Development of resynthesised rapeseed forms with low erucic acid character and their use in hybrid breeding

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Abstract

Resynthesised (RS) forms of rapeseed (*Brassica napus* L.; genome AACC, 2n = 38) generated from interspecific crosses between its diploid progenitors *Brassica rapa* L. (syn. *campestris*; genome AA, 2n = 20) and *Brassica oleracea* L. (CC, 2n = 18) are getting interest in the development of rapeseed hybrids. İntensive breeding work during the last hundred years led to the narrow genetic base in this crop species. At the beginning the traditional quality of present rapeseed cultivars limited the use of rapeseed as plant oil. But the discovery of low erucic acid mutants in the cultivars Liho and Oro and the low glucosinolate character in the cultivar Bronowski give rise to the development of so called canola quality rapeseed. The low erucic acid character was found in the gene pool of rapeseed and transferred to other cultivars via crossing and backcrossing. During the screening of *Brassica* species *B. oleracea* genotypes with low erucic acid character were selected. The aim of this work is to develop 0-quality rapeseed forms over interspecific crosses with 00-*B. rapa* forms. We know that the low yield capacity of RS lines limits their use in yield breeding. On the other hand, the *B. oleracea* and *B. rapa* genotypes used in this study are winter forms. Rapeseed hybrids using RS-lines were developed before, but they were RS-lines with spring character and traditional fatty acid composition. At the end of this study 0-quality RS-lines will be developed via interspecific crosses and it will be the first time that rapeseed hybrids based on RS-lines will be developed using 0-uality RS-lines. These developed hybrids will further be tested for their yield capacity at field conditions.

Key words: *Brassica napus* – resynthesised rapeseed – hybrid breeding

Introduction

The gene pool of oilseed rape breeding material has been considerably narrowed by the emphasis on specific quality traits, genetic variability is restricted with regard to many valuable characters. On the other hand the development of synthetic *Brassica napus* forms provides important basic germplasm for further improvements of seed yield, disease and pest resistance as well as relevant seed quality traits (Chen & Heneen 1989a, Lühs et al. 2002).

Regarding seed quality traits some examples of introgression breeding are documented, such as the resynthesis approach to create new genetic variation for low glucosinolate content (Gland 1982, Raney & Rakow 1995), the approaches developing of yellow-seeded rapeseed *via* interspecific crosses (Chen et al. 1988, Chen & Heneen 1992, Rashid et al. 1994, Meng et al. 1998, Rahman 2001) and the development of quality RS lines (Seyis et al., 2005; Lühs et al., 2003).

Experiments were conducted by Chen & Heneen (1989b), Heath & Earle (1995, 1997), Rahman et al. (1996), Lu et al. (2001) and Rahman (2002) using interspecific *Brassica* hybridization in order to modify the fatty acid composition of oilseed rape. We know, that current double-low breeding material seems to be closely related and intensive quality breeding - using 'Liho'/'Oro' and 'Bronowski' spring rapeseed as unique donors for quality improvement - has also contributed to narrow the genetic base of oilseed rape. Using zero-erucic *B. oleracea* mutants (Lühs et al. 2000) resynthesised *B. napus* were developed to provide novel genetic resource for both quality and yield improvement of oilseed rape (Seyis et al., 2005).

Furthermore, the potential for hybrid rapeseed cultivars is well documented (Schuster and Michael, 1976; Sernyk & Stefansson, 1983; Grant & Beversdorf, 1985; Lefort-Buson, 1987; Brandle & Mc Vetty, 1989). For this aim different pollination systems were developed (Buzza, 1995). CMS-systems (cytoplasmatic male sterility systems) like the Ogu/INRA CMS-System (Renard et al., 1997), and the MSL-System (male sterility system) (Paulmann & Frauen, 1998).

As oilseed rape breeders today are seeking for genetic diversity in their hybrid breeding programmes resynthesised *B. napus* (RS lines) forms are gaining interest (Lühs et al., 2002; Girke, 2002; Seyis, 2003). Howevere, because of the low yield potential of RS lines their use is more directed to deve¬loping semi-synthetic rapeseed forms (cf. Kräling, 1987; Friedt et al., 2003) or backcross breeding procedures to introgress the novel genetic diversity.

The present work deals with the development of quality resynthesised rapeseed forms using 00-*B. rapa* forms and low erucic *B. oleracea* mutants and their use in developing rapeseed hybrids. This project is an ongoing project at it's beginning and therefore we will present some results and the main object of this project.

Materials and Methods

Individual zero-erucic *B. oleracea* plants belonging to the three accessions 'Kashirka 202' and 'Ladozhskaya' (Lühs *et al*. 2003, 2000; Seyis, 2004) and Eisenkopf were crossed with different *B. rapa* quality types, namely '15080' and '15591', kindly provided from the Institute of Crop Science and Plant Breeding I, Justus-Liebig-University. The fatty acid composition of this material is shown in Table 1.

The efficiency of interspecific crosses (Fig. 1) will be aided by embryo rescue protocols as described earlier (Lühs & Friedt, 1994; Seyis et al. 2005). Cuttings from obtained hybrids will be treated with colchicine in order to obtain amphidiploid *B. napus* plants (C_0) and will be then artificially vernalised for 8 to 10 weeks at 5 °C.

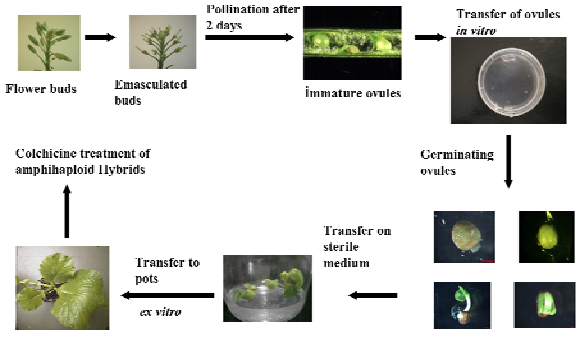

Fig. 1: *İn ovulum* method in producing resynthesised *B. napus* (Seyis et. al, 2005)

Results

As described earlier the fatty acid composition of used material can be seen in Table 1. These values were determined in seed samples of these genotypes.

Table 1: Fatty acid composition of investigated genotypes

Genotype	Oleic	Linoleic	Linolenic	Erusic
PI 263058 Kashirka	61,6	19,2	11,4	0,5
PI 263072 Ladozshkaya	62,6	18,6	9,7	0,2
PI 246111 Eisenkopf	46,5	32,1	11,5	0,1
00-15991 *B. rapa*	56,89	22,03	16,34	0,00
00-15080 *B. rapa*	55,43	28,33	16,23	0,00

Therefore seeds of these genotypes were directly sown in pods and were vernalised as described before. Unfortunately, the *B. oleracea* genotypes Kashirka and Ladozshkaya did not flower and all plants from the *B. rapa* genotype 00-15591 died during vernalization. Therefore, only crosses could be made with Eisenkopf and 00-15080 (Table 2). İn this table only obtained immature pods and transferred ovules are given in numbers. Only one plant were obtained from the crosses 00-15080×PI 246111. This plant were multiplicated using cuttings and will be now vernalized under defined conditions.

Discussion

Resynthesised rapeseed forms were developed earlier (Lühs et al., 2003; Seyis et al., 2005). But because of the high glucosinolate content of the *B. oleracea* parents the developed hybrids showed mainly 0+-quality.

The *B. rapa* genotypes used in this work display both glucosinolate contents of 2-3 µmol. Of course, the developed RS-lines will have high amounts of glucosinolates, but if they were used in developing rapeseed hybrids, we hope to reduce this character for a further extent.

Table 2: Performed interspecific crosses

Crossing combination	İmmature pod	Obtained ovules
PI 246111-4 x 15080-16	17	406
PI 246111-6 x 15080-16	25	498
15080-3 x PI 246111-9	7	36
15080-4 x PI 246111-9	18	139
15080-5 x PI 246111-9	22	165
15080-7 x PI 246111-9	15	133
15080-10 x PI 246111-9	21	139
15080-11 x PI 246111-9	3	6
Totally	128	1522

Conclusions

RS-lines were used before in the development of rapeseed hybrids (Seyis et al., 2003; Girke, 2002). But the used RS-lines were either spring forms or have traditional quality characters. İn our case, we hope to develop RS-lines with both winter character and low erucic acid content to use them in the development of rapeseed hybrids

The traditional seed quality of resynthesised *B. napus* is limits often the broad use of this novel gene pool in modern double-low rapeseed (canola) breeding prorammes. With the development of above described genotypes we hope to

overcome this problem.

Acknowledgements

TUBİTAK (Türkiye Bilimsel ve Tekonolojik Araştırma Kurumu = The Scientific & Technological research Council of Turkey) is funding this work. The authors thank Institute of Crop Science and Plant Breeding I, Justus-Liebig-University for providing seed material.

References

Brandle J., McVetty P.B.E. (1989). Heterosis and combining ability in hybrids derived from oilseed rape cultivars and inbred lines. Crop. Sci. **29**, 1191-1195.

Buzza G.C. (1995). Plant Breeding. In: Kimber, D., Mc Gregor D.J. (Eds.), *Brassica* Oilseeds – Production and Utilization. CAB Int., Wallingford, UK.

Chen B.Y., Heneen W.K. (1989a). Resynthesized *Brassica napus* L.: A review of its potential in breeding and genetic analysis. Hereditas **111**, 255-263.

Chen B.Y., Heneen W.K. (1989b). Fatty acid composition of resynthesized *Brassica napus* L, *B. campestris* L. and *B. alboglabra* Bailey with special reference to the inheritance of erucic acid content. Heredity **63**, 309-314.

Chen, B.Y., Heneen, W.K. (1992). Inheritance of seed colour in *Brassica campestris* L. and breeding for yellow-seeded *B. napus* L. Euphytica **59**, 157-163.

Chen, B.Y., Heneen, W.K., Jönsson R. (1988). Resynthesis of *Brassica napus* L. through interspecific hybridization between *B. alboglabra* Bailey and *B. campestris* L. with special emphasis on seed colour. Plant Breeding **101**, 52-59.

Friedt W., Lühs W., Müller M., Ordon, F. (2003). Utility of winter oilseed rape (*Brassica napus* L.) cultivars and new breeding lines for low-input cropping systems. German J. Agron. **2**, 49-55.

Girke A. (2002). Neue Genpools aus resynthetisiertem Raps für die Hybridzüchtung. Dissertation. FB Agrarwissenschaften. Georg-August-Universität Göttingen.

Gland A. (1982). Gehalt und Muster der Glucosinolate in Samen von resynthetisierten Rapsformen. Z. Pflanzenzüchtg. 88, 242-254.

Grant I., Beversdorf W.D. (1985). Heterosis and combining ability estimates in spring-planted oilseed rape (*Brassica napus* L.). Can. J. Genet. Cytol. **27**, 472-478.

Heath D.W., Earle E.D. (1995). Synthesis of high erucic acid rapeseed (*Brassica napus* L.) somatic hybrids with improved agronomic characters. Theor. Appl. Genet. **91**, 1129-1136.

Heath D.W., Earle E.D. (1997) Synthesis of low linolenic acid rapeseed (*Brassica napus* L.) through protoplast fusion. Euphytica **93**, 339-343.

Kräling K. (1987) Utilization of genetic variability of resynthesized rapeseed. Plant Breeding **99**, 209-217.

Lefort-Buson M., Guillot-Lemoine B., DatteeY. (1987). Heterosis and combining ability estimates in spring oilseed rape (*Brassica napus* L.). Can. J. Gent. Cytol. **27**, 472-478.

Lu C.M., Zhang, B., Kakihara, F., Kato, M. (2001). Introgression of genes into cultivated *Brassica napus* through resynthesis of *B. napus* via ovule culture and the accompanying change in fatty acid composition. Plant Breeding **120**, 405-410.

Lühs W., Friedt, W. (1994). Present state and prospects of breeding rapeseed (*Brassica napus*) with maximum erucic acid content for industrial applications (in German). Fat Sci. Technol. **96**, 137-146.

Lühs W., Seyis, F., Baetzel, R., Friedt, W. (2003). Genetic diversification of *Brassica* napus seed quality by wide hybridisation. 11th Intern. Rapeseed Congr., 6-10 July 2003, Copenhagen, Denmark, Vol. **2**, pp. 375-377

Lühs, W., Seyis, F., Voss, A., Friedt, W. (2000). Genetics of erucic acid content in *Brassica oleracea* seed oil. Czech. J. Genet. Plant Breed. **36**, 116-120.

Lühs W., Seyis, F., Snowdon, R., Baetzel, R., Friedt, W. (2002). Genetic improvement of *Brassica napus* by wide hybridisation. GCIRC Bull. **18**, 227-234.

Meng J., Shi, S., Gan, L., Li, Z., Qu, X. (1998). The production of yellow-seeded *Brassica napus* (AACC) through crossing interspecific hybrids *B. campestris* (AA) and *B. carinata* (BBCC) with *B. napus*. Euphytica **103**, 329-333.

Paulmann W., Frauen, M. (1998). Erfahrungsbericht zur Entwicklung und Saatguterzeugung von restaurierten Winterraps-Hybridsorten. Bericht über die 48. Tagung der Arbeitsgemeinschaft der Saatzuchtleiter. BAL Gumpenstein 25.-27. November 1997

Rahman M.H., (2001) Production of yellow-seeded *Brassica napus* through interspecific crosses. Plant Breeding **120**, 463-472

Rahman M.H. (2002) Fatty acid composition of resynthesised *Brassica napus* and trigenomic *Brassica* void of genes for erucic acid in their A genomes. Plant Breeding **121**, 357-359

Rahman M.H, Stølen, O., Sørensen, H., Rahman, L. (1996). Recurrent backcross: a method for transferring erucic acid allele into *Brassica* oilseed cultivars, *B. campestris* L. Yellow sarson as an example. Acta Agric. Scand., Sect. B, Soil and Plant Sci. **46**, 68-73.

Raney P., Rakow, G. (1995). A new *Brassica napus* genotype with superior yellow seed colour and very low alkenyl glucosinolate content. Proc. 9th Intern. Rapeseed Congr., 4-7 July 1995, Cambridge, United Kingdom, Vol. 4, pp. 1154-1156.

Rashid, A., Rakow, G., Downey, R.K. (1994) Development of yellow seeded *Brassica napus* through interspecific crosses. Plant Breeding **112,** 127-134.

Renard M., Delourme, R., Vallee, P., Pierre, J. (1997). Hybrid rapesed breeding and production. Acta Hortculturae **459,** 583-591.

Sernyk J.L., Stefansson, B.R. (1983). Heterosis in summer rape. (*Brassica napus* L.). Can. J. Plant Sci. **63**, 407-413.

Schuster, W., Michael, J. (1976). Investigations into breeding depressions and heterosis effects in rape (*Brassica napus oleifera*). Z. Pflanzenzüchtung, **80**, 277-298.

Seyis, F., Friedt, W., Lühs, W. (2003). Resynthesised *Brassica napus* as genetic resource in rapeseed improvement for quality and agronomic performance. In: H. Knüpffer and J. Ochsmann (eds.), Rudolf Mansfeld and Plant Genetic Resources. Proceedings of a symposium dedicated to the 100th birthday of Rudolf Mansfeld, Gatersleben, Germany, 8-9 October 2001. Schriften zu Genetischen Ressourcen 19, pp. 336-340. ZADI/IBV, Bonn

Seyis, F., Friedt, W., Voss, A., Lühs, W. (2004) İdentification of İndividual *Brassica oleracea* plants with low erucic acid content. Asian Journal of Plant Science **3 (5)**: 593-596.

Seyis, F., Friedt, W., Lühs, W. (2005). Development of Resynthesized Rapeseed (*Brassica napus* L.) Forms with Low Erucic Acid Content Through in ovulum Culture. Asian Journal of Plant Science **4 (1)**: 6-10.