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Morphological and genetic diversity among and within common bean (*Phaseolus vulgaris* L.) landraces from the Campania region (Southern Italy)

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ABSTRACT

The common bean (*Phaseolus vulgaris* L.) is arguably the most important food legume and a fundamental source of proteins especially for rural societies. In several countries, this species is characterized by a number of locally adapted landraces and many of them are at risk of extinction. In Italy, common bean cultivation has always been a typical element of rural economies especially in the Southern regions. We carried out an investigation of the morphological and genetic diversity in 25 common bean populations cultivated in the Campania region (Southern Italy). We analyzed 26 qualitative and 11 quantitative traits following the IPGRI descriptors. Furthermore, 10 SSRs were employed to examine genetic polymorphism, differentiation and population structure. Molecular and morphological data distinguished all the landraces under investigation. A considerable phenotypic diversity among landraces was observed for many characters, including some related to agronomical performance. At molecular level, all the SSRs were polymorphic, with an average of 8.5 alleles per locus. Moreover, the vast majority of the landraces (92%) displayed intra-varietal differences. Our work indicated the presence of a wide-ranging variation among and within cultivated common bean landraces. Moreover, it provided evidence that the implementation of measures for their on-farm conservation, management and promotion should be useful also to preserve genetic variability.

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is a world-wide cultivated legume of agricultural interest in many countries (Lioi and Piergiovanni, 2013). In Italy, it represents the main grain legume for direct human consumption. Common bean was introduced into Italy from the New World in the 16th century (Bitocchi et al., 2012; Piergiovanni and Lioi, 2010). The first documentation dates to 1532 and refers to a donation of seeds from the Spanish Emperor Charles V to the Pope Clemente VII (Lioi and Piergiovanni, 2013). Since its introduction into Europe, this species has experienced an adaptive radiation being, for instance, cultivated for the production of dry seeds, shell seeds and green pods (Angioi et al., 2010; Lioi and Piergiovanni, 2013). Bean diversification can be ascribed to different factors such as the need for adaptation to the soil type and climatic conditions of new environments, the geographical isolation of several growing areas, the cultivation technique (e.g. the frequent consociation with local maize varieties) as well as aesthetical and organoleptic preferences of specific areas. In Mediterranean countries, common bean cultivation is present nearly in all the traditional agricultural settings (Lioi and Piergiovanni, 2013; Negri and Tosti, 2002). The strong link between rural agriculture and common bean has given rise to landraces that are frequently associated to restricted areas (Negri and Tosti, 2002; Piergiovanni and Lioi, 2010).

During the last decades, in Italy as well as in other developed European countries, the cultivated area of *P. vulgaris* has declined because of economic (e.g. low competitiveness, decreasing price on the international market), social (e.g. larger availability of animal proteins, its strong identification with a rural diet) and technical reasons (e.g. diffusion of fertilizers, mono-culture and mechanical harvesting). This reduction has been particularly evident for elite cultivars that replaced traditional varieties after WWII, while in several Italian cropping areas, farmers still maintain in cultivation common bean landraces (Lioi et al., 2005; Marotti et al., 2007; Negri and Tosti, 2002; Piergiovanni and Lioi, 2010).

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Currently, modern nutritional recommendations inspired by the traditional dietary patterns of Greece, Spain, Portugal and Southern Italy have increased the value of legumes as healthy source of proteins. Moreover, consumers are more attracted by agricultural and food products that are distinguished from others by certain characteristics, qualities or reputations that derive from their geographical origin. For all these reasons, traditional common bean varieties are receiving a growing attention from both consumers and policy makers (Piergiovanni and Lioi, 2010). The primary policy goal for conservation and exploitation of agricultural biodiversity should focus on the assessment of the existing diversity not only between but also within landraces (Esquinas-Alcazar, 2005). This effort is essential to prospect strategies for the implementation of adequate on-farm conservation schemes and it is as a prerequisite for the possible development of breeding programs (Esquinas-Alcazar, 2005; Huang et al., 2010).

The evaluation of morphological traits is a traditional, important method for the description and the determination of relationship among common bean landraces (Skroch and Nienhuis, 1995). In addition, molecular analysis provides additional information that is independent of environmental effects and in *P. vulgaris*, it has proved to be a valuable tool for association mapping and the study of genetic diversity, population structure and phylogenetic relationships (Beebe et al., 1995; Bitocchi et al., 2012; Blair et al., 2009; Metais et al., 2000; Shi et al., 2011). Moreover, DNA analyses of plant genetic resources are valuable to identify duplicate accessions in core collections and possible cases of homonymy and synonymy in cultivated landraces (Corrado et al., 2014; Rao et al., 2009).

In Italy, the common bean cultivation has always been a typical element of rural economies especially in the Southern regions (Piergiovanni and Lioi, 2010). In those areas, common bean is probably the horticultural crop with the highest number of landraces (Perrino et al., 1984). The germplasm of different Italian regions has been characterized (Lioi et al., 2012; Mercati et al., 2013; Piergiovanni and Lioi, 2010; Raggi et al., 2013), but despite the long history of cultivation, the number of accessions (Hammer et al., 1989) and the presence of some well-known landraces that excel for product quality (e.g. “di Controne”, “Dente di Morto”), the germplasm of the Campania region (Southern Italy) has been poorly investigated.

The aim of this study was to collect, characterize and evaluate the diversity of traditional common beans that are cultivated in the Campania region (Southern Italy) using morphological and molecular data.

2. Materials and methods

2.1. Plant material and its description

The study took place at the Genetics’ Experimental Station of the Faculty of Agricultural Science, University of Naples Federico II (Portici, Italy), during the 2012–2013 period. The investigation was performed on 25 common bean landraces (*P. vulgaris* L.) from different geographic areas of the Campania region (Table 1). Collected seeds were not multiplied before trials. The morphological characterization was done according to the IPGRI recommendations, including the number of replications (IPGRI, 1982). Briefly, we recorded a number of highly hereditable characters selected as being easily seen and expressed in all environments (IPGRI, 1982). In total, we analyzed 26 qualitative and 11 quantitative traits. Traits under investigation, along with their IPGRI code, are reported in Supplementary Tables 2 and 3.

Table 1
List of the landraces investigated, their code and site of collection/growing area. AV: Avellino; BN: Benevento; NA: Naples; SA: Salerno.

Code	Name	Site of collection/growing area	Province
BM	Bianco	Montefalcone	BN
C	di Controne	Controne	SA
DM	Dente di Morto	Marigliano	NA
EU	Dente di Morto	Acerra	NA
F	a Formella	Visciano	NA
MI	Mustacciello	Ischia	NA
MP	Mustacciello	Pimonte	NA
OC	Occhio nero	Oliveto Citra	SA
OS	Occhio nero	alta valle del Sele	SA
P1	Sel Pirolo 1	Acerra	NA
P2	Sel Pirolo 2	Acerra	NA
P3	Sel Pirolo 3	Acerra	NA
P4	Sel Pirolo 4	Acerra	NA
P5	Sel Pirolo 5	Acerra	NA
R	della Regina	Casalbuono	SA
RG	della Regina (di Gorga)	Stio	SA
RL	della Regina	San Lupo	BN
RO	Cannellino Romano	Agro-acerrano-nolano	NA
SI	Screziato impalato	Castellabate	SA
TBC	Tondino bianco	Caposele	SA
TC	Tondo	Caposele	SA
TV	Tondino	Villaricca	NA
V	Cannellino Viscardi	Agro-acerrano-nolano	NA
ZI	Zampognaro d’Ischia	Ischia	NA
ZO	Zolfariello	Visciano	NA

2.2. Analysis of morphological data

Qualitative characters were transformed into dummy binary attributes as reported (Romesburg, 2004). Pairwise similarities between landraces were calculated by using the Jaccard similarity coefficient (Sneath and Sokal, 1973). Variability of the quantitative traits between and within landraces was evaluated calculating the coefficient of variation (i.e. the absolute value of the standard deviation of the mean/mean). This coefficient ranges from 0 to 1. For continuous variables, pairwise dissimilarity coefficients were computed using squared Euclidean distances after standardization of mean values (Sneath and Sokal, 1973). To calculate the standardized value Z_{ij} for the ith attribute and jth object, we subtracted the mean of the values of the ith attribute from the corresponding value X_{ij} in the data matrix and divided the result by the standard deviation of the values of the ith attribute. On the basis of each resemblance matrix, landraces were clustered by the unweighted pair-group method with arithmetic averages (UPGMA) (Sneath and Sokal, 1973). A cophenetic value matrix (Sneath and Sokal, 1973) of the UPGMA clustering was also used to test for the goodness-of-fit of the clustering to the resemblance matrix on which it was based, by computing the product-moment correlation coefficient (r) with 1000 permutations (Rohlf and Fisher, 1968). These analyses were conducted using the NTSYSpc v. 2.1 package (Rohlf, 1998).

2.3. SSR analysis

Total DNA was isolated from leaves using a previously reported procedure (Caramante et al., 2009). We analyzed three plants per landrace. Ten SSR loci were used for PCR amplification (Buso et al., 2006; Gaitan-Solis et al., 2002; Grisi et al., 2007; Hanai et al., 2010; Yu et al., 2000). SSR features, primer sequences and annealing temperature (T_a) are reported in Supplementary Table 1. PCR was performed in a 25 μl volume containing 20 ng genomic DNA, 1.5 mM $MgCl_2$, 100 mM dNTPs, 0.2 mM fluorescently labeled forward primer and unlabeled reverse primer, and 0.5 U Taq DNA polymerase (Promega) in 1× PCR buffer. The amplification conditions were: one denaturing step at 94 °C for 5 min, followed by 35 cycles of 94 °C for 45 s, T_a °C for 45 s and 72 °C for 1 min 30 s. After