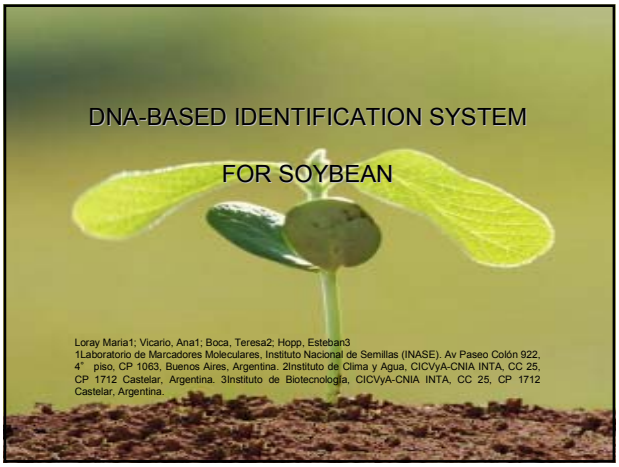

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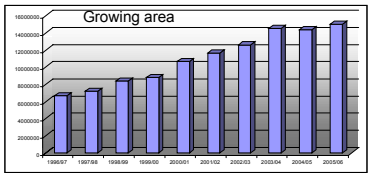
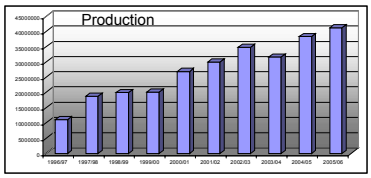
INTRODUCTION





INTRODUCTION

Previous analysis

Identification	**250 varieties, 5 bulked plants**	**30 SSR (5 AFLP; 22 RAPDs)**
Uniformity	**7 varieties, 15 plants each**	**15 SSR**
Stability	**7 varieties, 5 bulked plants, 4 years**	**32 SSR**

- **Select SSR as the appropriate markers for soybean characterization**
- **When analyzed with SSR, the number of off-type plants obtained is larger compared with the accepted number of off-type plants using the traditional system.**
- **SSR patterns may vary over the years**

These uniformity and stability variations may be due to:
- **Errors in purity maintenance**
- **Cross-pollination**
- **SSR mutation**
- **Internal variability not represented in the analysis**

INTRODUCTION

The aim of this study is to test, in a large sample, the heterogeneity of Argentine soybean varieties and determine the number of plants to be analyzed to obtain a detailed allelic profile feasible to be used for identification when comparing an unknown sample with data from already characterized varieties.

- **3 commercial varieties**
- **90 individual plants twice (samples 1 and 2)**
- **up to 8 SSR**

RESULTS AND DISCUSSION

TABLE 1

VARIETY	SSR	Sample 1	Sample 2	TOTAL
A	231	1 and 2 (0.988 / 0.012)	1 and 3 (0.983 / 0.017)	1, 2 and 3 (0.986 / 0.014/ 0.014)
	226	1	1	1
	294	1	1	1
	168	1	1	1
	42	1	1	1
	173	1 and 2 (0.461 / 0.539)	1 and 2 (0.551 / 0.449)	1 and 2 (0.506 / 0.494)
	175	1	1	1
	353	1	1	1
B	173	1	1	1
	175	1	1	1
	353	1	1	1
	294	1, 2, 3 and 4 (0.011 / 0.005 / 0.139 / 0.844)	1, 2, 3 and 4 (0.011 / 0.017 / 0.051 / 0.92)	1, 2, 3 and 4 (0.011/ 0.011/ 0.095 / 0.882)
	168	1	1, 2 and 3 (0.988 / 0.005 /0.005)	1, 2 and 3 (0.994 / 0.003 / 0.003)
C	175	1, 2 and 3 (0.06 / 0.927 / 0.012)	2 (1.00)	1, 2 and 3 (0.029 / 0.964/ 0.006)
	294	1 and 2 (0.566 / 0.434)	1 and 2 (0.517 / 0.483)	1 and 2 (0.530 / 0.470)
	168	1 and 2 (0.975 / 0.025)	1	1 and 2 (0.988 / 0.012)

RESULTS AND DISCUSSION

- **Then, if the sample to be compared against the reference varieties in a data base is small, there could be the possibility of diagnosing a rare allele as predominant.**
- **The analysis of large samples made on individual plants, is cost and time consuming.**
- **In order to reduce this, it was decided to pool DNA samples from different plants and perform a single PCR detection.**
- **A test on PCR sensitivity was performed to determine the proper size of the pool to be analyzed for which only the major allele would be found.**

RESULTS AND DISCUSSION

TABLE 2

Sample preparation

one target allele (belonging to a plant containing it as single rare allele) diluted in DNA from plants having a solely predominant allele.

VARIETY	SSR	AMPLIFICATION (at different dilutions)				
		1/10	1/20	1/30	1/40	1/50
A	173	Yes	Yes	Yes	Yes	Yes
A	231	Yes	Yes	No	No	No
B	294	Yes	No	No	No	No
B	168	Yes	No	No	No	No
C	294	Yes	Yes	Yes	Yes	Yes
C	175	Yes	No	No	No	No
C	168	Yes	No	No	No	No

RESULTS AND DISCUSSION

•Then, in order to obtain a profile based on the most frequent allele/s, the identification system for soybean cultivars protection purposes could be carry out on:

• 100 bulked seeds, avoiding the rare alleles detection due to the competence for the PCR reactants;

•to generate 3 or 4 independent samples of 5-10 plants from which DNA should be extracted and pooled to perform PCR analysis. If more than one allele is detected, only those that are shared in the 3 or 4 samples should be taken in consideration for genotyping the variety.

CONCLUSIONS

- **Even working with self-pollinated varieties, that have been tested for homogeneity of morphological traits, it is possible to find alleles at very low frequencies, that could be detected or not in the PCR reactions depending on the sample size.**
- **This will certainly be a problem if fixing a DNA pattern for each variety is the objective for genotyping.**
- **This is because an important proportion of the background genome may still be segregating. Other factors like purity maintenance, cross-pollination or SSR mutation may also affect the appearance of new alleles.**

CONCLUSIONS

- **A way to reduce the influence of the latter factors is to include DNA-based markers during the breeding processes.**
- **By now, in order to obtain a profile based on the most frequent allele/s, the identification system for soybean cultivars protection purposes could be carry out on:**
  - **100 bulked seeds;**
  2. **3 or 4 independent samples of 5-10 plants from which, only those alleles that are shared should be taken in consideration for genotyping the variety.**
- **Any of these procedures will allow getting an individual identification pattern, avoiding the consideration of infrequent alleles that may alter the similarity among samples and protected varieties.**