Identification of proteaginous pea cultivars (*Pisum sativum* L.) using microsatellite molecular markers

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1. Introduction

Conventionally morphological descriptors are routinely used for establishing the identity of varieties. This kind of descriptors has some disadvantages, namely most of them are quantitative, controlled by several pairs of genes, and their expression is influenced by environmental factors. Molecular markers have a potential to facilitate this procedure, increase the reliability of decisions, and substantially save the time and space needed for experiments.

In this study we intended to identify 20 cultivars of proteaginous pea (*Pisum sativum* L.), registered in the Community Catalog of Varieties, by microsatellites molecular markers (Table 1).

Table 1 - *Pisum sativum L.* cultivars studied.

	Cultivar	*Country of admission and number of responsible for the maintenance*	*Cycle type*
1	ALEZAN	FR 8174	Spring
2	ALHAMBRA	ES 225	Autumn
3	ARTHUR	FR 11539	Spring
4	AUDIT	FR 13262	Intermediate
5	CARTOUCHE	FR 9295, UK 182	Autumn
6	CORRENT	IT 2	Intermediate
7	CHEROKEE	FR 11553	Autumn
8	ENDURO	FR 8444	Autumn
9	GREGOR	DE 147, FR 9295, UK 6136	Spring
10	GRISEL	PT 2	Autumn
11	GUIFILO	ES 9	Spring
12	GUIFREDO	IT 332	Autumn
13	IDEAL	ES 2041	Intermediate
14	ISARD	FR 9504	Autumn
15	JAMES	FR 9295	Intermediate
16	KLEOPATRA	DE 7627	Spring
17	LIVIA	FR 8451	Spring
18	LUMINA	FR 13262	Spring
19	ONIX	FR 9295	Spring
20	PIXEL	PT 2	Autumn

2. Material and Methods

Seven SSR markers, originated from a pea genetic map constructed by Loridon et al. (2005) were studied to detect polymorphisms in pea cultivars (Table 2).

PCR amplifications were conducted and the resulting fragments were separated on an 3,5% MS-8 agarose gel in TBE buffer, at 90V/h.

Results were analyzed based on the presence (1) or absence (0) of amplified fragments (amplicons) for each *locus* and cultivar. The resulting binary data matrix was processed wih the software NTSYS-pc version. 2.2 (Rohlf F. J. 1998). The data were processed using the SIMQUAL module and Jaccard similarity coefficient, followed by UPGMA cluster analysis.

The polymorphic index content (PIC) simplified from Anderson et al. (1993) was estimated for each marker from the formula: $PIC_i = 1 - \Sigma p^2_{i,}$ where p_i is the frequency of the i^{th} allele.

Table 2. Number of alelles and PIC generated by PCR using primers for seven pea (*Pisum sativum* L.) *loci.*

S S marker	*R Primer F*	*Primer R*	*Number of alleles*	*PIC*
A9	GTGCAGAAGCATTTGTTCAGAT	CCCACATATATTTGGTTGGTCA	2	0,42
AA205	TACGCAATCATAGAGTTTGGAA	AATCAAGTCAATGAAACAAGCA	1	0,00
AA355	AGAAAAATTCTAGCATGATACTG	GGAAATATAACCTCAATAACACA	4	0,46
AB25	TTTTCACTCAAAACACTCGGCT	GATGCCATTGCTGAAGGAGATT	2	0,46
AB53	CGTCGTTGTTGCCGGTAG	AAACACGTCATCTCGACCTGC	5	0,58
AD61	CTCATTCAATGATGATAATCCTA	ATGAGGTACTTGTGTGAGATAAA	3	0,56
AD270	CTCATCTGATGCGTTGGATTAG	AGGTTGGATTTGTTGTTTGTTG	3	0,52
Total			20	
Average			2,86	0,43

3. Results and Discussion

Within the investigated group of 20 peas cultivars the number of alleles ranged from 1 (AA205) to 5 (AB53), detecting altogether 20 alelles (Table 2). The average number of alleles per *locus* was 2,86. PIC ranged from 0,42 (A9) to 0,58 (AB53), on average 0,43.

In the AA205 locus only one allele was detected and is value as a marker for distinguishing cultivars is null. It was in AB53 and AA355 *loci* where a large number of alleles were detected, respectively 5 and 4.

In spite of the use of a relatively simple separation system, the six polymorphic *loci* studied provide precise identification of almost all cultivars (Figure 1).

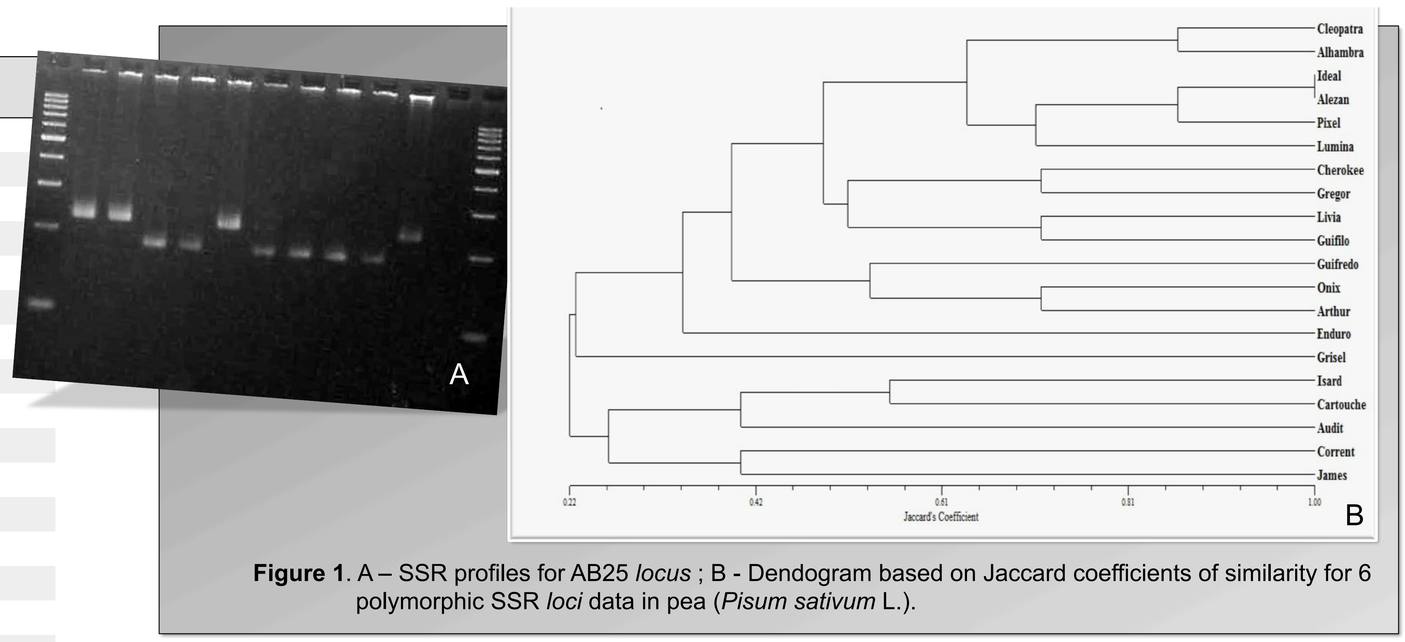

Figure 1. A – SSR profiles for AB25 *locus* ; B - Dendogram based on Jaccard coefficients of similarity for 6 polymorphic SSR *loci* data in pea (*Pisum sativum* L.).

Only cultivars Ideal and Alezan have an identical set of bands profiles, presumably they might have been derived from the same pedigree. The similarity coefficients ranged from 0,083 to 0,857.

The cluster analysis of SSR markers separated the pea genotypes into two distinct clusters. The first cluster included the five cultivars: Isard, Cartouche, Audit, Corrent and James. The second cluster included the remaining fifteen cultivars and was further divided in two subclusters. The first subcluster had the Portuguese genotype Grisel and second subcluster contained the remaining fourteen cultivars. In this subcluster Ideal and Alezan had 100 percent similarity.

A high stability of cultivars was found, which can be assign to the high degree of self-pollination in pea, as previously observed by Smýlak et al. 2008.

4. Conclusions

Molecular markers are useful in germplasm management and genetic diversity studies as well as a tool for rapid variety identification.

In this study the molecular identification of 20 cultivars of pea protein crops with microsatellites was carried out. By combined analysis of six polymorphic *loci* it was possible to distinguish almost all cultivars. However, the complete identification of 20 cultivars implies the study of a few additional *loci*. For the seven loci studied we detected 20 different alleles being AB53 and AD61the most informative *loci* for genetic variability.

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