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SSR MARKERS IN BRAZILIAN SOYBEAN

Document prepared by experts from Brazil

1. The characterization of soybean varieties based on morphologic characteristics for registration and plant variety protection (intellectual propriety protection), in Brazil, frequently cannot distinguish between new varieties, and registered/protected varieties. That is because morphological characteristics are limited in number, and present low variability in elite varieties. However, the number of molecular markers is practically unlimited. Molecular markers can identify variability including for elite varieties.

2. Our research group has used SSR markers frequently for soybean variety characterization, including judicious demand for applications for plant variety protection. In our routine, we use genotyping in agarose gels, and automatic sequencer (capillary gel).

3. Agarose gels are less precise, and can only distinguish alleles with more than 10 base pairs difference. But sufficient variability can be observed between Brazilian soybean varieties using agarose gels. It is possible to distinguish soybean genotypes, including varieties with a high level of coefficient of parentage. Using agarose gels, we differentiated two soybean varieties (CD 201 and CD 208), with 99.22% of parentage coefficient (Vieira et al., 2009). In this work, 23 soybean varieties were genotyped with 283 SSR markers, and 111 markers were polymorphic (39.2%). Two markers presented four alleles (1.8%), 25 markers presented three alleles (22.5%), and 84 markers (75.7%) presented two alleles. A set of 53 markers with alleles easily visualized in agarose gels, were used to genotype 53 soybean

varieties. In these set of 53 markers, 15 presented three alleles (28.3%) and 38 presented two alleles (71.7%).

4. Agarose gels are practice to be used in low scale routine. But their low resolution (10bp), does not allow identification of all of the alleles in each marker. But it is possible to differentiate soybean varieties using this genotyping system. One limitation for the use of agarose gel in genotyping SSR, is the impossibility of identifying the length of the alleles, and the results are not comparable from one study to another. One alternative is to use known samples in each gel, as a marker for each allele. In one of our studies (Vieira et al., 2009), the frequencies of all the alleles identified in 53 Brazilian soybean varieties were presented, and one reference variety for each allele. In this case, using new varieties, in another independent study, it is possible to compare with these 53 varieties, only using the reference varieties to identify the alleles. In the same paper, the frequencies for each variety were presented, and can be used to estimate the identity probability between unknown samples.

5. In Oliveira et al (2010), we presented a database of 48 SSR markers used to genotype a set of 32 soybean varieties from Coodetec, genotyped in automatic sequencer (ABI 3130xl). In that study, all the markers were polymorphic, because it was chosen using the previous work (Vieira et al., 2009). One marker (2.1%) was observed with seven alleles, two markers (4.2%) with six alleles, seven markers (14.6%) with five alleles, 15 markers (31.2%) with four alleles, 18 markers (37.5%) with three alleles and five markers (10.4%) with two alleles.

6. To genotype SSR markers in automatic sequencer has the advantage to determine with elevated precision the length of the alleles. The results can be stored and new samples (varieties) can be genotyped in independent work, and the results can be combined, based on these data.

7. The genotyping method depends of the objective of the molecular analysis, and both, agarose gel an automatic sequencer, can be used to distinguish soybean varieties. But for precise characterization, genotyping in automatic sequencer is recommended.

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