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BMT/13/33
ORIGINAL: English
DATE: December 8, 2011

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES, AND DNA-PROFILING IN PARTICULAR

Thirteenth Session
Brasilia, November 22 to 24, 2011

DEVELOPMENT OF AN INTERNATIONAL SEED TESTING ASSOCIATION (ISTA) DNA-BASED APPROACH FOR TESTING VARIETY IDENTITY

Document prepared by an expert from VARCOM

1. Morphological characteristics are used for identification, registration and plant variety protection. This type of characteristics are instable and in an insufficient number and variability. For these reasons, the use of more stable and efficient characteristics as molecular markers has been recommended.

2. One of the most recommended molecular markers for variety identification is the microsatellite (SSR). This marker is multialelic, co-dominat, specie-specific and cover the entire genome. There is sufficient number of SSR markers developed for different crops and they are amenable to automation.

3. The genotyping of varieties can be done using different systems which include agarose, polyacrilamide and capillary eletrophoresis. The first two methods are less expensive while the capillary eletrophoresis requests investment in an automatic sequencer.

4. Molecular markers for variety identification has been used by several breeding and seed companies. Their use has been consider and discussed in the International Seed Testing Association. Reproducibility and validation tests are necessary for a method recommendation.

5. Reproducibility tests have been conducted by the Variety Committee DNA working group in a round of three Comparative Tests (CT's). To this purpose four crops and a set of SSR markers were chosen and tested by several laboratories in different countries (Argentina, Brazil, Canada, France, Italy and Taiwan). Maize, rice, soybean and wheat varieties of breeding programs were used, representing different genealogies. The maize inbreeds used were public ones. In the first CT a set of eight SSR markers in average was used to genotyping eight varieties of each crop. The markers sets were chosen based on the highest polymorphic information content (PIC value). In this round only maize inbreeds were totally differentiated and a set of markers were selected. For wheat null alleles were observed when *T. aestivum* and *durum* varieties were compared. The markers that presented a clear allelic pattern were selected. In the second round the number of varieties of each crop was increased and new markers were included to differentiate rice, soybean and wheat varieties. The same allelic pattern was observed in one soybean variety from Argentina and another one from Brazil. Rice and wheat varieties continue not been totally differentiated. In the third round other laboratories were included (other laboratories from Argentina, Canada and new ones from USA).

6. Different protocols, reactants and genotyping systems (agarose, polyacrilamide and capillary electrophoresis) were used in all rounds, with the purpose to evaluating the results reproducibility.

7. For rice and soybean a fourth CT was conducted as the set markers of each crop was not sufficient to differentiating all varieties. More markers and varieties of new genealogies were included. The results are been processed.

8. Polyacrilamide and capillary electrophoresis presented the most similar results for all variety crops. These two genotyping methods are more precise for variety identification.

9. The high reproducibility of the SSR markers was confirmed. These markers can be recommended for variety identification.

10. Reference materials, statistical analysis and a future proficiency test are been organized.

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