mountain areas. The genetic analysis showed that the Italian and Venetian bean populations came from both domestication centers, Andean and Mesoamerican. Of all the accessions, 56% are from the Andean center of origin, and 39% from Mesoamerica. Two accessions, 5%, are *P. coccineus* that is originated from Mexico (Salinas, 1988). Thus, at least three different and independent introductions of *Phaseolus spp.* have occurred in Veneto. The proportion of Andean and Mesoamerican accessions in Veneto is close to the one found by Angioi et al. (2010) in Europe: 67% Andean and 33% Mesoamerican. However, this proportion is slightly different when compared to their results of Italian landraces: 75% Andean and 25% Mesoamerican. The data obtained in this study are also in agreement with Angioi et al. (2010) in terms of hybridization since his data showed that 44% of the European *P. vulgaris* landraces were derived from hybridization between Andean and Mesoamerican gene pools, however, in Spain and Italy the distribution of hybrids were low. The genetic data of the present study showed that 90 of 92 entries had a membership of almost 100% to their cluster, the only two entries that were not, were from populations 4 and 22, both *P. coccineus*. The low hybridization between Andean and Mesoamerican in these landraces must be due to two facts: i) the geographic isolation in the Venetian mountains and ii) the fact that *P. vulgaris* is highly self-pollinated. As an opposite example, *P. coccineus* had higher rates of mixing since it outbreeds more frequently.

In total, the six SNPs markers evaluated spanned 3,271 nucleotides and in the DNA sequences of *P. vulgaris* 28 SNPs, and 3 indels were found. Dividing these 3,271 nucleotides by the 28 SNPs, we had the SNP frequency of 116 bp. This value is relatively high when compared to other legumes like *Medicago truculata* (Branca et al., 2011) and *Glycine max* (Lam et al., 2010), indicating that the regions chosen for these analyses have a high polymorphism rate. It is also important to highlight that, although these primers were for *P. vulgaris*, it was also compatible with the *P. coccineus*. A total of 19 haplotypes were detected using these markers, five in cluster A and 14 in cluster B. From those, only three were found only in 5% of the accessions on cluster B. This results show that the cluster B have a greater variability than cluster A, which is in agreement with

Kwak and Gepts (2009) studies, which have shown that the Mesoamerican (cluster B) common beans have a greater genetic diversity when compared to the ones from South America (cluster A). In terms of functional haplotypes, cluster A had three, while cluster B had seven, also indicating a greater phenotypic variation.

The great genetic diversity among the populations assessed also showed great phenotypic plasticity when cultivated in different environments. The accumulated growing degree days (GDD) are a great tool to model and predict different crops. However, the same accessions cultivated in two environments with entirely different weathers had different GDD demands. On average, the total cycle of these climbing accessions lasted 130 days in MT and 128 days in SL, and dwarf accessions 123 days in MT and 121 days in SL. Due to the fact that SL is located in the low plain and its weather is much hotter compared to MT's mountainous weather, the accumulated GDD during the cultivation period was almost triple, 1,800 vs. 600 GDD. Another fact that can explain this difference is that the GDD in this study was calculated using the most common base temperature, 10°C for common beans (Jenni et al., 2000). However, each accession may have its own minimum base temperature. Since most of them are adapted to the mountainous weather, a maximum base temperature should be added to the equation as well. Jenni et al. (2000) showed that by changing the minimum base temperature from 10 to 0°C, bean cultivars would increase their growing degree days from sowing to maturity from about 600 to about 1,200. These results also show that these mountainous accessions are likely to have not only a minimum base temperature, but also a maximum base temperature, limiting plant growth above it.

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The dwarf accessions vegetative and flowering stage lasted about the same time in both locations, leading to the same time of fresh and ripening pods. These accessions did not have a significant difference in production per plant when comparing both locations, producing, on average, about 55 g of dry seed per plant. For the climbing accessions, this difference was significant. The fresh pods stage is important in achieving greater yields since the seeds are filled and developed, having a direct impact on yield. The response of these accessions to the SL weather conditions, shortening the fresh pods phase, and making the flowering and pod ripening stages longer is probably one of the reasons for the significant effect of the environment in the seed production. In MT, the seed production per plant of the landraces was more than double than in SL, and the precommercials production was a little less than double in MT.

Considering a spacing of 0.5 m x 0.5 m for each climbing plant in a typical Veneto bean production system, there are about 4,000 plants per hectare. Thus, the seed yield in Asiago was about 840 kg of dry beans per hectares for the landraces. This value is reduced to 380 kg per hectare in Legnaro. Accessions as the 23, 24, 30, and 36, Gialet, Posenati, Seclé, and Maron achieve greater yields in Asiago, 1,200, 1,300, 1,080, and 1080 kg per hectare, respectively. In 2004, Piergiovanni et al. called attention to the risk of the disappearance of these typical Veneto ecotypes, highlighting that in terms of yield, landraces such as Gialet do not reach the quantities needed to compete with Lamon beans (4,000 kg ha-1 vs. < 2,000 kg ha-1). However, due to the diversity of the characteristics of the seeds and the quality, it is common to find these ecotypes being sold in farmers' markets throughout Veneto at prices higher than common commercial beans. Prices are usually over 10 euros per kilogram and can reach almost 20 euros, in the case of

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accession 22, Fasolo del Diavolo. The Gialet from Belluno was sold from 18 to 25 euros per kg and the Lamon bean IGP from 20 to 25 euros per kg in 2017, according to the Treviso-Belluno Chamber of Commerce (Camera di Commercio Treviso-Belluno, 2017). However, most of these landraces are commercialized in very specific and small areas and are likely to be replaced by the ones with higher prices and better yields. This fact increases the importance of conserving them in germplasm and also knowing and describing their genetic, agronomic, morphologic, and nutraceutical characteristics.

Also, the cultivation of beans is a cultural tradition, often seen in small gardens in the back of the houses, and often the exchange or commercialization is made between friends or known neighbors. This is one of the reasons why all Veneto landraces are climbing and have indeterminate growth. These characteristics facilitate manual harvesting and allow harvest staggered for weeks or even months of fresh beans.

The high genetic and phenotypic diversity of the landraces and other accessions assessed in the Valebio Project is also present in the morphologic characteristics of the seeds and the structure of the seeds. In addition to different shapes and colors, seed dimensions and weights were also varied among the accessions. The seed length ranged from 10.2 to 17.4 mm, the width from 5.2 to 11.3, and the thickness from 4.9 to 8.3 among the 26 accessions that completed their cycles in both locations. The weight of 100 seeds ranged from 24.68 g to 95.02 g and the volume of 100 seeds from 15 mL to 77 mL, among the accessions that completed their cycle in both locations. Accession 22 was the one with the greatest seeds' dimensions: 25 mm x 12 mm x 9 mm. The seeds dimensions of the accessions assessed in this study have a greater range than the ones described by (Giurcã, 2009), and the weight of 100 seeds is close to the weight of Venetian agro-

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ecotypes presented by Piergiovanni et al. (2004). In 2019, Nicoletto et al. assessed the effect of different Venetian environments on two Venetian landraces seed properties and did not find significant effects of the environment, just of the accessions on 100 seeds weight, seed volume, and seed density. In this study, more accessions were assessed and a significant difference between the different environments was observed for the 100 seeds weight, all the seeds dimensions, seed volume, and seed density. The seeds from MT had greater weight, length, width, thickness, and density. The longer pods filling periods in MT may be a plausible reason to explain these significant differences in the seeds' morphological characteristics.

As it was mentioned previously, this study only performed the nutraceutical characterization of dry seeds, which is not how the beans are normally consumed. Soaking the bean seeds make the cells softer, which can solubilize bound polyphenols that can be absorbed by the water, reducing the content in the bean as showed by Boateng et al. (2008). Rocha-Guzmán et al. (2007) found out that cooking beans under pressure can reduce by 90% the phenolic content in the seed coat, their results are in agreement with Barroga et al. (1985) that boiled another legume, the mung bean (*Vigna radiata*), for 30 minutes and found that it reduced by 73% the phenolic content. Other authors, however, have shown an increase in the content of phenols (Fernández et al., 1982, Vidal-Valverde et al., 1994).

Nicoletto et al. (2019), however, found a significant effect of the environment on the total phenols and total antioxidant capacity of Venetian beans. Of the accessions assessed in this study, a small difference was found between the two locations, however, not significant. The accessions' effect on the total phenols and antioxidant capacity was significant.. Akond et al. (2011) assessed the total polyphenols and antioxidant capacity of 29 common beans from Mexico, the USA, Brazil, and India. The total polyphenols ranged from 5,870 to 14,140 mg of GAE kg⁻¹. Garretson et al. (2018) assessed the total phenols and antioxidant capacity of five raw heirloom beans seeds and the amounts ranged from 4,800 to 9,600 mg of GAE kg⁻¹. In this study, the total phenols ranged from 3,900 to 4,791 mg of GAE kg⁻¹ among the accessions that completed their cycles in both locations, indicating that Italian beans have a lower total phenols content when compared to the ones cultivated in the Americas. Nicoletto et al. (2019) assessed the accessions Lingua di Fuoco and Gialet (accessions 8 and 23 in this study) and found a total phenol of 1,240 and 1,047 mg GAE kg⁻¹, respectively, amount much lower than the ones found in this study, 3,335 and 3,468 mg GAE kg⁻¹, respectively. Accession 22, Fasolo del Diavolo, had the greatest total phenols, 6,488 mg GAE kg⁻¹.

When compared to other legumes, Gutiérrez-Uribe et al. (2011) found lower values for the whole seed of cowpeas, 755.7 mg GAE kg⁻¹. Kumar et al. (2010) compared the total phenols content of soybean accession of different colors: yellow, green, and black. They found lower values in the accessions green and yellow, ranging from 960 to 2,890 mg GAE kg⁻¹. However, the range found in this study was within the range found by them for soybean accessions with black seeds, from 810 to 5,890 mg GAE kg⁻¹.

The antioxidant capacity ranged from 3,267 mg Fe⁺² kg⁻¹ to 13,472 mg Fe⁺² kg⁻¹ among the accessions cultivated in both locations. Accession 22, however, had the greatest antioxidant capacity, 38,365 mg Fe⁺² kg⁻¹. The total antioxidant capacity of the Lingua di Fuoco and Gialiet assessed by Nicoletto et al. (2019) was 3,482 and 2,133 mg Fe⁺² kg⁻¹, respectively, also lower than the content obtained in this study, 4,287 and 7,986 mg Fe⁺² kg⁻¹, respectively. Accessions 6, 7, 17, and 18 had low contents of total phenols and antioxidant capacity and their seed colors were white or yellowish with, in the case of accession 18. Whereas accessions 33, 34, 36, 8, 22 had the greatest contents of total phenols and antioxidant capacity. These accessions seeds' colors were light brown and dark brown, golden brown, brown, purple/white and purple, and purple and black, respectively. Thus, this study confirms the relationship of seed coat color and seeds' total phenols and antioxidant capacity stated by Díaz-Batalla et al. (2006), Oroian and Escriche (2015), and Ombra et al. (2016).

Guzmán-Maldonado et al. (2000) characterized 70 wild and weedy common beans from Durango and Jalisco (Mexico) and found that the protein content ranged from 18.0 to 33.0%. The protein content of 59 accessions from North Spain and five commercial cultivars ranged from 19.3 to 25.2% (Escribano et al., 1997), and 73 South Brazil landraces ranged from 19.0 to 31.0% (Pereira et al., 2016). The protein content of the Italian common beans assessed in this study ranged in agreement with the studies mentioned before, from 19.35% to 33.55%. The environment had a positive effect on the protein content of climbing varieties. On average, seeds from Legnaro had a protein content of 29.03%, while the seeds from the mountain protein content was 26.45%. The starch content had a wider range, from 14.12% to 35.25%, and the environment had a significant effect on the starch content of the dwarf accessions that was significantly greater in the moutain. The accession and the environment had a low effect on the content of most of amino acids.

5. CONCLUSIONS

The introduction of beans into Veneto occurred in several events, with 56% of these accessions from the Andean domestication center. Accessions from the Mesoamerican domestication center had a higher diversity and very low hybridization between the two centers of origin was found.

Accessions had a high diversity on seeds' physical and nutraceutical characteristics. There was a wide range of protein, starch, antioxidant capacity and total phenols content. The environment did not affect the content of the last two. However, seeds produced in SL had 10% higher protein content than those produced in MT. The environment also affected seeds' physical characteristics since MT increased weight and density of seeds.

Accessions also had different agronomic performance and it was highly affected by the environment. In general, landraces and pre-commercial accessions produced more than two times more seeds per plant in MT, than SL. Accession 36, for example, produced twenty times more in MT. Thus, this show a strong connection and adaptation of these landraces to the Venetian mountainous area. The characterization of accessions conducted in this study will be essential to conserve these accessions and provide valuable information for the development of new varieties

LITERATURE CITED

Adler-Nissen, J. (1986). Enzymatic Hydrolisis of Food Protein. New York: Elsevier.

- Agbor, G. A., Oben, J. E., Ngogang, J. Y., Xinxing, G., and Vinson, J. A. (2005).
 Antioxidant capacity of some herbs/spices from Cameroon: A comparative study of two methods. *J. Agric. Food Chem.* 53, 6819–6824. doi:10.1021/jf050445c.
- Akond, A. S. M. G. M., Khandaker, L., Berthold, J., Gates, L., Peters, K., Delong, H., et al. (2011). Anthocyanin, Total Polyphenols and Antioxidant Activity of Common Bean. Am. J. Food Technol. 6, 385–394.
- Albala, K. (2007). "Phaseolus vulgaris: Mexico and the World," in *Beans: a History* (London: Bloomsbury Academic), 240.
- Angioi, S. A., Rau, D., Attene, G., Nanni, L., Bellucci, E., Logozzo, G., et al. (2010).
 Beans in Europe: Origin and structure of the European landraces of Phaseolus vulgaris L. *Theor. Appl. Genet.* 121, 829–843. doi:10.1007/s00122-010-1353-2.
- Arnoldi, A., Zanoni, C., Lammi, C., and Boschin, G. (2015). The Role of Grain Legumes in the Prevention of Hypercholesterolemia and Hypertension. *CRC. Crit. Rev. Plant Sci.* 34, 144–168. doi:10.1080/07352689.2014.897908.
- Barroga, C. F., Laurena, A. C., and Mendoza, E. M. T. (1985). Polyphenols in Mung Bean (Vigna radiata (L.) Wilczek): Determination and Removal. J. Agric. Food Chem. 33, 1006–1009. doi:10.1021/jf00065a056.
- Beebe, S., Rengifo, J., Gaitan, E., Duque, M. C., and Tohme, J. (2001). Diversity and 78

Origin of Andean Landraces of Common Bean. *Crop Sci.* 41, 854–862. doi:10.2135/cropsci2001.413854x.

- Beebe, S., Skroch, P. W., Tohme, J., Duque, M. C., Pedraza, F., and Nienhuis, J. (2000).
 Structure of Genetic Diversity among Common Bean Landraces of Middle
 American Origin Based on Correspondence Analysis of RAPD. *Crop Sci.* 40, 264–273. doi:10.2135/cropsci2000.401264x.
- Benzie, I. F. F., and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* 239, 70–76. doi:10.1006/abio.1996.0292.
- Bishnoi, S., and Khetarpaul, N. (1993). Variability in physico-chemical properties and nutrient composition of different pea cultivars. *Food Chem.* 47, 371–373. doi:10.1016/0308-8146(93)90179-J.
- Boateng, J., Verghese, M., Walker, L. T., and Ogutu, S. (2008). Effect of processing on antioxidant contents in selected dry beans (Phaseolus spp. L.). *LWT - Food Sci. Technol.* 41, 1541–1547. doi:10.1016/j.lwt.2007.11.025.
- Branca, A., Paape, T. D., Zhou, P., Briskine, R., Farmer, A. D., Mudge, J., et al. (2011).
 Whole-genome nucleotide diversity, recombination, and linkage disequilibrium in the model legume Medicago truncatula. *Proc. Natl. Acad. Sci. U. S. A.* 108, E864– E870. doi:10.1073/pnas.1104032108.
- Calles, T. (2016). Preface to special issue on leguminous pulses. Plant Cell, Tissue Organ

Cult. 127, 541–542. doi:10.1007/s11240-016-1146-7.

- Camera di Commercio Treviso-Belluno (2017). Indicazioni di mercato sui prezzi medi dei prodotti tipici bellunesi. 1. Available at: http://webcache.googleusercontent.com/search?q=cache:FNHTLhECyJ0J:www.bl.c amcom.it/UserFile/File/Statistica_Protesti/listino%2520prodotti%2520tipici%2520b ellunesi/06_giugno%25202017(1).pdf+&cd=2&hl=pt-BR&ct=clnk&gl=us [Accessed June 8, 2020].
- Courty, P. E., Smith, P., Koegel, S., Redecker, D., and Wipf, D. (2015). Inorganic
 Nitrogen Uptake and Transport in Beneficial Plant Root-Microbe Interactions. *CRC*.
 Crit. Rev. Plant Sci. 34, 4–16. doi:10.1080/07352689.2014.897897.
- Díaz-Batalla, L., Widholm, J. M., Fahey, G. C., Castaño-Tostado, E., and Paredes-López,
 O. (2006). Chemical Components with Health Implications in Wild and Cultivated
 Mexican Common Bean Seeds (Phaseolus vulgaris L.). *J. Agric. Food Chem.* 54,
 2045–2052. doi:10.1021/jf0517061.
- Díaz, L. M., and Blair, M. W. (2006). Race structure within the Mesoamerican gene pool of common bean (Phaseolus vulgaris L.) as determined by microsatellite markers. *Theor. Appl. Genet.* 114, 143–154. doi:10.1007/s00122-006-0417-9.
- Escribano, M. R., Santalla, M., and De Ron, A. M. (1997). Genetic diversity in pod and seed quality traits of common bean populations from northwestern Spain. *Euphytica* 93, 71–81. doi:10.1023/A:1002908224793.

- Evanno, G., Regnaut, S., and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol. Ecol.* 14, 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x.
- Fernádez, R., Elís, L. G., Braham, J. E., and Bressani, R. (1982). Trypsin Inhibitors and Hemagglutinins in Beans (Phaseolus vulgaris) and Their Relationship with the Content of Tannins and Associated Polyphenols. J. Agric. Food Chem. 30, 734–739. doi:10.1021/jf00112a027.
- Garretson, L., Tyl, C., and Marti, A. (2018). Effect of Processing on Antioxidant Activity, Total Phenols, and Total Flavonoids of Pigmented Heirloom Beans. J. *Food Qual.* 2018, 1–6. doi:10.1155/2018/7836745.
- Giovanelli, G., and Buratti, S. (2009). Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. *Food Chem.* 112, 903–908. doi:10.1016/j.foodchem.2008.06.066.
- Gutiérrez-Uribe, J. A., Romo-Lopez, I., and Serna-Saldívar, S. O. (2011). Phenolic composition and mammary cancer cell inhibition of extracts of whole cowpeas (Vigna unguiculata) and its anatomical parts. *J. Funct. Foods* 3, 290–297. doi:10.1016/j.jff.2011.05.004.
- Guzmán-Maldonado, S. H., Acosta-Gallegos, J., and Paredes-López, O. (2000). Protein and mineral content of a novel collection of wild and weedy common bean (Phaseolus vulgaris L). *J. Sci. Food Agric.* 80, 1874–1881. doi:10.1002/1097-0010(200010)80:13<1874::AID-JSFA722>3.0.CO;2-X.

- Hidalgo, R. (1988). "The Phaseolus World Collection," in *Genetic Resources of Phaseolus Beans*, ed. P. Gepts (Dordrecht: Springer), 67–90. doi:10.1007/978-94-009-2786-5_4.
- Hjalmarsson, S., and Akesson, R. (1983). Modern Kjeldahl Procedure. Int. Lab. 3, 70-76.
- IBPGR (1982). International Board for Plant Genetics Resources. Phaseolus vulgaris descriptors. 1–37. Available at: https://www.bioversityinternational.org/fileadmin/_migrated/uploads/tx_news/Phase olus_vulgaris_descriptors_160.pdf [Accessed January 14, 2020].
- Johns, M. A., Skroch, P. W., Nienhuis, J., Hinrichsen, P., Bascur, G., and Muñoz-Schick, C. (1997). Gene Pool Classification of Common Bean Landraces from Chile Based on RAPD and Morphological Data. *Crop Sci.* 37, 605–613. doi:10.2135/cropsci1997.0011183X003700020049x.
- Jones, A. (1999). PHASEOLUS BEAN: Post-harvest Operations. *FAO*, 1–25. Available at: http://www.fao.org/3/a-av015e.pdf [Accessed January 13, 2020].
- Kumar, V., Rani, A., Dixit, A. K., Pratap, D., and Bhatnagar, D. (2010). A comparative assessment of total phenolic content, ferric reducing-anti-oxidative power, free radical-scavenging activity, vitamin C and isoflavones content in soybean with varying seed coat colour. *Food Res. Int.* 43, 323–328. doi:10.1016/j.foodres.2009.10.019.
- Kwak, M., and Gepts, P. (2009). Structure of genetic diversity in the two major gene

pools of common bean (Phaseolus vulgaris L., Fabaceae). *Theor. Appl. Genet.* 118, 979–992. doi:10.1007/s00122-008-0955-4.

- Lam, H. M., Xu, X., Liu, X., Chen, W., Yang, G., Wong, F. L., et al. (2010).
 Resequencing of 31 wild and cultivated soybean genomes identifies patterns of genetic diversity and selection. *Nat. Genet.* 42, 1053–1059. doi:10.1038/ng.715.
- Lu, X., Wang, J., Al-Qadiri, H. M., Ross, C. F., Powers, J. R., Tang, J., et al. (2011).
 Determination of total phenolic content and antioxidant capacity of onion (Allium cepa) and shallot (Allium oschaninii) using infrared spectroscopy. *Food Chem.* 129, 637–644. doi:10.1016/j.foodchem.2011.04.105.
- Moghaddam, M., and Mehdizadeh, L. (2015). Variability of total phenolic, flavonoid and rosmarinic acid content among Iranian basil accessions. *LWT Food Sci. Technol.* 63, 535–540. doi:10.1016/j.lwt.2015.03.068.
- Nelson, M. E., Hamm, M. W., Hu, F. B., Abrams, S. A., and Griffin, T. S. (2016).
 Alignment of Healthy Dietary Patterns and Environmental Sustainability: A
 Systematic Review. *Adv. Nutr.* 7, 1005–1025. doi:10.3945/an.116.012567.
- Nicoletto, C., Zanin, G., Sambo, P., and Dalla Costa, L. (2019). Quality assessment of typical common bean accessions cultivated in temperate climate conditions and different growth locations. *Sci. Hortic. (Amsterdam)*. 256, 108599. doi:10.1016/j.scienta.2019.108599.
- Ombra, M. N., D'acierno, A., Nazzaro, F., Riccardi, R., Spigno, P., Zaccardelli, M., et al.

(2016). Phenolic Composition and Antioxidant and Antiproliferative Activities of the Extracts of Twelve Common Bean (Phaseolus vulgaris L.) Endemic Ecotypes of Southern Italy before and after Cooking. *Oxid. Med. Cell. Longev.* 2016, 1–12. doi:10.1155/2016/1398298.

Oroian, M., and Escriche, I. (2015). Antioxidants: Characterization, natural sources, extraction and analysis. *Food Res. Int.* 74, 10–36. doi:10.1016/j.foodres.2015.04.018.

Palumbo, F., and Barcaccia, G. (2018). "Critical aspects on the use of microsatellites markers for assessing genetic identity of crop plant varieties and authenticity of their food derivatives," in *Rediscovery of Landraces as a Resource for the Future*, ed. O. Grillo (London: IntechOpen), 129–160. Available at: https://books.google.it/books?hl=pt-

BR&lr=&id=7GiQDwAAQBAJ&oi=fnd&pg=PA129&dq=(2018)+Critical+Aspects +on+the+Use+of+Microsatellite+Markers+for+Assessing+Genetic+Identity+of+Cro p+Plant+Varieties.&ots=EvQasWiEoj&sig=EwfSnM1SZb0qUnroLPcD86Da6gA& redir_esc=y#v=onepage&q=(2018) Critical Aspects on the Use of Microsatellite Markers for Assessing Genetic Identity of Crop Plant Varieties.&f=false [Accessed May 21, 2020].

Peakall, R., and Smouse, P. E. (2012). GISAID Global Initiative on Sharing All Influenza Data. Phylogeny of SARS-like betacoronaviruses including novel coronavirus (nCoV). *Bioinforma. - Oxford* 28, 2537–2539. doi:10.1093/BIOINFORMATICS.

- Peakall, R., and Smouse, P. E. (2006). Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* 6, 288–295. doi:10.1111/j.1471-8286.2005.01155.x.
- Peix, A., Ramírez-Bahena, M. H., Velázquez, E., and Bedmar, E. J. (2015). Bacterial Associations with Legumes. CRC. Crit. Rev. Plant Sci. 34, 17–42. doi:10.1080/07352689.2014.897899.
- Pereira, T., Coelhor, M. M. C., Bogo, A., Guidolin, A., and Miquelluti, D. J. (2016).
 Long time no see rediscovery of peculiar ephemeral fern Anogramma leptophylla
 (L.) Link in Croatia. *Acta Bot. Croat.* 76, 91–94. doi:10.1515/botcro-2016-0021.
- Piergiovanni, A. R., Cerbino, D., and Brandi, M. (2000). The common bean populations from Basilicata (Southern Italy). An evaluation of their variation. *Genet. Resour. Crop Evol.* 47, 489–495. doi:10.1023/A:1008719105895.
- Piergiovanni, A. R., and Laghetti, G. (1999). The common bean landraces from Basilicata (Southern Italy): an example of integrated approach applied to genetic resources management. *Genet. Resour. Crop Evol.* 46, 47–52. doi:10.1023/A:1008641731573.
- Piergiovanni, A. R., and Lioi, L. (2010). Italian Common Bean Landraces: History, Genetic Diversity and Seed Quality. *Diversity* 2, 837–862. doi:10.3390/d2060837.
- Piergiovanni, A. R., Taranto, G., Losavio, F. P., and Sanson, S. (2004). The Agroecotypes of Common Bean (Phaseolus vulgaris) from Val Belluna (Veneto Region).

- Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of Population Structure Using Multilocus Accession Data. *Genet*. 155, 945–959. Available at: http://www.stats.ox.ac.uk/pritch/home.html. [Accessed June 7, 2020].
- Rendón-Anaya, M., Montero-Vargas, J. M., Saburido-Álvarez, S., Vlasova, A., Capella-Gutierrez, S., Ordaz-Ortiz, J. J., et al. (2017). Genomic history of the origin and domestication of common bean unveils its closest sister species. *Genome Biol.* 18, 60. doi:10.1186/s13059-017-1190-6.
- Rocha-Guzmán, N. E., González-Laredo, R. F., Ibarra-Pérez, F. J., Nava-Berúmen, C. A., and Gallegos-Infante, J. A. (2007). Effect of pressure cooking on the antioxidant activity of extracts from three common bean (Phaseolus vulgaris L.) cultivars. *Food Chem.* 100, 31–35. doi:10.1016/j.foodchem.2005.09.005.
- Rodiño, A. P., Santalla, M., De Ron, A. M., and Singh, S. P. (2003). A core collection of common bean from the Iberian Peninsula. *Euphytica* 131, 165–175. doi:10.1023/A:1023973309788.
- Salinas, A. D. (1988). "Variation, Taxonomy, Domestication, and Germplasm
 Potentialities in Phaseolus coccineus," in *Genetic Resources of Phaseolus Beans*, ed.
 P. Gepts (Springer, Dordrecht), 441–463. doi:10.1007/978-94-009-2786-5_18.
- Shimelis, E. A., and Rakshit, S. K. (2005). Proximate composition and physico-chemical properties of improved dry bean (Phaseolus vulgaris L.) varieties grown in Ethiopia. *LWT - Food Sci. Technol.* 38, 331–338. doi:10.1016/j.lwt.2004.07.002.

- Singleton, V. L., Orthofer, R., and Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* 299, 152–178. doi:10.1016/S0076-6879(99)99017-1.
- Spagnoletti Zeuli, P. L., Baser, N., Riluca, M., Laghetti, G., Logozzo, G., Masi, P., et al. (2004). Valorisation and certification of Italian bean agro-ecotypes (Phaseolus vulgaris) (in Italian). in *Ecotipi Vegetali Italiani: una preziosa risorsa di variabilità* genetica (Roma), 19–19.
- Tohme, J., Gonzalez, D. O., Beebe, S., and Duque, M. C. (1996). AFLP Analysis of Gene Pools of a Wild Bean Core Collection. *Crop Sci.* 36, 1375–1384. doi:10.2135/cropsci1996.0011183X003600050048x.
- Veneto Inside Venetian geography (in Italian). Available at: https://www.venetoinside.com/it/scopri-il-veneto/geografia/ [Accessed June 6, 2020].
- Venora, G., Grillo, O., Ravalli, C., and Cremonini, R. (2009). Identification of Italian landraces of bean (Phaseolus vulgaris L.) using an image analysis system. *Sci. Hortic. (Amsterdam).* 121, 410–418. doi:https://doi.org/10.1016/j.scienta.2009.03.014.
- Vidal-Valverde, C., Frias, J., Estrella, I., Gorospe, M. J., Ruiz, R., and Bacon, J. (1994). Effect of Processing on Some Antinutritional Factors of Lentils. J. Agric. Food Chem. 42, 2291–2295. doi:10.1021/jf00046a039.

Yeh, F., Yang, R., Boyle, T., Ye, Z., and Mao, J. (1997). POPGENE, the user-friendly shareware for population genetic analysis. *Mol. Biol. Biotechnol. Cent. - Univ. Alberta*. Available at: https://www.scienceopen.com/document?vid=49ae41b2-21fb-4d6a-ad75-cb515c37c969 [Accessed May 21, 2020].

Zeven, A. C. (1997). The introduction of the common bean (Phaseolus vulgaris L.) into Western Europe and the phenotypic variation of dry beans collected in the Netherlands in 1946. *Euphytica* 94, 319–328. doi:10.1023/A:1002940220241.

TABLES

Id	Name	Туре	Growth		Id	Name	Туре	Growth	
1	Mangiatutto rampicante	Italian pre- commercial	Indeterminate (climbing)	-16-	23	Gialet	Venetian 1andrace	Indeterminate (climbing)	
2	Borlotto nano A	Italian elite line	Determined (Dwarf)		24	Posenati	Venetian 1andrace	Indeterminate (climbing)	25
3	Borlotto nano B	Italian elite line	Determined (Dwarf)		25	Semi- rampicante abruzzese	Venetian landrace	Indeterminate (climbing)	
6	Fagiolo nano creso	Italian elite line	Determined (Dwarf)	Ŵ	26	Fasol dela nonna	Venetian landrace	Indeterminate (climbing)	32°
7	Bblue lake sel. Gia	Italian pre- commercial	Indeterminate (climbing)	-	27	Maseleta rossa	Venetian landrace	Indeterminate (climbing)	<u>وي</u>
8	Anellino di Trento	Italian elite line	Determined (Dwarf)		28	Zia Orsolina	Venetian landrace	Indeterminate (climbing)	÷
9	Anellino giallo	Italian pre- commercial	Indeterminate (climbing)		29	Meraviglia di Venezia	Venetian 1andrace	Indeterminate (climbing)	<i>R</i>
11	Bortollo lingua di fuoco 3	Italian pre- commercial	Indeterminate (climbing)	Ś	30	Secle	Venetian 1andrace	Indeterminate (climbing)	888C
12	Blue lake a grano nero	Italian pre- commercial	Indeterminate (climbing)	1000	31	Della Clorinda	Venetian landrace	Indeterminate (climbing)	æ
17	Fagiolo nano valdarno	Italian elite line	Determined (Dwarf)	·z	32	Pegaso	Venetian landrace	Indeterminate (climbing)	S.
18	Coco nain blanc precoce	Italian elite line	Determined (Dwarf)	-	33	SC-iosela	Venetian landrace	Indeterminate (climbing)	
19	Tondino abruzzese	Italian pre- commercial	Indeterminate (climbing)	5.5	34	D'oro (val di fiemme)	Venetian landrace	Indeterminate (climbing)	÷
20	Verdone del piave	Italian elite line	Determined (Dwarf)	*	36	Maron	Venetian 1andrace	Indeterminate (climbing)	*

Table 2.1. List and pictures of the seeds of the 26 accessions assessed in this study

Table 2.2. Analysis of variance (ANOVA) table with the effects of accession, environment, and the interaction of accessions and environment on climbing and dwarf accessions phenology, seed production, seeds' physical characteristics, and seeds' nutraceutical properties.

	Accession		Environ	Environment		vironment
	Climbing	Dwarf	Climbing	Dwarf	Climbing	Dwarf
		Phe	enology			
Vegetative phase	**	**	n.s.	n.s.	n.s.	n.s.
Flowering phase	***	***	***	n.s.	n.s.	n.s.
Fresh pods	***	***	***	***	n.s.	n.s.
Dry pods	***	***	n.s.	***	n.s.	n.s.
Total cycle length	***	**	n.s.	n.s.	n.s.	n.s.
		Seed p	roduction			
g of dry seeds per plant	***	***	**	n.s.	**	n.s.
	Seed	ds' physica				
Length	***	***	**	**	**	n.s.
Width	***	***	***	**	***	n.s.
Thickness	*	***	***	**	***	n.s.
Weight of 100 seeds	***	***	***	***	***	n.s.
Volume of 10 seeds	**	***	***	*	***	n.s.
Density	***	n.s.	***	**	n.s.	n.s.
	ì	Nutraceutie	cal properties-			
Total phenols	*	**	n.s.	n.s.	n.s.	n.s.
Antioxidant capacity	***	***	n.s.	n.s.	n.s.	n.s.
Protein	***	***	**	n.s.	n.s.	n.s.
Starch	***	***	n.s.	**	n.s.	n.s.
Essential amino acids	*	*	n.s.	n.s.	n.s.	n.s.
Non-essential amino acids	*	*	n.s.	n.s.	n.s.	n.s.

*** p < 0.001; ** p < 0.01; * p < 0.05; n.s. = non-significant effect (p > 0.05).

Table	2.3.	Effects	of the	environn	nent on tl	ie length	of the	vegetativ	e phase,	flower	ing
phase,	fres	h pods	period,	dry pods	periods,	and the	duratio	n of total	cycle of	dwarf	and
climbi	ing a	ccessio	ns.								

	Vegetative Phase		Flow	vering	Fresh Pods Dry Po		Pods	Total	Cycle	
	Mountain	Sea level	Mountain	Sea level	Mountain	Sea level	Mountain	Sea level	Mountain	Sea level
Dwarf	37 n.s.	38 n.s.	24 n.s.	23 n.s.	45 a	29 b	18 b	31 a	123 n.s.	121 n.s.
Climbing	44 n.s.	46 n.s.	18 b	31 a	49 a	29 b	19 n.s.	21 n.s.	130 n.s.	128 n.s.
Different letters after the length of the period indicate a significant difference within										

climbing and/or dwarf accessions. n.s. = non-significant environmental effect. $\alpha = 0.05$.

Table 2.4. Effects of the accession on average dry seed production per plant of dwarf accessions.

Accession	2	3	6	8	17	18	20
Seed production	53.8 abc	42.1 abc	82.7 a	59.1 abc	27.7 bc	67.4 ab	20.3 c

Different letters indicate a significant difference between accessions. $\alpha = 0.05$.

		Weight of	Volume	Seed			
		100 seeds	of 10	density	Length (mm)	Width (mm)	Thickness (mm)
		(g)	seeds (mL)	$(g mL^{-1})$			
	1	83 abcdef	6.8 abcd	1.22 abcd	17.3 abc	8.9 ghijkl	7.0 bcdefghijklmn
	7	27 rs	2.0 m	1.25 abcd	13.0 fghijklm	6.4 no	5.5 klmnopq
	9	68 ghij	5.3 ef	1.29 abcd	16.5 abcde	9.5 defghijk	6.9 cdefghijklmn
	11	77 bcdefghi	6.8 abcd	1.12 bcd	15.1 abcdefghij	10.9 abcde	6.8 defghijklmno
	12	30 qrs	2.5 klm	1.21 abcd	13.2 fghijklm	5.8 o	4.7 q
	19	44 mnopq	3.3 jkl	1.33 abc	10.5 m	8.9 ghijkl	7.0 cdefghijklmn
	23	47 mnop	3.5 ijk	1.33 abc	12.2 ijklm	8.4 hijklm	6.5 fghijklmnopq
Ц	24	90 ab	7.2 ab	1.26 abcd	15.8 abcdefg	11.0 abcd	8.6 abcd
ai	25	40 mnopqr	3.0 jklm	1.32 abc	12.5 hijklm	9.0 efghijk	5.3 mnopq
fut	26	46 mnop	3.3 jkl	1.39 a	11.3 klm	8.7 hijkl	6.7 efghijklmnop
lol	27	69 fghi	5.2 efg	1.34 ab	12.1 jklm	9.8 cdefghi	7.9 abcdefgh
Z	28	90 ab	7.0 abc	1.28 abcd	15.4 abcdefghi	10.8 abcdef	8.4 abcde
	29	39 nopqrs	3.2 jklm	1.23 abcd	14.1 cdefghijkl	7.8 klmn	4.8 pq
	30	71 defghi	5.8 cdef	1.21 abcd	14.5 abcdefghijk	9.8 cdefghij	7.6 abcdefghij
	31	64 hijk	5.0 efg	1.28 abcd	16.1 abcdef	10.2 bcdefgh	6.5 efghijklmnopq
	32	82 abcdefg	6.8 abcd	1.20 abcd	17.6 a	9.6 defghijk	6.1 hijklmnopq
	33	94 a	7.5 a	1.25 abcd	15.8 abcdefgh	11.8 ab	8.4 abcdef
	34	85 abcde	6.2 bcde	1.37 ab	14.1 cdefghijkl	10.2 bcdefgh	8.9 ab
	36	91 ab	7.3 ab	1.25 abcd	17.0 abcd	12.2 a	9.4 a
	1	79 bcdefgh	7.0 abc	1.13 bcd	14.9 abcdefghij	8.0 jklmn	5.5 lmnopq
	7	25 s	2.2 lm	1.23 abcd	12.4 hijklm	6.7 mno	5.2 nopq
	9	46 mnop	4.0 ghij	1.15 abcd	14.1 cdefghijkl	8.0 ijklmn	5.8 jklmnopq
	11	81 abcdefg	7.5 a	1.08 cd	17.4 ab	11.0 abcd	8.8 abc
	12	37 opgrs	3.2 jklm	1.15 abcd	13.7 efghijklm	7.0 klmno	5.0 opg
	19	45 mnop	3.7 hijk	1.24 abcd	11.5 klm	9.5 defghijk	7.8 abcdefghi
	23	44 mnopq	3.5 ijk	1.25 abcd	12.6 ghijklm	7.9 klmn	6.3 ghijklmnopq
	24	74 cdefghi	6.2 bcde	1.21 abcd	15.4 abcdefgh	10.3 abcdefgh	7.4 bcdefghijkl
5	25	39 nopqrs	3.3 jkl	1.17 abcd	11.9 jklm	7.7 klmn	5.6 klmnopq
eve	26	49 lmno	4.0 ghij	1.23 abcd	11.0 lm	9.6 defghijk	7.4 bcdefghijk
a le	27	63 ijkl	5.2 efg	1.22 abcd	13.2 fghijklm	10.2 bcdefgh	7.8 abcdefghi
Se	28	85 abcd	7.2 ab	1.18 abcd	14.2 cdefghijklm	10.7 abcdefg	8.0 abcdefg
•1	29	34 pqrs	3.2 jklm	1.07 d	13.1 fghijklm	7.8 abcdefg	5.5 lmnopq
	30	54 jklm	4.7 fghi	1.16 abcd	13.3 efghijklm	8.9 fghijk	6.9 defghijklm
	31	54 jklm	4.8 fgh	1.12 bcd	13.2 fghijklm	8.4 hijklm	6.0 ijklmnopq
	32	87 abc	7.7 a	1.14 abcd	17.3 abc	9.8 cdefghij	7.1 bcdefghijklm
	33	73 cdefghi	6.2 bcde	1.19 abcd	15.8 abcdefg	11.6 abc	7.3 bcdefghijkl
	34	53 klmn	4.0 ghij	1.31 abcd	13.8 defghijklm	8.8 ghijkl	8.1 abcdefg
	36	70 efghi	5.7 def	1.24 abcd	13.8 defghijklm	9.0 efghijk	7.1 bcdefghijklm

Table 2.5. Effect of accession and environment interaction on climbing accessions seeds' physical characteristics.

Different letters indicate a significant difference between compared groups. $\alpha = 0.05$.

	Weight 100 seeds (g)	Volume of 10 seeds (mL)	Seed density (g mL ⁻¹)	Length (mm)	Width (mm)	Thickness (mm)
		En	wironment			
Mountain	44.8 A	3.6 A	1.26 A	13.4 A	7.6 A	6.4 A
Sea level	37.7 B	3.2 B	1.14 B	12.4 B	7.0 B	5.8 B
		/	Accession			
2	60.3 a	5.4 a	1.11 n.s.	14.5 ab	9.5 a	7.0 ab
3	61.7 a	5.3 a	1.15 n.s.	15.3 a	9.0 a	7.7 a
6	30.2 d	2.5 c	1.21 n.s.	12.5 c	5.9 cd	5.5 cd
8	36.2 c	2.9 c	1.24 n.s.	13.1 bc	6.4 c	5.8 c
17	19.2 e	1.6 d	1.22 n.s.	10.2 d	5.2 d	4.8 d
18	35.3 c	2.8 c	1.29 n.s.	10.4 d	7.5 b	6.3 bc
20	46.0 b	3.9 b	1.78 n.s.	14.1 ab	7.5 b	5.9 c

Table 2.6. Effects of environments and accessions on seeds' physical characteristics of dwarf accessions.

Different uppercase letters indicate a significant difference between environments. Different lowercase letters indicate a significant difference between accessions. n.s. = non-significant genotypic effect. $\alpha = 0.05$.

	Starch (% DM)	Protein (% DM)						
	Environment							
Climbing								
Mountain	25.55 N.S.	26.45 B						
Sea level	25.24 N.S.	29.03 A						
	Dwarf							
Mountain	23.31 A	26.68 N.S.						
Sea level	20.87 B	26.19 N.S.						
	Accession							
	Climbing							
1	23.58 bcd	28.63 ab						
7	19.31 d	26.80 bc						
9	27.64 bc	29.08 ab						
11	20.76 cd	28.59 ab						
12	21.95	27.47 bc						
19	28.05 ab	23.77 d						
23	21.17 cd	30.15 a						
24	31.05 a	26.73 bc						
25	21.68 cd	28.02 b						
26	29.72 ab	24.74 cd						
27	31.57 a	27.72 b						
28	30.01 a	26.7 bc						
29	18.87 d	30.36 a						
30	30.95 a	27.05 bc						
31	25.58 bc	26.67 bc						
32	22.12 cd	31.05 a						
33	26.65 bc	28.32 ab						
34	23.75 bcd	27.75 bc						
36	28.06 ab	27.42 bc						
	Dwarf							
2	20.7 bc	28.32 a						
3	23.85 ab	27.52 ab						
6	25.23 a	26.63 bc						
8	21.39 b	26.71 bc						
17	17.32 c	22.62 d						
18	24.05 ab	25.67 c						
20	22.44 b	27.59 ab						

Table 2.7. Effects of the environments and of the accessions on starch and protein content of climbing and dwarf accessions.

Different uppercase letters indicate a significant difference between environments. N.S. = non-significant environmental effect. Different lowercase letters indicate a significant difference between accessions. $\alpha = 0.05$.
	Histidine ^{ns} Methionine [*] Phenylalanine ^{ns}		Isoleucine	Isoleucine ^{ns} Leucine ^{ns}		Threonine*	Tryptophan ^{ns}	Valine ^{ns}			
Climbing (mg 100 g ⁻¹)											
1	675	321a	1492	1053	1985	2612	1261a	175	1161		
7	762	253c	1380	968	1875	2499	1197ab	188	1048		
9	839	292abc	1455	1027	2021	2938	1178ab	169	1116		
11	497	273abc	1202	836	1658	1816	1091ab	174	947		
12	531	256c	1234	850	1671	1939	1081ab	165	949		
19	467	256c	1133	806	1531	1750	970ab	184	906		
23	678	263bc	1264	937	1841	2233	1026ab	198	1031		
24	803	293abc	1402	1022	1986	2795	1181ab	195	1136		
25	524	261bc	1099	817	1595	1760	899b	174	893		
26	445	256c	1068	775	1470	1657	958ab	210	881		
27	521	282abc	1227	868	1694	1888	962ab	175	970		
28	495	277abc	1207	868	1648	1787	990ab	177	973		
29	511	260bc	1348	1003	1932	2111	1042ab	199	1100		
30	436	267abc	1174	847	1619	1728	911b	172	960		
31	529	280abc	1244	885	1705	1959	945ab	180	1009		
32	659	283abc	1521	1084	2086	2621	1093ab	185	1214		
33	631	277abc	1371	981	1891	2403	1015ab	194	1084		
34	612	284abc	1378	991	1871	2295	1027ab	178	1094		
36	425	310ab	1263	907	1730	1821	1000ab	171	1042		
	Histidine	^{ns} Methionine ^{n.s}	^b Phenylalanine ^{ns}	Isoleucinens	Leucine ^{ns}	Lysine ^{ns}	Threonine*	Tryptophan ^{ns}	Valine ^{ns}		
Dwarf (mg 100 g ⁻¹)											
2	491	272	1332	913	1796	2032	1179a	169	1052		
3	546	279	1199	817	1569	1774	1003c	156	917		
6	551	239	1155	793	1535	1807	1013c	169	901		

Table 2.8. Effects of the accession on the essential amino acids profile of climbing and dwarf accessions.

1023c 1045c 1100b Different letters indicate a significant difference between accessions. n.s. = non-

1131ab

significant genotypic effect. $\alpha = 0.05$.

	Aspartic acid ^{ns}	Glutamic acid ^{ns}	Serine ^{ns}	Glycine ^{ns}	Arginine ^{ns}	Alanine ^{ns}	Tyrosine [*]	Proline ^{ns}	Cysteine ^{ns}		
Climbing (mg 100 g ⁻¹)											
1	3396	5113	1742	1064	1778	1146	811a	1372	289		
7	3006	4714	1539	992	1437	1073	726ab	1209	216		
9	3326	5253	1660	1031	1774	1131	778ab	1326	260		
11	3075	4730	1529	930	1468	1044	614ab	1425	273		
12	2936	4322	1453	928	1355	1014	629ab	1347	238		
19	2653	4062	1314	863	1227	942	572ab	1288	226		
23	3053	4811	1507	919	1564	1037	658ab	1938	229		
24	3361	5187	1651	993	1480	1134	734ab	1915	229		
25	2811	4585	1359	863	1615	969	616ab	1581	214		
26	2617	3829	1306	837	1189	934	569b	1461	222		
27	2961	4592	1475	913	1616	1013	644ab	1658	227		
28	2961	4631	1457	889	1516	1009	615ab	1621	242		
29	3293	5189	1644	1032	1710	1133	706ab	1666	198		
30	2943	4245	1388	890	1553	981	589ab	1551	222		
31	2966	4501	1422	935	1487	1025	616ab	1564	206		
32	3497	5466	1738	1071	1966	1180	785ab	1702	221		
33	3130	4839	1523	970	1682	1081	682ab	1624	213		
34	3130	4919	1545	950	1406	1067	704ab	1598	236		
36	3181	4883	1543	956	1543	1074	686ab	1667	212		
	Aspartic acid ^{ns}	Glutamic acid ^{ns}	Serin*	Glycine*	Arginine ^{ns}	Alanine ^{ns}	Tyrosine ^{n.s.}	Proline ^{ns}	Cysteine ^{ns}		
Dwarf (mg 100 g ⁻¹)											
2	3191	4683	1611a	1004a	1520	1104	679	1415	223		

Table 2.9. Effects of the accession on the non-essential amino acids profile of climbing and dwarf accessions.

Dwarf (mg 100 g ⁻¹)									
2	3191	4683	1611a	1004a	1520	1104	679	1415	223
3	2733	4047	1363ab	852c	1277	941	658	1314	210
6	2656	3966	1334b	863bc	1218	943	585	1125	225
8	3135	4526	1549a	973abc	1495	1062	684	1381	227
17	2913	4388	1433a	916abc	1435	996	609	1559	188
18	2832	4170	1406ab	898abc	1260	994	590	1410	215
20	3218	4778	1567a	986ab	1483	1097	673	1485	257

Different letters indicate a significant difference between accessions. n.s. = nonsignificant genotypic effect. $\alpha = 0.05$.

FIGURES



Fig.2.1. Average daily maximum and minimum temperatures in Asiago (mountain) and Legnaro (sea level) during the bean production cycle.



Fig. 2.2. First, Dendogram with all the accessions separated in clusters. In the middle, ancestral membership percentage estimated by the STRUCTURE analysis for k = 2. Last, allele combinations characteristics of the corresponding sample. Each bar is divided in six, each representing the molecular marker written in the left. Dashed blocks = missing data.



Fig. 2.3. Effects of accession on the length of the vegetative phase, the flowering phase, the fresh pods period, the ripe pods period, and on the total cycle duration of dwarf (on the top) and climbing (on the bottom) accessions. Different letters inside the bars of each period indicate a significant difference on the length of this period. Different letters outside, on the right of the bars, indicate a significant difference on the total cycle length. $\alpha = 0.05$.



Fig. 2.4. Relationship of the plant development (BBCH-scale) and the accumulated growing degree day (GDD) for climbing accessions at sea level (a) in the mountains (c) and dwarf accessions at the sea level (b) and in the mountains (d).



Fig. 2.5. Average seed production per plant of each climbing accession in the mountain (black bars) and at the sea level (grey bars), and average seed production in the mountain (solid line) and at the sea level (dash-dotted line). Different letters indicate a significant difference between compared groups. $\alpha = 0.05$.



Fig. 2.6. Effects of the accessions on the seeds' total phenols content of climbing (on the left) and dwarf (on the right) accessions. Different letters indicate a significant difference between accessions. $\alpha = 0.05$.



Fig. 2.7. Effects of the accessions on the seeds' antioxidant capacity of climbing (on the left) and dwarf (on the right) accessions. Different letters indicate a significant difference between accessions. $\alpha = 0.05$.

CHAPTER 3: RESPONSE OF VENETIAN BEAN (*Phaseolus spp.*) LANDRACES TO DROUGHT CONDITIONS ON DIFFERENT PLANT DEVELOPMENT STAGES

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ABSTRACT

Currently, most of the beans are cultivated in areas under high risk of facing drought conditions. For this reason, it is important to screen bean accessions for drought tolerance. This study assessed the response of six landraces from Veneto (Italy) when drought conditions were imposed during different growth stages: never stressed (NES), always stressed (ALS), stressed before flowering (SBF), and stressed after flowering (SAF). Imposing drought conditions after flowering significantly affected yield of five accessions. Gialet's ALS and SAF plants yields were reduced by about 40% and 30%, respectively, however, this difference was not significant when compared to NES and SBF. Secle's SBF yields were significantly greater than NES, ALS, and SAF, which had increased by 80% the seed production per plant compared to NES. Imposing drought conditions did not significantly affect the seeds' content of total phenols, antioxidant capacity, protein, phosphorus, magnesium, calcium, and zinc. Among accessions, D'oro and Maron had significantly greater antioxidant capacity and total phenols content than Gialet. Gialet and Maron had significantly greater Zn content (~ 60 ppm), showing potential to be explored in mineral biofortification breeding programs. Therefore, Venetian landraces assessed in this study had different responses to drought conditions imposed in different growth stages, and drought conditions did not affect nutraceutical properties.

Keywords: drought conditions; bean landraces; genetic conservation; genetic diversity; biofortification.

Abbreviations:

- DAS Days after sowing
- DM Dry matter
- $DTI-Drought \ tolerance \ index$

DW – Dry weight

- LT-Leaf temperature
- $PNM-Potential\ nitrogen\ mineralization$
- YRR Yield reduction rate

1. INTRODUCTION

Legumes are essential to improve global food safety and security (FAO, 2016; Jimenez-Lopez et al., 2020). Common bean (*Phaseolus vulgaris*) is the most important directly consumed legume on human diet (Blair, 2013) and the principal food legume for 250 million in South and Central America and for 400 million people in eastern Africa (McClean and Raatz, 2017), providing essential food components to combat malnutrition in these regions (Katungi et al., 2009). More than 40,000 varieties of beans are cultivated in all continents, except Antarctica, in different crop systems and in a wide range of environments. Beans are considered one of the major crops with the highest levels of variation in growth habit, seeds characteristics, maturity, and adaptation (Jones, 1999). Beans also play an important role in achieving a more sustainable food production system, being able to fix atmospheric nitrogen biologically and reduce the input of synthetic N, improve nutrient cycling, provide other services in multiple cropping systems, such as improving soil physical conditions and its biodiversity (Courty et al., 2015; Peix et al., 2015; Calles, 2016).

According to Food and Agriculture Organization (2009) world population will grow from 6.8 billion to 9.1 billion people from 2009 to 2050, mainly in developing countries. This will require an increase of 70% in the world food production. Against this background, climate change will make growing food even more challenging. It is estimated that, currently, most of the beans are grown in areas at risk of facing drought conditions throughout its life cycle (Beebe et al., 2008). The area of beans cultivated under these conditions is likely to increase in the future due to desertification and land degradation, caused by cropland expansion and intensification (United Nations, 2020).

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Thus, screening beans' germplasm banks for drought condition tolerance is considered decisive for breeding programs to obtain superior accessions for adaptation to drought (Gonçalves et al., 2019).

Common beans domestication was unique in two independent centers of origin Mesoamerican and Andean (Beebe et al., 2001; Bitocchi et al., 2013) with introduction into Western Europe around 1500, in the 16th century (Zeven, 1997). Although Italy is not among the world's major producers (FAOSTAT, 2017), beans created a long tradition that allowed the evolution of many landraces that are adapted to microclimates in restricted areas. It represents cultures and traditions that are strongly connected with the recent history of Italy, where they are used by low impact environmental agriculture (Venora et al., 2009). In Veneto, the diffusion of common bean occurred quickly, and today, the cultivation of this pulse is of great economic relevance in the Belluno region (Piergiovanni and Lioi, 2010) and many local landraces are still commonly found at local markets (Nicoletto et al., 2019). However, although those accessions have original morpho-agronomical and nutritional characteristics, with high organoleptic qualities, they have been gradually substituted by genetic uniform commercial varieties (Spagnoletti Zeuli et al., 2004; Venora et al., 2009). For this reason, the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) from the University of Padova (UNIPD) is establishing a bean germplasm to conserve and characterize Italian and, mainly, Venetian bean landraces.

This study aimed to assess the effect of drought conditions during different growth stages of six Venetian bean landraces on agronomic performance, plant morphology, and seed physical and nutraceutical characteristics. Fasolo del Diávolo, Gialet, Posenati, Secle, D'oro, and Maron were selected from 48 accessions of the DAFNAE/UNIPD bean germplasm, based on their genetic homogeneity, agronomic performance, cultivation tradition, and seeds' nutraceutical characteristics and visual traits of commercial interest. Four different irrigation regimes were imposed on these accessions: never stressed, always stressed (from sowing to harvesting), stressed before flowering, and stressed after flowering. The effect of these different irrigation regimes was assessed on the yield, shoots biomass, number of leaves, leaf temperature, seeds' antioxidant capacity, total phenols, phosphorus, zinc, calcium, and magnesium content, protein content, and seeds' dimensions.

2. MATERIALS AND METHODS

2.1. Plant materials

Of 48 accessions from the DAFNAE/UNIPD bean germplasm six climbing Venetian landraces with indeterminate growth were selected for this study. According to a genetic characterization performed with this material (not published data), these accessions were grouped in three main clusters: Mesoamerican, Andean, and *P. coccineus*. Fasolo del Diavolo is a *P. coccineus*, Gialet and Posenati are both from the Mesoamerican domestication center and Secle, D'oro and Maron are from the Andean domestication center. The last two being grouped in the same subcluster. The genetic similarity among the landraces are presented in table 3.1.

The factors used to select these landraces were their agronomic performance in field trials performed in 2019 in two different Venetian environments, Asiago (Venetian

mountains, 997 m altitude) and Legnaro (sea level, 7 m altitude), seeds nutraceutical characteristics and visual traits of commercial interest (not published data). Fasolo del diavolo (F.D.), Devil's bean, was chosen due to its high performance in Asiago, its high total phenols and antioxidant activity, and seed characteristics appealing for commercial purposes. Gialet (G), yellowish in Veneto dialect, had a good agronomic performance in both locations and is a PGI landrace from Belluno, Veneto; thus, has a good commercial potential. Posenati (P), from the Posina, in Vicenza, Veneto, had a great agronomic performance in both locations and have high total phenols and antioxidant capacity. Although its seeds are not colorful, they are visually similar to the traditional Lamon bean, so it can have commercial appeal in the region. Seclè (S), had a great performance in both locations and high contents of total phenols and antioxidant activity. Its purple seeds are visually appealing and can increase its commercial value. D'oro, (D) of gold, had a great performance in both locations and its seeds have good commercial characteristics. Maron (M), brown, had a good performance in Asiago, high contents of total phenols and antioxidant activity and its seeds characteristics can increase its commercial value (Fig. 3.1.)

2.2. Experimental design and environmental conditions

Pot experiments were performed in a rain-out shelter located at 33° 46' 53.8248" N, 83° 19' 49.7028" W. Each landrace was subjected to four treatments: never stressed (NES), always stressed (ALS), stressed before flowering (SBF), and stressed after flowering (SAF). From sowing until flowering, NES and SAF received a normal irrigation regime and ALS and SBF received a reduced irrigation regime. After

flowering, NES and SBF got a normal irrigation regime, whereas ALS and SAF got a reduced irrigation regime. Pots were arranged in a randomized complete block design with four replications of each treatment. To ensure that the pots were not getting rainwater, the rain-out shelter was covered on the top and sides with clear plastic. A Decagon Em50 data logger (Meter Group Inc.) was placed in the middle of the shelter and connected to a VP-4 Humidity/Temperature/Barometer sensor (Meter Group Inc.) to monitor and record the air temperature and relative humidity. Four 5TM Moisture/Temperature soil probes were placed at a 5 cm depth into different treatment pots to monitor hourly soil water content. Four reference pots without plants were placed randomly and weighted before and after each irrigation, in order to monitor water evaporation. After this experiment, sensors were calibrated in the lab using the same soil with different water contents. Gravimetric water content was determined and converted to volumetric water content using the soil density. Unprocessed data was converted into volumetric water content (VWC, m^3 of water per m^3 of soil) (calibration curve $R^2 =$ 0.9342), and the hourly average volume of water per pot of each treatment before and after flowering were calculated. The field capacity (FC) and the permanent wilting point (PWP) of this soil were 46 and 16% (VWC), respectively, or 1.62 and 0.56 L of water per pot. Plant available water is the difference between water content and permanent wilting point (Fig. 3.2.).

Each pot with a 20 cm diameter and 3.8 L volume and filled with 3 kg of certified organic soil compost (Soil Cube, Greenville, South Carolina) and two seeds were sowed on March 30th 2020 followed by 500 ml of water to saturate the soil. The soil used had a high organic matter content, 32% loss of ignition, pH 6.8 in 0.01M CaCl₂ (1:2) and 7.3 in

distilled H₂O (1:2), bulk density of 0.85 g cm⁻³, 22.8 mg/kg of potentially mineralizable nitrogen by hot KCl methods, 57.5 mg/kg of plant available nitrogen and CEC of 22.8 meq 100 g⁻¹. After five days, all plants emerged and at three days after emergence one plant was thinned from each pot, ensuring that all plants within each accession were as homogenous as possible. All plants bloomed between 35 and 52 DAS.

2.3. Analysis

2.3.1. Plant phenology and morphology

Every ten days each plant development stage was monitored following the bean BBCH scale proposed by Feller et al. (1995). This scale has nine different growth stages, in this study it was summarized in four main growth stages:

- i) vegetative phase: from germination until first flower opened,
- ii) flowering: from the first flowers open until the first pods are visible,
- iii) fresh pods: from the first green pod visible until the first pod started to dry and ripe,
- iv) dry pods: from when the pods and seeds ripe until the plant's senescence.

The number of leaves of each plant was counted just before the first plant bloomed, at 40 DAS, just after the last plant bloomed, at 55 DAS, and during the fresh pods period, at 100 DAS. At 40 DAS and 100 DAS the third leaf of each plant had its temperature measured with a HTI Ht-02 thermal imaging and visible light camera IR resolution 3600 pixels (Hti - equipments). In the last day of its life cycle, each plant was collected and placed in paper bags. Paper bags were dried in an oven at 65°C for 48h, until constant weight. Dried plants were weighed in a 3-decimal analytical balance to determine shoots dry matter.

At harvesting, the number of pods and seeds harvested per plant were recorded. Seeds were weighed and dry seed produced by plant and weight of 10 seeds were determined. A digital caliper was used to measure the length, width, and thickness of five seeds from each plant. The number of plants that produced seeds was also recorded. The yield reduction rate (YRR) and drought tolerance index (DTI) were calculated using the Eq. 1 and Eq. 2, respectively Fischer and Maurer (1978). The ratio of seeds:biomass was calculated by diving the weight of seeds produced by the plant by the plant shoots dry matter (Eq. 3).

$$YRR = \frac{(Yield of NES-Yield of stressed treatment)}{Yield of NES}$$
(Eq. 1)

$$DTI = \frac{(Yield of NES*Yield of stressed treatment)}{(Yield of NES*Yield of NES)}$$
(Eq. 2)

$$Yield: Biomass = \frac{Yield}{Shoots dry matter}$$
(Eq. 3)

2.3.2. Soil characterization

Soil samples were collected before the experiment started and from each pot after the plants were harvested and stored at -4°C. Three grams of each sample was extracted using 20 mL of 2 M KCl at room temperature for 30 minutes (cold extraction) (Maynard and Kalra, 1993) and at 100°C water bath for 4 hours (hot extraction) (Picone et al., 2002). The NH₄⁺ - N content of the cold and hot extraction, and the NO₃⁻ - N of the cold extraction were measured using a TECAN Infinite 200 Pro spectrophotometer using the salicylic acid method (Kempers and Zweers, 1986) and vanadium (III) chloride method (Doane and Horwáth, 2003), respectively. The potentially mineralizable nitrogen was calculated by the difference between the NH_4^+ - N content of the hot extraction and the NH_4^+ - N content of the cold extraction (Dahal et al., 2020). The plant available nitrogen was calculated by the sum of the NH_4^+ - N and the NO_3^- - N of the cold extraction. Soil pH was determined by mixing 10 g of soil in 20 mL distilled H_2O or CaCl₃ and the loss of ignition was estimated by placing dried samples into a muffle furnace at 550°C for eight hours.

2.3.3. Seeds' nutraceutical characterization

Dry seeds were ground to fine powder and approximately 0.2 g of each sample was added to a 50 mL Falcon tube and homogenized in 20 mL of methanol. Samples were filtered in Whatman 4 grade filter papers, and appropriate aliquots of extracts were assayed by Folin Ciocalteau assay for total phenol content and by Ferric Reducing Antioxidant Power assay for total antioxidant activity.

The content of total phenols was measured on the basis of mg of Gallic Acid Equivalent per kg (mg GAE kg⁻¹ dw) of dry bean powder (Singleton et al., 1999). Bean extract was mixed with 20% sodium carbonate solution and Folin-Ciocalteau reagent (Fischer Scientific). After 2 h at room temperature, the absorbance of the colored reaction product was measured at 765 nm.

FRAP reagent was prepared fresh so that it contained 1mM 2,4,6 tripyridyl-2triazine and 2mM ferric chloride in 0.25M sodium acetate at pH 3.6 (Benzie and Strain, 1996). A 100 μ L aliquot of the methanol extract prepared as above was added to 1900 μ L of FRAP reagent and accurately mixed. After leaving the mixture at 20 °C for 4 min, the absorbance at 593 nm was determined. FRAP values were calculated as mg mL⁻¹ ferrous ion (ferric reducing power) from three determinations and are presented as mg of Fe²⁺E (ferrous ion equivalent) kg⁻¹ dw.

Approximately 0.2 g of dried ground bean powder were digested on two cycles of 200°C for one hour and at 375°C for two and a half hours, with 3 mL sulfuric acid being add at each cycle. Catalyst was added in the first cycle only. Total protein was estimated from total nitrogen measured from the Kjeldhal digestion (Hjalmarsson and Akesson, 1983) and total phosphorus determined using the method described by Jirka et al. (1976). Calcium, zinc, and Magnesium were measured in a Perkin Elmer AAnalyst200 atomic absorption unit using as reference standard curves obtained by diluting 1000 ppm stock solutions (Fischer Chemical) in 2% nitric acid.

2.4. Statistical analysis

All data collected was digitized and processed on Microsoft Excel. A summary spreadsheet with the data of each variable of each experimental unit was made and input into JMP Pro 15. Analysis of variance (ANOVA) considering the main effects of treatment, accession, and their interaction was conducted on JMP Pro 15. Tukey test was used to separate means at p < 0.05. Principal component analysis (PCA) was performed on JMP 15 Pro. Linear regressions were performed on SigmaPlot 11.0, which was also used to prepare graphs.

3. RESULTS

3.1. Plant cycles

Treatments, accessions, and the interaction between treatments and accessions had a significant effect on the length of the vegetative, flowering, fresh pods, and dry pods periods, and in the total cycle length (Table 3.2.). Posenati plants ALS, SBF, and SAF, and Secle plant's NES had the shortest vegetative phase, 35 days, whereas Fasolo del Diavolo NES and SBF had the longest vegetative phase, 52 days. Fasolo del diavolo had the longest flowering phase. Secle ALS and Gialet NES had the shortest flowering phase, 6 and 5 days, respectively. Fresh pods periods ranged from 6 (Secle ALS) to 55 days (Gialet SBF). Gialet and Secle SBF, and Posenati NES and SBF, fresh pods period was significantly longer. On the other hand, dry pods period ranged from 13 (Posenati NES) to 59 days (D'oro ALS). The total cycle length ranged from 96 (Secle SAF) to 134 days (D'oro ALS) (Table 3.3.).

3.2. Leaves and shoots

Accessions and treatments had a significant effect on shoots dry matter, number of leaves at 40, 55, and 100 days after sowing. The interaction between accession and treatment did not have a significant effect on these variables. Leaf temperature at 40, 55, and 100 DAS was not significantly affected by accession, treatment and accession and treatment interaction (Table 3.2.). NES and SBF plants had significantly greater shoots biomass. At 40 and 55 DAS, NES and SAF plants had a significantly higher number of leaves. At 100 DAS, SBF and NES had a significantly higher number of leaves (Table 3.4.). Secle and D'oro had a significantly greater shoots dry matter when compared to Fasolo del Diavolo. At 40 DAS, Secle and Fasolo del Diavolo had the highest amount of leaves. At 55 DAS, Secle had significantly more leaves than Posenati, D'oro, and Maron. At 100 DAS, D'oro and Maron number of leaves was significantly higher than Fasolo del Diavolo (Table 3.4.).

3.3. Yield components, yield, and yield indexes

Seeds' dimensions, yield, weight of 10 seeds, seeds per pod, and pods per plant were significantly affected by both, the accession and the treatment, and by their interaction, as well. The number of seeds per plant was not significantly affected by the interaction between accession and treatment. The percentage of plants producing seeds was only affected by the accession (Table 3.2.).

The number of pods per plant ranged from 1 (D'oro SAF) to 5 (Secle SBF). The number of seeds per pods ranged from 1 (D'oro SAF) to 6.7 (Gialet NES). Posenati NES and Secle SBF had the greatest weight of 10 seeds, 7.9 and 7.8 g, respectively, whereas Gialet ALS, SBF, and SAF had the lowest weight of 10 seeds, 3.8, 3.3, and 2.9 g, respectively. Seeds' length ranged from 9 (D'oro SAF) to 14.8 mm (Posenati SBF), width from 6.5 (D'oro NES and SBF) to 10.8 mm (Maron SBF), and thickness from 4.8 (D'oro ALS) to 9 mm (Maron SBF) (Table 3.5.).

Posenati SBF (12.6 g) and NES (12.3 g), and Secle SBF (12.0 g) yields were significantly greater than all ALS and SAF plants, unless Gialet SAF. Considering the effect of the treatment within each accession, Gialet was the only one that did not have a significant difference among treatments, was not significantly affected by treatments

under drought conditions (Fig. 3.3.). The yield reduction rate of Posenati, Secle, and D'oro SBF were positive. Secle SBF was significantly the greatest, 78%, whereas Maron ALS was the lowest, -100% (Fig. 3.4, (i)). Secle SBF was also the one with greatest drought tolerance index, 1.78, significantly greater than all other accessions under different drought conditions, except D'oro SBF (1.1) (Fig. 3.4. (ii)).

3.4. Soil nitrogen

Accessions significantly affected the final PNM and plant available nitrogen. Treatments had a significant effect on plant available nitrogen. The interaction between accessions and treatments did not have a significant effect on these variables (Table 3.2.). SAF plant available N (29.3 ppm) was significantly higher than ALS (23.5 ppm). Fasolo del Diavolo, Gialet, and Secle had significantly greater PNM than D'oro and Maron. The last two had significantly greater plant available nitrogen (Table 3.6). This section needs to be updated.

3.5. Seeds nutraceutical characteristics

The interaction between accession and treatments only had a significant effect on seeds' zinc content. Treatment had no significant effect on any of the variables and accessions had a significant effect on total phenols, antioxidant capacity, Ca, and Zn content (Table 3.2.).

D'oro (4,288 mg GAE kg⁻¹) and Maron (4,128 mg GAE kg⁻¹) had significantly greater total phenols content than Posenati and Secle. Gialet was the accession with the significantly lowest total phenols content 2,455 mg GAE Kg⁻¹. Maron had the greatest

antioxidant capacity, 4,545 mg Fe^{+2E} kg⁻¹ whereas Gialet had the lowest 2,505 mg Fe^{+2E} kg⁻¹. Gialet (1,280 mg kg⁻¹) and Posenati (1,118 mg kg⁻¹) calcium content were significantly greater than Secle (996 mg kg⁻¹) and D'oro (945 mg kg⁻¹) (Table 3.7.). Zn content ranged from 37.1 ppm (Posenati SBF) to 69.2 ppm (Maron NES) (Table 3.8.).

3.6. Principal component analysis of yield/plant and seed quality parameters

Considering the plants and seeds morphology, soil plant available nitrogen and PNM, yield, and yield components a principal component analysis on covariance was performed. Component 1 represents 49.5% of the variability and component 2 24.8%. Seeds per plant, pods per plant, yield, weight of 10 seeds, length of the fresh pods period, seeds' width, length, and thickness were positively related to the component 1 and negatively related to component 2. The length of the period of dry pods was negatively related to the component 1 and positively related to the component 2 (Fig. 5 (i)). Since fresh pods period length and yields were closely related and dry pods period length was inversely related to these parameters, linear regressions with yield x fresh pods periods and yield x dry pods periods were performed for each Gialet, Posenati, Secle, and D'oro, accessions.

Yield was proportional to the length of the period that the plant had fresh pods for Gialet ($R^2 = 0.0841$), Posenati ($R^2 = 0.7392$), Secle ($R^2 = 0.7745$), and D'oro (0.7876). Gialet had the lowest relationship and for each extra day, yield is increased by 0.0347 g per plant. Posenati's yield, however, is highly influenced by the length of the fresh pods period, increasing by 0.3324 g of dry seeds per plant each day. Yield was inversely proportional to the length of the period that the plant had dry pods. For each day of this

period, Gialet's yield was reduced by 0.0505 g ($R^2 = 0.1092$), Posenati's by 0.259 g per day ($R^2 = 0.232$), Secle's by 0.0841 ($R^2 = 0.0952$), and D'oro's by 0.0995 ($R^2 = 0.7823$). For Posenati, Secle, and D'oro, the length of the fresh pods period had a relatioship with yield stronger thant the length of the dry pods period (Fig. 6).

Considering the nutritional and nutraceutical parameters' relationship with the yield a principal component analysis on covariance was also performed. Component 1 represented 83.6% of the variability and component 2 15.7% of the variability. Antioxidant capacity is positively related to both components. Phosphorus content, total phenols and protein are positively related to the component 1, and negatively related to component 2. Calcium and yield are negatively related to component 1 and positively related to component 2. Zinc and magnesium are negatively related to both components (Fig. 5 (ii)).

4. DISCUSSION

Phenotypic plasticity is the ability of a plant, as an individual, to alter its morphology/physiology in response to different environmental conditions and the same plant has different responses to the same stress at different development stages (Schlichting, 1986). In this study, accessions had different responses to drought conditions applied on different growth stages. Drought escape is a phenological adaptation by early maturity that can increase plant growth rate and, normally, anticipates the flowering, "saving" water for the reproductive stages (Osakabe et al., 2014; Bodner et al., 2015). However, none of the landraces` flowering dates were not affected by drought conditions. Drought escape is also observed when bean plants are under water stress during reproductive stages, with early maturity and rapid mobilization of photosynthates to the seeds (Acosta-Díaz et al., 2004; Beebe et al., 2013b). Landraces under stress during the reproductive stage (ALS and SAF) tended to shorten the seed filling period, anticipating, and extending dry pod period, and shortening the plant's life cycle. In this case, drought escape had a negative effect on yield since length of dry pods periods were inversely related to yield (Fig. 7 (ii)). Gialet, Posenati and Secle's plants that received normal irrigation regime during the reproductive phase (NES and SBF) significantly extended the fresh pods periods and their life cycle lengths, when compared to plants under stress in the reproductive period (Table 3). The extension of fresh pods periods had a positive impact on yield (Fig. 7 (ii)).

Fasolo del Diavolo did not developed pods in this experiment and it can be explained by two reasons: i) *P. coccineus* has a high degree of allogamy, needing the presence of specific pollinators. We speculate that the rain-out shelter was not conducive to the presence of these pollinators or conditions within the rain-out shelter resulted in the reduced incidence of proper plant-pollinator contact (Giurcă, 2009); ii) *P. coccineus* tolerates temperatures up to 30°C and in this experiment the temperature inside the rain-out shelter were commonly greater than 35°C. Thus, to assess the effects of drought conditions on this accession, further research with an environment with cooler temperature would be recommended. This also indicates that the cultivation of this accession should be limited to the mountainous environment, where it originates, with milder temperatures throughout the growing period.

Polania et al. (2016) results showed that Mesoamerican accessions tend to be more tolerant to drought conditions. The superior performance under drought stress of these accessions was associated to a better canopy biomass at mid-pod filling, that can be related to a deeper root system and effective use of water, an efficient remobilization of photosynthates and grain filling. Dipp et al. (2017) results confirmed that Andean accessions have a higher susceptibility to drought conditions. In this study, Gialet and Posenati represented the Mesoamerican gene pool. Drought conditions did not have significant effects on Gialet's yield, YRR and DTI. Gialet was also the accession with lowest \mathbb{R}^2 to the correlation of fresh pods period (0.0841), showing that the phenological response to water stress had lower effect on yields, indicating that this accession drought resistance is more likely to be related to drought avoidance mechanisms (Beebe et al., 2013b), that slows plant growth, by closing stomata to reduce photosynthesis and cell metabolism, minimizing water loss (Shavrukov et al., 2017). Maron and D'oro, two of the Andean landraces had higher susceptibility to drought stress, however, with different responses. Maron ALS plants were more affected, whereas D'oro's SAF plant were more susceptible (Fig. 3 and Fig. 4). Dipp et al. (2017) found that highly susceptible accessions can have yields reduced by about 70% and they considered that accessions with yield reduction lower than 40% are tolerant to drought conditions. Smith et al. (2019) also found that drought conditions can significantly reduce yield by 70%. The yields of Gialet and Secle, for example, were reduced by 42 and 40%, respectively, under drought conditions during the whole cycle, indicating that these accessions can be tolerant to drought conditions.

Stressing the plant before flowering and applying a normal irrigation regime during the reproductive stage increased yield of Posenati and D'oro, however not significantly, and significantly increased by 80% Secle's yields. Imposing stress before flowering on beans cultivation is a tactic recommended by Italian bean landraces and commercial varieties (Albanesi; Giardinaggio; Orto). According to the Italian seed company Moon Garden in the label of commercial bean landraces seeds, irrigating before flowering stimulates the plant to produce more leaves and shoots dry matter, which can affect negatively the seed production (Moon-Garden, 2016). Accessions with high root plasticity and deep roots can also be positively affected by drought stress before flowering (Ho et al., 2005), stimulating roots to grow deeper and increasing the absorption area as a drought avoidance and tolerance mechanism (Beebe et al., 2013b; Polania et al., 2016b).

Seeds' physical and morphological characteristics and yield components were affected by the accessions and by the treatments. Determining seeds dimensions is important to facilitate the design of cleaning, sizing and grading machinery in case of commercial production (Fıratlıgil-Durmus et al., 2009). Seeds dimensions and weight of 10 seeds were also closely related to yield (Fig. 5). ALS and SAF treatments reduced by 10% the weight of 10 seeds, less than the results of Smith et al. (2019) that reduced by 25%. Darkwa et al. (2016) results for yield components agree with the results obtained in this study. Plants under drought stress tended to reduce the weight of 10 seeds and produce less pods per plant and less seeds per pods.

Smith et al. (2019) also evaluated the effect of drought conditions on the macro and micronutrients content on bean seeds. Drought conditions did not affect the content of phosphorus, calcium, zinc, and magnesium. Results obtained in this study concur with Smith et al. (2019), with some exceptions. In this study, accession and treatments interaction had a significant effect on seeds zinc content. Posenati and D'oro ALS seeds had significantly higher Zn content than SBF. Nicoletto et al. (2019) assessed the effect of two different environments (30 m and 351 m altitude) on seed qualities of two Venetian landraces, Gialet and Lingua di Fuoco, and their results showed that the environment significantly affected antioxidant capacity, total phenols, and calcium, magnesium and phosphate content on seeds. Although the growth environment affected these parameters, drought conditions did not have significant effects on seeds' nutraceutical characteristics. Accessions, in contrast, had a significant effect on seeds' nutraceutical characteristics. D'oro and Maron had significantly greater total phenols content and Maron also had greatest antioxidant capacity, whereas Gialet had significantly the least amount of total phenol content and antioxidant capacity among the accessions. Most of the antioxidants and total phenols of beans are located in the seed coat and seed color is related to high contents of these compounds (Díaz-Batalla et al., 2006a; Oroian and Escriche, 2015; Ombra et al., 2016), confirming the data obtained in this study, since Gialet seeds are yellowish and pale, whereas, D'oro and Maron have strong golden and brownish colors. Nicoletto et al. (2019) results for antioxidant capacity and total phenols of Gialet seeds were lower than the ones obtained in this study, however, as they also found, the environment can play an important role on the content of these compounds. These results are based on the weight of dry seeds. Previous studies have shown that soaking and cooking beans can reduce by 90% the total phenols content

and antioxidant capacity (Barroga et al., 1985; Rocha-Guzmán et al., 2007; Boateng et al., 2008).

Blair et al. (2009) considered a Mesoamerican accession with 42.8 ppm Zn content on the seeds as low content and an Andean accession with 66.7 ppm as high Zn content. This study obtained a similar range, from about 42.2 ppm (Posenati) to 59.3 and 59.9 ppm, Maron and Gialet, respectively. Paredes et al.(2010) assessed the macro and micronutrient content of 52 Chilean bean accessions that had magnesium content ranged from 1,300 to 1,800 ppm, about five times greater than the ones obtained in this study and Smith et al. (2019) obtained magnesium contents closer to the ones from this study, about 500 ppm. However, in terms of calcium content, this study results are closer to Paredes et al. (2010), which ranged from 1,000 to 2,100 ppm, whereas Smith et al. (2019) calcium content was about 500 ppm. Paredes et al. (2010) used an atomic absorption unit to determine Ca and Mg, as it was used in this study and Smith et al. (2019) used inductively coupled plasma (ICP).

5. CONCLUSIONS

Venetian landraces assessed in this study showed high diversity in terms of seeds visual traits, physical and nutraceutical characteristics. Drought conditions on different development stages did not affect nutraceutical characteristics of these seeds. Different irrigation regimes significantly affected these landraces phenology. Plants that were subjected to stress after flowering (SAF and ALS) had shorter fresh pods periods and longer the dry pods periods (when plant is filling the seeds). Principal component

analysis and linear regression showed that for these accessions yield was closely related to the length of the fresh pods period and inversely related to the length of dry pods period. Gialet was the only accession that was not significantly affect by any of the drought treatments. All other accessions had yield reduced when subjected to ALS (from 39% to 100%) and SAF (from 21% to 89%). Stressing plants before flowering significantly increase the yield of Secle's plants. Therefore, this study showed that among Venetian landraces, there are accessions interesting in a nutraceutical point of view, as Maron and D'oro, for example, or than can be used in biofortification breeding programs to increase the Zn, antioxidant capacity and total phenolic content. In addition to that, Gialet showed to be tolerant to drought conditions and stressing the plants before flowering can be a useful tool to help growers increasing the productivity when cultivating Secle.

- Acosta-Díaz, E., Trejo-López, C., Ruiz-Posadas, L. M., Padilla-Ramírez, J. S., and Acosta Gallegos, J. A. (2004). Adaptation of common bean to drought stress during the reproductive stage. *Terra Latinoam.* 22, 49–58. Available at: https://agris.fao.org/agris-search/search.do?recordID=US201300990780 [Accessed September 7, 2020].
- Albanesi Coltivazione dei fagioli Coltivazione dei borlotti Albanesi.it. Available at: https://www.albanesi.it/ambiente/orto/coltivazione-fagioli.htm [Accessed September 7, 2020].
- Barroga, C. F., Laurena, A. C., and Mendoza, E. M. T. (1985). Polyphenols in Mung Bean (Vigna radiata (L.) Wilczek): Determination and Removal. J. Agric. Food Chem. 33, 1006–1009. doi:10.1021/jf00065a056.
- Beebe, S. E., Rao, I. M., Blair, M. W., and Acosta-Gallegos, J. A. (2013). Phenotyping common beans for adaptation to drought. Front. Physiol. 4 MAR. doi:10.3389/fphys.2013.00035.
- Beebe, S. E., Rao, I. M., Cajiao, C., and Grajales, M. (2008). Selection for Drought Resistance in Common Bean Also Improves Yield in Phosphorus Limited and Favorable Environments. Crop Sci. 48, 582–592. doi:10.2135/cropsci2007.07.0404.
- Beebe, S., Rengifo, J., Gaitan, E., Duque, M. C., and Tohme, J. (2001). Diversity and Origin of Andean Landraces of Common Bean. Crop Sci. 41, 854-862. doi:10.2135/cropsci2001.413854x.
- Benzie, I. F. F., and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem. 239, 70-76. 128

doi:10.1006/abio.1996.0292.

- Bitocchi, E., Bellucci, E., Giardini, A., Rau, D., Rodriguez, M., Biagetti, E., et al. (2013).
 Molecular analysis of the parallel domestication of the common bean (Phaseolus vulgaris) in Mesoamerica and the Andes. *New Phytol.* 197, 300–313.
 doi:10.1111/j.1469-8137.2012.04377.x.
- Blair, M. W. (2013). Mineral biofortification strategies for food staples: The example of common bean. J. Agric. Food Chem. 61, 8287–8294. doi:10.1021/jf400774y.
- Blair, M. W., Astudillo, C., Grusak, M. A., Graham, R., and Beebe, S. E. (2009).
 Inheritance of seed iron and zinc concentrations in common bean (Phaseolus vulgaris L.). *Mol. Breed.* 23, 197–207. doi:10.1007/s11032-008-9225-z.
- Boateng, J., Verghese, M., Walker, L. T., and Ogutu, S. (2008). Effect of processing on antioxidant contents in selected dry beans (Phaseolus spp. L.). *LWT - Food Sci. Technol.* 41, 1541–1547. doi:10.1016/j.lwt.2007.11.025.
- Bodner, G., Nakhforoosh, A., and Kaul, H. P. (2015). Management of crop water under drought: a review. *Agron. Sustain. Dev.* 35, 401–442. doi:10.1007/s13593-015-0283-4.
- Calles, T. (2016). Preface to special issue on leguminous pulses. *Plant Cell, Tissue Organ Cult.* 127, 541–542. doi:10.1007/s11240-016-1146-7.
- Courty, P. E., Smith, P., Koegel, S., Redecker, D., and Wipf, D. (2015). Inorganic
 Nitrogen Uptake and Transport in Beneficial Plant Root-Microbe Interactions. *CRC*.
 Crit. Rev. Plant Sci. 34, 4–16. doi:10.1080/07352689.2014.897897.
- Dahal, S., Franklin, D., Subedi, A., Cabrera, M., Hancock, D., Mahmud, K., et al. (2020). Strategic Grazing in Beef-Pastures for Improved Soil Health and Reduced Runoff-

Nitrate-A Step towards Sustainability. *Sustainability* 12, 558. doi:10.3390/su12020558.

Darkwa, K., Ambachew, D., Mohammed, H., Asfaw, A., and Blair, M. W. (2016).
Evaluation of common bean (Phaseolus vulgaris L.) accessions for drought stress adaptation in Ethiopia. *Crop J.* 4, 367–376. doi:10.1016/j.cj.2016.06.007.

Decagon Devices Inc., P. W. Decagon Em50 data logger. Available at: http://publications.metergroup.com/Manuals/20452_Em50_Manual_Web.pdf [Accessed June 4, 2020].

Díaz-Batalla, L., Widholm, J. M., Fahey, G. C., Castaño-Tostado, E., and Paredes-López,
O. (2006). Chemical components with health implications in wild and cultivated
Mexican common bean seeds (Phaseolus vulgaris L.). *J. Agric. Food Chem.* 54, 2045–2052. doi:10.1021/jf0517061.

- Dipp, C. C., Marchese, J. A., Woyann, L. G., Bosse, M. A., Roman, M. H., Gobatto, D. R., et al. (2017). Drought stress tolerance in common bean: what about highly cultivated Brazilian accessions? *Euphytica* 213, 1–16. doi:10.1007/s10681-017-1893-5.
- Doane, T. A., and Horwáth, W. R. (2003). Spectrophotometric Determination of Nitrate with a Single Reagent. *Anal. Lett.* 36, 2713–2722. doi:10.1081/AL-120024647.

FAO (2009). How to Feed the World in 2050. Rome.

FAO (2016). Pulses contribute to food security. Rome Available at: http://www.fao.org/fileadmin/user_upload/pulses-2016/docs/factsheets/FoodSecurity_EN_PRINT.pdf [Accessed August 30, 2020].

FAOSTAT (2017). Beans, dry. Available at: http://www.fao.org/faostat/en/#data/QC

[Accessed January 7, 2020].

- FELLER, C. . H., BLEIHOLDER, L., BUHR, H., HACK, M., HESS, R., KLOSE, U., et al. (1995). "Phenological stages of development of vegetables: II. Fruit vegetables and legumes, cited by:," in *Growth stages of mono-and dicotyledonous plants BBCH Monograph Federal Biological Research Centre for Agriculture and Forestry* (2001), ed. U. Meier (BBCH working group), 141–144.
- Fischer, R. A., and Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. *Aust. J. Agric. Res.* 29, 897–912. doi:10.1071/AR9780897.
- Fischer Scientific Folin and Ciocalteu's Phenol Reagent, 2.0N, MP Biomedicals 500mL:Chemicals | Fisher Scientific. Available at: https://www.fishersci.com/shop/products/folin-ciocalteu-s-phenol-reagent-2-0n-mpbiomedicals-500ml/icn19518690 [Accessed September 5, 2020].
- Fıratlıgil-Durmus, E., Šárka, E., Bubník, Z., Schejbal, M., and Kadlec, P. (2009). Size properties of legume seeds of different varieties using image analysis. *J. Food Eng.* 99, 445–451. doi:10.1016/j.jfoodeng.2009.08.005.
- Giardinaggio Fagiolo Phaseolus vulgaris Orto Fagiolo Phaseolus vulgaris Orto. Available at: https://www.giardinaggio.it/orto/singoleorticole/fagiolo/fagiolo.asp [Accessed September 7, 2020].
- Giurcă, D. M. (2009). Morphological and phenological differences between the two species of the Phaselous genus (Phaseolus vulgaris and Phaseolus Coccineus). *Cercet. Agron. în Mold.* XLII.
- Gonçalves, J. G. R., de Andrade, E. R., da Silva, D. A., de Fátima Esteves, J. A., Chiorato, A. F., and Carbonell, S. A. M. (2019). Drought tolerance evaluated in
common bean accessions. *Cienc. e Agrotecnologia* 43, 1719. doi:10.1590/1413-7054201943001719.

Hjalmarsson, S., and Akesson, R. (1983). Modern Kjeldahl Procedure. Int. Lab. 3, 70-76.

- Ho, M. D., Rosas, J. C., Brown, K. M., and Lynch, J. P. (2005). Root architectural tradeoffs for water and phosphorus acquisition. *Funct. Plant Biol.* 32, 737. doi:10.1071/FP05043.
- Hti equipments HT 02 Thermal Imager (60×60) Hti. Available at: https://htiinstrument.com/products/ht-02-thermal-imager [Accessed June 4, 2020].
- Jimenez-Lopez, J. C., Singh, K. B., Clemente, A., Nelson, M. N., Ochatt, S., and Smith,
 P. M. C. (2020). Editorial: Legumes for Global Food Security. *Front. Plant Sci.* 11, 926. doi:10.3389/fpls.2020.00926.
- Jirka, A. M., Carter, M. J., May, D., and Fuller, F. D. (1976). Ultramicro Semiautomated Method for Simultaneous Determination of Total Phosphorus and Total Kjeldahl Nitrogen in Wastewaters. *Environ. Sci. Technol.* 10, 1038–1044. Available at: https://pubs.acs.org/sharingguidelines [Accessed September 8, 2020].
- Jones, A. (1999). PHASEOLUS BEAN: Post-harvest Operations. *FAO*, 1–25. Available at: http://www.fao.org/3/a-av015e.pdf [Accessed January 13, 2020].
- Katungi, E., Farrow, A., Chianu, J., Sperling, L., and Beebe, S. (2009). Common bean in Eastern and Southern Africa: a situation and outlook analysis. *Int. Cent. Trop. Agric.*, 1–61. Available at: http://www.icrisat.org/what-we-do/impi/projects/tl2-publications/regional-situation-outlook-reports/rso-common-bean-esa.pdf [Accessed January 8, 2020].
- Kempers, A. J., and Zweers, A. (1986). Ammonium determination in soil extracts by the 132

salicylate method. *Commun. Soil Sci. Plant Anal.* 17, 715–723. doi:10.1080/00103628609367745.

- Maynard, D. G., and Kalra, Y. P. (1993). "Soil Sampling and Methods of Analysis," in Soil Sampling and Methods of Analysis, ed. M. R. Carter (Boca Raton, FL, USA: CRC Press), 25–38. Available at: https://books.google.com/books?hl=en&lr=&id=54lYLsV49zIC&oi=fnd&pg=PA25 &dq=Nitrate+and+exchangeable+ammonium+nitrogen&ots=K3nO_CM_xS&sig=Q Xcc-DbNRzM1x9cFyN5fzhdQ_tc#v=onepage&q=Nitrate and exchangeable
- McClean, P. E., and Raatz, B. (2017). "Common Bean Genomes: Mining New

ammonium nitrogen&f=false [Accessed September 5, 2020].

Knowledge of a Major Societal Crop," in *The Common Bean Genome*, eds. M. P. de
la Vega, F. Marsolais, and M. Santalla (Gewebestrasse, Switzerland: Springer
International Printer), 295. doi:10.1007/978-3-319-63526-2.

Moon-Garden (2016). Irrigazione del Fagiolo. Seed Co. label.

Nicoletto, C., Zanin, G., Sambo, P., and Dalla Costa, L. (2019). Quality assessment of typical common bean accessions cultivated in temperate climate conditions and different growth locations. *Sci. Hortic. (Amsterdam)*. 256, 108599. doi:10.1016/j.scienta.2019.108599.

Ombra, M. N., D'acierno, A., Nazzaro, F., Riccardi, R., Spigno, P., Zaccardelli, M., et al. (2016). Phenolic Composition and Antioxidant and Antiproliferative Activities of the Extracts of Twelve Common Bean (Phaseolus vulgaris L.) Endemic Ecotypes of Southern Italy before and after Cooking. *Oxid. Med. Cell. Longev.* 2016, 1–12. doi:10.1155/2016/1398298.

- Oroian, M., and Escriche, I. (2015). Antioxidants: Characterization, natural sources, extraction and analysis. *Food Res. Int.* 74, 10–36. doi:10.1016/j.foodres.2015.04.018.
- Orto Quando irrigare le piante di fagioli. Available at: https://www.ortodacoltivare.it/domande/irrigare-fagioli.html [Accessed September 7, 2020].
- Osakabe, Y., Osakabe, K., Shinozaki, K., and Tran, L. S. P. (2014). Response of plants to water stress. *Front. Plant Sci.* 5, 86. doi:10.3389/fpls.2014.00086.
- Paredes, M., Becerra, V., and Tay, J. (2010). Inorganic Nutritional Composition of Common Bean (Phaseolus vulgaris L.) Accessions Race Chile. *Chil. J. Agric. Res.* 69, 486–495.
- Peix, A., Ramírez-Bahena, M. H., Velázquez, E., and Bedmar, E. J. (2015). Bacterial Associations with Legumes. CRC. Crit. Rev. Plant Sci. 34, 17–42. doi:10.1080/07352689.2014.897899.
- Picone, L. I., Cabrera, M. L., and Franzluebbers, A. J. (2002). A Rapid Method to Estimate Potentially Mineralizable Nitrogen in Soil. *Soil Sci. Soc. Am. J.* 66, 1843– 1847. doi:10.2136/sssaj2002.1843.
- Piergiovanni, A. R., and Lioi, L. (2010). Italian Common Bean Landraces: History, Genetic Diversity and Seed Quality. *Diversity* 2, 837–862. doi:10.3390/d2060837.
- Polania, J., Rao, I. M., Cajiao, C., Rivera, M., Raatz, B., and Beebe, S. (2016).
 Physiological traits associated with drought resistance in Andean and Mesoamerican accessions of common bean (Phaseolus vulgaris L.). *Euphytica* 210, 17–29. doi:10.1007/s10681-016-1691-5.

- Rocha-Guzmán, N. E., González-Laredo, R. F., Ibarra-Pérez, F. J., Nava-Berúmen, C. A., and Gallegos-Infante, J. A. (2007). Effect of pressure cooking on the antioxidant activity of extracts from three common bean (Phaseolus vulgaris L.) cultivars. *Food Chem.* 100, 31–35. doi:10.1016/j.foodchem.2005.09.005.
- Schlichting, C. D. (1986). The Evolution of Phenotypic Plasticity in Plants. Ann. Rev.
 Ecol. Syst 17, 693. Available at: www.annualreviews.org [Accessed September 7, 2020].
- Shavrukov, Y., Kurishbayev, A., Jatayev, S., Shvidchenko, V., Zotova, L., Koekemoer,F., et al. (2017). Early flowering as a drought escape mechanism in plants: How can it aid wheat production? *Front. Plant Sci.* 8. doi:10.3389/fpls.2017.01950.
- Singleton, V. L., Orthofer, R., and Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* 299, 152–178. doi:10.1016/S0076-6879(99)99017-1.
- Smith, M. R., Veneklaas, E., Polania, J., Rao, I. M., Beebe, S. E., and Merchant, A. (2019). Field drought conditions impact yield but not nutritional quality of the seed in common bean (Phaseolus vulgaris L.). *PLoS One* 14, e0217099. doi:10.1371/journal.pone.0217099.
- Soil Cube (2020). Soil³ Organic Matter and Humus Compost Soil Delivered. Available at: https://soil3.com/ [Accessed September 1, 2020].
- Spagnoletti Zeuli, P. L., Baser, N., Riluca, M., Laghetti, G., Logozzo, G., Masi, P., et al. (2004). Valorisation and certification of Italian bean agro-ecotypes (Phaseolus vulgaris) (in Italian). in *Ecotipi Vegetali Italiani: una preziosa risorsa di variabilità* genetica (Roma), 19–19.

- United Nations (2020). 2020 Desertification and Drought Day | UNCCD. Available at: https://www.unccd.int/actions17-june-desertification-and-drought-day/2020desertification-and-drought-day [Accessed August 30, 2020].
- Venora, G., Grillo, O., Ravalli, C., and Cremonini, R. (2009). Identification of Italian landraces of bean (Phaseolus vulgaris L.) using an image analysis system. *Sci. Hortic. (Amsterdam).* 121, 410–418. doi:https://doi.org/10.1016/j.scienta.2009.03.014.
- Zeven, A. C. (1997). The introduction of the common bean (Phaseolus vulgaris L.) into
 Western Europe and the phenotypic variation of dry beans collected in the
 Netherlands in 1946. *Euphytica* 94, 319–328. doi:10.1023/A:1002940220241.

TABLES

Table 3.1. Genetic similarity within and among populations of Fasolo del Diavolo (F.D.), Gialet (G), Posenati (P), Secle (S), D'oro (D), and Maron (M).

	F.D.	G	Р	S	D	М
F.D.	0.98					
G	0.80	0.97				
Р	0.75	0.8	0.99			
S	0.72	0.77	0.90	0.99		
D	0.7	0.74	0.85	0.81	0.99	
М	0.72	0.75	0.86	0.83	0.94	0.95

	Accessions	Treatments	Accessions x Treatments
	Phenology		
Vegetative phase	***	*	***
Flowering phase	***	***	***
Fresh pods period	***	***	***
Dry pods period	***	***	***
Total cycle	***	***	***
	Leaves and show	ots	
Shoots DM (g)	*	***	n.s.
LT 40 DAS	n.s.	n.s.	n.s.
LT 55 DAS	n.s.	n.s.	n.s.
LT 100 DAS	n.s.	n.s.	n.s.
N of leaves 40 DAS	***	***	n.s.
N of leaves 55 DAS	***	***	n.s.
N of leaves 100 DAS	***	**	n.s.
	Soil		
Potential nitrogen mineralization	*	n.s.	n.s.
Plant available nitrogen	**	**	n.s.
	Seeds' dimensi	ons	
Length (mm)	***	***	**
Width (mm)	*	***	**
Thickness (mm)	***	***	**
	Yield component	nts	
Pods per plant	*	***	*
Seeds per pod	***	*	**
Seeds per plant	**	***	n.s.
Weight of 10 seeds (g)	***	***	***
Yield (g per plant)	***	***	*
% of plants producing seeds	*	n.s.	n.s.
	Yield indexes-		
Yield reduction rate (%)	**	***	**
Drought tolerance index	**	***	**
Seeds:Biomass ratio	***	n.s.	n.s.
	Seeds' nutraceutical	properties	
Total phenols (mg GAE Kg ⁻¹ dw)	***	n.s.	n.s.
Antioxidant capacity (mg Fe ^{+2E} kg ⁻¹ dw)	**	n.s.	n.s.
Protein content (%)	n.s.	n.s.	n.s.
Total phosphorus content (mg kg ⁻¹)	n.s.	n.s.	n.s.
Mg content (mg kg ⁻¹)	n.s.	n.s.	n.s.
Ca content (mg kg ⁻¹)	***	ns	ns
Z_n content (mg kg ⁻¹)	***	11.0. D. C.	**
Zn content (mg kg ')	-11-	n.s.	ጥጥ

Table 3.2. Analysis of variance (ANOVA) table with the effects of accessions, treatments, and the interaction of accessions and treatments on this study's variables.

*** p < 0.001; ** p < 0.01; * p < 0.05; n.s. = non-significant effect (p > 0.05).

Table 3.3. Effects of accession and treatment interaction on the length of plants' development phases and total cycle. Treatments were 1) never stressed (NES), 2) always stressed (ALS), 3) stressed before flowering (SBF), and 4) stressed after flowering (SAF).

Accession	Treatment	Vegetative phase (days)	Flowering phase (days)	Fresh pods (days)	Dry pods (days)	Total cycle (days)
	NES	52 a	70 ab	-	-	122 cde
Fasolo del	ALS	50 a	74 a	-	-	124 bcde
Diavolo	SBF	52 a	73 a	-	-	125 abcde
	SAF	50 a	71 ab	-	-	121 de
	NES	50 a	5 f	22 efg	46 b	123 bcde
Cialat	ALS	49 a	10 def	17 fg	45 b	121 de
Glalet	SBF	40 bc	20 c	55 a	18 ef	133 ab
	SAF	51 a	10 def	16 g	49 b	126 abcd
	NES	40 bc	20 c	48 abc	13 f	121 de
Decenati	ALS	35 c	10 def	30 d	33 c	108 fg
rosenau	SBF	35 c	17 cd	51 ab	30 cd	133 ab
	SAF	35 c	14 cd	26 de	34 c	109 fg
	NES	35 c	13 cd	27 de	45 b	120 de
Sacla	ALS	40 bc	6 ef	6 h	53 ab	105 fgh
Secle	SBF	40 bc	15 cd	53 a	24 cdef	132 abc
	SAF	40 bc	15 cd	21 efg	20 def	96 h
	NES	50 a	10 def	40 c	33 c	133 ab
D'ara	ALS	45 ab	15 cd	20 efg	54 ab	134 a
D 010	SBF	45 ab	15 cd	40 c	15 f	115 ef
	SAF	40 bc	14 cd	20 efg	59 a	133 ab
	NES	45 ab	15 cd	40 c	22 def	122 cde
Moron	ALS	45 ab	15 cd	40 c	-	100 gh
Maron	SBF	45 ab	10 def	45 bc	27 cde	127 abcd
	SAF	45 ab	15 cd	40 c	21 def	121 de

Means followed by different letters indicate a significant difference between compared groups. n.s. = non-significant difference. $\alpha = 0.05$.

Table 3.4. Main effects of treatments and accessions on the shoots dry matter (DM), leaf temperature at 40 and 100 days after sowing, and on the number of leaves at 40, 55, and 100 days after sowing. Treatments were 1) never stressed (NES), 2) always stressed (ALS), 3) stressed before flowering (SBF), and 4) stressed after flowering (SAF).

	Shoots DM (g)	N of leaves 40DAS	N of leaves 55DAS	N of leaves 100DAS
	Treatm	ents		
NES	12.13 A	8.7 A	10.2 A	14.5 A
ALS	5.88 B	6.7 B	7.3 B	10.9 B
SBF	10.93 A	6.7 B	7.1 B	14.7 A
SAF	5.58 B	8.7 A	10.1 A	11.1 B
	Access	ions		
Fasolo del Diavolo	- 6.4 b	9.0 a	9.0 ab	10.5 b
Gialet	8.8 ab	7.4 b	9.3 ab	12.4 ab
Posenati	8.9 ab	6.3 b	7.4 b	12.5 ab
Secle	9.6 a	9.6 a	10.7 a	12.3 ab
D'oro	10.0 a	7.0 b	7.9 b	14.1 a
Maron	9.0 ab	6.8 b	8.1 b	13.3 a

Means followed by different uppercase letters indicate a significant effect of the treatment. Means followed by different lowercase letters indicate a significant effect of the accession. $\alpha = 0.05$.

Accession	Treatment	Pods per plant	Seeds per pod	Weight of 10 seeds (g)	Length (mm)	Width (mm)	Thickness (mm)
	NES	3.3 abc	6.7 a	4.1 fgh	12.5 abcde	7.8 bcde	6.3 cde
alet	ALS	2.7 abc	5.0 bc	3.8 ghi	10.3 ef	7.3 cde	5.0 ef
Gi	SBF	4.3 abc	5.2 ab	3.3 hi	11.8 bcdef	8.0 bcde	6.3 cde
	SAF	4.0 abc	5.4 ab	2.9 i	11.3 cdef	7.8 bcde	5.5 def
·=	NES	4.3 abc	3.6 bcde	7.9 a	13.3 abc	8.5 bcde	5.5 def
enat	ALS	2.0 c	4.1 bcd	6.9 bc	13.3 abc	7.8 bcde	5.8 def
OS6	SBF	4.5 ab	3.9 bcd	7.1 ab	14.8 a	8.0 bcde	6.8 bcd
Н	SAF	2.3 bc	3.4 cde	6.2 cd	12.8 abcde	8.3 bcde	6.3 cde
	NES	4.0 abc	2.4 def	7.2 ab	12.8 abcde	9.0 abc	6.8 bcd
cle	ALS	2.0 c	3.4 cde	6.1 cd	12.5 abcde	7.5 cde	6.8 bc
Se	SBF	5.0 a	3.1 def	7.8 a	13.0 abcd	9.8 ab	7.3 bc
	SAF	2.0 c	3.8 bcde	5.3 de	12.5 abcde	8.8 abcd	6.8 bcd
~	NES	2.3 bc	4.1 bcd	4.9 ef	9.8 f	6.5 e	5.3 ef
orc	ALS	2.0 c	2.5 def	5.1 def	9.8 f	6.8 de	4.8 f
D,	SBF	3.5 abc	3.0 def	5.0 def	13.3 abc	6.5 e	6.0 cdef
	SAF	1.0 c	1.0 f	4.9 ef	9.0 f	7.0 cde	7.0 bc
	NES	3.0 abc	3.7 bcde	7.0 ab	12.0 bcdef	7.8 bcde	6.8 bcd
ron	ALS	-	-	-	-	-	-
Ma	SBF	2.7 abc	3.3 def	7.2 ab	15.0 a	10.8 a	9.0 a
	SAF	3.0 abc	3.0 def	6.8 bc	14.3 ab	9.8 ab	8.0 ab

Table 3.5. Effects of accession and treatments interaction on yield components and seeds' physical characteristics. Treatments were 1) never stressed (NES), 2) always stressed (ALS), 3) stressed before flowering (SBF), and 4) stressed after flowering (SAF).

Means followed by different letters suggest a significant difference between compared groups. $\alpha = 0.05$.

Table 3.6. Effects of treatments and accessions on the soil potential nitrogen mineralization and plant available nitrogen. Treatments were 1) never stressed (NES), 2) always stressed (ALS), 3) stressed before flowering (SBF), and 4) stressed after flowering (SAF).

	PNM (ppm)	Plant available N (ppm)
	Treatments	
NES	20.8 n.s.	27.4 AB
ALS	21.9 n.s.	23.5 B
SBF	17.9 n.s.	27.1 AB
SAF	22.2 n.s.	29.3 A
	Accessions	
Fasolo del Diavolo	23.2 a	22.5 с
Gialet	23.1 a	26.6 b
Posenati	21.0 ab	25.9 b
Secle	23.3 a	25.8 b
D'oro	18.1 b	31.0 a
Maron	15.7 b	29.9 a

Means followed by different uppercase letters suggest a significant effect of treatment. Means followed by different lowercase letters suggest a significant effect of the accession n.s. = non-significant difference. $\alpha = 0.05$.

	Total phenols (mg GAE Kg ⁻¹ dw)	Antioxidant capacity (mg Fe ^{+2E} kg ⁻¹ dw)	Protein content (% dw) ^{n.s.}	Total phosphorus content (mg kg ⁻¹ dw) ^{n.s.}	Mg content (mg kg ⁻¹ dw) ^{n.s.}	Ca content (mg kg ⁻¹ dw)
Gialet	2455 с	2505 с	30.9	1395	297.0	1280 a
Posenati	3368 b	3718 b	30.3	1386	287.8	1118 a
Secle	3326 b	3256 bc	28.2	1439	292.7	996 b
D'oro	4288 a	3736 b	32.3	1422	296.3	945 b
Maron	4128 a	4545 a	27.1	1379	281.3	1081 ab

 Table 3.7. Effects of accessions on seeds' nutraceutical characteristics.

Means followed by different letters suggest a significant difference between compared accessions. n.s. = non-significant difference. $\alpha = 0.05$.

Accession	Treatment	Zn content (mg kg ⁻¹)
	NES	58.4 abcd
ulet	ALS	56.8 abcde
Ę	SBF	61.8 abc
	SAF	65.6 ab
	NES	43.9 efg
anal	ALS	47.4 defg
OSE	SBF	37.1 g
Ч	SAF	38.7 fg
	NES	47.9 cdefg
cle	ALS	49.2 bcdefg
Še	SBF	58.3 abcd
	SAF	50.9 bcdefg
0	NES	49.4 bcdefg
ore	ALS	56.2 abcdef
Ó	SBF	41.2 fg
-	SAF	37.5 fg
_	NES	69.2 a
ron	ALS	-
Ma	SBF	58.3 abcd
	SAF	51.4 abcdefg

Table 3.8. Effects of accession and treatment interaction on seeds' zinc content. Treatments were 1) never stressed (NES), 2) always stressed (ALS), 3) stressed before flowering (SBF), and 4) stressed after flowering (SAF).

Means followed by different letters suggest a significant difference between compared groups. $\alpha = 0.05$.

FIGURES



Fig. 3.1. Seeds of landraces used in this study, showing the high diversity of visual traits and potential for commercial interests.



Fig. 3.2. Hourly average of liters of water per pot in each treatment before flowering (black, BF) and after flowering (grey, AF). Field capacity (FC) is represented by the dash-dot line and permanent wilting point is represented by the solid line. Plant available water is the difference between the volume of water in the pot and the permanent wilting point. Treatments were 1) never stressed (NES), 2) always stressed (ALS), 3) stressed before flowering (SBF), and 4) stressed after flowering (SAF).



Fig. 3.3. Effects of accession and treatments interaction on yield (g of dry seeds per plant). Different letters indicate a significant difference between compared groups. $\alpha = 0.05$. Treatments were 1) never stressed (NES), 2) always stressed (ALS), 3) stressed before flowering (SBF), and 4) stressed after flowering (SAF).



Fig. 3.4. Effects of accession and treatment interaction on yield reduction rate (YRR) on top (i) and on drought tolerance index (DTI) on the bottom (ii). Different letters suggest a significant difference between compared groups. $\alpha = 0.05$. Treatments were 1) never stressed (NES), 2) always stressed (ALS), 3) stressed before flowering (SBF), and 4) stressed after flowering (SAF).



Fig. 3.5. Principal component analysis (PCA) of parameters related to yield and seeds physical characteristics (i) and to seeds' nutraceutical and nutritional characteristics (ii).



Fig. 3.6. Regression equations (on top) showing the relationship between yield and length of fresh pods period (i), and length of dry pods period (ii).

CHAPTER 4: FINAL REMARKS

For centuries, these landraces were cultivated in a very close distance, however, isolated by geographic or cultural barriers. Each landrace was a result genetic resources, cultural uses, and environmental conditions. These landraces created a strong connection to a specific city or microregion. This is evidenced as some of these landraces, for example, Giálet Bellunese, Fagiolo di Lamon della Vallata Bellunese, D'oro di Val Fiemme, Mamme Bianche di Bassano, are cultivated in specific Veneto microregions and cultivation of the beans landraces became an important social-cultural and economic activity during the summer, mainly in the mountains.

In the last decades, as transportation and connectivity technologies increased, these geographical barriers began to decrease. Consequently, local farmers and/or bean growers are having access to commercial cultivars that can achieve higher yields and have a more economical interest. This is only one of many reasons some of these landraces are under threat and cultivation is declining. This has already happened in other regions around the world with beans and other crops. The Valebio project at the University of Padova has the main objective of conserving this precious and diverse genetic material.

In addition to 21 pre-commercial Italian accessions supplied by the seed company Blumen S.A., 27 landraces were collected on small farms or at seed exchange fairs. The Valebio project performed the agronomic, nutraceutical, morphological, and genetic characterization of this material. This characterization further exposed the genetic and phenotypic diversity of these accessions. The cultivation of these landraces in the 'pianura' (sea level) reduced yields by more than half, demonstrating that besides that cultural connection, these Veneto landraces are highly adapted to the mountainous environment. The genetic characterization of these landraces confirmed all these agronomic, morphological, and nutraceutical diversities. Veneto beans came from the two domestication centers of *P. vulgaris*. Besides, very little hybridization has occurred in this region over the centuries, unlike other European regions. This demonstrates how local farmers have kept this material in-situ for centuries.

Regarding the morphology of plants and seeds, only two characteristics are shared among all landraces: climbing and indeterminate growth. However, the diversity is clear when taking into account the colors of the flowers, the shapes of the leaves, the design of the plants, the sizes and shapes of the seeds, the colors of the seeds, the shape and length of the pods. Each landrace has its own morphological characteristics. These accessions are also diverse with their nutraceutical characteristics. Differences in protein content, starch, total phenols, antioxidant capacity, and amino acid profile, make each accession have its own nutraceutical composition and even when cultivated in an environment warmer and with less fertile soil, there was no difference in its nutraceutical properties. Therefore, the characterization and description of these accessions that will be converted into the germplasm of the University of Padova are extremely important for the possible availability of genetic materials for breeding programs in the future.

One of the biggest challenges of agriculture is to increase food production and nutritional quality to provide enough food to the growing world population, in the same area, however, adapting to increasingly warmer air temperatures and fluctuating moisture conditions due to climate change. Currently, mostly of the beans cultivated worldwide are at risk to being exposed to drought conditions during its cultivation cycle. In these same areas, for example, eastern Africa, people are suffering of micronutrients deficiency, like zinc. This situation tends to get worse, since desertification and degradation are expected to expand affected areas. Unlike chapter two, which focused on unraveling the connection of these accessions to a specific region in Italy and conserving this local genetic material, chapter three had a more comprehensive objective, aiming to address these food security issues previously mentioned.

Gialet is a typical Veneto accession, was found to be tolerant to drought conditions, and was not significantly affected by reduced irrigation during the whole cycle, before or after flowering and it also had a high content of zinc (59.9 ppm) and calcium (1,280 ppm). Thus, this is a accession that has a potential to be explored by breeding programs, not only to increase drought tolerance, but also for mineral biofortification strategies. D'oro and Maron, besides great agronomic performance in the mountains on Chapter 2, also had high antioxidant capacity and total phenols content, which can also be explored by breeding programs. On Chapter 3, Posenati showed best agronomic performance, even under stress, although it was reduced by about 50% when compared non stressed plants. In the context of Venetian mountains, Chapter 3 also brought results that are interesting for growers of Secle. Inducing stress before flowering significantly increased final yields by 80%, thus, with this information, farmers can reduce or eliminate irrigation of Secle's plant until flowering in order to increase its seed production, and not affect its nutraceutical properties. The conservation of genetic resources and diversity is essential for sustainable agricultural development. In Veneto, only one of 20 Italian regions, an enormous variety of bean accessions was found and the germplasm that DAFNAE is establishing will play a very important role in that. In a short time, many important results have already been obtained for local producers and breeding agencies around the world. However, this germplasm still has the potential to be studied and explored further.