



Effect of number of parents on some important biometric genetic parameters in rapeseed (*Brassica napus* L.)

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SUMMARY

Half F₂ diallel crosses of eight spring cultivars of rapeseed were used in partial circulant diallel analyses to estimate biometric genetic parameters for phenological traits, yield components and seed yield. The greatest variation in the GCA-to-SCA mean square ratio was related to days to flowering, and its lowest variation to days to maturity. A high coefficient of variation of the narrow-sense heritability estimate was obtained for days to maturity, followed by plant height and seed yield. For diallel analysis with a low number of parents, the differences of the heritability estimates of the traits were small; therefore, for precise estimation of heritability, a high number of diallel crosses will be preferred.

Key words: diallel crosses, narrow-sense heritability, seed yield.

1. Introduction

In breeding programs, it is necessary to use adequate methodologies to deal with phenotypic data in order to correctly understand the estimates of genetic parameters and hence to rank the genotypes correctly. Diallel crossing is a widely used method by which the performance of a group of parents can be evaluated on an individual basis and also in hybrid combinations; it can also be used for determining the gene action of quantitative traits (Singh et al. 2010). Many extensions to Griffing's model have been proposed to extract more precise effects from the diallel. These include decomposing specific combining ability (SCA) into dominance, heterosis, and epistasis components (Hayman 1957; Gardner and Eberhart 1966) and into reciprocal effects (Griffing 1956),

and their further decomposition into maternal and paternal effects (Cockerham and Weir 1977; Zhu and Weir 1996). Conversely, interest in obtaining general combining ability (GCA) with a reduced number of crosses has motivated variants of the design such as the half-diallel (Griffing 1956) and the partial diallel (Kempthorne and Curnow 1961), among others (Lynch and Walsh 1998), which have themselves led to nominal innovation (Greenberg et al. 2010). Knowledge of genetic control of agronomic traits is very important for plant breeders to formulate a proper breeding strategy for the improvement of new oilseed brassica varieties. Singh and Chaudhry (2004) state that the heritability of a trait approaches its maximum in successive generations following hybridization. In addition, the presence of additive gene effects for a trait indicates that selection may be successful for the trait (Mather and Jinks 1982; Kearsley and Pooni 1996). Estimation of the genetic constitution of the parents for seed yield and its components can be important for indirect selection for high seed yield in rapeseed (Downey and Rimer 1993; Nassimi et al. 2006; Singh et al. 2010; Teklewold and Becker 2005; Yadav et al. 2005). Although combining ability studies in oilseed *Brassica* spp. are scanty, most such studies have emphasized the preponderance effect of GCA for yield and its components, indicating the importance of additive gene action (McGee and Brown 1995; Wos et al. 1999; Rameeh 2011). Khan et al. (2006) reported that variation for both GCA and SCA was responsible for dry matter yield and other quantitative traits in *B. napus*. Significant GCA and SCA effects were reported for pods per main raceme, pods per plant, pod length, number of seeds per pod, 1000-seed weight and seed yield in *B. napus* (Thakur and Sagwal 1997; Mahmud et al. 2009), but in another study (Singh et al. 1995) the importance of additive genetic effects for pods per plant and 1000-seed weight was emphasized. Singh and Yadav (1980) and Thakur and Sagwal (1997), in examining the genetic control of seed yield in rapeseed, found both additive and non-additive gene effects to be involved. Khan and Khan (2005) performed a complete diallel cross analysis in rapeseed and reported that number of pods per plant, 1000-seed weight and seed yield per plot had moderate narrow-sense heritability. A limiting factor for the use of diallel crosses is the large number of parents that have to be evaluated in some cases. An alternative is to evaluate only

a sample of these parents in a so-called circulant diallel method (Alan et al. 2012). The circulant diallel suggested by Kempthorne and Curnow (1961) justifies special consideration because it permits the use of a reduced number of parents, although questionable estimates of GCA and SCA may result when the number of crosses per parent is too small. There have been only a few studies dealing with these issues (Murty and Anand 1966; Murty et al. 1967).

The objective of the present study was to estimate biological genetic parameters for phenological traits, plant height, seed yield and its components in eight rapeseed breeding lines using partial circulant diallel analysis.

2. Materials and methods

Eight rapeseed (*Brassica napus* L.) spring genotypes including L41, Zafar, L56, L31, L22, LF2, L420 and L401 (all of the genotypes were F₇ generation with coefficient of inbreeding one (F=1) and were selected based on a pedigree method in a northern province of Iran) were crossed in a half diallel mating design during 2011–12. The hand cross-pollinated flowers on the inflorescences were isolated using paper to produce an F₁ generation. Twenty-eight F₁ crosses along with their parents (36 in total) were planted in a randomized complete block design with three replications at Bayekola Agriculture Research Station, located in Neka, Iran (13° 53' E longitude and 43° 36' N latitude, 15 m above sea level) during autumn in 2012–13. In both years the genotypes were sown on October 10. The soil was classified as a deep loam soil (Typic Xerofluents, USDA classification), and contained an average of 280 g clay kg⁻¹, 560 g silt kg⁻¹, 160 g sand kg⁻¹ and 22.4 g organic matter kg⁻¹, with a pH of 7.3. Soil samples were found to have 45 kg ha⁻¹ of mineral nitrogen (N) in the upper 30 cm profile. The plots consisted of four rows 5 m long and 40 cm apart, and the distance between plants in each row was 5 cm. The site had been planted with wheat in the previous year. Phosphorus (P) and potassium (K) were applied at 100 kg ha⁻¹ and 150 kg ha⁻¹ using potassium sulfate (48% K₂O) and triple super phosphate (48% P₂O₅) respectively. Nitrogen was applied at 180 kg ha⁻¹, which included 391 kg ha⁻¹ urea (46%N). Management of the trial involved the broadcasting of a third of the

nitrogen and the whole of the phosphorus and potassium fertilizers along with spraying of trifluralin (a,a,a-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) herbicide at 2 lit/ha, followed by harrowing to incorporate the pre-emergent herbicide and fertilizers. Seeds were sown on the plots by hand with a 2 cm depth on October 29, 2012. A third of the nitrogen was applied at the time of sowing, a third at the beginning of stem elongation and the rest at the flowering period. The plots were hand weeded during the season. Maximum and minimum temperatures and precipitation were recorded at 35 °C, -4 °C and 485 mm respectively. All plant protection measures were applied to ensure that the crop was free of insects. Seed yield (adjusted to kg ha⁻¹) was recorded based on the two middle rows of each plot, and plant height and yield components were measured based on 10 randomly selected plants in each plot.

Combining ability was analyzed using partial circulant diallel analysis with different numbers of parents (N=4, 5, 6, 7, 8) and their F₁ generations using Griffing's method 2 with mixed-B model (Griffing 1956). The linear model of observations in this method is of the form:

$$X_{ij} = \mu + g_i + q_j + s_{ij} + \frac{1}{b} \sum_{k=1}^b e_{ijk}$$

($i=j=1, \dots; k=1, \dots, b$), where μ - the population mean; g_i - the general combining ability effect of the i th parent; q_j - the general combining ability effect of the j th parent; s_{ij} - the specific combining ability effect of the cross between the i th and j th parents, such that $s_{ij} = s_{ji}$; and e_{ijk} - the environmental effect associated with the ijk th observation.

$$V_{(GCA)} = \frac{MS_{(GCA)} - MS_E}{n+2} = \frac{1+F}{4} V_A$$

$$V_{(SCA)} = MS_{(SCA)} - MS_E$$

$$V_{(SCA)} = COV_{F,S} - 2COV_{H,S} = \frac{1+F}{2} (V_A + V_D) - 2 \frac{1+F}{4} V_A = \frac{1+F}{2} V_D$$

$$\text{If } F=1: V_{(GCA)} = \frac{1}{2}V_A \text{ and } V_{(SCA)} = V_D$$

where $V_{(GCA)}$ = variance of general combining ability, $MS_{(GCA)}$ = mean square of general combining ability, MS_E = mean square error, n = number of parents, F = coefficient of inbreeding, V_A = additive variance, $V_{(SCA)}$ = variance of specific combining ability, $MS_{(SCA)}$ = mean square of specific combining ability, $COV_{F.S}$ = covariance between full-sibs, $COV_{H.S}$ = covariance between half-sibs, and V_D = dominance variance. All analyses were performed using MS-Excel and SAS software (Zhang and Kang 1997).

3. Results and discussion

The results of diallel analysis using Griffing's method 2 with mixed-B model for traits including days to flowering, days to maturity, plant height, first branching height, number of branches and seed yield for parent numbers 4, 5, 6, 7 and 8 are presented in Tables 1, 2, 3, 4 and 5. Significant mean squares of GCA for the parents and also SCA for their crosses were obtained for the traits in most of the analyses, indicating the importance of additive and non-additive genetic effects for these traits. Similarly, significant GCA and SCA effects have been reported for some important agronomic traits in *Brassica napus* L. (Downey and Rimer 1993; Rameeh 2010) and other brassica species (Nassimi et al. 2006). Due to the use of different numbers of parents in partial circulant diallel analyses, the SCA mean square of the traits was more affected than the GCA mean squares. The SCA mean square was not significant for first branching height and number of branches in the diallel analysis with number of parents $N=7$, similarly for plant height with $N=6$, for pods on main raceme with $N=5$, and for days to flowering, first branching height and pods on main raceme with $N=4$.

Table 1. Diallel analysis of phenological traits, plant height, yield components and seed yield of eight parents (N=8) of rapeseed (*Brassica napus* L.) based on Griffing's method 2

S.O.V.	df	M.S.						
		Days to flowering	Days to maturity	Plant height	First branching height	Number of branches	Pods on main raceme	Seed yield
Replication	2	16.1	4.7	275.3*	906.9**	1.60	79.9	1571000**
Crosses	35	913.3**	424.8**	593.7**	610.0**	1.91**	359.4**	387481**
GCA	7	3979.2**	798.8**	1526.9**	1469.8**	4.77**	1181.7**	975122**
SCA	28	146.8**	331.3**	360.4**	395.1**	1.20*	153.9**	240570**
Error	70	7.6	2.7	151.1	178.0	0.72	27.5	64580

*, ** Significant at $p < 0.05$ and 0.01 , respectively, GCA: general combining ability, SCA: specific combining ability

Table 2. Diallel analysis of phenological traits, plant height, yield components and seed yield of seven parents (N=7) of rapeseed (*Brassica napus* L.) based on Griffing's method 2

S.O.V.	df	M.S.						
		Days to flowering	Days to maturity	Plant height	First branching height	Number of branches	Pods on main raceme	Seed yield
Replication	2	25.2*	4.4	260.8	307.7	1.58*	53.1	1392000**
Crosses	27	600.1**	271.4**	426.6**	488.9**	1.86**	290.4**	367177**
GCA	6	2489.8**	431.7**	1059.4**	1177.5**	5.83**	894.0**	886969**
SCA	21	60.2**	225.7**	245.8*	292.2	0.73	117.9**	218665**
Error	54	7.4	1.6	125.6	167.2	0.49	28.7	65840

*, ** Significant at $p < 0.05$ and 0.01 , respectively, GCA: general combining ability, SCA: specific combining ability

Table 3. Diallel analysis of phenological traits, plant height, yield components and seed yield of six parents (N=6) of rapeseed (*Brassica napus* L.) based on Griffing's method 2

S.O.V.	df	M.S.						
		Days to flowering	Days to maturity	Plant height	First branching height	Number of branches	Pods on main raceme	Seed yield
Replication	2	23.3*	1.2	163.3	326.5	0.40	29.3	920920**
Crosses	20	50.6**	55.7**	360.7**	502.9**	2.09**	266.5**	397724**
GCA	5	136.1**	139.7**	1053.1**	1218.6**	5.88**	703.4**	1079000**
SCA	15	22.0**	27.8**	130.0	264.4*	0.82*	120.8**	170686**
Error	40	5.9	1.7	109.6	136.0	0.37	28.0	54272

*, ** Significant at $p < 0.05$ and 0.01 , respectively, GCA: general combining ability, SCA: specific combining ability

Table 4. Diallel analysis of phenological traits, plant height, yield components and seed yield of five parents (N=5) of rapeseed (*Brassica napus* L.) based on Griffing's method 2

S.O.V.	df	M.S						
		Days to flowering	Days to maturity	Plant height	First branching height	Number of branches	Pods on main raceme	Seed yield
Replication	2	17.2	1.4	24.0	131.0	0.05	25.0	487678**
Crosses	14	37.7**	12.9**	396.7**	467.1**	2.26**	89.7**	446317**
GCA	4	110.9**	23.9**	1179.0**	1183.7**	5.67**	243.3**	1282000**
SCA	10	8.4*	8.5**	83.8*	180.4*	0.90*	28.3	112185**
Error	28	5.1	1.4	41.7	89.9	0.35	21.6	31374

*, ** Significant at $p < 0.05$ and 0.01 , respectively, GCA: general combining ability, SCA: specific combining ability

Table 5. Diallel analysis of phenological traits, plant height, yield components and seed yield of four parents (N=4) of rapeseed (*Brassica napus* L.) based on Griffing's method 2

S.O.V.	df	M.S						
		Days to flowering	Days to maturity	Plant height	First branching height	Number of branches	Pods on main raceme	Seed yield
Replication	2	19.7	0.83	61.9	152.9	0.08	21.3	264710**
Crosses	9	45.3**	9.89**	541.5**	700.5**	2.52**	89.8**	351221**
GCA	3	121.3**	15.13**	1379.1**	1641.6**	5.90**	221.4**	856083**
SCA	6	7.4	7.26**	122.7*	230.0	0.84**	24.0	98791**
Error	18	7.0	1.24	54.5	121.2	0.11	23.4	27475

*, ** Significant at $p < 0.05$ and 0.01 , respectively, GCA: general combining ability, SCA: specific combining ability

Biometric genetic parameters

The GCA-to-SCA mean square ratio ($MS_{(GCA)}/MS_{(SCA)}$), which indicates the prime importance of additive genetic effects, ranged from 20.85 to 2.85, for days to flowering and days to maturity respectively (Table 6). The greatest variation of this genetic parameter was related to days to flowering, and its lowest variation to days to maturity. Due to the reduction of the number of parents in diallel analysis, except for days to flowering and days to maturity, for the other traits the mean square ratio ($MS_{(GCA)}/MS_{(SCA)}$) increased. The greatest amounts of variation of this index were related to days to flowering, days to maturity, plant height and seed yield.

Additive variance (V_A) as affected by the different numbers of parents in the diallel analysis, and its trend, varied between the traits. A high value of the

coefficient of variation (CV) of additive variance (V_A) for the use of different numbers of parents was recorded for days to flowering and days to maturity (Table 6). Singh and Chaudhry (2004) state that the heritability of a trait approaches its maximum in successive generations following hybridization. In addition, the presence of additive gene effects for a trait indicates that selection may be successful for the trait (Mather and Jinks 1982; Kearsley and Pooni 1996).

For all traits, as the number of parents in the diallel analysis was reduced, dominance variance (V_D) greatly decreased. Large variation of V_D was found for days to flowering, days to maturity, plant height and pods on main raceme. Dominance variance ranged from 0.49 to 119,471 for number of branches and seed yield respectively. The greatest reduction of V_D for a low number of parents ($N=4$) in diallel analysis was obtained for days to flowering.

Heritability, a measure of the phenotypic variance attributable to genetic causes, has a predictive function in crop breeding. The higher the heritability estimates, the simpler are the selection procedures (Mather and Jinks 1982). High narrow-sense heritability was estimated for days to flowering and pods on main axis. Khan and Khan (2005) performed a complete diallel cross analysis in rapeseed and reported that number of pods per plant, 1000-seed weight and seed yield per plot had moderate narrow-sense heritability. The greatest fluctuation of narrow-sense heritability estimates obtained from partial circulant diallel analyses was detected for days to maturity, followed by plant height and seed yield (Table 6). For diallel analysis with a low number of parents ($N=5, 6$) the differences of the heritability estimates of the traits were low; therefore, for precise estimation of heritability, a high number of diallel crosses will be preferred. In a simulation study, Pederson (1972) concluded that circulant diallels yield good estimates of heritability. Results reported by other authors (Pederson 1972; Ferreira et al. 2004) have shown that when a small number of crosses is used, the heritability values are less reliable because the estimates tend to be farther away from the parametric values. Results reported in the literature concerning the number of crosses per parent (s) in circulant diallels are quite different.

The degree of dominance ranged from 0.27 to 1.11, for days to flowering and days to maturity respectively. High variation in the degree of dominance due to the use of different numbers of parents was determined for days to flowering, plant height and pods on main raceme; therefore, a high number of parents should be used in diallel crosses for greater precision as regards the degree of dominance of these traits.

Table 6. Biometric genetic parameters of phenological traits, plant height, yield components and seed yield of rapeseed (*Brassica napus* L.) based on Griffing's method 2

Biometric genetic parameters	Number of parents	Days to flowering	Days to maturity	Plant height	First branching height	Number of branches	Pods on main raceme	Seed yield
MS(GCA)/ MS(SCA)	N=8	27.11	2.41	4.24	3.72	3.98	7.68	4.05
	N=7	41.36	1.91	4.31	4.03	7.99	7.58	4.06
	N=6	6.19	5.03	8.10	4.61	7.17	5.82	6.32
	N=5	13.20	2.81	14.07	6.56	6.30	8.60	11.43
	N=4	16.39	2.08	11.24	7.14	7.02	9.23	8.67
	mean	20.85	2.85	8.39	5.21	6.49	7.78	6.91
CV (%)	65.81	44.50	51.37	29.60	23.52	16.59	45.89	
V _(A)	N=8	766.48	93.50	233.30	214.94	0.71	205.56	146910
	N=7	539.91	45.78	180.80	196.73	1.13	172.47	148512
	N=6	28.53	27.98	230.78	238.55	1.27	145.65	227079
	N=5	29.29	4.40	312.91	286.66	1.36	61.43	334233
	N=4	37.97	2.62	418.80	470.53	1.69	65.80	252431
	mean	280.44	34.86	275.32	281.48	1.23	130.18	221833
CV (%)	124.66	107.08	33.82	39.41	29.00	49.46	35.34	
V _(D)	N=8	139.20	328.60	209.30	217.10	0.48	126.40	175990
	N=7	52.80	224.10	120.20	125.00	0.24	89.20	152825
	N=6	16.10	26.10	20.40	128.40	0.45	92.80	116414
	N=5	3.30	7.10	42.10	90.50	0.55	6.70	80811
	N=4	0.40	6.02	68.20	108.80	0.73	0.60	71316
	mean	42.36	118.38	92.04	133.96	0.49	63.14	119471
CV (%)	136.96	125.92	81.92	36.47	36.13	89.09	37.74	
h ² _N	N=8	0.84	0.22	0.39	0.35	0.37	0.57	0.38
	N=7	0.90	0.17	0.42	0.40	0.61	0.59	0.40
	N=6	0.56	0.50	0.64	0.47	0.61	0.55	0.57
	N=5	0.78	0.34	0.79	0.61	0.60	0.68	0.75
	N=4	0.84	0.27	0.77	0.67	0.67	0.73	0.72
	mean	0.78	0.30	0.60	0.50	0.57	0.62	0.56
CV (%)	16.86	42.75	31.42	27.28	20.33	12.39	30.67	
d/a	N=8	0.30	1.33	0.67	0.71	0.58	0.55	0.77
	N=7	0.22	1.56	0.58	0.56	0.33	0.51	0.72
	N=6	0.53	0.68	0.21	0.52	0.42	0.56	0.51
	N=5	0.24	0.90	0.26	0.40	0.45	0.23	0.35
	N=4	0.07	1.07	0.29	0.34	0.47	0.07	0.38
	mean	0.27	1.11	0.40	0.51	0.45	0.38	0.55
CV (%)	61.49	31.31	51.74	28.56	20.06	57.75	35.20	

V_(A): additive variance, V_(D): dominant variance, h²_N: narrow-sense heritability, d/a: degree of dominance

4. Conclusions

All biometric genetic parameters were affected by a reduction in the number of parents used in diallel analysis. The greatest variation in $MS_{(GCA)}/MS_{(SCA)}$ was related to days to flowering, and the lowest variation to days to maturity. Due to reduction of the number of parents in diallel analysis, except for days to flowering and days to maturity, for the other traits $MS_{(GCA)}/MS_{(SCA)}$ increased. The greatest fluctuation of narrow-sense heritability estimates obtained from partial circulant diallel analyses was detected for days to maturity, followed by plant height and seed yield. With a low number of parents in diallel analysis the differences in the heritability estimates of the traits were low; therefore, for precise estimation of heritability, a high number of diallel crosses will be preferred.

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