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ADDENDUM

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

Document prepared by experts from Canada
Evaluation of simple sequence repeat (SSR) markers on the Canadian reference potato DNA collection

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Background

• Project carried from 2006 to 2008 by 4 DUS testing stations;
  • SASA United Kingdom
  • BSA, Germany
  • COBORU, Poland
  • Naktuinbouw, the Netherlands

• Resulted in the collection of morphological descriptions representing 733 varieties
Background

• UK and NL had selected 9 microsatellite markers and generated DNA reference samples and SSR profiles representing 900 varieties

• Not counting known mutants, only 8 pairs with identical profile were observed
  • 3 of which were suspected to be either mislabelled, the same or mutant
  • 5 unexplained

In Canada...

• Plant Breeders rights are granted on DUS data provided by the applicant – no government DUS station

• CFIA Ottawa Plant Laboratory receives potato tubers, leaves or in vitro plantlets for variety verification.
  • Possible mix up during propagation of plantlets
  • Seed certification inspections

• Since 1997 CFIA is using a molecular method (AFLP) for the verification of potato varieties.
Potato variety verification using AFLP

Canadian potato reference DNA collection

- Reference DNA was produced for the validation of the AFLP technique
  - 150 potato varieties originated from two sources
  - 26 and 41 varieties originated from one of the two sources
  - > 700 reference DNA extracts representing 217 varieties
  - DNA extraction procedure CTAB

- Two DNA samples per variety per source were extracted and tested using AFLP.
  - When there was a discrepancy between the fingerprints of the same variety, another set of plantlets was requested from both sources.
Why SSRs?

- Existing potato reference DNA collection
- For testing, SSRs are:
  - Easier to transfer for one laboratory to another
  - Easier and faster to use for diagnostics than AFLP
  - Easier to automate the scoring process
- Another step toward international harmonisation of molecular identification of crop varieties
- Very promising results as demonstrated at the BMT11

Objectives

- To help evaluate the use of DNA markers to possibly supplement phenotypic characteristics in the distinctness assessment of the future.
- Assist in the establishment of international guidelines for management and harmonization of molecular information for potatoes
- The SSR profiles representing potato varieties registered in Canada can be use as reference instead of live material to support the VRO
- The SSR markers will provide an improved method for the identification of potato varieties
Method

• List of SSRs used
  - 0019 *
  - 2005 *
  - 2028 *
  - 3009 *
  - 3012 *
  - 3023 *
  - 5136 **
  - 5148 **
  - SSR1 ***

* Milbourne et al. 1998
** Ghislain et al. 2004
*** Kawchuk et al. 1996

1st step
- Optimize, test and compare the data from a set of reference varieties common to UK and Canada (34 varieties)

2nd step
- Test all reference DNA extracts from the Canadian reference collection representing 217 varieties
Analysis of 34 varieties common to the United Kingdom and Canada

• All SSR profiles generated from the Canadian references DNA matched closely the U.K profiles
• There were 4 pairs that were not matching
  • 2 were explained by the fact that the U.K. samples were matching other varieties in their database.
  • 2 were cases of different varieties with the same name but registered at different time.
• Overall discrepancies for the presence/absence scoring on 3 alleles
  • E and H for marker 0019
  • D for marker 3009

Analysis of the Canadian reference collection representing 217 potato varieties

• SSRs differentiated most of the varieties from the Canadian DNA collection
  There were 11 groups that had identical profiles
  • 6 pairs and 1 group of 5 suspected or confirmed to be mutants
  • 1 pair and 1 group of 3 shared common parents
  • 2 pairs unexplained

  There was 1 pair segregating together with 97% similarity corresponding to one allele differences
Analysis of the Canadian reference collection representing 217 potato varieties

- The reference DNA of the 150 varieties from 2 sources mostly generated identical profiles
- There are 2 varieties for which there was 2 profiles corresponding to 1 allele difference
- There were 7 situations where the profile was different between the two sources.
  - 4 pairs from which, one of the two sources was identical to the profile of another varieties and potentially mislabelled.
  - 3 pairs discrepancies still unresolved

Conclusions

- The SSR method established by the EU laboratories was successfully used by the Canadian
- The method differentiated 217 varieties in the Canadian reference DNA collection except 9 confirmed groups
  - The 7 groups are likely mutant and 2 have common parent(s)
Conclusion

- Further investigation is required to assess DNA extraction procedure relative to presence/absence or relative intensities of certain alleles
- Further investigation is required to establish rules to further harmonize the allele scoring
- The method was successfully used to fulfill Canadian Food Inspection Agency official genotyping request

References

- BMT/11/10 (2008) Construction of an integrated microsatellite and key morphological characteristic database of potato varieties on the EU common catalogue. Part II: The database
- Kawchuck et al. (1996) Molecular and General Genetics 259: 233-245