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Inheritance of microspore embryogenic ability in *Brassica* crops

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Abstract Inheritance of microspore embryogenic ability in oilseed rape (*Brassica napus* L.) and Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*) was examined by 4 × 4 diallel crosses using cultivars showing a different response. In both species, embryo yields of most F_1 hybrids were similar to, or over, the high responsive parent and some F_1s showed intermediate embryo yields between their parents. Diallel analysis showed that both additive and dominant effects were significant at the 1% level for the genetic control of microspore embryogenic ability in both species. Dominant genes had positive effects on microspore embryogenesis. In oilseed rape, the additive effects were important, while in Chinese cabbage the dominant effects were largely contributed. The broad- and narrow-sense heritabilities were 0.972 and 0.811 in oilseed rape, and 0.959 and 0.659 in Chinese cabbage, respectively. From the results of the segregation of embryo yields in the F_2 population of 'Lisandra' × 'Kamikita', it is considered that the microspore embryogenic ability is controlled by two loci with additive effects in oilseed rape.

Keywords Diallel analysis · Heritability · Microspore embryogenesis · Oilseed rape (*Brassica napus* L.) · Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*)

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Introduction

Microspore-culture technique is well known for its potential applications in plant genetic research and breeding. In the genus *Brassica*, since the successful isolated microspore culture of *Brassica napus* was reported by Lichter (1982), many studies have been carried out to improve and to utilize this technique, such as the identification of factors influencing embryogenesis, the characterization of the embryogenic process and the utilization of this system in plant breeding (reviewed by Takahata 1997). However, as described in many other tissue-culture works, success in the microspore embryogenesis of *Brassica* crops is genotype-dependent and a large genotypic variation in embryo yields was reported (Chuong and Beversdorf 1985; Ohkawa et al. 1987; Cao et al. 1993; Guo and Pulli 1996; Kuginuki et al. 1997). As an approach to the use of this technique for breeding programs in a wide extent of varieties, the transfer of high microspore-culture responsiveness to low or no responsive genotypes having important agronomic value is indispensable. However, the genetic factors controlling microspore embryogenic ability are not clear.

Genotypic variation in embryogenesis and regeneration ability was observed in many crops, and genetic factors controlling the ability for tissue culture have been reported in several crops. The inheritance of callus-formation ability in anther culture of rice was reported by several workers (Miah et al. 1985; Quimio and Zapata 1990; Imuta et al. 1991). The regeneration capacity of recalcitrant lines was reported to be improved by crossing with high responsive cultivars in potato and maize (Wenzel and Uhring 1981; Beckert and Qing 1984; Hodges et al. 1986; Armstrong et al. 1992). In *Brassica* crops, little work has been reported on the inheritance of tissue-culture ability, except for shoot regeneration from the cotyledonary expant of oilseed rape (*B. napus*) (Ono and Takahata 2000).

In the present study, genetic factors involved in microspore embryogenic ability were examined by diallel analysis in oilseed rape and Chinese cabbage.

Materials and methods

Plant material

Diallel crosses were made among four cultivars of 'Topas', 'Lisandra', 'Kizakino' and 'Kamikita', in oilseed rape (*B. napus* L.) and among four,'Ho Mei', 'Hisfu early 30 days', '269' and '181', in Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*). The parent cultivars were chosen on the basis of previous work in which the four cultivars showed a different ability for microspore embryogenesis. 'Topas', 'Lisandra', 'Ho Mei' and 'Hisfu early 30 days' are high responsive cultivars (Ohkawa et al. 1987; Kuginuki et al. 1997), while 'Kizakino', 'Kamikita', '269' and '181' are low- or non-responsive ones (Takahata; Zhang, unpublished data). '269' and '181' were self-incompatible pure lines bred by the Beijing Vegetable Research Center. 'Ho Mei' and 'Hisfu early 30 days' were provided from the National Research Institute of Vegetable Ornamental Plants and Tea, and selfed for several generations. The F_2 seeds of 'Lisandra' × 'Kamikita' were also produced by selfing.

All plants of parents, F_1s and the F_2 were grown in an uncontrolled-environment glasshouse. Plants were fertilized with 5-10-5 Hyponex weekly after bolting.

Microspore culture

Microspore culture was carried out as previously described by Wakui et al. (1994) and Takahata (1997). Two–three mm-length flower buds of Chinese cabbage and 3–4 mm ones of oilseed rape were collected and surface-sterilized in sodium hypochlorite (2% active chlorite) for 15 min and then rinsed three times with sterile distilled water. After the buds were macerated in a mortar containing B5 medium (Gamborg et al. 1968) supplemented with 13% sucrose at pH 6.0 (B5–13), microspores were obtained by filtration through Miracloth and then washed three times with B5–13 medium by centrifuging at 1,000 rpm for 3 min. The microspores were suspended at a density of 1×10^5/ml in 1/2 NLN-10 medium for Chinese cabbage (Wakui et al. 1994) and 5×10^4/ml in 1/2 NLN-13 medium for oilseed rape (Takahata 1997). Two milliliters of the microspore suspension were cultured in a 60×15-mm plastic Petri dish. The Petri dishes were incubated at 32.5°C for 1 day in Chinese cabbage and for 4 days in oilseed rape prior to maintenance at 25°C in darkness. After 3 weeks of culture, the embryo yields were examined.

Experimental design and data-analysis for diallel analysis

Three plants of each parent or F_1s and 80 F_2 plants of 'Lisandra' × 'Kamikita' were used for microspore culture, and four dishes per plant were cultured. For statistical analysis, direct and root-transformed data were used. Diallel analyses were performed according to the method of Hayman (1954a, b) and Griffing (1956) using the computer program ''DIALL'' developed by Ukai (1989). Broad- and narrow-sense heritabilities were calculated after Mather and Jinks (1971).

Results

Diallel analysis for microspore embryogenic ability

The number of embryos produced in microspore culture of the four parents and their F_1s for oilseed rape and Chinese cabbage is shown in Table 1. Significant differences among parents were observed in both species . 'Topas' and 'Lisandra' of oilseed rape, and 'Ho Mei' and 'Hisfu early 30 days' of Chinese cabbage were much more responsive to microspore embryogenesis than 'Kizakino' and 'Kamikita', and '269' and '181', respectively. Large variation in embryo yields was also observed among their F_1s. The F_1s produced different embryo yields varying from 41 in 'Kamikita' × 'Kizakino' to 2,537 in 'Lisandra' × 'Topas' for oilseed rape and ranging from 22 of '181' × '269' to 800 of '269' × 'Ho Mei' in Chinese cabbage. Generally, F_1s between high-responsive cultivars produced high embryo yields and F_1s between low-responsive ones produced low yields. In oilseed rape, F_1s between high-responsive cultivars showed the highest embryo yields and those between high- and low-responsive cultivars showed a similar ability to the high-responsive parents or were intermediate between their parents. In Chinese cabbage, F_1s of '269' crossed with high-responsive cultivars showed predominant embryogenic ability; the embryo yields of F_1s between

Table 1 Embryo yields from microspore culture in four parents and their F_1 hybrids of oilseed rape (a) and Chinese cabbage (b). The underlined figures represent parental values

Female	Male			
	1	2	3	4
a. Oilseed rape				
1. Topas	924	1,800	626	375
2. Lisandra	2,537	720	1,031	494
3. Kizakino	1,048	712	8	80
4. Kamikita	198	490	41	0
b. Chinese cabbage				
1. Ho Mei	359	446	753	100
2. Hisfu	281	314	397	50
3. 269	800	368	4	62
4. 181	107	52	22	0

Table 2 Analysis of variance of the diallel table for microspore embryogenesis, after Hayman (1954 a)

Crop	Source[a]	*df*	SS	MS	*F* value
Oilseed rape	a	3	2,295.42	765.14	117.99**
	b	6	488.73	81.45	12.56**
	b1	1	296.93	296.93	45.79**
	b2	3	160.80	53.60	8.27**
	b3	2	30.99	15.50	2.39
	c	3	43.23	14.41	2.22
	d	3	49.91	16.63	2.57
	Error	30		6.49	
Chinese cabbage	a	3	740.57	245.86	65.03**
	b	6	350.82	58.47	15.40**
	b1	1	80.37	80.37	21.16**
	b2	3	220.52	73.51	19.36**
	b3	2	49.96	24.98	6.58**
	c	3	4.57	1.53	0.40
	d	3	11.96	3.99	1.05
	Error	30		3.79	

**: significant at 1% level

[a] a, additive effect; b, dominant effect; b1, mean dominance deviation; b2, dominance deviation due to each parent; b3, dominance deviation due to each crossing combination; c, maternal effect; d, reciprocal differences not ascribable to ''c''

Table 3 Genetic components of variation for microspore embryogenesis in a four-parent diallel cross of oilseed rape and Chinese cabbage

Components	Oilseed rape	Chinese cabbage
D	241.305	94.790
F	–114.466	8.116
H_1	173.498	144.202
H_2	150.751	109.821
hh	218.444	57.760
E	6.485	3.796
Average degree of dominance $(H_1/D)^{1/2}$	0.808	1.233
Proportion of dominance genes $(H_2/4H_1)$	0.360	0.517
Balance of positive and negative alleles $\{(4DH_1)^{1/2}+F\}\{(4DH_1)^{1/2}-F\}$	0.217	0.190
Broad-sense heritability	0.972	0.959
Narrow-sense heritability	0.811	0.659

'Ho Mei' and '269' were especially higher than that of the high-responsive parent 'Ho Mei'.

The embryo yields were significantly different among genotypes; therefore, statistical analysis was carried out to estimate the effect of variances associated with genetic factors. As two calculations using direct and root-transformed data gave similar results, only the later are described in this paper. Analysis of variance of the diallel table revealed that both additive (a) and dominant effects (b) were significant at the 1% level, whereas maternal effects (c and d) were not significant, in both species (Table 2). A dominant effect due to each crossing combination (b3) was not significant in oilseed rape, but was significant in Chinese cabbage.

The graph of Vr and Wr for microspore embryogenic ability is shown in Fig. 1. Cultivars located close to the origin have more dominant alleles, while those far from the origin have more recessive ones. In oilseed rape, no parents were located near the origin, indicating the absence of a completely dominant parent (Fig. 1a). 'Kizakino' was located at a position away from the origin, indicating an excess of recessive genes. 'Lisandra', 'Topas' and 'Kamikita' were distributed intermediate between the two ends. The regression line passed above the origin, indicating the influence of incomplete dominance. In Chinese cabbage, 'Ho Mei', 'Hisfu early 30 days' and '181' occupied positions near the origin, indicating that they had a relative excess of dominant genes (Fig. 1b). '269', located at a position away from the origin, indicated that it carried an excess of recessive genes. The correlation coefficient between Vr + Wr and the parental value were –0.325 and –0.467 in oilseed rape and Chinese cabbage, respectively. The negative correlation indicated that dominant genes are involved in microspore embryogenesis.

Genetic components of variation and genetic information are presented in Table 3. In oilseed rape, additive genetic variance (D) was larger than the dominance genetic variances (H_1 and H_2). The average degree of dominance was estimated to be 0.808, indicating incomplete dominance. Broad- and narrow-sense heritabilities were

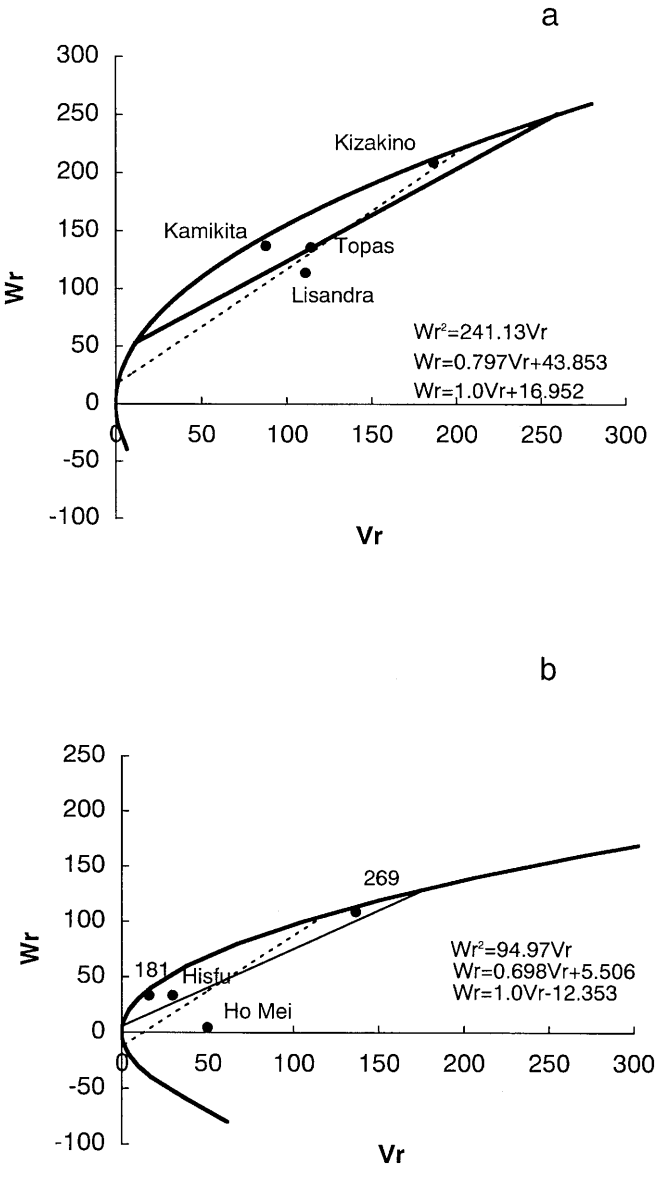

Fig. 1 Vr, Wr graph of the four-parent diallel analyses for microspore embryogenic ability in oilseed rape (**a**) and Chinese cabbage (**b**)

0.972 and 0.811, respectively. In Chinese cabbage, additive genetic variance (D) was lower than the dominance genetic variances (H_1 and H_2). The average degree of dominance was estimated to be 1.233, indicating super-dominance. Broad- and narrow-sense heritabilities were 0.959 and 0.659, respectively. The reduction of narrow-sense heritability is considered to be due to relatively large dominant effects.

Segregation in the F_2 population of 'Lisandra' × 'Kamikita' for embryogenic ability

The distribution of embryo yields in 80 F_2 plants of 'Lisandra' × 'Kamikita' and the analysis of segregation

Table 4 Analysis of segregation of embryo yields in an F_2 population of 'Lisandra' × 'Kamikita'

No. of embryos	No. of plants		χ^2	p
	Observed	Expected (1:4:6:4:1) [a]		
>700	4	5	1.683	$0.70 < p < 0.80$
101–700	16	20		
16–100	31	30		
1–15	23	20		
0	6	5		
Total	80	80		

[a] Expected ratio is based on two multiple genes with additive effects and incomplete dominance

pattern are given in Table 4. The embryo yields of F_2 plants varied from 0 to 1,378. They were classified into five groups based on their embryo yields. The results of the χ^2-test showed that the segregation of embryo yields in the F_2 population fitted the expected ratio of 1:4:6:4:1, indicating that microspore embryogenic ability may be controlled by two loci with additive effects.

Discussion

A large genotypic variation in microspore embryogenesis was reported in *Brassica* species (reviewed by Takahata 1997; Palmer and Keller 1999). In this study, we confirmed that the microspore embryogenic ability is controlled by genetic factors, and elucidated the quantitative inheritance parameters involved in microspore embryogenesis.

The F_1s from the 4×4 diallel crosses showed various embryo yields, and many F_1s produced higher or similar embryo yields than or to the high responsive parents. These results are consistent with those observed in the F_1 of 'Topas' × 'Isuzu' in *B. napus* (Ohkawa et al. 1987) and in the F_1 of 'Ho Mei' × 'Nozaki No. 2' in Chinese cabbage (Kuginuki et al. 1997).

Both additive and dominant effects were significant for microspore embryogenic ability. This is in agreement with the results obtained from the genetic analysis of in vitro regeneration in barley, rice and rapeseed (Komatsuda et al. 1989; Taguchi-Shiobara et al. 1997; Ono and Takahata 2000), somatic embryogenesis in wheat (Ou et al. 1989) and anther culture in rice (Miah et al. 1985; Quimio and Zapata 1990). Compared with oilseed rape, the dominant effect was relatively larger in Chinese cabbage. Especially, the F_1s between '269' and other parents showed superdominance. Such a relatively large dominant effect was also observed in the regeneration ability of rice (Peng and Hodges 1989; Taguchi-Shiobara et al. 1997). On the other hand, maternal effects were not detected in this study, suggesting that embryogenic ability was controlled only by nuclear genes.

In this study, the heritability of microspore embryogenesis is shown to be quite high. This indicated that embryogenic ability could be transferred from high-responsive genotypes to low- or non-responsive ones by crossing. Such high heritabilities of the in vitro responsiveness were also reported in regeneration from the anthers and seeds of rice (Quimio and Zapata 1990; Taguchi-Shiobara et al. 1997) and from the cotyledons of *B. napus* (Ono and Takahata 2000).

Genetic factors estimated for microspore embryogenic ability were similar in many points between oilseed rape and Chinese cabbage; namely, (1) dominant genes had positive effects on microspore embryogenic ability, (2) both additive and dominance genetic effects were significant, (3) no significant maternal effects were observed, and (4) the heritability was high, especially broad-sense heritability was higher than 0.9. These results suggest that microspore embryogenic ability is controlled by similar genetic factors in different species of *Brassica*; whereas the following points were inconsistent between two species: (1) though significant additive and dominant effects were found in both species, a larger contribution of dominant effects for microspore responsiveness was observed in Chinese cabbage, and (2) The narrow-sense heritability was higher in oilseed rape than Chinese cabbage, indicating that additive gene effects were more important in oilseed rape.

The results of F_2 segregation in embryo yields suggest that the genetic mode of microspore embryogenesis in oilseed rape is not too complex, and is considered to be mainly controlled by two multiple gene loci with additive effects. These results are consistent with those reported by Cloutier et al. (1995) and Ajisaka et al. (1999), who found that two putative chromosomal regions were associated with microspore-culture responsiveness in *B. napus* and *B. campestris*, respectively.

The results of the present study suggest that microspore-culture response can be improved genetically in *Brassica* crops. The detection of molecular markers involving QTLs (quantitative trait loci) for microspore embryogenesis will be useful for identification of the genes and the selection of high responsive genotypes.

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