Characteristic of winter rapeseed double low restorer lines for cms *ogura* system

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ABSTRACT

Breeding of oilseed rape hybrid varieties in Poland is based on cms *ogura* hybridization system. In the beginning, the major problem was the breeding of double low restorer lines containing less than 15 μM/g of seeds of glucosinolates. At present double low restorer lines have been selected and are used to produce double low restored F_1 hybrids. However, the selection of genotypes with homozygous alleles of the restorer gene and low glucosinolate content using molecular markers is still a problem. In some restorer lines, the introgression of the *Rfo* gene from radish is modified and the allele *Pgi-2* is lost. Especially in populations of restorer lines with extremely low glucosinolate contents, the selection by the use of isozyme PGI-2 marker is not effective. Because of the above difficulties, different RAPD markers have been tested on restorer lines to find markers for the restorer gene existing in different modifications of radish genome introgression into rapeseed genome. The obtained results showed that in genome of restorer lines with extremely low glucosinolate content and lost PGI-2 marker, PCR-RAPD molecular marker OPC 02 is not lost with the fragment of DNA responsible for high glucosinolate content.

Key words: *Brassica napus* – cms *ogura* - restorer gene – molecular markers – glucosinolates

INTRODUCTION

The lack of restorer lines with appropriate qualitative and agronomical traits was the factor limiting the utilization of CMS *ogura* system in breeding of restored hybrid varieties of double low winter oilseed rape (*Brassica napus* L.). Restorer gene *Rfo* has been introduced to the rapeseed genome from radish genotype (*Raphanus sativus*) (Heyn 1976) through intergeneric hybridisation. The obtained recombinants retained too much genetic information from radish and therefore they posses some undesirable traits: low seed set and close linkage of restorer alleles with the genes determining hight glucosinolate content. Investigations conducted by Delourme et *al*. (1995) revealed that the improvement of these traits and elimination of radish genetic information is possible by backcrosses with double low lines. The studies concerning the restorer gene markers revealed that this gene is close linkage with isozyme marker phosphoglucomutase PGI-2 (Delourme and Eber 1992) and four RAPD markers (Delourme et *al.* 1994). However, the selection of genotypes with homozygous alleles of the restorer gene and low glucosinolate content using molecular markers is still a problem. In some restorer lines, the introgression of the *Rfo* gene from radish is modified and the allele *Pgi-2* is lost (Delourme et *al*.1999). Especially in populations of restorer lines with extremely low glucosinolate content, the selection by the use of isozyme PGI-2 marker is not effective.
The aim of investigations undertaken by Oil Crop Department of IHAR was to obtain restorer lines with low glucosinolate content and good yielding ability.

MATERIALS AND METHODS

Investigations of double low restorer lines for CMS *ogura* in F_6 generations were carried out. The lines were selected from the crossing between low glucosinolate male sterile lines CMS *ogura* (4,1 – 11,8 μM/g nasion) and starting restorer line R with glucosinolates content of about 60 μM/g of seeds. Selection of genotypes with restorer gene alleles was carried out on the phenotypic expression of this trait and with the use of isozyme marker PGI –2 (Delourme and Eber 1992). The presence of molecular marker of restorer gene RAPD-OPC 02 (Delourme et *al*. 1994) was investigated in lines characterized by low glucosinolate content with PGI-2 marker and with lost PGI-2 marker.

The analyses of glucosinolates were performed with the method of gas chromatography of silyl derivatives of desulfoglucosinolates.

RESULTS

The selected plants in F_6 generation were characterized by low glucosinolate content in range 1,4 - 19,3 μM/g of seeds (Table 1.) About 87% of 102 restorer lines selected from F_6 progeny were characterized by glucosinolate content below 15 μM/g of seeds (Polish norm for sowing material). After the examination with the use of PGI-2 marker it was observed that 59,9% of restorer lines characterized by glucosinolate content in range 3,8 – 18,8 μM/g of seeds lost the radish *Pgi-2* allele (Fig.1.). The remaining 40,1% of investigated lines maintaining PGI-2 marker originating from radish were characterized by glucosinolate content in range 1,7 – 19,3 μM/g of seeds (Fig.1.). However, in the class of plants with very low glucosinolate content below 10 μM/g of seeds the majority of plants lost PGI-2 marker from their genotype. RAPD – OPC 02 marker carried by radish introgression was tested on all restorer lines. The presence of OPC 02 marker was observed in all low glucosinolate genotypes, independently of the presence or absence of *Pgi-2* allele of radish (Fig.2.).

Table 1. Glucosinolate content in restorer lines of F_6 progeny with PGI-2 marker and with lost PGI-2 marker

	Sum of glucosinolates in restorer lines of F_6 progeny (μM/g of seeds)	
	Plants with marker PGI-2	Plants with lost marker PGI-2
Number of lines (102)	41 (40,1%)	61 (59,9%)
Mean	12,2	9,9
Maximum	19,3	18,8
Minimum	1,7	3,8
Standard deviation	4,19	3,82
Coefficient of variability	34,33	38,76

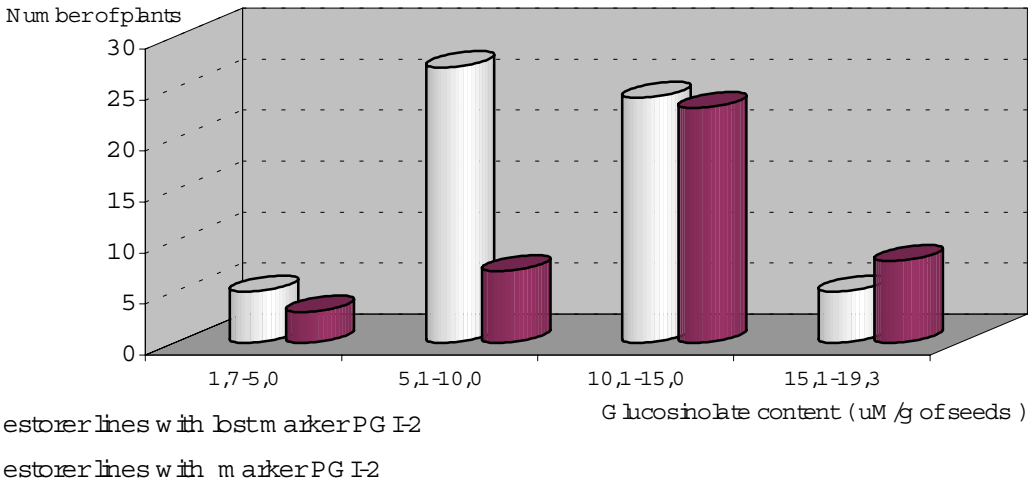

Fig. 1. Glucosinolate content in restorer lines of F_6 progeny with PGI-2 marker and with lost PGI-2 marker

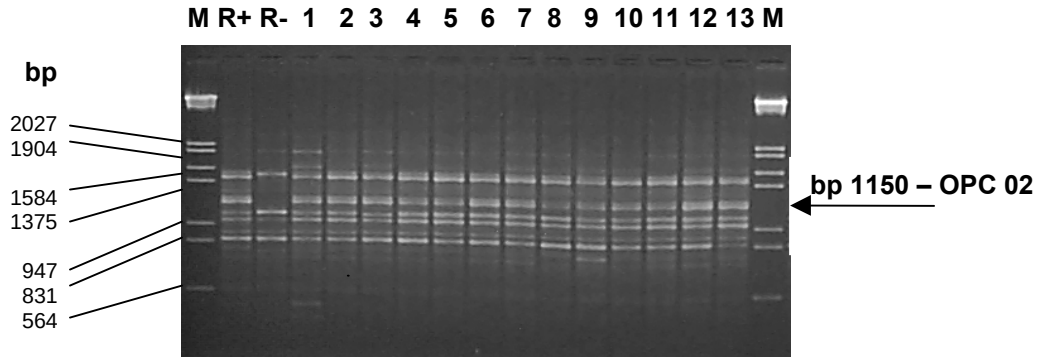

Fig. 2. 1.8% agarose gel electrophoresis of PCR-RAPD products obtained with the use of primer OPC 02; R+ - model plant with restorer gene, R- - model plant without restorer gene, 1-13 – plants tested for the presence of restorer gene marker (arrow indicate polymorphic band): 1-7 – plants with PGI-2 marker, 8-13 – plants without PGI-2 marker, M – molecular size marker (in base pairs).

DISCUSSION

It was stated on the basis of the conducted study that after the double low winter rapeseed genome was introduced to genome of restorer lines there may emerge recombinants without DNA fragments determining high glucosinolate content: with and without PGI-2 marker. The break of linkage between the restorer gene and radish isozyme PGI-2 marker occurred in the majority of investigated restorer lines. It indicates that the use of PGI-2 marker in selection for low glucosinolate restorer lines in very low glucosinolate populations was not effective, whereas in F_2 population with high and medium glucosinolate content the linkage of this marker was very high and appeared in average in about 98,8% individuals (Bartkowiak-Broda, Poplawska 2001). In the aim to verify if the linkage between gene restorer and RAPD marker OPC 02_{1150} stated by Delourme et *al*. (1999) appears also in population of low glucosinolate restorer lines with genotype of winter rapeseed bred in Poland this marker has been tested on the whole population of restorer lines. Investigated marker RAPD – OPC 02 closely linked to the restorer gene is not lost in extremely low glucosinolate recombinants together with the fragment of DNA responsible for high glucosinolate content. This marker can be used effectively in breeding programmes for low glucosinolate restorer lines for cms *ogura* system.

REFERENCES

Bartkowiak-Broda I, Poplawska W. 2001: Introgression of *Brassica napus* genome into restorer lines for CMS-*ogura* estimated with the use of molecular markers. Biotechnologia 1(52),112-118.

Delourme R., Eber F. 1992: Linkage between an isozyme marker and a restorer gene in radish cytoplasmic male sterility of rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 85, 222-228.

Delourme R., Eber F., Renard M. 1995: Breeding double low restorer lines in radish cytoplasmic male sterility of rapeseed (*Brassica napus* L.). Proc. 9th - Intern. Rapeseed Congress, Cambridge, UK, 4-7.07.1995, vol. 1, 6-8.

Delourme R., Horvais R., Vallé P., Renard M., 1999: Double low restored F_1 hybrids can be produced with the Ogu-INRA CMS in rapeseed. Proc. 10th Intern. Rapeseed Congress, Canberra, Australia, 26 –29.09.1999, CD ROM

Heyn F.W. 1976: Transfer of restorer genes from *Raphanus* to cytoplasmic male sterile *Brassica napus*. Cruciferae Newsletter, Eucarpia, 1, 15-16.