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Production of asymmetric hybrids between *Arabidopsis thaliana* and *Brassica napus* utilizing an efficient protoplast culture system

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Abstract Application of the protoplast culture method developed for *Brassica* protoplasts to protoplasts of *Arabidopsis thaliana* has increased the opportunities for interspecific hybridizations involving *Arabidopsis*. A more-efficient and much-simpler method was established compared to the earlier-reported protocol developed for *A. thaliana* protoplasts in which alginate beads were utilized. Mesophyll protoplasts of *A. thaliana* (ecotypes 'Landsberg *erecta*' and 'Wassilewskija') were cultured in the modified 8p liquid medium, which had been developed for *Brassica* protoplasts. For comparison, protoplasts were cultured in sodium alginate beads supplied with B5 medium according to the protocol for *A. thaliana*. The protoplasts divided with high frequencies in the 8p medium, and calli proliferated more rapidly than in the sodium alginate beads. High frequencies of shoot differentiation and regeneration were observed in calli of both ecotypes, from about 30% in the ecotype 'Wassilewskija' to about 60% for 'Landsberg *erecta*'. The more-rapidly the calli developed, the higher the regeneration frequencies were. Asymmetric hybrids between *A. thaliana* and *Brassica napus* were obtained by treating the protoplasts of *A. thaliana* with iodoacetamide (IOA) and *B. napus* protoplasts with UV-irradiation before fusion with polyethylene glycol (PEG). By using the culture procedure developed for *Brassica* protoplasts, calli developed and plants were regenerated. Although most of the plants regenerated after cell fusion were *A. thaliana*-like and were judged to be escapes from IOA treatment, more than ten plants showed hybrid features of both morphological and molecular characters. Among the hybrids that have flowered so far, both male-fertile and male-sterile plants have been obtained. Backcrossings to *A. thaliana* are now in progress as is morphological and molecular characterization of the plants.

Keywords Asymmetric somatic hybrid · Protoplast culture · *Arabidopsis thaliana* · *Brassica napus*

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Introduction

New tools recently developed in genome research offer many possibilities to unravel the genetic composition, and exploit the genetic variation, stored in natural germplasms (Koornneef and Stam 2001). Wide crosses and interspecific hybridization, followed by consecutive backcrosses and analysis using linkage maps based on molecular markers, enable the identification, mapping and investigation of individual loci that control specific traits (Tanksley and Nelson 1996; Alfonso-Blanco and Koornneef 2000). Thus, the new paradigm in plant breeding is to look for the genes coding for certain traits rather than to look for the phenotype, which has been the traditional approach to screening a germplasm for a specific character with phenotypic expression (Tanksley and McCouch 1997).

One of the most-useful species for genetic analysis is *Arabidopsis thaliana*. The complete sequence of the nuclear, mitochondrial and chloroplast genomes has been published recently (The Arabidopsis Genome Initiative 2000; Unseld et al. 1997; Sato et al. 1999). Since *A. thaliana* is largely amenable to genetic analysis, it has been used in several interspecific hybridization experiments both via sexual hybridization (Nasrallah et al. 2000) and via somatic hybridization (Glimelius 1999). The potential to exploit exotic germplasms can be realized by somatic hybridization since sexual crossing barriers are bypassed. For instance, a useful application of somatic hybrids would be to combine *A. thaliana* as the receptor genome with a partial-donor genome, for example derived from a *Brassica* species, to facilitate the identification and isolation of important donor genes.

The first somatic hybrids, including the genome of *A. thaliana* combined with the *Brassica* genome, were obtained more than 20 years ago (Gleba and Hoffmann 1980). However, these somatic hybrids displayed morphological and cytological abnormalities and could not be established in soil. In order to obtain fertile and more-vigorous hybrids, asymmetric somatic hybridizations have been performed (Bauer-Weston et al. 1993; Siemens and Sacristán 1995; Forsberg et al. 1998a, b).

However, a problem of using *A. thaliana* in protoplast fusions has been the difficulty in regenerating plants from the calli. Several protocols for protoplast culture of *A. thaliana* have been established (Damm and Willmitzer 1988; Karesch et al. 1991; Masson and Paszkowski 1992), but all these protocols rely on rather-complicated culture procedures. The problems with cell- and tissue-culture procedures have been a clear drawback to the investigation of cell genetics and genetic engineering using *A. thaliana*.

In order to improve the possibility of using *A. thaliana* for interspecific hybridizations we have established an improved protoplast culture procedure based on the culture method developed for *Brassica* (Glimelius 1984; Glimelius et al. 1986). Significantly, we found that *A. thaliana* regenerates into plants with high frequency using our protocol. In addition, we also report the production of asymmetric hybrids between *A. thaliana* and *Brassica napus*, in which *A. thaliana* was used as the receptor genome and *B. napus* as the donor genome. Selection for asymmetric hybrids was accomplished by inhibiting growth of the parental materials by iodoacetamide (IOA)-treatment of *A. thaliana* and UV-treatment of *B. napus* protoplasts. The culture procedure and the characteristics of the hybrids are described here.

Materials and methods

Protoplast culture of *A. thaliana*

Ecotypes of *A. thaliana*, 'Landsberg *erecta*' transformed with the *bar* gene and the ecotype 'Wassilewskija', were used for protoplast isolation. The hypocotyls and leaves of 2-week-old seedlings grown aseptically in Petri dishes were cut into small pieces in CPW solution (Banks and Evans 1976), and treated with enzymes. Protoplast isolation and washing were performed according to Forsberg et al. (1994).

The density of protoplasts was adjusted to 2×10^5/ml, and they were cultured in modified 8p liquid medium with 0.4 M glucose according to Glimelius et al. (1986). The medium contained 1 mg/l of 2, 4-D, 0.5 mg/l of BAP and 0.1 mg/l of NAA. For the first 3 weeks, protoplasts were cultured in darkness, and then transferred to 16 h daylength at 25 °C. The cell suspensions were diluted with an equal volume of modified 8p medium without hormones every 7 to 10 days. Along with protoplast culture using the 8p medium, we also tested the protocol of Damm and Willmitzer (1988) with the modifications of Masson and Paszkowski (1992), which were exploited for the culture of *A. thaliana* protoplasts.

After 4 weeks, the culture suspension containing micro-calli was transferred to the K3 medium for callus development, which included 0.1 M sucrose and 0.15% agarose. The medium also contained 0.25 mg/l of 2, 4-D, 0.125 mg/l of BAP and 0.025 mg/l of NAA. After 3 weeks of culture calli larger than 2 mm in diameter were transferred to shoot-differentiation media. Calli smaller than 2 mm were subcultured to fresh medium of the same composition, and transferred 3 weeks later to shoot regeneration medium.

For shoot regeneration, three media were tested (B5, K3 and MS). All of them contained 0.5% sucrose, 0.4% agarose, 0.5 mg/l of BAP, 0.5 mg/l of zeatin and 0.1 mg/l of IAA. The number of calli with shoots was counted and the shoots were transplanted to MS medium containing 1% sucrose, but without hormones, for root induction. All of these cultures were at 25 °C.

Cell fusion between *A. thaliana* and *B. napus*

Protoplasts were isolated from leaves of *B. napus* cv 'Hanna', grown in vitro, (Glimelius 1984). The protoplasts were exposed to UV irradiation (Forsberg et al. 1998a) at a dose estimated to be 4,680 J/m^2. Protoplasts from the *A. thaliana* ecotype 'Landsberg *erecta*' with the *bar* gene were isolated by the procedure of Forsberg et al. (1994) and were treated with 2 mM of IOA (iodoacetoamide) for 15 min at room temperature.

After the pretreatments the protoplasts were fused with polyethylene glycol (PEG) according to Forsberg et al. (1994), and washed as described by Sundberg and Glimelius (1991) and cultured as described above. Plants were transplanted to soil and grown in a growth chamber.

Morphological and molecular characterization of regenerated plants after fusion treatment

The morphology of the regenerated plants in the cell-fusion experiment were judged with respect to hybrid characters. The plants which flowered in the growth chamber were self-pollinated, while those that did not develop pollen were crossed with *A. thaliana* as pollinator.

Total DNA was isolated from leaves of 35 regenerated shoots by the method of Dellaporta et al. (1983). Each shoot chosen was derived from a different callus and had developed well in vitro, thus providing more than 1 g of leaves which was required for the molecular analyses. PCR and Southern hybridization were applied to characterize the nuclear and organellar DNAs. The nuclear genome was probed with two species-specific probes of repetitive DNA sequences isolated from the parental species, as described by Forsberg et al. (1994). The chloroplast genome was determined by the use of PCR with primers selected from two chloroplast genes *trnL* and *trnF*. In order to study mitochondrial DNA constitution, two primer pairs specific to *apt1* and *orfB* were used for PCR analysis. The RFLP patterns of the *atp6* and *atp9* genes were also investigated by Southern hybridization.

Results

Plant regeneration from protoplasts of *A. thaliana*

The culture procedure developed for the protoplasts of *Brassica* species (Glimelius 1984; Glimelius et al. 1986), in which modified 8p medium was used for cell proliferation, was also efficient for the culture of *A. thaliana* protoplasts. In Fig. 1, growth of calli and shoot regeneration of the ecotype, 'Wassilewskija', 2 months after protoplast isolation, are compared with the results obtained when culturing protoplasts according to Damm and Willmitzer (1988) and Masson and Paszkowski (1992). Shoots had already regenerated from larger calli using the culture system developed for *Brassica*, while only small calli were formed in the other culture conditions. The protoplasts of 'Wassilewskija' proliferate much faster in the 8p medium than in the B5 medium used for the bead culture procedures.

Table 1 Effects on the regeneration frequency of shoots from calli derived from protoplasts of *A. thaliana* (ecotypes 'Wassilewskija' and 'Landsberg *erecta*') cultured in 8p medium

[a] –; calli larger than 2 mm were transferred to regeneration media 7 weeks after protoplast isolation. +; calli smaller than 2 mm were subcultured for another 3 weeks in fresh medium, and thereafter transferred to regeneration media

Ecotype	Subculture in callus media[a]	Regeneration medium	No. of calli (A)	No. of calli with shoots (B)	% (B/A)
Wassilewskija	–	B5	237	101	42.6
	–	K3	147	39	26.5
	+	B5	172	12	7.0
	+	K3	171	22	12.9
	+	MS	171	15	8.8
Landsberg *erecta*	–	B5	157	105	66.9
	–	K3	156	91	58.3
	+	B5	63	9	14.3
	+	K3	63	4	6.4
	+	MS	64	5	7.8

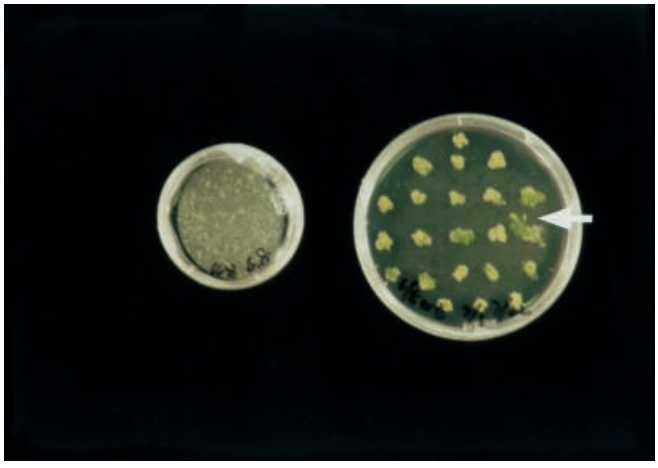

Fig. 1 Development of calli and shoots derived from protoplasts of *A. thaliana* (ecotype 'Wassilewskija') 2 months after isolation. Left: protoplasts cultured in sodium alginate beads supplied with B5 medium. Right: protoplasts cultured in the modified 8p medium and transferred to callus and regeneration media. The *arrow* shows a regenerated shoot

Using the protocol for *Brassica*, we compared the effects of K3 and B5 regeneration media on the frequency of shoot regeneration (Table 1). After 3 weeks of culture, calli which had grown to a size larger than 2 mm in diameter were transferred to regeneration medium. High frequencies of shoot regeneration were observed. In the ecotype 'Wassilewskija' the percentage of shoot regeneration was 43% and 27% depending on the basic medium used for regeneration. Calli of the ecotype 'Landsberg *erecta*' regenerated in even higher frequencies, 67% and 58%. In contrast, calli that were smaller than 2 mm and were sub-cultured for another 3 weeks in fresh callus culture medium, resulted in drastically lowered shoot regeneration frequencies of about 10% in both ecotypes when transferred to regeneration medium (Table 1). Although the regeneration medium affected regeneration frequency, with B5 being slightly better than K3, the differences were not as significant as the effect of time in callus culture, or rather the rate of cell proliferation during callus growth.

Table 2 Effects of iodoacetamide (IOA) on colony formation of *A. thaliana* protoplasts (ecotype 'Landsberg *erecta*')[a]

IOA concentration (mM)	No. of calli per Petri dish	Percent (%)
0	124	100.0
1	28	22.6
1.5	7.5	6.0
3	0	0

[a] Protoplasts were treated with IOA for 15 min at room temperature

Somatic hybridization between *A. thaliana* and *B. napus*, and morphology of the regenerants

In order to obtain asymmetric somatic hybrids, we performed IOA-treatment of *A. thaliana* and UV-irradiation of *B. napus* protoplasts. From preliminary experiments, we found that treatment with 1.5 mM of IOA for 15 min suppressed callus formation to less than 10% of the control and that 3 mM completely inhibited the division of protoplasts from 'Landsberg *erecta*' (Table 2). We applied 2 mM of IOA treatment before cell fusion. Protoplasts from the other parent were exposed to UV irradiation for 30 min, which corresponded to a dose of 4,680 J/m^2. This dosage effectively suppressed cell division of *B. napus* protoplasts (data not shown).

After the pretreatments, the protoplasts of the two species were fused and cultured according to the same procedure as described above. From 1,520 calli transferred to the shoot regeneration medium, 155 regenerated shoots. The shoots were transferred to MS medium: 109 rooted in vitro and were transplanted to soil. However, in total only 68 plants could be established in soil.

The regenerated shoots of *A. thaliana* bolted soon after regeneration in our culture condition because of the long daylength used (16 h). Therefore, it was easy to judge whether the regenerated plants expressed the characteristics of *A. thaliana* or *B. napus*. Among the 155 regenerated shoots, 138 possessed the characteristics of *A. thaliana*. This fact suggested that a large number of protoplasts from *A. thaliana* had escaped the selection pressure of IOA treatment, although the possibility that some of them are cybrids or partial hybrids between the two

Fig. 2 Leaf morphology of *A. thaliana* (left), somatic hybrid (No. 34) and *B. napus* (right)

Fig. 3 Flowers from *A. thaliana* (left), hybrids (No. 119, 34, 13 from left to right) and *B. napus* (right). *Arrows* indicate the abnormal anther development in the hybrids

parents cannot be outruled completely. The other 17 plants did not bolt in the regeneration and root-inducing media, and showed a morphology unique from both parents. One example of the leaf morphology is demonstrated in Fig. 2. Among 68 plants that were established in the culture chamber, 56 plants, all of which had the same flower size as *A. thaliana*, produced seeds after self-pollination, while the other 12 plants either did not flower or failed to set seeds. Three plants were obtained with a novel morphology and aberrant stamens without pollen (Fig. 3). Interestingly, the degree of abnormality correlated with the size of the petal; the smaller the petals were, the shorter the stamens were.

Molecular characteristics of the regenerants

Of 35 shoots which according to morphological investigations were *A. thaliana*-like (27) or displayed a novel morphology (8), and developed sufficient leaf tissue for DNA isolation using the method of Dellaporta et al. (1983), the nuclear and organellar genome constitutions were analysed by Southern hybridization and PCR analysis. From these analyses we found that 11 regenerants (31.4%), three of which having the morphology of *A. thaliana*, showed different characteristics from the two parental species for at least one marker (Table 3). The other 24 regenerants were judged to have the characteristics of *A. thaliana* from all the molecular markers adopted in this study.

The Southern hybridization of the repetitive nuclear DNA revealed that seven of the 11 regenerants possessed nuclear DNA from both parents showing that they contained hybrid nuclear genomes. Among the four remaining ones, all had the nuclear DNA of *A. thaliana*, and three showed mitochondrial DNA which differed from the parents. One had a novel morphology. The chloroplast DNA evaluated by PCR of the *trnL* and *trnF* genes was either *A. thaliana* or *B. napus* type (Table 3, Fig. 4). No hybrid pattern was detected. Mitochondrial DNA was

Table 3 Molecular and mophological characteristics of the regenerated plants showing hybrid features between *A. thaliana* and *B. napus*. Abbreviations: nd, not determined; BN, *B. napus*; A, *A. thaliana*; R, rearranged mt DNA; Novel, different morphology from the parents

Hybrid no.	Repetitive nuclear DNA	Mitochondrial DNA[a]				Chroloplast DNA	Morphology	Male fertility
		atp6	*atp9*	*atp1*	*orfB*			
1	BN+A	BN	BN	BN	BN	BN	Novel	nd
13	BN+A	BN	BN	BN	BN	BN	Novel	Sterile
34	BN+A	R	R	R	R	BN	Novel	Sterile
36	BN+A	nd	nd	BN	BN	BN	Novel	nd
37	BN+A	R	A	R	R	BN	Novel	nd
47	A	A	A	A	A	A	Novel	nd
57	BN+A	R	A	R	A	BN	Novel	nd
100	A	R	R	A	A	A	A	Fertile
119	BN+A	R	A	R	R	BN	Novel	Sterile
151	A	A	A	R	A	A	A	Sterile
155	A	A	A	R	A	A	A	Fertile

[a] *Atp6* and *atp9* were analysed by the Southern hybridization, while *atp1* and *orfB* were studied by PCR

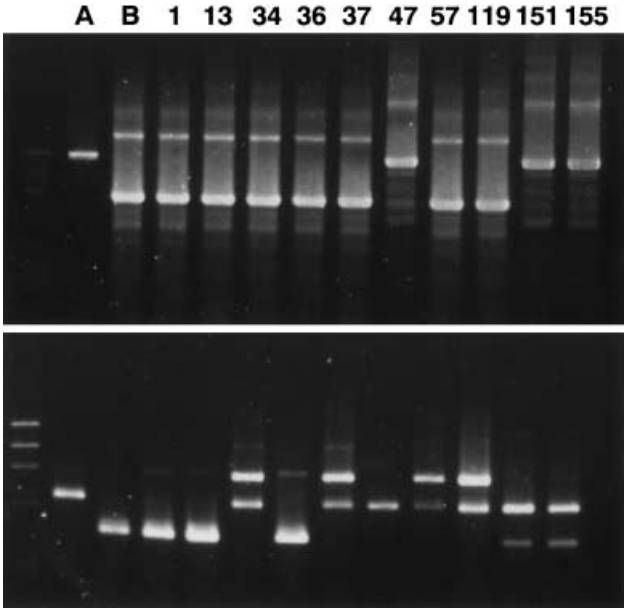

Fig. 4 PCR amplification patterns of chloroplast DNA (*trnL* and *trnF*: above), and mitochondrial DNA (*atp1*: below). *A*; *A. thaliana* (ecotype 'Landsberg *erecta*'); *B*, *B. napus* (cv 'Hanna'); 1–155, somatic hybrids (see Table 3)

analyzed by Southern hybridization and RFLP patterns of the *atp6* and *atp9* genes, or by PCR analysis of *atp1* and *orfB*. Seven regenerants exhibited a rearranged DNA pattern, three were identical to *B. napus* and one to *A. thaliana*, respectively (Table 3, Fig. 4). Of the three male-sterile plants, all of which had hybrid nuclear genomes and strongly suppressed anther development, two (No. 34 and No. 119) had rearranged mtDNA, and one (No.13) possessed the mtDNA of *B. napus* (Table 3).

Morphological observations and DNA analyses showed that most of the regenerants were derived from protoplasts of *A. thaliana* that had escaped the selection procedure *per se*. These regenerants indicate that the IOA concentration was not high enough to completely inactivate the protoplasts of *A. thaliana*, and clearly illustrate the efficiency of the culture system for *A. thaliana*. The results definitely showed that somatic hybrids were obtained between *A. thaliana* and the UV-irradiated *B. napus* even though in low frequency.

Discussion

In this study we report the production of asymmetric somatic hybrids between *A. thaliana* and *B. napus*. Attempts to produce symmetric somatic hybrids between the genomes of *Arabidopsis* and *Brassica*, "Arabidobrassica", as first reported by Gleba and Hoffmann (1980), have been followed up by several groups describing the production of asymmetric hybrids. Among these, Bauer-Weston et al. (1993) and Forsberg et al. (1998a, b) obtained hybrids between X-irradiated or UV-irradiated *A. thaliana* and non-treated *B. napus*. O'Neill and Mathias (1995) and Siemens and Sacristan (1995) made efforts to produce asymmetric hybrids combining the species in the reciprocal direction. They fused *A. thaliana* with UV-irradiated *B. napus* (O'Neill and Mathias 1995) or with X-irradiated *B. nigra* (Siemens and Sacristan 1995). Some asymmetric hybrids were obtained from these fusions. However, the *A. thaliana*-like hybrids of O'Neill and Mathias (1995) did not root, and those of Siemens and Sacristan (1995) resulted only in *B. nigra*-like hybrids. Thus, asymmetric hybrid plants containing fragments of the *Brassica* genome in a complete genome of *A. thaliana* have not been established until now. In this study hybrid features were demonstrated for more than ten plants, even though the degree of asymmetry has not been evaluated yet. Backcrossing the somatic hybrids with pollen of *A. thaliana* is in progress.

To obtain asymmetric hybrids we treated the protoplasts of *A. thaliana* with iodoacetamide (IOA) and irradiated *B. napus* protoplasts with UV before cell fusion. Even though we used a high concentration of IOA (2 mM), we obtained a large number of escapes. Terada et al. (1987) found that protoplasts treated with IOA of much higher concentration than required for inactivation can survive and regenerate after cell fusion treatment, which they considered to be due to 'nurse-effects' of non-treated protoplasts. It is not clear whether the 'nurse-effects' also worked in our investigation because the protoplasts of the other partner (*B. napus*) had been UV-irradiated, but it is clear that a higher concentration than 3 mM is necessary to inhibit the regeneration of *A. thaliana* after cell fusion. In contrast, UV irradiation of 4,680 J/m^2, which is the highest dose that Forsberg et al. (1998a) used for *A. thaliana*, was effective in inactivating divisions of the protoplasts of *B. napus*, since no regenerated plants of *B. napus* were obtained after cell fusion.

This study clearly shows that the modified 8p medium developed for protoplast culture of *Brassica* species (Glimelius 1984) is also effective for *A. thaliana*. After the report on protoplast isolation and culture protocol by Damm and Willmitzer (1988), several modifications have been described. For example, Masson and Paszkowski (1992) tested the growth conditions of donor plants, and O'Neill and Mathias (1993) induced direct embryogenesis in lieu of callus development. However, these reports basically used the same method as Damm and Willmitzer (1988); namely, they embedded the protoplasts into beads formed by sodium alginate. This protocol requires several additional steps which are unnecessary in the 8p system. In the modified 8p medium callus proliferation occurred earlier than with the sodium alginate beads supplied with B5 medium. Moreover, regeneration frequency from the calli was equally as high as those obtained in the experiments using the protocol of Damm and Willmitzer (1988) and Karesch et al. (1991). Our finding thus leads to a simplified method for the culture of *A. thaliana* protoplasts, beneficial not only for the development of somatic hybrids between *A. thaliana* and other species, but also for the production of

transgenic *A. thaliana* using the protoplasts as targets for DNA uptake via electroporation or PEG treatment.

Of clear interest and importance for further study is the production of plants showing male-sterile phenotypes. As reported in this study three plants were male-sterile. They displayed hybrid morphology and had either a recombined mitochondrial DNA pattern or *B. napus* mtDNA. By recurrent backcrosses to *A. thaliana* it should be possible to obtain alloplasmic plants of *A. thaliana*, displaying a novel cytoplasm. These plants will most probably express male sterility and aberrant flower modifications due to the alloplasmic constitution of the nuclear genomes of *A. thaliana* and the mt-DNA of *B. napus*. Thus, they constitute excellent materials for further molecular studies of CMS-inducing genes and genes causing aberrations of the flower morphology, especially when considering the fact that both the nuclear and mitochondrial DNA of *A. thaliana* are sequenced. Furthermore, the male-sterile cybrids represent the reciprocal combination of the *Arabidopsis* and *Brassica* nuclear and mitochondrial genomes, to that of our earlier studies, in which we obtained combination of the nuclear genome of *B. napus* with the mitochondrial genome of *A. thaliana* (Forsberg et al. 1998a, b). In this combination, alloplasmic CMS lines were also obtained, which expressed phenotypic modifications of the floral organs similar to those of the asymmetric hybrids produced in this study.

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References

Alfonso-Blanco C, Koornneef M (2000) Naturally occurring variation in *Arabidopsis*: an underexploited resource for plant genetics. Trends Plant Sci 5:22–29

Banks MS, Evans PK (1976) A comparison of the isolation and culture of mesophyll protoplasts from several *Nicotiana* species and their hybrids. Plant Sci Lett 7:409–416

Bauer-Weston B, Keller W, Webb J, Gleddie S (1993) Production and characterization of asymmetric somatic hybrids between *Arabidopsis thaliana* and *Brassica napus*. Theor Appl Genet 86:150–158

Damm B, Willmitzer L (1988) Regeneration of fertile plants from protoplasts of different *Arabidopsis thaliana* genotypes. Mol Gen Genet 213:15–20

Dellaporta SJ, Wood J, Hicks JB (1983) A plant DNA mini-preparation: version II. Plant Mol Biol Rep 1:19–21

Forsberg J, Landgren M, Glimelius K (1994) Fertile somatic hybrids between *Brassica napus* and *Arabidopsis thaliana*. Plant Sci 95:213–223

Forsberg J, Dixelius C, Lagercrantz U, Glimelius K (1998a) UV-dose dependent DNA elimination in asymmetric somatic hybrids between *Brassica napus* and *Arabidopsis thaliana*. Plant Sci 137:65–76

Forsberg J, Lagercrantz U, Glimelius K (1998b) Comparison of UV-light, X-ray and restriction enzyme treatment as tools in production of asymmetric somatic hybrids between *Brassica napus* and *Arabidopsis thaliana*. Theor Appl Genet 96:1178–1185

Gleba YY, Hoffmann F (1980) "Arabidobrassica": a novel plant obtained by protoplast fusion. Planta 149:112–117

Glimelius K (1984) High growth rate and regeneration capacity of hypocotyl protoplasts in some Brassicaceae. Physiol Plant 61:38–44

Glimelius K (1999) Somatic hybridization. In: C. Gómez-Campo (ed) Biology of *Brassica* coenospecies. Elsevier Science B.V. Amsterdam, The Netherlands, pp 107–148

Glimelius K, Djupsjöbacka M, Fellner-Feldegg H (1986) Selection and enrichment of plant protoplast heterokaryons of *Brassicaceae* by flow sorting. Plant Sci 45:133–141

Karesch H, Bilang R, Potrykus I (1991) *Arabidopsis thaliana*: protocol for plant regeneration from protoplasts. Plant Cell Rep 9:575–578

Koornneef M, Stam P (2001) Changing pardigms in plant breeding. Plant Physiol 125:156–159

Masson J, Paszkowski J (1992) The culture response of *Arabidopsis thaliana* protoplasts is determined by the growth conditions of donor plants. Plant J 2:829–833

Nasrallah M, Yogeeswaran K, Snyde S, Nasrallah JB (2000) *Arabidopsis* species hybrids in the study of species differences and evolution of amphiploidy in plants. Plant Physiol 124:1605–1614

O'Neill CM, Mathias RJ (1993) Regeneration of plants from protoplasts of *Arabidopsis thaliana* L. cv Columbia (C 24), via direct enbryogenesis. J Exp Bot 44:1579–1585

O'Neill CM, Mathias RJ (1995) Somatic fusions for the transfer of agronomically important traits into *Arabidopsis thaliana* from *Brassica* spp. Proc 9th Int Rapeseed Cong:1110–1112

Sato S, Nakamura Y, Kaneko T, Asamizu E, Tabata S (1999) Complete structure of the chloroplast genome of *Arabidopsis thaliana*. DNA Res 6:283–290

Siemens J, Sacristán MD (1995) Production and characterization of somatic hybrids between *Arabidopsis thaliana* and *Brassica napus*. Plant Sci 111:95–106

Sundberg E, Glimelius K (1991) Effects of parental ploidy level and genetic divergence on chromosome elimination and chloroplast segregation in somatic hybrids within *Brassicaceae*. Theor Appl Genet 83:81–88

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplams into elite breeding lines. Theor Appl Genet 92:191–203

Terada R, Yamashita Y, Nishibayashi S, Shimamoto K (1987) Somatic hybrids between *Brassica oleracea* and *B. campestris*: selection by the use of iodoacetamide inactivation and regeneration capacity. Theor Appl Genet 73:379–384

The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796–815

Unseld M, Marienfeld JR, Brandt P, Brennicke A (1997) The mitochondrial genome of *Arabidopsis thaliana* contains 57 genes in 366,924 nucleotides. Nature Genet 15:57–61