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BMT/12/11
ORIGINAL: English
DATE: April 7, 2010

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

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

Twelfth Session
Ottawa, Canada, May 11 to 13, 2010

EVALUATION OF SIMPLE SEQUENCE REPEAT (SSR) MARKERS FOR IDENTIFICATION OF PEAS VARIETIES REGISTERED IN CANADA

Document prepared by experts from Canada

EVALUATION OF SIMPLE SEQUENCE REPEAT (SSR) MARKERS FOR IDENTIFICATION OF PEAS VARIETIES REGISTERED IN CANADA

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INTRODUCTION

1. Peas are an economically important pulse crop for Canada and it is increasingly difficult to identify varieties in the field, therefore additional tools for this task would prove to be valuable to the Canadian Food Inspection Agency (CFIA). Currently in Canada, during Seed certification inspections, complaint and inquiry samples are sent by the CFIA inspector for identification by the Ottawa plant lab, Seed Science Unit. The samples are then tested in field plots for identification using morphological characteristics. However, as the number of varieties increases, so does the difficulty of identifying varieties, variants, off-types and impurities based on these morphological characteristics. Therefore a molecular method was sought by the Canadian Food Inspection Agency for confirmation of pea varieties as well as to provide more rapid responses to these complaints and inquiries. SSRs, or microsatellite markers, for peas have been described (Loridon *et al*, 2005) and some were used to analyze the genetic diversity of pea varieties available in Canada (Tar'an *et al*, 2005). Therefore, to address the need for pea varieties molecular identification, our laboratory has selected a series of microsatellite markers described by Loridon *et al* (2005) to test their efficacy to differentiate all varieties of peas currently registered in Canada (approximately 110 varieties).

2. It was then proposed to further evaluate the use of microsatellite markers for the differentiation of varieties of peas. 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 peas. Furthermore, the microsatellite genotype dataset representing pea varieties registered in Canada can be used in addition to reference seed to support the Canadian Variety Registration Office - CFIA Seed Program. In addition, the SSR markers will provide a pea variety identification method allowing delivery of diagnostic results in a timely manner during the Canadian Seed Certification process.

MATERIALS AND METHODS

3. Reference seeds representative of all pea varieties registered in Canada were obtained from the Ottawa Plant Lab Seed Science Unit, which manages the reference seed sample submitted by the breeder (or representative) at the time of registration. For each pea variety tested, DNA was extracted from half a seed using Qiagen DNeasy kit. Two sets of 5 individual seeds were extracted for each variety for a total 10 individual extracts per variety (110 varieties in total).

4. In a first step, 38 markers were selected on quality, number of alleles, and sizes for possible multiplexing. Eleven varieties were used to test these markers and, from there, a final set of 12 markers were selected for their high quality signal, ease of scoring, high number of alleles and their robustness.

5. In a second step, all DNA extracted from 10 individual seeds from all pea varieties registered in Canada (110 varieties) were tested using the 12 selected markers multiplexed in 2 sets of 6 markers using Phusion (New England Biolabs) enzyme. The PCR products were run on a capillary DNA analyzer ABI 3100 (Applied Biosystem). Alleles were scored in a binary format in Access and imported into BioNumerics (Applied Maths) for analysis.

RESULTS AND DISCUSSION

6. Currently, the 12 markers produce a total of 130 possible alleles to be scored. The range of number of possible alleles per markers was 3 to 19. All alleles were scored by the analyzer software. Generally there is one allele seen per marker for each sample but very rarely two alleles were recorded (0.05%). Analysis of 10 individuals representing 110 reference pea varieties registered in Canada showed that all varieties can be distinguished using the 12 markers, except 4 groups. However, many varieties displayed a broad range of genotypes but generally segregating together therefore allowing the use of this set of markers for variety identification. Of the 4 groups of varieties that could not be differentiated, 2 groups of 2 varieties shared the same genotype(s), therefore were indistinguishable from each other; 1 variety had one of its two genotype variants identical to another variety and another variety had 2 of its 4 genotype variants identical to 2 different varieties. It is unknown at this point if we are looking at mutants or common lineage. Mislabelling could also be a possibility, but is unlikely. It remains to be investigated further.

7. The genotypes generated per variety were often complex as many individuals had a unique allele set but still segregated within the variety. Of the 110 varieties tested, 50 presented a main genotype from which 16 had 1 or 2 individuals with a unique genotype. Some 28 varieties presented 2 main genotypes with one or 2 individuals with a unique genotype. The remainder of the varieties (32) presented more genotype variants as the level of unique genotype increased. No less than 10 varieties have shown at least 7 different genotypes. We have examples of varieties that present as many genotypes as the number of individual tested (10/10 and 12/12). Furthermore, more individual seeds or plants were tested for 2 highly variable varieties and it was possible to record 16 genotypes for 20 individuals and 25 genotypes on 92 tested individuals.

8. The percentage of similarity varies greatly between varieties and within varieties. One variety can be as close as 92% similar to another variety and an individual of the same variety can have a similarity as low as 61%.

CONCLUSION

- The SSR markers method could successfully differentiate pea varieties as most genotypes generated were co-segregating.
- The method successfully differentiated 110 varieties of peas registered in Canada except 4 groups;
- It is not yet determined if more markers are needed to further differentiate the 4 groups
- Most intra-varietal variations do co-segregate.
- The method has already successfully being used to fulfill genotyping requests from the CFIA Seed Science unit.

Next steps

- Investigate further those varieties that co-segregate.
- Prepare the procedure for future transfer of the method to other laboratories.

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