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RESEARCH ARTICLE

Biochemical responses of brown sarson (*Brassica campestris* L.) genotypes to water stress under mid hill conditions

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SUMMARY

The drought tolerance of *Brassica campestris* genotypes *viz.,* KBS-3, KDH-06, KS-101, KLM-40, KLM-1 and KLM-4 was investigated after exposure to drought stress at various growth stages in a pot experiment. Water stress imposed at branch initiation, flower initiation and siliquae formation stages. Data of various biochemical parameters (total chlorophyll content, chlorophyll stability index, total free proline and total oil content) was recorded which revealed significant differences among the various *Brassica campestris* genotypes for chlorophyll content and proline accumulation. Total chlorophyll content of all the *Brassica campestris* genotypes declined due to drought stress at all the growth stages. Genotype KBS-3 showed least reduction in chlorophyll content during branch initiation and siliquae formation stage. There was significant increase in osmo-regulating substance proline under water stress and KBS-3 accumulated highest proline. Drought treatment at different growth stages reduced grain yield significantly. Greater reduction in grain yield was observed when stress was imposed at siliquae formation stage. Average yield was found greater in KBS-3 and least in KLM-4. The better osmoregulation ability under drought stress conditions in KBS-3 proves it as drought tolerant cultivar. The findings of the present research investigation recommended the growing of KBS-3 in the drought prone areas to obtain high economic yield even in adverse condition.

Key Words : Brown sarson, Drought, Chlorophyll content, Proline

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Brassica campestris L. is one of the most important agricultural oilseed crop belongs to the family Brassicaceae that is grown for its edible oil. India is an important rapeseed and mustard growing country ranking second in area and third in production in the world. The total world production of rapeseed is around 62.0 million metric tons. China is the largest producer which accounts for 13.5 million metric tons followed by Canada (11.8 million metric tons) and India (7.3 million metric tons) (Anonymous, 2009).

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The average productivity of rapeseed mustard in India is (1190 kg/ha) which is much lower than the world's average productivity (1730 kg/ha). Though, rapeseed mustard occupies prime position in both area and production yet the productivity of these crops is very low due to large number of factors *viz.,* its cultivation under marginal conditions and losses caused by various biotic and abiotic stresses (Toor and Atwal, 2007).

Brassica campestris commonly known as brown sarson is one of the most important oilseed crop of rapeseed mustard group and grown in Northern part of India. Indian mustard (*Brassica juncea*), brown sarson, yellow sarson and toria (*Brassica campestris*), karan sarson (*Brassica carinata*) and gobhi sarson (*Brassica napus*) are some of the most important types of *Brassica* grown in India. They are grown in tropical as well as temperate agroclimatic zones and are best adapted to areas having a relatively cool, moist climate during the growing season and dry harvest periods with 25-40 cm of rainfall. The oil obtained from their seeds is the main cooking oil in Northern India. Seeds are also used as condiments in the preparation of pickles, flavouring curries and vegetables. Rapeseed mustard oil is used in soap making as mixtures with mineral oil for lubrication and in medicines as plasters and poultices to alleviate inflammation and pain. Its cake is used as cattle feed and manure in most of the Indian villages. *Brassica campestris* mainly grown in rainfed areas, where water availability is one of the most important limiting factors affecting plant growth and development. Exposure of plants to a water-limiting environment during various developmental stages appears to activate various physiological and developmental changes, which contribute towards adaptation to such unavoidable environmental constraints. The response of plants to stresses depends on many factors such as phenological stage, genotype, time and strength of stresses (Torres *et al*., 2006). So, aim of the study was to identify *Brassica campestris* genotypes for selective drought tolerance based on biochemical parameters.

MATERIAL AND METHODS

The trial was carried out at Department of Biology and Environmental Sciences in the College of Basic Sciences,CSKHPKV, Palampur. The site is located at 32.6^0 N latitude, 76.3^0 S longitude and an altitude of 1290 m above mean sea level. Seeds of six genotypes of brown sarson were procured from Shivalik Agriculture Research and Extension Centre, Kangra (H.P) and were planted in October, 2011 in pots having 30 cm diameter in Completely Randomized Design with four replications. The pots were prepared by mixing soil, vermicompost and sand in the 3:2:1 ratio along with addition of N, P and K in the ratio of 12:32:16. Nirogen was applied in two doses, one at time of sowing and other at the time of flowering. Stress was created at three stages *i.e.* branch initiation stage (45 DAS), flower initiation stage (60 DAS) and siliquae formation stage (100 DAS) by withholding water till the appearance of slight wilting symptoms and control/ unstressed plants were watered regularly in the net house and hand weeding was done regularly. Biochemical parameters included were chlorophyll content calculated by Dimethyl sulphoxide (D.M.S.O.) method given by Hiscox and Israelstam (1979). The method of chlorophyll stability index (CSI) was followed as suggested by Murty and Majumdar (1962). Total free proline was estimated by the method of Bates *et al.* (1973). Total oil content was estimated from seeds (AOAC 1970). Chlorophyll content, chlorophyll stability index and proline were estimated after 30 days of stress imposition and correlation analysis was made with the data of 30 and 60 days of stress imposition. Drought susceptibility index was calculated by Fischer and Maurer (1968).

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussions have been presented under following sub heads:

At branch initiation (T_1) stage :

At branch initiation stage, genotype KBS-3 showed minimum reduction whereas genotype KLM-1showed maximum reduction in chlorophyll content and chlorophyll stability index under stressed condition followed by KLM-4 (Table 1). Genotype KDH-06 showed maximum increase whereas KLM-4 showed minimum increase in total free proline under stressed condition at branch initiation (T_1) stage whereas genotype KLM-4 showed maximum reduction in total oil content at T_1, T_2 and T_3 stage. Chlorophyll stability index showed least reduction at (T_1) stage in comparison to T_2 and T_3 stage *i.e.* initially it was more stable and at later stages there was decrease in its stability.

At flower initiation (T_2) stage :

At flower initiation stage, genotypes KDH-06 and KBS-3 showed minimum reduction whereas KLM-4 showed maximum reduction in total chlorophyll content, chlorophyll

Table 1 : Water stress at branch initiation (T_1) stage

Genotypes	Total hlorophyll content (µg/g)			Chlorophyll stability index (%)			Total free proline (µg/g)			Total oil content (%)		
	S	US	RP %)	S	US	RP (%)	S	US	RP (%)	S	US	RP (%)
KBS-3	12.8	13.8	7.10	97.1	98.1	0.90	206.3	78.26	-163.6	43.0	46.0	6.50
KDH-06	11.8	13.1	9.80	86.0	94.4	6.10	203.7	76.25	-167.2	39.5	44.5	11.2
KS-101	12.8	16.8	23.6	84.7	93.7	9.50	206.2	105.1	-96.12	32.0	40.0	20.0
KLM-40	12.3	13.5	8.80	85.0	94.4	9.80	204.9	101.1	-102.5	33.5	42.0	20.2
KLM-1	10.5	14.1	25.5	71.3	95.2	25.1	206.7	103.1	-100.5	32.0	40.5	20.9
KLM-4	11.6	15.2	23.3	84.3	92.6	8.90	170.0	139.2	-22.17	27.5	36.5	24.6
Mean	12.0	13.9		84.7	94.1		199.6	100.5		34.5	41.5	
C.D. 0.05)												
Stress	0.34			0.09			0.59			0.26		
Genotypes	0.59			0.15			0.59			0.32		
Interaction	0.84			0.22			0.83			0.65		

S- Stress, US- Unstressed, RP- Reduction percentage stability index and total oil content. Genotype KBS-3 showed maximum increase whereas genotype KLM-4 showed minimum increase in total free proline under stressed condition (Table 2).

At siliquae formation (T_3) stage :

At siliquae formation stage, genotype KBS-3 showed minimum reduction whereas KLM-4 showed maximum reduction in total chlorophyll content, chlorophyll stability index and total oil content under stressed condition. Genotype KDH-06 showed maximum increase followed by KBS-3 whereas KLM-1 showed minimum increase followed by KLM-4 in total free proline under stressed condition. Among three stages siliquae formation (T_3) stage was found to be most sensitive to total oil content reduction (Table 3).

Chlorophyll content showed a remarkable effect under water stressed conditions in brown sarson genotypes. Plant under water stressed conditions accumulates less chlorophyll content than the unstressed plants. Chlorophyll content in *B.campestris* genotypes shows varied response to water stress. In *B.campestris* genotypes KBS-3 and KDH-06 showed less reduction in chlorophyll content under stressed conditions than genotypes KLM-40, KS-101, KLM-1 and KLM-4. Values of chlorophyll content declined at siliquae formation stage as chlorophyll content in leaves depends upon the age of the plant. Naidu *et al.* (2001) reported that the increase in chlorophyll content in tolerant genotype is due to higher leaf area duration, high relative water content and high net assimilation rate. Singh *et al.* (2003) studied mustard genotypes under irrigated and rainfed conditions and found that chlorophyll content decreased during all the growth stages under rainfed. The chlorophyll content in the leaves of all genotypes decreased significantly under drought. PS-20 showed maximum reduction in chlorophyll content during vegetative and maturity stage. They found that the genotypes with high chlorophyll content at flowering and maturity produced high yield under rainfed. The decrease in chlorophyll content in drought-stressed plants might possibly be due to

Table 2 : Water stress at flower initiation (T_2) stage

Genotypes	Total chlorophyll content (µg/g)			Chlorophyll stability index (%)			Total free proline (µg/g)			Total oil content (%)		
	S	US	RP(%)	S	US	RP(%)	S	US	RP(%)	S	US	RP(%)
KBS-3	13.4	15.0	10.6	32.2	36.0	10.5	275.2	105.4	-160.9	41.5	46.0	9.70
KDH-06	17.0	17.7	3.50	29.0	35.2	17.6	289.6	145.8	-98.60	39.5	44.5	10.1
KS-101	11.8	12.3	3.70	27.9	38.0	26.5	245.0	174.1	-40.70	32.0	40.0	10.0
KLM-40	12.4	13.6	8.60	29.5	39.6	25.5	260.0	155.0	-67.40	33.5	42.0	16.6
KLM-1	11.5	13.8	16.6	18.9	30.2	37.4	242.2	172.7	-40.20	32.0	40.5	14.8
KLM-4	7.50	10.4	29.5	12.6	35.0	64.0	240.2	188.9	-27.10	27.5	36.5	23.2
Mean	12.3	13.5		25.0	36.6		258.7	157.0		34.5	41.5	
CD(5%)												
Stress	0.06			0.05			0.42			0.26		
Genotypes	0.11			0.09			0.42			0.32		
Interaction	0.16			0.13			0.59			0.65		

S- Stress, US- Unstressed, RP- Reduction percentage

Table 3 : Water stress at siliquae formation (T_3) stage

Genotypes	Total chlorophyll content (µg/g)			Chlorophyll stability index (%)			Total free proline (µg/g)			Total oil content (%)		
	S	US	RP(%)	S	US	RP(%)	S	US	RP(%)	S	US	RP(%)
KBS-3	12.0	12.4	3.90	29.0	31.9	8.90	418.1	215.2	-94.30	39.5	46.0	14.1
KDH-06	11.5	12.2	6.10	27.9	31.0	10.0	427.1	198.2	-115.5	37.5	44.5	15.7
KS-101	9.10	12.5	27.2	18.9	32.3	41.5	356.2	245.0	-45.40	30.5	40.0	23.7
KLM-40	11.4	11.8	3.90	22.8	29.5	22.6	403.2	216.0	-86.60	31.0	42.0	26.2
KLM-1	10.8	14.7	26.5	21.0	32.2	34.8	301.0	222.5	-35.20	31.5	40.5	22.2
KLM-4	9.20	12.9	28.6	13.7	30.1	54.4	308.5	206.5	-49.40	24.5	36.5	32.8
Mean	10.6	12.9		22.2	31.2		369.1	217.2		32.4	41.5	
CD(5%)												
Stress	0.03			0.06			0.46			0.26		
Genotypes	0.06			0.10			0.46			0.32		
Interaction	0.08			0.15			0.66			0.65		

S- Stress, US- Unstressed, RP- Reduction percentage changes in the lipid protein ratio of pigment–protein complexes or increased chlorophyllase activity (Iyengar and Reddy, 1996; Parida *et al*., 2004). Parida *et al*. (2007) found that the degree of decrease in chlorophylls, carotenoids and protein contents under drought was higher in the sensitive genotype (Ca/H 631) as compared to the moderately tolerant genotype (GM 090304).The data of chlorophyll stability index revealed that genotypes with maximum chlorophyll stability index showed drought tolerance as compared to unstressed plants. Genotypes KBS-3, KDH-06 and KLM-40 showed maximum chlorophyll stability index than genotypes KS-101, KLM-1 and KLM-4 under stressed condition. Gadallah (1995) studied the effect of dehydration stress on chlorophyll content, chlorophyll stability index, leaf relative water content in cotton plant (*Gossypium barbadens*) and reported that the membrane of water stressed plants was less stable than that of unstressed plants. He also reported that proline either alone or in combination with ABA significantly enhanced chlorophyll content as well as chlorophyll stability index, leaf relative water content especially at low water potential, and it was observed that the leaf relative water content was significantly higher in ABA treated stress level. Reddy *et al.* (2003) screened six groundnut cultivars for drought tolerance based on chlorophyll stability index (CSI) and ash content. CSI and ash content showed significant and positive correlation with pod yield under moisture stress conditions.

Total free proline increased under water stressed conditions at every stage in all the genotypes of *B. campestris* .The accumulation of proline under stressed environment is a water stress tolerant trait of the genotypes. All the six genotypes of *B. campestris* showed significantly more increase in the total free proline under stressed as compared to unstressed plants. In this study, genotypes KBS-3, KDH-06 and KLM-40 showed maximum free proline than all other genotypes of brown sarson. Genotype KLM-4 showed significantly less accumulation of free proline. Proline was considered to be the principal solute that may allow plants to overcome drought effect through osmotic adjustment, and serves as storage forms of nitrogen and carbon for future use under less stressful conditions. (Sundaresan and Sudhakaran, 1995).

Phutela *et al.* (2002) observed that in *Brassica juncea* cultivars seeds when treated with a stress of -0.3 MPa at sowing time or a stress of -1.0 MPa after germination by using PEG 6000 showed increased proline content with greatest increase in cv. Varuna under both stressed conditions. Martinez *et al.* (2004) reported that proline is one of the compatible solutes that accumulate in response to water stress and the accumulation of these osmolytes represents an important adaptive response to salt and water stress. Fanaei *et al*. (2011) found that the water stress increased proline and soluble carbohydrate accumulation in the leaves of *Brassica* sp. In non stressed condition (control) in different growth stages, proline was lower than water-stressed plants and leaf proline content decreased significantly after irrigation.

Singh and Singh (1991) studied *B.juncea* cultivars when water stress imposed for two weeks between siliquae initiation and siliquae formation stages and found that oil content decreased by 5.2 per cent compared with control. There was reduction in the quantity of oil in Indian mustard (*B. juncea)* cultivars grown under both salinity and drought (Chauhan and Chauhan, 2005). Germchi *et al.* (2010) results showed that on rapeseed when initial drought occurred during green flower bud stage, reduced the seeds oil content. Shirani *et al.* (2012) reported that water deficit stress reduced the seed oil percentage (oil content) and the oil yield by 2.6 per cent and 25 per cent, respectively in comparison with the normal irrigation treatment.

The lowest drought susceptibility value is shown by the drought tolerant genotype while highest drought susceptibility index is recorded in drought susceptible genotype. Drought susceptibility index showed decreasing trend in all the genotypes from branch initiation stage to siliquae formation stage. Genotype KBS-3 showed significantly less value of DSI than genotypes KDH-06, KLM-40, KS-101and KLM-1 and KLM-4 showed highest DSI (Table 4). Sadaqat *et al*. (2003) and Chauhan *et al*. (2007) reported that drought tolerant varieties showed minimum drought susceptibility index. Based on biochemical responses of all the genotypes of brown sarson (*Brassica campestris* L.) under

Table 4 : Drought susceptibility index (DSI) of different genotypes of *Brassica campestris* at T_1, T_2 and T_3 stages of stress treatments

Genotypes	Drought susceptibility index		
	T_1	T_2	T_3
KBS-3	0.087	0.081	0.080
KDH-06	0.260	0.213	0.123
KS-101	0.315	0.238	0.173
KLM-40	0.313	0.215	0.125
KLM-1	0.485	0.373	0.332
KLM-4	0.522	0.402	0.205
Mean	0.330	0.254	0.173
C.D. (0.05)	0.19	0.87	0.32

water stress it was concluded that genotypes KBS-3 and KDH-06 proved to be tolerant to water stress. Genotype KLM-40 was moderately drought tolerant, whereas genotypes KS-101 and KLM-1 were moderately susceptible and KLM-4 was highly susceptible to water stress. Water stress at siliquae formation stage was found to be more sensitive than branch initiation stage and flower initiation stage.

Correlation analysis of seed yield with biochemical parameters in *Brassica campestris* genotypes :

At branch initiation stage :

At branch initiation stage, seed yield was positively correlated with all biochemical parameters. Total chlorophyll content and chlorophyll stability index (CSI) was positively correlated with total free proline content at both stress intervals. Oil content was positively correlated with all other biochemical parameters except total free proline content at 75 DAS (Table 5).

At flower initiation stage :

Seed yield was positively correlated with all the biochemical parameters at flower initiation stage except with total chlorophyll content at 90 DAS. Chlorophyll stability index (CSI) and total free proline content was positively correlated with all biochemical parameters except total chlorophyll content at 120 DAS. Total free proline content and oil content was positively and significantly correlated with each other (Table 6).

At siliquae formation stage :

At siliquae formation stage, seed yield was positively correlated with total chlorophyll content, CSI, total free proline and oil content. Total free proline was negatively correlated with total chlorophyll content at 130 DAS and with CSI at 160 DAS (Table 7).

The correlation analysis of various biochemical parameters with seed yield in *Brassica campestris* genotypes revealed that the parameters which are positive and significant

Table 5: Correlation analysis of biochemical parameters with seed yield at branch initiation stage

		Total chlorophyll content		Chlorophyll stability index		Total free proline		Oil content
		75	105	75	105	75	105	
Seed yield		0.031	0.504*	0.361	0.370	0.627*	0.311	0.504*
TCC	75		0.106	0.256	0.499	0.488	0.319	0.299
	105			0.452	0.159	0.434	0.626*	0.488
CSI	75				0.228	0.612*	0.516*	0.384
	105					0.413	0.535*	0.030
TFP	75						0.677*	-0.517
	105							0.266

* Indicate significance of value at P=0.05

Table 6: Correlation analysis of biochemical parameters with seed yield at flower initiation stage

		Total chlorophyll content		Chlorophyll stability index		Total free proline		Oil content
		90	120	90	120	90	120	
Seed yield		-0.064	0.513*	0.238	0.237	0.609*	0.518*	0.348
TCC	90		0.251	0.626*	0.358	0.464	0.440	0.112
	120			-0.393	0.371	-0.158	-0.088	0.051
CSI	90				0.224	0.421	0.503*	0.305
	120					0.437	0.592*	0.481
TFP	90						0.933*	0.351
	120							0.124

* Indicate significance of value at P=0.05

Table: 7: Correlation analysis of biochemical parameters with seed yield at siliquae formation stage

		Total chlorophyll content		Chlorophyll stability index		Total free proline		Oil content
		130	160	130	160	130	160	
Seed yield		0.364	0.589*	0.535*	0.510	0.691*	0.526*	0.448
TCC	130		0.578*	0.561*	0.437	-0.285	-0.416	0.309
	160			0.413	0.362	0.652	0.684	0.056
CSI	130				0.625*	0.624	0.566	0.092
	160					-0.379	-0.477	0.140
TFP	130						0.679*	0.339
	160							0.262

* Indicate significance of value at P=0.05

were best correlated with seed yield. This shows that all these parameters contributed significantly in determining yield. Singh *et al.* (2002) found that seed yield per plant was strongly and positively associated with number of seeds and number of pods per plant as well as with chlorophyll content and chlorophyll stability index.

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