Analysis of winter oilseed rape (*Brassica napus* L.) genomic DNA region linked to restorer gene for CMS *ogura* – transformation of an RAPD marker into a SCAR marker

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ABSTRACT

Fertility restorer lines for gene-cytoplasmic male sterility *ogura* system applied for oilseed rape hybrid varieties breeding can be characterized on molecular level with the use of molecular markers. OPC-02 is an RAPD marker linked to restorer gene and used for routine analyses. In order to characterize it as well as to transform RAPD/OPC-02 marker into a SCAR marker, genomic DNA was isolated from a restorer line for CMS *ogura* system. 1149 bp OPC-02 amplified fragment was cloned and sequenced. NCBI sequence alignment revealed the presence of a short sequence similar to an *A.thaliana* expressed protein of unknown function. A pair of primers was designed on the basis of C-02 nucleotide sequence and PCR reaction conditions were established to obtain a SCAR marker. Application of the new marker to analysis of over 80 lines revealed that it is characteristic for restorer gene and can be used instead of the RAPD marker.

Key words: winter oilseed rape (*Brassica napus* L.), restorer gene, DNA markers

INTRODUCTION

Cytoplasmic male sterility (CMS) is a maternally inherited trait encoded by the mitochondrial genome and characterized by the inability of plant to produce functional pollen. It is applied in breeding programmes for producing of F_1hybrid crops. In Poland, oilseed rape hybrid breeding is based on gene-cytoplasmic CMS *ogura* system which fully ensures cross-pollination [Bartkowiak-Broda I., 1998; Bartkowiak-Broda *et al.*, 2001]. Nuclear fertility restorer genes (Rf) suppressing the male sterile phenotype and restoring fertility of plants carrying the CMS mitochondrial genome have been introduced into *B.napus* from *R.sativus* genome *via* intergeneric crosses [Heyn, 1976] resulting in fully restored oilseed rape lines which are components of F_1 hybrid varieties [Pelletier *et al*., 1987; Delourme et. al., 1995]. Selection of restorer lines as well as restored hybrids is accompanied by the use of molecular markers which is a powerfull tool enabeling identification of a trait of interest at the early developmental stages. A dominant isozyme *PGI-2* marker linked to restorer gene [Delourme and Eber, 1992] has been succesfully used for identifying *Rf* alleles. However, in some very low-glucosinolate lines the linkage of the marker and restorer gene has been broken and in such a case other markers were usefull. OPC-02 belongs to a group of RAPD markers linked to restorer gene [Delourme*et al.*, 1994] and it is used for routine analyses at the Plant Breeding and Acclimatization Institute in

Poznań [Mikołajczyk *et al*., 1998], especially for selection of low glucosinolate restorer lines [Bartkowiak-Broda *et al*., 2003].

The aim of this work was to analyse genomic DNA region amplified by RAPD OPC-02 primer and linked to an Rf gene, as well as to transform an RAPD marker into a SCAR marker.

MATERIALS AND METHODS

Plant Material: Restorer lines and restored hybrids of winter oilseed rape obtained from breeding materials of the Plant Breeding and Acclimatization Institute, Poznań Branch as well as of Plant Breeding Strzelce Ltd., Co. – Division at Borowo were used for research.

DNA Isolation: Genomic DNA was isolated from 10-days old leaves with the use of the method described by Doyle [1990].

DNA Electrophoresis: Genomic DNA as well as PCR reaction products were analysed by 0,8% and 1,8% agarose gel electrophoresis in 1xTBE buffer, respectively.

DNA Cloning and Sequencing: *B.napus* restorer line genomic DNA fragment linked to Rfo gene was PCR amplified with the use of Operon Technologies OPC-02 primer [Delourme *et al*., 1994]. The obtained polymorphic band was gel eluted and used as a template in re-PCR amplification. It was then cloned by means of TOPO T/A Invitrogen cloning system. Plasmid DNA of positive clones was isolated with the use of Qiagen kit. Sequencing reaction was performed authomatically on both strands with the use of DTCS (Beckman Coulter) i BigDye v3.1 (Applied Biosystems) reagents as well as CEQ2000XL i ABI Prism 3130XL sequencers. Nucleotide sequences obtained were further analysed using NCBI BLASTN alignment tools.

Transformation of the OPC-02 RAPD Marker into a SCAR Marker: A pair of primers was designed on the basis of the 5' and 3' nucleotide sequence regions of C-02 fragment and PCR reaction conditions were established in order to obtain a SCAR marker, characteristic for Rfo genotypes. It was then used for testing 75 restorer lines and hybrids of different genetic background – *i.e*. homozygous or heterozygous restorers, restored F_1 hybrids as weel as new homo- or heterozygous restorers.

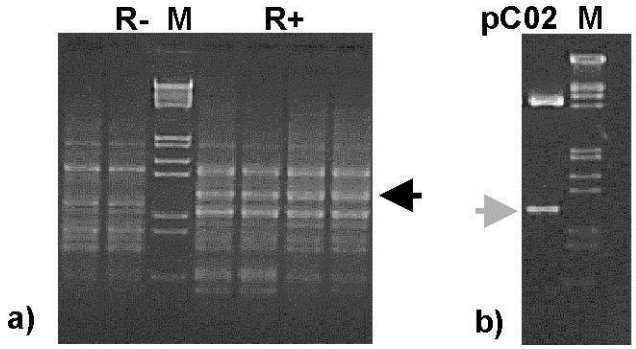

Figure 1. Agarose gel electrophoresis of a) RAPD OPC-02 products; R^- – DNA of oilseed rape plants without restorer gene, R^+ – DNA of oilseed rape plants with restorer gene; b) plasmind DNA containing a cloned C-02 insert (pC-02); M – size marker: λ phage DNA digested with *Eco RI* and *Hind III* endonucleases

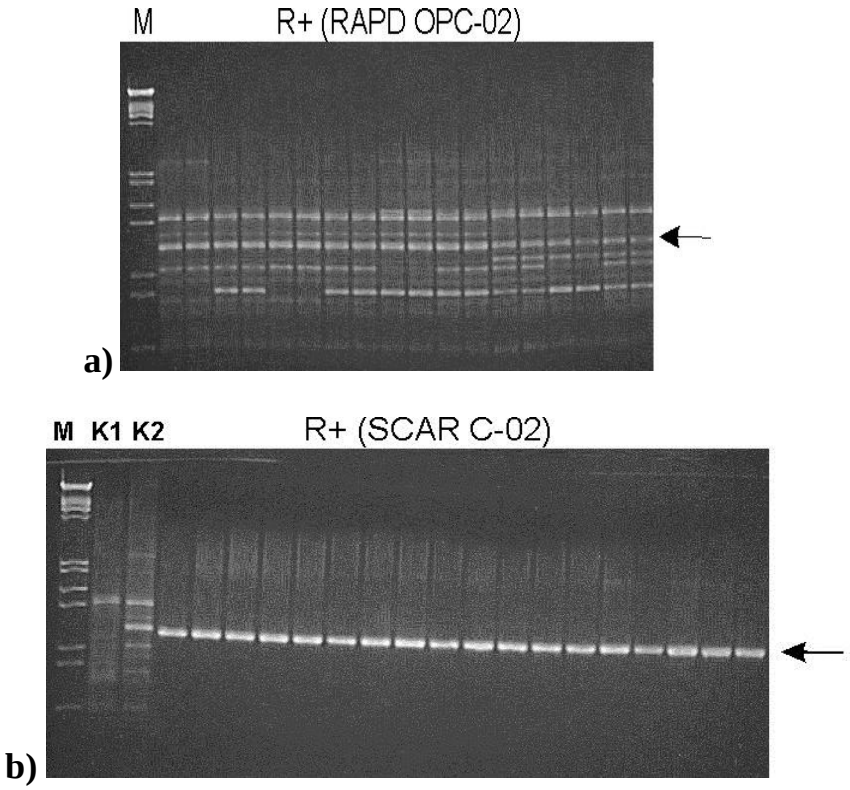

Figure 2. Agarose gel electrophoresis of PCR products obtained from: a) RAPD OPC-02, b) SCAR (C-02); K1 and K2 – PCR/RAPD OPC-02 amplification of genomic DNA obtained from oilseed rape plants without- and with Rfo gene, respectively; M – DNA size marker: phage λ DNA digested with endonucleases *Eco RI* and *Hind III*; arrows indicate C-02 band

RESULTS AND CONCLUSIONS

1. *B.napus* genomic DNA region of 1149 bp, linked to a fertility restorer gene, was amplified with the use of RAPD OPC-02 primer, cloned and sequenced (Fig. 1)
2. Alignment with NCBI nucleotide revealed the presence of three regions of high similarity to records from databases and representing expressed sequences (not shown); possible expression of the regions is under study
3. A pair of primers was designed on the basis of C-02 nucleotide sequence and PCR reaction conditions were established to obtain a SCAR marker
4. Analyses with the use of the marker revealed that it is characteristic for the presence of restorer gene and can be used equvalently to RAPD OPC-02 marker (Fig. 2)
5. Application of the obtained marker for routine analyses will make the selection of oilseed rape plants with a fertility restorer gene for CMS *ogura* more effective

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