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BMT-TWA/Potato/1/5
ORIGINAL: English
DATE: June 16, 2004

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR POTATO

First Session
Poznań, Poland, June 28, 2004

OVERVIEW OF EXPERIENCE GAINED IN FRANCE ON THE USE OF MOLECULAR MARKERS FOR SEED POTATO CERTIFICATION AND RESPECTIVE INTEREST OF SSR MARKERS AND VISUAL INSPECTION

Document prepared by experts from France

Key words: Genetic fingerprint, Microsatellites, Polymorphism, *Solanum tuberosum tuberosum*

Abstract

The certification of seed potatoes guarantees both health quality and the characteristics of a variety; so, it implies to check both the identity and the purity of the seed lots. The identification of varieties is currently still based on phenotypic characters but molecular markers may be used as complementary tools or to quickly check the identity of a variety. Different techniques have been tested on potato in France and Simple Sequence Repeats (SSR) markers appeared to be the most adapted to such purposes. The genetic fingerprints obtained with five SSR loci allowed discrimination between all the 207 tested varieties but were not able to distinguish variants. The patterns are registered in a database. The reproducibility of SSR markers has been assessed by ring tests and the technique has been officially approved and should be introduced in the certification scheme in combination with field inspections.

Introduction

The continuous increase in the number of varieties (over 200 potato varieties are registered in France) represents a high risk of exchange and mixing of lots. Despite these risks, identification is still based upon morphological characters (shape and colour of the tuber, sprout colour, growth habit, etc…) that are often difficult to assess and sometimes vary due to environmental influences. All these reasons argue for a reliable tool for variety identification.

Identification may be based on the polymorphism of isozymes and/or total proteins, but the data may be influenced by the developmental stage of the plant as well as by growing conditions. Furthermore, the patterns must be compared to a control.

For years, several molecular approaches have been evaluated for potato variety diagnosis, including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), inter simple sequence repeats polymerase chain reaction (ISSR-PCR), amplified fragment length polymorphism (AFLP) and simple sequence repeats PCR (SSR-PCR).

Potato is vegetatively propagated by rapid *in vitro* methods and a key point is to check the varietal conformity from the first generations of prebasic seed. As the phenotype may be physiologically or genetically altered by *in vitro* culture, it may be difficult to check the morphological traits from the 1st year and complementary tools such as molecular markers would also be useful at this stage.

Presentation of the studies on potato identification by molecular markers

This paper presents the results of the experience obtained recently in French seed potato laboratories on the subject. RAPD markers were first tested to distinguish potato varieties. 23 varieties were successfully distinguished using 3 RAPD markers (Garry et al, 1999). ISSR type markers were tested and compared to RAPD markers on 37 varieties of the French catalogue. ISSR revealed more polymorph patterns than RAPD markers (Moisan Thiéry et al 2001). By this time, SSR were developed on potato and the sequence of primers were published (Provan et al 1996, Kawchuk et al 1996, Milbourne et al 1998). 30 pairs of primers from Kawchuk et al 1996 and Milbourne et al 1998 were selected to study their ability to distinguish 207 varieties of the French catalogue (Moisan Thiéry et al, in press). 5 SSR were chosen in accordance to the number of alleles, the amplification products sizes and the polymorphism they revealed: SSR1, STM2005, Lemalx, STM1097 and STM2020 (table 1).

The 207 potato varieties analysed were obtained from the national seed potato collection maintained at Hanvec by the FNPPPT. Two plants were analysed per variety. DNA was extracted from young leaves or tuber chips from the cortical zone, according to a Sodium Bisulfite and a CTAB procedure.

Protocols are detailed in listed publications.

Table 1. SSR loci used to distinguish the 207 potato varieties.

LOCUS	Motif	Origin	Localisation	Primers	Sequence 5' → 3'	Fragment size (bp)	No. of alleles	No. of profiles
SSR1[1]	(TCAC)n	S. tuberosum	Chr. VIII[3]	SSR19 SSR20	GAT GAG ATG AGA TAT GAA ACA ACG CGC AAT TCT CTT GAC ACG TGT CAC TGA AAC	210-252	10[5]	47[5]
STM2005[2]	(CTGTTG)n	S. tuberosum	Chr. XI	STM2005F STM2005R	TTT AAG TTC TCA GTT CTG CAG GG GTC ATA ACC TTT ACC ATT GCT GGG	160- 93	5[5]	14[5]
LEMALX[2]	(ATT)n	L. esculentum	*Chr. V*	LEMALXF LEMALXR	CTC ACC CAC AAA GAA AAT TC CTA ACA AAC ATT GTA CAA CAA TAA TC	126-135	4[()]	13[5]
STM1097[2]	(CGTTT)n	S. tuberosum	Chr. XII[4]	STM1097A STM1097B	GTT CAC AGC CTT CGT GAA CG ATT CAA ACT CAG CCA GCA GC	252-307	6[6]	12[6]
STM2020[2]	(TAA)n	S. tuberosum	Chr. I	STM2020F STM2020R	CCT TCC CCT TAA ATA CAA TAA CCC CAT GGA GAA GTG AAA ACG TCT G	162	7[7]	10[7]

[1] Kawchuk (1996), [2] Milbourne (1998). Associated genes : [3] Starch synthase, [4] Sucrose synthase. Number of cultivars used : [5] 207, [6] 66, [7] 15.

More recently, various techniques (SSR, AFLP and cDNA-AFLP) have been compared on a selection of varieties from various origins and on some variants (originated from INRA Ploudaniel), in combination with visual inspection in a national behaviour field.

Results

The two independent extractions carried out on two plants per variety allowed the consistency of banding profiles in the same clone to be assessed.

70% of the 207 varieties could be distinguished with the three loci SSR1, STM2005 and Lemalx. STM1097 has been registered for the remaining 66 varieties and among these varieties, 15 were genotyped using STM2020. A database was built containing the allele composition of 207 varieties (table 2).

Table.2. Database extract showing the allele composition of some varieties for four SSR loci.

VARIETY	SSR1	STM2005	Lemalx	STM1097
Ackersegen	3-5-7	4-5	2-3-4	4-7
Adora	3-7-10	2-4-5	3	ND
Agata	3-5-7	2-4-5	2-3-4	ND
Agria	3-5-7-10	1-2-4	3-4	3-4-7
Aïda	3-5	1-2-5	2-3	ND
Alaska	3-7	2-4-5	2-3	7
Alcmaria	5-7-10	2-4-5	2-3	ND

The method is currently used routinely in two officially-approved laboratories of the French seed potato growers, at the INRA Ploudaniel lab and also under experimentation at the Fraud Repression lab. Two independent samples of each variety to be tested are simultaneously extracted and amplified. It is possible to multiplex PCR for two markers and we also multiplex markers for the acrylamide electrophoresis. Silver nitrate staining is used to reveal SSR polymorphism. The allele mobility of the unknown samples are compared to our own standards i.e. well characterised varieties representing the entire allele panel of the database. The next step is to determine the allele composition of the sample and to compare it to our database. The method was successfully tested from frozen, dried and fresh leaves and also from tubers. Some problems of reproducibility were solved by testing different DNA extraction methods and appropriated DNA dilutions . The database is gradually updated.

An interlab experiment was organised in 2003 by the SOC (Official Inspection Service). Tuber samples from 10 cultivated varieties were distributed to the labs of the French seed potato growers with only the certificate number. The aim was to determine the variety's identity and to test if the labs results were in agreement with each other. This first assay was successful and it was conducted again on 20 varieties this year. An additional lab was included in the test. Results are under evaluation.

None of the techniques were able to distinguish the variants from the original variety, even if the morphological differences were quite clear.

Discussion

RAPD is a cost-effective and easy method that may discriminate potato varieties but is not as reproducible as other molecular techniques based on DNA amplification. ISSR has the disadvantages to present / lack of faint bands. AFLP is a powerful technique for DNA fingerprinting of potato varieties but its cost, the level of skills required, and database construction have to be taken into account. Therefore, SSR markers appeared from these experiences as the most adapted for the needs of potato industry as they are highly polymorphic, demonstrate co-dominant banding patterns, show a high repeatability of band profiles and the analysis needs less than 2 days.

As a database of SSR alleles of French potato varieties was established, any unknown variety can now be identified on the basis of its DNA pattern unless it has not yet been registered in the database. The database will be up-dated every year with newly registered French varieties and with foreign varieties. It would be interesting to standardise the technique and markers used in the different countries and to have an international database on potato varieties.

Some additional experiments are needed to validate our method. For example we will test its sensitivity in order to know how far we can go in pooling our samples. We also plan to test the PCR products in sequencing systems.

The limits of fingerprint analyses appear when a variety is a sport or somatic mutation of a previously-released variety. The same limit would be found if a new variety was a result of genetic engineering. None of the markers we investigated up to now have been able to discriminate between such variants. The French certification has recently introduced a

behaviour field to compare all first generations and to check the varietal identity and trueness to type, in cooperation between breeders and inspectors.

Molecular markers for variety identification could be also useful for Plant Variety Protection. Morphological characteristics should remain the official procedure for varietal DUS but molecular markers could be used by mutual agreement or as a complementary tool to obtain quick results for seed producers, growers and end users to check the identity and purity of produce and protect the breeders' rights from any unauthorized exploitation of their new varieties. So, UPOV could introduce the method, besides guidelines for morphological examination and / or recommend a definite procedure for crops like potatoes, for which molecular markers seem powerful.

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Acknowledgements

This work was financially supported by the French Federation of Seed Potato Growers, by the Education, Research and Technology Ministry, by the Agriculture Ministry (Bureau des Semences / CTPS) and by the Bretagne Regional Council.

References

1 Garry G., Le Hingrat Y., Kerlan MC., Bozec M. (1999). Identification de variétés de pomme de terre par des marqueurs moléculaires de type RAPD. Proceedings of the EAPR'99, Sorrento, Italy, 586-587.

2 Moisan-Thiéry et al (2001) Potato cultivars identification using molecular markers. Proc. Int. Symp. On molecular markers Eds. Doré, Dosba et Baril. Acta Hort.546, 471-476.

3 Moisan-Thiéry M., Kerlan MC, Gokelaere T., Le Hingrat Y. Potato cultivar identification using simple sequence repeat markers (SSR). Potato research, in press.

4 Provan et al (1996) Microsatellite analysis of relationships within cultivated potato (*Solanum tuberosum*) TAG 92: 1078-1084.

5 Kawchuk et al (1996). Characterization of *Solanum tuberosum* simple sequence repeats and application to potato cultivar identification. American Potato Journal 73, 325-335.

6 Milbourne et al. (1998). Isolation, characterisation and mapping of simple sequence repeat loci in potato. MGG 259: 233-245.

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