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REPEATABILITY AND DISCRIMINATION POWER OF SSR DATA IN THE VEGETATIVELY REPRODUCED POTATO VARIETIES: IMPACT OF "WEAK ALLELES"

Document prepared by experts from France

REPEATABILITY AND DISCRIMINATION POWER OF SSR DATA IN THE VEGETATIVELY REPRODUCED POTATO VARIETIES: IMPACT OF "WEAK ALLELES."

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1. During the first session of the UPOV BMT Ad Hoc crop subgroup on molecular techniques for potato, held in Poland on June 28, 2004, five documents were presented reporting on the potential of the SSR markers for the potato variety identification and outlined the necessity for harmonization of methods and exchange of data (documents BMT-TWA/Potato/1/2, BMT-TWA/Potato/1/3, BMT-TWA/Potato/1/4, BMT-TWA/Potato/1/5, BMT-TWA/Potato/1/6).

2. The variations observed in DNA profiles for repeated analyses within clones was reported as evidences for intra-clone variation (genetic instability), or for mixture of varieties, or for variety mislabelling. Such factors can be considered only for some samples when variations are observed at several loci, but can not explain variations observed for a large set of varieties on a reduced number of markers. We addressed the built-in variability of the SSR technology *per se* as being most likely responsible for such variations in potato.

3. In this document, repeated analyses of different sets of varieties for the last three successive years are presented.

4. DNA extraction and quantification were carried out on field grown tubers or *in vitro* plantlets. Standard PCR reactions were performed using primers with fluorescent labelling. Amplification products were analysed by capillary electrophoresis on an ABI3100 sequencer (Applera) using a 400bp size standard. The determination of allelic sizes for each sample and each SSR marker was realized using Genotyper (Applera).

5. For each selected set of varieties and markers, a matrix of genetic distances was evaluated (Nei & Li) and the corresponding dendrogram was constructed (Group Average calculation method) using the software Java Genetic Distance (Agrogene).

6. Some alleles were easily scored due to strong signals for some varieties but generated weak signals for other varieties. Furthermore, given a specified allele and a specified variety, different intensities have been observed in different samples or distinct experiments.

7. Then, in repeated analyses of the same variety, few alleles were either present, although intensities were low, or absent. Scores were “1” or “0” accordingly. These alleles, which we have called “weak alleles”, proved to be responsible for SSR built-in, residual, undesirable variability.

8. Different allele intensities may be observed due to several factors: (i) size competition during DNA amplification leading to a weaker amplification of the largest alleles (in base pairs); (ii) differences in the copy number of the different alleles and (iii) variation of the PCR efficiency. Further PCR optimisation may be necessary for some markers in order to increase amplification efficiency and reliability, and to reduce the occurrence of artefacts or stutter bands.

9. In our variety set, the “weak alleles” are observed for a limited number of markers but their frequencies within the variety population are quite high.

10. Seeking the best primer pairs (showing no “weak alleles”) or seeking the right alleles to be scored (no occurrence of weak amplification in any of the varieties analysed), or a combination of both, to improve the repeatability of the SSR analyses, resulted in rapid reduction in DNA polymorphism within the variety pool we analysed. Furthermore, we believe that such an approach might not give any warranty that new “weak alleles” would not be revealed in repeated analyses of larger or different variety pools. Evidence for that is that, although each research group who presented SSR results in the former UPOV-BMT Ad Hoc Potato Subgroup did its best to select a small set of 5 to 10 markers (for repeatability and variety discrimination) from a single original set of SSR markers (Milbourne & al. (1998)), it was quite significant that only very few markers were found in common.

11. For variety identification, then, would be better not to put too much emphasis on discarding these “weak alleles” but rather to accept them for scoring as they are informative. A similarity index threshold can be clearly identified to characterize residual SSR variability inherent in the technology (around 5% for the panel of 7 -10 markers used for this study). We also had nice repeatability in our variety pool when scoring “0” in case of “weak allele” (weak or no amplification in repeated analyses one variety) and “1” in case of a clear-cut amplified allele (“strong allele”).

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