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A new RAPD marker identifying restorer lines for CMS *ogura* system

Nowy marker typu RAPD identyfikujący linie restorery dla systemu CMS *ogura*

Key words: winter oilseed rape, restored hybrids, CMS *ogura, Rfo* restorer gene, molecular markers, RAPD

78 winter oilseed rape lines used in the breeding of hybrid varieties (31 CMS *ogura* lines and 47 restorer lines) were subjected to research on their DNA polymorphism. The owner of investigated lines is Plant Breeding Company Strzelce Ltd. RAPD method with the use of 27 arbitrary 10-bp-long oligonucleotides as primers (Operon Technologies) was applied to the analysis of this breeding material. The investigations aimed at evaluation of genetic distance between all lines mentioned above (the results will be published separately). At the same time they made it possible to search for new markers of restorer gene for CMS *ogura*. A new band developed by OPY-15 primer, which appears in restorer lines was discovered. Among 188 polymorphic products of amplifications it was the only characteristic of all restorer lines, apart from the marker OPC-02_{1150} already known and applied to selection. The band generated by OPY-15 primer probably can be a new marker of restorer gene, however it should be verified in further investigations.

Słowa kluczowe: rzepak ozimy, mieszańce zrestorowane, CMS *ogura*, gen restorer *Rfo*, markery molekularne, RAPD

Badaniom polimorfizmu DNA poddano 78 linii rzepaku ozimego wykorzystywanych w hodowli odmian mieszańcowych (31 linii CMS *ogura* oraz 47 linii restorerów), będących własnością Spółki Hodowla Roślin Strzelce. Analizy tego materiału dokonano za pomocą metody RAPD przy użyciu 27 starterów firmy Operon Technologies. Badania prowadzone w celu oceny odległości genetycznej pomiędzy wymienionymi liniami (wyniki zostaną opublikowane oddzielnie), pozwoliły jednocześnie na wykrycie nowego, charakterystycznego tylko dla linii restorerów prążka, wygenerowanego przy użyciu startera OPY-15. Wśród otrzymanych 188 produktów amplifikacji różnicujących badany materiał roślinny był to jedyny prążek występujący tylko u linii restorerów, oprócz znanego już i stosowanego w selekcji markera OPC-02_{1150}. Przypuszczalnie może to być nowy marker alleli genu restorera, co jednak wymaga potwierdzenia w dalszych badaniach.

Introduction

Hybrid varieties of winter oilseed rape have become a very important part of oilseed rape cropping due to the heterosis effect occuring in seed yield in F_1 progeny.

Breeding of oilseed rape hybrid varieties in Poland is based on CMS *ogura* hybridization system developed in INRA – France, called also Ogu – INRA. The advantage of this system is total stability of male sterility expression (Bartkowiak-Broda et al. 1979). However, the lack of double low restorer lines with good yielding ability is the factor limiting progress in breeding of restored hybrids.

The problem with the development of valuable restorer lines is connected with the origin of restorer gene (*Rfo*) for CMS *ogura.* The *Rfo* gene was introduced by Heyn (1976) to rapeseed genome from radish genotype (*Raphanus sativus)*. The region of the radish genome introgression was bigger than the locus of the restorer gene which resulted in the disturbances of the first meiotic behaviour in PMC and thus it affected the female fertility and yielding ability of restorer lines (Pellan-Delourme and Renard 1988). The restorer gene in the obtained initial recombinant was tightly linked to genes responsible for high content of undesirable compounds — glucosinolates, therefore the obtainment of high yielding restorer lines with low glucosinolate content was difficult (Delourme et al. 1995, 1999).

Partial elimination of the redundant DNA segment was possible by backcrosses of restorer lines with genetically stable double low lines characterized by high yielding ability. Then double low recombinants with restorer gene were selected (Delourme et al. 1995, Popławska 2000, Popławska et al. 2001, Bartkowiak-Broda et al. 2003). The selection lasting usually for several generations is difficult and time-consuming and therefore must be assisted by molecular markers in order to shorten and advance this process.

In the investigations conducted on restorer lines in the Plant Breeding and Acclimatization Institute in Poznań (Popławska et al. 1999, Popławska 2000, Popławska et al. 2001, Bartkowiak-Broda et al. 2003), selection of genotypes with restorer gene alleles was assisted by isozyme marker PGI-2 (Delourme and Eber 1992) closely linked to restorer gene. The break of linkage between the restorer gene and high glucosinolate content and further selection in some recombinants was followed by the break of linkage between *Rfo* gene and *Pgi-2* alleles (Delourme and Eber 1992, Delourme et al. 1999, Popławska et al. 2001). So, the selection by the use of PGI-2 marker became not sufficient, especially in populations of restorer lines with extremely low glucosinolate content. This fact could indicate a possibility of modification occuring in the introgression of radish genome region. These changes consisted in the loss of *Pgi-2* alleles characteristic for the radish, together with closely linked DNA fragment determining high glucosinolate content (Popławska and Bartkowiak-Broda 2001).

Taking advantage of the study carried out by Delourme et al. (1994) the investigations in the RAPD markers were undertaken. An RAPD OPC-02 marker completely linked to *Rfo* gene was adapted and applied for routine analyses at the Plant Breeding and Acclimatization Institute in Poznań by Mikołajczyk et al. (1998). Tight linkage between RAPD marker $OPC\text{-}02_{1150}$ and *Rfo* gene was

observed even in genotypes with extremely low glucosinolate content (Bartkowiak-Broda et al. 2003) which lost the *Pgi-2* allele.

Determining the largest possible number of markers around the *Rfo* gene will allow to get to know better the introgression of radish genome fragment.

Material and methods

Plant material

Investigations were carried out on 31 male sterile CMS *ogura* lines and 47 restorer lines belonging to Plant Breeding Company Strzelce Ltd. This company makes use of investigated lines for breeding of winter oilseed rape hybrid varieties, based on CMS *ogura* hybridization system.

DNA analysis

Isolation of DNA from young leaves of 10 plants for each line was performed according to the modified method by Doyle and Doyle (1990). The quality and concentration of obtained DNA templates were evaluated on 0,8% agarose gel in 1x TBE buffer.

Restorer lines are bred on sterile cytoplasm *ogura* type. It allows to verify the purity of selected proper lines with the use of a mtDNA marker, specific for CMS *ogura* cytoplasm. This verification was performed according to the method elaborated and applied for routine analyses at Plant Breeding and Acclimatization Institute in Poznań by Mikołajczyk et al. (1998) on the basis of the results obtained by Krishnasamy and Makaroff (1993) and also Sigareva and Earle (1997).

RAPD analysis

RAPD markers were tested for their ability to detect polymorphism of DNA samples using Williams et al. (1990) method. Male sterile and restorer lines were investigated by 27 arbitrary 10-bp-long oligonucleotides as primers (Operon Technologies): OPA-07, OPA-08, OPA-18, OPC-02, OPC-18, OPF-01, OPF-04, OPF-14, OPG-04, OPG-05, OPL-12, OPN-02, OPN-07, OPN-18, OPP-03, OPP-05, OPP-09, OPP-14, OPW-05, OPW-09, OPY-01, OPY-02, OPY-04, OPY-05, OPY-10, OPY-13, OPY-15. These primers were chosen on the ground of their arrangement in the genetic map which was elaborated for rapeseed by Lombard and Delourme (2001) and also based on results obtained by Nowakowska et al. (2004) and by Fürguth (data not published yet). The selected primers differentiated the investigated lines most of all. The DNA amplifications were performed in Biometra and Eppendorf thermocyclers, under the following conditions: initial denaturation for 30 s at 95°C; then 45 cycles of 30 s at 95°C, 1 min. at 35°C and 2 min. at 72°C followed by final amplification for 5 min. at 72°C. Reaction mixture, in a final

volume of 12,5 µl, contained: PCR reaction buffer [1 x conc.], $MgCl_2$ [2 mM], dNTP [0,1 mM], primer [0,2 µM] and 0,4 enzymatics units of *Taq* DNA polymerase from MBI Fermentas. Amplification products were resolved by 1,8% agarose gel electrophoresis and visualised under UV light after staining with ethidium bromide.

Results and discussion

The result of RAPD reactions were 188 polymorphic products of amplifications. Only two were characteristic for restorer lines out of all these markers. The first one was already known and applied to the selection marker $OPC\text{-}02_{1150}$ (Fig. 1). The second one was discovered as a new marker developed by OPY-15 primer (Table 1).

Table 1

The result of RAPD reactions with the use of OPC-02 and OPY-15 primers obtained for all investigated CMS *ogura* and restorer lines. The minus and plus signs point respectively to absence and presence of the $OPC\text{-}02_{1150}$ marker and also new marker developed with OPY-15 primer — *Wynik reakcji RAPD z zastosowaniem starterów OPC-02 oraz OPY-15 otrzymany dla wszystkich badanych linii CMS ogura i linii restorerów. Znaki minus i plus odpowiednio wskazują brak i obecność markera* $OPC\text{-}02_{1150}$ *oraz nowego markera uzyskanego za pomocą startera OPY-15.*

CMS *ogura* lines *Linie CMS ogura*	OPC-02 primer	OPY-15 primer	Restorer lines *Linierestorery*	OPC-02 primer	OPY-15 primer
MS 3	–	–	R 15	+	+
MS 16	–	–	R 16	+	+
MS 18	–	–	R 29	+	+
MS 62	–	–	R 50	+	+
MS 65	–	–	R 51	+	+
MS 69	–	–	R 52	+	+
MS 70	–	–	R 53	+	+
MS 77	–	–	R 54	+	+
MS 78	–	–	R 59	+	+
MS 83	–	–	R 64	+	+
MS 96	–	–	R 67	+	+
MS 104	–	–	R 68	+	+
MS 108	–	–	R 69	+	+
MS 109	–	–	R 70	+	+
MS 110	–	–	R 71	+	+
MS 119	–	–	R 72	+	+
MS 120	–	–	R 73	+	+
MS 126	–	–	R 74	+	+
MS 130	–	–	R 76	+	+
MS 171	–	–	R 77	+	+

MS 220	–	–	R 81	+	+
MS 222	–	–	R 83	+	+
MS 227	–	–	R 133	+	+
MS 236	–	–	R 137	+	+
MS 238	–	–	R 228	+	+
MS 242	–	–	R 250	+	+
MS 262	–	–	R 277	+	+
MS 263	–	–	R 310	+	+
MS 264	–	–	R 508	+	+
MS 265	–	–	R 512	+	+
MS 266	–	–	R 526	+	+
			R 572	+	+
			R 610	+	+
			R 642	+	+
			R 643	+	+
			R 659	+	+
			R 762	+	+
			R 778	+	+
			R 791	+	+
			R 817	+	+
			R 919	+	+
			R 926	+	+
			R 940	+	+
			R 1046	+	+
			R 1050	+	+
			R 1058	+	+
			R 1059	+	+

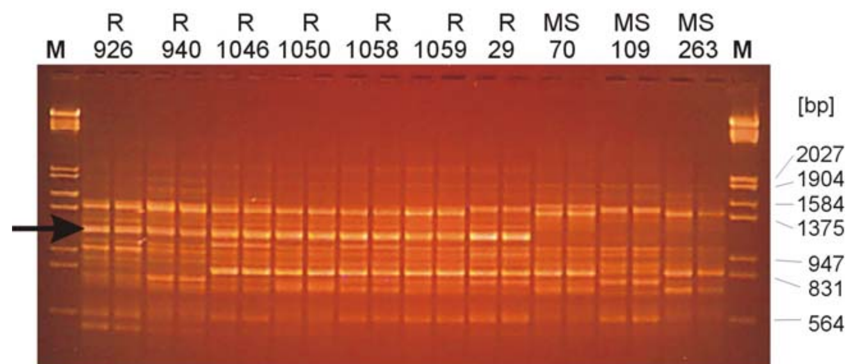

Fig. 1. 1.8% agarose gel electrophoresis of RAPD products obtained with the use of OPC-02 primer. Arrow indicates the OPC-02_{1150} marker completely linked to restorer gene. Signs above the photo: for explanation see Fig. 2 — *Eletroforetyczny rozdział na 1,8% żelu agarozowym produktów reakcji RAPD z zastosowaniem startera OPC-02. Strzałką zaznaczono marker OPC-02_{1150} sprzężony całkowicie z genem restorerem. Oznaczenia na górze fotografii – objaśnienia przy obrazie nr 2*

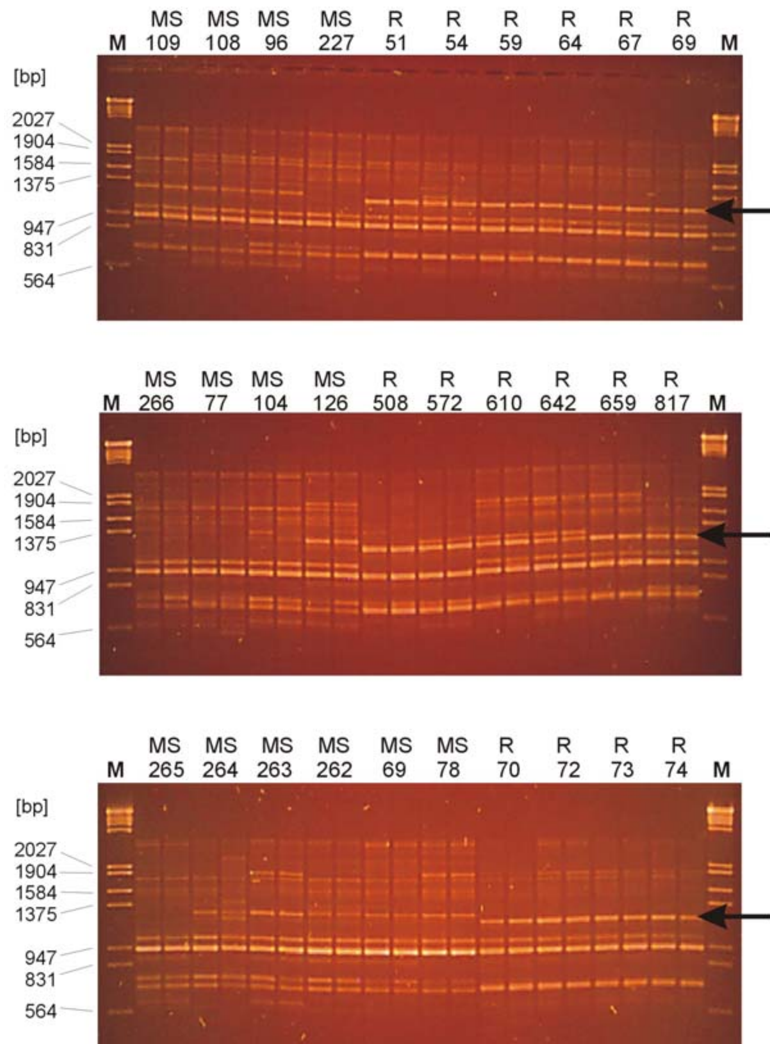

Fig. 2. 1.8% agarose gel electrophoresis of RAPD products obtained with the use of OPY-15 primer as a result of three reactions carried out independently of themselves. Signs above the photos: MS and R with the numbers describe the studied male sterile CMS *ogura* and restorer lines respectively, M – molecular size marker (in base pairs): phage lambda DNA digested with endonucleases Eco RI and Hind III. Arrows indicate the polymorphic band characteristic for restorer lines for CMS *ogura* system — *Elektroforetyczny rozdział na 1,8% żelu agarozowym produktów trzech przeprowadzonych niezależnie od siebie reakcji RAPD, otrzymanych przy zastosowaniu startera OPY 15. Oznaczenia na górze fotografii: MS i R wraz z numerami oznaczają odpowiednio badane linie CMS ogura oraz linie restorery, M – marker wielkości (w parach zasad): DNA faga λ trawione enzymami restrykcyjnymi Eco RI i Hind III. Strzałki wskazują polimorficzny prążek charakterystyczny dla linii restorerów systemu CMS ogura*

The size of the generated polymorphic DNA fragment is estimated as about 1250 bp on the basis of the molecular size marker (Fig. 2). In the case of RAPD markers the exact nucleotides sequence is not known, so the number of bp is given in approximation. Only cloning and sequencing of DNA fragment associated with the given marker allow to evaluate the precise size of a polymorphic band. It makes possible the transforming of a RAPD marker into a more effective SCAR marker as it has already been done with the marker OPC-02 (Mikołajczyk et al. 2005).

In the Figure 2 three different gels are presented in order to show a reproducibility and stability of the new marker, which is visible on all the gels. The pictures show the results of three RAPD reactions carried out independently of themselves at different times

The double low restorer lines for the CMS *ogura* system has been already developed, however the number of such restorer lines with improved yielding ability is very low. In the development of valuable restorer lines for CMS *ogura* system, components of hybrid varieties, every marker identyfying these lines and suitable to be applied to selection is of great value. A new band characteristic for restorer lines, which was developed with OPY-15 primer in RAPD reactions can be one of such markers. The large number of analysed genotypes (78) and the significant number of applied primers (27) may indicate that this band is the new RAPD marker assiociated with the restorer gene alleles. This hypothesis will be verified in further investigations.

Conclusion

The new RAPD marker developed with OPY-15 primer has been discovered. This fact marks the progress in the research on the introgression of radish genome fragment including the *Rfo* restorer gene.

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