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USE OF MOLECULAR MARKERS FOR DISTINGUISHING POTATO VARIETIES

Document prepared by experts from the United Kingdom

Use of Molecular Markers for Distinguishing Potato Varieties

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INTRODUCTION

The Scottish Agricultural Science Agency (SASA), based at East Craigs, Edinburgh, is an executive agency of the Scottish Office Agriculture and Fisheries Department (SOAFD), providing statutory testing and scientific advice for the Government. As part of this role, SASA is the UK centre for distinctness, uniformity and stability (DUS) testing of new potato varieties. This takes the form of two year trials during which 50 botanical characters of candidate cultivars are compared with control cultivars. This results in a description of the candidate cultivar and a statement on its DUS. SASA also co-ordinates trials for value for cultivation and use (VCU) which are carried out in conjunction with NIAB in Cambridge. As the leading UK centre for varietal testing of potatoes, we are interested in the potential use of molecular markers in potato DUS and VCU testing. As potato is a vegetatively propagated crop, it could be argued that it lends itself to this type of technique, since intra-cultivar variation should be non-existent. This paper outlines our views on the use of such techniques, based on work carried out at SASA or elsewhere on our behalf.

At SASA, molecular marker research has looked at the possibility of fingerprinting cultivars and detection of somaclonal mutation. The latter has particular importance for our work, because one of SASA's functions is to propagate nucleus clones of seed potato, which are checked annually for trueness to type. This work is also of direct relevance to DUS because some cultivars that are somoclonal mutants of another have been registered and more may be submitted in the future.

RAPDs

The methodology of RAPDs has been well documented elsewhere, so I will confine my comments to our experiences with the technique.

In common with others, we found that variations in the number or size of RAPD fragments could result from factors such as the model of thermal cycler used, the type of thermostable polymerase, and even the brand of micro-tube. In our experience it is possible to produce consistent results in our laboratory, however the fact that variation can be generated by seemingly trivial factors raises serious questions about the transferability of RAPD results between laboratories.

Work carried out at SASA has shown that a single RAPD primer can distinguish between 6 randomly chosen potato cultivars. However, 145 primers could not distinguish between the cultivars Estima and Famosa. Famosa arose as a somatic mutant of Estima. This illustrates another limitation of RAPDs - and probably other

molecular marker methods - in distinguishing potato cultivars. We were also unable to distinguish between the cultivar Pentland Squire and 7 somatic mutations derived from it. However, other workers have succeeded in finding polymorphisms between some somatic mutations and their parent cultivars (Demeke *et al*, American Potato Journal, **70**, 561-570,1993 and Hosaka *et al*, American Potato Journal, **71**, 535-546, 1994)). This apparent discrepancy may be explained by the fact that somatic mutation can arise through a number of genomic events, ranging from fairly large chromosomal rearrangements to single base pair changes.

The smaller the difference between two genomes, the less chance there is of a molecular marker technique picking up the difference. The odds of a 3 Kb fragment illustrating a 3 Kb change in the genome (2,000,000Kb) is 1/666,666th. A million primers would be required to give reasonable odds of picking up the difference - and some changes reulting in somatic mutation may be smaller than this.

So here we have a situation where phenotypic changes large enough for detection by DUS testing do not have corresponding detectable genotypic changes. This is the opposite problem to that usually raised with regard to molecular markers, i.e. that genotypic differences may not be related to phenotypic ones.

Work is continuing at SASA on assessing the ability of a range of marker techniques to detect a range of different somoclonal mutations. This will include the possibility of detecting somaclonal mutations arising from the activity of transposable elements.

SIMPLE SEQUENCE REPEATS (SSRs)

The problems with RAPDs outlined above have led us to investigate other molecular marker methods. Most importantly, a database of current national list potato varieties is being prepared for SASA by the Scottish Crop Research Institute in Dundee using this DNA profiles based on simple sequence repeats (SSRs). The database will be passed to SASA in 18 months time and will provide a means of distinguishing between most cultivars with results that will be transferable between laboratories. However, it is unlikely that SSRs will improve on the ability to distinguish between somatic mutations and parent cultivars.

GENETICALLY MODIFIED VARIETIES

GM varieties present much the same problems as somatic mutations - small changes in the genome are unlikely to be detected by existing molecular marker methods. As it may be useful to be able to detect whether a tuber sample has a genetic insert, a library of primers is being complied on our behalf, again by SCRI, which will allow us to detect common marker genes.

DISCUSSION

RAPDs, SSRs and other molecular marker techniques can be used to show differences between most potato cultivars. The lack of intra-cultivar variation makes potato an attractive crop for the application of molecular markers. However, the advantage of the lack of intra-cultivar variation is offset by the disadvantage caused

by the use of somoclonal mutants in generation of new potato varieties, which may not be detectable by existing molecular marker methods.

It is unlikely, in our view, that molecular markers will be used to determine distinctness in the near future. This would require agreement on the circumstances in which they could be used and on methodology. We believe that the variation of RAPD profiles between laboratories makes this method particularly unsuitable for this task.

The registration of genetically modified varieties is regarded by some as an instance where the use of nucleic acid based tests may be useful, as it may not be possible to distinguish between the modified cultivar and the parent cultivar using the characteristics usually used in DUS testing. However, where the genetic insert confers a phenotypic trait of agronomic importance, such as pest/disease resistance or increase in content of a particular chemical, it would be better to use a special test for the response, as this will confirm the expression of the insert rather than just its presence in the genome.

Where there may be a role for molecular marker methods is in certification. If a DNA profile was included in the description of a cultivar used in registration, then this DNA profile could be used to check purity during the multiplication stage covered by certification. In the case of stable genetically modified varieties, then this could take the form of the presence of a defined PCR band generated when insert-specific primers were used.

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