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Intergeneric somatic hybrid production through protoplast fusion between *Brassica juncea* and *Diplotaxis muralis*

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Summary. The need to transfer genetic traits from *Diplotaxis muralis* (2n = 42) to *Brassica juncea* (2n = 36), a major oil seed crop of the tropical world, was realised. Since the two plant types are sexually incompatible, attempts were made to evolve parasexual hybrids as the result of protoplast fusion. Protoplasts of hypocotyl-derived calli of two cultivars of *B. juncea* were fused with normal and γ-irradiated mesophyll protoplasts of *Diplotaxis muralis*. Regeneration of 110 plants from the fused products was successfully achieved. Upon analysis of some of them, we realised that true somatic hybrids and partial somatic hybrids had been generated. Thus the primary goal of evolving intergeneric hybridisation products between these two plant types was fulfilled.

Key words: Intergeneric somatic hybridisation – *Brassica* – *Diplotaxis* – Somatic hybrid

Introduction

Although there are many success stories in plant genetics with regard to sexual crosses between distantly related plant species, most plant breeders would agree that much greater potential exists in exotic germ plasm than has actually been tapped. Indeed, solutions of some of the problems improving the oil yielding *Brassica* crops of the tropical world are believed to lie in introgression of these genetic resources from their wild relatives. Genus *Diplotaxis* is a rich source of such desirable characteristics for oil yielding *Brassica* crops. Thus introgression of genetic characteristics contained in *Diplotaxis* in *B. juncea* is highly desirable, not withstanding the fact that recovery of sexual hybrids between members of *Diplotaxis* and *Brassica* is very difficult, if not impossible. A few records of past success producing such intergeneric hybrids exist (Mizushima 1972; Hinata et al. 1974; Hinata and Konno 1979), but the potential of the earlier successes was not fully realised. Consequently, the necessity for expanding the pool of accessible genes for *Brassica* by overcoming natural isolating mechanisms remained unfulfilled.

Brassica juncea is the major oil yielding crop of the Indian subcontinent. There have been no reports of intergeneric sexual hybrids between *Diplotaxis* species and *Brassica juncea*. Somatic cell fusion technique was considered suitable enough to generate hybrids in such a situation. Through this technique, several intergeneric somatic hybrid plant productions were achieved in the past (Melchers et al.1978; Gleba and Hoffmann 1980; Hoffmann and Adachi 1981; Krumbiegel and Schieder 1981; Dudits et al. 1980; Nagao 1982; Gleba et al. 1984; Pental et al. 1986; Kushnir et al. 1987; Toriyama et al. 1987). However in *Brassica*, interspecific somatic hybrids (Schenck and Robbelen 1982; Sundberg and Glimelius 1986; Taguchi and Kameya 1986; Terada et al. 1987; Robertson et al. 1987) as well as intergeneric cybrids (Pelletier et al. 1983) and intraspecific cybrids (Yarrow et al. 1986; Barsby et al. 1987; Menczel et al. 1987; Morgan and Maliga 1987) were obtained earlier. Most of these somatic hybrids were produced using selection systems such as biochemical mutants, antimetabolite-resistant strains and also counter-selectable markers.

This paper deals with our success in recovering somatic hybrids of *Brassica juncea* and *Diplotaxis muralis*. High morphogenic ability of the fused cellular products helped in recovering a large number of plants in the absence of any selectable markers. Morphogenic characters, meiotic chromosomal behaviour, isozyme analysis and isoelectric focusing patterns of the RuBP carboxylase of the hybrids have established their characteristics.

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Materials and methods

Experimental design

The experimental strategy used for generation of plants from fused products of protoplasts of *Brassica juncea* and *Diplotaxis muralis* contained the following features:

(1) The plating density of the protoplasts in fusion experiments was kept high by making available four times as many protoplasts of *Diplotaxis* as of *B. juncea*. In such a situation ca 60% of *B. juncea* protoplasts underwent intergeneric fusion. Protoplasts of *D. muralis* at high plating density (1×10^5 protoplasts/ml) failed to divide and showed abysmally low plating efficiency, whereas the plating efficiency of *B. juncea* protoplasts remained unaffected.
(2) Plant regeneration from the protoplast-derived microcolonies was carried out through a regeneration medium, where organogenesis was achieved in protoplast-derived cells of *B. juncea*. This step ensured that plants regenerated would either be from protoplasts of *B. juncea* or from the intergeneric fused protoplasts, in cases where the genetic potentiality of *B. juncea* cells to respond to the specific regeneration medium is dominant over that of *D. muralis*.
(3) Additionally in a parallel setup, protoplasts of *D. muralis* were exposed to gamma irradiation at LD_{50} and LD_{80} levels before they were fused with *B. juncea* protoplasts. Irradiation was offered to inactivate nuclear function of the *D. muralis*. Other experimental steps remained unaltered to recover plants from such fusion attempts. In such a situation, the following likely consequences were expected to be achieved: (a) production of cybrids in case of total inactivation of the *D. muralis* nucleus; (b) possibility for the production of partial somatic hybrids in case of destablisation of the chromosomes of *D. muralis*; (c) promotion of morphogenic ability of the fused cells as the result of genetic complementation and/or elimination of the inhibitory factor(s) as the consequence of irradiation.
(4) Initial selection of the regenerated plants was carried out on the basis of their morphological characters. All plants which appeared not to fully resemble any one of the parents were set aside. Thereafter characterization of these plants was carried out through chromosome analysis, isozyme pattern of esterase, and isoelectric focusing characteristics of RuBP carboxylase.

Plant materials and cultural conditions

Two varieties of *Brassica juncea* (L) Czern (2n = 36), viz, cv T-59 and cv B-85, and *Diplotaxis muralis* (2n = 42) formed the experimental materials. Seed culture of the plant materials was developed in aseptic conditions (Sikdar et al. 1987). Hypocotyls from 8-day old seedlings of T-59 and B-85 were cut into pieces (cv 0.5 cm long) and cultured in callus induction medium in the dark [$CaH_4(PO_4)_2 \cdot H_2O$ minus K_3 basal medium of Kao et al. (1974) (MK_3) with 0.25 mg^{-1} 2,4-D + 0.25 mg^{-1} NAA + 0.5 mg^{-1} BAP + 10% (v/v) CM + 2% (w/v) sucrose + 0.8% agar, pH 5.8]. The freshly induced calli were utilised as the source for protoplasts of *B. juncea*. In the case of *D. muralis*, 15-day old seedlings were utilised for apical shoot tip culture. Cultural conditions were similar as indicated earlier (Chatterjee et al. 1985). The apical shoot tips were subcultured every 3 weeks and leaves of such 3-week old cultures were used as the source of protoplasts.

Protoplast isolation: Mesophyll tissue (1 g fresh weight) of *D. muralis* were stripped into 2 mm pieces in washing solution [0.6 M mannitol + 0.2% (w/v) $CaCl_2 \cdot 2\,H_2O$] and then incubated in 20 ml enzyme mixture containing 0.25% (w/v) Cellulase (Onozuka R-10) + 0.5% (w/v) Pectinase (Serva) + 0.6 M mannitol + 0.2% (w/v) $CaCl_2 \cdot 2\,H_2O$, pH 5.8 for 15½ h at 27 ± 1 °C. Protoplasts were isolated from freshly induced hypocotyl calli of both the varieties of *B. juncea* using an enzyme mixture (15 ml/g fresh weight of tissue) containing 1% (w/v) Cellulase (Onozuka R-10) + 1% (w/v) Driselase (Kyowa Hakko Kogyo + 1% (w/v) Pectinase (Serva) + 0.6 M mannitol + 0.2% (w/v) $CaCl_2 \cdot 2\,H_2O$, pH 5.8, at 27 ± 1 °C for 17 h. Purification of the protoplasts was subsequently carried out (Sikdar et al. 1987).

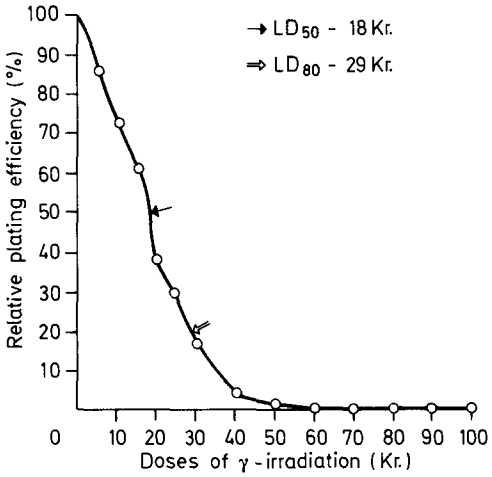

Fig. 1. Estimation of LD_{50} and LD_{80} of γ-irradiation for the inactivation of *Diplotaxis muralis* protoplasts

Gamma irradiation of protoplasts: Varying doses of gamma irradiation of mesophyll protoplasts of *D. muralis* were administered from a ^{60}Co source (Gamma chamber 900, Isotope division, BARC), which delivered an average dose rate of 1.5 Kr/min. The survivability of the irradiated protoplasts was judged by their relative plating efficiency (relative plating efficiency was calculated on the basis of plating efficiency at the particular dose/control plating efficiency × 100). The radiation doses necessary for LD_{50} and LD_{80} effects were ascertained from our background results (Fig. 1).

Protoplast fusion and culture

Protoplast fusion was carried out between hypocotyl callus protoplasts (hcp) of each of the two cultivars of *B. juncea* and mesophyll protoplasts (mp) of *D. muralis*. Hereafter, hypocotyl callus-derived protoplasts of *B. juncea* cv T-59 and B-85 will be referred to as hcp T-59 and hcp B-85 respectively, and mesophyll protoplasts of *D. muralis* as mp Dm in this text. Furthermore, the irradiated mesophyll protoplasts of *D. muralis* at LD_{50} and LD_{80} will be referred to as mp Dm50 and mp Dm80 respectively. Thus, for each of the cultivars of *B. juncea*, the following sets of experiments were carried out:

1. hcp + mp Dm
2. hcp + mp Dm50
3. hcp + mp Dm80
4. hcp self-fusion
5. mp Dm self-fusion
6. mp Dm50 self-fusion
7. mp Dm80 self-fusion

The protoplasts were suspended at 1×10^6/ml density. For fusion, hcp and mp were mixed in 1:4 ratio. Following is the protocol adopted for protoplast fusion. Four droplets each containing 50 µl of the mixed protoplast suspension were placed separately on a 35 mm sterile plastic petriplate and allowed to

Table 1. Results of intergeneric protoplast fusion between *B. juncea* and *D. muralis*

Fusion partners	Total no. of calli developed	No. of plants regenerated	% of plant regeneration [1]	Nature of regenerated plants		Plants not identical to either of the parents
				Plants identical to either of the parents		
				Brassica looking	*Diplotaxis* looking	
hcpT-59 + mpDm	140	8	5.71	2	–	6
hcpT-59 + mpDm50	192	39	20.31	10	–	29
hcpT-59 + mpDm80	94	16	17.02	2	–	14
hcpT-59 self fusion	157	16	10.19	16	–	–
hcpB-85 + mpDm	164	8	4.87	4	–	4
hcpB-85 + mpDm50	116	25	21.55	8	–	17
hcpB-85 + mpDm80	80	14	17.5	3	–	11
hcpB-85 self fusion	132	12	9.09	12	–	–
mpDm self fusion	2	–	–	–	–	–
mpDm50 self fusion	–	–	–	–	–	–
mpDm80 self fusion	–	–	–	–	–	–

[1] Single plant/callus

settle for 20 min. 50 µl polyethylene glycol (PEG) fusing solution [30% (w/v) PEG, MW 1540 + 0.2 M glucose + 10 mM $CaCl_2 \cdot 2\,H_2O$ + 0.7 mM KH_2PO_4, pH 5.8] was added gradually to each of the droplets and incubated at 25 ± 1 °C for 10 min. Gradual elution of PEG was carried out by adding 25 µl of freshly prepared eluting solution [freshly prepared by mixing 1 part solution A (0.5 M Glycine-NaOH buffer, pH 10.5) with 9 part solution B (0.4 M glucose + 60 mM $CaCl_2 \cdot 2\,H_2O$ + 10% (v/v) DMSO] to each droplet four times at 5 min intervals. The whole suspension was finally replaced by the washing solution and incubated for 5 min. This step of washing was repeated three times. Finally, the fusion products were cultured in 2 ml MK_3B_2 protoplast culture medium (MK_3 basal medium + 0.6 M glucose + 0.2 mg^{-1} 2,4-D + 0.2 mg^{-1} NAA + 0.25 mg^{-1} BAP + 0.1 mg^{-1} GA_3, pH 5.8) per 35 mm petriplate for 7 days in the dark at 24 ± 1 °C. After 7 days the old medium was replaced by the fresh medium and the plates were subjected to 16 h of photoperiod (illumination 2,000 lux) at 25 ± 1 °C and 60% relative humidity. After 2 weeks of incubation, the culture medium was replaced by liquid MK_3A_5 medium (MK_3 basal medium + 0.4 M glucose + 0.0583 M sucrose + 0.1 mg^{-1} 2,4-D + 0.1 mg^{-1} NAA + 1.0 mg^{-1} BAP + 0.1 mg^{-1} GA_3, pH 5.8). At this stage illumination was increased from 2,000 lux to 2,500 lux. We then replaced the 21 day-old culture by solid MS-A_6 medium (MS basal medium + 0.1 mg^{-1} 2,4-D + 0.1 mg^{-1} NAA + 2.0 mg^{-1} BAP + 0.1 mg^{-1} GA_3 + 0.0583 M sucrose + 0.5% agar, pH 5.8).

Plant regeneration

After 4 weeks when calli reached 0.5–1 mm diameter, they were transferred to MS-S regeneration medium (0.5-strength MS basal medium + 1.0 mg^{-1} NAA + 1.0 mg^{-1} BAP + 0.5 mg^{-1} GA_3 + 0.0583 M sucrose + 0.8% agar, pH 5.8). After one week, initiation of plant regeneration through organogenesis could be observed. The regenerated plants were finally transferred to hormone-free MS medium for root initiation. The regenerated plants flowered within 6–8 weeks of culture.

Chromosome analysis

Meiotic chromosome analysis of the regenerated plants was carried out following the standard method of aceto-carmine staining. Characteristic of chromosomal configuration at diakinesis was considered to be adequately stringent for judging nuclear hybridity.

Isozyme analysis

Plants with reconstructed chromosome content were further analysed for their nuclear-coded isozyme patterns of esterase. Cell-free extracts were prepared from leaves of exponentially growing shoots (2 months after regeneration) following the method of Cammaerts and Jacobs (1980). Polyacrylamide gel electrophoresis (PAGE) was performed under non-denaturating conditions according to the method of Davis (1964) using 10% separating gel overlayed with 3% spacer gel. The slab gel system of Studier (1973) was used and gels of 18 × 16 × 0.2 cm size were prepared. The running time was 7 h at a constant voltage of 140 volt at 4 °C. Banded patterns of isozymes were detected on the gels after staining for esterases (Brewbaker et al. 1968).

Analysis of ribulose biphosphate carboxylase (RuBP Case)

Isoelectric focusing of RuBP Case was carried out according to the method of Cammaerts and Jacobs (1980), with broad range ampholine (pH 3.5–10) at a constant voltage of 500 volt for 12 × 12 × 0.2 cm gel. The running time was 30 h at 15 °C.

Results

Table 1 documents the results of protoplast fusion between hcp T-59/hcp B-85 and mp Dm. The plating efficiency ranged between 0.039% to 0.008%, the maximum occurring in self-fusion of hcp protoplasts and the minimum in fusion between hcp and non-irradiated mp Dm. The irradiated protoplasts showed to have partially restored the plating efficiency, which seemed to decline where intergeneric fusions were carried out. Plant regeneration capacity, however, was observed to have significantly improved in cases of intergeneric fusions where one of the partners (mp Dm) was irradiated. The hetero-

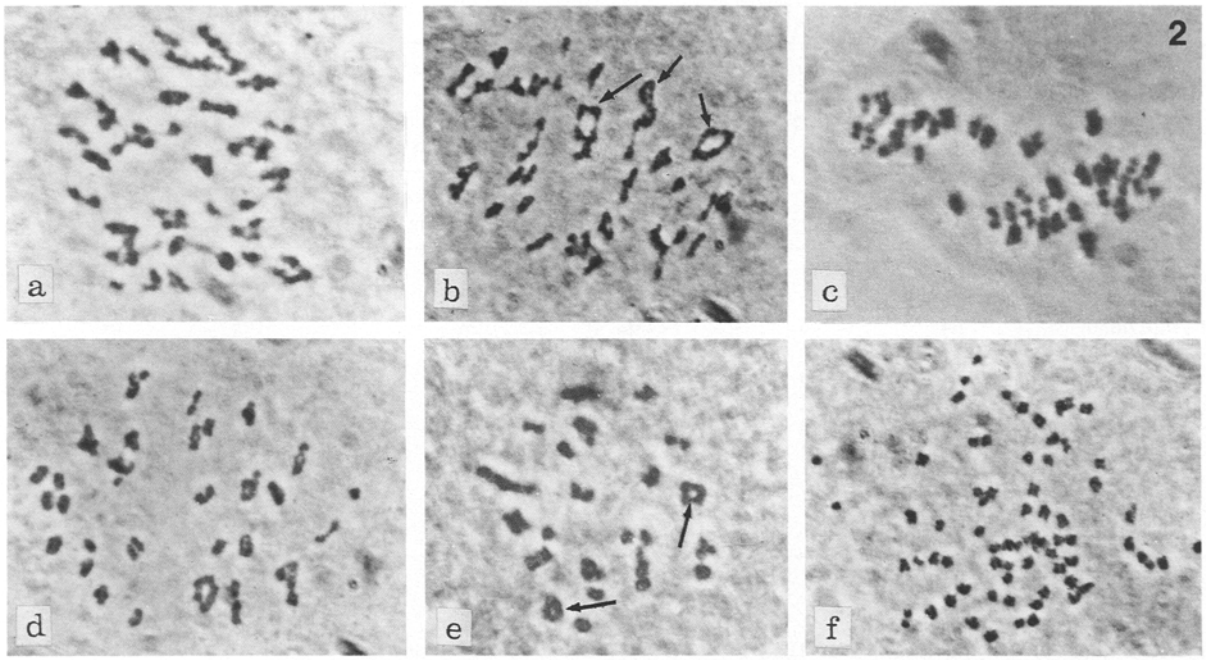

Fig. 2a–f. Light micrograms of **a** metaphase I of line No. BI/BjDm 2/82 showing 39 bivalents (2n = 78); **b** metaphase I of line No. BI/BjDm 1/56 showing three quadrivalents and 33 bivalents (2n = 78); **c** metaphase II (equatorial view) of line BI/BjDm 1/53 showing 39 chromosomes of one of the two daughter nuclei; **d** metaphase I of line BI/BjDm 2/137 showing 34 bivalents and one hexavalent (2n = 74); **e** metaphase I of line BI/BjDm 1/72 showing 31 bivalents and two quadrivalents (2n = 70); **f** metaphase II (polar view) of both the daughter nuclei together of line BI/BjDm 1/16 showing chromosomes (2n = 70). (Arrow indicates quadrivalent)

zygous state created by the protoplast fusion was not enough for improved morphogenic potentiality of the fused cells. On the other hand, irradiated protoplasts of *D. muralis* seemed to create a situation which encouraged morphogenic potentiality of the protoplast-derived calli.

It was possible to regenerate a total of 110 plants, of which 81 appeared to be morphologically different from the *B. juncea* parent. No regenerated plant resembled completely that of *D. muralis* parent. Alteration of morphological features like growth pattern, smooth stem, glabrous leaf, and flower characters were the common characteristics pronounced in these 81 plants. Pollen fertility ranged from complete fertility to very low fertility. To date, 26 such plants have been analysed (Table 2).

Chromosomal analysis (Fig. 2) revealed that both true somatic hybrids (Fig. 3) and partial somatic hybrids could be recovered. The true somatic hybrids at diakinesis revealed formation of three quadrivalents and 33 bivalents. That the two genomes have homology between at least three linkage groups could be established. In some cases, presence of one hexavalent could also be seen (Fig. 2d). Formation of hexavalent indicates that the homology contained in one chromosome in one genome is distributed in two chromosomes in the other genome. This may be the result of translocation in the course of evolution. Quadrivalent formation varied in cases of partial somatic hybrids between 1 and 3. However, in no case was univalent formation observed. Generation of all partial somatic hybrids occurred in cases when irradiated protoplasts of *D. muralis* was used. The chromosome number of the partial somatic hybrids ranged from 68–74. No plant appeared to contain more chromosomes than the summation of the diploid status of the two parents.

The esterase isozyme patterns of the somatic hybrids (Fig. 4) and partial somatic hybrids were looked into. The somatic hybrids had distinct characteristic patterns of both the genotypes. The esterase pattern of the various partial hybrid lines did not fully resemble the somatic hybrid characteristics, nor were they totally identical among themselves. Nevertheless, the patterns were different from any of the parents.

Isoelectric focusing pattern of SS of RuBP Case of the two plant types *B. juncea* and *D. muralis* have distinct patterns of their own. The somatic hybrids and in some cases the partial somatic hybrids revealed their hybridity in relation to the genus concerned (Fig. 5).

Since the isoelectric focusing characteristic of the large subunit of RuBP Case of *Brassica juncea* and *Diplotaxis muralis* is similar (Uchimiya and Wildman 1978), it was not possible to analyse the cytoplasmic characteristics of the regenerated plants. We are actively pursuing our efforts to throw light on this aspect by adopting suitable techniques for resolving this issue. However a

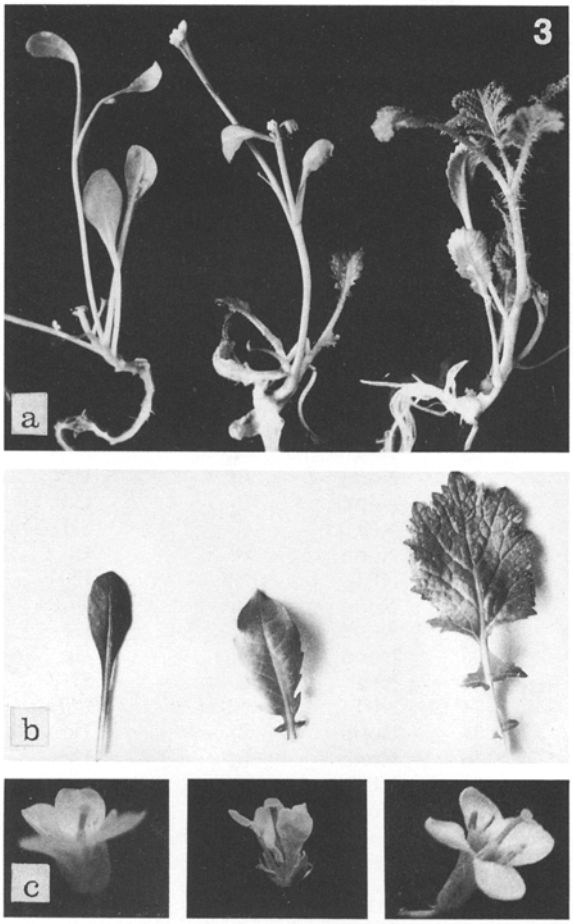

Fig. 3a–c. Morphological features of whole plants (**a**), leaves (**b**), and flowers (**c**), of *B. juncea* cv. T-59 (right), *Diplotaxis muralis* (left) and their somatic hybrid, BI/BjDm 1/56 (middle)

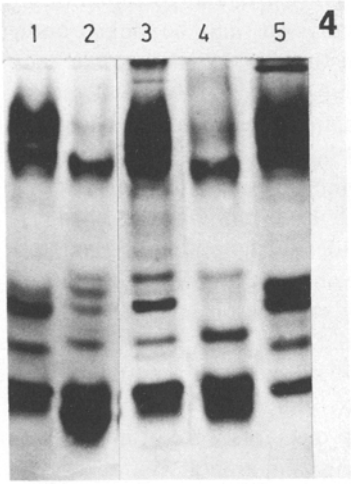

Fig. 4. Esterase isozyme pattern of 1. SH (BI/BjDm 1/56), 2. *B. juncea* cv. T-59, 3. SH (BI/BjDm 2/82), 4. *B. juncea* cv. B-85, and 5. *D. muralis*

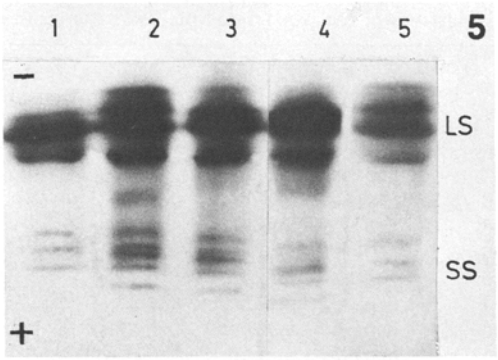

Fig. 5. Isoelectric focusing pattern of RuBPCase of *B. juncea*, *D. muralis* and their somatic hybrids (SH) 1. *B. juncea* cv. B-85, 2. SH (BI/BjDm 1/56), 3. SH (BI/BjDm 2/82), 4. *D. muralis*, and 5. *B. juncea* cv. T-59

number of plants, although they had nonparental morphological features, contained 36 chromosomes typical of *B. juncea*. These plants originated only in cases when irradiated protoplasts of *D. muralis* were used as one of the fusion parents. Nevertheless we could not establish that the variation was the result of fusion. Such plants were grouped for the present as unclassified, since the tests employed in the present study failed to explain the reasons for their nonparental morphological characteristics. The wide range of variations amongst these plants may not be the result of somaclonal variation as the non-irradiated protoplast fusion products did not show such variation. The genetical basis of their nonparental nature will be looked into in future.

Discussion

The potential of somatic hybridization of plants is well recognised (Cocking et al. 1981; Harms 1983), nevertheless there are at least two major problems which generally impede the development of this technique as regards application. Regeneration of plants from the fused products is not generally an easy proposition. After all, plant regeneration from protoplasts or from whole cells has not been achieved in many of the crop species (Flick et al. 1983; Vasil 1983). The second problem lies with the ability to identify the heterokaryons from the homokaryons and the unfused parental protoplasts. Hence, detection of the protoplast fusion products has commonly been based on expression of genetic markers or growth requirements. In absence of any clear marker characteristics, hybrids have also been selected using regeneration capability or hybrid vigour (Austin et al. 1985; Schieder 1982). In the present experimental design, the inability of *Diplotaxis* protoplasts at high plating density to regenerate, and their inability to develop into whole plants in a defined growth condition were turned to advantage.

Table 2. Characteristics of the regenerated plants as the result of protoplast fusion between *B. juncea* and *D. muralis*

Code no.	Nature of fusion experiments	Diakinetic configuration (predominantly)	2n	Esterase isozyme analysis	Analysis of SS of RuBP case	Nomenclature
BI/BjDm 2/5	hcpB-85+mpDm50	18_{II}	36	Norm	Bj	Uc
BI/BjDm 2/10	hcpB-85+mpDm50	18_{II}	36	Norm	Bj	Uc
BI/BjDm 1/16	hcpT-59+mpDm50	$2_{IV}+31_{II}$	70	NPT	NS	PSH
BI/BjDm 2/30	hcpB-85+mpDm50	18_{II}	36	Norm	Bj	Uc
BI/BjDm 2/46	hcpB-85+mpDm50	18_{II}	36	Norm	Bj	Uc
BI/BjDm 1/53	hcpT-59+mpDm	$3_{IV}+33_{II}$	78	NPT	C	SH
BI/BjDm 1/55	hcpT-59+mpDm50	18_{II}	36	Norm	Bj	Uc
BI/BjDm 1/56	hcpT-59+mpDm	$3_{IV}+33_{II}$	78	NPT	C	SH
BI/BjDm 2/61	hcpB-85+mpDm50	18_{II}	36	Norm	Bj	Uc
BI/BjDm 2/63	hcpB-85+mpDm50	$1_{IV}+32_{II}$	68	NPT	NS	PSH
BI/BjDm 1/66	hcpT-59+mpDm80	18_{II}	36	Norm	Bj	Uc
BI/BjDm 1/72	hcpT-59+mpDm50	$3_{IV}+29_{II}$	70	NPT	NS	PSH
BI/BjDm 2/77	hcpB-85+mpDm50	18_{II}	36	Norm	Bj	Uc
BI/BjDm 2/80	hcpB-85+mpDm50	18_{II}	36	Norm	Bj	Uc
BI/BjDm 2/82	hcpB-85+mpDm	$3_{IV}+33_{II}$	78	NPT	C	SH
BI/BjDm 1/95	hcpT-59+mpDm80	18_{II}	36	Norm	Bj	Uc
BI/BjDm 2/99	hcpB-85+mpDm50	$2_{IV}+31_{II}$	70	NPT	NS	PSH
BI/BjDm 1/106	hcpT-59+mpDm80	18_{II}	36	Norm	Bj	Uc
BI/BjDm 1/113	hcpT-59+mpDm80	18_{II}	36	Norm	Bj	Uc
BI/BjDm 1/122	hcpT-59+mpDm50	18_{II}	36	Norm	Bj	Uc
BI/BjDm 2/129	hcpB-85+mpDm50	$3_{IV}+33_{II}$	78	NPT	C	SH
BI/BjDm 2/137	hcpB-85+mpDm50	$2_{IV}+33_{II}$	74	NPT	NS	PSH
BI/BjDm 2/138	hcpB-85+mpDm80	18_{II}	36	Norm	Bj	Uc
BI/BjDm 2/145	hcpB-85+mpDm80	18_{II}	36	Norm	Bj	Uc
BI/BjDm 1/151	hcpT-59+mpDm50	$2_{IV}+32_{II}$	72	NPT	NS	PSH
BI/BjDm 1/155	hcpT-59+mpDm80	18_{II}	36	Norm	Bj	Uc

Norm – normal *B. juncea* type; NPT – nonparental type; NS – not studied; C – combined type; Bj – *B. juncea* type; Dm – *D. muralis* type; SH – somatic hybrid; PSH – partial somatic hybrid; Uc – unclassified ones

These, as well as the selection procedure based on the ability to distinguish the hybrids at the morphological level, bore certain similarities with earlier works (Adams and Quiros 1985; Kinsara et al. 1986). Likewise the type of protoplasts used and the methods followed in the present fusion experiments had similarities with the recent report of Terada et al. (1987).

Results of the present study indicate that the experimental strategies set out worked well. The somatic hybrids generated, the allotetraploids, appeared to be stable as revealed through their chromosomal status after a number of passages and also their performance in the outdoor condition in terms of fertility when backcrossed with either of the parents. They are likely to produce reconstructed genetic makeup (Kao 1977; Power et al. 1980) of *B. juncea* when strategical use of these plants is made in advanced generations (Leelavathi et al. 1987). Diakinetic configurations indicate at least three chromosomes may undergo crossing over, helping in the process of genetic reconstruction. The sexual cross between *B. oleracea* and *D. erucoides* formed four quadrivalents (Mizushima 1972). *B. oleracea* contains C genome which *B. juncea* is devoid of (U N 1935).

Thus the homology in *B. juncea* and *Diplotaxis* as seen in the present study indicates that *Diplotaxis* genome may contain genes common to the progenitor of the *Brassica* speciation and would be a good source of discovering primitive forms of genes, some of which may turn out to be useful. The partial somatic hybrids would additionally be a good source for production of genetic variabilities, essentially along the same line as that of the somatic hybrids. The present use of the γ-irradiation in producing partial somatic hybrids may be looked upon in the same manner as in the case of mammalian cells (Pontecorvo 1971). Destabilization of the genome results in elimination of chromosomes after fusion has been experienced (Dudits et al. 1980). However in the present case, elimination of chromosomes has not been extensive. The partial somatic hybrids contained 68 chromosomes at the lower end. Ideally the potential plant hybrids would have been the ones with complete genome of *B. juncea* and only a few chromosomes of *D. muralis*. This could not be achieved. Such plants might have been lost during the process of selection or they might not have been recognised as hybrids.

The unclassified group of plants were of interest although the nature of their nonparental characteristics could not be assigned to any causal reasons. The possibility for cytoplasmic reconstruction remained high, which could not be detected with our present method-

ology. The other possibility for creation of such variation may be through somatic transformation as observed earlier (Pandey 1980; Jinks et al. 1981) in somewhat similar situations.

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