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TOWARDS THE USE OF DNA PROFILES FOR THE IDENTIFICATION AND THE DISTINCTNESS OF THE POTATO VARIETIES

Document prepared by an expert from France

TOWARDS THE USE OF DNA PROFILES FOR THE IDENTIFICATION AND THE DISTINCTNESS OF THE POTATO VARIETIES

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1. During the first session of the *Ad Hoc* Crop Subgroup on Molecular Techniques for Potato (Crop Subgroup for Potato), held in Poznan, Poland on June 28, 2004, the ninth session of the Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular (BMT), held in Washington D.C., United States of America, from June 21 to 23, 2005, and the tenth session of the BMT held in Seoul, Republic of Korea, from November 21 to 23, 2006 information was presented on the potential of different types of molecular markers, AFLP, SSR and SNP, for the analyses of different potato gene pools.

Consensus on the use of DNA technologies

2. Two major applications were considered that could be of interest in the management of the potato collections and the DUS testing.

3. Firstly: the possibility to identify *groups of varieties* that are related to different geographical origins, reflecting different breeding backgrounds. The higher the number of markers used, the higher the significance of the resulting classification. Even within a limited collection of private varieties, groups based on a similarity index can describe (but not always) the genetic relationship such as parent-child, full-sib and half-sib.

4. The groups of varieties based on the above approach did not reflect similarity of phenotypes according to several morphological traits (e.g. skin color, flesh color, maturity group) that used for DUS trials. Furthermore, no correlation was found between a set of AFLP markers and the DUS descriptors for a limited number of varieties (GEVES-unpublished).

5. Secondly: the possibility to DNA-fingerprint each single variety in such an efficient way, whatever the technology used, such that *variety identification* could be unequivocally established through a limited number of selected markers:

- all varieties that passed the DUS trials or clones that had a unique phenotype were clearly and reliably distinguished;
- varieties known to be mutants or genetically modified varieties could not be distinguished from the original variety, but differences in their phenotypes were reported or documented;
- cases of repeated analyses produced slight differences in the DNA profiles of a single variety.

6. These results are not really new. The Max Planck Institute (MPI) (Germany) and the International Potato Centre (CIP) (Peru) published similar results with the “old” RFLP technology in the early 1990’s:

- phylogeny trees of the potato species were nicely built;

- DNA-profiles of 136 varieties of the German variety list were produced. All but six were checked distinct. The six formed three distinct pairs of varieties, each one being

composed of two registered varieties in different years, bred by different breeders: according to the descriptions in the Catalogue, only slight, minor phenotypic differences were observed.

- High frequency of wrong pedigree claims for the potato varieties (personal communication)…

7. Recently, new reports were presented at the 2006 EAPR-EUCARPIA meeting in Carlow (Ireland) that confirmed the previous communications:

- Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) (Germany) made the proposal of a set of SSR markers simultaneously applicable to cultivated, natives and wilds of the Gross Luesewitz potato collection. A few markers are in common with the ones of CIP and SASA.

- The Scottish Agricultural Science Agency (SASA) (United Kingdom) used AFLP to describe the Peruvian origin and the phylogeny of the cultivated potato. In the meantime, they demonstrated that the genetic diversity within the cultivated varieties was as large as the genetic diversity within wild species! They also used both AFLP and SSR in combination to describe the increase of both the genetic diversity and the level of heterozygosity in potato varieties and breeding clones. Using AFLP, minimum genetic dissimilarity (Nei) between two varieties was around 25%, which is quite high!

- The *Instituto Vasco de Investigación y Desarrollo Agrario* (NEIKER) (Spain) used 19 SSR markers to report molecular groups of local potato varieties that reflected more-or-less the different islands of the Canary Islands.

- The University of South Bohemia (Czech Republic) investigated SSR, ISSR and retro-transposon based markers (RBIP) for the identification of potato varieties cultivated in the Czech Republic. Researchers found all the tested methods were utilizable but were of the opinion that, for identification of a wide range of varieties, it would be best to use a combination of molecular and morphological markers.

- The Genetic Resource Centre LVMI (Latvia) tested the resolving power of 15 SSR markers to fingerprint a small set of Latvian cultivars.

- ARC Seibersdorf Research (Austria) reported that SNP genotyping provided additional potential for resolution of genetic variation based on individual specific discrimination of allelic variants.

<u>Reliability and Repeatability of the DNA profiles</u>

8. Other PCR-based markers were previously proposed by the scientific community, but they have not been widely used because of the unreliability of the results (e.g. RAPD).

9. Although RFLP is considered as a basic reliable technology, high costs and low flow have restricted its use to more specific research on DNA.

10. AFLP has had great success in many crops. However, most of the expertise is likely to be located in the private company Key Gene, which owns the patent and runs most of the

works for consortiums of private breeders. Some repeatability problems related to the "digestion" of the DNA by restriction enzymes have been reported. That was also recently reported for the potato crop.

11. Alternatively, SSR markers have been reported as being more reliable because they do not require DNA-digestion. Most of the reports in UPOV meetings did not mention any repeatability problem.

12. However, in potato, weak alleles have been reported to be responsible for a slight variation in the SSR profiles of samples of a single variety. One must also consider that although most of the SSR work on potato used a common set of original primers, only a few of them are shared in common by different teams. Several factors might explain that situation:

- SSR markers used to detect polymorphism are not numerous enough to be representative of the whole potato genome. Therefore, each set of a limited number of varieties will get a "specific" set of markers to reveal this limited DNA polymorphism;

- choice of the technology to reveal the markers, either electrophoresis on acrylamide / starch gels or DNA sequencers, might also play a role;

- DNA amplification variability: inter-allelic competition etc.;

- technical skill may vary from one laboratory to another.

One must acknowledge that there are problems still to be addressed to improve repeatability and reliability of the SSR markers.

<u>Experts are required for the legal implementations of the DNA technologies</u>

13. The fascinating progresses of the DNA technologies that have been made for the last two decades have resulted in the widespread view that DNA markers could serve as the ultimate tools to identify and to ensure distinctness of the varieties for many crops. In the extreme, controlling the genotype at the DNA level could avoid the need for further work on the phenotype!

14. However, it soon became clear that legal consequences of such an approach for the registration of the varieties on the national lists and for the integrity of Plant Breeders' Rights might be deleterious if adequate markers and decision making rules were not properly set.

15. Considerations have been discussed for years, trying to define a unique set of general principles and rules to be used for all crops. Recently, it was agreed that a crop-by-crop approach would be best to make significant progress in solving the variety of questions raised. Indeed, molecular data should be analyzed with respect to the biology and the traditional breeding methods of the crop.

16. Considering potato, one can assume that the probability of a high rate of genetic similarity is zero for two varieties bred by sexual crossing because:

- varieties are tetraploid: epsitasy and dominance help to maintain the allelic diversity; recombinants and genetic diversity of gametes;

- the potato genome is highly heterozygous: a relatively high number of different alleles are found at the same locus;

- there is a large genetic diversity within the cultivated gene pool: similar to the wild gene pool, there are high values of dissimilarity index;

- long recombination breeding cycles: 5-10 years between two meiosis prevents genetic erosion of the germplasm;

- multi-trait screening;

- strong inbreeding depressive effect: breeders will not select for low heterozygosity;

- turn-over of varieties is slow;

- vegetative reproduction of the seed material: genetically fixed.

17. However, we did observe identical or slightly different DNA profiles for a few clones. Those cases were found to be due to:

- mutants;
- genetically modified organisms (GMOs);
- mislabelling or local names;
- misappropriation;
- residual random variability of the technology (artefacts).

18. The first two cases illustrate clearly that *similarity of the DNA profiles does not necessarily imply identical varieties*. Phenotypes are known to differ by at least one trait; therefore, the variety identification and distinctness is not challenged. *However, one has to consider that essential derivation, as defined by UPOV, may be considered at the DNA level.*

19. In the third case, the variety identification and distinctness is not challenged. One has to consider the importance of "cleaning" the breeding and reference collections, and the official registration lists.

20. In the last two cases, the identity or the distinctness (and the ownership) of a variety may be challenged. In particular, the last case that illustrates that d*issimilarity in the DNA-profile does not necessarily imply distinct varieties.* These cases should be a major concern for persons in charge of legal administrations for variety listing, seed certification, intellectual property rights, fraud, commercial litigations etc…

21. However, none of these five cases would have a strong negative impact when the end users of the results are scientists, experts or technicians specialised in the crop, because they will consider other information before reaching a conclusion.

22. From the point of view geneticists and breeders, it is not worthwhile to wait for a perfect DNA technology which would predict the phenotype precisely enough to replace all phenotypic evaluations, because:

- most nuclear DNA has no known function;
- most DNA markers are neutral, being located in no-coding areas of the genome;
- a single gene may have several functions (pleiotropy);
- a single function may require several interacting genes (epistasy) or alleles (dominance);
- undetectable DNA mutations (e.g. a single base pair of an amino-acid) may result in large phenotype variation;
- environment and genotype x environment interactions are constitutive parts of the phenotype.

23. Biochemists and DNA specialists are aware of the built-in strengths and weaknesses of each technology used to produce raw DNA data sets. Each raw data set is nothing more than a "fingerprint" that has some (small) variance due to the variability in a specific biochemical environment and that does not correlate with phenotype. In that sense, it is more like a "DNA phenotype" than a true genotype which the breeders are willing to use.

24. The same is true for the statisticians that are aware of the built-in strengths and weaknesses of each method to analyze these data sets. One obvious point is that the larger is the number of markers, the less will be the relative weight of a slight uncontrolled variation of a single one for the interpretation of DNA profiles. In that respect, AFLP nowadays are likely to be the best technology that we should consider again (patent is not for ever!). SSR may give acceptable results provided that the number of markers is high enough.

25. At the very end of the process, the repeatability and reliability of the DNA analyses will always have a level of doubt that results in risks of misinterpretation. This level may vary according to the technologies and laboratories, but it should not be ignored, underestimated, or overestimated!

26. It has long been acknowledged that the description of the phenotype may be affected by the environment and, therefore, requires accredited protocols and experts of the crop to make DUS decisions. In the same way, most DNA markers should be acknowledged as descriptors which are unrelated to the phenotype and which need accredited protocols and experts of the crop to make the decisions.

27. With that provision, one might consider that DNA markers have sufficient background in the potato crop to be used along with phenotype of expressed genes in variety identification and DUS testing for officials purposes. Bearing in mind that genetic unifrmity and stability are assured by the vegetative reproduction of the seed material, only distinctness needs to be considered:

- Varieties that have passed DUS testing have a low similarity between DNA profiles: databases are meaningful. Low similarity between new and existing profiles might have a high relative weight versus phenotype to assess distinctness of new varieties;

- High similarity between profiles would require further investigations by DUS experts and DNA experts of the potato to check:

  - Repeatability of the DNA profiles;
  - Phenotypes of Mutants or GMOs;
  - Essential derivation

28. What is a low or a high similarity between DNA-profiles should be decided by potato experts, not by computer!

29. The current rule of at least one difference in phenotype for distinctness ought to be kept.

30. The DNA-profiles might help a lot in the management of the potato reference collections to:

- eliminate redundancy and rapidly resolve the current mislabelling;
- ascertain annually the variety identity of controls and material provided by maintainers;
- establish a balance between the various species, origins, old publics varieties etc., to be maintained as living material.

31. DNA-profiles currently provide a weak contribution today to check parentage of varieties when that is desirable, although one might consider that selected AFLP markers might have good potential.

32. Apart from the mutant and GMO cases, as already discussed, the DNA-profiles provide no help to organize the evaluation of phenotypes (no correlation). A small set of phenotypic traits provided by the breeders, which is currently the situation, will continue to be the best way to proceed.

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