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Genetic analysis of shoot regeneration from cotyledonary explants in *Brassica napus*

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Abstract Genetic analysis of shoot regeneration from cotyledonary explants of *Brassica napus* was carried out by 7×7 diallel crosses using cultivars showing a different ability for regeneration. Both additive and dominant effects were significant, with the additive effect being more important than the dominant one. Dominant genes had a positive effect on shoot regeneration. Non-allelic interaction and average maternal effects were not detected, while specific the maternal one was significant. In the 5×5 sub-diallel table, the maternal effect became nonsignificant. The mean degree of dominance was 0.759. Broad- and narrow-sense heritabilities were 0.973 and 0.819, respectively, indicating that shoot regeneration ability can be easily transferred into economically important cultivars showing a low or an unresponsive ability.

Key words Diallel analysis · Regeneration ability · Cotyledonary culture · *Brassica napus* · Heritablity

Introduction

Efficient plant regeneration via organogenesis or embryogenesis is indispensable to the application of tissue culture technology for plant improvement. However, great variation among genotypes in the ability of plant regeneration was observed in many plants. Such variation can be an obstacle to utilizing tissue culture for practical breeding. Genetic factors controlling the ability of tissue culture have been reported in several plants such as rice (Abe and Futsuhara 1991; Taguchi-Shiobara et al. 1997), barley (Komatsuda et al. 1989), maize, (Petolino and Thompson 1987) and wheat (Lazar et al. 1984). Recently, the loci involved in regeneration ability have been identified using molecular markers in several plants (Armstrong et al. 1992; Koornneef et al. 1993; Komatsuda et al. 1995; Yamagishi et al. 1996).

In *Brassica* crops various kinds of plant regeneration systems have been developed (Palmer and Keller 1994), but little work has been carried out on the genetic factors involved in the regeneration ability. Shoot regeneration systems using cotyledonary explants have been developed in some Brassicas and employed for genetic transformation, due to their high regeneration ability (Narasimhulu and Chopra 1988; Moloney et al. 1989; Hachey et al. 1991; Babic et al. 1998). We recently reported that the ability for shoot regeneration from cotyledonary explants was strongly influenced by genotype in rapeseed (*Brassica napus*) (Ono et al. 1994) and Chinese cabbage (*Brassica campestris*) (Zhang et al. 1998). In the present study, we have attempted to obtain genetic information on shoot regeneration from cotyledonary explants of *B. napus* by diallel analysis.

Materials and methods

Seven cultivars of rapeseed (*B. napus*) (see Table 1), were chosen, based on the results of a previous study in which they showed different abilities for shoot regeneration from cotyledons (Ono et al. 1994). A diallel set of crosses was performed among the seven cultivars. The ability of shoot regeneration from cotyledonary explants was examined for 42 reciprocal crosses and seven selfed parents. Tissue culture conditions and medium composition were performed as previously described (Ono et al. 1994). Seeds were sterilized in sodium hypochlorite (1.0% active chlorite) with 1–2 drops of Tween-20 for 20 min. After rinsing in sterile distilled water three times, the seeds were placed in 15×90 mm Petri dishes containing growth regulator-free MS agar (0.7%) medium (Murashige and Skoog 1962) at density of 20 seeds per dish. Cotyledonary explants excised from 4-day old seedlings were embedded into the regeneration media which was that composed of MS medium supplemented with 4 mg/l of 6-benzylaminopurine. Ten explants were cultured in a 15×90 mm Petri dish. After 3 weeks of culture, the adventitious shoots formed on the explants were counted. The regeneration frequency (number of explants with shoots/total number of explants) was averaged for at least three replications.

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Statistical analysis using either direct or arcsin-transformed data was performed according to the methods by Hayman (1954a, b) and Jinks (1954). Calculation in the diallel analysis was carried out using the computer program ‘DIALL’ developed by Ukai (1989). The narrow- and broad-sense heritabilities were calculated according to the method of Mather and Jinks (1971).

Results and discussion

The frequency of shoot regeneration for the seven parents ranged from 0% to 96.7% (Table 1). ‘Arabella’, ‘Doral’ and ‘Westar’ showed a high frequency of regeneration, while ‘Topas’, ‘Bridger’ and ‘Cascade’ showed a low frequency and ‘Norin 30’ could regenerate no shoots. Among 42 F_1 combinations, the regeneration frequencies of many F_1s were similar to those of the high-responsive parent. The regeneration ability of less-responsive cultivars could be improved by crossing with high-responsive ones. Heterotic combinations were found in those between ‘Bridger’ and ‘Topas’ and between ‘Bridger’ and ‘Norin 30’.

Table 1 Shoot regeneration frequency (%) from cotyledon explants in seven parents and their F_1 hybrids of *B. napus*. The underlined figures represent parental values

Female	Male						
	1	2	3	4	5	6	7
1 Arabella	96.7	90.0	86.7	73.3	90.0	83.3	93.3
2 Doral	96.7	93.3	43.3	78.5	78.5	86.7	80.0
3 Westar	76.7	70.0	86.7	50.0	43.3	43.3	40.0
4 Topas	53.3	46.7	70.0	13.3	20.0	6.7	20.0
5 Bridger	83.0	86.7	33.3	36.7	6.7	3.3	16.7
6 Cascade	83.3	78.5	30.0	13.3	6.7	6.7	6.7
7 Norin 30	93.3	73.3	70.0	6.7	33.3	0.0	0.0

Table 2 Analysis of variance of a 7×7 diallel table for shoot regeneration, after Hayman (1954a)

Source	*df*	SS	MS	*F*
a	6	39569.200	6599.370	232.16**
b	21	11155.700	531.222	18.69**
b1	1	600.095	600.095	21.11**
b2	6	1998.800	333.133	11.72**
b3	14	8556.760	611.197	21.50**
c	6	136.932	22.822	0.80
d	15	1815.060	121.004	4.26**
Error	96		28.426	

*, ** Significant at the 5% and 1% levels, respectively

Table 3 Analysis of variance of 5×5 sub-diallel table for shoot regeneration, after Hayman (1954a)

Source	*df*	SS	MS	*F*
a	4	29260.000	7314.990	320.07**
b	10	6896.980	689.698	30.18**
b1	1	2025.300	2025.300	88.62**
b2	4	321.871	80.468	3.52*
b3	5	4549.810	909.962	39.82**
c	4	54.972	13.743	0.60
d	6	247.024	41.171	1.80
Error	48		22.854	

*, ** Significant at the 5% and 1% levels, respectively

As two calculations for statistical analysis using direct and arcsin-transformed data gave the similar results, only the former are described in this paper. Analysis of variance of a diallel table for shoot regeneration revealed that both additive (a) and dominant (b) effects were significant (Table 2). This is consistent with the results obtained from genetic analysis of the regeneration ability of other crops such as rice (Peng and Hodes 1989; Abe and Futsuhara 1991; Taguchi-Shiobara et al. 1997), barley (Komatsuda et al. 1989) and maize (Petolino and Thompson 1987). The average maternal effect (c) was not significant, while the specific maternal one (d) was. The existence of maternal effects on regeneration ability were contrary to studies using other crops. This may be due to differences in the genotype employed. The relatively high maternal effects were detected in the combinations using ‘Westar’ and ‘Topas’ as a parent. Therefore, these two cultivars were eliminated from the diallel table to make a 5×5 sub-diallel table. In the analysis of the sub-diallel table, additive and dominant effects were significant, but the maternal one became nonsignificant (Table 3).

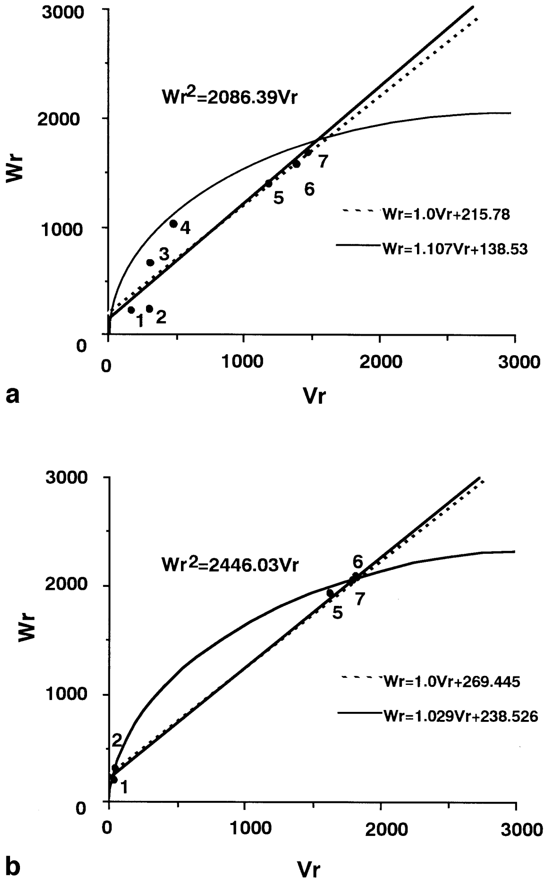

Fig. 1 Vr, Wr graph for shoot regeneration from a 7×7 diallel table (**a**) and a 5×5 sub-diallel table (**b**). *1* ‘Arabella’, *2* ‘Doral’, *3* ‘Westar’, *4* ‘Topas’, *5* ‘Bridger’, *6* ‘Cascade’, *7* ‘Norin 30’

Table 4 Estimates of genetic parameters for shoot regeneration derived from 5×5 sub-diallel table

Component	Estimated value±SE
D	2461.690±67.851
F	417.148±158.912
H_1	1440.510±154.597
H_2	1393.540±151.322
h^2	1299.170±162.243
E	22.854
$(H1/D)^{1/2}$ [a]	0.759
$H_2/4H_1$ [b]	0.383
$\{(4DH_1)^{1/2}+F\}\{4DH_1)^{1/2}-F\}$ [c]	0.299
h^2 (ns) [d]	0.819
h^2 (bs) [e]	0.973

[a] Mean dominance
[b] Proportion of dominant genes
[c] Balance of positive and negative alleles
[d] Heritability, narrow sense
[e] Heritability, broad sense

The graph of Vr and Wr for shoot regeneration is shown in Fig. 1. There were no significant differences in the slope of the regression of Wr on Vr from unity, and the homogeneity of Wr-Vr suggested the absence of non-allelic interaction. Cultivars located close to the origin have more dominant alleles, while those far from the origin have more recessive ones. ‘Arabella’ and ‘Doral’ carried exclusively dominant alleles, and ‘Bridger’, ‘Cascade’ and ‘Norin 30’ possessed an excess of recessive ones. The analysis of sub-diallels indicated that ‘Arabella’ and ‘Doral’ are completely dominant parents and ‘Cascade’ and ‘Norin 30’ are completely recessive parents (Fig. 1b). The regression line that intercepted the Wr-axis was above the origin, indicating that incomplete dominance was observed. The correlation coefficient between Vr+Wr and the parental value was r=–0.919, suggesting that the action of dominant genes was positive for shoot regeneration. The same results were found in regeneration from the seed-derived callus of rice (Abe and Futsuhara 1991; Taguchi-Shiobara et al 1997). In contrast, a negative effect of dominant genes for regeneration was reported in the regeneration from the leaf disc of tomato (Frankenberger et al. 1981) and anther culture (Quimio and Zapata 1990), and a seed-derived cell suspension culture (Tsukahara et al. 1995) of rice. This may be due to differences in the materials employed, the culture conditions and the explant tissues.

The genetic components of variation and the genetic information estimated from the sub-diallel table are presented in Table 4. Additive genetic variance (D) was larger than the dominance genetic variances (H1 and H2). The mean degree of dominance was 0.759, indicating incomplete dominance. This confirmed the results of the graphical analysis as shown in Fig. 1. The broad- and narrow-sense heritabilities were 0.973 and 0.819, respectively. Such a high narrow-sense heritability is considered to be due to the major contribution of additive gene effects to genetic variation in shoot regeneration ability.

The inheritance of in vitro traits have been mainly studied in cereal crops such as rice, barley, wheat and maize. To our knowledge, this is the first report clarifying genetic factors for regeneration ability in *Brassica* crops. The present results indicated that the ability of shoot regeneration from a cotyledonary explant is mainly controlled by dominant nuclear genes, and that such genes can be accumulated into agronomically important cultivars. To identify the molecular makers linked with the genes related to shoot regeneration ability, the production of recombinant imbred lines between ‘Arabella’ and ‘Norin 30’ is currently in progress.

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