EVALUAREA UNOR GENOTIPURI DE CĂPȘUN UTILIZATE IN PROGRAMUL DE AMELIORARE PENTRU CREȘTEREA REZISTENȚEI LA BOLI

ASSESSING SOME STRAWBERRY GENOTYPES USED IN BREEDING PROGRAMME FOR INCREASING RESISTANCE TO DISEASES

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Abstract

The experiments were conducted at Research Institute for Fruit Growing Pitesti during 2016-2017 to identify the suitable strawberry genotypes with good resistance to the specific diseases. In Pitesti conditions, the most tolerant genotypes on the *Mycosphaerela fragariae* - leaf spot of strawberry attack were: 8-14-42, 8-15-3 (DISINC% and DISEV% 0.67-1.00) and Kupava (DISINC%=1.33, DISEV%=0.67). The molecular analyzes results performed with the two markers to the two resistance genes show the presence of the two *Rpf1* (*Phytophthora fragariae*) and *Rca2* (*Colletotrichum acutatum*) in four genotypes (Benton, Real, 08-14 -42, 08-14-8). Due their good behavior to fungal pathogens attack and the presence of the two resistance genes, studied genotypes will be intensively used in the strawberry breeding program.

Cuvinte cheie: căpșun, boli fungice, gene de rezistență , programul de ameliorare
Key words: strawberry, fungal diseases, resistant genes, breeding program

1. Introduction

The climate change trends (high amplitudes of temperature in a very short period, increased frequency and damages caused by late frosts, or even early ones, drought and warm waves in the summer time), favored the changes in the biological cycle of the pathogens, that becoming more aggressive for the strawberry crops (Moretti et al., 2010). In Romania, strawberry plants are usually affected by a number of pathogens including *Mycosphaerella fragariae* (Tul.) Lindau (leaf spot), *Diplocarpon earliana* (Ell. & Ev.) Wolf (leaf scorch),*Colletotrichum acutatum* Simmonds (anthracnosis) and *Phytophthora fragariae* Hickman (red stele). To maintain the attack below the minimum biological threshold for damages, every year a large number of chemical treatments of the crops are needed to control of many of these diseases. These are expensive and often ineffective due to the limited range of available plant protection products. Many cultivars have resistance to some of the more common diseases (Haymes et al. 2000, Averre et al. 2002, Lerceteau-Kohler et al. 2005). The breeding programs follow the improvement in fruit quality, yield and disease resistance/tolerance (Simpson 2014, Masny et al, 2016). To induce resistance to these diseases using conventional methods is difficult, time consuming and affected by epistatic interactions between genes. Conventional plant breeding takes time and depends on the ambient conditions. The breeders are very interested in new technologies, such as molecular markers, that could make this procedure more efficiently and faster (Sturzeanu et al, 2015).

The aim of this study was to identify the suitable genotypes with good resistance/tolerance to the specific diseases for use in breeding program.

2. Material and methods

The research was conducted in 2016-2017, at the Research Institute for Fruit Growing Pitesti in randomized blocks with three replicates (eight plants per each replicates). The experimental study in the plots and the biotechnological method for markers-assisted selection regarding two resistance genes were carried out the following genotypes: Benton, Mira, Queen Elisa, Real, Krasnyi Bereg, Kupava, 08-14-6, 08-14-8, 08-14-42, 08-15-3, 08-15-13, 08-18-1, 13-12, 8-20, 6-10-05, 1-18-05, 2-23-05.

Observations and assessments were done on the disease incidence (DISINC%) and the attack severity (DISSEV%) in the field, under natural conditions of infection for two diseases affecting frequently the foliage of the strawberries: *Mycosphaerella fragariae* - leaf spot and *Diplocarpon earliana* - leaf scorch.

The biotechnological method for markers-assisted selection regarding two resistance genes ***Rpf1*** (*Phythophtora fragariae*) and ***Rca2*** (*Colletotrichum acutatum*) by PCR (Polymerase Chain Reaction) was carried out.

Total DNA isolation was performed using the kit extraction Isolate II Plant DNA (Bioline) from the leaves of the strawberry fresh grinded with liquid nitrogen, and the sample was distributed into the tube of 2 ml (100 mg) and kept at -80°C.

The amplification reaction of DNA was carried out for evidence of gene ***Rpf1*** using the MyTaq™ Red Mix in 0.2 ml tubes with 20 µl final reaction volume and 18-20 ng of genomic DNA. The primer used is: SCAR-R1A Forward: 5'-TGC ATC ATT AAT GTA GAA GTC TTT-3' and Reverse: 5′-TGA TGC GAC ATA CAA AAA TAT TAG-3' (Haymes et al., 2000); RPF1NF1 Forward: 5'-CGGTTCCCCAAAAGATAGTTAC-3' and Reverse: 5′-GTTCTACGCATTAAGATGCACTTGC-3' (Rugienius et al., 2006).

Amplification was performed in a termocycler Applied Biosystem 9600 after the following programme: Initial denaturation for 1 min. at 95°C; -30 cycles, each consisting of 15sec. at 95°C, 15 sec. at 60°C, 10 sec. at 72ºC, and 5 min. at 72°C. The PCR products were evaluated by electrophoresis on a 2% agarose gel and 0.5 X TBE buffer, stained with ethidium bromide and visualization to combine BioPrint - Vilber Lourmat combine.

The amplification reaction of DNA was carried out for evidence of gene ***Rca2*** using the MyTaq™ Red Mix in the tubes of 0.2 ml containing 20µl final volume reaction and 18-20 ng of genomic DNA. The primer used is: STS-Rca2_240 CAC_240_2F 5'-GCC ACG TCA CTA GTC AAA TTC AA-3', CAC_240_2RB 5'-TCA TGG ACA GTG GTC TCA GC-3' (Lerceteau-Köhler et al. 2005).

Amplification was performed in a thermocycler Applied Biosystem 9600 after the following programmer: Initial denaturation for 1 min. at 95°C; -35 cycles, each consisting of 15sec. at 95°C, 15 sec. at 64°C, 10 sec. at 72ºC, and 5 min. at 72°C. The PCR products were evaluated by electrophoresis on a 2% agarose gel and 0.5 X TBE buffer, stained with ethidium bromide and visualization to combine Bio Print - Vilber Lourmat combine.

The data were collected on three replicates ranged and processed using Microsoft Office Excel 2010 and its graphical and statistical facilities.

3. Results and discussions

The resistance to the leaf spots and leaf scorch is of polygenic nature and breeding strawberry for the resistance or low susceptibility is very difficult.

Assessment of the figure 1 reveal that under the conditions of the years 2016 - 2017, selections 13-12, 8-20, 6-10-05, 1-18-05, 2-23-05, showed no symptoms of *Mycosphaerella fragariae*, the disease incidence DISINC% and severity DISEV% being zero. Very good behavior had the elites 8-14-42, 8-15-3, on which, the disease incidence DISINC% and severity DISEV% 0.67-1.00. Among the cultivar evaluated the best behavior was noticed on Kupava DISINC%=1.33, DISEV%=0.67, Krasnyi Bereg, DISINC%=2.00, DISEV%=0.67, and Real DISINC%=4.67, DISEV%=1.33.

Evaluation of the figure 2 reveal that under the conditions of the years 2016 - 2017, all strawberry varieties and elites studied showed no symptoms of *Diplocarpon earliana* pathogen attack, except the elite 08-18-1, on which the pathogen incidence DISINC% was 0.67 and disease severity DISSEV% was 1.67.

The symptoms of strawberry plant infection by *Mycosphaerella fragariae* observed on the young leaves of some genotypes in the form of single small spots with a purple border and grey centre were likely caused by the weather conditions also been reported by Carisse et al. (2000).

The minor symptoms of *Diplocarpon earliana* pathogen attack were observed at 08-18-1 genotype rather on older leaves than on newly expanded ones also been reported by Zheng and Sutton (1994).

In strawberry, *Rpf1* is a dominant gene for resistance to *Phythophtora fragariae* (Van de Weg, 1997a, Van de Weg et al., 1997b) and *Rca2* is a dominant gene for resistance to anthracnose pathogenicity group 1 of *Colletotrichum acutatum* (Denoyes and Baudry 1995, Lerceteau-Kohler et al. 2005).

The molecular analyses performed for evidence the alleles of genes *Rpf1* and *Rca2* in the seventeen genotypes showed that these genes are found together in some genotypes and others separately. The synthesis of the results is presented in Figures 3 and 5 as well as in Table 1.

Figure 3 shows the presence of the product PCR (285 bp) associated with the *Rpf1* gene resistance allele at seven genotypes (Benton, Mira, Real, 08-14-42, 08-14-8, 08-18-1) of the seventeen studied.

Figure 4 shows the presence of the product PCR (400 pb) associated with the Rpf the sensitivity allele. This is present in sixteen genotypes of those studied. Thus, the marker SCAR-R1 is dominant for the resistance genes, and marker RPF1NF is dominant for gene of the sensitivity. That shows the resistant heterozygous genotypes are: Benton, Mira, Real, 08-14-42, 08-14-8, 08-18-1.

The molecular analyses performed with the STS-Rca2-240 marker (Figure 5) in the seventeen genotypes revealed the presence of the product PCR (240 bp) associated with the anthracnose resistance allele, *Rca2*, in seven genotypes (1-18-05, Kupava, Benton, Real, 08-14-42, 08-14-8, 08-15-3).

Given the nature of these dominant markers, we can not to specify whether these genes are homozygous (RR) and which are heterozygous (Rr).

The presence of the markers indicated monogenic control of resistance to strawberry red stele root rot and strawberry anthracnose have been confirmed the results reported by Van de Weg (1997), Haymes et al. (2000), Denoyes-Rothan et al. (2005), Lerceteau-Kohler et al. (2005).

The molecular analyses results performed with the two markers for the two genes resistant show the presence of the two *Rpf1* (possibly heterozygous and homozygous) and *Rca2* (possibly heterozygous and homozygous) in 4 genotypes (Benton, Real, 08-14 -42, 08-14-8). Therefore, it can be said that these genotypes contains resistance genes at red stele root rot *Rpf1* (RR or Rr) and anthracnose *Rca2* (RR or Rr).

4. Conclusions

The most tolerant genotypes on the *Mycosphaerela fragariae* - leaf spot of strawberry attack were: 8-14-42, 8-15-3 and Kupava.

The strawberry genotypes: Benton cv., Real cv., 08-14-42 selection and 08-14-8 selection may successfully be used in future breeding program for resistance to the *Phythophtora fragariae* and *Colletotrichum acutatum* diseases.

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References

1. Averre CW, Jones RK, Milholland RD, 2002. Strawberry diseases and their control. In: Creswell TC (reformatted) Plant Pathology Extension. College of Agriculture and Life Sciences—North Carolina State University. www.ces.ncsu.edu/depts/pp/notes/oldnotes/fd5.htm.
2. Carisse O, Bourgeois G, Duthie JA, 2000. Influence of temperature and leaf wetness duration on infection of strawberry leaves by *Mycosphaerella fraga*riae. Phytopathology 90:1120–1125.
3. Denoyes B, Baudry A., 1995. Species identification and pathogenicity study of French *Colletotrichum* strains isolated from strawberry using morphological and cultural characteristics. Phytopathology 85:53–57.
4. Haymes K.M, W.E. Van de Weg, P. Arens J.L. Maas, B. Vosman, A.P.M. Den Nijs, 2000. Development of SCAR markers linked to a *Phytophthora fragariae* resistance gene and their assessment in European and North American strawberry genotypes, J. Amer. Soc. Hort. Sci.125(3): 330–339, http://journal.ashspublications.org/content/125/3/330.full.pdf .
5. Hancock J.F., Finn C.E., Luby J.J., Dale A., Callow P.W., Serçe S., 2010. Reconstruction of the strawberry, *Fragaria* × *ananassa*, using genotypes of *F. virginiana* and *F. chiloensis*. HortScience, *45*: 1006–1013.
6. Lerceteau-Kohler E., Guerin G., Denoyes-Rothan B., 2005. Identification of SCAR markers linked to *Rca2* anthracnose resistance gene and their assessment in strawberry germplasm. Theor. Appl. Genet. 111 (5): 862–870.
7. Masny A., Masny S., Zurawicz E., Pruski K., Madry W., 2016. Suitability of certain strawberry for breeding of new cultivars tolerant to leaf diseases based on their combining ability. Euphytica 210:341-366, doi: 10.1007/s1081-016-1690-6.
8. Moretti C.L., Mattos L.M., Calbo A.G., Sargent S.A., 2010. Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: A review, Food Research International 43:1824–1832.
9. Rugienius R., Siksnianas T., Stanys V., Gelvonauskiene D., Bendokas V., 2006. Use of RAPD markers for indentification of strawberry genotypes carrying red stele (*Phytophthora fragariae*) resistance gene *Rpf1.*
10. Simpson DW., 2014. Strawberry breeding and genetics research in North West Europe. Acta Hortic 1049:107–111.
11. Sturzeanu M., Coman M., Ciuca M., Ancu I., Cristina D., Turcu A.G., 2016. Molecular characterization of allelic status of the *Rpf1* and *Rca2* genes in six cultivars of strawberries**.** III Balkan Symposium on Fruit Growing, Volume 1, Acta Horticulturae: 1139: 107-111.
12. Xue A.G., Sutton J.C., Dale A., 1997. Comparative assessments of seedling runner and adult-plant resistance to leaf scorch in strawberry. Canadian Journal of Plant Science, 77: 279–281.
13. Van de Weg WE, 1997. Resistance to Phytophthora fragariae var. fragariae in strawberry: the Rpf2 gene. Theor Appl Genet.94:1092–1096.

14. Van de Weg, W.E., B. Henken, and S. Giezen. 1997a. Assessment of the resistance to *Phytophthora fragariae* var. *fragariae* of the USA and Canadian differential series of strawberry genotypes. J. Phytopathol. 145:1–6.
15. Van de Weg, W.E., H.J. Schouten, and B. Henken. 1997b. Resistance to *Phytophthora fragariae* var. *fragariae* in strawberry: the *Rpf1* gene, p. 71–76. In: W.E. Van de Weg (ed.). 1997. Gene-for gene relationships between strawberry and the causal agent of red stele root rot, *Phytophthora fragariae* var. *fragariae*. PhD thesis. Waginingen Agr. Univ. CPRO-DLO, Wageningen.
16. Zheng, J., and Sutton, J. C. 1994. Inoculum concentration, leaf age, wetness duration, and temperature in relation to infection of strawberry leaves by *Diplocarpon earlianum*. Can. J. Plant Pathol. 16:177-186.

Figures and Tables:

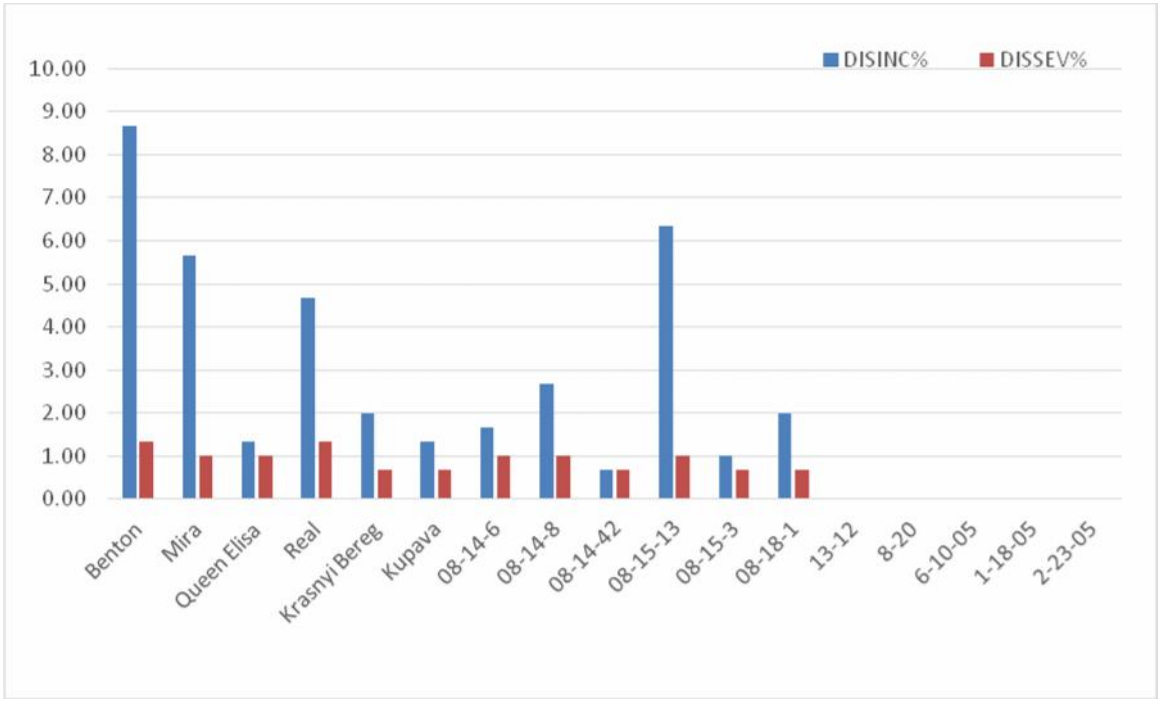

Fig. 1. Behavior of some strawberry genotypes at *Mycosphaerella fragariae* pathogen attack (RIFG Pitesti, Romania 2016-2017)

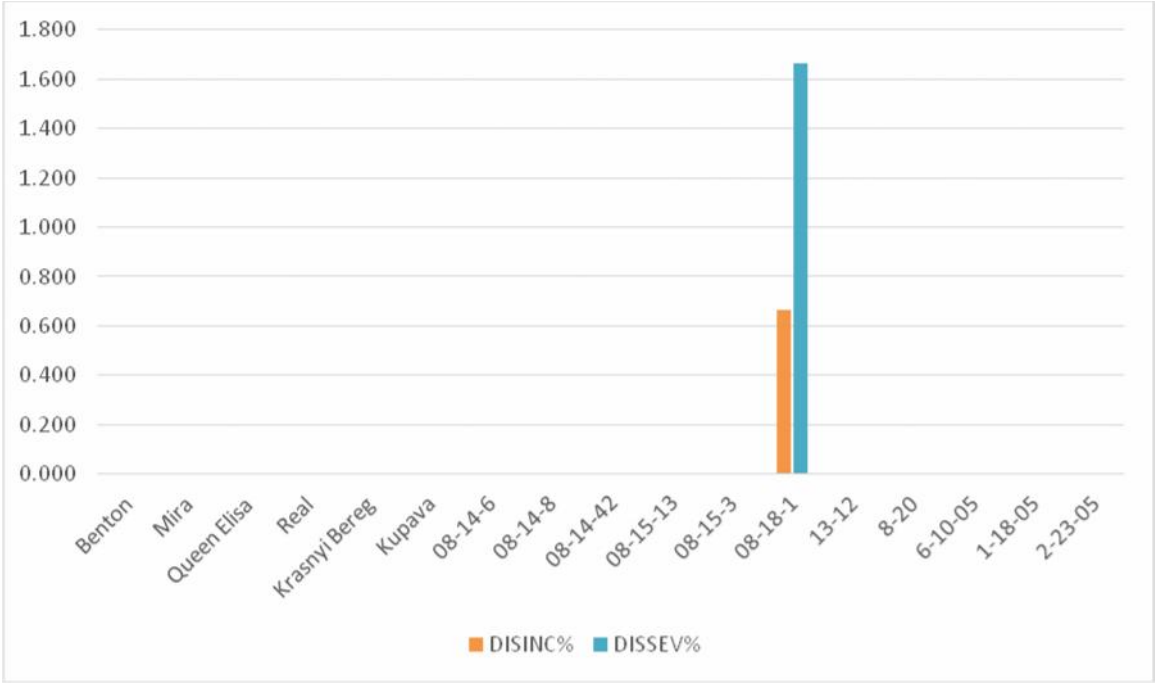

Fig. 2. Behavior of some strawberry genotypes at *Diplocarpon earliana* pathogen attack (RIFG Pitesti, Romania 2016-2017)

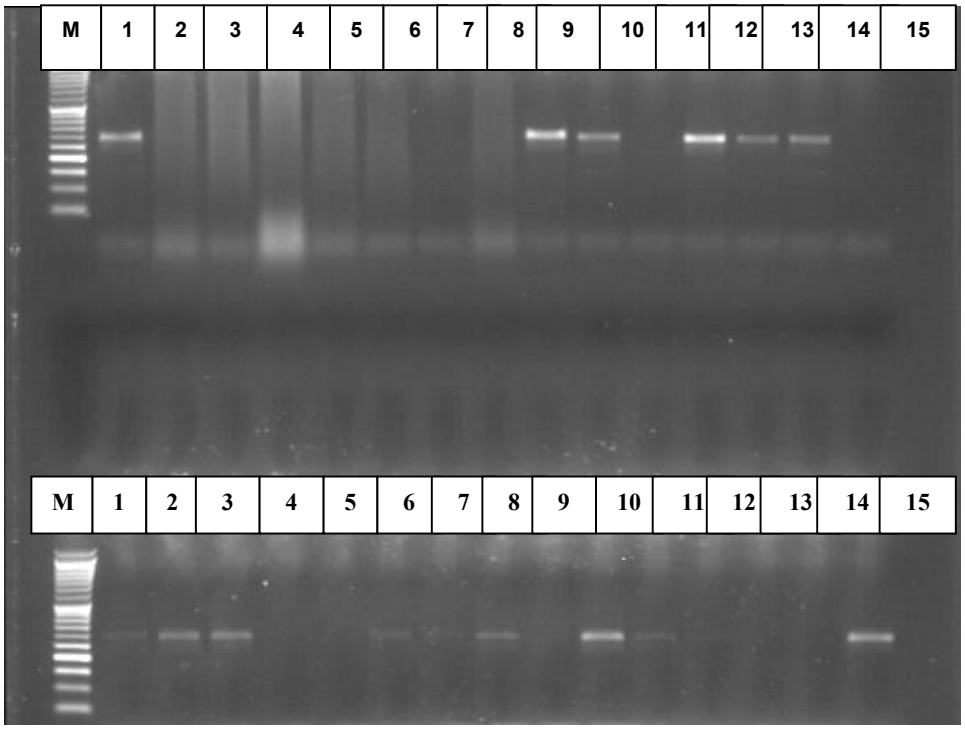

Fig. 3. Electrophoretic profile obtained by SCAR-R1A marker

I M. Ladder 50 pb, 1. Benton; 2. 13-12; 3. 8-20; 4. 6-10-05; 5. 1-18-05; 6. 2-23-05; 7. Krasnyi Bereg; 8. Kupava; 9. Benton; 10. Mira; 11. Queen Elisa; 12. Real; 13. 08-14-42; 14.08-14-08; 15.08-15-13; **II M.** Ladder 50 pb, 1. 08-14-6; 3. 08-15-3; 4.08-18-1

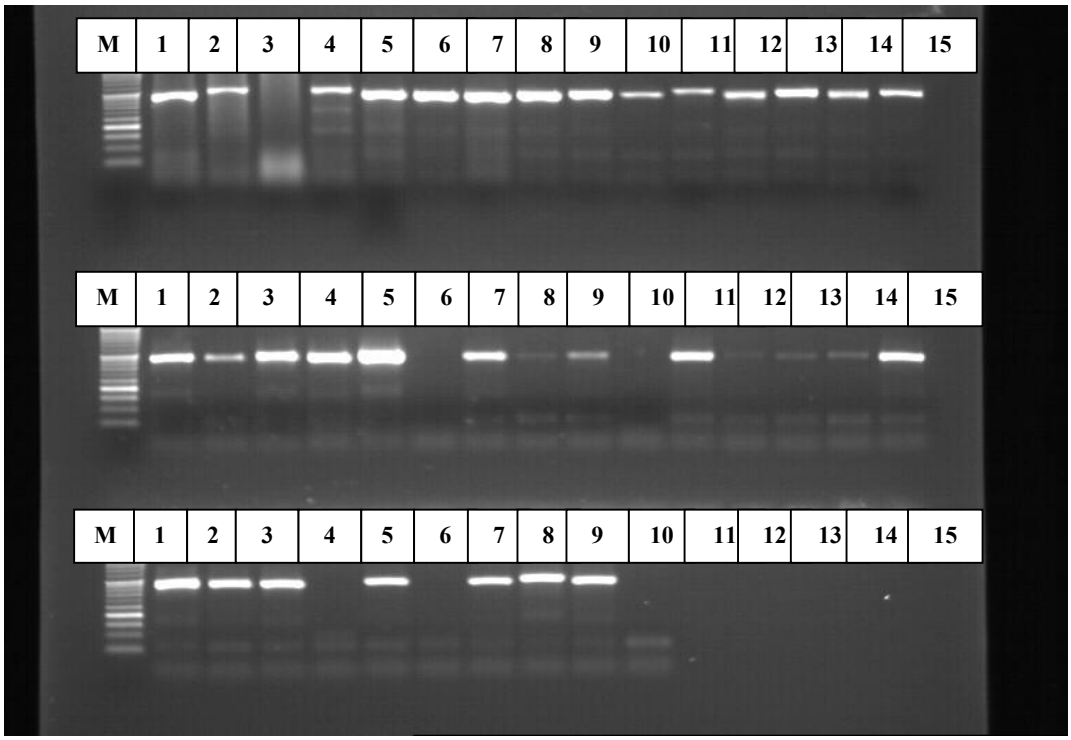

Fig. 4. Electrophoretic profile obtained by RPF1N marker

I M. Ladder 50 pb, 1. Benton; 2. 13-12; 3. 8-20; 4. 6-10-05; 5. 1-18-05; 6. 2-23-05; 7. Krasnyi Bereg; 8. Kupava; 9. Benton; 10. Mira; 11. Queen Elisa; 12. Real; 13. 08-14-42; 14.08-14-08; 15.08-15-13; **II M.** Ladder 50 pb, 1. 08-14-6; 3. 08-15-3; 4.08-18-1

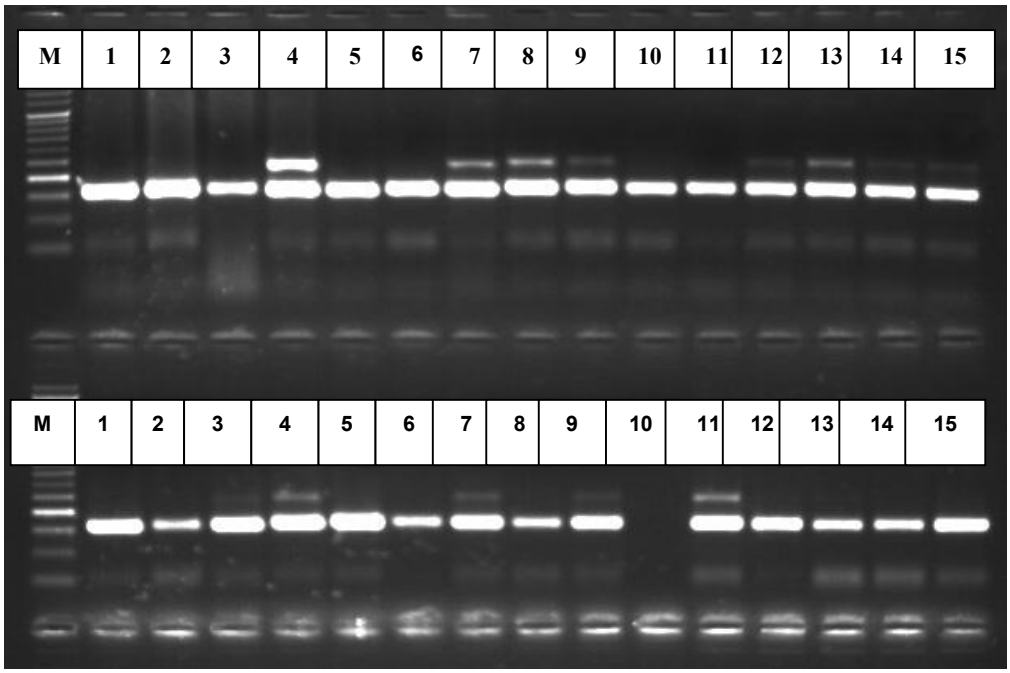

Fig. 5. Electrophoretic profile obtained by STS-Rca2_240 marker
I M. Ladder 50 pb, 1. 13-12; 2. 8-20; 3. 6-10-05; 4. 1-18-05; 5. 2-23-05; 6. Krasnyi Bereg; 7. Kupava; 8. Benton; 9. Benton; 10. Mira; 11. Queen Elisa; 12. Real; 13. Real 14. 08-14-42; 15. 08-14-08;
II M. Ladder 50 pb, 1. 08-15-13; 2. 08-14-6; 4. 08-15-3; 5. 08-18-1

Table 1. Genotype and cultivar names, their parents and their status for the SCAR markers (+ present; - absent)

Genotype	Parents	Origin	SCAR-R1A	RPF1N	STS-Rca2_240
Benton	OSC 2414 x Vale	USA	+	+	+
Mira	Scott × Honeoye	Canada	+	+	-
Queen Elisa	Miss x USB 35 (Lateglow x Seneca)	Italia	-	+	-
Real	Premial x Brio	Romania	+	+	+
Krasnyi Bereg	Venta x Tenira	Belarus	-	+	-
Kupava	Red Gauntlet x Krasnyi Bereg	Belarus	-	+	+
08-14-6	Mira x Real	Romania	-	+	-
08-14-8	Mira x Real	Romania	+	+	+
08-14-42	Mira x Real	Romania	+	+	+
08-15-3	Mira x Queen Elisa	Romania	-	+	+
08-15-13	Mira x Queen Elisa	Romania	-	+	-
08-18-1	Queen Elisa x Mira	Romania	+	+	-
13-12	Dukat x Venta	Belarus	-	+	-
8-20	Slavutich	Belarus	-	-	-
6-10-05	Kent	Belarus	-	+	-
1-18-05	Venta x Elkat	Belarus	-	+	-
2-23-05	Venta x Rosinka	Belarus	-	+	-