Theor Appl Genet (1988) 75:309–318

THEORETICAL AND APPLIED GENETICS

Interspecific hybrids between *Brassica napus* L. and *B. oleracea* L. developed by embryo culture*

M. H. Quazi**

Crop Research Division, DSIR, Lincoln, New Zealand

Received November 15, 1985; Accepted August 19, 1987
Communicated by R. Riley

Summary. Interspecific hybrids between *Brassica napus* and *B. oleracea* are difficult to produce, and previous attempts to transfer economic characters from one species to the other have largely been unsuccessful. In these studies, oilseed rape cv. Tower (2n : 38) (*B. napus*) was crossed with broccoli and kale (2n : 18) (*B. oleracea*), and hybrid plants were developed from embryos in culture by either organogenesis or somatic embryogenesis. In rape × broccoli, F_1 plants were regenerated from hybrid embryos and the plants produced viable selfed seeds. F_5 plants (2n : 38) homozygous for white flower colour were selected for high oil content (47%) and Line 15; a selection from these plants produced fertile hybrids with rape, broccoli and kale without embryo culture. In reciprocal crosses between oilseed rape cv. Tower and an aphid resistant diploid kale, 28 and 56 chromosome F_1 hybrid plants were regenerated from somatic embryos. The 56 chromosome plants were self-fertile and it was concluded from F_2 segregation ratios that a single dominant gene controls resistance to cabbage aphid in kale. The 28 chromosome F_1's were self-sterile, but these and the 56 chromosome F_1's could be backcrossed to rape and kale. A cross between the F_1 (2n : 56) and a forage rape resulted in the selection of a cabbage aphid (*Brevicoryne brassicae* L.) resistant line (Line 3). Both Line 15 and Line 3 can serve as bridges for gene interchange between *B. campestris, B. napus* and *B. oleracea,* which has not been possible hitherto. Hybridisations between rape and tetraploid kale produced F_1 plants with 37 chromosomes. One F_2 plant possessed coronal scales and the inheritance was shown to be controlled by a single recessive gene unlinked to petal colour.

Key words: *Brassica* – Embryo culture – Somatic embryogenesis – Cabbage aphid resistance – *Brevicoryne brassicae* L.

Introduction

Brassica napus (2n : 38) is an alloploid from *B. oleracea* (2n : 18) × *B. campestris* (2n : 20) (U 1935). *B. napus* and *B. campestris* cross naturally and produce fertile hybrids (Catcheside 1934; Palmer 1962) and have probably introgressed extensively.

Spontaneous hybrids between *B. oleracea* and *B. campestris* have not been reported, and most authors (except Rudorf 1950) have reported very few hybrids from many thousands of pollinations. The cross has been slightly more successful at the tetraploid level (Frandsen 1947; Olsson 1960). *B. napus* includes only a small part of the range of *B. oleracea,* so it is possible that the *B. napus* arose spontaneously only a very few times.

Characters can now be readily transferred from *B. oleracea* to *B. napus* by re-synthesising *B. napus* from the cross *B. campestris* × *B. oleracea* using embryo culture (McNaughton 1963). Backcrossing the re-synthesised *B. napus* to *B. campestris* could transfer genes from *B. oleracea* to *B. campestris.*

Until now there has been no method by which genes could be transferred routinely from *B. napus* or *B. campestris* to *B. oleracea,* in spite of attempts made by several workers (Roemer 1935; Honma and Summers 1976; Chiang et al. 1977).

* This paper is dedicated to Mr. T. P. Palmer, a colleague and close friend who retired from the DSIR as Assistant Director of the Crop Research Division in September 1984

** Present address: NWFP Agricultural University, Peshawar, Pakistan

This paper reports the first large scale production of reciprocal hybrids between *B. napus* and three varieties of *B. oleracea*, and the successful transfer of several characters including resistance to cabbage aphid, from kale to rape.

Materials and methods

Plants of oilseed rape (*B. napus* cv 'Tower', 2n = 38), broccoli (*B. oleracea* var. *italica* Line G1117B, 2n = 18), marrowstem kale (*B. oleracea* var. *acephala* cv 'Rawara', 2n = 18 and Line OL418, 2n = 36), and curly kale (*B. oleracea* var. *fimbriata* Line OL419, 2n = 36) were grown in a glasshouse maintained at 18 ± 3 °C and 16 h day light. Kale plants were kept at 5 °C under low light intensity and 16 h day length for 8 weeks in order to induce flowering.

About 500 unsuccessful crosses between rape and broccoli (including reciprocals) were made in June 1978 and repeated in October the same year. All crosses were made one or two days before anthesis with freshly collected pollen. The development of pods, ovules and embryos were recorded at weekly intervals for three weeks.

In June 1979 reciprocal crosses were made between rape and kale, and the embryos were cultured (Table 1). Pods were removed from female parents 22 days after pollination and surface sterilised in 70% ethanol for 1 min and in 3.2% sodium hypochlorite for 20 min prior to rinsing in sterile water. Ovules that appeared to contain embryos were dissected under sterile conditions, and the embryos excised and cultured on sterile filter paper bridges half submerged in liquid nutrient medium in test tubes. Mature embryos were also cultured on the same medium containing potato dextrose agar ($7 g l^{-1}$).

The nutrient medium combined the inorganic salts from the Murashige and Skoog (1962) medium with the amino acids and vitamins of Jensen's (1976) C17 and 6% sucrose. Citric acid (500 mg dissolved in 60 ml water with pH adjusted to 5.3) and tri-potassium citrate ($300 mg l^{-1}$) were added to the medium. The pH of the medium was adjusted to 5.5 with 0.5 M NaOH, and it was then autoclaved at 121 °C for 5 min.

The cultures were maintained at 22 ± 3 °C under diffuse light, 16 h day length for the first four weeks, when light intensity was increased to 10,000 lx. After three months the plants were transplanted into soil in pots. Chromosomes were counted from root rips using the Feulgen method.

The technique of Davis (1964) was used for the electrophoresis of *Brassica* glucosinolase isozymes from seed. Pollen fertility was determined according to the double staining methods of Owczarzak (1952). For determining oil content, seeds were analysed by the low-resolution nuclear magnetic resonance (NMR) technique originally used by Conway and Earle (1963).

Cabbage aphids (*Brevicoryne brassicae*) were collected from *Brassica* crop in the field during March and April 1982 and were multiplied on rape plants in the glasshouse. The aphids caused leaf-curling damage to susceptible plants. For screening plant material, five to ten aphids were placed on 14-day-old plants and the leaf-curling response was recorded 7 days after infestation.

For field testing, seeds were precision sown on October 16, 1984, by Wintersteiger plotspider in 5 m rows with 60 cm between rows. After 95 days the trial was grazed for one week by approximately 50 sheep. Damage to the regrowth by cabbage aphid was assessed on April 23, 1985.

Table 1. Types of crosses made between various cultivars and breeding lines of *Brassica napus* and *B. oleracea*

Oilseed Rape cv Tower (2n = 38) × broccoli line G1117B (2n = 18)
cv Tower × Kale cv Rawara (2n = 18)
cv Tower × Kale line OL418 (2n = 36)
cv Tower × Curly Kale line OL419 (2n = 36)
cv Tower × line 15 [F_5 cv Tower × line G1117B] (2n = 38)
cv Rawara × cv Tower
F_1 (cv Tower × line G1117B) (2n = 28) × cv Tower
F_1 (line 15 × line OL419) (2n = 37) × cv Tower
F_1 (cv Tower × cv Rawara) (2n = 28) × cv Tower
F_1 (cv Tower × cv Rawara) (2n = 56) × cv Tower
F_1 (cv Rawara × cv Tower) (2n = 28) × cv Tower
F_1 (cv Rawara × cv Tower) (2n = 56) × cv Tower
F_1 (cv Tower × cv Rawara) (2n = 28) × forage rape cv Rangi
F_1 (cv Tower × cv Rawara) (2n = 56) × cv Rangi
F_1 (cv Rawara × cv Tower) (2n = 28) × cv Rangi
F_1 (cv Rawara × cv Tower) (2n = 56) × cv Rangi
F_1 (cv Tower × cv Rawara) (2n = 28) × line 15
F_1 (cv Tower × line 418) (2n = 37) × line 15
F_4 (cv Tower × line 418) × line 15
Line 15 × line G1117B
Line 15 × line OL419
Line 15 × cv Rawara

Results

Embryo culture

Table 2 shows development of the pods and the number of healthy ovules per pod in the rape (♀) × broccoli (♂) crosses. On day 22 after pollination, about 98% of the ovules had collapsed inside the pods. The ovules had no endosperm and the testa were shrivelled. These degenerated ovules were removed from the pods and dissected under the microscope. Healthy embryos at various stages of development were found in 22% of the ovules; these were isolated and cultured (Fig. 1 A–C). No embryos were recovered from the reciprocal crosses because the embryo sacs had degenerated within two weeks of pollination, in spite of the development of bigger pods and the presence of endosperm.

The numbers of embryos isolated and cultured from various crosses, their reciprocals and the numbers of hybrid plants developed from these embryos are shown in Table 3. In liquid culture embryos from all the

Table 2. Pod size and number of healthy ovules per pod in 20 pods resulting from the rape (♀) × broccoli (♂) crosses

Days after pollination	Average pod size (mm)		No. healthy ovules per pod
	Length	Breadth	
7	24.6 ± 1.1[a]	1.9 ± 0.1	28.4 ± 0.3
14	36.8 ± 2.1	2.6 ± 0.1	13.4 ± 2.1
21	38.5 ± 1.9	2.9 ± 0.1	1.9 ± 0.7

[a] SE of mean

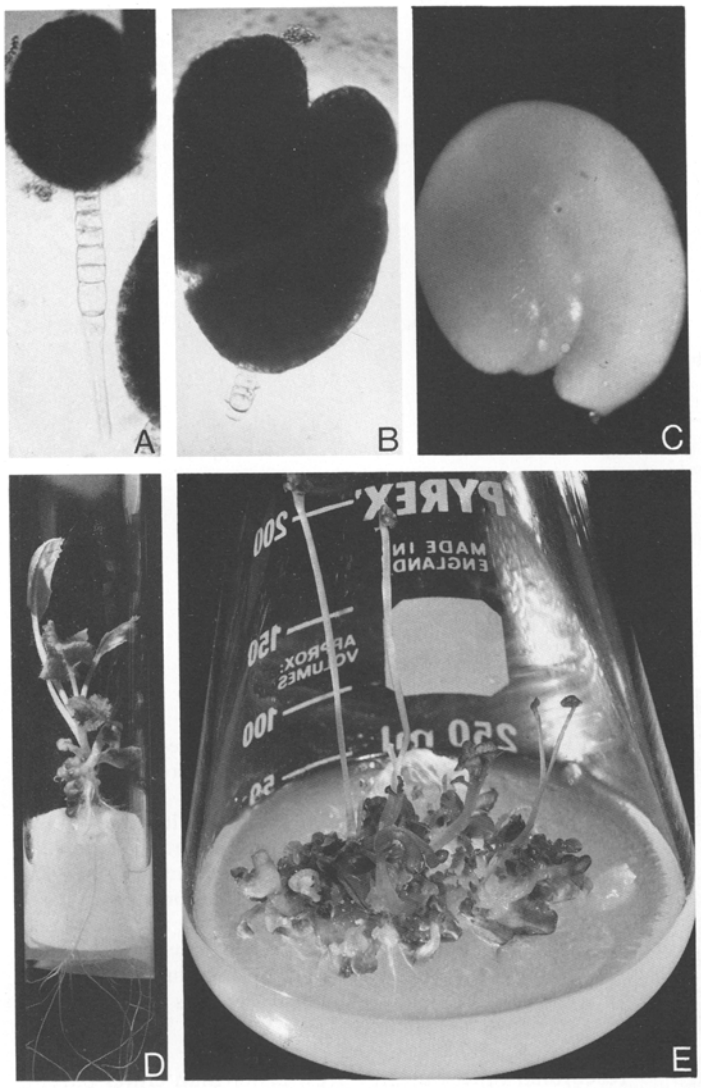

Fig. 1. A–C Embryos at globular, intermediate and mature stages of development isolated from crosses between *Brassica napus* oilseed rape cv, 'Tower' and *B. oleracea,* broccoli line G1117B (**A** × 130, **B** × 66, **C** × 44). **D** Plantlets developed from the mature embryos in liquid culture on a filter-paper bridge. **E** Shoots developing from callus of embryos cultured on agar-based medium

Table 3. The production of F_1 plants by embryo cultures from hybridisation of *B. napus* L. and *B. oleracea* L.

Cross combination	Polli-nations	Embryos cultured	Embryos differen-tiated	Plants produced
Rape cv Tower × broccoli line G1117B (2n = 18)	144	182	49	30
Rape cv Tower × Kale cv Rawara (2n = 18)	111	117	57	34
Kale cv Rawara (2n = 18) × Rape cv Tower	73	35	17	8
Rape cv Tower × Kale line OL418 (2n = 36)	91	16	14	7
Kale line OL418 (2n = 36) × Rape cv Tower	160	54	35	13
Rape cv Tower × Curly Kale line OL419 (2n = 36)	73	17	15	8

crosses produced a single shoot (Fig. 1 D), but in the agar-based medium the embryos formed a green callus structure from which multiple shoots arose (Fig. 1 E).

Detailed study of the embryos from the cross *B. napus* cv Tower × *B. oleracea* cv Rawara showed that in agar-based medium the embryos not only formed green calluses, they also produced somatic embryos (Fig. 2 A). These have all the organs of a normal zygotic embryo, but lack the characteristic shape and folding of the cotyledons and have no vascular connection with the callus from which they were derived. The somatic embryos are straight, the cotyledons small unequal primordia with meristematic region between, the hypocotyl and radicle contain a normal and continuous

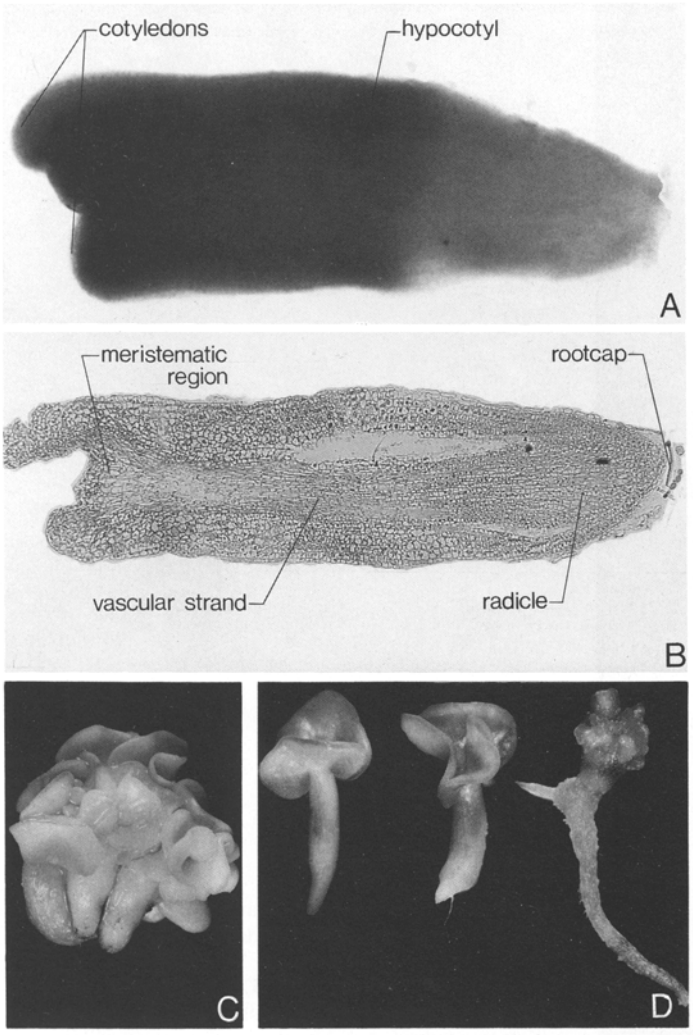

Fig. 2. **A** A young somatic embryo isolated from callus of a zygotic embryo after six weeks in culture from the cross between *Brassica napus,* oilseed rape cv Tower and *B. oleracea,* kale cv Rawara (×38). **B** A longitudinal section of the somatic embryo showing a continuous vascular strand connecting the cotyledons and the radicle (×38). **C** A cluster of mature somatic embryos on an agar-based medium. These embryo developed from a single zygotic embryo of a cross between *Brassica napus* cv Tower and *B. oleracea* cv Rawara (×11). **D** The cluster of somatic embryos split into individual embryos that were regenerated into plants (×11)

vascular strands, and the radicle has a root cap (Fig. 2B). Figure 2C shows a cluster of somatic embryos, each embryo with a well developed radicle and multiple cotyledonary leaves, arising from a single zygotic embryo cultured on agar-based medium. All clusters were split into separate embryos which grew into plants (Fig. 2D). In liquid culture somatic embryogenesis occurred in the same way as observed in the solid medium, but the green callus was small and few shoots formed, possibly because of the limited space in the test tube.

Interspecific hybrids and backcrosses

The F_1 hybrids developed from *B. napus* cv Tower and the various varieties of *B. oleracea* are characterised and compared with both parents in Table 4.

1 Rape×broccoli hybrid

Thirty F_1 hybrid plants were raised from the rape cv Tower×broccoli Line G1117B. Juvenile F_1 plants resembled rape, but they grew faster than either parent. The stems were thick and had internodes like those of broccoli; the inflorescence and the shape of the petal resembled rape, but the flowers were white like the broccoli parent. The hybrids contained glucosinolase isozymes derived from the male parent.

The F_1 hybrid plants had $2n=28$ chromosomes. The plants flowered profusely for six months and had 27% normal staining pollen grains. Study of pollen mother cell meiosis revealed nine pairs and ten univalent chromosomes. The plants produced 250 seeds from selfing and 158 from backcrosses to rape. Chromosome counts from root tips of ten selfed F_2 plants showed five plants having 32 chromosomes, two with 33, two with 34, and one with 45 chromosomes. For petal colour, 89 F_2 plants segregated in a 3 : 1 ratio (67 : 22) for white versus yellow. The backcross produced 44 white and 43 yellow flowered plants from 87 B_1 seeds sown. The hybrid plants and their progenies were self-fertile, and pollen viability rose from 27% in F_1 to 100% in F_5. Thirty seven F_4 white flower plants were analysed for

Table 4. Description of F_1 hybrids between *Brassica napus* and *B. oleracea* and of both parents

Characters	Female parent		Male parent		F_1 hybrids		
	B. napus		*B. oleracea*		*B. oleracea*		
	Rape cv. Tower	Broccoli line G1117B	Curly Kale line OL419	Marrowstem Kale line OL418	Rape × broccoli	Rape × Curly Kale	Rape × marrowstem Kale
Chromosome no. (2n)	38	18	36	36	28	37	37
Genome	aac c	cc	cccc	cccc	ac c	ac cc	ac cc
Pollen stainability (%)	100	95.2	84.3	84.2	27.4	54.9	56.1
Stem colour	green	green	pink	green	green	red	green
Basal leaf, lamina	thin, flat	thick, flat	thick, curly	thick, flat	intermediate flat	intermediate curly	intermediate flat
colour	slightly glaucous	glaucous	strongly glaucous	strongly glaucous	glaucous	glaucous	glaucous
veins	white	white	pink	white	white	slightly pink	white
Upper leaf	⅔ clasping	⅓	⅓	⅓	½	½	½
Flowering	annual	annual	biennial	biennial	annual	annual	annual
Petal, colour	yellow	white	pale yellow	pale yellow	white	pale yellow	pale yellow
breadth	6.72 ± 0.141[a]	6.94 ± 0.106	12.23 ± 0.150	12.80 ± 0.091	8.15 ± 0.083	9.20 ± 0.270	8.40 ± 0.145
length	7.23 ± 0.090	8.30 ± 0.167	13.30 ± 0.165	16.11 ± 0.133	8.80 ± 0.075	10.40 ± 0.150	10.40 ± 0.137
B/L ratio	0.93 ± 0.020	0.83 ± 0.014	0.92 ± 0.012	0.79 ± 0.009	0.92 ± 0.008	0.89 ± 0.022	0.81 ± 0.009
Short stamen	spreading	erect	erect	erect	suberect	suberect	suberect
Beak	slender	slightly swollen	slightly swollen	slightly swollen	slightly swollen	slightly swollen	slightly swollen

[a] SE of mean based on 20 observations

Table 5. Cross compatibility of F_5 rape – broccoli hybrid with cultivars from *B. campestris, B. napus* and *B. oleracea*

Test cultivar	Polli-nations	No. pods developed	Seeds	Seeds per pod
Broccoli line G1117B (♂)	20	20	27	1.3
Rape cv Tower (♀)	12	12	200	16.6
F_1 Tower × Rawara hybrid (2n = 28) (♀)	136	71	9	0.1
Kale cv Rawara (2n = 18) (♂)	32	23	14	0.6
Curly Kale line OL419 (2n = 36) (♂)	30	15	10	0.6
F_1 Tower × line OL418 hybrid (2n = 37) (♀)	36	26	495	19.0
Turnip line ECDO4 (2n = 20) (♂)	82	64	749	11.7
Turnip line ECDO4 (2n = 20) (♀)	60	28	59	2.1

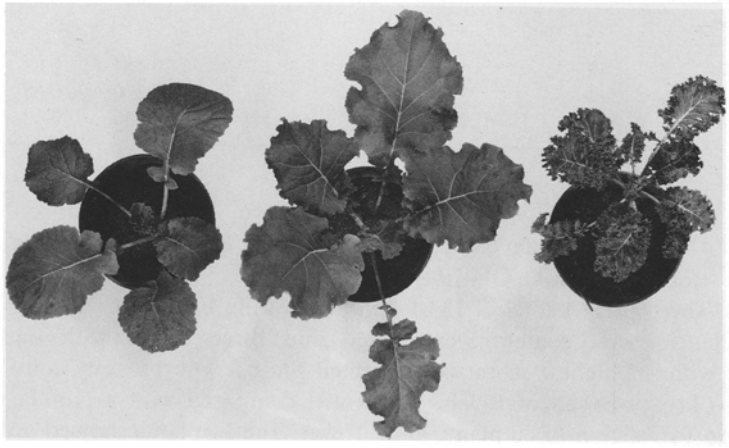

Fig. 3. Three to five week old plants of the F_5 rape-broccoli hybrid line 15 (♀) on the *left*, the curly Kale line OL419 (♂) on the *right*, and the hybrid between the two lines in the *center*

Table 6. The reciprocal hybrids between *Brassica napus* cv Tower and *B. oleracea* cv Rawara, their chromosome number, mean length and breadth of petals, pollen viability and seed set in self and backcrosses to *B. napus* for 24 plants

	Plant no.	Chromosome no.	Petal measurement[a]			Pollen viability (%)	Seed set	
			L	B	B/L		Self	Backcross
cv Tower × cv Rawara	1[b]	56	11.50	11.80	1.03	98.2	704	928
	2	–	8.80	8.40	0.95	6.8	–	–
	3	–	8.95	7.40	0.83	7.4	–	–
	4[b]	28	9.70	8.20	0.84	8.5	–	–
	5	–	9.20	8.00	0.87	13.7	–	8
	6	–	8.10	7.50	0.93	9.5	–	–
	7[b]	28	9.90	8.30	0.84	16.3	–	–
	8	–	8.90	8.20	0.92	21.8	–	25
	9	–	9.60	8.50	0.89	13.4	–	–
	10[b]	28	9.60	7.60	0.79	12.7	–	–
	11	–	9.40	8.10	0.86	10.3	–	15
	12	–	8.80	6.70	0.76	5.8	–	–
	13	–	8.80	7.70	0.87	13.0	4	34
	14	–	9.30	7.80	0.84	11.3	–	–
	15[b]	28	9.40	7.30	0.78	8.9	–	–
	16	–	8.80	8.00	0.91	17.2	2	2
	17	–	9.30	7.60	0.82	3.1	–	–
	18	–	9.20	7.40	0.81	13.4	–	–
	19	–	8.50	6.50	0.76	16.3	–	34
	'*t*' value (3df)[c]		8.0[d]	7.4[d]	6.2[d]			
cv Rawara × cv Tower	1	56	9.30	8.30	0.90	94.3	360	220
	2	28	9.00	7.10	0.79	30.4	–	18
	3	28	7.80	6.60	0.85	31.5	1	41
	4	28	7.80	6.80	0.87	17.6	–	4
	5	28	8.10	6.40	0.79	19.9	–	30
	'*t*' value[c]		1.8 NS	4.7[b]	1.62 NS			
	SE		0.139	0.118	0.013			

[a] Mean of 20 petals
[b] These plants are derived from embryoids developed on callus of the same zygotic embryo
[c] '*t*'-test for difference between 56 chromosome plant and mean of four 28 chromosome plants
[d] Significantly different at 1% probability level

Table 7. Evidence of single dominant gene resistance to Cabbage aphid (*Brevicoryne brassicae*) in *Brassica oleracea* and the hybrid *B. napus* × *B. oleracea*

Species	Common name	No. of plants		Expected ratio	χ^2	P*
		Resistant	Susceptible			
Brassica oleracea L. cv Rawara	Giant Kale	41	0	–	–	–
B. napus L. cv Tower	Oilseed Rape	0	35	–	–	–
cv Rangi	Fodder Rape	0	22	–	–	–
B. napus × *B. oleracea*						
cv Tower × cv Rawara – F_2	–	53	20	3 : 1	0.164	0.69
F_1 Tower-Rawara × cv Tower – B_1	–	11	12	1 : 1	0.000	0.68
F_1 Tower-Rawara × cv Rangi – B_1	–	198	209	1 : 1	0.246	0.62

* Probability under null hypothesis of observed value of χ^2 being exceeded

oil content and two plants with the highest score of 47% were selected. Both these plants were homozygous for white flower colour and had 2n : 38 chromosomes. The progeny of these plants was designated as Line 15. Plants from Line 15 were tested for cross compatibility with cultivars of *B. napus, B. oleracea* and *B. campestris* (Table 5). They gave hybrids with all three species. An F_1 hybrid developed from seed of the cross Line 15 × curly kale Line OL419 is shown in Fig. 3.

A total of 134 F_5 progeny of the F_4 plants with 47% oil content were tested and three plants with the highest score were selected for F_6. The progeny comprised of 165 F_6 plants was compared with a population of 60 plants of cv Tower. The F_5 plants showed no

Fig. 4. Plants of cv Rangi defoliated by cabbage aphid attack in the field. Plants of line 3a selection from the (Tower × Rawara) F_1 × Rangi cross remain undamaged. April 1985

improvement in oil content over the F_4's, but the F_6 plants had a mean oil content of 42.5% (S.E. 0.9) which was 5.5% greater than cv Tower.

2 Rape × kale hybrids

Rape × diploid kale crosses. From reciprocal crosses between rape cv Tower (2n : 38) and the diploid kale cv Rawara (2n = 18), 42 F_1 hybrids were produced. Table 6 shows petal measurement, pollen viability, chromosome number, and seed production of 24 of these plants. All these plants had coloured stems, flat and glaucous basal leaf laminae and light coloured veins. The upper leaf was "half clasping". The petals were yellow, the short stamens were erect and the pods had swollen beaks. The reciprocal hybrids had a rosette growth habit, and none of the eight plants flowered without cold treatment. Chromosome counts from root-tips of five F_1 plants and five reciprocal hybrids showed in each case four plants with 2n = 28 and one plant with 2n = 56 chromosomes. All the 28 chromosome plants were self sterile but set seeds with the two parents and also with Line 15. Although the morphological characters of cv Rawara were similar to that of the tetraploid marrowstem kale described in Table 4, it was resistant to cabbage aphids. The F_1 Tower × Rawara hybrids could not be tested, but both 56 chromosome plants produced sufficient seeds to make screening for resistance to cabbage aphids possible in the F_2.

Table 7 shows reaction to cabbage aphids of cv Rawara and cv Rangi, and of the F_2 Tower × Rawara hybrids, and the backcross of F_1 (2n = 56) to rape, cv Tower and cv Rangi. The number of resistant to susceptible plants did not differ significantly from the expected ratio of 3 : 1 in the F_2 and 1 : 1 in the backcrosses provided evidence for single gene inheritance of resistance to cabbage aphid (Table 7). The F_2 plants had 56 chromosomes, but 15 plants from the backcross to rape cv Rangi were found to be aneuploid with chromosome numbers ranging from 42 to 50.

Ten lines from the backcross to cv Rangi were grown in the field during May–September 1983 with forage rape cultivars. All the lines bolted earlier than the standard cultivars and were discarded, except line 3 which had more biennial plants and comparable dry matter yields. This line was compared with the cabbage-aphid susceptible parent cv Rangi in a replicated field trial during October 1984–April 1985. The trial was grazed on January 21. The grazed plants were less than 5 cm tall with a very low level of residual growth. The trial was allowed to regrow till April 23, during which time the peak flights of cabbage aphid occurred. The cv Rangi plants became infected with cabbage aphids and leaves developed severe distortion, whereas Line 3 had visually similar aphid infestation but remained healthy with plainer leaves and no distortion (Fig. 4). In a separate experiment 30 plants of Rangi and of Line 3, grown in wooden boxes during February–April 1985, were compared at 12 weeks of age. The Rangi plants had 466 ± 214 aphid and Line 3 had 8 ± 8 aphids per plant. Plants of Line 3 had fleshy, flat leaves with glaucous leaf surfaces similar to kale, but short internodes like Rangi rape, and could therefore be described as 'bushy kale'.

Rape × tetraploid kale crosses (Table 4). Seven F_1 plants were developed from embryos of the crosses between cv Tower and Line OL418, and 13 plants from the reciprocals. Eight F_1 hybrid plants were obtained from embryos of rape × curly kale OL419 crosses. All the F_1 hybrids had a diploid number of 37 chromosomes, and in F_2 root tips from four rape × curly kale hybrids all

Fig. 5. Coronal scales in the F_2 hybrid between *Brassica napus* cv 'Tower' and *B. oleracea,* tetraploid Kale line OL418

showed 38 chromosomes and four plants from the backcross to rape had 56 chromosomes.

One plant in the F_2 from rape × kale (Line OL418) was observed with coronal scales (Fig. 5). This plant was selfed and ten seeds were obtained which were sown and produced ten F_3 plants. Only seven of these exhibited the annual flowering character and four had petals with coronal scales. Three plants with coronal scale petals and one plant without coronal scale petals were selfed and seeds were collected. Forty-two F_4 plants grown from the selfed seeds of the three plants produced flowers with the coronal scale petals only. These plants, with yellow flower colour and coronal scale petals were crossed with the white flower rape × broccoli Line 15 plants which lacked the coronal scales.

Thirty-two F_1 plants thus produced all had white petals without coronal scale. These plants produced selfed seed from which 120 F_2 plants were grown. Sixty-eight plants flowered and segregated for flower colour and the coronal scale as follows: petals white, coronal scales absent – 43; petals white, coronal scales present – 11; petals yellow, coronal scales absent – 12; petals yellow, coronal scales present – 2.

After performing χ^2 analysis on this data, it was found that there was no significant deviation from a 9 : 3 : 3 : 1 ratio ($P = 56$) and thus no evidence for linkage of the two characters. Furthermore, there was no significant difference from a 3 : 1 ratio of white vs yellow petals ($P = 40$) or absence vs presence ($P = 31$) of coronal scales. From these data it was concluded that a single recessive gene governs the presence of coronal scales.

Discussion

Embryo development and regeneration of F_1 hybrids

The 100 plants developed from embryos of crosses between *B. napus* and *B. oleracea* (Tables 3 and 4) were hybrids as shown by the expression of paternal morphological characters in the F_1 plants, for instance white flowers in the rape × broccoli or curly leaf in the rape × curly kale hybrids, and from studies of isozymes and cytological analyses. The ovules from which the embryos were isolated were characterised by an absence of endosperm and degeneration of testa. Chiang et al. (1977) may have disregarded these ovules in their studies and cultured instead embryos from healthy ovules that produced matromorphic plants. Because the hybridity of these ovules was suspect, we chose not to culture embryos from the few healthy ovules recorded in Table 2. Embryos in broccoli × rape cross were not found because the embryo sacs had degenerated by the time of ovule dissection 22 days after pollination. Many workers have experienced difficulties in obtaining hybrids between *B. oleracea* and *B. napus,* and it has been suggested that cultivars with genome 'c' are poor female parents compared to those with genome 'a' or 'ac' (Chiang et al. 1977). Hybrid plants produced from *B. oleracea* × *B. napus* and reciprocals were roughly proportional to the number of pollinations and number of embryos cultured. These data do not support the notion of *B. oleracea* perse being a poor parent. Similarly, crosses between *B. campestris* × *B. napus* have been reported as unsuccessful by some workers (Becker 1950; Hoffmann and Peters 1958; McNaughton 1963) and fertile by others (Palmer 1962; Lammerink 1970) with the reciprocals usually fertile. Because hybrids between the diploid and tetraploid kales and rape have been successfully developed (Table 3), the difficulty in producing broccoli × rape hybrids could be a varietal effect and not necessarily produced by genome 'c'.

Somatic embryogenesis occurs in numerous plant species (Tisserat et al. 1979). In *Brassica,* embryoids have been developed from mesophyll cell protoplasts (Li and Kohlenbach 1982), pollen (Keller et al. 1975), epidermal cells of hypocotyl (Thomas et al. 1976; Crouch 1982), and calli (Pareek and Chandra 1978; Leelavathi et al. 1984). Here somatic embryos were obtained from cultures of 22 day-old zygotic embryos rescued from *B. napus* × *B. oleracea* cross. In the present paper only the morphology, histology and cytogenetics of the embryoids developed from cultures of interspecific hybrid embryos of rape and diploid kale are reported, but embryoid formation was also observed in the other crosses given in Table 3, and in both liquid and agar-based media.

Cytogenetics of the hybrids

The F_1 hybrids between rape cv Tower ($2n = 38$) and broccoli Line G1117B ($2n = 18$) had 28 chromosomes, probably deriving 19 chromosomes from rape and 9 from broccoli. The presence of 9 pairs and 10 univa-

lents at the pollen mother meiosis was expected because rape, *B. napus,* is an amphidiploid of *B. campestris* (2n = 20) and *B. oleracea* (2n = 18). Thus the 10 univalents appear to be the chromosomes of *B. campestris* which contributed to the aneuploidy observed in the F_2 plants. The presence of white flowered plants among the F_2 population was evidence that gene transfer had taken place between homologous *B. oleracea* chromosomes. From the progenies of these plants Line 15 was selected for oil content which was homozygous for white flower colour, had 2n : 38, and was compatible with both the rape and broccoli parents.

In cv Tower (2n = 38) × cv Rawara (2n = 18) both the F_1 and the reciprocal hybrids regenerated via somatic embryogenesis had either 2n = 28 or 56 chromosomes. None of the plants developed from embryoids of the rape × broccoli and rape × kale crosses were aneuploid. A triploid was produced by rape × tetraploid kale. The zygotic embryos with 2n = 28 in the reciprocal crosses between cv Tower × cv Rawara produced embryoids in culture from which plants developed with 2n = 28 and 56 chromosomes as shown in Table 6. Engvild (1974) suggested a number of mechanisms for polydiploidisation in culture, but in these studies it is likely to have arisen from endomitosis.

Phenotypic variations such as multiple branching of stem, abnormal cupped leaves, exceptionally slow growth, and failure to flower were observed in progenies of plants developed from embryo cultures of all the interspecific crosses. The inheritance of these characters was not pursued except that the coronal scales observed in the F_2 rape × kale hybrid plants were investigated. Data collected showed that coronal scales are inherited by a single recessive gene which is independent of petal colour. The coronal scales have not been recorded in *Cruciferae,* and although reported in plants of *Caryophyllaceae* (Chater and Walters 1964) their mode of inheritance was unknown. The role of in vitro processes in the emergence of this sudden heritable character is perhaps worthy of further investigation.

Seed setting

All the F_1 hybrids were partially or completely self-sterile except the 56 chromosome hybrids developed from the cross cv Tower × cv Rawara, which produced numerous F_2 and backcrossed seeds (Table 6). The 28 chromosome cv Tower × cv Rawara F_1 hybrids set only a few seeds, although its pollen viability score was comparable to that of the cv Tower × Line G1117B F_1 hybrids, which produced 250 selfed seeds from 30 plants. The rape × tetraploid kale F_1 hybrids were mainly sterile although the pollen viability scores were greater than the rape × broccoli F_1 hybrids (Table 4). Kales are biennial, predominantly outcrossing and self-incompatible while broccoli is annual and self-pollinating type. Obviously these differences are reflected in their ability to form fertile F_1 hybrids with *B. napus.*

Practical application

The purpose of crossing a number of cultivars from *B. oleracea* with *B. napus* cv Tower was to establish a model system for transferring to rape known and identifiable characters such as white flower from broccoli (Anstey 1955) and curly leaf from curly kale. Once the technique for transfer of flower colour from broccoli to rape was established, then cultivars such as Rawara and Line OL419 with resistance to cabbage aphid and clubroot, respectively, were included in these studies. The main objective was to transfer these characters to forage rapes, but attempts to hybridize rape cultivars such as cv Rangi, cv Wairangi and cv Moana with the kale group failed. The oilseed rape cv Tower has now been successfully hybridized with many varieties of *B. oleracea,* and the F_1 hybrids thus produced are compatible with the two parents and also with forage rapes. One of these hybrids is the homozygous white-flowered Line 15 selected for high oil content from the rape × broccoli cross. This line is potentially an oilseed crop, but more importantly it served as a bridge between *B. napus* and *B. oleracea.* The presence of a marker gene for white flower and the ability to set seeds with *B. napus, B. oleracea* and *B. campestris* makes Line 15 a useful breeding resource (Table 5).

Clubroot (*Plasmodiophora brassicae* Wor.) is a common problem in the genus *Brassica* L. (Crete and Chiang 1967; Honma and Summers 1976). Natural immunity to the pathogen has been demonstrated in *B. napus* and *B. campestris* but does not appear to be common in *B. oleracea* (Crute et al. 1980). Line 15 has now been successfully crossed with the clubroot resistant lines of turnip ECD04 and rape (Lammerink 1985) and the resulting hybrids could be crossed with *B. oleracea.* Recently resistance to the herbicide atrazine was transferred from rape to broccoli and kale using Line 15 as a bridge and without embryo culture. Similarly, the transfer of resistance to cabbage aphid from kale to forage rape was achieved by using the F_1 Tower × Rawera hybrid as a bridge because forage rape cv Rangi and cv Rawera do not cross.

Palmer (1960) developed a cabbage aphid resistant forage rape, but this resistance was overcome following the appearance of a new biotype causing leaf-curling damage in the resistant rape (Lammerink 1968). Claridge (1972) observed that kales were least affected by aphid attack and cv Rawara was found to be

resistant to the leaf-curling damage caused by cabbage aphid (Palmer, pers. commun.). Data presented in Table 7 shows that the resistance in cv Rawara to leaf-curling caused by cabbage aphid infestation is controlled by a single dominant gene, and this character can be transferred across the species barrier. The resistance in progeny from rape×kale hybrids was tested in the F_2 from Tower×Rawara and in the backcrosses to cv Tower and the forage rape cv Rangi, first in the glasshouse and then confirmed both in the glasshouse and in field experiments (Fig. 4). Thus cabbage aphid resistant Line 3 has now been selected for evaluation as forage rape from the cross F_1 (Tower× Rawara)×cv Rangi.

Acknowledgements. Thanks are expressed to M. K. Williams for technical assistance, E. J. Beuzenberg for chromosome counts, Dr. J. A. K. Farrell for aphid manipulation, A. Wallace for statistical analysis, and R. Lamberts and W. F. Rennie for photography. The assistance of R. D. Banfield in the field trials and that of D. B. MacGibbon in electrophoresis is highly appreciated.

References

Anstey TH (1955) Inheritance of white petal in green sprouting broccoli. Can J Agric Sci 35:573–578

Becker T (1950) Siebenjährige blütenbiologische Studien an den Cruziferen *Brassica napus* L., *Brassica oleracea* L., *Raphanus* L., und *Sinapis* L. Z Pflanzenzücht 29:222–240

Catcheside DG (1934) The chromosomal relationship in the swede and turnip groups of *Brassica*. Ann Bot 48:601–633

Chater AO, Walters SM (1964) Silene. In: Tutin TG, Haywood VH, Burges NA, Valentine DH, Walters SM, Webb DA (eds) Flora Europaea, vol 1. Cambridge University, pp 159–181

Chiang MS, Chiang BY, Grant WF (1977) Transfer of resistance to race 2 of *Plasmodiophora brassicae* from *Brassica napus* to cabbage (*B. oleracea* var. *'capitata'*). I. Interspecific hybridization between *B. napus* and *B. oleracea* var. *'capitata'*. Euphytica 26:319–336

Claridge JH (1972) Arable farm crops of New Zealand. In: The Department of Scientific and Industrial Research in association with Reed AH and AW (eds). Wellington Auckland Christchurch Sydney Melbourne, pp 193–195

Conway TF, Earle FR (1963) Nuclear magnetic resonance for determining oil content of seeds. J Am Oil Chem Soc 40:265–268

Crete R, Chiang MS (1967) Screening tests of crucifers for resistance to clubroot in organic soils of Quebec. Plant Dis Rep 51:991–992

Crouch ML (1982) Non-zygotic embryos of *Brassica napus* L. contain embryo-specific storage protein. Planta 156: 520–524

Crute IR, Gray AR, Crisp O, Buczacki ST (1980) Variation in *Plasmodiophora brassicae* and resistance to clubroot disease in *Brassicas* and allied crops – A critical review. Plant Breed Abstr 50:91–104

Davis BJ (1964) Disc electrophoresis. II. Method and application to human serum proteins. Ann NY Acad Sci 121: 404–427

Engvild KC (1974) Plantlet ploidy and flower-bud size in tobacco anther cultures. Hereditas 76:320–322

Hoffmann W, Peters R (1958) Versuche zur Herstellung synthetischer und semisynthetischer Rapsformen. Züchter 28: 40–51

Frandsen KJ (1947) The experimental formation of *Brassica napus* L. var. *oleifera* D.C. and *Brassica carinata* Braun. Dan Bot Ark 12:1–16

Honma S, Summers WL (1976) Interspecific hybridization between *Brassica napus* L. (Napobrassica group) and *B. oleracea* L. (Botrytis group). J Am Soc Hortic Sci 101: 299–302

Jensen CJ (1976) In: Gaul H (ed) Barley genetics, vol III. Thiemig, München, pp 316–345

Keller WA, Rajhathy T, Lacapra J (1975) In vitro production of plants from pollen in *Brassica campestris*. Can J Genet Cytol 17:655–666

Lammerink J (1968) A new biotype of cabbage aphid (*Brevicoryne brassicae* L.) on aphid resistant rape (*Brassica napus* L.). N Z J Agric Res 11:341–344

Lammerink J (1970) Interspecific transfer of clubroot resistance from *Brassica campestris* L. to *B. napus* L. N Z J Agric Res 13:105–110

Lammerink J (1985) Identification of an eight race of *Plasmodiophora brassicae* Worn. N Z J Agric Res 29 (in press)

Leelavathi S, Reddy VS, Sen SK (1984) Somatic cell genetic studies in *Brassica* species. I. High frequency production of haploid plants in *B. alba* (L.) H f & T. Plant Sci Lett 3: 102–105

Li L, Kohlenbach HW (1982) Somatic embryogenesis in quite a direct way in cultures of mesophyll protoplasts of *Brassica napus* L. Plant Cell Rep 1:209–211

McNaughton IH (1963) The scope and problems involved in synthesising new amphidiploids and autotetraploid fodder brassicas in the group *B. napus* L., *B. campestris* L. and *B. oleracea* L. Scottish Plant Breeding Station, Record 1963, pp 48–68

Olsson G (1960) Species crosses within the genus *Brassica*. II. *Artifical Brassica napus* L. Hereditas 46:351–386

Owczarzak A (1952) A rapid method for mounting pollen grains with special regard to sterility studies. Stain Technol 27:249–251

Palmer TP (1960) Aphis resistant rape. N Z J Agric 101: 375–376

Palmer TP (1962) Population structure, breeding system, interspecific hybridisation and allopolyploidy. Heredity 17:278–283

Pareek LK, Chandra N (1978) Somatic embryogenesis in leaf callus from cauliflower (*Brassica oleracea* var. *Botrytis*). Plant Sci Lett 11:317–322

Roemer W (1935) Fruchtbarkeits- und Vererbungsstudien *Brassica* – Artkreuzungen. Z Pflanzenzücht 20:337–616

Rudorf W (1950) Über die Erzeugung und die Eigenschaften synthetischer Rapsformen. (On the production and characteristics of synthetic forms of rape). Z Pflanzenzücht 29: 35–54

Thomas E, Hoffmann F, Potrykus I, Wenzel G (1976) Protoplast regeneration and stem embryogenesis of haploid androgenetic rape. Mol Gen Genet 145:245–247

Tisserat B, Esan EB, Murashige T (1979) Somatic embryogenesis in angiosperms. Hortic Rev 1:1–78

UN (1935) Genome-analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn J Bot 7:389–452

Note added in proof

Lines 15 and 2 have also been successfully hybridized with *B. juncea* and *B. carinata*.