



Combining ability of yield attributes traits, oil and protein contents in oil seed rape (*Brassica napus*) under normal and restricted nitrogen application

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ABSTRACT

Combining ability of yield associated traits has been studied quite frequently in oilseed rape (*Brassica napus* L.) but it was hardly ever studied based on nitrogen levels variations. Six parents along with their 15 F₂ half diallel crosses were evaluated in two nitrogen (N) levels including N₀ and N₊. Analysis of variance revealed significant mean squares of general and specific combining abilities (GCA and SCA) for all the traits studied. The high narrow-sense heritability estimates were found for seed yield and harvest index, implying the more importance of additive genetic effects for and consequently high selection efficiency for improving these traits. Due to low narrow-sense heritability estimates for oil and protein contents and therefore more importance of non-additive genetic effects, selection for improving these traits should be done in later segregating generations. The GCA × N level interaction was significant only for oil content which is indicating of more differences of GCA effect of this trait at two nitrogen levels. Significant N × SCA effect mean squares for harvest index and oil content, revealed significant variation of SCA effects of these traits in nitrogen levels application. Most of the crosses with high seed yield at N₀ and N₊ had at least one parent with positive GCA effect at both N levels. Among the six parents, PF7045/91 appeared to be the best combiner for seed yield at the N₀. This variety also showed high GCA at the N₊ but its low oil content at the N₊ is certainly disadvantageous as the quality of the hybrid is often intermediate between the two parents.

Key words : Additive, Diallel, Environment, Narrow-sense heritability, Oilseed rape

Plant stress is a major limitation to crop yield. The breeding of crop varieties tolerant to environmental stresses is the most effective economical means to improve and stabilize yield under different stress conditions. High rates of nitrogen fertilizer are usually applied to oilseed crops in order to obtain maximum seed yields. A number of investigations showed that nitrogen fertilizers gave substantial oil seed rape (*Brassica napus* L.) yield increases even in diverse and contradicting conditions (Maroni *et al.* 1994, Colnenne *et al.* 1998). However, fertilizer nitrogen requirements can differ significantly according to soil type, climate, management practices, timing of nitrogen application and cultivars used (Holmes and Ainsley 1977, Kalkafi *et al.* 1998). A great variation in nitrogen uptake in oil seed rape has been reported (Holmes 1980). When compared to cereals, oil seed rape requires more nutrients and available nitrogen frequently restricts seed yield. Colnenne *et al.* (1998) proposed that oil seed rape has a higher critical N demand for biomass formation than wheat. While a substantial amount of N is provided by conversion of previous crop residues and soil

organic matter into soluble soil N, additional mineral N is a prerequisite for high yields (Rathke *et al.* 2005).

Eventhough most of the seed is produced by open-pollinated oil seed rape cultivars, it is well known that canola has adequate heterosis to justify efforts to develop hybrid cultivars (Rameeh 2012). Information on general and specific combining ability effects (GCA and SCA) related to yield components, seed yield and quality traits, viz. oil and protein contents of Brassica is very important in conducting a successful breeding program to achieve sufficient heterosis (Malik *et al.* 2004). In addition, information about the nature of gene action involved in expression of quantitative and qualitative traits of economic importance is also required to develop desirable lines. Seed yield is a complex trait that includes various components and finally results in a highly plastic yield structure (Jeromela *et al.* 2007). Yield per area is the product of population density, the number of pods/plant, the number of seeds/pod and the individual seed weight. While examining the genetic control of grain yield in oilseed rape both additive and non-additive gene effects have been found to be involved (Marjanovic-Jeromela *et al.* 2007, Singh *et al.* 2010). Most of the studies showed significant GCA and SCA effects for yield and its component characters indicating

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that both additive and non-additive gene action were important in the inheritance of these traits (Mahto and Haider 2004, Huang *et al.* 2010, Azizinia 2011). Earlier breeders concluded in their research that with the changes in environment gene effects for different traits contributing to yield or yield itself changes in oil seed rape therefore for different environment one has to suggest different selection criteria for the improvement in the yield. For those traits that are controlled by additive gene action, simple selection in early segregating generation is suggested, whereas for those traits controlled by non-additive gene action, selection in later segregating generation would be more effective (Cheema and Sadaqat 2004).

Though F_1 data of diallel crosses is mostly used to estimate the genetic parameters in Griffing's method (1956), but it is usually difficult to obtain sufficient F_1 seeds especially for multi location testing in rape. Due to production of large quantity of F_2 seeds, many researchers use F_2 generation for diallel analysis to estimate combining abilities (Rameeh 2010). These researchers all reported that F_2 diallel analysis provide reliable and better information than F_1 generation. Multi locations or conditions testing of GCA and SCA effects and other genetic parameters will reveal the stability of these parameters for selection criteria (Mahter and Jinks 1982).

Although diallel analysis are frequently used in oil seed rape breeding to assess general and combining abilities for traits but the most of these studies were conducted at normal application of nitrogen levels. The objectives of this study were therefore (i) to evaluate whether F_2 oil seed rape hybrids utilize nitrogen more efficiently than pure lines at low and high N levels, and (ii) to identify genetic parameters for N utilization among a set of adapted cultivars.

MATERIALS AND METHODS

Six spring cultivars of oil seed rape including RGS003, Option500, RW008911, RAS-3/99, 19H and PF7045/91, selected based on their different agronomic characters were crossed in half diallel method during 2004-05. In order to produce F_2 progenies, fifteen F_1 s were selfed at Baikola Agriculture Research Station, Neka, Iran during winter 2005-06. F_2 progenies along with six parents were grown in a randomized complete block design (RCBD) with four replications at two experiments including N_0 (without nitrogen) and N_+ (150 kg nitrogen per hectare) during 2006-07. The plots related to each experiment were consisted of four rows 5 m long and 40 cm apart. The distance between plants on each row was 5 cm resulting in approximately 400 plants/plot, which were sufficient for F_2 genetic analysis in each experiment. The soil was classified as a deep loam soil (Typic Xerofluents, USDA classification) contained an average of 280 g clay/kg, 560 g silt/kg, 160 g sand/kg, and 22.4 g organic matter/kg with a pH of 7.3. Soil samples were found to have 45 kg/ha (mineral N in the upper 30-cm profile).

Fertilized experiment (N_+) received 150 kg/ha N as urea (50 kg N at planting time, beginning of stem elongation, and at initial of flowering stage) while unfertilized experiment (N_0) received no N. All the plant protection measures were adopted to make the crop free from insects. Twenty plant samples selected from middle part of each row were used to measure the traits including biological yield and harvest index. Seed yield of three middle rows of each plot, excluding the border area, was measured at physiological maturity and yield was adjusted to 12.5% seed moisture content. Oil content was estimated with the help of nuclear magnetic resonance spectrometry (Madson 1976). For analysis of nitrogen content, 100 mg of dried seed was digested with sulphuric acid and o-phosphoric acid in Kjeldhal digestion unit estimated by the method of Lemaire and Meynard (1997).

Analysis of variance for the crosses was based on Griffing's method 2, model 1 for fixed genotypes (Griffings 1956) and the linear model (Singh and Chaudhary 1985). The analysis was performed for individual as well as pooled over environments using the diallel-SAS programme (Zhang and Kang 1997). A *t*-test was used to test whether the GCA and SCA effects were different from 0 (Mather and Jinks 1982).

RESULTS AND DISCUSSION

Combining ability analysis in *Brassica* spp is normally conducted to find out suitable parents and more appropriately a cross with greater potential as hybrid or to produce desirable recombinant lines. Significant mean squares of two nitrogen levels (N_0 and N_+) for seed yield, biological yield, harvest index, oil and protein contents traits indicated that all the trait were significantly affected by nitrogen application (Table 1). Significant mean squares of GCA and SCA effects were indicating the importance of additive and non-additive genetic effects for these traits. Hence both types of gene effects may be employed in developing the superior genotypes. The high narrow-sense heritability estimates were found for seed yield and harvest index, implying the more importance of additive genetic effects and consequently high selection efficiency for improving these traits. The traits which are controlled by additive gene action, simple selection in early segregating generation is suggested (Cheema and Sadaqat 2004). Due to low narrow-sense heritability estimates for oil and protein contents and therefore more importance of non-additive genetic effects, selection for improving these traits should be done in later segregating generations and also one of the improvement strategies might be hybrid breeding (Sabaghnia *et al.* 2010, Azizinia 2011).

General combining ability and mean performances of the parents

The GCA \times N level interaction was significant only for oil content which indicated more differences of GCA effect of this trait at two nitrogen levels of application (Table 1).

Table 1 Combined analysis of variance for seed yield, biological yield, harvest index, oil and protein percentages of oil seed rape (*Brassica napus*) based on Griffing's method 2 at two nitrogen application levels (N_0 and N_+)

S.O.V	df	MS				
		Seed yield	Biological yield	Harvest index	Oil%	Protein%
Nitrogen (N)	1	27607326**	792818162**	199.31**	610.28**	392.60**
N(R)	6	269432	6966515	0.82	5.775	5.68
Treat	20	134484**	44290693**	142.18**	18.05**	18.55**
GCA	5	1238146**	69847918**	328.12**	17.90**	7.06**
SCA	15	1326241**	35739865**	77.42**	36.12**	22.65**
N xTreat	20	144202	4195099	8.45**	3.24**	1.52
N xGCA	5	99034	5081649	6.40	8.34**	1.92
NxSCA	15	162230	3832864	9.10**	2.46**	0.72
Pooled error	120	113421	2862715	3.45	1.73	1.63
	σ^2_A	374908	22328401	108	5	2
	σ^2_D	214669	58192556	131	31	37
	σ^2_{ph}	702998	83383672	243	68	41
	σ^2_g	58957	80520957	239	66	39
	h^2_N	0.53	0.38	0.83	0.09	0.05

*, ** Significant at $P < 0.05$ and 0.01 , respectively.

PF7045/91 had significant positive GCA effect for seed yield, therefore, this parent had high yield performance in crossing with different parents (Table 2). This implied that favorable genes for seed yield were present in PF7045/91 and using this line as a parent may increase this trait. Both additive and non-additive effects were also found to influence

oil content in *B. napus* (Lefort-Buson and Dattee 1982, Cheema and Sadaqat, 2004). Wang *et al.* 2010 indicated that oil content was mainly influenced by dominant and additive effects, with dominant effect the most important player. This parent had also the most seed yield performance at two nitrogen levels of application (Table 3). RAS-3/99 had positive

Table 2 Estimates of GCA effects for seed yield, biological yield, harvest index, oil and protein percentages of oil seed rape (*B. napus*) at two application nitrogen levels (N_0 and N_+)

Parents	Seed yield		Biological yield		Harvest index		Oil%		Protein%	
	N_0	N_+	N_0	N_+	N_0	N_+	N_0	N_+	N_0	N_+
RAS-3/99	70.86	42.75	271	754**	-0.56**	-1.05**	-0.73*	-0.55	0.35	0.47**
RW008911	-76.78	-31.98	-1173**	-1941**	3.48**	3.84**	-0.77*	-0.48	0.42	0.66**
19H	-46.84	-80.93	-193	-613*	-0.14	0.48	0.42	0.13	-0.27	-0.48**
RGS 003	-38.12	55.05	-653**	-268	1.82**	0.76**	0.43	0.64*	-0.16	-0.29
Option 500	-215.15**	-173.48*	100	733**	-3.08**	-2.83**	-0.34	0.63*	-0.51	0.12
PF7045/91	306.02**	188.60*	1649**	1336**	-1.51**	-1.20**	0.99**	-0.37	0.17	-0.49**

*, ** Significant at $P < 0.05$ and 0.01 , respectively.

Table 3 Means of seed yield, biological yield, harvest index, oil and protein percentages in six parents of oil seed rape at N_0 and N_+

Parents	Seed yield (kg/ha)		Biological yield (kg/ha)		Harvest index		Oil%		Protein%	
	N_0	N_+	N_0	N_+	N_0	N_+	N_0	N_+	N_0	N_+
RAS-3/99	1 650.0	2 635.5	7 365.8	14 579.6	22.40	18.33	38.48	42.20	20.19	22.88
RW008911	1 370.3	2 505.8	3 977.8	7 934.6	34.56	31.67	38.00	43.50	19.38	23.75
19H	1 705.0	2 447.9	5 810.4	9 867.3	29.45	25.98	39.25	45.25	18.35	21.89
RGS 003	1 720.0	2 971.8	5 413.0	11 652.4	31.76	25.49	40.00	46.20	18.33	21.54
Option 500	1 171.3	2 218.6	4 846.8	10 729.3	24.25	20.69	39.50	47.13	17.38	23.02
PF7045/91	2 165.0	3 131.9	8 673.2	14 213.9	25.04	22.07	39.75	41.43	21.37	23.18
LSD($\alpha=0.05$)	326.0	437.4	1 413.2	2 348.4	1.73	2.46	1.43	1.57	1.42	1.52
LSD($\alpha=0.01$)	402.5	540.1	1 744.7	2 899.3	2.14	3.04	1.76	1.94	1.75	1.88

Table 4 Estimates of SCA effects for seed yield, biological yield, harvest index, oil and protein percentages in the half diallel crosses of six parents of oil seed rape at N_0 and N_+

Parents	Seed yield		Biological yield		Harvest index		Oil%		Protein%	
	N_0	N_+	N_0	N_+	N_0	N_+	N_0	N_+	N_0	N_+
RAS-3/99 × RW008911	591.6**	411.3*	35	-500	6.64**	4.05**	-1.9**	-1.5*	1.68**	1.39**
RAS-3/99×19H	21.7	95.3	574	-804	-1.35**	1.62*	2.1**	2.5**	-1.91**	-1.50**
RAS-3/99×RGS 003	649.2**	351.8	2892	2696**	-1.49**	-2.02**	-0.6	-0.3	0.15	0.67
RAS-3/99 ×Option500	-79.9	-178.9	280	-490	-1.23*	-0.16	-1.4*	-1.3*	0.81	1.19**
RAS-3/99×PF7045/91	29.2	71.1	-279	-1200	1.41**	2.32**	2.3**	1.9*	-1.69**	-1.21**
RW00891 × 19H	294.4*	75.1	2699**	1400	-5.81**	-3.10**	2.7**	1.5*	-1.26**	-1.95**
RW008911 × RGS 003	375.6**	249.5	111	-422	3.90**	3.20**	-0.6	-1.2	1.05*	1.38**
RW008911×Option 500	-269.8*	-44.9	-375	820	-4.18**	-2.90**	-2.5**	-3.3**	2.45**	1.84**
RW008911 ×PF7045/91	189.2	20.3	2035**	906	-4.71**	-2.55**	3.6**	3.6**	-2.97**	-3.12**
19H × RGS 003	-651.7**	-115.5	-2368**	-603	-1.51**	-0.14	0.1	-1.7*	0.83	0.78
19H × Option 500	485.2**	285.7	2377**	3084**	-0.73	-2.50**	-1.1	-2.7**	1.75**	0.66
19H ×PF7045/91	481.5**	290.6	1477**	574	0.97	0.76	-0.3	-1.6*	0.83	0.72
RGS 003 ×Option 500	365.1**	164.3	2742**	610	-3.49**	0.02	4.5**	2.6**	-2.45**	-3.62**
RGS 003 ×PF7045/91	-102.1	-522.8**	335	-815	-2.62**	-2.32**	-1.3*	-1.3	1.13**	0.95
Option500× PF7045/91	524.9**	493.5**	2834**	3292**	-0.14	-0.47	0.5	0.8	1.13**	-1.24**

*, ** Significant at $P < 0.05$ and 0.01 , respectively.

GCA effect for seed yield at two nitrogen levels of application and RGS003 had negative and positive GCA effects at N_0 and N_+ , respectively. PF7045/91 had significant positive GCA effect of biological yield at N_0 and N_+ and RAS-3/99 had positive and significant positive GCA effect of this trait at N_0 and N_+ , respectively. The mean of this trait at N_+ was about two times of its amount at N_0 . PF7045/91 and RAS-3/99 with 8673.2 and 14579.6 kg/ha of biological had the highest amount of this trait at N_0 and N_+ , respectively. In earlier study (Rathke *et al.* 2005) was also reported that the mean of biological yield was increased at the N_+ due to nitrogen important role for biomass formation and increasing vegetative growth stage. RW008911 and RGS003 with significant positive GCA effects for harvest index were considered as good combiners for this trait. Both of these parents had significant GCA effect for two related traits of harvest index including seed yield and biological yield. RW008911 and RGS003 had also high mean performance of harvest index at N_0 and N_+ . For oil content, different parents had different combinations at N_0 and N_+ . RGS003 and Option 500 had significant positive GCA effects for oil content at N_+ but PF7045/91 had significant positive GCA effect of this trait at N_0 . The parents including 19H, RGS003 and Option500 with 45.25, 46.2 and 47.13 oil percentage had high amount of this trait at N_+ . High amounts of protein content of parents were observed at N_+ . RAS-3/99 and RW008911 with significant positive GCA effects for protein content were good combiners for this trait.

Specific combining ability and mean performances of the crosses

The result of SCA effects of the crosses for all the traits

is presented in Table 4. Out of 15 crosses, 7 crosses had significant positive SCA effects for seed yield at N_0 but only two crosses had significant positive SCA effects for this trait at N_+ , therefore recognition of superior cross combination is more easier at N_0 than N_+ . The crosses including RAS-3/99 × RW008911, RAS-3/99 × RGS003, 19H × PF7045/91 and Option500×PF7045/91 had high amount of seed yield at N_0 and N_+ (Table 5). Most of the crosses with high seed yield at N_0 and N_+ had at least one parent with positive GCA effect at N_0 and N_+ . In other studies (Azizinia 2011) similar results were reported. Maximum difference of seed yield at N_0 and N_+ was related to 19H×RGS003. Significant positive correlation was detected between seed yield at N_0 and N_+ (Table 6) indicating most of the crosses with high seed yield at N_0 had also high amount of this trait at N_+ . Out of 15 crosses, 6 and 3 crosses had significant positive SCA effects for biological yield at N_0 and N_+ , respectively. Significant positive correlation of seed yield at N_0 with biological yield at N_0 and N_+ , indicating biological yield at N_0 and N_+ is good indicator for seed yield prediction at N_0 . Biological yield was varied from 5362 to 13158.3 kg/ha related to 19H×RGS003 and Option 500 × PF7045/91, respectively at N_0 and also it ranged from 10 288.3 to 18 282.3 kg/ha related to RW008911× RGS 003 and Option 500 × PF7045/91, respectively at N_+ . The cross combinations including RAS-3/99 × RW008911, RAS-3/99 × PF7045/91 and RW008911× RGS003 with significant positive SCA effects for harvest index were considered merit combinations for improving this trait. Significant SCA effects of cross combinations were reported in other studies for oilseed rape (Marjanovic-Jeromela *et al.* 2007) and other *Brassica* species (Mahto and Haider 2004). Due to significant positive correlation between

Table 5 Means of seed yield, biological yield, harvest index, oil and protein percentages in the half diallel crosses of six parents of oil seed rape at N_0 and N_+

Parents	Seed yield (kg/ha)		Biological yield (kg/ha)		Harvest index		Oil%		Protein%	
	N_0	N_+	N_0	N_+	N_0	N_+	N_0	N_+	N_0	N_+
RAS-3/99 × RW008911	2 700.0	3 347.5	7 708.9	11 233.5	35.07	30.18	36.75	41.38	21.46	24.72
RAS-3/99×19H	21.95	19.74	42.10	41.25	23.38	24.84	14217.1	11508.5	2956.7	2855.0
RAS-3/99 × RGS 003	2 796.3	3 375.0	11 085.8	16 103.0	25.28	21.03	39.25	43.75	19.36	23.05
RAS-3/99 × Option500	1 890.0	2 615.8	9 226.6	13 919.0	20.65	19.30	37.65	42.75	19.67	23.98
RAS-3/99 × PF7045/91	2 520.3	3 225.0	10 216.8	13 811.0	24.85	23.41	42.75	45.00	17.85	20.98
RW00891 × 19H	2 285.0	2 505.8	9 909.3	11 765.3	23.04	24.56	42.50	45.08	17.89	20.44
RW008911 × RGS 003	2 375.0	2 887.5	6 860.6	10 288.3	34.71	31.14	39.13	42.88	20.31	23.96
RW008911×Option 500	1 552.5	3 197.9	7 127.4	12 532.3	21.74	21.45	36.50	40.75	21.37	24.82
RW008911 ×PF7045/91	2 532.5	2 675.0	11 086.9	13 221.2	22.78	23.43	44.00	46.75	16.63	19.26
19H × RGS 003	1 377.5	3 099.5	5 362.0	11 435.1	25.68	24.44	41.13	43.00	19.41	22.21
19H × Option 500	2 337.5	2 784.2	10 859.2	16 123.6	21.57	18.49	39.20	42.00	19.98	22.5
19H ×PF7045/91	2 855.0	2 956.7	11 508.5	14 217.1	24.84	23.38	41.25	42.10	19.74	21.95
RGS 003 ×Option 500	2 226.0	3 320.8	10 764.4	13 995.5	20.76	21.29	44.75	47.90	15.9	18.41
14- RGS 003×PF7045/91	2 280.0	2 971.3	9 906.3	13 173.2	23.20	20.59	40.25	42.93	20.15	22.37
15- Option 500×PF7045/91	2 730.0	2 643.3	13 158.3	18 282.3	20.79	18.84	41.25	45.10	17.37	20.59
LSD ($\alpha=0.05$)	326.0	437.4	1 413.2	2 348.4	1.73	2.46	1.43	1.57	1.42	1.52
LSD ($\alpha=0.01$)	402.5	540.1	1 744.7	2 899.3	2.14	3.04	1.76	1.94	1.75	1.88

harvest index at N_0 and N_+ , most of crosses with high amount of harvest index at N_0 had also high amount of this trait at N_+ . The crosses including RAS-3/99× RW008911 and RW008911× RGS003 had high amount of harvest index at N_0 and N_+ . Out of 15 crosses, 5 crosses had significant positive SCA effects for oil percentage at N_0 and N_+ . Significant positive correlation was detected between oil content at N_0 and N_+ , therefore most of the genotypes with high oil content at N_0 had high oil content at N_+ . The crosses including RW008911× PF7045/91, RGS003× Option500 and RAS-3/99×PF7045/91 had high oil content at N_0 and N_+ . The crosses including RAS-3/99 × RW008911, RW008911× RGS003 and RW008911× PF7045/91 had significant positive

SCA effects for protein percentage at N_0 and N_+ , therefore these cross combinations are suitable for improving this trait. Most of the crosses with high protein content at N_0 and N_+ had at least one parent with significant positive or positive GCA effects at N_0 and N_+ .

In general from a breeder point of view, the creation of good hybrids to be cultivated in low-input systems at low N level will be made possible by the high GCA effects observed. The GCA × N level interaction indicates that results classically obtained at high N level would not be enough to recognize parents for low nitrogen management and that specific experiment at low N level will be necessary to meet this objective. Among the six parents, PF7045/91 appeared to be

Table 6 Correlation among the traits in half diallel crosses of six parents of oil seed rape at N_0 and N_+

Parents	Seed yield		Biological yield		Harvest index		Oil%		Protein%	
	N_0	N_+	N_0	N_+	N_0	N_+	N_0	N_+	N_0	N_+
Seed yield	N_0	1								
	N_+	0.42*	1							
Biological yield	N_0	0.83*	0.27	1						
	N_+	0.57**	0.26	0.82**	1					
Harvest index	N_0	-0.09	0.05	0.60**	-0.69**	1				
	N_+	-0.04	0.06	-0.51**	-0.78**	0.90**	1			
Oil%	N_0	0.32	0.07	0.49*	0.23	-0.38	-0.15	1		
	N_+	-0.07	-0.27	0.06	-0.09	-0.14	-0.02	0.70**	1	
Protein%	N_0	-0.03	0.27	-0.24	-0.06	0.31	0.14	-0.79**	-0.95**	1
	N_+	-0.27	0.03	-0.48*	-0.26	0.41	0.22	-0.94**	-0.76**	0.86**

*, ** Significant at $P < 0.05$ and 0.01 , respectively.

the best combiner for seed yield at the both N levels. This variety also showed high GCA at the N_+ but its low oil content at the N_+ is certainly disadvantageous as the quality of the hybrid is often intermediate between the two parents. Due to significant $N \times SCA$ effect for harvest index and oil content, good cross combinations varied based on nitrogen levels.

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