Production of improved self-incompatible lines of winter oilseed rape (*Brassica napus* L.) with convenient seed quality for hybrid breeding using of microspore culture technique

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Abstract

Doubled haploid (DH) regenerants were derived via improved microspore culture technique from crosses between the donor of "00" quality OP 2051 (pollinator) and initial self-incompatible (SI) line Tandem 6/85 with high Glucosinolate (GSL) and erucic acid content in seed. The average GSL content in this set of regenerants was 84.68 μmol/g of dry matter of seeds and the average erucic acid content was 22.84%. In the second cycle of quality improvement, two selected DH SI lines were crossed with donors of quality (two breeding materials and two commercial cultivars). The percentage of obtained SI plants was 38.2%, i.e. 87 out of 228 derived fertile DH regenerants. 72 plants were selected for the detection of erucic acid content. The average erucic acid content in the set of DH regenerants was 14.20%. Nine plants with erucic acid lower than 2% were analyzed for glucosinolate content by high-pressure liquid chromatography (HPLC) method. Five out of the selected nine plants had GSL content lower than the limit 18 μmol/g at 9% seed moisture. The results of analyses showed that in the case of SI DH regenerants from the second cycle of quality improvement the critical factor of seed quality was erucic acid content contrary to GSL content. The DH method proved to be applicable for the seed quality improvement of DH lines together with maintaining of SI reaction. The method based on the spraying of inflorescences with 5% NaCl to enable reproduction of SI lines was verified and used in a large scale. In 2006, F_1 seed from 44 crosses between improved DH SI lines and high yielded lines have been produced and will be included in station trials to evaluate agronomic performance in 2007.

Key words: oilseed rape (*Brassica napus* L. var. *napus*), hybrid breeding, self-incompatibility (SI), seed quality, glucosinolates, erucic acid, doubled haploids (DH)

Introduction

Hybrid vigor in rapeseed (*Brassica napus* L.ssp. *napus* f. *biennis*) was noted and verified by several authors (Thompson, 1983; Grant and Beversdorf, 1985; Lefort-Buson *et al*., 1987; Paul *et al*., 1987; Paulman and Frauen, 1991, Mohring et al., 1998). In addition to cytoplasmic male sterility (CMS) and genic male sterility (GMS) also self-incompatibility (SI) has been utilized in practical breeding of hybrid cultivars. In the Czech Republic, hybrids on the basis of male sterility systems MSL (*Mänlicher Sterilität Lembke*) and Ogu-INRA are planted and registered at present time. SI system is defined as the inability of a fertile hermaphrodite seed plant to produce zygotes after self-pollination. Sporophytic SI system in the *Brassica* family is controlled by multialelic S locus, which determines specificity of interaction between pollen and cells of stigma (Nettancourt, 1977). S-alelles in *Brassicaceae* according to classical genetic analysis, based on phenotype effect, are divided to the allele group with a high level of dominance and to the group of recessive alleles (Nasrallah *et al*., 1991). Gemmell et al. (1989) found nine different S alelles controlling sporophytic SI in *Brassica napus.* Schweiger and Rudloff (1981) observed natural occurrence of SI in winter rapeseed and they found about 1 % SI plants in populations of oilseed rape. Esch and Wricke (1995) noted the frequency of SI plants in oilseed rape populations about 0.08 %. Havel (1994, 1996) detected the frequency of SI plants in several oilseed rape cultivars about 1 % but the 90 % of the progeny from the SI plants was self-compatible (SC). From large set of genotypes the six stable SI lines were found. Four of the detected lines had different S alleles, in the two another lines identification of S alleles has not been carried out. The initial SI plants were with high GSL and erucic acid content (Havel 1994). SI reaction after quality improvement using of backcoss by fertile lines was lost. Therefore, the use of the method of microspore cultures for obtaining improved SI lines was considered. The occurrence of SI plants from the whole amount of DH regenerants derived from F_1 hybrids SI lines with 00 quality donors was 10 – 27 %. Ziemborska and Harney (1986) also detected the number of self-compatible plants 71 – 93 % in DH regenerants from anther cultures of *Brassica napus var. napobrassica.* Havel (1996) searched SI lines by means of fluorescence and seed-set methods. Fluorescence microscopic test, based on observing pollen tubes growth in UV light have been used for detection of SI plants (Nettancourt, 1977) combining with seed-set test. In final the seed-set test provided more accurate results. The occurrence of SI plants in population of low erucic acid and GLS content cultivars was from 0.3 to 3.9 % in dependence on the year (Havel 1994). The procedure of maintenance and reproduction of SI lines is aimed at overcome SI reaction in the open flowers after self-pollination. As one of possibility to overcome SI reaction, using of increased concentration of CO_2 has been recommended (Nakanishi et al. 1973). Fu et al. (1992), Mohring et al. (1998) demonstrated overcoming of self-incompatibility in oilseed rape by spraying inflorescences with the 2 – 10 % NaCl solution. In the field conditions, 5 % concentration of NaCl

solution was applied.

Material and Methods

Production of SI lines with improved seed quality by the method of production doubled haploid regenerants

The initial line Tandem 6/85 with high GSL and erucic acid content in seed was obtained at the workplace OSEVA PRO Ltd. Research Institute of Oilseed Crops at Opava (RIOC). Doubled haploid regenerants derived from the F_1 hybrids of SI line Tandem 6/85 and „00" quality donor OP-2051 after the first cycle of crossing were produced in the year 2000 at the Research Institute of Crop Production (RICP) Praha – Ruzyně. The seed-set test were used as the main method for selection lines with stable SI reaction. Assessment by minimizing methods (gas chromatography) of antinutritional substances in seed of DH regenerants was carried out at RIOC Opava. In the second cycle of improvement crossing five combinations of the best two SI DH lines OP-23 AI/3 and OP-23 AI/6 with 00 quality donors for DH production were created in 2002. The rapeseed lines originated from RIOC (OP-BN-03, OP-571/00) and registered varieties (Rasmus and Lisek) were used as the male parents. From these crosses, 72 DH SI regenerants were derived and analyzed for erucic acid content (figure 1) in 2004 and 2005. From these amount, 18 SI DH lines were with erucic acid content lower than 2 % in oil.

Assessment of anti-nutritional substances in seeds of SI plants

Individual fatty acid (FA) assessment in seeds of oilseed rape was carried out according to the „Method of single fatty acid assessment in rapeseed oil by the method of Gas Chromatography (GC) "modified for the analysis of single seed sample (Kolovrat, 1985). Methylesters of FA were analysed on gas chromatograph CHROM 5. Single glucosinolate assessment in seeds of oilseed rape was carried out according to the "Method of glucosinolate content assessment in rapeseed by High Pressure Liquid Chromatography (HPLC). " The method that is deduced from the International Norm ISO 9167-1:1992 (E) (1992) was partially modified for the purposes of single seed analysis (Kolovrat, 1998). The analyses was carried out on liquid chromatograph Spectra-Physics, type SP 8100 XR with the detector SP 8440 XR UV/VIS and the integrator SP 4200.

Production of double-haploid regenerants from microspore cultures

Doubled haploid regenerants were produced by microspore culture method at the RICP Praha (Vyvadilová and Zelenková, 1992). The method was optimized for routine usage in winter oilseed rape and cruciferous vegetables breeding programmes (Klíma et al., 2004).

Seed multiplication of SI lines by NaCl treatment

Seed production procedure of SI lines by 5 % NaCl solution was verified on SI lines OP-23 AI/3 and OP-23 AI/6. The spraying was carried out 3 times per week during the flowering period. The number (percentage) of produced seeds of SI lines after NaCl treatment were counted by the method of mean weight of SI plants seeds compared with the produced seeds of plants OP-23 AI/3 and OP-23 AI/6 crossed with fertile line OP-2051. Mean weight of created seeds of F_1 plants was calculated as 100 %.

Results and Discussion

Production of SI lines with improved seed quality by the method of doubled haploid regenerants production

After the first crossing of initial SI lines with donor of „00" quality, decrease of GSL and erucic acid content was achieved. From 10 derived lines only two lines (OP-23 AI/3 and OP-23 AI/6) had distinctly lower erucic acid content than the initial SI material Tandem 6/85. The range of GSL content in set of first regenerants was ranged from 71.00 to 102.06 µmol/ g of dry matter of seeds. Erucic acid content was ranged from 3.20 to 33,98 %. After first crossing and producing of DH lines, no line with convenient „00" seed quality were obtained. Therefore, the production of DH microspore regenerants from the second cycle of hybrids of SI and quality donors was started from 2003. In total, 468 regenerants were derived in 2004 and 48.7 % of them (228 plants) were with fertile flowers. From the whole number of fertile DH regenerants 38.2 % (87 plants) showed to be with SI reaction. The occurrence of SI plants from the total number of fertile DH regenerants ranged from 32.4 to 45.5 % in accordance with the origin. The earlier results (Havel, 1994) showed that in F_2 generation derived from SI hybrids the segregation ratio SI to SC plants was 1: 15. This indicates recessive self-incompatibility controlled by two allelic pairs. In the population of 228 DH regenerants, obtained in the year 2004, the segregation ratio SI:SC plants was 1:1.62 which did not correspond with either monogenic or digenic base of observed SI. Expected ratio of SI to SC regenerants is approximately 1:1. However, considerable shift to self-compatibility was observed, probably due to gametic selection against SI genotypes in microspore culture (Kučera et al., 2002). In addition, modifier genes (Hinata et al., 1983) could influence expression of self-incompatibility. Therefore, a suitable genetic background should be found for each of S allele in which is strongly and stable expressed (Mohring et al. 1997). From the analysed set of 72 SI DH regenerants in 2004 and 2005, 18 regenerants showed erucic acid content lower than 2 % and the rest of 54 regenerants over 2 % (figure 1). The mean of erucic acid content in the set of DH regenerants was 14.20 %. All of selected five plants analysed by HPLC method had GSL content lower than the limit 18 µmol /g by 9 % of seed moisture. The results of analyses indicate that in the case of SI DH regenerants from the second cycle of crossing with successive DH deriving the critical factor of seed quality was erucic acid content contrary to GSL content. As reported by Havel (1994, 1996) it is more progressive to achieve lines with convenient GSL and erucic acid content by method of producing DH after crossing with donor of quality. The method proved to be applicable for the seed quality improvement of DH lines together with maintaining of SI reaction.

Seed multiplication of SI lines by NaCl treatment

The obtained results of spraying inflorescences of rape plants with 5% NaCl solution for seed production of SI lines are consistent with the statements of Fu et al. (1992) and Mohring et al. (1998). The seed production of two SI genotypes varied from 14.3 % to 16.4 %. The average production of seed of the both lines was 4.12 g/plant.

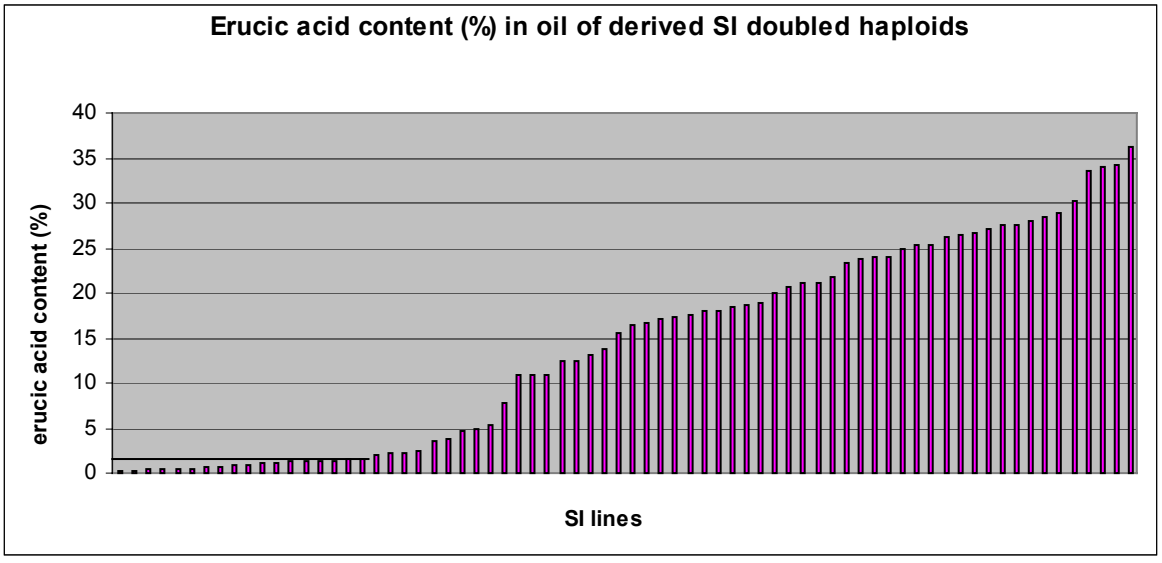

Figure 1: Derived SI doubled haploids with analyzed oil quality

Conclusion

Two populations of SI DH regenerants after crossing initial SI lines with donor of quality were derived. 18 SI lines with desirable seed quality derived by method of production doubled haploids from microspore cultures after the second crossings of with seed quality donors were obtained. The average erucic acid content in these group of 72 analyzed SI DH regenerants was 14.20 % (in oil). In each of the following cycle of DH regenerants production, GSL and erucic acid content in seed decreased. According to the results of analyses, GSL content were not limited factor of seed quality in SI DH group. Field multiplication of SI lines using of NaCl solution was verified. Obtained SI lines with improved quality have been used for self-incompatibility research purposes and for creating new experimental SI hybrids after crossing with high yielding fertile lines.

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