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YIELD OF *BRASSICA NAPUS* L. HYBRIDS DEVELOPED USING RESYNTHESISED RAPESEED MATERIAL

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ABSTRACT: Resynthesised (RS) rapeseed genotypes developed through interspecific crosses between diverse *B. rapa* and *B. oleracea* genotypes have the potential to significantly increase the available gene pool and provide important basic germplasm for further improvements of seed yield, seed quality traits, disease and pest resistance. Furthermore, RS rapeseed is potentially of great interest for hybrid breeding, since heterosis effects are higher in crosses of genetically distant materials. On the other hand, previous studies with RS material showed that raw amphidiploids derived from interspecific hybridisations are usually rather low yielding. The utilisation of such basic germplasm in commercial variety-breeding programmes is restricted to the development of intraspecific hybrids, so-called semi-synthetic rapeseed, and backcross progenies thereof. In order to determine the general combining ability (GCA) of different rapeseed material, nine high-erucic acid RS lines and three old spring rapeseed cultivars were used as pollinators of male-sterile double-low spring rapeseed lines (MSL system). Yield performance of the single-cross hybrids were tested in a two year field trial in Rauischholzhausen/Germany that revealed a high yield potential of semi-synthetic rapeseed hybrids based on RS lines. Developed test hybrids showed 1-15 % more yield compared with the control cultivar Senator. The use of such resynthesised rapeseed forms in developing hybrids and their possible use in future oilseed breeding is discussed based on two year results.
Key words : *Brassica napus*, resynthesised rapeseed, hybrid, breeding

TÜRLERARASI MELEZ KOLZA MATERYALİ KULLANILARAK GELİŞTİRİLEN *BRASSİCA NAPUS* HİBRİTLERİNİN VERİMİ

ÖZET Farklı *B. rapa* ve *B. oleracea* genotipleri arasında yapılan türler arası melezlemeler sonucunda geliştirilen türlerarası kolza melezleri mevcut gen havuzunu önemli derecede zenginleştirme ve tane verimi, tane kalite özellikleri, hastalık ve zararlılara dayanıklılık yönünde yapılacak ıslah çalışmaları için önemli genetik materyal sağlama potansiyeline sahiptirler. Bundan başka, türlerarası melez kolza genotipleri hibrit ıslahı için de önemlidirler, çünkü genetik olarak farklı materyalin melezlenmesi sonucu ortaya çıkan heterosis etkileri daha yüksek olmaktadır. Diğer taraftan, türler arası melez kolza bitkileri ile yapılan çalışmalar türlerarası melezlemelerden gelen ilk generasyon bitkilerin düşük tane verimine sahip olduğunu göstermiştir. Bu gibi temel materyalin ticari ıslah programlarında kullanılması tür içi hibritlerin – yani yarı sentetik hibritler – geliştirilmesi ve onların geriye melezlenmeleri ile sınırlıdır. Farklı kolza materyalinin genel kombinasyon yeteneğini belirlemek için 9 adet yüksek erusik asit içeriğine sahip türlerarası melez kolza ve üç eski yazlık kolza çeşidi 00 kalitesine sahip erkek kısır hatların melezlenmesinde kullanılmıştır. Geliştirilen deneme hibritleri tane verimleri yönünden Rauischholzhausen/Almanya da yürütülen iki yıllık tarla denemeleri sonucunda türlerarası melez kolza bitkileri kullanılarak geliştirilen kolza hibritlerinin yüksek verim potansiyeline sahip olduğu görülmüştür. Geliştirilen kolza hibritlerinden kontrol çeşidi olan Senatöre göre % 1-15 kadar daha fazla verim elde edilmiştir. Türlerarası kolza melezlerinin hibritlerin geliştirilmesinde kullanılması ve bu materyalin kolza ıslah programlarında kullanılması iki yıllık veriler kullanılarak tartışılacaktır.
Anahtar Sözcükler : *Brassica napus*, türlerarası melez, hibrit, ıslah

1. INTRODUCTION

The availability of genetically distant plant material is of great importance for successful hybrid-breeding programmes because breeders want to exploit the expected heterosis effects. This effect is higher in cases where the parents are relatively different on genetic base. An advantage of hybrids compared to open-pollinated cultivars is the higher yield stability under unfavourable conditions due to their heterozygous genotype (Wricke and Weber, 1986; Léon 1991). Also in case of rapeseed, heterosis effects are generally higher in crosses of genetically distant materials. Lefort-Buson *et al.* (1987) determined higher yields in spring rapeseed hybrids, which were developed from genetically divergent spring rapeseed cultivars compared with traditional rapeseed varieties. The advantage of hybrid cultivars over cultivars derived from inbred lines is not only the use of heterosis for increasing yield, also there is a higher yield stability due to the heterozygous character of hybrids (Paulmann and Frauen, 1991). The superiority of hybrids specially becomes obvious in unfavourable conditions. Becker (1987) showed based on three year results that the relative level of heterosis in the unfavourable year was two fold higher compared to the high yielding year.

The potential for hybrid rapeseed cultivars is well documented (Schuster and Michael, 1976; Sernyk and Stefansson, 1983; Grant and Beversdorf, 1985; Lefort-Buson, 1987; Brandle and Mc Vetty, 1989). For this aim different pollination systems were developed

(Buzza, 1995). Cytoplasmatic male sterility (CMS systems) like the Ogu/INRA CMS-System (Renard *et al.*, 1997), and the Male sterility system (MSL-system) (Paulmann and Frauen, 1998).

In order to prevent self-pollination of the female line emasculation by hand is required, raising costs and labor expenses of seed production. The utilization of naturally occurring cytoplasmatic male-sterility (cms) is a more efficient approach. Mostly correlated with changes in the mitochodrial or chloroplast DNA, in some lines of distinct species like maize (Laughnan and Gabay-Laughnan, 1983), oilseed rape (Jarl *et al.* , 1988), rice (Kodowaki *et al.* ., 1988) and sugar beets (Hallden *et al.*, 1988) the anthers do not develop viable pollen, consequently the flowers only contain fertile female reproductive organs making cross-pollination obligate. Such pollination systems allows the breeder to develop hybrids easily.

Due to this development hybrids were later developed and commercially available. The necessity of such systems were underlined with the work of Sernyk and Stefansson (1983), who investigated heterosis in spring rapeseed and reported that the successfull development of a hybrid cultivar in rapeseed depends on the availability of a suitable cytoplasmatic, genetic or chemical pollination system.

Further Lefort-Buson (1987) investigated the yield of oilseed *B. napus* in hybrids derived from lines with similar or different origin. Hybrids derived from inbred lines with similar origin showed lower heterosis values compared to hybrids from genetically different crosses. Between spring rapeseed cultivars the highest heterosis effect was obtained in crosses between distinctly related parents, for example between the Canadian spring rapeseed cultivar 'Regent' and the Australian spring rapeseed cultivar 'Marnoo' (Brandle and Mc Vetty, 1989; Diers *et al.* , 1995).

In the past it was assumed that cultivars which are used as parents to develop hybrids must be completely homozygous (Grant, 1984), but the work of Brandle and Mc Vetty (1989) showed that oilseed *B. napus* cultivars are in fact heterogenous.

On the other hand, Butruille *et al.,* (1999) investigated the possibility to increase yield of spring material by using winter germplasm. In other reports only spring material with spring material (Sernyk and Stefansson, 1983; Grant and Beversdorf, 1985; Brandle and Mc Vetty, 1990; Enqvist and Becker, 1991) or winter material with winter material was crossed (Leon, 1991; Ali *et al.*, 1995). 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). Because of the low yield potential of RS lines their use is more directed to developing semi-synthetic rapeseed forms (Kräling, 1987; Friedt *et al.*, 2003) or backcross breeding procedures to introgress the novel genetic diversity. In this study intraspecific hybrids were developed and tested under field conditions to estimate the general combining abilities (GCA) of selected RS lines for yield.

2. MATERIALS AND METHODS

2.1. Plant material

Nine selected RS lines - derived from interspecific crosses between different cauliflower (*B. oleracea* ssp. *oleracea* convar. *botrytis* var. *botrytis*) cultivars (BK2256, BK2287, BK3094, BK3096 and Venus) and Yellow Sarson (Y.S., *B. rapa* ssp. *trilocularis*) (Lühs, 1996; Lühs and Friedt, 1999) as well as three old spring cultivars were used as pollinators in order to develop intraspecific hybrids (Table 1). For hybrid seed production the male sterile double-low lines 'MSL-506C' and 'MSL-510C' (Male Sterility Lembke-System, Norddeutsche Pflanzenzucht, Hohenlieth, Germany) were grown in 2000 under isolation cages together with the male lines; pollination was facilitated by bumble bees. The semi-synthetic rapeseed hybrids (TH1-TH18) based on RS lines as well as the conventional test hybrids (TH19-TH24) were tested in the years 2001 and 2002 for their yield performance at the Field Research Station of Rauischholzhausen (RH) near Marburg/Hesse. The experimental design was a 5 x 5 Lattice Square with 2 replications using the spring open-pollinated cultivar 'Senator' and the hybrid 'PF 8242/96' as controls. The statistical analysis was carried out according to Schuster and von Lochow (1992). Estimates for GCA effects were calculated according to Wricke and Weber (1986).

Table 1: Pedigree of experimental hybris (TH1-TH24) developed through intraspecific crosses (MSL-line x Pollinator)

Female Parent		Male Parent (Pollinator)	
MSL-510c	**MSL-506c**	**RS- Line / Cultivar**	**Pedigree of RS-Line**
TH1	TH2	RS 578d	BK 2256 x Y.S.
TH3	TH4	RS 55	Y.S. x BK 2256
TH5	TH6	RS SF 301	Y.S. x BK 3094
TH7	TH8	RS SF 306	Y.S. x BK 3094
TH9	TH10	RS SF 390	Y.S. x BK 3094
TH11	TH12	RS SF 279	Y.S. x BK 3096
-	TH14	RS 232a	BK 2287 x Y.S.
TH15	TH16	RS 16S/5b	Y.S. x BK Venus
TH17	TH18	RS 239b	BK 2287 x Y.S.
TH19	TH20	Janetzkis Sommerraps	Natural rapeseed
TH21	TH22	Bronowski	Natural rapeseed
TH23	TH24	Svaloefs Gulle	Natural rapeseed

3. RESULTS

In genetically different crosses the offspring displays a higher yielding potential compared to the mean yield of its parents (Becker, 1993).

Table 2: Yield values of experimental hybrids for the location RH 2001-2002

Genotype	MSL-parent	Pollinator	Yield (dt/ha)	Rel YV. (%)	Yield (dt/ha)	Rel YV. (%)
			2001*		2002*	
TH2	MSL506c	RS 578d (BK 2256 x Y.S.)	34.8a	127		115
TH18	MSL506c	RS 239b (BK 2287 x Y.S.)	34.2ab	125	22.1abc	109
TH4	MSL506c	RS 55 (Y.S. x BK 2256)	34.0ab	124	21.1b-e	105
TH24	MSL506c	Svaloefs Gulle	33.3abc	121	21.1b-e	105
TH6	MSL506c	RS sF301 (Y.S. x BK 3094)	33.3abc	121	21.1b-e	105
TH14	MSL506c	RS 232a (BK 2287 x Y.S.)	33.0abc	120	20.5b-f	101
TH8	MSL506c	RS sF 306 (Y.S. x BK 3094)	32.6a-d	119	20.5b-f	102
TH20	MSL506c	Janetzki	32.6a-d	119	20.1c-g	99
TH1	MSL510c	RS 578d (BK2256 x Y.S.)	31.7b-e	115	17.0j	84
TH22	MSL506c	Bronowski	31.5b-f	115	21.6a-d	107
PF8242/96			31.0c-f	113	22.6ab	112
TH17	MSL510c	RS 239b (BK2287 x Y.S.)	30.9c-g	113	17.8hij	88
TH10	MSL506c	RS sF 390 (Y.S. x BK 3094)	30.1d-h	110	20.1c-g	99
TH9	MSL510c	RS sF 390 (Y.S. x BK 3094)	30.0d-h	109	18.5f-j	92
TH12	MSL506c	RS sF 279b (Y.S. x BK 3096)	29.9d-h	109	21.6a-d	107
TH16	MSL506c	RS 16S/5b (Y.S. x Venus)	29.6e-h	108	21.6a-d	107
TH11	MSL510c	RS sF 279 (Y.S. x BK 3096)	29.4e-h	107	19.2e-I	95
TH19	MSL510c	Janetzki	29.4e-h	107	17.8hij	88
TH15	MSL510c	RS 16S/5b (Y.S. x Venus)	29.3e-h	107	17.8hij	88
TH5	MSL510c	RS sF 301 (Y.S. x BK 3094)	28.7f-h	104	17.3ij	85
TH7	MSL510c	RS sF 306 (Y.S. x BK 3094)	28.6f-h	104	17.0ij	84
TH3	MSL510c	RS 55 (Y.S. x BK 2256)	28.1gh	102	18.1g-j	89
TH23	MSL510c	Svaloefs Gulle	27.8h	101	18.6g-j	92
Senator			27.5h	100	20.2c-g	100
TH21	MSL510c	Bronowski	23.6i	86	20.1c-g	99
		Average	30.6		19.9	
			LSD %1 = 2,91		LSD % 1 = 2,13	

* Numbers in a column followed by different letters indicate significant differences at P<0.01.

Based on the determined genetic divergence of resynthesised rapeseed compared to conventional rapeseed material (Becker et al., 1995; Song et al., 1995; Seyis et al., 2003) spring rapeseed hybrids were developed and tested for their yield capacity at the location Rauischholzhausen/Hesse in the years 2001 and 2002. The yields of all investigated genotypes can be seen in Table 2. In 2001 nearly all hybrids – exclusively TH 21 – showed higher yield values compared with the controll cultivar Senator.All test hybrids developed with the female parent MSL 506C showed higher yield values compared to the corresponding test hybrids with the same pollinator, but MSL 510C as female parent. This gives reason to a special combination ability of given resynthesised rapeseed lines.

Statistical analysis revealed a high significance regarding yield in 2001 (Table 3). Mean yield of the investigated 25 genotypes was 30,6 dt/ha (Table 2). TH2 showed the highest yield value of 34,8 dt/ha, which is corresponding to relative yield of 127 compared to the yield of Senator (27,5dt/ha = 100%) and a yield difference of 7,3 dt/ha. The relative yield values ranged between 86-127 %, whereas as mentioned only one test hybrid - TH 21 (MS L510c x Bronowski)- showed a lower yield value compared with Senator (Table 2).

In 2002 the test hybrids TH2 and TH18 showed the highest yields (Table 2), but the yield values were much lower in 2002 compared to 2001. A yield difference of 11.5 dt/ha can be seen in case of TH2. Because of the low yield in this year the highest relative yield was only 115 compared to the seed yield of Senator. The same phenomena could be seen in 2002. All test hybrids with MSL 506C as female parent have higher yield values in comparison to test hybrids with the same pollinator, but MSL 510C as female parent. In 2002 the yield differences were again high significant (Table 4). Mean yield of all 25 genotypes was 19,9 dt/ha.

If we compare both years we can see high significant differences between genotypes and years (Table 5) regarding seed yield.

Table 3: Variance analysis of experimental hybrids (TH1-TH18) based on resynthesised *B. napus* pollinator lines compared to natural rapeseed including conventional test hybrids (TH19-TH24) and the check varieties 'Senator' and 'PF 8242/96' at the location Rauischholzhausen 2001 with 3 replications

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F
Total	74	757,6641		
Replication	2	130,8778		
Genotypes	24	454,9297	18,955	11,077**
Blocks	12	110,2528	9,1877	1,63
Error	36	61,3038	1,7112	

** P<0.01

Table 4: Variance analysis of experimental hybrids (TH1-TH18) based on resynthesised *B. napus* pollinator lines compared to natural rapeseed including conventional test hybrids (TH19-TH24) and the check varieties 'Senator' and 'PF 8242/96' at the location Rauischholzhausen (2002) with 3 replications

Source of Variation	*Degree of Freedom*	*Sum of Squares*	*Mean Square*	*F*
Total	74	528,8770		
Replication	2	213,5944		
Genotypes	24	256,0026	18,955	19,40**
Blocks	12	24,1123	2,0094	2,057
Error	36	35,1677	0,9769	

** P<0.01

Table 5: Variance analysis of experimental hybrids (TH1-TH18) based on resynthesised *B. napus* pollinator lines compared to natural rapeseed including conventional test hybrids (TH19-TH24) and the check varieties 'Senator' and 'PF 8242/96' at the location Rauischholzhausen (2001-2002) with 3 replications

Source of Variation	*Degree of Freedom*	*Sum of Squares*	*Mean Square*	*F*
Genotype (G)	24	552.29	23,01	4,13**
Replication (R)	2	30.13	15.07	
R x G	48	136.45	2,84	
Year (Y)	1	4314.80	4314,8	775,21**
R x Y	2	314.35	157,18	
G x Y	24	158.67	6,61	

** P<0.01

Fig.1 is showing the calculated general combining ability (GCA) values of used male and female parents. The calculation of GCA (general combining ability) values showed that the male sterile line MSL 506C has a better general combining ability compared with MSL 510C. Further the RS-lines RS578d and RS239b have better general combining abilities compared with the other RS-lines and conventional cultivars.

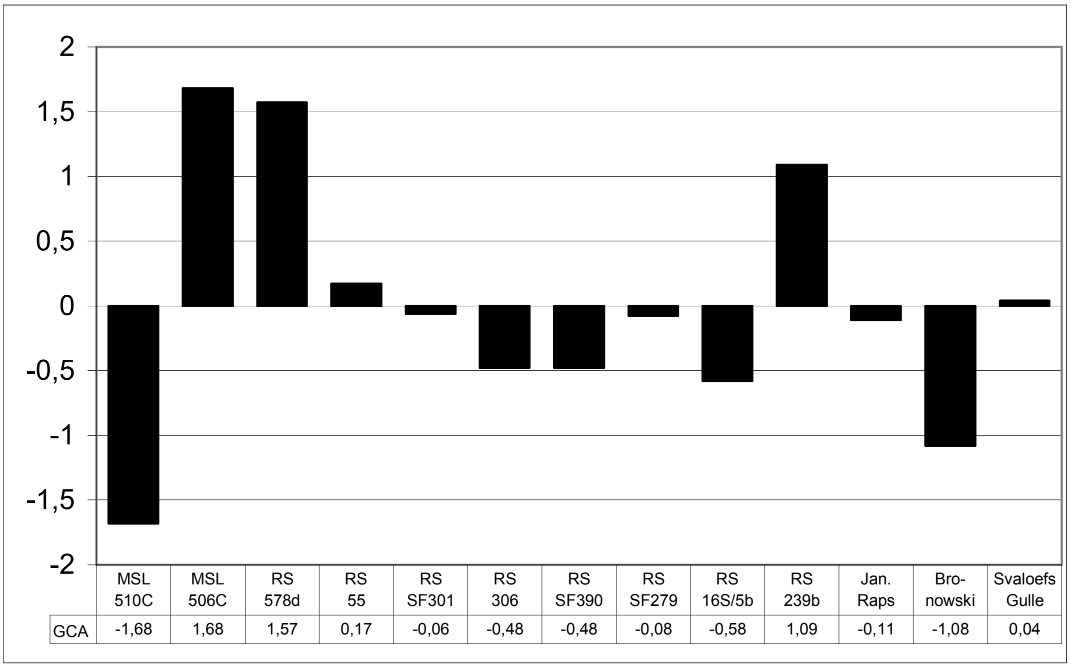

Figure 1: General combining ability (GCA) regarding seed yield of nine selected RS lines, three old spring cultivars (Janetzkis Sommerraps‘, ‘Bronowski‘ and ‘Svaloefs Gulle‘) and two male sterile MSL-lines (‘MSL-506c‘ and ‘MSL-510c‘). Data from field trials in 2001-2002 at one location (RH).

4. DISCUSSION AND CONCLUSION

Brassica napus is a relatively young species that derived only some 500-1000 years ago, in a limited geographic region, from spontaneous hybridisations between turnip rape (*B. rapa*) and cabbage (*B. oleracea*) genotypes (Kimber and Mc Gregor, 1995). The gene pool of elite rapeseed breeding material has been further eroded by an emphasis on specific quality traits. As a consequence, genetic variability in this important oilseed crop is restricted with regard to many characters of value for breeding purposes. Resynthesis of novel genotypes through artificial crosses between the diploid parents, assisted by embryo rescue techniques, is a useful strategy for broadening the genetic basis of breeding material, particularly in terms of increasing heterotic potential through hybrid development. Both progenitor species exhibit an extremely broad genetic and phenotypic diversity that gives the potential for a huge variety of different resynthesized rapeseed forms (Chen and Heneen, 1989; Becker *et al.*, 1995).

Regarding quality considerable breeding efforts were achieved in rapeseed (*B. napus* L.) in the last decades. With the creation of erucic acid free cultivars rapeseed oil established to a valuable food with a broadening demand. Through the strong reduction in the glycosinolate content existing limitations for rapeseed meal in livestock feeding was cancelled (Makowski, 2000), whereas breeding practices in these both directions limited the genetic base in this crop plant.

Resynthesising *B. napus* was systematically used to create new variation for the present gene pool of this crop plant, but the conventional quality (high erucic, high glycosinolates) of the new developed forms limited the use of such forms for quality and yield breeding in rapeseed (*B. napus*).

Successful breeding programmes based on genetically divergent populations. The determined diversity of resynthesised forms can be used as allel sources for agronomic traits and quality characters. Correspondingly, the test hybrids used in this investigation give us an insight to the use of resynthesised rapeseed in hybrid breeding.

Regarding the yield potential of hybrids developed through single plant crossings in rapeseed (*B. napus* L.) heterosis values about 40-70 % were often published (Schuster and Michael, 1976; Sernyk and Stefansson, 1983; Grant and Beversdorf, 1985; Lefort-Buson *et al.*, 1987).

But about the use of resynthesised rapeseed in developing hybrids not much works were present. Girke and Becker (2002) obtained in a yield trial a heterosis value of 22% and some test hybrids have reached the level of the involved hybrid cultivars These results are confirming the assumed potential of resynthesised rapeseed material in hybrid breeding (Becker and Engqvist, 1995; Girke *et al.*, 1999).

Further, Kräling (1987) tested the general combining ability of 8 resynthesised rapeseed forms with 3 tester cultivars. The hybrid cultivars were clearly superior with regard to single plant yield compared to plants developed using resynthesised rapeseed forms. But with creating three way-hybrids it could be shown, that with reduction of the resyn-genome yield can be increased. The highest yield values were observed in the test hybrids TH2 and TH18 in both years (Table 2). Only in 2001 the test hybrid TH2 (MSL506c x [Res 578d (BK2256 x Y.S.)] overranged the open pollinated cultivar Senator for 10,4 dt/ha, although this cultivar is counted among the best yielding spring rapeseed cultivars (Anonymous, 2000). This both test hybrids and their male parents are promising material for further investigations. The higher yield of this mentioned hybrids can be underlined with the high general combining abilitiy of their male parents (RS 578d = 1,57 and RS 239b = 1,09).

Interesting were the lower yields of hybrids based on spring cultivars, which have negative general combining abililities (Fig. 1). The fact, that developed hybrids with a RS-line background give higher yields compared with hybrids, which have conventional spring rapeseed cultivars like Bronowski, Janetzki und Svaloefs Gulle in their pedigree, indicated the potential of resynthesised rapeseed in hybrid breeding.

Hybrids developed with the female parent 'MSL 506C' tended to give higher seed yields compared to hybrids from 'MSL 510C' (Table 2).This can be explained by the general combing ability values of the female parents (MSL 506C = 1,68 and MSL 510C = -1,68). MSL 506C had a better general combining ability compared with MSL510C. The yield differences in both years can be explained also through the rainfall differences (Fig. 2 and 3). In the second year, the rainfall in June during the development phase was lower compared to 2001. Again in 2002, the rainfall was higher in July and August. This leads to poor seed set and prolongation of the vegetation period of tested hybrids.

The yield potential of RS-lines is so low that the use of such forms must be well directed regarding quality and yield to enable the integration of such material to high yielding breeding material. Further, the mentioned conventional ++-quality of resynthesised rapeseed limits the development of a new gene pool based on Resyn-material. This option must be regarded as a long time perspective.

In addition, the discovery of low erucic acid mutants in cabbage (B. oleracea) for example (Lühs et al. , 2000a) and the development of resynthesised rapeseed through interspecific crosses with 0-00 quality B. rapa forms, will offer the possibility to use resyn-material in quality and yield breeding (Lühs et a.., 2000b; Seyis et al. , 2001)

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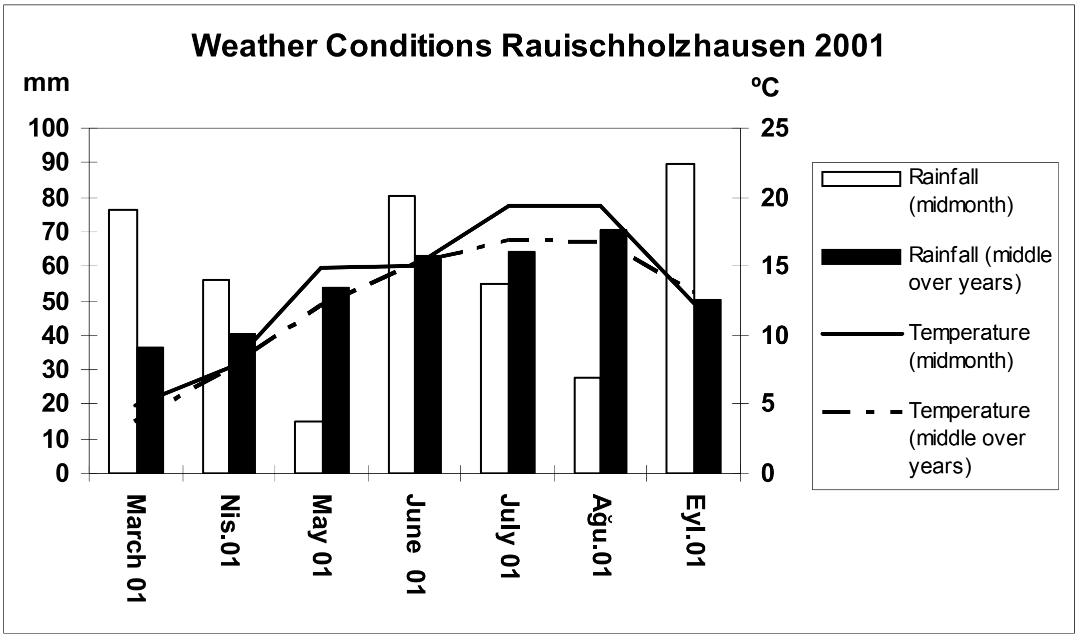

Fig. 2 : Weather conditions in 2001 (Rauischholzhausen)

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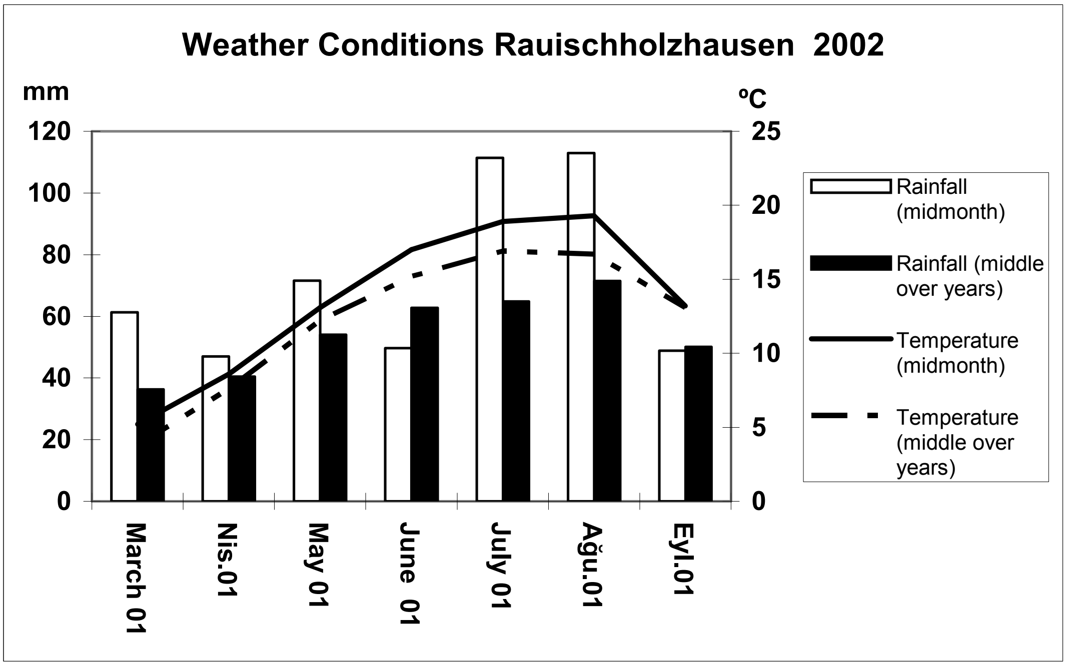

Fig. 3 : Weather conditions in 2002 (Rauischholzhausen)

It must be considered that in this case resyn-material with spring character were used in the development of intraspecific hybrids. Resyn-material with winter character and adequate quality characters (0-or 00-quality) will play in future a more important role in rapeseed breeding because of the expected higher heterosis and yield increase. The enormous potential of RS-lines in rapeseed breeding will highlight works in future which are dealing with germplasm conservation and well directed use of the diploid parents.

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