

HETEROTIC PATTERNS IN RAPESEED (*BRASSICA NAPUS* L.): CROSSES BETWEEN SPRING-TYPE AND WINTER-TYPE GENOTYPES

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Abstract: Genetic improvement in a crop, viz. *Brassica* species, through plant breeding essentially requires the presence of adequate genetic diversity within the gene pool. Winter rapeseed is known to be genetically different from spring rapeseed and can therefore be used for extending genetic diversity in the progenies produced via crossing methods. Using line×tester analyses of two spring types of testers and six lines of winter-type rapeseed varieties, heterotic patterns of phenological traits, plant height and seed yield were estimated. Significant mean squares between parents and crosses revealed significant heterosis for all the traits. Line×tester mean squares, indicating the non-additive genetic effects, were significant only for plant height and seed yield. High narrow-sense heritability estimates for phenological traits underline the importance of additive genetic effects and thus the efficiency of selection for improving these traits. Based on the significant and positive expression of heterosis effects for phenological traits and plant height in the winter parents, it was concluded that the F1 progenies had earlier and shorter maturity than the winter parents. F1 progenies were also early flowering, early maturing and taller than the spring-type parents. Significant positive heterosis of the crosses for seed yield was observed in 75% and 42% of F1 progenies compared to the spring and winter parents, respectively, indicating a higher yield potential of the F1 hybrids than the spring and winter parents.

Key words: heritability, line×tester, phenological traits, seed yield.

Introduction

As the second most important source of oil production in the world, *Brassica* oilseeds have been significantly improved through common and modern breeding methods (Sabaghnia et al., 2010; Bennett et al., 2012; Ofori et al., 2012). Exploiting genetic variability of *Brassica* species is considered critical for genetic improvement of phenological traits, yield components and seed yield (Rahman, 2013; Gourrion et al., 2020). The limited geographic range of *Brassica* species

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along with intensive quality breeding resulted in a narrow genetic basis of this species (Seyis et al., 2005; Inamullah et al., 2006). Rapeseed cultivars are categorized as winter or spring types according to their vernalization requirements for flower initiation. Due to the restricted genetic basis of spring rapeseed, the winter type of rapeseed can be a suitable candidate for increasing yield potential and genetic variation of spring type in winter×spring type combinations (Qian et al., 2007; Kebede et al., 2010). Gourrion et al. (2020) investigated the possibility of increasing the yield of spring material by using winter germplasm. In many other reports, each spring type (Rameeh, 2010; Rahman et al., 2016) or winter type (Amiri-Oghan et al., 2009) was used separately in breeding programs. The line×tester analysis is a suitable method of evaluating a large number of genotypes as well as providing information on the relative importance of genetic parameters for determining the genetic basis of important plant traits (Singh and Chaudhury, 2001). Phenological and yield-associated traits were studied for heterosis and heritability estimates were made using this method (Shen et al., 2002; Wang et al., 2007). Several studies on spring cultivars of rapeseed have shown the important role of heterosis effects in high narrow-sense heritability estimates for phenological traits (Huang et al., 2010; Rameeh 2011). Likewise, studies with winter rapeseed cultivars (Diepenbrock, 2000; Amiri-Oghan et al., 2009; Sabaghnia et al., 2010; Rameeh, 2011) show that both additive and dominant gene effects play a significant role in the inheritance of phenological traits. With an appropriate level of heterosis, commercial development of F₁ hybrids would be justified (Nassimi et al., 2006; Bus et al., 2011). When breeding for high grain yield, the most promising crop type was both late maturing and early flowering (Dong et al., 2007; Radoev et al., 2008; Cuthbert et al., 2009). Heterosis is used commercially in rapeseed (*Brassica napus* L.) and its potential use has been demonstrated in turnip rape (*B. rapa* L.) and Indian mustard (*B. juncea* L.) for most agronomic traits (Teklwoold and Becker, 2005; Zhang and Zhu, 2006). Significant negative mid-parent and better-parent heterosis was reported for days to 50% flowering and physiological maturity (Nassimi et al., 2006). In this study, mid-parent heterosis for days to 50% flowering and physiological maturity ranged from -0.04 to -2.78 and from -0.01 to -3.06, respectively, and its high parent heterosis for these traits also ranged from -0.92 to -2.78 and from -0.01 to -4.08, respectively. A significant correlation was found between the post-anthesis duration and some important agronomic traits, including the number of pods per plant, 1000-seed weight and oil yield (Marjanovic-Jeromela et al., 2007). For seed yield, an average high parent heterosis of 30% with a range of 20–50% was observed in spring rapeseed hybrids, while an average high parent heterosis of 50% with a range from 20 to 80% was reported for winter rapeseed hybrids, as reviewed by McVetty (1995).

Although line×tester analysis has been widely used in rapeseed breeding to assess genetic parameters for yield-associated traits, several studies have focused

on heterosis of winter or spring parents. Therefore, the objectives of this study were to compare heterosis of phenological traits, plant height and grain yield in winter and spring parents, and to identify narrow-sense heritability for these traits.

Material and Methods

Six winter rapeseed (*B. napus* L.) cultivars, including Zarfam, Licord, Talayeh, Okapi, Modena and Opera as lines were crossed with two rapeseed spring-type testers, including H308 and H401 in 2006–2007, based on the line×tester crossing method (Table 1).

Table 1. The origin and some characteristics of rapeseed genotypes.

Genotypes	Origin	Growth habit	Seed quality
1-H308	Iran	Spring	Double zero*
2-H401	Iran	Spring	Double zero
3-Zarfam	Iran	Winter	Double zero
4-Licord	Hungary	Winter	Double zero
5-Talayeh	Germany	Winter	Double zero
6-Okapi	France	Winter	Double zero
7-Modena	Denmark	Winter	Double zero
8-Opera	France	Winter	Double zero

*Double zero: the fatty acid content of the oil is less than 2% and the glucosinolates in the meal is less than 30 µM/g.

In winter 2007–2008, twelve F_1 progenies were grown together with their parents in a randomized complete block design with three replications at Biekol Agriculture Research Station, located in Neka, Iran (13°53' E longitude and 43°36' N latitude, 15 m above sea level). Each plot consisted of four rows, 5 m long and 40 cm apart. The distance between plants in each row was 5 cm, resulting in approximately 300 plants per plot, which was sufficient for the F_1 genetic analysis in each experiment. The soil which was classified as a deep loam soil (Typic Xerofluents, USDA classification) contained an average of 285 g clay kg^{-1} , 562 g silt kg^{-1} , 165 g sand kg^{-1} , and 21.4 g organic matter kg^{-1} at a pH of 7.1. Soil samples contained 40 kg ha^{-1} (mineral N in the upper 30-cm profile). Fertilizers were applied at the rates of 100: 50: 90 kg ha^{-1} of N: P: K, respectively. All plant protection practices were adopted to free the crop from insects. Seed yield (adjusted to kg ha^{-1}) was recorded based on the three middle rows of each plot. The data were recorded on ten randomly selected plants of each entry of each replication for days to flowering, days to complete flowering, duration of flowering and days to maturity and plant height.

Data for the genotypes were subjected to line×tester analysis (Singh and Chaudhury, 2001) to estimate general heritability and other genetic parameters.

The difference between the hybrid and the mean of each spring-type and winter-type parent was computed separately to estimate heterosis of phenological traits, plant height and seed yield over winter and spring parents. A least significant difference (LSD) test was used as the statistical test for heterosis.

Results and Discussion

Analysis of variance for line×tester design

There were significant differences among the crosses for all the traits except flowering duration, indicating the existence of genetic variability among the crosses (Table 2). The variance between the spring genotypes used as testers was greater than that of the winter genotypes, indicating some paternal effects on the studied traits. Line×tester variance was significant for plant height and seed yield, indicating the importance of non-additive genetic effects for these traits. High narrow-sense heritability estimates for phenological traits indicate the key role of additive genetic effects for these traits. Similarly, various studies on spring (Huang et al., 2010, Rameeh, 2011) and winter (Sabaghnia et al., 2010) cultivars of rapeseed have shown the important role of additive genetic effects for phenological traits.

Table 2. Line ×tester analysis of variance for phenological traits, plant height and seed yield of rapeseed genotypes.

S.O.V	df	M.S					
		Days to flowering	Days to complete flowering	Flowering period	Days to maturity	Plant height	Seed yield
Replication	2	76.32	2.52	105.87	0.62	4.58	1947658.84
Treatment	19	231.34**	203.69**	64.68**	134.22**	765.25**	808909.56**
Parents	7	565.05**	311.43**	48.00**	287.09**	1503.12**	704977.64
Parents vs crosses	1	127.21**	1488.40**	745.34**	251.67**	2822.40**	4422301.14**
Crosses	11	28.45**	18.33*	13.42	26.27**	108.69**	546557.91**
Lines	5	29.61**	13.89	2.94	23.47*	41.32	1748.27
Testers	1	138.81**	114.00**	112.81**	119.81**	452.82**	3144397.13**
Line × tester	5	5.23	3.64	4.03	10.36	107.23**	571799.71**
Error	38	7.05	8.41	12.69	8.77	24.83	109944.88
V _A		85.16	53.86	34.44	58.33	5.33	1713.43
V _P		91.60	60.68	44.25	67.64	59.22	171343.22
H ² _N		0.93	0.89	0.78	0.86	0.09	0.01

*, ** Significant at p=0.05 and 0.01, respectively. V_A: Additive variance, V_P: Phenotypic variance, H_{2N}: Narrow-sense heritability.

Heterosis of spring and winter parents

Most of the cross combinations had higher seed yield than the spring-type parents (Table 3). Significant negative winter parent heterosis for days to flowering and days to complete flowering was observed in all the combinations (Table 4), but all combinations had significant positive results of spring parent heterosis for days to flowering. Out of 12 crosses, 5 crosses had significant positive spring parent heterosis for days to complete flowering (Table 5). Early flowering in Brassica can provide adequate time for the grain formation process and can certainly cause early maturity and higher yields, therefore, negative heterosis is desirable for flowering. The cross combinations including Opera×H308, Licord×H308, Talayeh×H308 and Modena×H308 with a mean value for days to flowering of 158, 157, 157 and 157, respectively (Table 3), also had high significant negative winter parent heterosis for days to flowering and they were found to be good combinations for improving this trait. The crosses including Opera×H308 and Talayeh×H308, with mean values of 180 and 182 days to complete flowering (Table 3), also had highly significant negative winter parent heterosis for this trait (Table 4).

Table 3. The mean performance of rapeseed genotypes for phenological traits, plant height and seed yield.

Genotypes		Days to flowering	Days to complete flowering	Flowering period	Days to maturity	Plant height (cm)	Seed yield (kg ha ⁻¹)
Testers	1-H308	142	177	35	218	130.00	3300.00
	2-H401	130	167	37	210	126.67	3033.33
Lines	3-Zarfam	157	190	33	225	169.97	4208.33
	4-Licord	166	193	27	236	184.63	3457.00
	5-Talayeh	163	194	31	233	173.23	3697.33
	6-Okapi	165	194	29	235	176.59	3349.18
	7-Modena	167	194	27	234	178.93	4334.20
	8-Opera	167	193	26	236	169.50	3105.76
Crosses	9-Zarfam × H401	149	174	25	221	154.50	4350.69
	10-Licord × H401	153	177	24	222	150.07	3470.10
	11-Talayeh × H401	151	177	26	222	151.87	4050.10
	12-Okapi × H401	153	177	24	222	157.17	3877.88
	13-Modena × H401	155	178	23	224	154.61	4566.87
	14-Opera × H401	152	174	22	223	144.30	4466.73
	15-Zarfam × H308	151	176	25	220	147.08	3250.20
	16-Licord × H308	157	177	20	225	141.60	4233.77
	17-Talayeh × H308	157	182	25	227	160.27	3894.54
	18-Okapi × H308	157	181	24	228	142.45	4183.33
	19-Modena × H308	157	180	23	227	145.87	4572.32
	20-Opera × H308	158	177	19	229	146.50	4461.21
LSD (0.05)		4.38	4.79	5.88	4.89	8.22	547.15
LSD (0.01)		5.85	6.39	7.85	6.53	10.98	730.98

The significant positive correlation between days to flowering and days to complete flowering (0.85**) indicates that the genotypes with high mean performance in days to flowering also had high mean performance in days to complete flowering (Table 6). Out of 12 crosses, 5 crosses had significant negative winter parent heterosis for the duration of flowering and all combinations had significant negative spring parent heterosis for this trait. Spring rapeseed genotypes mature earlier than winter rapeseed varieties, so significant positive spring parent heterosis was observed for days to flowering and days to maturity. Significant negative winter parent heterosis of days to flowering and days to maturity was observed for most of the combinations, suggesting that early flowering and early maturity were controlled by dominant spring-type genes of the parents. This finding is in agreement with Nassimi et al. (2006), who found significant negative mid-parent and better-parent heterosis for days to 50% flowering and physiological maturity. Most of late-maturity genotypes had low mean flowering duration; therefore, a significant negative correlation was found between these two traits. F₁ progenies of winter×spring varieties were taller than spring-type varieties but shorter than winter-type parents. Therefore, significant negative and positive effects of heterosis on plant height were observed in spring-type and winter-type parents, respectively.

Table 4. Heterosis over winter parents of rapeseed genotypes for phenological traits, plant height and seed yield.

Traits Genotypes	Days to flowering	Days to complete flowering	Flowering period	Days to maturity	Plant height	Seed yield
10-Zarfam × H401	-8.00**	-16.00**	-8.00**	-4.00	-15.47**	142.36
12-Licord × H401	-13.00**	-16.33**	-3.33	-14.00**	-34.57**	13.10
18-Talayeh × H401	-12.00**	-17.00**	-5.00	-10.67**	-21.37**	352.77
16-Okapi × H401	-11.67**	-16.67**	-5.00	-13.00**	-19.42**	528.70*
14-Modena × H401	-12.00**	-16.00**	-4.00	-10.00**	-24.32**	232.67
20-Opera × H401	-15.33**	-19.00**	-3.67	-13.00**	-25.20**	1360.98**
11-Zarfam × H308	-6.00**	-14.00**	-8.00**	-5.00*	-22.88**	-958.13**
13-Licord × H308	-9.00**	-15.67**	-6.67*	-11.00**	-43.03**	776.77**
19-Talayeh × H308	-6.00**	-12.00**	-6.00*	-6.00*	-12.97**	197.21
17-Okapi × H308	-7.67**	-12.67**	-5.00	-7.00**	-34.14**	834.15**
15-Modena × H308	-10.00**	-14.00**	-4.00	-7.00**	-33.07**	238.12
21-Opera × H308	-9.00**	-16.00**	-7.00*	-7.00**	-23.00**	1355.45**

*, ** Significant at p=0.05 and 0.01, respectively.

Among the gene pools of *Brassica* species, the winter type of *B. napus* proved to be genetically different from the spring types, while alleles of the winter type introduced into a spring type demonstrated great potential for increasing seed yield

in hybrid and open-pollinated cultivars (Huang et al., 2010; Kebede et al., 2010). Out of 12 crosses, 5 crosses had significant positive heterosis effects of grain yield on spring-type parents (Table 5).

Table 5. Heterosis over spring parents of rapeseed genotypes for phenological traits, plant height and seed yield.

Traits Genotypes	Days to flowering	Days to complete flowering	Duration of flowering	Days to maturity	Plant height	Seed yield
10-Zarfam × H401	7.00**	-2.67	-9.67**	3.00	24.50**	1050.69**
12-Licord × H401	11.00**	0.00	-11.00**	4.00	20.07**	170.10
18-Talayeh × H401	9.00**	0.33	-8.67**	4.33	21.87**	750.10**
16-Okapi × H401	11.00**	0.33	-10.67**	4.00	27.17**	577.88*
14-Modena × H401	13.00**	1.33	-11.67**	6.00**	24.61**	1266.87**
20-Opera × H401	10.00**	-2.67	-12.67**	5.00**	14.30**	1166.73**
11-Zarfam × H308	20.67**	9.00	-11.67**	10.00**	20.42**	216.87
13-Licord × H308	26.67**	10.33**	-16.33**	15.00**	14.93**	1200.43**
19-Talayeh × H308	26.67**	15.00**	-11.67**	17.00**	33.60**	861.21**
17-Okapi × H308	26.67**	14.00**	-12.67**	18.00**	15.78**	1150.00**
15-Modena × H308	26.67**	13.00**	-13.67**	17.00**	19.20**	1538.99**
21-Opera × H308	28.00**	10.00**	-18.00**	19.00**	19.83**	1427.88**

*, ** Significant at $p=0.05$ and 0.01 , respectively.

Table 6. The correlation of phenological traits, plant height and seed yield for rapeseed genotypes.

Traits	Days to flowering	Days to complete flowering	Flowering period	Days to maturity	Plant height	Seed yield
Days to flowering	1					
Days to complete flowering	0.85**	1				
Duration of flowering	-0.37	0.16	1			
Days to maturity	0.97**	0.88**	-0.25	1		
Plant height	0.82**	0.89**	0.02	0.82**	1	
Seed yield	0.18	-0.12	-0.57**	0.08	-0.03	1

*, ** Significant at $p=0.05$ and 0.01 , respectively.

The crosses Modena×H401, Opera×H401, and Modena×H308 gave 4566.87, 4466.73, and 4572.32 kg ha⁻¹ of seed yield and significant positive winter and spring parent heterosis for this trait, respectively, and were found to be good combinations for improving seed yield. Similarly, for grain yield, an average high parent heterosis of 30% with a range of 20–50% was observed in spring rapeseed hybrids, while an average high parent heterosis of 50% was reported for winter rapeseed hybrids, ranging from 20 to 80%, as reviewed by McVetty (1995).

Conclusion

In conclusion, all the phenological traits were more heritable than the other studied traits, so the efficiency of selection will be high for these traits. Most of the crosses had significant negative heterosis effects for phenological traits and also significant positive heterosis for plant height in spring-type parents. Therefore, F1 progenies of winter×spring rapeseed varieties were characterized by early flowering, early maturity and great plant height as compared to spring-type parents. The general crossing between spring and winter types of rapeseed genotypes allows high yields of F1 progeny with medium maturity, which can be used for improving seed yield.

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HETEROTIČNI OBRASCI KOD ULJANE REPICE (*BRASSICA NAPUS* L.): UKRŠTANJA IZMEĐU GENOTIPOVA JAROG I OZIMOG TIP

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Genetičko poboljšanje useva, kao što su *Brassica* vrste, putem oplemenjivanja biljaka, u suštini zahteva prisustvo adekvatne genetičke raznovrsnosti u okviru genskog pula. Poznato je da se ozima uljana repica genetički razlikuje od jare uljane repice i da se stoga može koristiti za proširenje genetičke raznovrsnosti kod potomstva dobijenog metodama ukrštanja. Koristeći linija×tester analize za dva jara tipa testera i šest linija ozimih sorti uljane repice, procenjeni su heterotični obrasci fenoloških osobina, visine biljke i prinosa semena. Značajni srednji kvadrati između roditelja i hibrida otkrili su značajan heterozis za sve osobine. Srednji kvadrati linija×tester, koji ukazuju na neaditivne genetske efekte, bili su značajni samo za visinu biljke i prinos semena. Procenjene visoke vrednosti heritabilnosti u užem smislu za fenološke osobine naglašavaju važnost aditivnih genskih efekata, a time i efikasnost selekcije za poboljšanje ovih osobina. Na osnovu značajnog i pozitivnog ispoljavanja heterotičkih efekata za fenološka svojstva i visinu biljke kod roditelja ozimog tipa, zaključeno je da su F1 potomci imali ranije i kraće sazrevanje od roditelja ozimog tipa. Potomci F1 su takođe ranije cvetali, ranije sazrevali i bili viši od roditelja jarog tipa. Značajan pozitivan heterozis hibrida za prinos semena uočen je kod 75% i 42% F1 potomaka u poređenju sa roditeljima jarog odnosno ozimog tipa, što ukazuje na veći potencijal prinosa F1 hibrida u odnosu na roditelje jarog i ozimog tipa.

Ključne reči: heritabilnost, linija×tester, fenološke osobine, prinos semena.

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