New Genetic Resources of B.napus by Resynthesis

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Within B.napus species little variation is known in content and pattern of glucosinolates. Other Brassica species, however, which are closely related to rapeseed, such as its diploid ancestors B.oleracea and B.campestris, show many differences not only in the total glucosinolate content but also in the proportion of specific glucosinolates (i.e. glucosinolate pattern) (ANJOU et al. 1977, GLAND et al. 1981).

Glucosinolate content and pattern of 708 forms and cultivars of the two diploid species B.oleracea and B.campestris were determined by gaschromatography (THIES 1979). For hybridization, 166 forms were selected according to their different contents and patterns of glucosinolates. Brassica species exhibit five major alkenyl glucosinolates: sinigrin, gluconapin, glucobrassicanapin, progoitrin, and gluconapoleiferin.

In the past five years, an intensive crossing programme was carried out, in our Institute, in order to create newly synthesized B.napus forms. Altogether, 20638 pollinations were made, from which 4436 pods have developed. Embryo culture was essential, 3311 embryo could be excised 28 days after pollination. Diploidization was most effectively done by submerging the vegetative plant apex in 0.05% colchicine solution with 1.5% DMSO for eight hours (JENSEN 1974, GLAND 1981). Finally 268 viable amphidiploid plants in 120 different parental combinations were received.

Regarding their vegetative growth, most of the new alloploids exhibited superior green matter production in comparison to natural rapeseed. In 1981, several new rapeseed forms were tested in a field trial, using one square metre plots. The trial included progenies of 15 different resynthesized rapeseed and 10 different Brassica species, generally used for fodder production. Table 1 shows the data of some morphological characteristics of resynthesized and natural rapeseed obtained from this field trial. In

Table 1: Comparison of data from a field trial between resynthesized and natural Brassica forms

Parents				Progeny	Plant height (cm)	Stem length (cm)	Fresh weight (kg)	Dry matter content (%)
B.oleracea ♀		B.campestris ♂						
Collection No.	Subspecies	Collection No.	Subspecies					
2169	capitata	2824	pekinensis	H 321	50	7.0	11.65	6.7
2823		2366		H 286	35	7.5	11.26	6.2
		2824		H 250	38	5.0	11.75	6.1
581	sabauda	2824		H 28	50	5.0	11.10	7.0
				H 44	35	4.0	10.70	7.7
585		29		H 176	25	2.0	7.70	6.5
587		2372		H 103	25	2.0	7.40	7.2
		2824		H 179	30	2.0	7.70	6.7
2179		2486	perviridis	H 80	50	5.0	11.85	8.2
2184				H 123	30	2.0	7.40	7.6
2152	acephala sabellica	383	chinensis	H 203	50	5.0	8.65	8.1
2154		2366	pekinensis	H 200	45	10.0	7.70	8.2
		29		H 210	40	20.0	9.22	6.6
613	gongylodes	170	narinosa	H 192	55	2.0	12.10	8.9
2288	medullosa			H 326	40	6.0	7.10	9.5

Cultivar	Species	Plant height (cm)	Stem length (cm)	Fresh weight (kg)	Dry matter content (%)
Grüner Angeliter	B.oleracea	60	45.0	6.73	10.1
Noko	B.campestris	130	130.0	10.50	8.5
Perko	B.campestris	50	2.0	10.30	8.2
Petranova	B.napus annua	85	70.0	6.15	10.6
Akela	B.napus biennis	60	30.0	6.95	11.5
Emerald	B.napus biennis	75	45.0	11.10	10.8
Gloria	B.napus biennis	65	25.0	11.95	9.4
Winfred	B.napus biennis	70	40.0	10.73	11.1
No. 2423	Raphanobrassica	55	15.0	10.35	9.3
Pegletta	Oil radish	120	120.0	9.60	10.6

general, plant height and stem length of the new alloploids were very low (i.e. 30 cm), while the natural rapeseed exhibited a plant height up to 85 cm. The fresh weight of resynthesized rapeseed was as high as or even higher than that of the cultivars, but they were inferior in dry matter content. Only one resynthesized progeny (H 192 from the combination of kale turnip and B.campestris ssp. narinosa) showed results similar to that of the best cultivars, i.e. 'Gloria'.

With respect to seed production, most of the resynthesized rapeseed forms exhibited various degrees of sterility even in later generations.

When resynthesized forms were used in crosses with cultivars or breeding lines the resulting heterosis in green matter productivity, winter hardiness, and seed yield was much higher than that usually encountered in conventional testcrosses.

Concentrations of glucosinolates varied in B.oleracea between 1 µmol/g defatted meal (broccoli; kale turnip) and about 200 µmol/g (fodder borekole). Some cultivars of broccoli (i.e. No. 2166) or kale turnip (i.e. No. 602) contained much less glucosinolate than the rapeseed cultivar 'Bronowski'. Also the pattern of glucosinolates was very different in the analyzed B.oleracea forms (GLAND et al. 1981), which could be expected from the predominant vegetable use of this species. Human selection often changed the flavour components of B.oleracea, thereby changing unconsciously their glucosinolate pattern. In fact, a continuous variation was ascertained in B.oleracea ranging from pure "sinigrin types", up to forms which contain more than 70 per cent progoitrin (figure 1).

Within the B.campestris group, the variation of glucosinolate content and pattern was also very high but clearly lower than in B.oleracea. One important distinction to B.oleracea was the total absence of sinigrin in the seeds of B.campestris (figure 1). In general, gluconapin was the dominating compound in B.campestris.

In seeds of the seed types of B.napus the glucosinolate pattern is rather uniform being usually composed of two thirds of progoitrin and of one quarter of gluconapin (figure 1). Only a few rutabag cultivars (i.e. 'York') are characterized by an extremely high progoitrin content (up to 85 per cent). No sinigrin was found in any of the investigated seed samples, thereby B.napus was confirmed to be the second Brassica species lacking this glucosinolate.

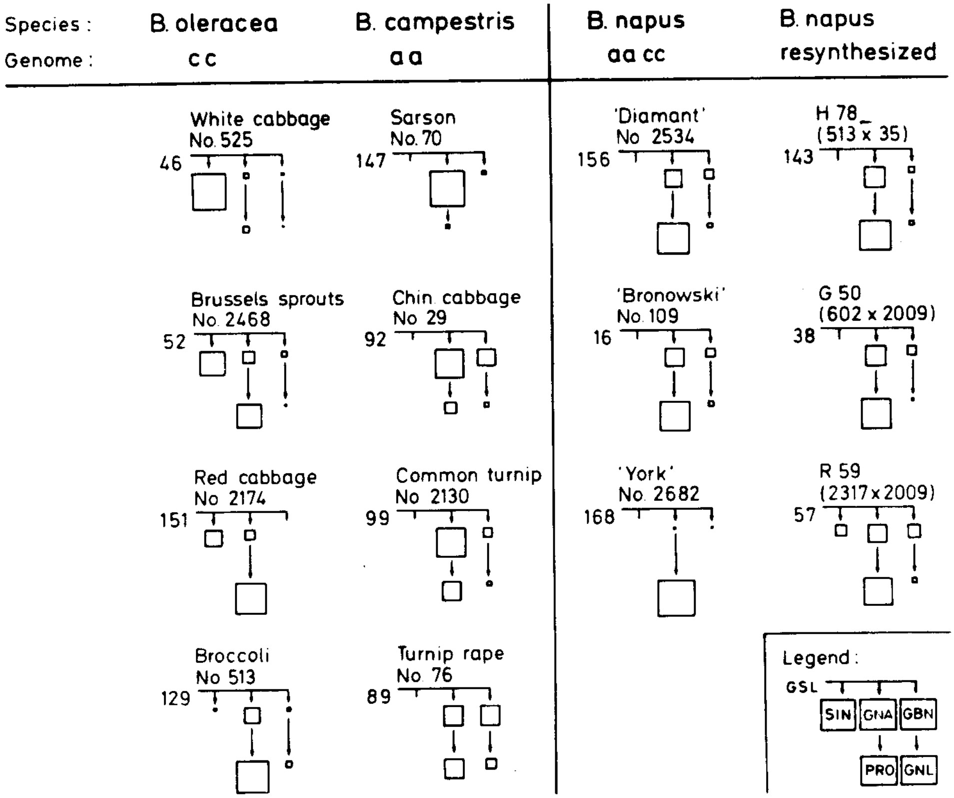

Fig. 1: Glucosinolate pattern of the diploid Brassica species B.oleracea and B.campestris and their amphidiploid progenies. The size of the quadratic facets symbolize the percentage proportion of the five specific glucosinolates within the total amount of glucosinolates. This amount expressed in µmol/g defatted meal is written on the left side of each diagram. The independence of the characteristics "pattern" and "total glucosinolate content" is exemplified by the similarity of the B.napus cultivars 'Diamant' (total 156 µmol/g) and 'Bronowski' (total 16 µmol/g). Numbers above diagrams represent collection number.

Specific interest was directed towards the creation of new amphidiploid genotypes with a low glucosinolate content both in the seed meal and in the green matter. But preselection within the B. oleracea and B.campestris parents did not always lead to the desired composition in the hybrid.

Table 2: Total glucosinolates (in µmol/g defatted meal) in seeds of Brassica oleracea and B.campestris and glucosinolate pattern (content of a specific glucosinolate in per cent of the total contents of analyzed glucosinolates) and total glucosinolates in seeds of 15 different resynthesized B.napus forms. The specific glucosinolates are: SIN = sinigrin, GNA = gluconapin, GBN = glucobrassicanapin, PRO = progoitrin, GNL = gluconapoleiferin.

Parents				Resynthesized rapeseed forms						
♀		♂		Progeny	Specific glucosinolates in %					Total glucosinolates
Collection No.	Total glucosinolates	Collection No.	Total glucosinolates		SIN	GNA	GBN	PRO	GNL	
B.oleracea ssp.		B.campestris ssp.								
2317	119.2	2362	82.3	H 226	7.0	24.0	8.2	57.1	3.7	117.7
		2009	7.2	R 59	9.2	23.7	13.6	51.7	1.7	57.3
525	45.6	2371	111.7	H 7	10.1	13.1	7.0	66.8	3.0	195.8
587	47.7	2486	115.2	H 48	10.1	18.0	7.4	59.0	5.5	115.4
		2824	36.4	H 180	13.4	27.4	1.9	56.3	1.0	113.8
		2009	7.2	R 38	11.5	16.8	3.3	67.7	0.8	39.9
2330	158.7	454	119.7	H 108	11.0	21.7	8.6	56.2	2.6	115.6
513	129.3	35	151.1	H 78	-	29.1	3.4	66.1	1.5	143.0
2166	0.9	2138	162.1	R 7	6.6	23.7	5.9	62.9	0.8	70.8
602	1.6	2009	7.2	G 50	-	29.4	5.5	64.4	0.7	38.5
613	25.1	170	86.1	H 192	7.4	18.1	3.8	68.2	2.2	142.0
2152	31.4	383	119.1	H 203	12.1	21.4	8.7	54.2	3.6	169.2
		2366	62.9	H 200	10.5	33.4	7.8	46.7	1.8	73.4
		2824	36.4	G 60	7.2	15.1	3.3	74.1	0.3	127.4
2154	88.7	29	91.5	H 210	19.4	10.5	6.7	59.7	3.8	87.0

Seeds of about 50 combinations of resynthesized diploid rapeseed have been analyzed. In these combinations the content of glucosinolates ranged from 38 to 196 µmol/g. With respect to the glucosinolate pattern the specific components were present in amounts similar to those found in natural rapeseed (figure 1). Surprisingly, most of the hybrids, except 'H 78' and 'G 50' contained sinigrin in amounts representing 7-19% of the total glucosinolate content (table 2). The glucosinolate content and pattern of two different hybrids, derived from the pollination of two B.oleracea cultivars by the B.campestris form 'No. 2009', are shown in figure 1 and table 2. Both female parents are extremely different in glucosinolate content. Nevertheless, their hybrids show a rather similar content and pattern of glucosinolates.

Although, the variation in glucosinolate content and pattern in seeds of diploid Brassica species is much higher than has been reported earlier, most of our resynthesized rapeseed forms exhibited a glucosinolate pattern similar to any natural rapeseed cultivars, a notable exception being a different proportion of sinigrin. When the parents were of the pure sinigrin or of the pure gluconapin-type or both contained additional progoitrin, no similarity with these characteristics of the progenitors was detected in the new rapeseed hybrids. These result indicates a complex genetic interaction between the two parental genomes, which evidently results in a uniform glucosinolate pattern, typical to the present day B.napus cultivars.

References

ANJOU, K., B. LÖNNERDAL, B. UPPSTRÖM, and P. AMAN: Swedish J. agric.Res. 7, 169-178 (1977)

GLAND, Astrid: Cruciferae Newsletter 7, 20-22 (1981)

- , G. RÖBBELEN, and W. THIES: Z.Pflanzenzüchtg. 87, 96-100 (1981)

JENSEN, C.J.: Proc.First Intern.Symp. Guelph, Ontario, Canada June 10-14, pp. 151-190 (1974)

THIES, W.: Naturwissenschaften 66, 364-365 (1979)