A new recombined double low restorer line for the *Ogu*-INRA cms in rapeseed.

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ABSTRACT

Getting double low restorer lines with a shorter introgression and a good agronomic value is always a major breeding objective for the *Ogu*-INRA cytoplasmic male sterility (cms) system in rapeseed (*Brassica napus* L.).

The development of low glucosinolate content (low GC) restorer lines often occurred through deletion of a part of the introgression. One of these lines has lost the radish *Pgi-2* allele expression, without recovering the one of rapeseed *Pgi-2* allele. It showed a defect in meiotic transmission of the restorer gene *Rfo* and a very poor agronomic value.

We initiated a program to force non-spontaneous recombination between this *Rfo* carrying introgression and the rapeseed homologous chromosome from a low GC *B. napus* line. Gamma ray irradiation was used to induce chromosome breakage just before meiosis aiming at such recombination. Low GC cms test plants were crossed with the pollen of irradiated plants, heterozygous for this introgression. F2 families were scored for their vigour, transmission rate of *Rfo* and for their female fertility. One family, 'R2000', showed an improved behaviour compared to the irradiated parent, regarding the three criteria. It appears stable over several selfed generations. Molecular markers were developed to characterise this new recombined genotype.

Key Words: Ogu-INRA, cytoplasmic male sterility, fertility restoration, γ rays irradiation

INTRODUCTION

Breeding restorer lines for the *Ogu*-INRA Cytoplasmic Male Sterility (cms) system in rapeseed (*Brassica napus* L.) has been a major objective during the past few years. Extensive backcross and pedigree breeding were necessary to improve their female fertility and to get double low restorer lines. However, some difficulties can still be encountered in breeding these lines (introgression rearrangements, possible linkage with negative traits) due to the large radish introgression. Our objective was then to get an improved double low restorer line with a good agronomic value and, if possible, a shorter introgression.

We choose one low GC spring homozygous restorer line, 'R211', already exhibiting deletions in the introgression (2a,2b,3). Several molecular markers are missing on either side of *Rfo*, such as spATCHIA (3), *spSG91 (4).* 'R211' lost the isozyme expression of the *Pgi-2* allele of the radish gene but also the one of *Pgi-2* allele of *B.oleracea* genome (1,2). Moreover, the homozygous 'R211' shows linked negative traits such as low vigour and very poor seed set. We hypothesised that these plant lack a rapeseed chromosomal segment. The fertile ratio in F2 progenies derived from this material is lower than expected (64% instead of 75%). We initiated the program from this 'R211' line and tried to force non-spontaneous recombination between the *Rfo* carrying introgression from this deleted line and the rapeseed homologous chromosome from a low GC *B. napus* line.

Ionising irradiation is known to induce chromosomal rearrangements by double strand breaks followed by aberrant rejoining of the ends (5.6). γ-ray irradiation was used on a heterozygous F1 derived from the 'R211' line to induce chromosome breaks, just before meiosis, aiming at a recombination of the deleted radish introgression in the rapeseed genome.

MATERIALS AND METHODS

Genotypes: The ‘R211’ line with a deleted radish insertion was crossed to the spring low GC rapeseed ‘Drakkar’ to produce a F1 progeny (’R211*Dk’). The spring low GC cms line ‘Wesroona’ (australian origin) was used for following crosses. Winter restored lines derived from ‘Samourai’ carrying the complete (‘RRH1’) or incomplete (‘R113’,) introgression as well as European radish line7, Asiatic restored radish D81 and wild radish were used as control in molecular analyses.

Gamma ray irradiation: Whole flowering plants were treated with gamma rays from a Co60 source in a controlled area. Dose flow was tested on the first inflorescence of sets of six plants. The chosen dose was used for further two experiments on 35 plants.

Testcrosses and F2 production: Irradiated plants were transferred in an insectproof greenhouse after removing flower buds larger than 2 mm. The irradiated F1 progeny was used to handpollinate the cms ‘Wesroona’ line, chosen for its molecular polymorphism compared to‘Drakkar’. The restored derived F1’ plants were allowed to produce F2 families harvested individually and precisely sown in a field assay along with non irradiated controls.

Phenotypic selection: Three visual criteria were scored (on a 1 to 5 scale) 1-Vegetative vigour 2- Normality of the ratio of fertile /sterile plants in the F2 segregation 3- Female fertility (pod aspect and seed set). Advanced selfed generations of the selected families were obtained either in field or greenhouse and produced homozygous lines (F4) for further analysis.

Isozyme analysis was performed as in (1) Marker development from (3): PCR products are validated by sequencing. Alignments were made using Blast NCbi and Uk Crop Net Brassica DB and the Multialin software INRA Toulouse.

RESULTS

Co60 irradiation flow doses of 55, 65, 90 and 110 Gray/hour were assayed in two different energy regime, either an hour (long irradiation LIR) or a tenth of this time, six minutes for plants (short irradiation SIR of a higher energy). The gamma ray sublethal dose flow of 65 Gray/hour has been determined as the maximum dose allowing pollen maturation from young buds irradiated prior meiosis.

F2 progeny analysis was made over three years in field assays. 1265 offsprings plus 57 controls (82 330 quoted plants) were scored. Phenotypic selection was made considering all the fertile plants (*Rfo/Rfo* and *Rfo*/-) in each F2. Very few families were at the best score for the three criteria. Only one, ’R2000’, issued from a SIR, proved to produce homozygous plants with a stable recovery of good agronomic traits, such as normal stem stiffness and a good female fertility, with a normal seed set compared to ‘R211’ (Tab1). Glucosinolate analysis confirmed its low content.

PGI isoenzyme analysis showed that fertile ‘R2000’ progeny expressed the rapeseed *Pgi-2* allele from *B. oleracea* genome. From the sequence of *Pgi-2* in BrassicaDB, we developed a PCR marker PGIol specific to the *B.oleracea* genome *Pgi-2* allele (248bp band in the MJB21-12 *Arabidopsis* locus) in the tested spring rapeseed lines. The ‘R211’ line doesn’t show any amplification while the ‘Drakkar’ maintainer and the F1 (‘R211’*Dk) show the PGIol band. We showed that the homozygous ‘R2000’ family has recovered the PGIol band.

The PGIol band sequences from all the tested genotypes were compared. It revealed a polymorphism at a restriction site due to one nucleotide substitution within the 248bp sequence between two winter genotypes (Samourai and a derived Darmor line) and the spring ones (‘Drakkar’, and Wesroona), The ‘R2000’ PGIol sequence is identical to the ‘Drakkar’ type. This can be visualised by restriction analysis (fig1). ‘R2000’ has recovered the PGIol gene from the spring genome, possibly from the Drakkar genome, present in the irradiated F1’ (R211.Dk).

Then we developed a co-dominant marker PGIint amplifying a longer part of the *Pgi-2* gene, in a conserved area allowing amplification in all the tested species. The band size allows the clear distinction between *Arabidopsis*, European or Asiatic radish, wild radish and *Brassica*. *B.rapa* differs from *B.oleracea* in their PGIint sequence. It shows that the tested restored rapeseed genotypes, but the ‘R211’ line, exhibit the European radish band together with the *Brassica* one, homologous to the *B.rapa* one. The ‘R2000’ plants don’t show the radish band, as ‘R211’but show the *Brassica* ones, homologous to the *B.oleracea* and *B. rapa* ones.

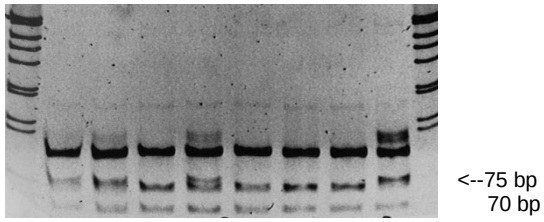
We used the SG129 PCR marker, as it is located closer to the *Rfo* gene (4). Its interest is that it co-amplifies distinct bands for the *B.oleracea* (950bp) and *B.rapa* (850bp) genomes in

B.napus. The previous restored rapeseed genotypes showed only the *B.rapa* band, suggesting that the *B.oleracea* ortholog of the target gene is absent or modified. We observed that 'R2000' homozygotes again recovered the *B.oleracea* band. Its sequence blasts with the common ends of two *Arabidopsis* clone contigs and also blasts with one *B.oleracea* clone, in BrassicaDB. The second *Arabidopsis* clone contig allowed to blast for another *B.oleracea* clone distant from about 300bp on the *Arabidopsis* map. We design a new marker, BolJon, between the two *B.oleracea* clones. It co-amplifies a band of a specific size for each tested species *Arabidopsis*, European or Asiatic radish, wild radish and *B.rapa* and *B.oleracea*. The *B.rapa* and the European radish bands are present in homozygous restored rapeseed including 'R211'. 'R2000' shows the two bands of 'R211' plus the recovered *B.oleracea* BolJon band.
The homozygous 'R2000' presents a unique combination of the PGIint and BolJon markers when compared with the different complete or incomplete rapeseed restorer analysed yet.

Tab1: Number of seeds per pod assessed on the best 'R2000' F4 families in self pollination (SP) and in testcrosses (TC) on cms 'Pactol' as well as on control

Genotype	SP	TC
Drakkar	29.3	
Pactol	23.1	
R211	11.2	25.5
R2000	26.5	27.0
	(24.0 – 31.1)	(24.0 – 28.7)

Fig1: Digestion of the PCR PGIch bands by Mse1 rest. enz. Samourai: 1, Darmor: 2, lines have a 75bp band; Drakkar 3, R211.Dk: 5 and R2000: 6,7 a 70bp one. (Acrylamlide 15%)



DISCUSSION

In this work, we selected a new recombined low GC restorer line that exhibits a good female fertility. It is characterised by the association of markers that are not linked in the parental lines. Our hypothesis is that in the pollen mother cell that gave rise to 'R2000' plants, a recombination event took place linking the deleted insertion radish segment to the normal 'Drakkar' homologous chromosome segment, carrying the *B.oleracea Pgi-2* gene.

The choice of the poor line 'R211' was determinant for the ability to, first, select in field for a rare recombination event and, secondly, to characterise the 'R2000' family. This family is the only one among 12 tested families issued from the same inflorescence to be different from the controls. Still, as a single event was screened among all tested families, it is difficult yet to connect this event directly to an irradiation effect in a pollen mother cell.

The pattern observed for BolJon suggests that the recombination event resulted in a duplicated region (originating from radish and *B. oleracea*) on the chromosome carrying the introgression in the 'R2000' family. At least, one gene is recovered in 'R2000' plant; more markers are required to investigate whether the already shorten 'R211' insertion has been modified or not by the rearrangement.

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