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GENETIC BACKGROUND ANALYSIS TO HELP SOLVING SOME SOYBEAN (*GLYCINE MAX* (L.) MERR.) REGISTRATION PROBLEMS

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GENETIC BACKGROUND ANALYSIS TO HELP SOLVING SOME SOYBEAN (*GLYCINE MAX* (L.) MERR.) REGISTRATION PROBLEMS

Loray María[1]; Vicario, Ana[1]; Mitidieri Ulises[2], Dalmau Fernanda[2], Labarta Marcelo[2]
[1]Laboratorio de Marcadores Moleculares. Dirección de Calidad. Instituto Nacional de Semillas (INASE). Av Paseo Colón 922, 4° piso, CP 1063, Buenos Aires, Argentina.
[2]Dirección de Registro de Variedades, Instituto Nacional de Semillas (INASE). Av Paseo Colón 922, 3° piso, oficina N° 347 - CP 1063, Buenos Aires, Argentina.

INTRODUCTION

1. During the last years, forty (40) new soybean varieties are annually submitted for registration in the National Seed Institute Office of Argentina. So, over four hundred and fifty (450) varieties were registered since twenty years ago.

2. But in some cases it become rather difficult to take a decision about the differentiation of a new cultivar, even though sixteen (16) relevant characteristics – morphological, phenological, physiological – are used for its description. The differentiation process begins with the comparison of the new variety and the rest by employing a computarized program – specially developed for that purpose, which compare eight (8) of these traits (distinctness strategy).

3. So, by reason of this difficulty it was necessary to carry out field trials during the last seven years to compare the new variety and the most similar ones and to complete the DUS testing for this species.

4. The aim was to analyze this similitude at microsatellite level among the varieties that seemed to be highly similar in morphological traits.

MATERIALS AND METHODS

Morphological analysis

5. The National Registration Office analyzed data for morphological, phenological, physiological, etc. traits among 16 soybean varieties. Nine are already registered (Var225, Var208, Var239, Var232, Var4, Var203, Var212, Var211 and Var226) and for the others seven DUS testing is being carried out (Var197, Var202, Var222, Var229, Var198, Var199 and Var200). In a first analysis, each new variety was studied by the analysis of the 8 most important morphological, phenological, physiological, etc. traits (data provided by the applicant) and compared to all others varieties previously characterized using a computer program. When it was not enough to distinguish them, the new varieties were planted at field and compared with the rest considering all the all morphological descriptors used for DUS testing.

Microsatellites analysis

6. DNAs from 16 soybean varieties were screened for microsatellite loci. Ten representative soybean microsatellites (SSR) containing "ATT" repeated sequence motifs were selected according to their distribution in the genetic map and reported polymorphism.

PCRs were performed as reported before (UPOV - BMT - TWA/soybean/1/2, September 2002). Amplification products were resolved by standard sequencing electrophoresis on denaturing polyacrylamide gels following Sambrook, et al (1989) protocols. Bands were revealed using a silver nitrate staining kit according to manufacturer instructions (Promega Biotech, USA).

7. Each microsatellite allele band was scored as either present (1) or absent (0) across all genotypes to create a binary matrix. Similarity values were calculated analyzing the binary matrix with the Jaccard association coefficient (Sneath and Sokal, 1973) using the NTSyS pc2.0 program.

RESULTS AND DISCUSSION

Morphological traits

8. The analysis of the first 8 most important morphological, phenological, physiological, etc. traits (Growth type; Plant type; Pubescence colour; Leaf shape; Flower colour; Peroxidase reaction; Hilum colour and Maturity group) showed that some new varieties were identical or highly similar to some others already registered. Taking into account those first results, varieties were distributed in 5 groups according to their similarities. Afterward, the complete morphological, phenological, physiological, etc. traits analysis for all varieties were carried out. (Table 1).

Table 1: DUS testing results on 16 soybean varieties. Results after comparing all morphological, phenological, physiological, etc. traits on the field among the varieties are shown. Groups of varieties with distinctness problems (1 to 5).

	Varieties on DUS testing process	Similar varieties at morphological traits level	Result after the complete field trials
Group 1	Var197	Var222	Not distinct
		Var225	Not distinct
	Var222	Var225	Not distinct
Group 2	Var229	Var208	Distinct
		Var239	Distinct
		Var232	Distinct
	Var198	Var208	NRR
		Var239	NRR
		Var232	NRR
		Var229	NRR
Group 3	Var199	Var4	Distinct
		Var212	Distinct
		Var197	Distinct
		Var222	Distinct
		Var225	Distinct
Group 4	Var200	Var211	Distinct
		Var203	Distinct
Group 5	Var202	Var226	Distinct

NRR: No reliable results

9. Varieties from group1 could not be differentiated after three years of field trials. For group 2, variety 229 was distinct from 208, 232 and 239. However, variety 198 had some growth problems along two years of field trials, so no reliable information could be recorded. The applicant provided data about var198 that has to be confirmed by the Registration Office using field trials. Var199 (group 3), var200 (group 4) and var202 (group 5) were properly distinguished from the others among group after field trial was completed.

Microsatellite data

10. In previous works, it was demonstrated the feasibility of generating efficient genetic fingerprints of commercial soybean varieties and landraces using microsatellite (SSR) markers (Giancola, 1998, Vicario, 2000). In the present work, soybean varieties that presented distinctness problems after the first morphological, phenological, physiological, etc. characterization were analyzed on its genetic background using 10 SSR. Table 2 shows all groups of varieties analyzed and similarity values obtained using SSR.

Table 2: Similarity values obtained using SSR.
Soybean varieties presenting distinctness problems were analyzed on its genetic background using 10 SSR. Similarity values calculated using the Jaccard association coefficient are shown.

	Analyzed varieties	Similar varieties at morphological traits level	Similarity values obtained using SSR
Group 1	Var197	Var222	1
		Var225	0.83
	Var222	Var225	0.83
Group 2	Var229	Var208	0.22
		Var239	0.57
		Var232	0.33
	Var198	Var208	0.375
		Var239	0.5
		Var232	0.44
		Var229	0.62
Group 3	Var199	Var4	0.22
		Var212	0.22
		Var197	0.22
		Var222	0.22
		Var225	0.16
Group 4	Var200	Var211	0.1
		Var203	0.37
Group 5	Var202	Var226	0.63

11. Var197 and var222 are very similar between them and resulted very similar to var225 also. In field trials it was not possible to differentiate them so they could not be registered up to date. In this case the genetic background confirms the field examination.

12. In the case of groups 2 (var229), 3 (var199), 4 (var200) and 5 (var202) microsatellite results show low similarities values. This is in accordance with the results got from morphological trait analysis. Var198 (group 2), which could not be properly characterized on the field, showed low similarities values compared to the other varieties in the group. This indicates different genetic background among these varieties.

CONCLUSIONS

- Considering all 16 varieties analyzed microsatellites the results are in accordance with those from traditional DUS testing.

- The similarity values found using 10 SSR allow us to think again in a possible threshold of 0.8 (or a value close to it), above which two plants would be considered the same variety.

- The SSR study may provide a useful tool for the Registration Office in solving distinctness problems.

- These preliminary results show that these markers could be applied for germplasm classifications and its intellectual protection.

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