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Review

Italian Common Bean Landraces: History, Genetic Diversity and Seed Quality

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Abstract: The long tradition of common bean cultivation in Italy has allowed the evolution of many landraces adapted to restricted areas. Nowadays, in response to market demands, old landraces are gradually being replaced by improved cultivars. However, landraces still survive in marginal areas of several Italian regions. Most of them appear severely endangered with risk of extinction due to the advanced age of the farmers and the socio-cultural context where they are cultivated. The present contribution is an overview of the state of the art about the knowledge of Italian common bean germplasm, describing the most important and recent progresses made in its characterization, including genetic diversity and nutritional aspects.

Keywords: genetic resources; germplasm; molecular markers; *Phaseolus vulgaris*; phaseolin

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1. Domestication and Dissemination Pathways of Common Bean

Over a time period of at least 7000 years, the common bean (*Phaseolus vulgaris* L.) has evolved from wild growing into a major leguminous crop. Before domestication, wild *P. vulgaris* had already diverged into two major gene pools, each with a proper geographical distribution. Domestication of wild common beans occurred independently in Mesoamerica and Andean South America [1] and gave rise to two major gene pools also within the cultivated forms [2]. Evidence supporting common bean organization into two major domesticated gene pools came from studies made on morphological, agronomical, and adaptation traits [3-5], phaseolin type [6-8], isozymes [9,10], and molecular markers [11]. Cultivars from Mesoamerica usually are small- or medium-seeded (<25 g or 25–40 g/100 seed weight, respectively) and have S phaseolin type. The South American counterparts have larger seeds (>40 g/100 seed weight) with T, C, H, and A phaseolin patterns [4,6]. Some limited germplasm exchange took place between the two gene pools in pre-Columbian age, while much more extensive seed flow occurred after the 1500s. Despite their partial reproductive isolation, the two gene pools still belong to the same biological species. Although viable and fertile progeny can be obtained and gene transfer could be attempted, still today breeding programs have only partially comprised systematic combinations of a wide range of genotypes from each gene pool [12,13].

The discovery of the Americas triggered a rapid exchange of crops between the Old and New World. The pathways of beans dissemination in Europe is still unclear and currently under discussion, since the initial input of common bean in Europe is largely unrecorded. It is likely that sailors and traders brought the nicely colored and easily transportable bean seeds already from the first trips towards the Americas in the 16th and 17th centuries. The initial common bean accessions were introduced probably in Europe from Mesoamerica, since Columbus arrived in Central America in 1492 and Cortes reached Mexico in 1518, while Pizarro, exploring Peru in 1528, gave the chance to introduce common bean from the Andes. The first European explorers certainly devoted great interest towards this species. For example, Gonzalo Fernandez de Oviedo, who explored Panama and Nicaragua in 1530, included in its travel reports detailed information on common bean cultivation techniques used by the American natives [14].

There are strong evidences that common bean reached France already in 1508, probably without value for human consumption at that time [15]. The first description of common bean in European herbals was done by Fuchs (1542–1543), who reported that the common bean had climbing habit, white or red flowers and red, white, yellow, skin-colored or liver-colored seeds with or without spots. However, it cannot be excluded that Fuchs reported a combination of traits belonging to both *P. vulgaris* and *Phaseolus coccineus* L. species. Further descriptions were done by Roesslin in 1550, by Oellinger in 1553 and by Dodonaeus in 1554 [15]. Fuchs and Dodonaeus only referred information about the climbing habit of the bean plant. A punctual selection of old manuscripts (1493–1774) mentioning *P. vulgaris* or its synonyms was recently reported by Krell and Hammer [16]. Since the Mesoamerican biotypes are not very frequent in Europe, McClean *et al*. [17] supposed that the germplasm dispersed into Europe was predominantly of Andean origin. However, after the introduction, a natural selection took place within bean germplasm for tolerance to long days, disease and pest resistance, stress tolerance and ability to survive, combined with a man-drive selection for plant habit, seed color, seed pattern type and also disease and pest resistance [15].

2. Common Bean Introduction in Italy

As for other European countries, common beans arrived in Italy mainly unregistered. Bean pictures are present in festoons adorning the myth of Psyche decorating Villa Farnesina in Rome, painted by Giovanni di Udine in 1515 [18]. Mentions in historical documents fixed 1532 as the year of common bean introduction in Italy. At that time, the humanist and literate Pierio Valeriano received a bag of bean seeds like compensation for work at the court of Pope Clemente VII. This last greatly supported the diffusion in the Italian peninsula of this new crop that he received from the Spanish Emperor Charles V [14]. Valeriano sowed the precious seeds in its fields located in Belluno province (Veneto region, Northeastern Italy). Subsequently, Valeriano described in a short poem in Latin entitled '*De Milacis Cultura*', the cultivation technique, the plant and seed morphology, and the supposed therapeutic properties of bean seeds [14]. The diffusion of common bean in Veneto region occurred quickly and still today the cultivation of this pulse has a great economic relevance in Belluno province.

The interest for common bean by literates living in Northeastern Italy may be perceived by the presence of citations regarding this pulse in some books that were published in Venice at the end of 16th century. In 1565, the physician and botanist Pietro Andrea Mattioli included in a translation and commentary of the works of the Greek botanist Dioscorides the description of common bean (Figure 1), together with those of other plants brought from explorations of the new lands in progress at that age [14].

Figure 1. Picture of common bean plant (called 'Fagiuoli') from *Commentarii in Sex Libros Pedacii dioscorides* of Pier Andrea Mattioli (1501–1577).

The physician Baldassarre Pisanelli in his 'Treatise of the nature of food and drinks' published in 1583 estimated that common beans were "much worse than faves, but among them the red are the best". Teofilo Folengo, a monk and poet of noble family, also reported some features of common bean in a treatise dealing with food published in 1562 [14].

As we go through the list of popular 16th century New World foods in the Old World, it became obvious why some crops throve and others struggled. Italians, as well as Europeans, did not recognize common bean as a foreign species. They thought that this plant was just a new variety of a crop that they had used to grow and eat since a very long time, the cowpea (*Vigna unguiculata* (L.) Walp.). The only ones to really notice the differences of New World beans were botanists, but the confusion between cowpea and common bean was usual in 16th and 17th centuries. It is noteworthy that in Italy the substitution of cowpea with common bean took place gradually over the time so that certainly the two species coexisted for a long period of time. Cultivation of cowpea landraces is yet practiced only in restricted areas of Central and Southern Italy [19,20].

An important aspect regards the introduction of common beans in the Italian consumer diet. Elite cookbooks published between the 16th and 17th centuries were reluctant to offer common bean recipes, which were known to be a sign of poverty and rustic living, as mentioned by the Florentine Luigi Alamanni in an ode to agriculture printed in 1546 [14]. Only in the early 18th century a recipe, which used dry bean, was reported in a cookbook by the Jesuit priest Francesco Gaudenzio [18]. Although in the 17th century, chickpea and broad bean were the pulses predominant in the diet, at the end of 18th century the main source of vegetable proteins for a large part of the population was represented by common bean [21]. For this reason, it was frequently defined the meat of paupers.

3. Italian Landraces and Safeguard Actions

The selective pressure operated by Italian farmers over the time on the pool of common bean accessions introduced in Italy produced a myriad of landraces. Adaptation to the soil type and climatic conditions of the new environments, the geographical isolation of several growing areas, the peculiar agro-technique (*i.e.*, the consociation with maize), aesthetical and organoleptic preferences have provided the prerequisites for the selection toward a large number of landraces. This process resulted in the establishment of different groups of landraces in each Italian region, though it is not unusual that some landraces are associated to sub-regional areas such as some close villages, a valley, or a small island. Generally, because of particular thermal, humidity and edaphic requirements, each landrace or group of landraces cannot be grown with success in areas different from that where they are traditionally cultivated. At the beginning of 20th century, Comes [22] published a book describing 472 Italian common beans. Successive publications confirmed the high degree of diversity associated to the most important traits both of plant and seed among Italian populations and attempted their classification [23,24]. A very small collection representative of common bean diversity available in Italy at the beginning of 20th century is presently stored at Vavilov Research Institute of Plant Industry (VIR), the Russian gene bank. Vavilov collected a number of samples belonging to several species during his trip in Italy (1924–1926), including 12 common bean varieties (Table 1).

Nowadays, common bean is widely cultivated in intensive agricultural systems and commercialized mainly as green pod and snap seed. In this frame, new cultivars completely displaced the old landraces. However, in the areas where traditional or low input agricultural systems are still present, Italian farmers still grow autochthonous varieties not only for personal consumption, but also for sale as niche products or specialties in farmer markets. This means that despite the lack of coordinated efforts, farmers have *de facto* practiced the *on farm* maintenance of this germplasm [19,25].

Table 1. List and brief description of Italian common beans collected by Vavilov and held at Vavilov Research Institute of Plant Industry (VIR).

Code	Collection year	Local name	Donor	Plant and seed description
VIR 2096	1924	Fagiolo Burro Rampicante	Ingegnoli, Milan	climbing bean; very late maturity; sphaericus x ellipticus; seeds large, dark-violet to black
VIR 2279	1925	Raparino Nano	Instituto Superiore Abburo, Bologna	bush bean; very late maturity; green pod; ellipticus; seeds pale reddish-brown with red stippling
VIR 2284	1925	Raparino Gigante	Instituto Superiore Abburo Bologna	semi-climbing bean; late maturity; ellipticus; seeds pale reddish-brown with red stippling
VIR 3559	1926	Fagiolo Metis	Ingegnoli, Rome	bush bean; middle maturity; flowers white; seeds bicolored: half is white and half black
VIR 3560	1926	Fagiolo Cento per uno	Ingegnoli, Rome	bush bean; middle maturity; pods flat; seeds small, pale brown
VIR 3562	1926	Regino Nano	Ingegnoli, Rome	late maturity; plant with curly top; pods green with striped purple; seeds pale reddish-brown with red stippling
VIR 3563	1926	Fagiolo	Orvieto, Umbria	bush bean; late maturity; flowers pale pink; green pod; seeds buff
VIR 3564	1926	No name	Orvieto, Umbria	semi-climbing bean; late maturity; flowers white; seeds small, white
VIR 3565	1926	No name	Orvieto, Umbria	semi-climbing bean; late maturity; flowers white; green pods; seeds slightly flattened, white
VIR 5009	1926	Fagioli scritti	Lucca, Tuscany	bush bean; late maturity; flowers pale pink; pods green and flat; seeds pale reddish-brown with pink stippling; productivity medium
VIR 5010	1926	Fagioli rossi scritti	Lucca, Tuscany	bush bean; late maturity; flowers pink; green pod; seeds reddish-brown with purple stippling; low productivity
VIR 5105	1926	Fagiolo Bianco comune	unknown	semi-climbing bean; very late maturity; flowers pink; seeds flat, white

Historical information dealing with cultivation area, description of agro-technique, traditional recipes, *etc*., are available only for some landraces. For example, it is proved that already in the 18th century, the garden pea cultivation was completely replaced by the common bean in the neighborhood fields of Lamon village, Belluno province (Figure 2). Bellati [26] reported how common bean had a relevant economic role for farmers of this province in the 19th century due to a commercialization of a large portion of annual harvest in neighboring regions.

Nowadays, common bean cultivation represents a lucrative activity for Lamon farmers and four appreciated landraces, generically named 'common bean from Lamon' are cultivated. It should be interesting to underline that the common names of three of these varieties probably relate with the beginning of common bean cultivation at Lamon. In fact, the names 'Spagnol', 'Spagnolet' and 'Spagnolon' are alterations of the Italian word 'spagnolo' (Spanish) and this circumstance recalls Valeriano's work, as previously mentioned. The high appreciation by consumers of these borlotto types (striped red seeds and striped pods), was recognized by the attribution of the Protected Geographical Indication (PGI) (GUCE 163/96 2 July 1996), one of the European Union marks attributable to traditional foods (E.C. reg. n. 2081/92 and 2082/92).

Figure 2. Map of Italy with provenance of some common bean landraces. (a) Piedmont; (b) Veneto; (c) Tuscany; (d) Marche; (e) Lazio; (f) Campania; (g) Basilicata; (h) Sardinia; (i) Sicily. Arrows indicate the landrace cultivation area.

Another landrace that has certainly been cultivated for a very long time is the common bean from Gradoli, (Figure 2) also named 'Fagiolo del Purgatorio', literally bean of Purgatory. According to historical sources, this white small-seeded common bean has been cultivated near the Lake of Bolsena since 17th century [27]. At that times, the brotherhood named 'Confraternita del Purgatorio' involved in the assistance of paupers, began the tradition to organize a lunch every year during Lent reserved for poor persons, consisting of dishes prepared with local common bean. The economic value attributed in the past centuries to this common bean is ascertained by the practice to reward the clergymen who celebrated religious rituals with bean seeds. Although the Lenten tradition is steadfastly maintained every Ash Wednesday, the 'Fagiolo del Purgatorio' is scarcely requested by consumers and consequently fated to extinction in a very short time.

Conversely, 'Billò' common bean is an appreciated landrace with a relatively short tradition of cultivation. Oral information collected by interview of elderly farmers indicated that the cultivation of this bean in Cuneo province, Piedmont region (Figure 2), started about 60 years ago. At that time, seeds from Lamon were introduced by some farmers in the area presently devoted to Billò cultivation. A recent study based on molecular markers has evidenced that several decades of cultivation in two different environments without exchange of material is producing a differentiation between the original material grown at Lamon and that collected in Cuneo province [28].

A detailed analysis of the recent scientific literature shown that *on farm* survival of local common bean varieties is a relevant phenomenon in Italy [28-34]. These findings assume a particular importance in the areas located along the Apennine ridge and in several small islands where agricultural systems still retain traditional forms. Unfortunately, the cultivation of these old varieties is generally practiced by elder farmers without any action of sustain by governmental or local institutions. These circumstances cannot assure the survival of this precious germplasm, so that a large fraction of common bean landraces is severely endangered with the risk of extinction. Recent European Community regulations introducing the possibility to attribute marks of origin and quality, PGI and PDO (Protected Designation of Origin), to local typical products, could give an important support to *on farm* survival of élite common bean landraces, but at same time, the abandonment of the less appreciated ones could be encouraged [35]. This discrepancy can be overcome through the application of both *on farm* and *ex situ* conservation to the same germplasm. Presently, only three PGI marks have been assigned to Italian common bean; they are 'Fagioli di Lamon', 'Fagioli di Sarconi' and 'Sorana' landraces.

As concerns the *ex situ* conservation of Italian germplasm, collections of about 1,500 accessions are held in the CNR, Institute of Plant Genetics (IGV, Italy) and the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK, Germany), while several small collections, consisting of at least 100 accessions, are maintained by some Italian Universities such as those of Turin, Perugia, Ancona, Basilicata, Viterbo, and Sassari. Moreover, the increasing attention towards the safeguard of biodiversity has encouraged some regions to create their own facilities for the *ex situ* conservation such as those created by the Tuscany and Friuli regions [36]. Nowadays, a very small fraction of Italian landraces is held in the regional gene banks. The knowledge about the levels of diversity present in these collections needs a robust scientific work with the aim of cataloguing and characterizing the stored material. These efforts constitute an important goal in prospecting adequate strategies for *ex situ* conservation management as well as for the utilization of this precious germplasm in breeding programs. At the same time, for *on farm* conservation, which can be seen as one of the most effective strategies applied to the safeguard of crop genetic resources, knowledge about the level of diversity within each landrace is fundamental in order to plan the most appropriate program for their survival.

Still today no systematic cataloguing of all common bean landraces cultivated in the Italian peninsula has been attempted. In the absence of appropriate financial resources and coordinate initiatives, only sporadic efforts have been tried. For example, some regional agricultural development agencies have published catalogues describing the common bean landraces grown in the own region. This is the case of the Agricultural Research Service of the Regions: Basilicata (ALSIA), Abruzzo (ARSSA), and Calabria (ARSSA) [37-39]. The description of some local common beans has been included in a number of publications on crop biodiversity at regional level edited by Friuli region [40] and Park of Dolomiti in Veneto region [41]. Web catalogues have been created within the frame of regional projects devoted to rural area development by the Department of Environmental Biotechnologies, University of Perugia and Agricultural Research Service of Lazio region (ARSIAL).

4. Characterization and Evaluation of Italian Common Bean Landraces

4.1. Phenotypic Variation

In spite of increasing use of DNA-based markers in investigating genetic diversity within germplasm collections, the evaluation of phenotypic variation is still crucial in determining adaptation, agronomic potential and breeding value of landraces.

A considerable diversity among the genotypes grown by Italian farmers has been observed in seed morphological traits: shape, size, coat color, and type and color of pattern. The first description of seed shape variation within Italian germplasm was that attempted by Comes [22] who analyzed 472 populations and grouped them into four classes: (1) kidney; (2) cylindrical; (3) elliptic; (4) round. Moreover, Comes [22] described a wide range of coat color from white to violet, in addition to a lot of bicolored, spotted and striped types using a visual inspection. It is surprising to observe that these classifications greatly agreed with the seed descriptors included in the official common bean descriptors released by IBPGR in 1982 [42]. More recently, a computer-aided image analysis system has been specifically designed for the characterization and discrimination of Italian common bean landraces by phenotypic traits [43]. The automatic acquisition of parameters such as size, shape, color, and texture of seeds gives scientists a reliable and quick tool for the identification of landraces (see also an extensive review by Dell'Aquila [44]). A survey of the scientific literature dealing with Italian landraces has evidenced that a large part of phenotypic variation described by Comes [22] has survived until now [30,34,37,45-51]. However, as a consequence of different aesthetical preferences of local farmers, the coat color variation is not homogeneously distributed in the Italian peninsula.

As shown in Table 2, white coat seed populations and borlotto types are present in all regions, while unusual coat types are associated to restricted areas indicating the prominent role played by aesthetical preferences of local farmers in the choice of the own set of local populations. For instance, black coat types are cultivated only in Central Italy and are named 'Marconi al palo', 'Stortino di Lucca' and 'Mangiatutto nano' grown in Lazio, Tuscany and Marche region, respectively (Figure 2). Two groups of landraces with yellow coat are described: the bush types named 'Zolfino' and 'Solfarino' cultivated in Tuscany and Lazio region, respectively [52,53], and the climbing ones named 'Gialet', and 'Cesarin' and 'Centut' grown in Veneto and Friuli region, respectively [54-56]. Presently, a study is in progress to ascertain if 'Gialet' and 'Cesarin' can be originated from the same genetic stock (F. Miceli Pers. Comm.). In contrast with the high phenotypic variation of seed traits among the populations, the literature reports a very low phenotypic variation at intra-population level. This trend is attributable to the custom of farmers to preserve only a few plants for each population to obtain seeds for the next season.

In regard to plant habit, a wide variation from bushy to aggressive climbing ability has been observed within Italian common bean landraces, though climbing types are widely predominant. It is known that the predominance of one growth habit type is related to ecological adaptation as well as to the cropping system. The practice, now abandoned, to grow common bean mixed with maize or alternatively, the presence of woods that assured abundant and cheap raw material to make the stakes, could have favored the predominance of climbing habit.

Table 2. Distribution of the seed coat types (according to IBPGR) within Italian common bean landraces as described in the literature.

Region	N. pop	Coat without pattern					Coat with pattern			Ref.
		White	Brown pale to dark	Yellow	Black	Other	bicolor	Borlotto type	Pattern around hilum	
Abruzzo	10	3	1					4	2	[28,47,49]
Basilicata	65	18	11			6	10	16	4	[30,37,117]
Calabria	1	1								[118,119]
Campania	3	2							1	[67,102]
Friuli	4			2				2		[56]
Lazio	19	6	4	1	1	1	1	5		[28,49,53,119]
Liguria	2	1	1							[119]
Marche	23	8	4		1		3	7		[120]
Piedmont	5	1	1					3		[119]
Sicily	4						3		1	[100,119]
Tuscany	24	11	2	1	1	1	2	4	2	[59,121]
Veneto	8		1	1		1		5		[34,54,122,123]
Total	*168*	*51*	*25*	*5*	*3*	*9*	*19*	*46*	*10*	

Currently, about 90 % of landraces cultivated in Basilicata region have climbing habit [30,45,46]. A similar frequency has been estimated in Cuneo province, Piedmont region [51]. Determinate and indeterminate growth type (40 *vs*. 60 %, respectively) were detected in Campania region [57] as well as within borlotto-like landraces of Marche region [58]. Variation of habit within one landrace has been also reported. As an example, the common bean named ‘Piattella Pisana’ or ‘San Michele’, cultivated in Tuscany exhibits a high variation of several morphological and physiological plant traits. Baldanzi and Pardini [59], analyzing 33 populations belonging to this landrace, observed that the climbing ability is highly variable. Similar results were reported for the common bean named ‘Decimino’ or ‘Turco della Garfagnana’, another landrace cultivated in Tuscany. The surveyor of material grown by farmers evidenced differences in habit, flower color, and seed shape within ‘Decimino’ [59]. Two distinct growth habits, determinate and indeterminate, that differ in plant height, number of nodes and pods, and yield, were detected also within the ‘Fagiolo del Purgatorio’ [61]. The presence of a different habit within these three landraces could due to farmer preferences since Tuscany and Lazio regions are geographically close (Figure 2). An aggressive climbing ability characterizes the common bean named ‘Fagiolo a pisello’ cultivated in Colle di Tora on the steep hillsides of Turano Lake. Plants of this common bean can been more than 3 m tall but flowers appear only in the upper part [62].

4.2. Seed Storage Proteins as Biochemical Markers

Unlike in other legumes, storage proteins in common bean are mainly constituted by vicilin, in this species called phaseolin. It accounts for 50% of the total proteins in mature seeds, consisting of a number of polypeptides in the Mr 54–44 kDa, and is considered a biochemical marker. Phaseolin electrophoretic analyses of wild-growing and domesticated materials supported the hypothesis of the presence of two major gene pools within common bean germplasm, the Mesoamerican and Andean ones [6]. Multiple domestication events are thought to be the cause of parallel geographic phaseolin patterns variation between wild and cultivated forms. The Mesoamerican wild forms showed S as well as a number of M phaseolin types, while S patterns predominated within the domesticated counterpart. Similarly, T, C, H, and A phaseolin types were found among wild and cultivated materials from the Andes [8]. It has been shown that phaseolin is a useful marker to follow the dispersal pathway of common bean from domestication areas into Europe. A higher frequency of Andean phaseolin types with respect to Mesoamerican ones was recorded in European germplasm first by Gepts and Bliss [7]. A more recent study on 544 European accessions confirmed that Andean phaseolin types T (45.6%) and C (30.7%) prevailed over the Mesoamerican S type (23.7%) [63]. Lioi [64], who analyzed a large Italian collection, showed the predominance of Andean genotypes (73%) over Mesoamerican ones, and that phaseolin C types had a higher frequency than T ones. The prevalence of the Andean gene pool within the Italian common bean germplasm stored in some international gene banks has been recently confirmed by Logozzo *et al.* [63], who reported 88% frequency of Andean types with a predominance of C type (51.5%), and only 12% of S types. Tiranti *et al.* [65] studied 159 common bean landraces collected from farmers and local markets in 10 Italian regions (six from Piedmont, 14 from Liguria, 11 from Friuli, 25 from Tuscany, four from Marche, 52 from Umbria, 25 from Lazio, 17 from Abruzzo, three from Basilicata, two from Sicily). The presence of the three major phaseolin types (C, T and S) was observed in seven regions, suggesting a homogeneous distribution of phaseolin types in the sampled regions. The observed frequencies were 40, 28, and 32% for Andean (C, T) and Mesoamerican (S) types, respectively. These results agree largely with Lioi's data [64], while the frequency of resulting S types was more than double the 12 % reported by Logozzo *et al.* [63].

Results of several studies carried out at regional level are summarized in Table 3. Despite a large variation in the number of analyzed populations and sampling strategies, among these investigations a prevalence of the Andean phaseolin types was observed.

At least for the more sampled regions such as Basilicata, Sicily, Campania and Sardinia (Figure 2), the frequency of each phaseolin type at regional level can be depicted by these studies. The clear predominance of C type has been observed for populations from Basilicata region [45,46]. Angioi *et al.* [66] recorded a high frequency of the C phaseolin among Sardinian common bean landraces (Table 3). Moreover, Mesoamerican types appear to be less represented in Sardinia; only one genotype with S phaseolin type was found only among 73 genotypes analyzed. A similar result has been recorded for the Campania region (Table 3), where only 9.2% of tested populations belonged to the Mesoamerican gene pool [57,67].

The predominance of C types has been observed also within Spanish common bean landraces by Rodiño *et al.* [68]. This similarity between Italian and Spanish germplasm strongly suggests that common bean may have been introduced in Italy mostly from Spain more than directly from America.

This is not surprising because several Italian regions were under Spanish rule in the 16th and 17th centuries.

Table 3. Distribution of the phaseolin pattern frequencies within Italian common bean landraces as described in the literature.

Region	**N pop**	**Phaseolin type**								**Ref.**
		T	**C**	**T+C**	**A**	**H**	**Mix Andean**	**S**	**Mix Andean-Mesoamerican**	
Abruzzo	9	1	1		1		3		3	[49]
Basilicata	66	18	38					8	2	[45,46,104]
Calabria	4		3						1	[118]
Campania	98			89				9		[57,67]
Lazio	17	4	1		1	4	3	2	2	[34,49]
Piedmont	5	1	3				1			[34,50]
Sardinia	73	21	51					1		[66]
Sicily	28	3	17				1	7		*
Tuscany	1							1		[104]
Veneto	12	4	5					3		[34,54,104,122]
Total	*313*	*49*	*119*	*89*	*2*	*4*	*8*	*31*	*8*	

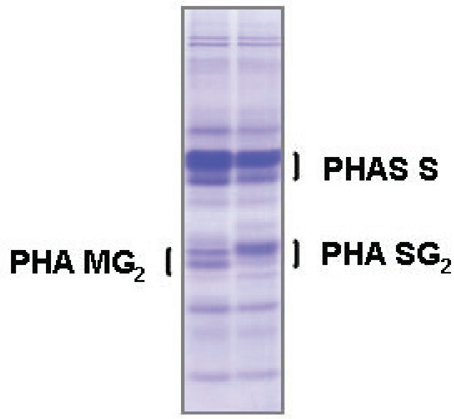
*Piergiovanni unpublished.

The presence of two or more phaseolin patterns within some populations from Abruzzo, Lazio, Piedmont and Sicily regions (Table 3) is indicative of the informal selection from which these landraces have originated. As shown in Table 3, the populations from Central Italy (Lazio and Abruzzo regions) showed the highest polymorphism of phaseolin protein fraction. In addition to the three main types (T, C, and S), within these populations phaseolin patterns uncommon in Europe, such as the Andean types A and H [47,49] were observed. This suggests that Central Italy retains higher germplasm variability than the other Italian regions, as was recently reported for the Italian lentil germplasm [69].

The existence of no small-seeded populations (100 seed weight > 40g) showing S phaseolin type has been evidenced by some authors [45,46,63]. These kinds of biotypes are uncommon within the European germplasm [70] and absent in the Mesoamerican gene pool. It has been hypothesized that they could be the result of a deliberate selection towards large-seeded genotypes within the Mesoamerican biotypes carried out by European farmers over time, being large-seeded types generally more accepted from consumers. Alternatively, when Mesoamerican and Andean genotypes began to be cultivated in proximity in the European environments, recombination resulted in increasing large-seeded types showing S phaseolin pattern [71].

Phytohemagglutin (PHA) is the second most abundant protein in common bean seeds. PHA exhibits a relatively limited heterogeneity but it may be used in addition to phaseolin for the characterization of landraces [72]. Although gene families coding for phaseolin and PHA are not linked, in cultivated materials there is a narrow association between specific phaseolin and PHA patterns [73]. As suggested by Gepts [74], these findings could be due to the establishment of two distinct gene pools, where specific alleles of different genes may be associated with one another more often than random assortments would predict. An extensive screening for PHA variation in Italian material was carried out by Lioi [75], who analyzed about 350 accessions. Of 10 different patterns detected, two (TG_2 and SG_2) were predominant and were generally co-present with Andean and Mesoamerican phaseolin patterns, respectively.

Figure 3. Electrophoretic patterns of phaseolin (PHAS) and phytohemagglitinin (PHA) in seeds of ‘Fagiolo del Purgatorio’ from Gradoli populations.



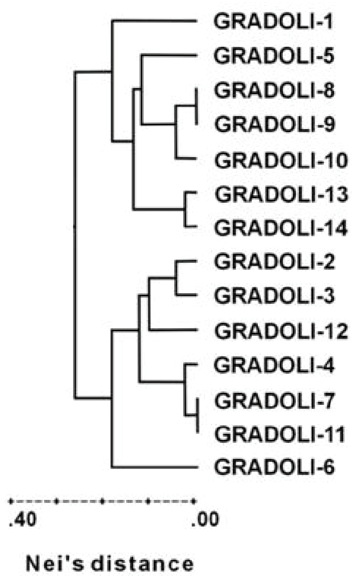
A more recent study also demonstrated that PHA could be utilized for the characterization of landraces. Lioi *et al.* [27] found an interesting variation of the PHA electrophoretic pattern within the populations of ‘Fagiolo del Purgatorio’. The PHA patterns, MG_2 and SG_2 (Figure 3), together with several different traits such as vegetative cycle, yield, and pest resistance, suggested that more than one constitutive nucleus has contributed to the genetic background of this landrace [76]. Therefore, an adequate number of populations should be preserved to ensure the retention of the diversity present in this landrace.

4.3. Molecular Diversity

Although phaseolin has been a valid tool in identifying geographical patterns of domestications and dispersion, its scarce polymorphism does not permit discrimination among closely related landraces. The advent of molecular techniques has greatly improved the ability to understand the genetic structure of common bean landraces. Studies of the level and structure of genetic diversity in common bean landraces are of fundamental importance in tracing reasonable patterns of dispersion and diversification of this species, and in understanding relationships among landraces, genetic resources management and plan safeguard actions for their preservation, improvement, and promotion. Although in the last decade the attention devoted by researchers to Italian common bean landraces has greatly increased, studies on this topic are far from being exhaustive.

A variety of PCR-based molecular markers are useful tools for the study of genetic diversity. In particular, SSRs (Simple Sequence Repeats) or microsatellites, are short (mostly 2–4 bp) tandem repeats of DNA sequence; their polymorphism originates from a different number of repetitive core motifs present at one locus [77]. They are useful genetic tools although their development is time consuming and requires the identification of SSRs. The first strategy used to search for microsatellite motifs was the screening of publically available DNA sequence databases developed first by Yu *et al.* [78,79] and then by Guerra-Sanz [80]. Another strategy used to enlarge the number of microsatellite loci in *P. vulgaris* was the sequencing of size-fractionated BAC clones [81]. A number of polymorphic microsatellite markers have been isolated from both cDNA and genomic libraries [82-85]. The number of gene-based microsatellites was recently increased through a cDNA library screening approach by a large scale library developed from multiple source tissues of an Andean gene pool genotype [86]. SSRs were used by several authors to construct genetic maps [79,87,88], to evaluate genetic diversity in commercial variety or lines or genotypes of common bean [89-91], or to separate more effectively wild and cultivated accessions as well as Andean and Mesoamerican gene pools [92,93].

Figure 4. UPGMA dendrogram of 14 'Fagiolo del Purgatorio' from Gradoli populations based on SSR molecular markers.



The first approach that applied SSRs to characterize Italian germplasm was performed by Masi *et al.* [94] on three common bean landraces from the Basilicata region. This study showed that the frequency of polymorphic SSRs was fairly high, with some examined loci being multi-allelic, confirming the usefulness of these markers to the study of genetic diversity in bean collections. Some studies on common bean landraces from different Italian regions analyzed by SSR markers revealed the presence of both gene pools with predominance of landraces of Andean origin [34,58]. A study conducted on 33 local populations belonging to seven Italian common bean landraces revealed that they retain a considerable level of heterogeneity. SSR markers used were highly variable depending on the locus, with detection of 53 alleles at 14 loci examined. Populations belonging to the same landrace were grouped in clearly distinguishable sub-clusters with only one exception, suggesting that different farmers maintain distinct materials. In particular, the ‘Fagiolo del Purgatorio’ populations were grouped in two sub-clusters (Figure 4) confirming biochemical and agronomic data and suggesting that more than one constitutive nucleus has contributed to the genetic background of this landrace [34].

A low level of diversity was detected in a Sardinian and a Ligurian common bean collection, characterized using SSR and RAPD markers [66,95], probably due to a strong founder effect. Despite a relatively low level of diversity, the landraces were clearly distinct from cultivated materials. A detailed study about the organization of the ‘Fagiolo a pisello’ diversity was reported by Tiranti and Negri [96]. The aim of this study was to define an appropriate *on farm* conservation strategy, which could be used as a model for other populations. Results showed a high level of genetic similarity suggesting that populations were closely related to each other, probably as consequence of a single introduction event. Nevertheless, all the populations were morphologically and genetically clearly differentiated, with a considerable within-population genetic variation.

Similar studies conducted at local level, and in particular on some landraces from Marche region, revealed the presence of genotypes intermediate between Andean and Mesoamerican gene pools, suggesting probable hybridization events which might generate additional variation [58]. Moreover, molecular investigations revealed that some landraces from northeastern Italy could have played a role in the genealogy of commercial varieties [56].

Another class of molecular markers, AFLPs (Amplified Fragment Length Polymorphisms), due to their capability to detect polymorphism at a great number of loci, were used to assess diversity among wild material, landraces and common bean cultivars [97,98]. From three to seven AFLP primer combinations were used to estimate genetic variation among common bean landraces maintained *on farm* from different Italian regions. They revealed a quite high percentage of polymorphism, with each accession showing a unique pattern. The branching patterns showed two main clusters, regrouping accessions belonging to Mesoamerican and Andean gene pools, respectively. Within each cluster, accessions were well separated from each other [34].

Detailed studies effectuated on some Central Italy landraces (Umbria, Lazio and Abruzzo regions) showed that genetic differences among landraces could be ascribed to individual farmer’s preference and selection as well as to different introductions occurring in each location [31-33].

In the last years, some studies were carried out devoted to the fingerprinting of specific landraces using different molecular markers such as AFLP, RAPD, semi-random, ISSR, and SSR. Some landraces of great interest such as: ‘Fagiolo Badda’ (Sicily) [99,100], ‘Occhio nero di Oliveto Citra’ and ‘Fagiolo di Controne’ (Campania) [101-103], ‘Fagioli di Lamon’ (Veneto), ‘Fagioli di Sarconi’ and ‘Fagioli di Rotonda’ (Basilicata), ‘Zolfino’ (Tuscany) [104], ‘Fagiolo a pisello’, and ‘Fagiolo del Purgatorio’ (Lazio) [29,32,105,106] were analyzed. Through the data obtained, relationships among landraces regarding problems related to the homonymy and distribution of the diversity within landraces have been clarified. They represent preliminary actions necessary for drawing up disciplinary rules for conservation consortia, as well as for the attribution of European PGI marks.

4.4. Seed Quality

The common bean is the world's second most important pulse after soybean. It is well known that dry seeds represent an affordable and inexpensive source of proteins despite being deficient in the sulfur amino acids. In addition, common bean seeds are rich in complex carbohydrates, dietary fiber, starch, minerals and vitamins. Like other legumes, common bean seeds contain a number of bioactive compounds including enzyme inhibitors, lectins, phytates, oligosaccharides and phenolic compounds, which play metabolic functions in humans or animals. These features may be regarded at the same time as disadvantageous (reduction of protein digestibility or mineral bioavailability, flatulence) or favorable (antioxidant effect or pre-biotic activity). As discussed in previous sections of this review, a wide range of genetic variation exists within the Italian local common bean varieties, so differences in compositional and physic-chemical traits may be predictable. Unfortunately, there are relatively scarce studies on the nutritional and nutraceutical value of the Italian landraces as well as on their comparison with the modern cultivars.

Table 4. Protein content relative to some Italian landraces derived from the literature. A range of values is reported when several populations belonging to the same landrace were analyzed.

Local name	Cultivation region	Protein (% dm)	Ref.
Northern Italy			
Billò	Piedmont	27.4	[50]
Bianco di Bagnasco	Piedmont	23.2	[27]
Spagnolet nano	Veneto	21.8–23.8	[27]
Gialet	Veneto	26.1–27.8	[123]
Bala rossa	Veneto	24.5	[54]
Bonei	Veneto	24.2	[54]
Lamon	Veneto	28.0	[108]
Central Italy			
Zolfino	Tuscany	21.5	[124]
Fagiolo del Purgatorio	Lazio	21.4–27.6	[27]
Fagiolo romanesco	Lazio	26.8	[125]
Fagiolo regina	Lazio	27.7	[125]
Fagiolina arsolana	Lazio	23.6–27.3	[125]
Cioncone	Lazio	24.4–25.9	[125]
Pallini	Lazio	23.4	[125]
A pisello peligni	Abruzzo		[125]
Pane aquilano	Abruzzo		[125]
Pane peligni	Abruzzo		[125]
Sourthern Italy			
Bianco di Rotonda	Basilicata	26.6	[34]
Ciuoto	Basilicata	20.8	[34]
Verdolino	Basilicata	22.2	[34]
Bianco del Pollino	Calabria	23.6–27.2	[118]
Badda bianco, Badda nero and Monaco Mussu Niuru	Sicily	26.8–27.1	[108,126]

Protein content is generally considered a very important trait to estimate the nutritional quality of common bean seeds. The evaluation of 21 local populations cultivated in Basilicata region for three consecutive growing seasons evidenced a broad variation of protein content (22.0–28.2% dm) within this pool of samples. The same study showed that seven tested landraces had an average protein content falling in the range 25.0–28.8% dm, the same recorded for six cultivars used as references [48]. A similar protein range (21.8–29.2% dm) was recorded by Perazzini *et al.* [107], who evaluated eight populations cultivated in Central Italy. Other landraces have been sporadically investigated, the information available in the literature is shown in Table 4. These studies suggest that a large fraction of Italian landraces have a good nutritional quality, at least in terms of total protein content.

More recently, the evaluation of minor seed components (starch, fat, fiber, macro and microelements, *etc.*) started, but this field is far to be sufficiently examined [48,108,109]. Data collected up to now suggest that a narrow range of variation among the landraces exists for these seed components, and that differences in soil type, adopted cropping technique and environmental conditions strongly affect these grain traits.

It is well known that common bean grains are relatively rich in trypsin inhibitors (TI), a class of low molecular weight proteins able to inhibit trypsin. In addition to this action, these inhibitors act as defensive agents against pests, regulators of germination, and reserve of sulfur-amino acids [110]. The range of TI variation within Italian landraces has been investigated, together with the year-to-year variation recorded only for a very narrow number of genotypes [111]. This study revealed not only a three-fold variation of TI values among the set of tested landraces (14.3–37.4 TIU mg^{-1} dm) but also a large effect of year-to-year climatic changes on these anti-nutritional compounds. The increase of TI expression might possibly be related to the drought stress suffered by plants during the vegetative growth stage.

Generally, researchers recognize that the pigments responsible for seed coat color in common bean are flavonoids and that these compounds could have positive health benefits as antioxidants. At the present, soybean is considered the most relevant source of flavonoids with beneficial health effects among legume crops. However, soybean is not a traditional crop in Italy, while common bean is usually consumed by a large part of the population. The scarce knowledge about the flavonoid content requires additional research work on all legume crops of the Mediterranean area, including Italian common bean landraces.

It is known that in the *Phaseolus* genus flavonols and other phenolic compounds are stored in seed coat because they have anti-pathogen and anti-feeding activities, assuring a good protection of seeds from external attacks. The flavonoid composition of three phenotypes of ‘Zolfino’ landrace was described by Romani *et al.* [112]. Dinelli *et al.* [113] characterized and quantified the flavonoid present in 23 Italian landraces traditionally grown in Basilicata, Tuscany and Veneto. Kaempferol and related conjugated forms were found in all the ‘Zolfino’ accessions and in the ‘Verdolino’ landrace from Sarconi, while they were absent in 'Fagioli di Lamon' as well as in the other landraces from Sarconi. These data reflect the different coat pigmentation of tested samples since ‘Zolfino’ and ‘Verdolino’ have a yellow and light green coat color, respectively, while Lamon common beans are borlotto types. Moreover, Dinelli *et al.* [113] evidenced a great variation of flavonoid content not only among the tested landraces (0.19–0.84 g/kg of seed fresh weight) but also for each landrace sampled in different years. The coefficient of variation observed in the period 2001–2003 ranged from ±18% to ±50%. Further experimental works is needed for a deep understanding of physiological mechanisms influencing the expression of these compounds.

A recent study has shown that different parts of dry beans exhibit antioxidant activity [114]. The anti-radicalic activity of dry beans belonging to 'Zolfino' (Tuscany), 'Poverella', 'Verdolino' and 'A'Marozzo' (Basilicata) landraces, grown in three different years and in different geographic areas, was quantify by Heimler *et al.* [115]. Authors recorded a wide range of antioxidant activity (from 39 to 2,810 EC_{50} for 'Verdolino' and 'Poverella', respectively) and attributed these results mainly to landraces and environmental conditions more than to total phenolics and total flavonoids detected in dry seeds.

With regards to the investigated physical seed properties (*i.e.*, cooking time, hydration index, swelling capacity and splitting of seeds during cooking) the results are strictly related to the commercial value of landraces. Very recently, a significant variation of hydration rate among Italian common bean germplasm has been described. The screening of 62 landraces belonging to different market classes revealed that they behaved differently in terms of hydration rate. Based on these data the tested landraces were classified into three hydration groups characterized by slow, intermediate and fast water uptake [116].

Finally, it should be mentioned that any scientific study has been reported in the literature regarding the tests on consumer approval of Italian common beans though the landraces are generically emphasized as preferred with respect to modern cultivars because of their sweet taste, soft texture, and cooked bean flavor.

5. Conclusions

In accordance with the literature presented in this review, Italian common bean germplasm is characterized by a high degree of genetic diversity. This is the consequence of five centuries of uninterrupted cultivation coupled to a capillary diffusion of this crop in all the regions. Although the overall number of Italian common bean accessions stored *ex situ* is remarkable, the lack of systematic investigations of these collections appears evident. Unfortunately, the chance of a systematic monitoring of the Italian common landraces is very low as a consequence of the lack of appropriate financial resources devoted to this aim. Taking into account the above mentioned studies, only a multidisciplinary approach can be fully effective to characterize each landrace and to help the efforts in planning adequate safeguard actions.

Moreover, available data demonstrated that wide differences between landraces and cultivars are detectable at several levels. As an example, if landraces are predominantly climbing, bush cultivars are widely used in the advanced agricultural systems by reason of their more appropriate features for mechanical harvesting. Similarly, the seed coat variation (color and pattern type) detected among landraces contrasts with the large predominance of white and borlotto types among the cultivars. This condition is a direct consequence of market demand, because white seed type is preferred for dry seed consumption while borlotto type is required as snap seed. From a practical standpoint, the safeguard of landraces assumes a strategic value to be able to answer multiple questions, though the implementation of adequate actions, and depends on the availability of economic resources and facilities as well as the sharing of goals among scientific, governmental, and local institutions.

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