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BMT-TWA/Potato/2/4
ORIGINAL: English
DATE: April 11, 2007

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR POTATO

Second Session
Quimper, France, April 17, 2007

USE OF SSR MARKERS FOR VARIETY IDENTIFICATION AND CERTIFICATION OF SEED POTATOES IN FRANCE

Document prepared by experts from France

Use of SSR markers for variety identification and certification of seed potatoes in France

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1. The continuous increase in the number of potato varieties registered in France presents a risk of exchange and mixing of seed lots. Despite those risks, variety identification is still based upon morphological characteristics, which are often difficult to assess and sometimes vary due to environmental influence. Our aim was to develop a reliable system of variety identification, based on molecular markers, which could be used at any stage in the plant cycle (Marhadour, Le Hingrat et al. 2004).

2. After testing different types of markers (RAPD, ISSR, AFLP, SSR) we chose the SSR system and developed a procedure which has been published (Moisan-Thiéry, Marhadour et al. 2005). 30 primer pairs were tested, of which 5 were selected (table 1) as being able to distinguish all potato varieties produced in France.

Table 1: Primer pairs used in our procedure

Locus	Motif	Fragment sizes (bp)	Alleles number	Number of profiles
SSR1(1)	$(TCAC)_n$	230 - 194	11	55 (3)
STM2005(2)	$(CTGTTG)_n$	193 - 160	6	13(3)
LEMALX(2)	$(ATT)_n$	140 - 120	4	15(3)
STM1097(2)	$(CGTTT)_n$	281 - 234	8	12 (3)
STM2020(2)	$(TAA)_n$	193 - 160	10	-

(1)(Kawchuk, Lynch et al. 1996), (2)(Milbourne, Meyer et al. 1998), (3) computed from at least 300 varieties

3. In each test, the allele mobility of the varieties to be tested (two independent DNA samples) is compared with our own standards. The allele composition of the sample is determined and compared to our database. The database contains more than 400 varieties and is gradually upgraded (table 2). We use silver-staining to reveal the profiles because this technique can be set up in a laboratory with standard equipment.

Table 2: Example of information contained in the database

Varietes	SSR1	SSR1_1	SSR1_2	SSR1_3	SSR1_4	SSR1_5	SSR1_6	SSR1_7	SSR1_8	SSR1_8'	SSR1_9	SSR1_10	SSR1_11	STM2005	STM2005_1	STM2005_1'	STM2005_2	STM2005_3	STM2005_4	STM2005_5	Lemaix	Lemaix_1	Lemaix_2	Lemaix_2'	Lemaix_3	Lemaix_4	STM1097	STM1097_3	STM1097_4	STM1097_5	STM1097_6	STM1097_6'	STM1097_7	STM1097_8	STM1097_9	STM2020
Magnum	7 11	0	0	0	0	0	0	1	0	0	0	0	1 2 4		0	0	1	0	1	0 1 3	1	0	0	1	0 3 4 7		1	1	0	0	0	1	0	0 .	
Manna	3 7	0	0	1	0	0	0	1	0	0	0	0	0 1 2 4 5		1	0	1	0	1	1 3	0	0	0	1	0 3 7		1	0	0	0	0	1	0	0 3 6	
Manon	3 5 7 11	0	0	1	0	1	0	1	0	0	0	0	1 2 4 5		0	0	1	0	1	1 2 3 4	0	1	0	1	1 3 7		1	0	0	0	0	1	0	0 1	
Manuéla	3 7 11	0	0	1	0	0	0	1	0	0	0	0	1 2 4		0	0	1	0	1	0 1 2 3	1	1	0	1	0 4 7		0	1	0	0	0	1	0	0 .	
Marabel	2 5 8	0	1	0	0	1	0	0	1	0	0	0	0 4 5		0	0	0	0	1	1 3	0	0	0	1	0 3 4 7		1	1	0	0	0	1	0	0 1 3 6	
Maranca	3 4 7	0	0	1	1	0	0	1	0	0	0	0	0 2 4 5		0	0	1	0	1	1 3 4	0	0	0	1	1 4 7		0	1	0	0	0	1	0	0 .	
Marathon	3 7 11	0	0	1	0	0	0	1	0	0	0	0	1 4 5		0	0	0	0	1	1 3	0	0	0	1	0 4 7		0	1	0	0	0	1	0	0 .	

4. The technique is now routinely used in 4 laboratories: two officially-approved laboratories of the French seed potato growers, at the National Institute for Agronomic Research (INRA) Ploudaniel laboratory and at the Fraud repression laboratory (DGCCRF laboratory). An interlaboratory experiment has been organized by the Official Inspection Service (SOC) each year since 2003. Tubers of cultivated varieties are distributed to the laboratories with their certificate numbers only. The number of varieties included in the test varied from 5 to 20 . The aim of the test is to determine the variety identity and the reproducibility between the laboratories.

5. The technique is used in cases of doubt for some seeds lots and to test the *in vitro* collections (the initial stocks used for multiplication) of the seed potato growers' laboratories. Any unidentified variety cultivated in France can now be identified, provided its profile has been registered in the database. We can also check the identity of a sample against a reference. The markers are also used to test breeders' collections and to genotype hybrids in selection.

6. As is well known, it is not possible to distinguish variants or mutants from a previously released variety using such a technique; the French certification authority, in cooperation with breeders and inspectors, has recently introduced a field trial to compare all first generation lots and to check the variety identity and trueness-to-type.

7. With the five selected markers, we can distinguish all the varieties in the database except a very limited number of pairs. Some of those pairs can be distinguished by using additional markers, but other pairs remain identical, even after testing with 14 additional markers.

8. We are currently testing additional markers in order to decrease the probability of finding different varieties with the same allelic profile. We also plan to test our markers using a sequencing system to be able to compare our data with other databases.

9. In conclusion, we propose the use of SSR markers as a complementary tool for producing rapid results for the protection of breeders against unauthorized exploitation of their protected varieties.

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.

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