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EVALUATION OF SIMPLE SEQUENCE REPEAT (SSR) MARKERS ON THE CANADIAN REFERENCE POTATO DNA COLLECTION

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EVALUATION OF SIMPLE SEQUENCE REPEAT (SSR) MARKERS ON THE CANADIAN REFERENCE POTATO DNA COLLECTION

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INTRODUCTION

1. The Canadian Food Inspection Agency (CFIA) has been involved for quite some time in the molecular identification of potato varieties both at the research and diagnostic level. Requests from CFIA inspectors and program officers for genotyping potato for variety verification purposes, originated due to possible mix up of plantlets in repositories which may have occurred during propagation. Requests also originated during seed certification inspections when the high level of variants was suspected to be due to off types. For each type of requests, morphological characteristics may not be sufficient for identification (plantlets and tuber) and a molecular analysis was sought for confirmation of variety. To respond to these requests occurring since the mid-nineties, research projects were initiated on the development of molecular identification of potato varieties which resulted in the production of a reference collection of DNA extracts representing some 217 potato varieties from Canadian repositories. Most of the reference DNAs was extracted from reference plantlets originating from 2 sources (150 varieties). Using this DNA collection, a reference collection of AFLP fingerprints (Amplified Fragment Length Polymorphism) representing the 217 potato varieties was generated as well. In the absence of characterized SSR markers, AFLP has been a very powerful method for distinguishing varieties. However the technique (AFLP) is more challenging technically than microsatellites (SSRs) and therefore not very suitable for high throughput or harmonization of the method from one laboratory to another. The testing and analysis is also very time consuming.

2. In 2006, the Community Plant Variety Office of the European Union (CPVO) funded a project with the purpose of constructing an integrated database that would include microsatellite genotypes and morphological characteristics specific to potato varieties in the European Union (EU) Common Catalogue. The four partners involved are responsible for DUS testing in Germany, the Netherlands, Poland and the United Kingdom. At the end of this project, approximately 900 varieties of potato were collected and genotyped using 9 microsatellite markers. Nearly all varieties (99.5%) had a unique genotype, except mutants (Document BMT/11/9). Both laboratories involved in the analysis scored identical genotypes for almost all varieties. The few differences were usually linked to a different interpretation of the presence/absence of an allele (Document BMT/11/9).

3. Canada proposed to further evaluate the markers that were used successfully by the EU group with the Canadian potato DNA collection at CFIA. The data obtained from this project will provide information to help evaluate whether the use of DNA markers could possibly be used to supplement or replace phenotypic characteristics in the distinctness assessment in the future. The data from this work will also provide scientific information to assist in establishing international guidelines for the management and harmonization of data sets of molecular information for potatoes. Furthermore, the microsatellite genotype dataset representing potato varieties registered in Canada can be used instead of live material to support the requirements of the Canadian Variety Registration Office - CFIA Seed Program.

In addition, the SSR markers will provide an improved potato varieties identification method allowing delivery of diagnostic results in a timely manner during the Canadian Seed Certification process.

MATERIALS AND METHODS

4. For each of the potato varieties tested, DNA was extracted from *in vitro* plantlets using the CTAB method (Doyle and Doyle, 1990). Most plantlets received from the PhytoDiagnostics (British Columbia, Canada) were extracted in 2002-03 and those from the Plant Propagation Centre in Fredericton (New Brunswick, Canada) were extracted in 2004. The DNA collection contains 150 varieties originating from both sources and 67 varieties originating from either repository. The method by Reid et al. (2008) describing the use of 3 sets of 3 microsatellite (SSR) markers from DNA extraction to identification was followed with a few modifications. The modifications were mostly at the level of DNA extraction method, relative concentration of primers, thermocyclers, TAQ enzyme and ABI DNA analyzer running conditions. The latter was already encountered by the Netherlands and the United Kingdom groups resulting in slight size differences of the PCR products, therefore the assignment of code letters was developed for scoring alleles instead of scoring using base pair length.

5. In a first step, a series of reference DNA extracts representing selected varieties common to Canada and the United Kingdom were selected for comparison purposes. A set of 34 potato varieties were tested after optimization of the method for our laboratory.

6. In a second step, DNAs extracted from all other Canadian varieties not already tested were genotyped (for a total of 217 varieties). Alleles were scored in a binary format and imported into BioNumerics (Applied Maths) for analysis (Document BMT/11/10).

RESULTS AND DISCUSSION

Analysis of 34 varieties common to the United Kingdom and Canada.

7. The method was quite reproducible as genotyping results were matching closely for each variety tested with the exception of 4 pairs. Of the 4 pairs, there were 2 United Kingdom varieties that were suspected to be of another variety; one pair was believed to be two different varieties registered at different times but with the same name. The discrepancy of the last pair scored was not resolved. Currently the method relies on a total of 95 possible alleles to be scored. Analyzing the genotyping data further, there were discrepancies between the two labs in scoring 3 alleles (E and H for marker 0019 and D for marker 3009) again due to different interpretations of the presence/absence of alleles. We will have to investigate if the DNA extraction protocol or DNA quality could be generating these differences and/or set a rule for calling these particular alleles.

Analysis of the Canadian collection of DNA representing 217 potato varieties

8. The 9 microsatellites provided a means to differentiate most of the Canadian reference potato varieties except for 8 (6 confirmed by two source materials) groups that generated identical genotypes. It is likely that the groups are of common parent or mutants, but this remains to be investigated as some of the varieties were from one origin and therefore not confirmed; mislabelling is a possibility as well. Furthermore, there are 3 other pairs (2 confirmed by two sources) segregating together at 97% similarity with only one allele

difference. These alleles are ones for which we, and other groups, had different interpretations of the presence/absence scores leading us to think that these varieties might be of identical genotypes once these differences in allele scoring are addressed.

9. Of the 150 varieties for which there was reference DNA from two sources, there were some variety genotype differences observed between the two sources which we mostly resolved by re-sampling and testing again. However, there are 3 varieties for which each source segregates on its own and 4 varieties for which one source genotype is identical to another variety but not the other source. All 7 situations remain to be investigated further. Furthermore, 4 pairs differed by 1 or 2 alleles between the two sources. It remains to be determined if these differences are due to a possible variation in the determination of presence/absence of alleles.

CONCLUSIONS

(a) As previously demonstrated by the EU laboratories, the SSR marker method was successfully used by the Canadian laboratory as most genotypes generated were identical.
(b) The method successfully differentiated 217 potato reference DNAs of the Canadian collection, except for 8 groups which are most likely mutants or have common parents
(c) Most intra-varietal variations were resolved by sub-sampling; remaining cases need to be investigated.
(d) The method was already successfully used to fulfill an official agency genotyping request.

Next steps

- Investigating if DNA extraction procedure and/or DNA quality influence some of the allele presence/absence scores or relative intensities.
- Establish rules for scoring some of the alleles to further harmonize the allele scoring between the laboratories and intra-variety variations.

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