Molecular markers as a tool for plant breeding and variety identification

University of South Bohemia in České Budějovice
Faculty of Agriculture
Biotechnological Centre
Faculty of Agriculture

Alena Nováková, Kateřina Šimáčková, Božena Kukolíková, Vladislav Čurn

Biotechnological Centre, University of South Bohemia, České Budějovice, Czech Republic

Molecular markers in potato variety identification

The traditional approach to variety identification is composed of the observation and recording of a range of morphological characters or descriptors. Such an approach is undoubtedly successful for DUS testing. However, it is less suitable when results are required rapidly, such as for the confirmation of tuber material identification. Furthermore, morphological characters are often multigenic, continuously expressed and influenced by environmental interactions, making it difficult to assess them quickly and objectively, and requiring replication of observation. Together with advances in molecular biology, several new molecular and biochemical marker techniques will be adopted. These techniques are a powerful tool for determining genetic distinctness and enable characterization of particular genotypes. Use of molecular markers for variety improvement of agricultural crops has been widely applied in the last decade to exactly determine genetic variation based on DNA analysis
.

In the year 2007 there were one hundred and seventy eight potato varieties present in the Czech List of Registered Potato Varieties and we need powerful tool for variety/tuber identification because the significant law no.110/1997 Sb. requires guarantee of variety declaration in commercial relation for food potato in Czech Republic.

Plant material. Selected set of registered potato varieties was use for analysis respectively Adéla (7), Adora (1), Agria (13), Asterix (18), Colette (2), Dali (8), Desirée (19), Ditta (14), Filea (15), Impala (3), Karin (9), Laura (16), Magda (4), Marabel (10), Rosara (5), Samantana (20), Santana (11), Secura (12), Solara (17) a Velox (6).

The DNA extraction was made from tuber juice by the Invisorb Spin Plant Mini Kit (INVITEK).

PCR-SSR analysis. SSR analyses were done using primers STM 2005, STM 1102, STM 3012, STM 1106, STM 3015, STWIN 12G and STG BBS.
PCR-ISSR analysis. ISSR were detected using primers P1, P2, P3, P4 a B1.
PCR-IRAP analysis. For PCR-RBIP analyses were chosen primers Tst101, 0š and 06.

The analyses were done according to the standard protocol – Biotechnological centre, Agriculture faculty, University of South Bohemia (http://www.eamos.cz/amos/bc/modules/low/kurz_obsah.php?kod_kurzu=bc_154)

Conclusion

We obtained patterns of seven SSR and three retrotransposons based markers for the set of twenty selected varieties registered in CR. The obtained polymorphism was appraised and the varieties were separated to the categories by the electrophoretic phenotype. Analyse of microsatellites (SSRs) is suitable method for rating variability and variety identification, however, the low detected polymorphism in individual SSR loci is disadvantage, ISSR analyse afford larger polymorphism, but using this method is connected with relevant disadvantage as is the instability of band pattern depending on age of DNA. IRAP markers generate sufficient level of polymorphism and allow distinguish all individual genotypes. This approach, utilization of retrotransposone-based markers, is utilizable for screening of large sets of potato samples but has also specific requirements.
Most of tested methods are utilizable for variety identification. But for wide range identification of variety it seems to be more suitable to use the complex set of molecular and morphological markers and descriptors.

The aims of this study was to introduce two model application of molecular markers:
(1) molecular markers in potato variety identification,
(2) molecular markers as selectable markers on hybrid breeding in oil seed rape.

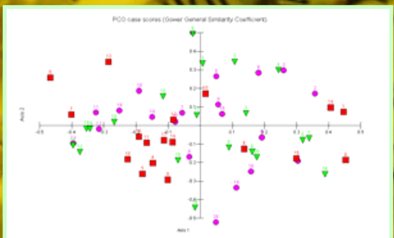


Fig. 1: Results of principal coordinates analyses – PCO plot obtained after analyses of 7 SSR markers (red square) and 3 retrotransposon based markers ISSR markers (green triangle) and we tested whole set of primers together too (lila circle).

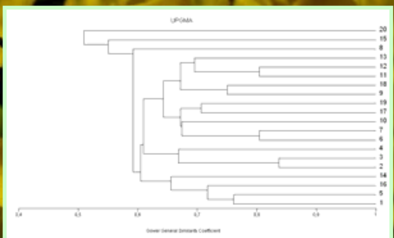


Fig. 2: Results of cluster analyses obtained after analyses of set of SSR and retrotransposon based markers.

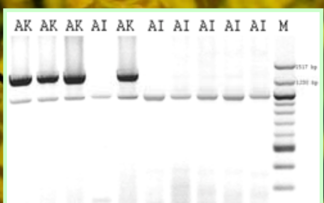


Fig. 3: Amplification of *SLG I* gene and segregation of SI and SC plants.
AI = SI, AK = SC

Molecular markers as selectable markers on hybrid breeding in oil seed rape

A segregating doubled haploid (DH) populations of oilseed rape (*Brassica napus*) was derived from four crosses between self-compatible (SC) cultivar 'Lisek' and self-incompatible (SI) line 'AIK 6', SC cultivar 'Rasmus' and SI line 'AIK 6', SC cultivar 'Rasmus' and SI line 'AIK 3', and finally SC line 'OP BN-03' and SI line 'AIK 3'. 'AIK 3' and 'AIK 6' SI lines were derived from SI line 'Tandem' with recessive type of self-incompatibility. This population was consisted of 118 plants.

Genomic DNA was extracted from young leaves of 2-week-old seedlings by the Invisorb Spin Plant Mini Kit (INVITEK).

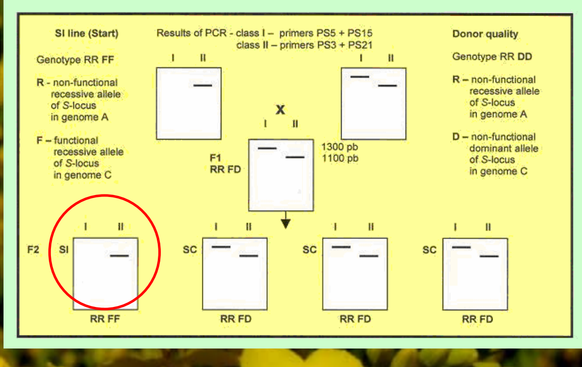
The PCR reaction was performed with class-I SLG-specific primers PS5 and PS15 (Nishio et al.1996). SCR gene was amplified with class-II SCR-specific oligonucleotide primers designed for functional allele originating from SI line 'Tandem' (5′-TTGGACTTTGACATATGTTC-3′ and 5′-CTCTGAAGTGG GTTTTACAG-3′).

Two marker genes were used for SI plants selection. PCR with class-I SLG-specific primers has resulted in approximately 1300 bp fragment. This fragment was specifically present in plants considered to be self-compatible. This marker gene has been detected in spectrum of naturally self-compatible oilseed rape cultivars whereas in self-incompatible lines not. The second marker system specifically targets allele of class-II SCR gene. This allele was found in self-incompatible lines derived from line 'Tandem'. Amplified fragment of class-II SCR gene allele was 280 bp long and specifically occurred in plants considered to be self-incompatible. The two marker systems segregated in ratio 1:1 as was expected and they exactly correlated each other.

Conclusion

The two marker genes were used to select self-incompatible plants from segregating doubled haploid populations of oilseed rape. The S-locus specific marker, allele of class-II SCR gene, and the universal marker, class-I SLG gene, exactly correlated with segregation ratio of self-incompatibility in doubled haploid population. Both marker systems would be used for marker-assisted selection in hybrid oilseed rape breeding. Model of utilization of molecular marker in selection of SI plants in hybrid breeding of oil seed rape was proposed.

Fig. 4: Proposed model of utilization of molecular marker in selection of SI plants.


SI line (Start)
Results of PCR - class I – primers PS5 + PS15
class II – primers PS3 + PS21
Donor quality
Genotype RR FF
R - non-functional recessive allele of S-locus in genome A
F – functional recessive allele of S-locus in genome C
Genotype RR DD
R – non-functional recessive allele of S-locus in genome A
D – non-functional dominant allele of S-locus in genome C
I II
X
F1
RR FD
1300 pb
1100 pb
F2
SI
SC
SC
SC
RR FF
RR FD
RR FD
RR FD

This study was supported by grants Grant Agency of the Czech Republic 521/08/H042, NAZV 1G46061, NAZV 1B44011 and MSM-60076658-06.