Molecular markers as a tool for potato varieties identification

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INTRODUCTION

Cultivated potato (*Solanum tuberosum* L.) belongs together with wheat, rice and maize to the tetrad of the most valuable world crops and it is the fourth most important food crop worldwide. Its importance can be seen not only as food crop, but potato is also widely used for livestock feeding and for industrial processing as feedstock for many industrial and food applications. Currently, there are more than 3,200 different potato varieties that are cultivated in over 100 countries all over the world (Hemester and Hils, 2003). In the year 2005 there was one hundred and fifty potato varieties present in the Czech list of registered potato varieties, over 70% of them come from European breeding subjects.

The classical morphometric characterisation is not effective for this number of varieties especially for identification at the level of tubers. Identification of potato varieties play the main role at all stages of potato cultivation and breeding and it is very important for breeders, growers, registration institution and trading companies. Czech law no.110/1997 Sb. requires guarantee of variety declaration in commercial relation for food potato.

In this study we analysed twenty potato varieties (*Solanum tuberosum* L.) by several methods as PCR-SSR, PCR-ISSR and PCR-RBIP. The goal of this study was the design of a molecular marker set for identification of potato varieties cultivated in the Czech Republic. We discovered that all of tested method are utilizable for variety identification, but for identification of wide set of varieties it seems to be more suitable to use the complex set of molecular and morphological markers.

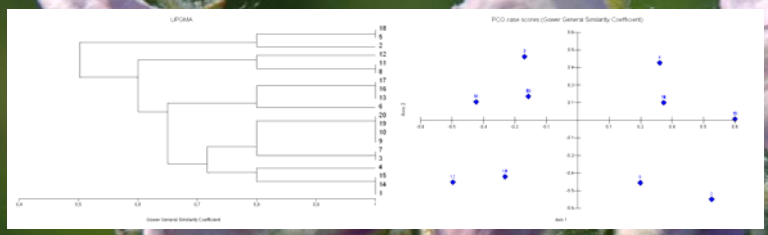

Fig. 1: Results of cluster (a) and coordinates analyses – PCO analysis (b) obtained by analyses of 5 SSR markers.

SSRs analyses. Polymorphism of SSR markers (Simple Sequence Repeats) was observed for twenty selected varieties after amplification with five primers pairs. Statistical evaluation appears from matrix of presence polymorphic bands. It was evaluated 5 from 10 possible positions of bands. Using of this method permit discrimination of 4 from 20 varieties (Colette (2), Magda (4), Velox (6) and Agria (12)).

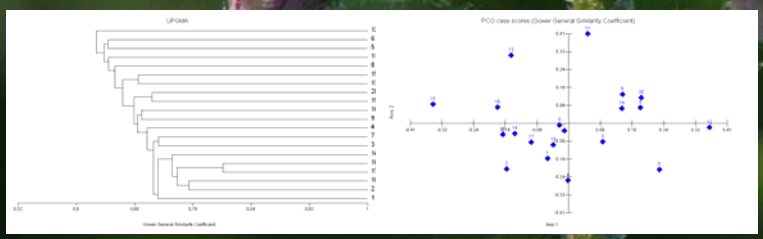

Fig. 2: Results of cluster (a) and coordinates analyses – PCO analysis (b) obtained by analyses of 5 ISSR markers.

ISSRs analyses. *Polymorphism of ISSR markers (Inter Simple Sequence Repeats) was observed for twenty selected varieties after amplification with five primers. Statistical evaluation appears from matrix of presence polymorphic bands. It was evaluated all 216 possible positions of bands. Using of this method permit discrimination each of varieties. The similarity was 65 – 80%.*

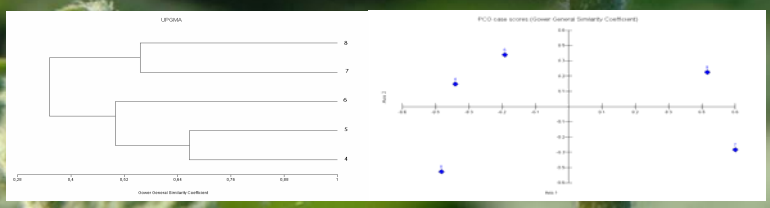

Fig. 4: Results of cluster (a) and coordinates analyses – PCO analysis (b) obtained by analyses of selected samples by RBIP marker.

RBIP analyses. The RBIP (Retrotransposon Based Insertional Polymorphism) analyse is relatively new. We are optimizing this method and the first results shown this method like useful. We expect the high level of polymorphism obtained by using this method. Bežo *et al*. (2006) got a sufficient level of polymorphism and products uniqueness, both suitable for potato and flax germplasm analysing and evaluating.

RESULTS

The whole data set obtained by the SSR, ISSR and RBIP analyses was evaluated by standard statistical methods. Figures 1 - 5 present the results of cluster analysis (UPGMA method) and coordinates analyses (PCO analysis). Genetic similarity was provided for each of samples.

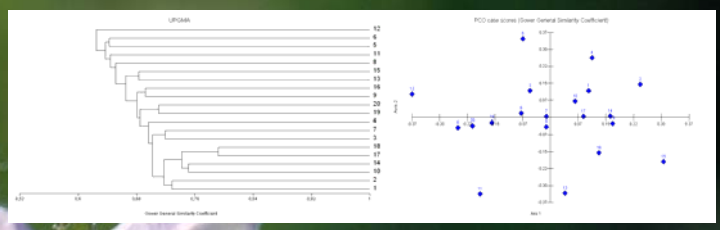

Fig. 3: Results of cluster (a) and coordinates analyses – PCO analysis (b) obtained by analyses of SSR and ISSR markers.

SSR and ISSR. Statistical evaluation appears from matrix of presence polymorphic bands obtained in both analyses. It was evaluated 221 from 226 possible positions of bands. Using of both this method permit discrimination each of varieties. The similarity was 62 – 80%.

Rate of difference among samples/varieties is sufficient and it enables explicit identification. Analogous order has the result of PCO analyses, there are not evident clusters of varieties and the individual samples are good distinguished. It is evident, the pattern of distribution SSR and ISSR markers is different. These two molecular markers systems appear as independent.

CONCLUSION

We obtained pattern of five SSR and five ISSR markers for the set of twenty selected varieties from the total set of 150 varieties registered in CR in the year 2005. It was appraised the obtained polymorphism and the varieties were separated to the categories by the electrophoretical phenotype. The methods of analyses of microsatellites (SSR and ISSR) are suitable methods for rating of variability and variety identification, however, the low detected polymorphism in SSR is disadvantage. These problems can be resolved by using of the other separating methods e.g. 10% PAGE or 3% Synergel.

Another suitable approach is the using of RBIP analysis. We are optimizing IRAP and AFLP analyses at present.

It is suitable using more molecular, morphological and biochemical markers for the prospective reliable distinguish of whole set of potato varieties registered in Czech Rep.

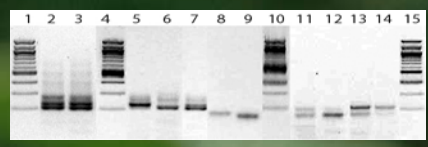

Fig.5. Example of electrophoreogram-3% agarose gel. SSR analyses: 2-3 primers pair STIIKA varieties Samantana and Secura, 5-7 primers pair STM 1052 varieties Impala, Magda and Secura, 8-9 primers pair STWIN12G varieties Marabel and Santana, 11-14 primers pair STM 1102 varieties Magda, Rosara, Karin and Santana, 1,4,10 and 15 DNA ladder marker 100bp.

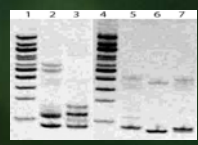

Fig.6. Example of electrophoreogram-10% PAGE. SSR analyses: 2-3 primers pair STM 1106 varieties Dali and Karin, 5-7 primers pair STWIN12G varieties Dali, Karin and Marabel, 1 and 4 DNA ladder marker 100 bp.

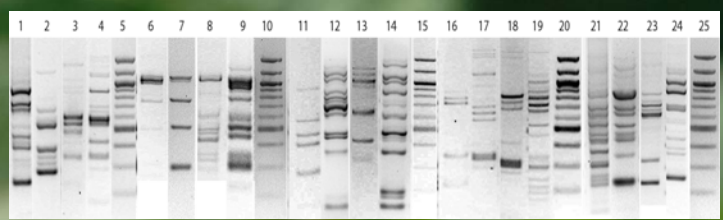

Fig.7. Example of electrophoreogram-2% agarose gel. ISSR analyses. 1-4 primer P1 varieties Adora, Colette, Marabel and Desireé, 6-9 primer P2 varieties Adora, Colette, Marabel and Desireé, 11-14 primer P3 varieties Adora, Colette, Marabel and Desireé, 16-19 primer P4 varieties Adora, Colette, Marabel and Desireé, 21-24 primer B1varieties Adora, Colette, Marabel and Desireé, 5,10,15,20 and 25 DNA ladder merker 100bp.

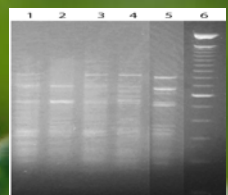

Fig.8. Examples of electophoreogram-2=agarose gel. RBIP analyses: 1-5 primer STS106 varieties Magda, Rosara, Velox, Adéla and Dali, 6 DNA ladder marker 100bp.

MATERIAL AND METHODS

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) and Velox (6). Material was rendered by ÚKZÚZ Lípa u Havlíčkova Brodu.

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

PCR-SSR analysis. For PCR-SSR analyses were chosen primer pairs STM 2005, STM 1102, STM 3012, STWIN and STG BBS.

PCR-ISSR analysis. For PCR-ISSR analyses were chosen primers P1, P2, P3, P4 a B1.

PCR-RBIP analysis. For PCR-RBIP analyses was chosen primer Tst106.

The analyses were done according to the standard protocol – Biotechnological centre, Agriculture faculty, University of South Bohemia (http://www2.zf.jcu.cz/public/departments/lenc/laboratory/metod_mb.pdf)

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