

COMBINING ABILITY STUDIES FOR BIOCHEMICAL TRAITS IN *BRASSICA RAPA* (L.) SSP. *DICHOTOMA* (ROXB.) HANELT

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Abstract

Combining ability was studied in 6 x 6 complete diallel cross in *Brassica rapa* (L.) ssp. *dichotoma* (Roxb.) Hanelt. Significant differences were observed for all the traits except protein and glucosinolate content. GCA mean squares were non-significant for all the traits. SCA and RCA components of variation were significant for all the traits with the respective exception of oleic acid and oil content. Prevalence of non-additive genetic control was detected for all the traits except oleic acid content for which maternal effects were more important. ACC-403, ACC-909 were good general combiners for oil content and linolenic acid; ACC-902, ACC-1500 for oleic acid and ACC-265 the worse general combiner for erucic acid. Based on desirable SCA effects and mean performance the cross combinations ACC-265 x ACC-909, ACC-403 x ACC-1500 (oil content); ACC-909 x ACC-902, ACC-909 x ACC-1500 (oleic acid content); ACC-403 x ACC-1500 (linolenic acid content) and ACC-265 x ACC-403; ACC-909 x ACC-1500; ACC-265 x ACC-902 (erucic acid content) were superior and can be further exploited.

Introduction

Rapeseed belongs to the family *Cruciferae* (Hatam & Abbasi, 1994) has about 3709 species and 338 genera (Warwick *et al.*, 2006) and is one of the ten most economically important plant families (Rich, 1991). The oilseed *Brassica* is third most important source on global scale (after palm and soybean) whereas second in Pakistan after cotton seed. These are also used as food, feed, in biodiesel (Marjanović-Jeromela *et al.*, 2007) and fertilizers production (French, 1977).

Pakistan has made improvement in the development of agriculture sector but the chronic shortage of edible oil has persisted and national requirement of edible oil is going to increase even further in the coming years due to high population growth rate and increase in per capita consumption (Farhatullah *et al.*, 2004). Despite the efforts made to boost the domestic edible oil production, there is no significant impact (Anjum *et al.*, 2005), which cost an enormous amount of foreign exchange (Amad *et al.*, 2013). Sunflower, canola, cottonseed, rapeseed and mustard are the major oilseed crops in Pakistan. Rapeseed and mustard were planted over 0.203 million hectares during the season 2010-11, resulted in a production of 0.176 million tons. Local edible oil production 0.696 million tons (from all sources) accounted for 34% of total edible oil requirement in the country, while the remaining 66% was imported at the expense of \$1,660.3 million (Anonymous, 2011-12). Research on *Brassica* germplasm could enhance the edible oil production and nutritional benefits of the crop (Zada *et al.*, 2013).

High yielding varieties with high oil percentage and reduced glucosinolates and erucic acid contents are required bridge the consumption production gap and reduce this exchequer. Germplasm collection from within the country and outside is a pre-requisite for developing high yielding and better quality genotypes of *Brassica* (Khan & Khan, 2003).

Diallel, line x tester, top cross and poly cross may be used by breeders to choose appropriate parents *via* combining ability analysis for hybridization schemes (Aghao *et al.*, 2010; Marjanović-Jeromela *et al.*, 2007;

Falconer & Mackay, 1996). A commonly used crossing scheme for inbred lines is the diallel, in which each line is crossed with every other line (Falconer & Mackay, 1996) to study mode of inheritance (Ahmad *et al.*, 2013). Combining ability is used as a tool for forecasting the performance of yet untested crosses (Simmonds, 1979). General combining ability (GCA) refers to average performance of an inbred in a series of cross combinations, and specific combining ability (SCA) is the deviation of inbred lines in hybrid combination from the individual average performance of the inbred lines (Sprague & Tatum, 1942). GCA and SCA variances are attributed to additive portion and non-additive portion (mainly dominance and epistatic deviations) of total variance respectively (Malik *et al.*, 2004). Non destruction of seed and small sample requirement makes NIRS (Near Infra-red Reflectance Spectroscopy) suitable for evaluating *Brassica* germplasm (Ali *et al.*, 2012).

The present study aimed at identification of the superior general combiners and their F₁ hybrids on the basis of GCA, SCA and reciprocal effects (RCA) for quality traits, the mode of gene action predominantly operative in expression of these traits.

Material and Methods

A 5 x 5 full diallel in *Brassica rapa* (L.) ssp. *dichotoma* (Roxb.) Hanelt (syn. *B. campestris* var. brown sarson) derived from parents (ACC-265, a plant introduction; ACC-403, collection from Swat (Pakistan), ACC-902 from Punjab (Pakistan), and ACC-909, ACC-1500 from Chitral (Pakistan), were planted during 2011-12 in RCB design with two replications, at The University of Agriculture, Peshawar during 2011-12. Each replication contained 25 sub-plots. Each sub-plot consisted of two rows with row length of four meters each. Row to row spacing was kept 50 cm. Plant to plant distance of 30 cm was maintained by thinning. Recommended cultural practices were followed from sowing till the harvesting. Data was recorded for oil, protein, glucosinolate, oleic acid, linolenic acid and erucic acid content using Near Infra red Reflectance

Spectroscopy facility at Nuclear Institute for Food and Agriculture (NIFA), Peshawar.

The analysis of variance was practiced according to Steel & Torrie (1980) using MS Excel. Combining ability analysis was conducted as outlined by Griffing (1956) Method-I, based on Eisenhart's Model-II using MS Excel to assess the genetic variances due to GCA, SCA and reciprocal effects.

Results and Discussion

Analysis of variance: Significant differences were observed for all the traits except protein and glucosinolate content (Table 1). Shen *et al.*, (2005); Qian *et al.*, (2007) reported highly significant differences for oil content. Turi *et al.*, (2010) reported highly significant differences for oil, glucosinolate,

protein, oleic acid and erucic acid content. These results are in agreement except for glucosinolate and protein content. Vaghela *et al.*, (2011) reported highly significant differences for oil, protein, oleic acid, linolenic acid and erucic acid content. Sabaghnia *et al.*, (2010) reported non-significant differences oil content in their experiment across two years.

Combining ability analysis: Combining ability analysis was practiced for traits exhibiting significant differences. GCA mean squares were non-significant for all the traits. SCA and RCA components of variation were significant for all the traits with the respective exception of oleic acid and oil content (Table 2). The various effects and predominant mode of genetic control are detailed as follow:

Table 1. Mean squares for oil, protein, glucosinolate, oleic acid, linolenic acid and erucic acid content in *B. rapa* (L.) ssp. *dichotoma* (Roxb.) Hanelt.

Plant traits	Mean squares		CV (%)
	Treatment (df=24)	Error (df=24)	
Oil content (%)	26.81*	11.83	7.9
Protein content (%)	17.68 ^{ns}	9.99	12.1
Glucosinolate content ($\mu\text{moles g}^{-1}$)	222.2 ^{ns}	170.3	15.4
Oleic acid content (%)	24.18*	12.43	7.7
Linolenic acid content (%)	3.336*	1.619	15.5
Erucic acid content (%)	29.82**	11.26	6.5

** Significant at $p \leq 0.01$, * Significant at $p \leq 0.05$, ns; non-significant

Table 2. Analysis of variance for combining ability for oil, oleic acid, linolenic acid and erucic acid content in *B. rapa* (L.) ssp. *dichotoma* (Roxb.) Hanelt.

Plant traits	Mean squares			
	GCA (df=4)	SCA (df=10)	RCA (df=10)	Error (df=24)
Oil content (%)	7.197ns	16.58*	12.71 ns	5.915
Oleic acid content (%)	8.227ns	9.057ns	16.67*	6.214
Linolenic acid content (%)	0.509ns	1.892*	1.907*	0.809
Erucic acid content (%)	9.001ns	18.46**	13.72*	5.632

** Significant at $p \leq 0.01$, * Significant at $p \leq 0.05$, ns; non-significant

Oil content: Positive combining ability effects for are desirable. Two out of five parental lines exhibited positive GCA effects. Maximum positive GCA effect was recorded for genotype ACC-403 (1.04) followed by for ACC-909 (0.58). Maximum negative GCA effect was achieved by genotype ACC-1500 (-0.31) (Table 3). Four out of 10 crosses exhibited positive SCA effects. Maximum positive SCA effects of 2.87 and 2.72 were noted for the hybrids ACC-265 x ACC-909 and ACC-403 x ACC-1500, respectively. Highest negative SCA effect was recorded for the cross combination ACC-909 x ACC-1500 (-3.94) (Table 4). RCA effects for five out of ten crosses were positive, the highest being 5.30 (ACC-403 x ACC-265) followed by 1.89 (ACC-902 x ACC-403) and ACC-902 x ACC-265 (1.34). Maximum negative RCA effect was noted for F₁ hybrid ACC-909 x ACC-902 (-4.43) (Table 5). Best general combiners (ACC-403; ACC-909), specific combiners (ACC-265 x ACC-909; ACC-403 x ACC-1500) also achieved good oil content and were among top three mean performers. Top reciprocal combiners (ACC-403 x

ACC-265 and ACC-902 x ACC-403) also achieved good oil content (Table 7). Significant SCA mean square (Table 2) indicated that non-additive genetic effects were operative which was supported by relative magnitudes of σ^2_{GCA} (-0.887), σ^2_{SCA} (6.348), σ^2_{RCA} (3.400) and $\sigma^2_{GCA}/\sigma^2_{SCA}$ (-0.140) (Table 6). Highly significant GCA and SCA mean squares for oil percentage were reported in some early studies in rapeseed (Rameeh, 2010; Sabaghnia *et al.*, 2010), Ethiopian mustard (Teklewold & Becker, 2005) and Indian mustard (Singh *et al.*, 2010; Vaghela *et al.*, 2011). These results are in agreement for SCA whereas in contrast for GCA effects. Shen *et al.*, (2005) highly significant GCA and non-significant SCA mean squares for oil content. Highly significant GCA, SCA and RCA effects for oil percentage were revealed (Suchindra & Singh, 2006; Turi *et al.*, 2010). Aher *et al.*, (2009) revealed prevalence of non-additive gene action in the expression of oil percentage. Huang *et al.*, (2010); Vaghela *et al.*, (2011) reported predominant role of additive genetic effects in inheritance of oil content.

Table 3. General combining ability effects oil content, oleic acid content, linolenic acid content and erucic acid content in *B. rapa* (L.) ssp. *dichotoma* (Roxb.) Hanelt.

Genotype	Oil content	Oleic acid content	Linolenic acid content	Erucic acid content
ACC-265	-1.15	-0.47	-0.18	-1.67
ACC-403	1.04	-1.29	0.18	0.29
ACC-902	-0.16	1.01	-0.25	0.45
ACC-909	0.58	0.14	0.28	0.27
ACC-1500	-0.31	0.60	-0.02	0.66
S.E(gi) ±	0.69	0.71	0.25	0.67
S.E(gi-gj) ±	1.09	1.11	0.40	1.06

Table 4. Specific combining ability effects oil content, oleic acid content, linolenic acid content and erucic acid content in *B. rapa* (L.) ssp. *dichotoma* (Roxb.) Hanelt.

Genotype	Oil content	Oleic acid content	Linolenic acid content	Erucic acid content
ACC-265 x ACC- 403	-3.54	-0.96	-1.63	-6.00
ACC-265 x ACC-902	-3.33	-0.17	1.23	-0.37
ACC-265 x ACC-909	2.87	-1.29	0.57	2.37
ACC-265 x ACC-1500	2.36	-1.21	0.38	0.54
ACC-403 x ACC-902	0.63	-0.82	0.11	0.32
ACC-403 x ACC-909	-1.18	2.56	-0.11	-1.54
ACC-403 x ACC-1500	2.72	-1.54	1.59	4.23
ACC-902 x ACC-909	-0.27	1.29	-0.71	0.47
ACC-902 x ACC-1500	-1.85	-0.05	-0.99	-0.26
ACC-909 x ACC-1500	-3.94	2.87	0.06	-3.94
S.E (Sij) ±	1.42	1.45	0.52	1.38
S.E (Sik-Skl) ±	1.88	1.93	0.70	1.84

Table 5. Reciprocal combining ability effects oil content, oleic acid content, linolenic acid content and erucic acid content in *B. rapa* (L.) ssp. *dichotoma* (Roxb.) Hanelt.

Genotype	Oil content	Oleic acid content	Linolenic acid content	Erucic acid content
ACC-403 x ACC-265	5.30	4.30	2.09	6.27
ACC-902 x ACC-265	1.34	-0.87	0.76	1.99
ACC-902 x ACC-403	1.89	-2.71	0.98	1.59
ACC-909 x ACC-265	-2.00	-1.05	0.69	2.16
ACC-909 x ACC-403	0.54	1.23	0.33	-2.18
ACC-909 x ACC-902	-4.43	3.84	-0.65	-2.91
ACC-1500 x ACC-265	-0.26	1.51	-0.96	0.05
ACC-1500 x ACC-403	-2.10	-5.01	0.88	-0.93
ACC-1500 x ACC-902	-1.00	2.05	-0.90	0.64
ACC-1500 x ACC-909	0.89	-2.81	0.34	1.92
S.E (rij) ±	1.72	1.76	0.64	1.68
S.E(rij-rkl) ±	2.43	2.49	0.90	2.37

Table 6. Estimates of variances due to general combining ability (σ^2_{GCA}), specific combining ability (σ^2_{SCA}), reciprocal combining ability (σ^2_{RCA}) and error (σ^2_e) for oil content, oleic acid content, linolenic acid content and erucic acid content in *B. rapa* (L.) ssp. *dichotoma* (Roxb.) Hanelt..

Plant traits	σ^2_{GCA}	σ^2_{SCA}	σ^2_{RCA}	σ^2_e	$\sigma^2_{GCA}/\sigma^2_{SCA}$
Oil content (%)	-0.887	6.348	3.400	5.915	-0.140
Oleic acid content (%)	-0.069	1.692	5.228	6.214	-0.041
Linolenic acid content (%)	-0.133	0.644	0.549	0.809	-0.207
Erucic acid content (%)	-0.885	7.634	4.044	5.632	-0.116

Table 7. Mean performance of hybrids and parents for the traits of oil content, protein content and glucosinolate content in *B. rapa* (L.) ssp. *dichotoma* (Roxb.) Hanelt.

Code	Oil content (%)	Protein content (%)	Glucosinolate content ($\mu\text{moles g}^{-1}$)
ACC-265	42.75	26.45	79.95
ACC-403	46.86	22.22	65.11
ACC-902	47.90	21.41	91.53
ACC-909	47.10	22.65	91.15
ACC-1500	43.49	26.53	69.35
ACC-265 x ACC-403	34.46	30.69	88.00
ACC-265 x ACC-902	37.43	30.85	95.71
ACC-265 x ACC-909	47.70	22.89	72.81
ACC-265 x ACC-1500	44.56	24.35	92.40
ACC-403 x ACC-265	45.06	24.56	76.80
ACC-403 x ACC-902	43.03	23.74	97.34
ACC-403 x ACC-909	43.31	27.28	94.00
ACC-403 x ACC-1500	48.95	21.50	63.35
ACC-902 x ACC-265	40.10	30.24	95.28
ACC-902 x ACC-403	46.80	24.47	92.29
ACC-902 x ACC-909	47.99	23.56	91.35
ACC-902 x ACC-1500	42.08	27.22	83.87
ACC-909 x ACC-265	43.71	27.16	68.58
ACC-909 x ACC-403	44.40	25.74	90.35
ACC-909 x ACC-902	39.13	29.08	75.45
ACC-909 x ACC-1500	38.85	30.14	98.61
ACC-1500 x ACC-265	44.04	25.49	80.70
ACC-1500 x ACC-403	44.75	26.45	88.29
ACC-1500 x ACC-902	40.08	29.44	86.27
ACC-1500 x ACC-909	40.62	29.23	89.43
LSD _(0.05)	7.009	-	-

Oleic acid content: Positive combining ability effects for oleic acid content are desirable for nutritional and industrial purposes. Parental lines ACC-902, ACC-909 and ACC-1500 exhibited positive GCA effects of 1.01, 0.14 and 0.60, respectively. Maximum negative GCA effect was recorded for genotype ACC-403 (-1.29) followed by ACC-265 (-0.47) (Table 3). Three out of 10 crosses exhibited positive SCA effects. The maximum positive effect was noted for the combination ACC-909 x ACC-1500 (2.87) followed by ACC-403 x ACC-909 (2.56) and ACC-902 x ACC-909 (1.29). Maximum negative SCA effect was noted for the hybrid ACC-403 x ACC-1500 (-1.54) (Table 4). RCA effects for five out of ten crosses were positive, ranged 1.23 to 4.30. Highest positive RCA effect was noted for F₁ hybrid ACC-403 x ACC-265 (4.30) followed by ACC-909 x ACC-902 (3.84). Maximum negative RCA effect was noted for ACC-1500 x ACC-403 (-5.01) (Table 5). Promising general combiners (ACC-902; ACC-1500)

and specific combiner (ACC-909 x ACC-1500) were among top three on the basis of mean performance. Superior reciprocal combiner (ACC-909 x ACC-902) was also among top three mean performers on mean basis (Table 8). Significant RCA effects (Table 2) indicated involvement of cytoplasmic genes. Prevalence of maternal effects was also evident from relative estimates of -0.069, 1.692, 5.228 and -0.041 for σ^2_{GCA} , σ^2_{SCA} , σ^2_{RCA} and $\sigma^2_{GCA}/\sigma^2_{SCA}$, respectively (Table 6). Schierholt *et al.*, (2001) reported highly significant GCA and non-significant SCA and prevalence of additive genetic effects for the trait under consideration. Vaghela *et al.*, (2011) revealed highly significant GCA and SCA effects and predominant role of additive genetic effects in inheritance of oleic acid percentage. These results are in contrast to the current study. Results for oleic acid are in conformity with Turi *et al.*, (2010). They reported Significant RCA; non-significant GCA and SCA effects.

Table 8. Mean performance of hybrids and parents for the traits of oleic acid content, linolenic acid content and erucic acid content in *B. rapa* (L.) ssp. *dichotoma* (Roxb.) Hanelt..

Code	Oleic acid content (%)	Linolenic acid content (%)	Erucic acid content (%)
ACC-265	48.21	7.28	51.51
ACC-403	43.70	8.58	54.95
ACC-902	47.30	8.03	52.14
ACC-909	40.36	8.93	54.57
ACC-1500	46.66	7.09	52.15
ACC-265 x ACC-403	38.50	4.47	37.73
ACC-265 x ACC-902	46.76	8.23	47.81
ACC-265 x ACC-909	44.94	8.17	50.19
ACC-265 x ACC-1500	42.93	9.33	50.88
ACC-403 x ACC-265	47.10	8.65	50.28
ACC-403 x ACC-902	47.14	7.23	50.86
ACC-403 x ACC-909	45.70	8.20	52.59
ACC-403 x ACC-1500	48.30	9.05	57.50
ACC-902 x ACC-265	45.02	9.75	51.78
ACC-902 x ACC-403	41.72	9.20	54.04
ACC-902 x ACC-909	44.12	8.15	55.48
ACC-902 x ACC-1500	45.03	7.82	51.61
ACC-909 x ACC-265	42.85	9.54	54.51
ACC-909 x ACC-403	48.16	8.86	48.24
ACC-909 x ACC-902	51.80	6.85	49.67
ACC-909 x ACC-1500	51.94	8.16	46.47
ACC-1500 x ACC-265	45.96	7.41	50.97
ACC-1500 x ACC-403	38.28	10.8	55.64
ACC-1500 x ACC-902	49.13	6.03	52.88
ACC-1500 x ACC-909	46.33	8.85	50.30
LSD _(0.05)	7.277	2.63	6.926

Linolenic acid content: Two out of five parental lines viz. ACC-403 and ACC-909 exhibited positive GCA effects of 0.18 and 0.28, respectively. Maximum negative GCA effect was recorded for ACC-902 (-0.25) (Table 3). Six out of 10 crosses exhibited positive SCA effects, ranged 0.06 to 1.59. Maximum positive effect was noted for the cross combination ACC-403 x ACC-1500 (1.59) followed by ACC-265 x ACC-902 (1.23) whereas maximum negative SCA effect was noted for the hybrid ACC-265 x ACC-403 (-1.63) (Table 4). RCA effects for seven out of ten crosses were positive, ranged 0.33 to 2.09. Highest RCA effect was noted for ACC-403 x ACC-265 (2.09) followed by ACC-902 x ACC-403 (0.98). Maximum negative RCA effect was noted for ACC-1500 x ACC-265 (-0.96) (Table 5). General combiners (ACC-403 and ACC-909) were also top mean performers for LA. Specific combiner ACC-403 x ACC-1500 performed well. Superior reciprocal combiners (ACC-403 x ACC-265; ACC-902 x ACC-403) exhibited average mean performance (Table 8). Involvement of non-additive and maternal effects was evident from significant SCA and RCA mean squares (Table 2). Relative magnitude of σ^2_{GCA} (-0.133), σ^2_{SCA} (0.644), σ^2_{RCA} (0.549) and $\sigma^2_{GCA}/\sigma^2_{SCA}$ (-0.207) indicated that both non-additive and cytoplasmic effects were predominant in control of the trait (Table 6). Vaghela *et al.*, (2011) revealed highly significant GCA and SCA effects for linolenic acid. These results are in contrast for GCA whereas in conformity for SCA effects found in the

current study. They also reported predominant role of additive genetic effects in inheritance of linolenic acid.

Erucic acid content: Negative combining ability effects for erucic acid content are desirable. Among the parental lines only ACC-265 exhibited negative GCA effect (-1.67). Maximum positive GCA effect was recorded for parent ACC-1500 (0.66) followed by ACC-902 (0.45) (Table 3). Five out of 10 hybrids displayed negative SCA effects. Maximum negative SCA effect was noted for the cross combination ACC-265 x ACC-403 (-6.00) followed by ACC-909 x ACC-1500 (-3.94) whereas maximum positive SCA effect was recorded for the hybrid ACC-403 x ACC-1500 (4.23) (Table 4). RCA effects for three out of ten crosses were negative, the highest being -2.91 for F₁ hybrid ACC-909 x ACC-902 followed by -2.18 (ACC-909 x ACC-403). Maximum positive RCA effect was noted for the hybrid ACC-403 x ACC-265 (6.27) (Table 5). Best desirable general combiner (ACC-265) also achieved desirable mean performance. Superior specific combiners (ACC-265 x ACC-403; ACC-909 x ACC-1500; ACC-265 x ACC-902) were also top three mean performers. Promising reciprocal combiners (ACC-909 x ACC-902; ACC-909 x ACC-403) also achieved desirable mean performance (Table 8). Significant SCA and RCA mean squares depicted that non-additive and maternal genetic effects were involved (Table 2). Preponderance of non-additive control was revealed by the relative magnitude of -0.885, 7.634, 4.044 and -0.116 for σ^2_{GCA} ,

σ^2_{SCA} , σ^2_{RCA} and $\sigma^2_{GCA}/\sigma^2_{SCA}$, respectively (Table 6). Results of the study are strengthened by earlier findings of Turi *et al.*, (2010). They reported non-significant GCA and highly significant SCA and RCA mean squares. Vaghela *et al.*, (2011) revealed highly significant GCA and SCA effects. Their results are in conformity for SCA mean squares. They also reported predominant role of additive genetic effects for the trait under reference.

Breeding methodologies based on type of gene action involved in trait expression are likely to fetch better results. Use of schemes like bi-parental mating design, diallel selective mating followed by recurrent selection is necessitated by prevalence of non-additive genetic control for all the traits except oleic acid content. Similar schemes were recommended in some earlier investigations (Suchindra & Singh, 2006; Vaghela *et al.*, 2011). Cross order should be considered while breeding for oleic acid content.

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