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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

 Canadian Food Inspection Agency / Agence canadienne d'inspection des aliments

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Evaluation of simple sequence repeat (SSR) markers on the Canadian reference potato DNA collection

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Canada


Our vision:
To meet as a consumer-based regulator, trusted and respected by Canadians and the international community.

Our mission:
Dedicated to safeguarding food, animals and plants which ensures the health and well-being of Canada's people, environment and economy.

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



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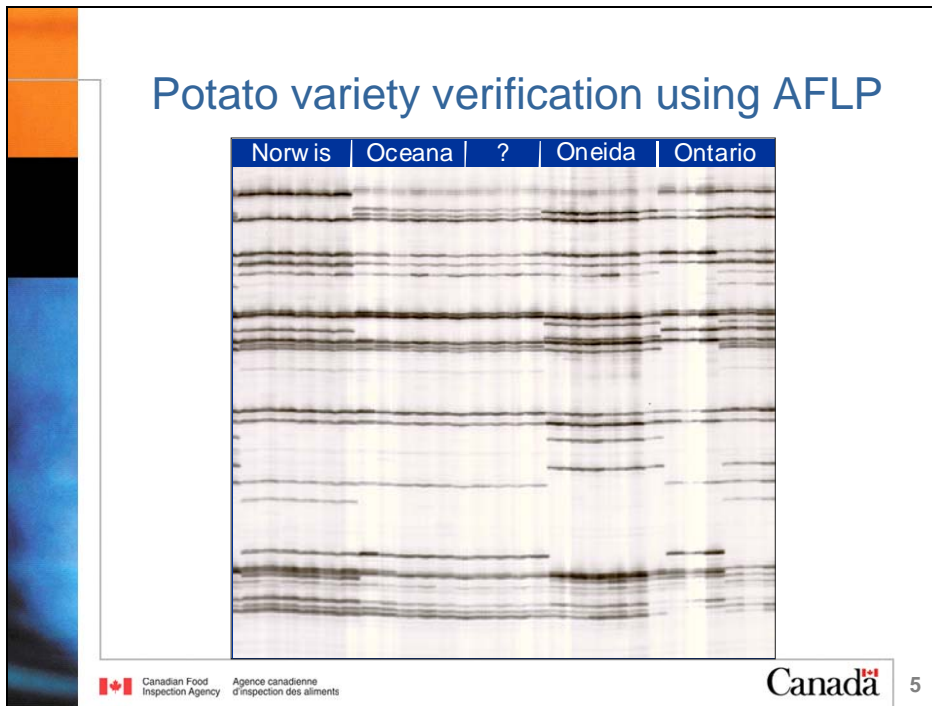
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Background

- 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.



- ### 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.
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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




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Method

- 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

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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

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