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Potential and Genetic Basis of Drought Tolerance in Canola (*Brassica napus*) II. Heterosis Manifestation in Some Morpho-Physiological Traits in Canola

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ABSTRACT

Heterosis expression over mid and better parents was estimated. The experiment comprised of crosses of four *B. napus* genotypes; two drought sensitive (Ester & Rainbow) and two-drought tolerant (Range & Shiralee) under two treatments (irrigated & drought) in canola for seedling, physiological and morphological traits. The expression of heterosis was observed in all crosses under both conditions for almost all traits studied. The magnitude and direction of heterosis varied with plant trait, cross combination and the treatments. Heterosis was observed in shoot length under normal as well as drought over mid as well as better parents. Fresh root weight showed range of heterosis over mid parent as well as better under normal and drought. Highest positive and significant better parent's heterosis under drought for water potential was observed in T x S. Heterosis over mid parent was observed for chlorophyll a in T x T under normal and drought and for oil contents in S x S and T x S under normal and drought. Under drought conditions T x T showed very high heterosis over mid and better parent. Cross combination T x T showed very high heterosis over mid parent in yield under normal conditions.

Key Words: *Brassica napus*; Heterosis; Irrigation; Drought; Oil contents; Phenology

INTRODUCTION

Plant breeders have recognized for many years that the progeny from a specific cross can out yield either of the two parents used in the cross. This is called heterosis. Accumulation of favorable dominants, interaction between non-allelic genes and heterozygosity *per se* are the suggested genetic mechanisms for the expression of heterosis. Heterosis provides (1) maximum performance under optimal growing conditions (2) Phenotypic stability under stress (3) joint improvement of traits negatively associated through linkage and (4) combination of harmonious set of traits in one individual from very diverse parents without necessarily disturbing the existing harmony.

For plant breeding, the observation of heterosis for yield has lead to the development of hybrid cultivars that exhibit a heterotic yield advantage. Over the years, progress was significantly higher for hybrid than for population breeding. This clearly reflects that hybrid breeding is genetically the most efficient method available. Obviously, the population varieties are ranked below the hybrid varieties for yield. Hybrids, has not only contributed to food security, but has also benefited the environment and are more attractive to farmers than the traditional populations because It is the basis of a billion-dollar agribusiness which has generated significant employment opportunities all over the world. This has been demonstrated by the success of hybrid varieties of maize, pearl millet, sorghum, sunflower, rice and cotton. Hybrid production of Brassicas in Pakistan could help reduce the edible oil deficit by bridging up the gap between potential and realized production. Presently, the breeding work in this domain does not much the required level of farmer's demand. To harvest the potential benefits of this technology, breeders need to produce hybrid cultivars locally.

One of the basic requirements for developing hybrid varieties in oilseed Brassica is the availability of proven heterosis. It is often observed that better heterosis could be expressed if involves parents of indigenous/adapted and exotic germplasm (Riaz *et al.,* 2001; Yu & Tuinstra, 2001). Research workers have reported the expression of heterosis in Brassicas. Agrawal and Badwal (1998) studied the heterosis for yield and other traits in 19 F_1 hybrids of *B. juncea* and compared to 5 commercial cultivars. Eighteen hybrids out yielded the best control variety RLM 514. Three of them were superior over the best control in seed yield by 81.19, 50.65 and 64.94%, respectively. Overall heterosis (taking all hybrids and checks into account) for seed yield was very high (59.69%). Qi *et al.* (2000) reported heterosis in hybrids of 6 cultivars of *B. campestris*. Significant heterosis for yield was found in some hybrids, with the highest being 96.4%. Most hybrids showed lower levels of heterosis, with the lowest being 1.4%. Katiyar *et al.* (2000) reported that in *B. juncea* 64 and 38 hybrids showed heterosis for seed yield over the better parent and standard cv. Varuna, respectively.

Significant heterosis for seed yield in oilseed rape has created interest in the development of hybrid cultivars but it needs to be explored using different combinations. The present studies investigate the potential of heterosis expression in three cross combinations of cultivars relatively tolerant and sensitive to drought under irrigation and drought conditions for some seedling, physiological, phenological traits, oil contents and yield and yield components.

MATERIALS AND METHODS

Experimental conditions. The experiments were conducted in the oilseeds laboratory, experimental area and green house of the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad during the year 1999-2002. The plant material and conditions of the experiments are described in Cheema and Sadaqat (2004, in this issue).

Seedling traits. Seeds of four parent and twelve generations were sown in plastic bags filled with sand. Temperature of glass house was noted from 16°C minimum to 30°C maximum. After saturation of pots, seeds were sown when there was some moisture in both treatments. Sowing was done on 23-10-2002. In each pot, five plants were maintained, others were rouged out. After germination of seeds, 3:1 irrigation supply was maintained to irrigated and non-irrigated bags. Irrigation water was given with the interval of 5 days. Three replications in each treatment were maintained. After 25 days of sowing data was collected. Shoot length was measured from root initiation to tip of the shoot and root length was taken from root initiation to tip of root in centimeters. Shoots and roots of each parent/generation were collected in already weighed glass vials, immediately weighed for fresh shoot and root weight and afterward kept in electronic oven for drying at 80 °C for twenty four hours and weighed for dry shoot and root weight.

Physiological parameters. Water potential of seedlings grown in green house was measured using water potential meter (ARIMAD 2, ELE International). Data regarding water contents were collected during December, 2001 and on conductance and chlorophyll contents during the last week of February, 2002. The data on physiological traits were recorded from already tagged plants. Data on leaf conductance were collected using porometer (AP4 Porometer ΔT Devices Ltd. Cambridge, England) on fifth node leaf from the base of stem. It was measured in cm/s.

Samples to determine chlorophylls and carotenoids were taken from the leaves using a 1.8 cm diameter cork borer excluding main veins from the same 5th node leaf, weighed quickly in pre-weighed clean glass vials and 5 cm^3 of 80% acetone was added to these samples. The leaf material was bleached and decanted off. The pigments were read at λ= 663, 646 and 470 nm using 80% acetone as blank in spectro-photometer (Spectronic Genesys-5 Milton Roy). Chlorophyll contents (μg g^{-1}), chlorophyll a (Ch a), chlorophyll b (Ch b) and carotenoids (Carots) were calculated according to Lichtenthaler and Wellburn (1983) using the following formulae.

$$\text{Chlorophyll a (Chl a)} = 12.21\ OD_{663} - 2.81\ OD_{646}$$

$$\text{Chlorophyll b (Chl b)} = 20.13\ OD_{646} - 5.03\ OD_{663}$$

$$\text{Carotenoids (Carots)} = (1000\ OD_{470} - 3.27 Chl.a - 104\ Chl.b) / 229$$

Samples from the same leaves were collected in other set of pre weighed glass vials weighed for fresh weight and dried in oven at 80°C for 24 h and weighed for dried weight, the tissue water contents were calculated as:

$$\text{Water contents \%} = 100 * \{(\text{Fresh weight} - \text{Dry weight}) / (\text{Fresh weight})\}$$

Biometrical approaches. The experiment in each case was treated as factorial in a randomized complete block design with three replications. Analysis of variance was performed using the GLM procedures of SAS software. Percent heterosis over mid and better parent was computed using formulae proposed by Falconer and Mackay (1996).

$$\text{Percent heterosis over mid parent (MP)} = 100 * (F_1 - MP) / MP$$

$$\text{Percent heterosis over better parent (BP)} = 100 * (F_1 - BP) / BP$$

$$MP = \text{Female parent } (♀) + \text{Male parent } (♂) / 2$$

Significance of heterosis over mid and better parent was tested using t-test (Wynne *et al*., 1970) as

$$t_{(Static)} = (F_1 - MP) / (3/8\ \sigma^2 E)^{1/2}$$

$$t_{(Static)} = (F_1 - MP) / (1/2\ \sigma^2 E)^{1/2}$$

RESULTS

Seedling traits. Heterosis was observed in shoot length under normal as well as drought condition over mid as well as better parents. Under normal condition significant heterosis over better parent was the highest in Rainbow x Ester (SxS) and in drought condition in Range x Ester (TxS). In root length under normal condition highest better parent heterosis was observed in Range x Ester (TxS) and under drought in Rainbow x Ester (SxS) (Table I). For fresh shoot weight better parent heterosis (%) was observed in Range x Shiralee (TxT) whereas, under drought no value was significant. Fresh root weight showed a very good Range of positive mid parent as well as better parent heterosis under normal as well as drought conditions. For fresh root weight Rainbow x Ester (SxS) showed highest negative better parent heterosis (%) under normal and highest positive heterosis under drought. Positive heterosis for dry shoot weight was not found. Positive and significant heterosis for dry root weight was the highest in Rainbow x Ester (SxS) under normal condition only. Highest positive and significant better parent heterosis under drought for water potential was observed in Range x Ester (TxS).

Physiological traits. Heterotic effects were not found under normal as well as drought conditions for conductance, tissue water contents, chlorophyll b and carotenoids (Table II).

Mid parent heterosis was observed for chlorophyll-a, in Range x Shiralee (TxT) under normal and drought conditions and for oil content in Rainbow x Ester (SxS) and Range x Ester (TxS) under normal and drought conditions. Whereas none of the crosses showed significant positive heterosis over better parent under normal as well as drought conditions.

Morphological traits. Heterosis in yield (Table III) under normal over mid parent ranged between 31.9% Rainbow x Ester (SxS) to 84.63% Range x Ester (TxS) but non-significant, and under drought Range x Shiralee (TxT) showed very high heterosis over mid parent and over better parent 71.06%.

No other heterosis, mid or better parent was useful under both conditions. Although it was significant in case of days to first bud and days to maturity but were positive.

Under normal heterosis for oil contents was observed in crosses Rainbow x Ester (SxS) and Range x Ester (TxS) under normal and drought conditions.

DISCUSSION

Presence of heterosis over mid and better parents under normal and drought conditions for seedling traits reflects predominance of over-dominance in these crosses. (YU & Tuinstra, 2001) had reported heterosis in various traits in seedling in sorghum.

Only a few value of heterosis of mid parent were significant for physiological traits. The existence of low values of heterosis in physiological traits indicated the presence of over dominance but influenced by environmental factors.

Range x Shiralee (TxT) showed high heterosis over mid (%) as well better parent (%) which could be exploited for genetic improvement in yield under drought condition for hybrid seed production and for looking high yielding

Table I. Heterosis Estimates in Seedling Traits of Canola

Traits	Normal			Drought		
	(TxT)	(SxS)	(TxS)	(TxT)	(SxS)	(TxS)
	Het. Het b	Het. Het b	Het. Het b	Het. Het b	Het. Het b	Het. Het b
Shoot length	15.59* -5.00	51.32** 31.80**	21.39** 14.38*	14.76* 0.87	26.51** 3.41	39.27** 32.07**
Root length	77.35** 56.96**	47.87** 28.62	79.53** 69.70**	12.53* 2.32	36.59** 23.25**	23.36** 2.32
Fresh shoot weight	47.63** 43.56**	13.81* 10.16	56.01** 42.25**	5.85 -3.91	8.46 0.0	6.58 -1.73
Dry shoot weight	-9.09 -24.24*	5 -4.55	-2.44 -9.09	-28.21** -33.34**	17.24** 0.0**	0.0 -9.52
Fresh root weight	40.74** 26.67**	-20.95* -33.33**	-18.52* -26.67**	29.41** 17.86**	60** 42.86**	88.24** 39.13**
Dry root weight	20 10.53	100** 56.52**	12.50 -5.26	7.95 2.15	5.26 2.58	5.59 2.41
Water potential	5.58 -7.11	31.41** 21.84**	22.79** 8.70	15.30** 5.49	23.96** -6.18	21.63** 14.79**

Table II. Heterosis Estimates in Physiological Traits of Canola

Traits	Normal			Drought		
	(TxT)	(SxS)	(TxS)	(TxT)	(SxS)	(TxS)
	Het. Het b	Het. Het b	Het. Het b	Het. Het b	Het. Het b	Het. Het b
Conductance	0.0 -20.69*	-10.50 -23.65	61.73 50	-16.28 -30.77	2.70 -13.64	2.56 -9.09
Water content	7.18 3.82	1.77 -0.29	6.79 3.48	6.55 .91	4.64 .69	3.39 1.10
Chlorophyll a	39.89 35.45	-9.41 -15.48	17.93 4.18	49.72** 39.52	-3.71 -16.91	21.77 14.68
Chlorophyll b	13.92 6.61	8.85 2.68	17.76 9.62	25.17 14.36	-5.57 -8.13	21.16 10.02
Carotenoids	40.63 20.54	-21.54 -38.18	32.57 20.11	74.50 52.94	-2.75 -27.89	57.19 44.20
Oil content	5.62 2.75	9.85** 3.43	8.12* 0.66	.87 -0.92	7.89* 3.02	8.04** -0.79

Table III. Heterosis Estimates in Morphological Traits of Canola

Traits	Normal			Drought		
	(TxT)	(SxS)	(TxS)	(TxT)	(SxS)	(TxS)
	Het. Het b	Het. Het b	Het. Het b	Het. Het b	Het. Het b	Het. Het b
Plant height	25.46* 13.46	-10.39 - 12.95	13.05 8.81	15.92 11.45	14.71 10.46	19.61 13.91
Primary braches	50.00 20.00	51.18 35.19	34.52 20.24	34.26 21.97	7.66 4.41	22.73 20.14
Secondary branches	50.80 14.83	18.30 4.74	40.09 18.12	62.91 38.20	43.09 34.94	20.95 14.16
Pods Silique per plant	40.28 18.65	14.36 10.63	15.63 10.85	32.74 16.45	7.10 2.83	-10.80 -18.56
Days to first bud	11.89 7.63	4.43 1.33	9.54 -3.30	14.72* 11.10	3.66 1.54	1.45 -0.88
Days to maturity	1.74 1.30	-0.86 -1.32	4.68 .52	4.95** 4.21*	-.02 -1.12	1.99 -2.18
Harvest index	2.97 6.29	16.11 4.71	-17.62 -21.74	3.32 -0.15	-0.72 -6.24	9.94 .10
Yield per plant	47.82* 20.98	31.90 17.11	84.63 75.47	94.64** 71.06*	26.21 10.28	44.21 39.34
Oil content	5.62 2.75	9.85** 3.43	8.12* 0.66	0.87 -0.92	7.89* 3.02	8.04** 0.79

* (P = 0.05) = significant; ** (P = 0.01) = highly significant; Upper value (Het) = heterosis over mid parent; lower value (Hetb) = heterosis over better parent's value; T x T = Drought tolerance x Drought tolerance; S x S = Drought susceptible x Drought susceptible; T x S = drought tolerance x Drought susceptible

proceeding in segregating generations. Agrawal and Badwal (1998), Qi, *et al*. (2000), Katiyar *et al*. (2000) and Raiz *et al.* (2001) also reported very high heterosis for yield in different crops. Under normal heterosis for oil contents was observed in crosses Rainbow x Ester (SxS) and Range x Ester (TxS) under normal and drought conditions. Mahto *et al*. (2000) reported positive heterosis for oil contents in *B. juncea* and therefore, supported the present results.

Heterosis in self-pollinated crops like the present one is generally low. Hybrid vigour is less in self-pollinated crops where additive gene action dominates. The reason for low heterosis in self-pollinated crops could be the effects of fixed genes due to conditions inbreeding (Falconer & Mackay, 1996).

CONCLUSION

The expression of heterosis was observed in all crosses under both conditions for almost all traits studied. The magnitude and direction of heterosis varied with plant trait, cross combination and the treatments. Heterosis over mid and better parents in yield was very clear under both treatments, which is good premonition of hybrid seed production in canola under normal as well as drought conditions.

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