Glucose-6-phosphate Isomerase as a Marker of a Fertility Restorer Gene in Rape

Jiří HORÁČEK and Martina AČANOVÁ

AGRITEC, Ltd., Šumperk, Czech Republic

Abstrakt: In breeding of winter rape (*Brassica napus* L. var. *napus*) using the OGU-INRA system, based on cytoplasmic male sterility (CMS), it is necessary to distinguish pollen-sterile lines, carrying CMS factors and the recessive fertility restorer alleles *rfrf*, from fertile lines, carrying at least one dominant restorer gene allele (*Rfrf* or *RfRf*). To grow plants till the flowering stage takes much time. The method was therefore modified using isozyme markers of glucose-6-phosphate isomerase (PGI) to distinguish male sterile (MS) from male-fertile lines in early stages. Since the restorer gene is tightly linked to the markers and the PGI isozymes can be distinguished by electrophoresis, the markers can be used to identify MS rape plants in early stages. Also, homozygous and heterozygous fertility-restored plants can be separated this way. In our work we tried to optimise the distinction of pollen-fertile and pollen-sterile rape plants with PGI isozyme markers, using vertical polyacrylamide gel electrophoresis (native-PAGE). The method will be used for the breeding of rapeseed with the OGU-INRA system.

Keywords: *Brassica napus*; winter rape; *Ogura* cytoplasmic male-sterility restorer gene; isozyme; PGI (glucose-6-phosphate isomerase)

Winter rape is the most spread oilseed plant in the Czech Republic and the second most cultivated crop after cereals. One of the most effective methods of winter rape breeding is the production of hybrid varieties, based on development and maintainment of MS lines and restorer lines. Several hybrid systems exist. One of the most advanced is the INRA/Ogura system patented as OGU-INRA. It is used to produce hybrid seeds with high heterosis effects on vitality and productivity and with declared characteristic features. In this system three kinds of lines are used: MS lines, carrying cytoplasmic MS factors and two recessive alleles of the male fertility restorer gene *rfrf*, (the interaction of the plasmotype with the recessive restorer gene alleles results in male sterility). The fertile lines carry one or two dominant restorer gene alleles and are either *Rfrf* or *RfRf*. The dominant allele *Rf* is tightly linked with high glucosinolate content. In hybrid rape breeding restorer lines with low glucosinolate content are desired for pollination of "00"-type MS lines (Vašák 1997; Poplawska *et al*. 1999). The male fertility restorer gene (*Rf*) was introgressed from radish (*Raphanus sativus* L.) into rapeseed by homologous recombination, substituting some rapeseed alleles. The *PGI* gene from radish was co-transferred with the *Rf* gene due to their tight linkage on the transmitted chromosome part (the radish isozyme allele at the Pgi-2 locus is tightly linked to the *Rf* gene, Delourme & Eber 1992).

Breeding of pollen fertile parental lines requires the recognition and elimination of MS plants. This had to be done so far in the bloom stage, several months after sowing. Since PGI isozymes (EC 5.3.1.9) can be used as a markers for different alleles of the fertility restorer gene, we tried to develop and optimise the methods of PGI analysis to allow the recognition of MS plants in early growth stages and to make the method suitable for the OGU-INRA breeding system. This would shorten the breeding process and make it cheaper.

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QE 0052.

MATERIALS AND METHODS

Plant material. A set of 200 winter rape plants from the greenhouse and from field trials was analysed. All originated from breeding programs for low glucosinolate content. Plants were purposely selected so, that both sterile and fertile lines were present in the sample. The 2nd true leaves of the young plants were analysed and the plants were then grow till flowering.

Sample preparation. 100 mg of leaf tissue was homogenised with 200 µl of extraction buffer (50mM Tris-HCl, 5mM 2-mercaptoethanol, 1mM EDTA, pH 7.4) and frozen at –20°C over night. Then, 100 µl of loading buffer (extraction buffer + 10% glycerol + 0.01% Bromophenol Blue) was added to the samples. The samples were centrifuged at 15 000 g (4°C) for 15 min and 10 µl of supernatant was loaded onto gel.

Electrophoretic analysis. Isozymes were separated using native discontinuous vertical electrophoresis on polyacrylamide gel (PAGE) (8% running gel, pH 6.4 and 4.5% stacking gel, pH 6.0) at 4°C. Immobilised glucose-6-phosphate dehydrogenase (EC 1.1.1.49, Harrison 1974) was added to the running gel as auxiliary enzyme. Tris-tricin buffer (pH 7.1) was used as the cathode solution, Tris-acetate buffer (pH 6.4) was used at the anode. Electrophoretic separations were performed at constant current of 50 mA for 4 hours.

Specific detection of PGI isozymes. Specific detection of PGI isozymes was performed according to the protocol of Harrison (1974). Staining was performed at 37°C for 1 hour. The stained gels were fixed, dried, scanned and saved as electronic files.

RESULTS AND DISCUSSION

We first analysed male-sterile plants without a *Rf* allele. The analyses were performed with tissue of the second true leaf of young plants, which were then grown till flowering. In all samples an identical PGI-2 isozyme pattern, consisting of three bands, was found (Figure 1). This pattern was expected, since PGI-2 is a dimeric enzyme with three possible combinations of two different subunits. It can be assumed, that any change in the PGI-2 isozyme spectrum indicates the presence a dominant *Rf* gene allele.

We analysed then 200 plants, a part of which was male-sterile and part male-fertile with the fertility restorer allele *Rf*. The plants differed in their electrophoretic patterns (in number, position and intensity of bands) as shown in Figure 2. We have found, that all deviations from the pattern of MS plants marked the presence of the *Rf* allele. The number of bands was in some cases greater, in some smaller than in MS plants (Figure 3). Some fertility-restored plants had two additional bands in the PGI-2 region, which were absent in MS plants. The increased number of bands can be explained by the presence of one radish allele *Pgi*-2, interacting with *Pgi*-2 alleles of rapeseed in the restored plants. As mentioned above, PGI is a dimeric enzyme. Therefore an additional allele results in two additional bands: a dimer encoded by the radish allele and an interallelic dimer encoded by this allele and a rapeseed allele. The second heterodimer of the radish allele and the rapeseed allele superposes the rapeseed isozymes (Delourme & Eber 1992). The presence of an additional allele probably decreases the amount of the most cathodic isozyme dimer. This is demonstrated by the lower intensity of the cathodic band. The explanation of a reduced number of bands is more difficult. It is probably caused by recombination with fertile lines without the PGI-2 marker (i.e. the *Rf* gene was introgressed without adjacent DNA and thus without Pgi-2). There was complete agreement between the isozyme patterns and the pollen sterility/fertility phenotype of the analysed plants. Thus, the use of PGI isozymes, determined by PAGE, as markers of the male-fertility restorer

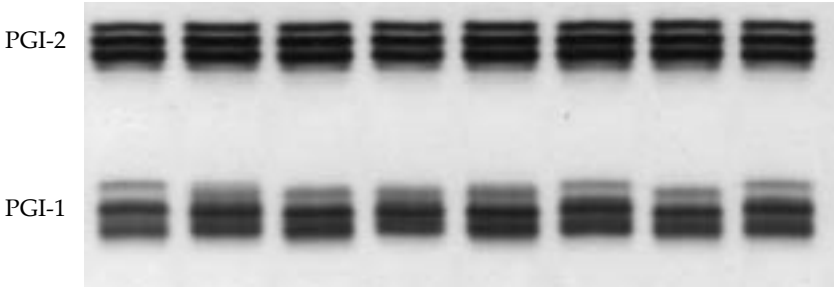

Figure 1. PGI electrophoreograms of rape without the *Rf* allele (male-sterile plants)

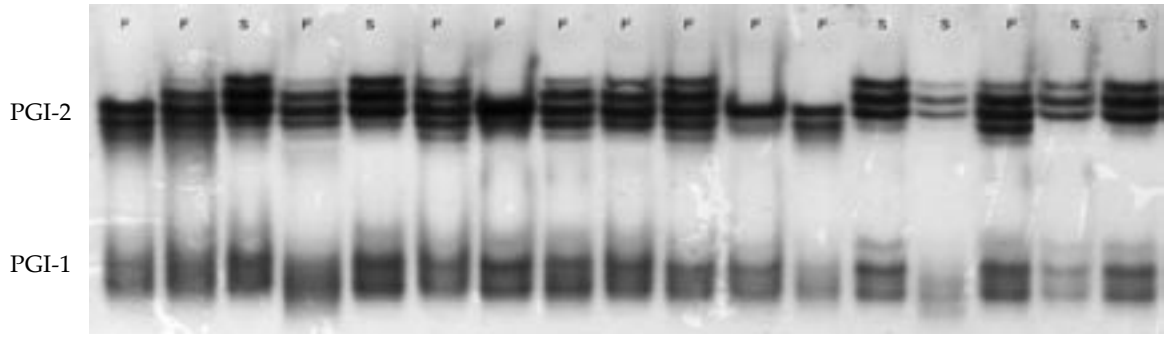

Figure 2. PGI electrophoreograms of male-sterile (S) and male-fertile (F) rape

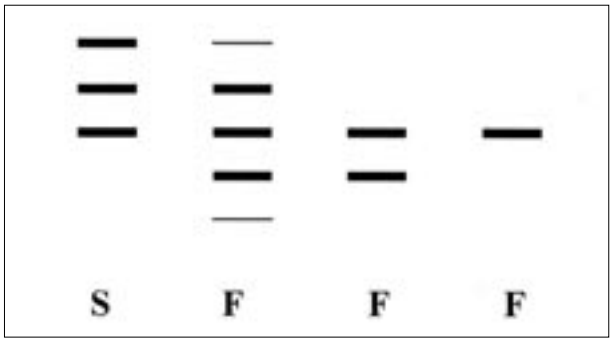

Figure 3. PGI-2 patterns of male-sterile (the left one) and male-fertile rape (all others). Only strong bands were considered for evaluation

gene can be presented as an optimised method, enabeling an easy separation of male-sterile from pollen-fertile plants.

PGI-2 isozymes are not the only markers of the fertility restorer gene. The use of RAPD markers was also described (Delourme *et al*. 1994; Hansen *et al*. 1997), based on the finding of some primers linked to the *Rf* gene. However, DNA-analysis is more laborious a expensive in comparison with the analysis of isozymes and storage proteins. Therefore, the analysis of PGI might be the method of choice.

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Received for publication March 6, 2003
Accepted after corrections August 4, 2003

Souhrn

Horáček J., Ačanová M. (2003): **Glukosa-6-fosfát isomerasa marker genu obnovy cytoplazmatické samčí fertility u řepky ozimé**. Czech J. Genet. Plant Breed., **39**: 130–133.

V průběhu šlechtění řepky ozimé (*Brassica napus* L. var. *napus*) systémem OGU-INRA, využívajícím cytoplazmatické samčí sterility, je důležité odlišení pylově sterilních linií, nesoucích kromě cytoplazmatických faktorů samčí sterility také recesivní alelu genu obnovitele samčí fertility *rfrf*, od pylově fertilních linií, obsahujících nejméně

jednu dominantní alelu téhož genu (*Rfrf* nebo *RfRf*). Pěstování rostlin do fáze květu je časově velmi náročné. Proto byla metoda pro odlišení jednotlivých linií řepky modifikována užitím isoenzymového markeru PGI (glukosa-6-fosfát isomerasa). Je známo, že mezi genem obnovitele samčí fertility a markerem je těsná vazba. Isoenzymy PGI, příslušející jednotlivým liniím řepky, se liší svými elektroforetickými spektry – je možné odlišit pylově fertilní a pylově sterilní rostliny v raných fázích vývoje. Práce se zaměřila na optimalizaci metodiky odlišení pylově sterilních a pylově fertilních rostlin řepky s použitím isoenzymového markeru PGI. Analýzy byly prováděny metodou vertikální elektroforézy na polyakrylamidovém gelu za nativních podmínek. Metodika bude využívána při šlechtění řepky systémem OGU-INRA.

Klíčová slova: *Brassica napus*; řepka ozimá; *Ogura* – gen obnovy cytoplaymatické samčí fertility; isoenzymy; PGI (glukosa-6-fosfát isomerasa)

Corresponding author:

Mgr. Jiří Horáček, Ph.D., AGRITEC, s. r. o., Zemědělská 16, 787 01 Šumperk, Česká republika
tel.: + 420 583 382 139, fax: + 420 583 382 999, e-mail: horacek@agritec.cz