

Full Length Research Paper

Biometric-genetic analysis of *in vitro* callus proliferation in rapeseed (*Brassica napus*)

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Accepted 23 November, 2011

The genetics/inheritance of callus induction and growth in terms of gene action, genetic variance components, and allied genetic parameters was determined in five pure lines of rapeseed (*Brassica napus* L.), Orakel, ACSN1, P504588, P704591 and Boanty by diallel analysis of F1 progenies from five parents. The parents and their F1 hybrids were grown on MS medium in Petri dishes. The data were collected after five weeks on percent of callus induction, callus diameter and callus fresh weight. The additive (D) and dominance (H) components were significant for all traits. H1 and H2 were higher than D, indicating that, the dominant component was larger than additive component. Epistasis was almost absent. Dominant alleles caused high and recessive alleles caused low expression of callus induction and growth. Because of better breeding value, high per-se performance, and desirable position in the $Wr - Vr$ graph, three parents Orakel, ACSN1 and Boanty were identified as potential genotypes to be exploited for callus growth improvement by hybridization followed by selection.

Key words: Callus growth, diallel analysis, inheritance, rapeseed, dominant alleles.

INTRODUCTION

Brassica napus is one of the world's most important sources of vegetable oil and protein, thus *B. napus* has become an object of extensive tissue culture (Turgut et al., 1998) and suspension culture (Biesaga-Koscielniak et al., 2008; Kharenko et al., 2011) studies and breeding. Information about the gene action of callus formation is needed for effective *in vitro* selection, exploitation of somaclonal variation and gene introgression (Mythili et al., 1997). *In vitro* characters can be used in combination with other agronomically important traits for crop improvement programs. This approach requires knowledge of the

genetic basis for 'in vitro aptitude' and will also help to predict the response of these characters to selection (Calligari et al., 1985; Powell et al., 1985). Exploration of the genetic variation for trait in a polymorphic species could lead to identification and transfer of favorable genes affecting morphogenesis (Dulieu, 1991). The influence of genotype on *in vitro* growth and differentiation patterns has been reported in a number of crop plants (Henry et al., 1994). The genetic basis of *in vitro* response has been analyzed in several plant systems including alfalfa (Wan et al., 1988; Kielly and Bowley, 1992; Crea et al., 1995), barley (Foroughi-Wehr et al., 1982; Komatsuda et al., 1989; Özgün et al., 2005), sunflower (Encheva et al., 2004), cotton (Gawel and Robacker, 1990), maize (Nesticky et al., 1983; Tomes and

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Smith, 1985), petunia (Dulieu, 1991), rice (Quimio and Zapata, 1990; Abe and Futsuhara, 1991; Yan et al., 1996; Kuroda et al., 1998), rye (Rakoczy–Trojanowska and Malepszy, 1993), tomato (Frankenberger et al., 1981; Koorneef et al., 1987; Soniya et al., 2001), wheat (Lazar et al., 1984; Ou et al., 1989; De Boyser et al., 1992; Lange et al., 1995; Dornelles et al., 1997; Abdel-Hady, 2006; Ozbay and Özgen, 2010), eggplant (Chakravarthi et al., 2010) and pearl millet (Mythili et al., 1997). Nesticky et al. (1983) noted significant reciprocal effects in the basic of combining ability and high values of reciprocal effects in corn tissue culture. They suggested that callus growth *in vitro* was controlled by two genetic systems, one located in the nucleus and another in the cytoplasm.

In Brassica, the influence of genotype on *in vitro* characters like growth rate has been reported previously (Buiatt et al., 1974; Ockendon and Sutherland, 1987; Aslam et al., 1990; Hanssen et al., 1999). Buitti et al. (1974) determined in wild cabbage (*Brassica oleracea* L.) *in vitro* characters was controlled primarily by additive gene effects.

The objective of this study was to analyze the genetic control of different callus characters and formation as revealed by 5 × 5 diallel analysis. We attempted to identify superior genotypes among the parental accessions for improving the *in vitro* response through selection. Also, we indicated that callus proliferation is under quantitative genetic control. To date, however, there have been no known studies on the quantitative genetic and the genetic basis of the *in vitro* characters in rapeseed.

MATERIALS AND METHODS

Plant material

Nine pure lines of *Brassica napus*, four explants and five different hormonal concentrations had been previously screened for their callus initiation frequency and growth (data not show). Of these, five genotypes, Orakel and ACSN1 with low callus induction, P504588, P704591 and Boanty, with high callus induction (hereafter referred to as P1, P2, P3, P4 and P5, respectively) which were previously selected for marked differences in their *in vitro* characters.

In vitro method

Culture condition for these accessions has been standardized and only the optimum conditions were used for genetic studies, that is, MS medium (Murashige and Skoog) supplemented with 1 mg L⁻¹ BAP and 0.5 mg L⁻¹ 2, 4-D for callus induction. 10 seeds from each parent and their hybrids were surface-sterilized in 1% calcium hypochlorite for 30 min, washed three times in autoclaved distilled water then were placed on growth regulator medium in 4 replication for callus initiation. Callus were removed from primary explants (hypocotyls) after 20 days and subcultured on fresh medium. The cultures were incubated at 25 ± 2°C and exposed to 16 h light and 8 h dark period. Growth rate of callus measured in terms of its fresh weight, diameter of callus and percentage of callus induction at the end of 6 weeks.

Experimental design

For the genetic analysis of *in vitro* characters, this present investigation with rapeseed is based upon 5 × 5 half diallel progenies involving five pure line of rapeseed (*Brassica napus* L.). The statistical analysis of data, averaged over samples, was done following the Hayman (Hayman, 1954) model as developed by Sharma (Sharma, 1998) for components of variance and allied parameters, including a Wr-Vr graph relating to all characters.

The adequacy of the additive dominance model was confirmed from the regression coefficient (Mather and Jinks, 1971). Graphs for Wr and Vr have been drawn by plotting the expected Wrei values against the respective array variances, and a straight line was drawn by excel program. The original array covariances and variances were noted on the graph. The point of interception of the regression line was calculated. If the intercept was negative, it was taken as an indication of over dominance. Epistasis was indicated by a non-significant deviation of the regression coefficient both from zero and unity. When the regression coefficient deviated significantly from zero but not from unity, it was taken as an indication of absence of epistasis (Singh and Chaudhary, 1979).

RESULTS

The absence of epistasis primarily confirmed the adequacy of the Hayman's additive model for the analysis of genetic variance and α -allied parameters the two tests of additively, namely F(wr-vr) which was significant for fresh weight, diameter of callus and percentage of callus induction and $b_{Wr/Vr}$ being > 0.5 and insignificant for all traits revealed absence of non-allelic interactions (epistasis) in controlling gene expression (Table 2). Both additive (D^*) and dominance (\hat{H}_1) components of genetic variance were significant for all traits. The (\hat{H}_1) was, however, more predominant than D^* . This observation was confirmed by the distribution of array points not clustered near the tangent of limiting parabola related to each trait (Figure 1). Moreover, a high degree of dominance effect was also reflected by the \hat{h}^2 , a sum total over all loci in heterozygous state. In comparison, the environmental component (\hat{E}), which was significant for all characters, indicates the results on variance components not to be nearer to the reality. Further, the dominance ratio (\hat{H}_1/D^*)^{0.5}, which was >1 revealed that overdominance was in control of all traits, Negative Y-intercept (a) parentally expressed over- dominance for all trait (Table 2). A close examination of the α -value, suggested that dominance was perhaps more responsible than overdominance for fresh weight and diameter of callus. The proportion or relative distribution of dominant and recessive alleles in parents was not symmetrical as the ratio $[(4D^*\hat{H}_1)^{0.5}+F]/[(4D^*\hat{H}_1)^{0.5}-F]$ was >1, indicating excess of dominant over recessive. This observation was also confirmed by $\hat{H}_1 \neq \hat{H}_2$ and the positive nature of F^* estimates for all characters. Besides, the positive direction of the estimates of (Wr+Vr) associated with all the parents proved that all parents probably have a high frequency of dominant alleles for all traits (Table 1). Among the dominant alleles, those with

Table 1. Performance, breeding value, and dominance relationship of parents of evaluated callus induction and growth.

Parent	Percent of callus induction			Callus diameter (mm)			Callus fresh weight (g)		
	\bar{P}	Wr + Vr	Order D	\bar{P}	Wr + Vr	Order D	\bar{P}	Wr + Vr	Order D
Orakel	100	27.73	II	8.07	0.783	III	0.174	0.0052	V
ACSN1	83.33	128.53	III	7.37	0.665	I	0.214	0.0035	II
P504588	100	11.73	I	6.40	1.916	IV	0.137	0.0041	III
P704591	84	146.13	IV	6.27	2.888	V	0.134	0.0044	IV
Boanty	76	243.73	V	7.70	0.701	II	0.190	0.0034	I

Order D, Dominance order.

Table 2. Genetic variance and estimates of allied parameters for callus induction and growth.

Variable	Percent of callus induction	Callus diameter (mm)	Callus fresh weight (g)
D^{\wedge}	171.57*	0.74*	0.0017*
F^{\wedge}	215.923*	0.996*	0.0017 ^{ns}
\hat{H}_1	188/011*	4.436*	0.0091*
\hat{H}_2	126.57*	4.038*	0.0015*
\hat{h}^2	254.07*	8.013*	0.0094*
\hat{E}	54.641*	0.526*	0.0019*
$(\hat{H}_1/D^{\wedge})^{0.5}$	1.0462	2.442	2.3406
$\hat{H}_2/4 \hat{H}_1$	0.1683	0.227	0.2247
$[(4D^{\wedge}\hat{H}_1)^{0.5}+F]/[(4D^{\wedge}\hat{H}_1)^{0.5}-F]$	4.0098	1.755	1.584
h_{ns}^2	0.9	0.5	0.5
h_b^2	0.42	0.67	0.56
F (wr-vr)	3.68 ^{ns}	0.26 ^{ns}	14.31 ^{ns}
r_{yr} (wr+vr)	0.98	0.91	0.46
a	-10.79	-0.87	-0.0016
bwr/vr	0.78 ^{ns}	0.87 ^{ns}	0.71 ^{ns}

*, Significant at the 0.05 probability levels; ns, non-significance. D, Additive effects; H1, overall dominance effect; H2, reflection of the proportion of genes with positive and negative effects; F, covariance of additive and dominance effects; h^2 , dominance effects of all loci in heterozygous phase; r, coefficient of correlation between parental order of dominance (Wr +Yr) and parental measurement (Yr).

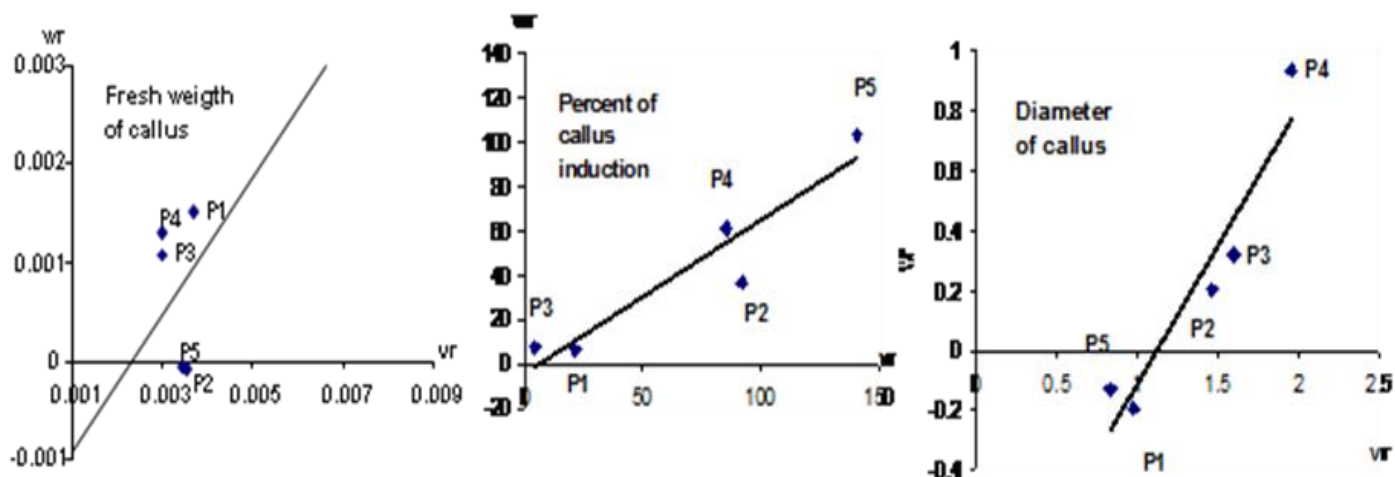


Figure 1. Wr /Vr relationship for percent of callus induction, diameter of callus and fresh weight of callus.

positive (high) effects on the expression of fresh weight and diameter of callus were more prevalent in parents than those with negative (low) effects, indicated by $\hat{H}_2/4\hat{H}_1 \neq 0.25$, although fresh weight and diameter of callus (with $\hat{H}_2/4\hat{H}_1 \sim 0.25$) appeared to have near symmetrical distribution. The positive value of $r_{yr}(wr + vr)$ was suggestive that the majority of dominant alleles tended to have positive effects. The individual parents Orakel (p1) and Pf 504588 (p3) for callus induction, Orakel (p1) and Boanty (p5) for diameter of callus, ACSN1 (p2) and Boanty (p5) for fresh weight of callus, which were close to the origin, possessed high frequency of dominant alleles, respectively, while the parents ACSN1 (p2) and PF704591 (p4) for callus induction, ACSN1 (p2) and PF504588 (p3) for diameter of callus, Orakel (p1), PF584588 (p3) and PF704591 (p4) for fresh weight of callus occupying an intermediate place from the origin, had an almost equal frequency of dominant and recessive alleles. Boanty (p5) for callus induction and PF704591 (p4) had a relatively high frequency of recessive alleles (Figure 1 and Table 1). This allelic distribution was corroborated by the dominance order of parents based on descending ($W_r - V_r$) values. Further, all the parents array points were scattered along the regression line for all traits, indicating considerably high genetic diversity among parents. For the pre-se performance (\bar{P}) in unison the parents manifested very high values of \bar{P} , showed low values of $W_r - V_r$.

DISCUSSION

Whereas, *B. napus* L., is especially useful for the production of seed oil then callus suspension cultures can provide a constant supply of relevant fresh material for analysing (Biesaga-Koscielniak et al., 2008) the effects of different culture conditions, including exogenously applied phytohormones or metabolic inhibitors, as well as mutagenic agents, on lipid metabolic pathways (Kharenko et al., 2011). Introducing the best genotype with favorable alleles for callus induction and growth can help us for subsequent study.

One of the assumptions underlying diallel analysis is the absence of epistatic gene action which has been satisfied for percent of callus induction, diameter of callus and fresh weight of callus in this study. Lack of epistatic interaction has been reported for several *in vitro* characters such as callus growth in rice (Abe and Futsuhara, 1991), mean callus diameter in barley (Komatsuda et al., 1989), plant regeneration rate in rice (Chu and Croughan, 1990) and total callus volume, fresh weight of callus and frequency of calli (Mythili et al., 1997). Both additive and dominance components of genetic variance were control all *in vitro* characters, although the dominant component was larger than additive component. Therefore, overdominance should actually be viewed as dominance in this study. Possibly, the additive

type of gene action can operate regardless of the degree of dominance (Mather and Jinks, 1971). Non-additive gene action for all traits which is indicative of either low selection potential or selection involving specific test crosses. Predominance of additive gene action was previously reported for callus induction in maize (Tomes and Smith, 1985), plant regeneration in wheat (Ou et al., 1989), shoot forming capacity in tomato (Frankenberger et al., 1981), in Brassica (Buiatti et al., 1974) and rice (Chu and Croughan, 1990). The negative y-intercept values (a), which supports the importance of over dominance for all traits. The dominant alleles were associated with positive effects causing high callus growth and induction while recessive alleles produced low callus growth. Contrary to this, Orakel contain the highest callus induction diameter because of the presence of a high propagation of dominant alleles with positive effects. For the fresh weight of callus p1, p3 and p4 showed partial dominance, p2 and p5 showed complete dominant. For diameter of callus p2, p3 and p4 showed partial dominance p1 and p5 showed overdominance. Among cereals, rice (Quimio and Zapata, 1990; Abe and Futsuhara, 1991) and barley (Komatsuda et al., 1989) cultivars have shown similar variation in the degree of dominance for some *in vitro* characters. Fresh weight and diameter of callus (with $\hat{H}_2/4\hat{H}_1 \sim 0.25$) indication of equal gene frequencies suggests that there was no selection against the favorable alleles in these materials. This would mean that the unfavorable alleles do not have a negative effect on fitness of the genotype and these genes may be favorably incorporated in breeding material. Since additive and dominance variances are both important for increased callus induction and growth, a breeding strategy involving hybridization followed by selection could be the most rewarding proposition because selection will utilized D^{\wedge} , while \hat{H}_1 will be exploited by hybridization among chosen parents in advance generation. Desirable parents could be chosen according to the distribution of array points (parents) in $W_r - V_r$ graph, the per-se performance and breeding values (General combining ability), which are the pragmatic criteria for choice of parents. Based on these criteria, only three parents namely Orakel, ACSN1 and Boanty were the best parents for callus proliferation. In this study, we indicated that callus proliferation in rapeseed is under quantitative genetic control to have near symmetrical distribution for positive and negative effects.

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