Anther-culture-derived 2x-Plants in Cabbage (*Brassica oleracea* L. ssp. *capitata* L.)

I. Meiotic abnormalities

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Accepted October 14, 1988

The most efficient way of producing haploid plants is through anther or pollen culture. One of the objectives of producing microspore derived haploid plants is to generate homozygous lines by their subsequent diploidization.

In *Brassica oleracea* vegetable group, haploid, diploid and polyploid plants of broccoli (*B. oleracea* L. var. *italica* Plenck) (Keller and Armstrong 1983), Brussel sprouts (*B. oleracea* L. var. *gemmifera* DC) (Ockendon 1984) and cabbage (*B. oleracea* L. ssp. *capitata* L.) (Chiang *et al.* 1985) were obtained from anther culture. Diploid plants recovered from this technique originated from haploid microspores and therefore were homozygous. However, a large number of diploid plants derived from anther culture were male sterile (unpublished data).

Mix *et al.* (1978) reported abnormal root tip mitosis and chromosome breakage in anther-culture-derived barley plants. Mercy and Zapata (1986) observed laggards and anaphase bridges in meiosis in diploid rice plants recovered from anther culture.

This paper reports the occurrence of various meiotic chromosomal abnormalities in diploid (2x) cabbage plants derived from anther culture.

Materials and methods

Twenty-nine 2x-plants derived from anther culture (Chiang *et al.* 1985) were used for this study. All plants were recovered from the anther donor plant, B-21, which was selected for its low content of glucosinolates and resistance to races 2 and 6 of the clubroot pathogen, *Plasmodiophora brassicae* Wor. (Chong *et al.* 1985). B-21 was originally derived from the interspecific cross between *B. napus* L. and cabbage. The dominant genes for resistance to clubroot pathogen were transferred from *B. napus* L. (Chiang *et al.* 1977). B-21 was vegetatively maintained by cuttings.

The anther-culture-derived plants were vernalized at 1°C for 12 weeks (12-13- leaf stage) then kept in a greenhouse from November to March under the following conditions: temperature, 20±2°C; photoperiod, 16h/8h; light intensity, 125 μ Em⁻²s⁻¹ at 15 cm above the plants. A 20-20-20 fertilizer solution was applied once weekly.

Flower buds for meiotic study were fixed in 3:1 v/v 95% ethanol: ferric acetate propionic acid solution. Slides were prepared by the standard acetocarmine squash method, and photomicrographs were taken with a Zeiss photomicroscope III. Chi-square test was used to compare the percentage of pollen mother cells (PMCs) with meiotic abnormalities between anther-culture-derived plants and anther donor (control) plants, using data of control plants as the expected value.

Results

Among the 29 2x-plants, 16 shed varying quantities of pollen and are herein referred to as anther-culture-derived male fertile (ACMF) plants. The other 13 plants had dried anthers, did not shed pollen, and are herein referred to as anther-culture-derived male sterile (ACMS) plants.

First meiotic division

Diakinesis-Metaphase I (M-I)

Besides the regular 9 bivalents (9II), univalents (Figs. 1 and 2), trivalents (Fig. 2) and quadrivalents (Figs. 1 and 3) were frequently observed. Mean percentages of PMCs showing 9II, stickiness and other meiotic abnormalities among ACMF, ACMS and control plant groups are presented in Table 1.

There was no difference in percentage of PMCs forming 9II (Fig. 4) between both ACMF, ACMS derived and control plants (Table 1). Corresponding data for chromosome stickiness (Fig. 5) were relatively high but also showed no difference between derived and control plants (Table 1). However, within ACMS group, the percentage of cells with other abnormalities, including univalents, lack of orientation (Fig. 6) and multivalents, was significantly higher (7.8%) than that of the control group (0.3%); there was no difference between the ACMF (0.5%) and the control group (Table 1).

Table 1. Mean percentage of PMCs with 9 bivalents and meiotic abnormalities during diakinesis and metaphase I in anther-culture-derived 2x-plants and anther donor plants of cabbage

Plant group ¹	9 bivalents		Stickiness		Other abnormalities ²	
	Mean±S.E.	χ^2 -value	Mean±S.E.	χ^2 -value	Mean±S.E.	χ^2 -value
ACMF	67.8 7.83	0.268	31.7 7.88	0.641	0.5 0.27	1.333
Control	72.2 2.58		27.5 2.48		0.3 0.19	
ACMS	62.4 8.81	1.330	29.8 8.70	0.192	7.8 5.70	187.500**

¹ ACMF: anther-culture-derived male fertile plants; Control: B-21, donor plants; ACMS: anther-culture-derived male sterile plants.

² Include cells with univalent, trivalent, quadrivalent and unoriented chromosomes.

** Significant at 1% level.

Anaphase I (A-I)–Telophase I (T-I)

While no anaphase bridge was observed in the control group, both ACMF and ACMS groups showed them with fragment (Fig. 7) or without fragment (Fig. 8). As indicated by significantly higher occurrence of laggards in both ACMF (11.6%) and ACMS (29.6%) groups in comparison to the control (1.0%) (Table 2), chromosome laggards (Figs. 10 and 11) and chromosome fragmental laggards (Fig. 9) were observed frequently. Similarly, abnormalities including 8–10 chromosome segregation (Fig. 12) occurred more frequently in the anther-culture-derived groups (ACMF, 5.2%; ACMS, 4.2%) in comparison with the control group (0.6%). The occurrence of normal 9–9 chromosome separation at T-I was significantly lower in the ACMS group (61.5%) than in the control group (92.3%); there was no corresponding difference between ACMF (78.6%) and the control (Table 2).

Second meiotic division

Persistent bridges were observed in plants of both ACMF and ACMS groups (Figs. 13 and 14) but not in those of the control. Laggards (Figs. 15 and 16) in A-II–T-II were observed

Figs. 1-12. First meiotic division of PMCs of anther-culture-derived 2x-plants except Fig. 4 which was from the anther donor plants B-21. Figs. 1-3. Diakinesis. 1, 1 IV (large arrow) + 6II + 2I (small arrows). 2, 2III + 5II + 2I. 3, 1 IV + 7II. Figs. 4-5. Metaphase I. 4, complete pairing of 18 chromosomes to form 9II. 5, two cells with sticky chromosomes. Fig. 6. Complete failure of homologous pairing. Figs. 7-11. Anaphase I. 7, anaphase bridge with freagment (small arrow). 8, anaphase bridge without fragment. 9, fragmented laggards. 10, two cells with laggards. 11, unequal chromosome distribution and laggards. Fig. 12. 8-10 chromosome distribution at T-I.

Table 2. Mean percentage of PMCs with meiotic abnormalities during A-I and T-I in anther-culture-derived 2x-plants and anther donor plants of cabbage

Plant ¹ group	Stickiness		Laggards		Bridges		Other abnormalities ²		9-9 segregation	
	Mean ± S.E.	χ^2 -value	Mean ± S.E.	χ^2 -value	Mean ± S.E.	χ^2 -value	Mean ± S.E.	χ^2 -value	Mean ± S.E.	χ^2 -value
ACMF	4.0 2.80	0.723	11.6 4.13	112.36**	0.5 0.28	—	5.2 2.51	35.266**	78.6 7.03	2 033
Control	6.1 0.26		1.0 0.41		0.0 0.0		0.6 0.36		92.3 0.78	
ACMS	3.3 2.27	1.285	29.6 7.41	817.96**	1.5 1.44	—	4.2 1.32	21.600**	61.5 8.01	10.278**

¹ See footnote under Table 1.² Include cells with unequal chromosome segregation.

** Significant at 1% level.

Table 3. Mean percentage of PMCs with meiotic abnormalities during M-II to T-II in anther-culture-derived 2x-plants and anther donor plants of cabbage

Plant ¹ group	Laggards		Bridges		Micronuclei		Other abnormalities ²		9-9-9-9	
	Mean ± S.E.	χ^2 -value	Mean ± S.E.	χ^2 -value	Mean ± S.E.	χ^2 -value	Mean ± S.E.	χ^2 -value	Mean ± S.E.	χ^2 -value
ACMF	5.7 2.15	0.519	0.3 0.25	—	4.7 2.77	11.782**	8.5 1.35	19.500**	80.8 7.09	0.773
Control	7.7 1.98		0.0 0.00		1.1 0.45		2.1 0.89		89.1 2.82	
ACMS	16.6 7.40	10.287**	0.2 0.24	—	22.3 7.59	408.582**	13.3 5.60	59.733**	47.6 10.43	19.329**

¹ See footnote under Table 1.² Include triploes and unequal chromosome segregation.

** Significant at 1% level.

in anther-culture-derived as well as in control plants, but significant differences were found only between ACMS (16.6%) and the control (7.7%) (Table 3). Other abnormalities, including triploles (Fig. 17), unequal chromosome distribution (Fig. 18) and sporads with dyads (Fig. 20) or triads (Fig. 21), were frequently observed in ACMF and ACMS groups. The percentages of PMCs with these abnormalities (ACMF, 8.5%; ACMS, 13.3%) were significantly higher

Figs. 13-21. Meiotic abnormalities of second meiotic division of PMCs of anther-culture-derived 2x-plants of cabbage. 13-14, A-II bridges and laggards. 15, A-II showing many lagging chromosomes. 16, T-II laggards. 17, triploles with unequal distribution of chromosomes. 18-19, T-II showing micronuclei formed in PMCs. Figs. 20-21. Abnormal sporads. 20, a dyad sporad. 21, a triad sporad.

than the control (2.1%) (Table 3). Cells forming micronuclei (Fig. 19) were common in the anther-culture-derived groups with occurrences (ACMF, 4.7%; ACMS, 22.3%) significantly higher than in the control group (1.1%). As a result of high incidence of A-II bridges, laggards, micronuclei and unequal chromosome segregations, the percentage of cells with 9-9-9-9 equal segregation at T-II were lower in both ACMF (80.8%) and ACMS (47.6%) groups in com-

parison with the control group (89.1%). However, Chi-square test indicated that only the ACMS group was significantly lower than the control (Table 3).

Discussion

The 2x-plants examined in this study were divided into 2 groups based on pollen shedding. Thirteen plants in the ACMS group did not shed pollen grains therefore produced no seed. Of the 16 plants in the ACMF group which were self pollinated, 2 produced no seed, 2 produced only 1 seed each and the remaining 12 produced seeds ranging from 6–172 with 6 of these plants producing 25 or more seeds (pollen and seed fertilities will be reported in a separate paper). Related investigations showed that selfed progenies within each “family” of these 6 plants segregated with regards to clubroot race 2 resistance and plant morphology varied slightly among the individuals within a “family”, indicating that these 6 plants of the ACMF group were heterozygous.

Keller and Armstrong (1983) reported that diploid plants recovered from anther culture of *Brassica* species originated from haploid microspores and therefore were homozygous. This indicated that 17 plants derived from anther culture in the present study (ACMS group, 13 plants; ACMF group, 2 plants producing no seed and 2 plants each producing 1 seed by selfing) may be homozygous.

Cabbage belongs to the cruciferous family, a naturally cross pollinated species, and will eventually suffer from inbreeding depression upon repeated self pollination. One of the phenomena of inbreeding depression is the reduction of fertility as reported by Currah and Ockendon (1987) in haploids of Brussel sprouts. The other consequence of inbreeding is the disturbance of meiotic chromosomal behavior. Dayal (1979) and Dayal and Singh (1985) reported that anaphase bridges, laggards, chromosome stickiness and desynapsis were found in inbred families of garden cress (*Lepidium sativum* L.) and radish (*Raphanus sativus* L. var. *radicola* Pers.). In rice (*Oryza sativa* L.), Mercy and Zapata (1986) found that 2.5% anther-culture-derived 2x-plants showed meiotic abnormalities which reduced pollen and spikelet fertilities by 69% and 80%, respectively.

In the present study, the anther donor plant B-21 and its cuttings had some disturbance in meiosis. However, these disturbances occurred mostly in anther-culture-derived plants with particularly high incidences in the ACMS group. The occurrence of zero fertility in ACMS group and low fertility in 4 plants of the ACMF group could be the result of 100% homozygosity induced by anther culture.

Abnormal meiosis observed in B-21 plants must be related to the nature of the origin of the interspecific hybrid between *B. napus* L. and cabbage. Since B-21 plants produced normal cabbage heads, it is assumed that the 9 pairs of cabbage chromosome of the *c* genome were recovered, except that one chromosome, either No. 1 or No. 7, had a small section which was transferred from *B. napus* L. carrying the gene responsible for clubroot resistance (Chiang *et al.* 1980). This small section of foreign chromosome translocated from *B. napus* L. could cause some genetic unbalance resulting in meiotic disturbance under heterozygous conditions. It will also cause a high incidence of disturbance in meiosis resulting in extremely low fertility under homozygous conditions.

Summary

Of 29 anther-culture-derived 2x-plants of cabbage (*Brassica oleracea* L. ssp. *capitata* L.), 45% were male sterile (ACMS) and 55% male fertile (ACMF). Chi-square test was used to compare the percentage of pollen mother cells (PMCs) with meiotic abnormalities in ACMF

or ACMS plants with those in anther donor (control) plants. In metaphase I, univalents, trivalents and quadrivalents were frequently observed but only in the ACMS group were these abnormalities significantly higher than in the control. In anaphase I to telophase I, there were significantly higher occurrences of laggards and other abnormalities including unequal chromosome segregation in both ACMF and ACMS groups; there were 2.6 time more laggards in the ACMS group. In anaphase II to telophase II, micronuclei, tripoles, unequal chromosome distribution and sporads with dyads and triads were frequently observed in both ACMF and ACMS but, in all cases, occurrences were markedly higher in the ACMS group accompanied by significantly lower 9-9-9 equal chromosome segregation. High incidence of sterility and chromosomal abnormalities in anther-culture-derived 2x-plants, particularly in the ACMS group, appears to be the result of homozygosity induced by anther culture.

References

- Chiang, M. S., Chiang, B. Y. and Grant, W. F. 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**: 219-336.
- Chiang, B. Y., Chiang, M. S., Grant, W. F. and Crête, R. 1980. Transfer of resistance to race 2 of *Plasmodiophora brassicae* from *Brassica napus* to cabbage (*B. oleracea* ssp. *capitata*) IV. A resistant 18-chromosome B_1 plant and its B_2 progenies. *Euphytica* **29**: 47-55.
- Chiang, M. S., Fréchette, S., Kuo, C. G., Chong, C. and Delafield, S. J. 1985. Embryogenesis and haploid plant production from anther culture of cabbage (*Brassica oleracea* var. *capitata* L.). *Can. J. Plant Sci.* **65**: 1033-1037.
- Chong, C., Chiang, M. S. and Crête, R. 1985. Studies on glucosinolates in clubroot resistant selections and susceptible commercial cultivars of cabbages. *Euphytica* **34**: 65-73.
- Currah, L. and Ockendon, D. J. 1987. Chromosome doubling of mature haploid Brussels sprout plants by colchicine treatment. *Euphytica* **36**: 167-173.
- Dayal, N. 1979. Cytogenetical studies in the inbred lines of radish (*Raphanus sativus* L. var. *radicola* Pers.) and their hybrids III. Meiotic abnormalities. *Cytologia* **44**: 1-5.
- and Singh, S. N. 1985. Inbreeding depression in chromosome behaviour of the garden cress (*Lepidium sativum* L.). *Cytologia* **50**: 289-300.
- Keller, W. A. and Armstrong, K. C. 1983. Production of haploid via anther culture in *Brassica oleracea* var. *italica*. *Euphytica* **32**: 151-159.
- Mercy, S. T. and Zapata, F. J. 1986. Chromosomal behavior of anther culture derived plants of rice. *Plant Cell Reports* **3**: 215-218.
- Mix, G., Wilson, H. M. and Foroughi-Wehr, B. 1978. The cytological status of plants of *Hordeum vulgare* L. regenerated from microspore callus. *Z. Pflanzenzüchtg.* **80**: 89-99.
- Ockenden, D. J. 1984. Anther culture in Brussels sprouts (*Brassica oleracea* var. *gemmeifera*) 1. Embryo yields and plant regeneration. *Ann. Appl. Biol.* **105**: 285-291.
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