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SSR Markers Distinguish Traditional Italian Bean (*Phaseolus vulgaris* L.) Landraces from Lamon

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Abstract

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In this study, 12 microsatellite markers (SSR) were evaluated for their applicability to protect from frauds and misuse the Italian Protected Geographical Indication (PGI) product "Common bean from Lamon". SSR analysis generated polymorphic alleles, with an average of 4 alleles per locus and all in the range of molecular weight between 181 and 284 bp. Twenty-nine variety-specific fragments were identified, which might be reasonably adopted for characterization and traceability purposes. Cluster analysis well outlined the relationships between the genotypes studied. Overall, our study underlines the use and usefulness of molecular markers to protect both farmers and consumers from frauds.

Keywords: fingerprinting; Protected Geographical Indication (PGI); varietal authentication

The common bean *(Phaseolus vulgaris* L.) is one of the most widely cultivated legume crops and it is consumed as a dietary staple worldwide. In Italy beans represent an important crop and in several areas growers still cultivate autochthonous landraces (Piergiovanni & Lioi 2010). These are locally adapted and represent regional specialties. Examples of quality products are four borlotto-type bean landraces cultivated in a restricted area of the Belluno province (Northern Italy), and sold under the brand name "Common bean from Lamon". To encourage its cultivation and to protect its name from misuse and frauds, the European Union attributed them the Protected Geographical Indication (PGI) (GUCE 163/96 2 July 1996). Frauds and counterfeits exist for this agriproduct, like the substitution of a similar, but cheaper landrace for one of these landraces. Given that, there is an increasing demand for the identification and labelling of such a high-quality product. Nowadays, molecular markers are routinely utilized worldwide for determining the authenticity of food matrices. Among them, microsatellites (SSRs) show high reproducibility and informativeness, are co-dominant, and can amplify the short target sequences which often characterize degraded DNA (Varshney *et al*. 2005). In the perspective of implementing actions addressing the protection of bean PGI products, we aimed to (1) better understand the genetic structure of four Italian common bean landraces included in the production specifications of "Common bean from Lamon" and (2) evaluate whether SSR markers can be used to provide a molecular basis to unequivocally distinguish "Lamon" bean landraces from those varieties generally used in fraudulent substitutions.

The plant material consisted of seeds belonging to the following four landraces: Climbing Spagnolit, Spagnol, Calonega and Canalino. The key phenotypic characteristics of these landraces are reported in Table 1. They are included in the product specifications approved by the EU for "Common bean from Lamon" production and were collected from independent plants. The landrace Dwarf Spagnolit, locally bred and derived from Climbing Spagnolit, was also included in the analysis. For each of the landraces, three accessions were collected from as

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Table 1. Morphological description of the four bean landraces included in the product specifications approved by the EU for "Common bean from Lamon" production

	Spagnol	Climbing Spagnolit	Calonega	Canalino
Seed shape	egg/subelliptic	round/barrel	slightly-flattened	ovoid
Seed colour	creamy with red vinous streaks	creamy with bright red streaks	creamy with bright red streaks	creamy with dark red/ black streaks
Seed skin	thin	very thin	fine	thick
Seed length (mm)	16.5	14.8	17	15.6
Seed thickness (mm)	8.8	8	7	8.7
Seed weight (average g)	1	0.75	0.65	1–1.3
Plant height at flowering (cm)	60–65	45–50	85–90	100–105
Maturity (DAF)	20–22	19–20	25–26	22–25

DAF – days after flowering

many as possible different farms (belonging to the grower association of "Common bean from Lamon") and numbered from one to three. As an outgroup, four commercial cultivars (Lingua di Fuoco, Teepee purple, Stregonta and Lamon Bean) were included since they are generally used in fraudulent substitutions. In addition, Lamon Bean was chosen as "Common bean from Lamon"-like due to its morphological similarities. For each accession, three seeds were germinated and obtained seedlings became the samples investigated here. DNA was isolated from seeds using the DNeasy plant mini kit (Qiagen, Valencia, USA). Based on the literature data, twelve SSRs were chosen among the most informative ones, namely: DROUGH1, AY1, AIA, PAX1, LIPOX, BETAG, BNG91-R, BNG91-R2 from Guerra-Sanz (2004); C42, C76, C130 from Wang *et al*. (2012); BM160 from Mercati *et al*. (2013). Molecular analyses were carried out using three biological replicates for each sample and PCR reactions were performed in triplicate, as described in Carputo *et al*. (2013). Totally, 81 reactions were executed. The number of alleles per locus (Na), observed heterozygosity (Ho), polymorphic information content (PIC) and the power of discrimination (PD) were calculated using GenAlex software (ver. 6.5). Cluster analysis was performed as described in Carputo *et al*. (2013).

All SSR loci amplified from seeds gave the highest value of amplificability (100%) and showed the total correspondence compared with leaf reference DNAs. These loci allowed the identification of 50 alleles (Table 2) and the differentiation of 9 different genotypes. On average, 4.42 alleles per locus were observed (Table 3), all in the range of molecular weight between 122 and 317 bp. Frequent self-pollination of common bean explains the low level of heterozygosity (on average 0.24). Nevertheless, five landraces and a commercial cultivar (Lamon Bean) showed genetic variability within their accessions (Table 3). Several hypotheses might explain such intra-accession variation, such as, among the others, the mixed-mating reproductive system of bean, with up to 10% outcrossing (Ibarra-Perez *et al*. 1997). The ability of our markers to distinguish between genotypes was estimated as the power of discrimination (PD) (Table 3). This was rather higher (greater than 0.94) for all the markers, except for BNG91-R2 and LIPOX, the only ones that did not give any polymorphic fragments in any samples. To test the level of informativeness of SSRs used we also estimated the PIC values, which peaked in BM160 (0.86). C42, BM160 and C130 harboured the maximum number of different alleles (12, 11 and 6, respectively) and showed, along with AIA, BETAG, C76 and AY1, a PIC value higher than 0.5. Therefore, these latter seven microsatellites proved to be the most discriminating and polymorphic markers for the rapid and unambiguous identification of all the samples analysed here. Such variability of SSR markers was previously reported also by Kwak and Gepts (2009) and Mercati *et al*. (2013). It might be speculated that the reduced number of alleles we observed could be the consequence of several factors, such as the small sample set tested, the self-pollinating system and the genetic bottleneck of European bean varieties compared to the American gene pool (Angioi *et al*. 2010).

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Table 2. Detected SSR alleles (bp) using 12 microsatellite markers on 9 bean varieties

Variety	AIA	AY1	BETAG	BM160	BNG91R	BNG91R2	C130	C42	C76	DROUGH1	LIPOX	PAX1
Dwarf Spagnolit	179, 219	221	224	**279**, **172***, **317**	209	181	278	122, **204**	256	235	218	**240**
Climbing Spagnolit	179, 219	221	224	**241***, 258	209	181	278	122, **196**	255	235	218	244
Calonega	179, 219	221*, **225**	224	**264**	197	181	**284**	176, 194	257	235	218	244
Canalino	179, 219	216	224	**237**, 256*	209	181	**268**	**152**, **283**	256	235	218	244
Spagnol	179, 170	216	224	256	197	181	269*, 276	122, **198**	257, **258***	235	218	**243**
Lamon Bean	179, 170	221	224, **239***	**247**	197	181	269*, **271**	122, 194	256	235	218	244
Stregonta	179, 219	216	**223**	258, **231**	209	181	278	**190**, **287**	255	235	218	244
Lingua di Fuoco	179, 219	220	222	256	209	181	278	176, **195**	256	235	218	244
Teepee purple	179, 219	220	222	**199**	209	181	276	122, **180**	255	**231**	218	244

Genotype-specific alleles are reported in bold; for each variety, alleles found in only one accession out of the three analysed are asterisked

Table 3. Genetic parameters of 12 microsatellite loci analysed

Locus	Na	Ho	PIC	PD
AIA	3.00	1.00	0.58	0.95
AY1	4.00	0.11	0.70	0.96
BETAG	4.00	0.00	0.54	0.95
BM160	11.00	0.26	0.86	0.96
BNG91-R	2.00	0.00	0.44	0.94
BNG91-R2	1.00	0.00	0.00	0.00
C130	6.00	0.00	0.73	0.95
C42	12.00	1.00	0.87	0.97
C76	4.00	0.00	0.65	0.95
DROUGH1	2.00	0.00	0.20	0.94
LIPOX	1.00	0.00	0.00	0.00
PAX1	3.00	0.00	0.37	0.95
mean	4.42	0.24	0.50	0.79

Na –number of alleles per locus; Ho – observed heterozygosity; PIC – polymorphic information content; PD – power of discrimination

Twenty-eight landrace/variety-specific alleles were identified, which are useful molecular tools for characterization and traceability purposes of food agriproducts (Adamo *et al.* 2012). We detected 12 alleles specific for the four landraces included in the "Common bean from Lamon" designation, and 16 in the other varieties (Table 3).

To better appreciate the relationships between landraces and varieties an UPGMA dendrogram was built (Figure 1). We distinguished each landrace sold under the designation and the derived ecotype Dwarf Spagnolit from its closer ancestor Climbing Spagnolit. In addition, data highlighted the likely common origin between the local bean landraces and the commercial variety Lamon Bean, which is often confused with the landraces included in the designation as well as with other two varieties of borlotto-type beans commonly commercialized.

In conclusion, DNA fingerprinting through SSR markers permitted to discriminate among landraces from Lamon and provided a detailed description of their genetic structure that appears to maintain a high level of variability. The presence of unique alleles in many genotypes allowed to unequivocally identify varieties and landraces and may be used as diagnostic markers to detect small distinct genomic regions within breeding materials with low polymorphism.

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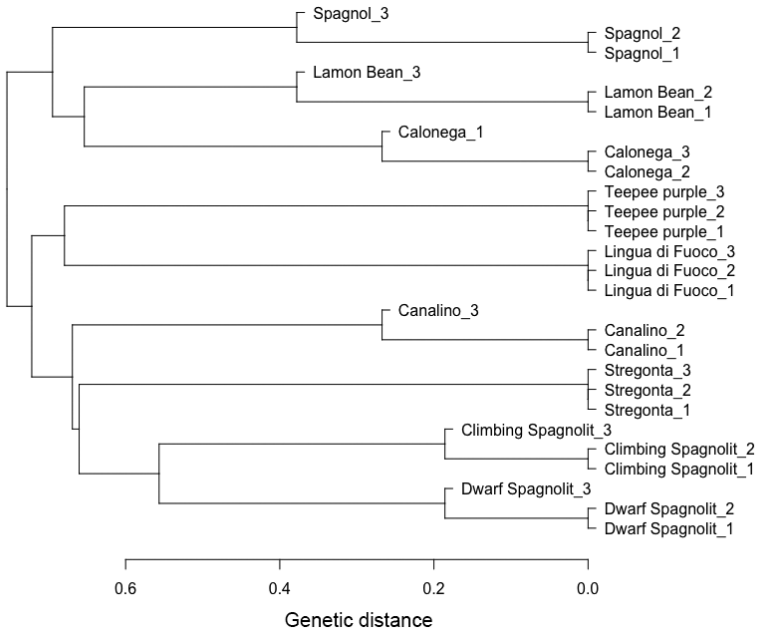

Figure 1. Dendrogram of nine genotypes of *Phaseolus vulgaris* using UPGMA cluster analysis of SSR marker data; DICE coefficient was used to estimate the degree of similarity of genotypes

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