Development of Fertility Restorers of Winter Oilseed Rape with Low Glucosinolate Content for the CMS Ogu-INRA System

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Abstract: We have bred low glucosinolate (GSL) winter oilseed rape lines carrying the fertility restorer for the CMS Ogu-INRA system. The original restorer line BO20 contained 31µmol/g GSL in seeds, but by crossing this line with various low GSL CMS lines, followed by repeated selection of fertile segregants, we were able to obtain fertile lines with a mean GSL content in seeds of 11.8 µmol/g. This result confirmed that the gene(s) controlling the GSL content are not closely linked to the fertility restorer gene. The results confirm, that the SCAR marker SG34 is closely associated with the fertility restoring allele, and facilitates so the selection of fertile segregants; however, the marker is unable to distinguish between the homozygous *RfRf* and the heterozygous *Rfrf* genotypes.

Keywords: CMS Ogu-INRA; fertility restorer lines; glucosinolates; molecular markers; winter oilseed rape

Hybrid winter oilseed rape (*Brassica napus*) cultivars enjoy a 15 to 20% yield advantage over conventional varieties, thanks to the expression of hybrid vigour (Brandle & McVetty 1989). A number of methods have been developed to maintain genetic male sterility, in order to enable a commercial hybrid seed production. One of the most recent of these is the Ogu-INRA Cytoplasmic Male Sterility (CMS) system, in which the male sterile parent is homozygous recessive for the fertility restorer gene (*rfrf*) and contains a male-sterile cytoplasm (S), while the restorer line is genetically *RfRf* (Delourme *et al.* 1998). The CMS trait was obtained from a protoplast fusion between an oilseed rape cultivar and a CMS line carrying radish (*Raphanus sativus*) cytoplasm (Pelletier *et al.* 1983). The fertility restorer gene *Rfo* was introgressed into oilseed rape from radish (Heyn 1976).

Genetic mapping has suggested that one or more gene controlling seed glucosinolate (GSL) content are linked to *Rfo* (Pellan-Delourme & Renard 1988; Renard *et al.* 1997; Delourme *et al.* 1998), an observation that has been confirmed by the difficulty experienced in improving the seed quality of fertility restorer lines (Bartkowiak-Broda & Popławska 1999). In particular, this linkage has hampered the exploitation of this CMS hybrid system for the creation of double zero hybrids (Delourme *et al.* 1995, 1998), although it has

been shown, that double-zero fertility restorers can be obtained by conventional breeding methods (Delourme *et al.* 1999; Pruvot *et al.* 1999). While conventional phenotypic selection may be effective for selecting restorer lines, which are double zero for GSL, the use of a genetic marker for *Rf* has been seen as a desirable means of boosting the selection efficiency. Isozyme markers have not been very effective to date in this regard (Delourme *et al.* 1999; Bartkowiak-Broda *et al.* 2003). It has been, therefore proposed, that DNA markers, in particular RAPDs, would be more as informative and user-friendly markers (Delourme *et al.* 1994; Bartkowiak-Broda *et al.* 2003). The RAPD assay, however, is compromised by its poor reproducibility, and needs to be converted to the SCAR format in order to become practically useful (Hu *et al.* 2007). Here, we describe our progress in creating double zero GSL fertility restorer lines appropriate for use in the CMS Ogu-INRA system by means of a single cross with a low GSL donor, followed by pedigree selection. In addition, we demonstrate the reliability of the SG34 SCAR marker as an indirect selection criterion for fertility restoration.

MATERIALS AND METHODS

Plant materials

The original *Rfrf* fertility restorer line B020 was obtained from INRA (France). Its seed GSL content of > 31 μmol/g (measured at 9% moisture content) exceeds the limit of 18 μmol/g required by the Czech Variety Office for "low glucosinolate" cultivars. Therefore, it was crossed with 13 low GSL CMS lines to obtain populations varying in seed GSL content. The mean seed GSL content of the donors was 13.2 μmol/g. The male sterile line A115 ((S)*rfrf*) was used as the test-cross parent to perform a fertility restoration test (FRT) in the F_1 generation.

Selection of fertility restorer lines for low GSL content

Restorer lines were planted and evaluated in 2.5 m^2 plots. Fertile selections were isolated from external pollen by covering them with a polypropylene isolation bag. Seed GSL content was assessed by HPLC (High Performance Liquid Chromatography) using the instrument SP 8100 XR Spectra-Physics, USA.

The glucotest method was used for estimating GSL content in the first year of experiments. This method is based on enzymatic decomposition of GSL in crushed seeds to glucose, which is then semi-quantitatively measured, using reagent paper strips.

Selection of low GSL fertility restorer lines by the fertility restoration test

To distinguish between *RfRf* homozygotes and *Rfrf* heterozygotes, we used a fertility restoration test (FRT) as follows:

The CMS line A115 was pollinated under isolation by the tested restorer line. Seeds of the obtained F_1 hybrids were sown in 5-row microplots. Restorer lines, whose hybrids consisted of 100 % fertile plants were considered *RfRf* homozygous, while those producing about 50% fertile hybrids were considered *Rfrf* heterozygous.

Heterozygous restorer lines meeting the 18 μmol/g limit were accepted in the first two years, while in the third year only *RfRf* homozygous lines meeting the limit were accepted.

Marker assisted selection of fertility restorer lines using SCAR SG34

Genomic DNA was extracted using Invisorb Spin Plant Kit (Invitek) affinity columns. The SG34 primer sequences were as published by Primard-Brisset *et al.* (2005). PCR products were separated by 1.5% agarose gel electrophoresis in TBE buffer, and visualised by ethidium bromide staining. At least 80 F_1 plants were analysed with the SG34 marker.

Statistical evaluation of experimental results

The STATISTICA package (StatSoft, Inc.,Tulsa, USA) was used for all statistical analyses. The selection differential (S) for GSL content was defined as the difference between the parental generation mean and selected progeny mean, and the response to selection by the difference between parental and next generation means. The intensity

of selection (IS) for GSL content was given by S/σ_p, where σ_p was the standard deviation of the progeny population.

RESULTS AND DISCUSSION

Selection of fertility restorers for low GSL content

The B020 (*Rfrf*) × CMS line (*rfrf*) F_1 generation segregated as *Rfrf* (male fertile) and *rfrf* (sterile). From the 95 fertile plants selected in 2000 (Table 1) 55 low GSL plants were selected using the glucotest method. In 2001, the stronger limit of 13 µmol/g GSL was applied and 38 fertile plants out of 178 were selected. In the following generation 22 selections were obtained with a mean GSL content of 12.4 µmol/g. Because of this large reduction in plant numbers, selection was relaxed, accepting 15 µmol GSL/g in the following year. From 96 fertile plants analysed in 2003, 65 were selected (IS = 0.55). The mean seed GSL content of this population had increased to 16.1 µmol/g, but this is still well below the officially required limit of 18 µmol/g. Variation in GSL content during the course of selection has been noted also by Rucker and Rőbbelen (1994), who ascribed low GSL content to the additive action of four or five recessive genes. Some of the variation from year to year can also be caused by climatic influence (Felde *et al.* 2006).

Selection of fertility restorers using the fertility restoration test

A set of 28 *Rf*-lines was selected in the first year of the FRT (IS = 0.21), and 15 of these showed a good level of fertility restoration. Five of the 15 lines produced seed with an acceptable GSL content (Table 2). In the second year of the FRT, 33 lines were selected (IS = 0.27), and of the 25 *RfRf* selections, 14 produced seed with an acceptable content of GSL. In the final year of the FRT, 21 restorer lines were selected, of which 19 produced seed with an acceptable content of GSL (IS = 0.05). Thus, we were able, in agreement with Delourme *et al.*

Table 1. The number of selected plants, the seed GSL content of their progeny (µmol/g) and the selection criteria imposed within each growing season

Year of harvest	No. of isolated plants/lines	GSL content in the population under selection				No. of selected plants	Selection criterion for GSL (µmol/g)
		mean	min.	max.	SD		
2000	95*	–	–	–	–	55	≤ 18
2001	178	17.54	3.57	32.17	5.07	38	13
2002	39	15.04	5.01	35.45	6.09	22	15
2003	96	12.40	4.20	40.40	5.83	65	15
2004	78	16.09	3.81	34.97	6.04	28	18
2005	72	15.49	5.13	36.54	5.52	33	18
2006	84	11.82	0.37	23.52	5.13	19	18

*Tested by the glucotest method; SD – standard deviation

Table 2. The occurrence of dominant homozygous restorer lines with acceptable seed GSL content

Year of fertility restoring test (FRT)	No. of dominant homozygous restorer lines (*RfRf*)	No. of dominant homozygous restorer lines with acceptable GSL content	Occurrence of lines with acceptable GSL content in the group of dominant homozygous restorers (%)
1	15	5	33.33
2	25	14	56.00
3	21	19	90.48
Total	61	38	–

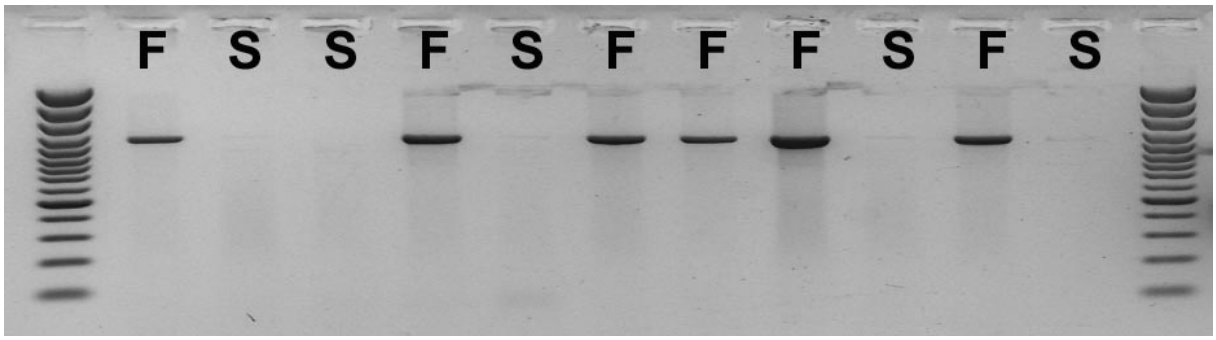

Figure 1. Association of male fertility with the presence of the SCAR marker SG34 (F – fertile plant, S – sterile plant)

(1999), Bartkowiak-Broda and Poplawska (1999) and Pruvot *et al.* (1999), through conventional pedigree breeding to generate restorer lines with desirable seed quality. The lack of a correlation between GSL content and fertility restoration is consistent with the report of Bartkowiak-Broda *et al.* (2003).

Marker assisted selection of fertility restorer lines using SG34 SCAR

No distinction was possible between *RfRf* and *Rfrf* segregants using the SG34 SCAR, as this is a dominant marker. Absence of the marker is indicative of an *rfrf* genotype. DNA from all 80 male fertile plants amplified the SG34 product (Figure 1).

CONCLUSION

We were able to verify that it is possible to select oilseed rape *Rf/Rf* fertility restorer lines with low seed GSL content, using conventional phenotypic selection. The SG34 SCAR marker facilitates the detection of the *Rf* allele among segregating individuals, although it does not discriminate *RfRf* homozygote and *Rfrf* heterozygote individuals.

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