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Full Length Research Paper

Analysis of the genetic callus induction and growth as revealed by diallel system

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Information about the genetics of callus formation is needed for efficient in vitro selection. Hybrid breeding has been proposed for genetic improvement of rapeseed but there isn't enough information on heterosis and gene action of callus growth characters in this crop. In the present study, genetic analysis and heterosis of three *in vitro* characters (percentage of callus induction, callus diameter and callus fresh weight) for five inbred lines of rapeseed, Orakel, ACSN1, P504588, P704591 and Boanty was investigated by a 5 × 5 diallel system using calli derived from mature embryo cultures in MS medium supplemented with 1 mg L^{-1} BAP and 0.5 mg L^{-1} 2,4-D.. Variance components due to specific combining ability (dominance variance) were larger than those of general combining ability (additive variance) for the traits under study. Positive heterosis was also noticed for all of the characters. High broad-sense (70.12 to 89.43) and low narrow-sense (3.49 to 23.01) heritability values suggested that dominance gene action was more important in the genetic control of the characters studied and therefore, exploiting this type of gene action for improving callus characters seems necessary. Among the five cultivars under study, Orakel was the best general combiner for callus induction and growth and may be used in the breeding programs for improving the callus characters in rapeseed.

Keywords: Callus induction and growth, combining ability, diallel, GCA and SCA, heritability, heterosis, rapeseed.

INTRODUCTION

Rapeseed (*Brassica napus* L.) is one of the world's most important sources of vegetable oil and protein. Therefore, this plant has become an object of extensive tissue culture studies and breeding (Turgut et al., 1998). In vitro characters can be used in combination with other agronomic important traits for crop improvement programs. Furthermore, tissue culture and in vitro selection is mainly associated with shorter time and lower cost as compared to conventional breeding methods (Abdel-Hady, 2006). This approach requires knowledge of the genetic basis for 'in vitro aptitude` which will help to predict the response of these characters to selection (Caligari et al., 1985 ; Powell et al., 1985).

The influence of genotype on in vitro growth and differentiation patterns was observed in a number of crop plants (Henry et al., 1994) including Brassicas (Buiatti et al., 1974 ; Ockendon and Sutherland, 1987 ; Aslam et al., 1990 ; Hanssen et al., 1999). Both additive and dominant effects were reported for the callus formation and plant regeneration in alfalfa (Wan et al., 1988) and anther culture of barley (Hou et al., 1994), wheat (Dağüstü, 2008) and sorghum (Can and Yushida, 1999). According to Wan et al., (1988) somatic embryogenesis in alfalfa was affected by two genes with complete dominance. Dominance genes were shown in plant regeneration from immature panicle of rice (Chu and Croughan , 1990) and non-embryonic callus in *Secale cereale* (Rakoczy and Malepszy, 1995). Moreover , heterosis was observed for

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callus induction, growth and regeneration competence in wheat (Haggag and El-Hennawy, 1991; Dornelles et al., 1997, Abdel- Hady, 2006 ; Dağüstü, 2008), corn (Tomes and Smith, 1985 ; Haggag and El-Hennawy, 1992 ; El-Shouny et al., 1999 ; Song et al., 2007), pearl millet (Mythili et al., 1997), tomato (Ohki et al., 1978), rice (Shigeru et al., 1998), barley (Özgen et al., 2005), pigeon pea (Kumar Suresh et al., 1985), tobacco (Keyes et al., 1981), petunia (Dulieu, 1991) and Brassicas (Buiatti et al., 1974 ; Ockedon and Sutherland, 1987 ; Aslam et al., 1990 ; Hansen et al., 1999). On the other hand, Buitti et al. (1974) showed the predominance of additive gene action for callus induction in wild cabbage (*Brassica oleracea* L.). Importance of additive genes was also indicated for callus induction in maize (Tomes and Smith, 1985), plant regeneration in wheat (Ou et al., 1989) and shoot forming capacity in tomato (Frankenberger et al., 1981) and rice (Chu and Croughan, 1990). Furthermore, Nesticky et al. (1983), Ou et al. (1989) and Özgen et al. (2001) reported significant maternal effects for callus formation and plant regeneration ability.

Information about the genetics and heterosis of callus characters is limited in rapeseed. The objectives of this study were, therefore, to analize the genetics callus induction and growth as revealed by a 5 × 5 diallel system and determine the amount of heterosis present among the studied rapeseed genotypes for the callus characters under investigation.

MATERIALS AND METHODS

Plant material

Five inbred lines of rapeseed (*Brassica napus* L.) were crossed to produce half diallel progenies. These lines were previously selected for callus characters (data not shown). Orakel and ACSN1 had low callus induction and P504588, P704591 and Boanty had high callus induction characteristics .

In vitro culture

Culture conditions for these accessions were optimized and only the optimum condition was used for the genetic studies. The optimum medium was MS (Murashige and Skoog, 1962) supplemented with 1 mg L^{-1} BAP and 0.5 mg L^{-1} 2, 4-D for callus induction. Ten seeds from each parent and hybrid were surface-sterilized in 1% calcium hypochlorite for 30 min and washed three times in autoclaved distilled water. Subsequently, mature embryos were placed on the growth regulator medium for callus initiation. Calli were detached from primary explants after 20 days and subcultured on fresh medium. All Cultures were kept at 25 ± 2 °C and exposed to 16/8 h photoperiod.

Experimental design

For the genetic analysis of *in vitro* characters, 10 seeds from each of the five parents and their 15 F1 diallel hybrids were used in a completely randomized design with four replications. Then, data were collected for callus fresh weight, callus diameter and percentage of callus induction at the end of six weeks.

Data analysis

After verifying the assumptions of equality of error variances within treatments and normality of error terms, data were analyzed according to Griffing's (1956) method 2, mixed model B. Then, general combining ability (GCA), specific combining ability (SCA), average degree of dominance, broad-sense and narrow-sense heritability were estimated using microcomputer programs (Burow and Coors, 1994; Johnson and King, 1998). The combining ability analyses were carried out under the assumptions of normal diploid segregation, absence of maternal effects, independent action of non-allelic genes, absence of multiple alleles, homozygous status of parents, independent distribution of genes between parents, and inbreeding coefficient of unity (Griffing, 1956 a,b). Mid-parent heterosis (MP) and heterobeltiosis (HP) were calculated as follows (Feyzian et al., 2009):

MP = (F1 - mean of Parents / mean of parents) × 100

HP = (F1 - better parent / better parent) × 100.

RESULTS

Mean squares of genotypes, GCA and SCA for percent of callus induction, callus diameter and callus fresh weight are presented in Table 1. Significant variations among genotypes were observed for all characters under study. GCA was only significant for percent of callus induction, while significant SCA were estimated for all callus characters.

Estimated GCA effects of parents for different callus characters are shown in Table 2. Significant GCA estimates were obtained for percent of callus induction in P504588 and for callus diameter in Orakel and P704591. ACSN1 and Orakel showed larger GCA effects for callus fresh weight; however, these values were not significantly different from zero. Negative GCA effects are related to the reduction in the callus induction capacity and growth rate, while positive effects are associated with the increased values in the progeny.

Mean values of parents and their F1 hybrids for all traits are shown in Table 3. Among the parents, Orakel and P504588 had maximum percent of callus induction, whereas, larger values of callus fresh weight and diameter were observed for Orakel, ACSN1, and Boanty. Estimates of SCA effects for callus induction and growth

Table 1. Analysis of variance in an incomplete 5 × 5 diallel system evaluated for percent of callus induction (PCI%), callus diameter (mm) (CD) and callus fresh weight (g) (CFW) in rapeseed

Source of variation	DF	PCI	CD	CFW
Crosses	14	170.89**	3.96**	0.0079**
Parents	4	323**	2.303**	0.0043***
Parents *vs* hybrids	1	874.8**	32.89**	0.037**
hybrids	9	25.07ns	1.49*	0.0062**
GCA	4	218.17*	1.33 ns	0.0032 ns
SCA	10	151.97*	0.016**	0.0098**
Experimental error	45	58.91	0.55	0.0018

* $p < 0.05$ and ** $p < 0.01$

Table 2. Estimates of general combining ability (GCA) effects for percents callus induction (PCI%), callus diameter (mm) (CD) and callus fresh weight (g) (CFW) in rapeseed

Parent	PCI	CD	CFW
Orakel	2.657	0.396	0.00885
ACSN1	-2.629	0.081	0.01253
P504588	3.229	-0.123	-0.0108
P704591	-0.771	-0.354	-0.00998
Boanty	-2.486	-0.002	-0.0006
SE (gi)	1.335	0.143	0.0074
SE (gi-gj)	2.111	0.226	0.01171

Table 3. Mean values of parents and their F1 hybrids for percent of callus induction (PCI%), callus diameter (mm) (CD) and callus fresh weight (g) (CFW) in rapeseed

Parent	PCI	CD	CFW
Orakel	100	8.13	0.175
ACSN1	82.5	7.31	0.208
P504588	100	6.56	0.144
P704591	88	6.46	0.139
Boanty	82	7.90	0.204
Orakel × ACSN1	94	9.82	0.30
Orakel × P504588	100	8.37	0.18
Orakel × P704591	100	9.47	0.25
Orakel × Boanty	100	8.45	0.24
ACSN1 × P504588	98	9.35	0.27
ACSN1 × P704591	100	8.65	0.21
ACSN1 × Boanty	100	7.72	0.15
P504588 × P704591	100	8.92	0.22
P504588 × Boanty	100	8.97	0.22
P704591 × Boanty	94	8.65	0.23
SE of the mean	3.83	0.37	0.02

characters are presented in Table 4. The hybrids Orakel × ACSN1, ACSN1 × P504588 and Orakel × P704591 showed larger SCA effects for callus fresh weight and diameter than other hybrids. However, for percentage of callus induction, higher SCA effects were observed for the crosses ACSN1 × P704591 and ACSN1 × Boanty.

Table 4. Specific combining ability (SCA) effects of the five parental lines for percent of callus induction (PCI%), callus diameter (mm) (CD) and callus fresh weight (g) (CFW) in rapeseed

Parents	PCI	CD	CFW
Orakel × ACSN1	-1.93	1.116	0.0613
Orakel × P504588	-1.78	-0.129	-0.0186
Orakel × P704591	2.21	0.989	0.0397
Orakel × Boanty	3.93	-0.178	0.0206
ACSN1 × P504588	1.50	1.161	0.0605
ACSN1 × P704591	7.50	0.479	-0.0022
ACSN1 × Boanty	9.21	-0.588	-0.0666
P504588 × P704591	1.64	0.959	0.0269
P504588 × Boanty	3.36	0.867	0.0195
P704591 × Boanty	1.36	0.560	0.0355
SE (sij-sik)	5.17	0.501	0.0287
SE (sij-skl)	4.72	0.457	0.0262

Table 5. Mid-parent (MP) and high-parent (HP) heterosis in five parent diallel crosses for percent of callus induction (PCI%), callus diameter (mm) (CD), callus fresh weight (g) (CFW) in rapeseed

Parent	PCI		CD		CFW	
	MP	HP	MP	HP	MP	HP
Orakel × ACSN1	3	-6	1.7	2.11	0.084	0.100
Orakel × P504588	0	0	0.25	1.03	0.013	0.029
Orakel × P704591	6	0	1.35	2.19	0.072	0.090
Orakel × Boanty	9	0	0.33	0.44	0.035	0.049
ACSN1 × P504588	6.75	-2	2.04	2.42	0.064	0.096
ACSN1 × P704591	14.7	12	1.34	1.77	0.0019	0.036
ACSN1 × Boanty	17.7	17.5	-0.18	-0.12	-0.053	-0.05
P504588 × P704591	6	0	1.37	2.42	0.072	0.074
P504588 × Boanty	9	0	1.08	1.75	0.014	0.044
P704591 × Boanty	9	6	0.75	1.47	0.031	0.063
Means of parents	90.5		7.27		0.174	
Means of hybrids	98.6		8.84		0.227	
Average Heterosis (%)	9		21.6		30.5	

Table 5 shows the estimates of mid-parent and high-parent heterosis for the traits under study. Positive heterosis was noticed for the callus characters and all hybrids demonstrated positive heterosis for callus fresh weight and diameter except ACSN1 × Boanty. Average heterosis ranged from 9% for percent of callus induction to 21.6% and 30.5% for callus diameter and callus fresh weight, respectively. Furthermore, dominance genetic variances were larger than additive genetic variances for all of the studied characters. The average degree of dominance ranged from 2, for percent of callus induction, to 6.4 and 6.7 for callus diameter and callus fresh weight, respectively.

Broad-sense heritability estimates were high (ranging from 70.12 to 89.48) but, narrow-sense heritability values were low (3.49 to 23. 01) (Table 6).

DISCUSSION

The high broad-sense heritability estimates for all of the studied characters indicated that callus induction and growth in B. napus is under genetic control and the environmental effects were small as compared to the genetic effects. High broad sense heritability estimates for the diameter and fresh weight of callus suggest that these characters can be considered as efficient indices for determining the callus growth rate. On the other hand, low narrow-sense heritability for these traits, larger dominance variance components and over-dominance gene action indicated the prominence of dominance effects in the control of callus characters. The presence of dominant genes were reported in controlling plant regeneration ability of alfalfa (Wan et al., 1988), shoot

Table 6. Estimates of genetic and environmental components, average degree of dominance and broad and narrow sense heritability (h^2_B and h^2_N, respectively) for percent callus induction (PCI%), callus diameter (mm) (CD) and callus fresh weight (g × 1000) (CFW) in rapeseed

Parent	PCI	CD	CFW
σ^2_{GCA}	5.69	0.028	0.044
σ^2_{SCA}	23.19	1.115	1.950
σ^2_A	11.38	0.055	0.088
σ^2_D	23.19	1.115	1.950
σ^2_e/r	14.73	0.138	0.480
$(2\sigma^2_D/\sigma^2_A)^{1/2}$	2.00	6.40	6.700
H^2_B (%)	70.12	89.48	81.87
H^2_N (%)	23.08	4.21	3.490

σ^2_{GCA}: Variance of general combining ability; σ^2_{SCA}: Variance of specific combining ability
σ^2_A: Additive genetic variance; σ^2_D: Dominance genetic variance; σ^2_e/r: error variance per entry mean.

forming capacity in the cotyledons of pigeon pea (Kumar Suresh et al., 1985) and ability of regeneration from protoplast in B. *oleracea* (Hansen et al., 1999). Both additive and non-additive effects of genes were also reported for plant regeneration in rice (Peng and Hodeg, 1989), callus growth in pigeon pea (Kumar Suresh et al., 1985), callus induction and plant regeneration in sorghum anther culture (Can and Yoshida, 1999) and callus induction and embryogenic callus induction frequency in wheat anther culture (Dağüstü, 2008). Some researchers have indicated the prevalence of additive gene action for plant regeneration in wheat (Ou et al., 1989; Dornells et al., 1997; Abdel-Hady, 2006), callus induction in maize (Tomes and Smith, 1985) and shoot regeneration in tomato (Frankenberger et al., 1981). The discrepancy of the results from different experiments may be related to sampling error and to different genotypes and growth media used in these studies.

As mentioned before, both mid-parent and high-parent heterosis for all *in vitro* characters of *B. napus* were observed in the hybrids. From the heterobeltiosis values of Table 5, it is seen that the degree of dominance ranged from incomplete dominance to over-dominance. However, over-dominance gene action was detected for most of the hybrids which resulted in the average degree of dominance of 2 for callus induction and above 6 for callus diameter and weight. High over-dominance value for callus characters may be the result of pseudo-over-dominance caused by correlated gene distributions among the parents, so that partial and/or complete dominance become pseudo-overdominant (Hayman, 1954; Dehghanpour et al., 1996).

Hybrids such as Orakel × ACSN1, ACSN1 × P504588, and Orakel × P704591 showed higher means and SCA effects for callus fresh weight and diameter, while for the percentage of callus induction, higher SCA effects were observed in the crosses ACSN1 × P704591 and ACSN1 × Boanty. Therefore, it may be stated that the gene(s) controlling callus induction are probably different from those controlling callus growth. Furthermore, the existence of heterobeltiosis in most of the hybrid combinations and large degrees of dominance in this study suggest the exploitation of dominance gene action for the improvement of callus characters. Several researches have stated that tissue culture technique can be used as a useful tool for early screening of combining ability and for the choice of the best parents in plant breeding programs (Haggag and El-Hennawy, 1992; El-Shouny et al., 1999; Abdel-Hady, 2006).

Among the parents, Orakel ranked as the best general combiner for callus diameter and was a promising source for the percentage of callus induction and callus fresh weight. Although, in a pilot experiment, Orakel showed a low potential for callus induction and growth in the media supplemented with growth regulators (data not shown), its proper callus formation ability in the media without these regulators indicated that endogenous hormones are probably higher in this cultivar which consequently inhibit the callus formation when applied exogenously. This cultivar could be used in the future breeding programs for genetic improvement of the callus induction and growth performance in rapeseed.

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