

EVALUATION OF HIGH YIELDING CANOLA TYPE *BRASSICA* GENOTYPES/ MUTANTS FOR DROUGHT TOLERANCE USING PHYSIOLOGICAL INDICES AS SCREENING TOOL

MUHAMMAD ALI KHAN*, M.Y ASHRAF¹, S.M. MUJTABA, M.U. SHIRAZI,
M.A. KHAN, A. SHEREEN, S. MUMTAZ, M. AQIL SIDDIQUI
AND G. MURTAZA KALERI

Plant Physiology Division, Nuclear Institute of Agriculture, Tandojam, Pakistan

¹*Nuclear Institute of Agriculture & Biology (NIAB), Faisalabad, Pakistan*

**Corresponding author: alipaec@hotmail.com*

Abstract

An experiment was conducted to assess the effect of various water regimes on growth and yield of canola genotypes/mutants using physiological indices as screening tool, in the year 2007-08. Three canola type genotypes viz., Con-III, Hyola-42 and Shiralee (Check) and two mutants of Rainbow viz., Rainbow-1 (R-75/1) and Rainbow-2 (R-100/6) (including Rainbow-Parent) were selected for the study. The mutants of Rainbow were developed by the *Brassica* Group of NIA, Tandojam. The experiment comprised of four water regimes i.e., W₁ (300 mm), three irrigations of 100 mm each at flowering, siliquae formation and at maturity stage; W₂ (200 mm), two irrigations of 100 mm each at flowering and siliquae formation stage; W₃ (100 mm), single irrigation of 100 mm at flowering stage and W₀ (no irrigation) except soaking one. Relative water contents (RWC), Osmotic potential (OP) and potassium contents were generally decreased whereas total greenness (Spad value) and proline contents increased under various water regimes as compared to control. The present study showed that the genotype Con-III and the mutant Rainbow-2 (R-100/6) were relatively tolerant to drought stress as compared to all other genotypes/mutants.

Introduction

Pakistan is deficient in edible oil and is continuously meeting the domestic requirement at the cost of precious foreign exchange. During 2006-07, 59.506 billion rupees were spent on the import of 1.787 million tons of edible oil. At present, edible oil requirement of the country is 2.764 million tons annually, of which 0.857 million tons (31%) comes from local resources and 1.907 million tons (69%) is being imported (Anon., 2006). To minimize this national loss and to meet the demands of ever-increasing population, the production of oil seed must be raised. Brassica species are widely cultivated for their edible oil and mostly grown as rainfed crop, depending upon the winter rains (Chopra & Prakash, 1996). Among brassica species rapeseed and mustards (*Brassica juncea*) contribute 21% towards national oil production but the quality of oil is low due to the presence of erucic acid and glucosinolates. Erucic acid decreases the taste and flavour while glucosinolates cause nutritional disorder. These chemicals adversely affect the growth and reproduction of animals if fed at significant level in diet (Vermorel *et al.*, 1986). Canola (*Brassica napus*) varieties on the other hand are low in these chemicals. Besides, this crop has lowest saturated fats, containing only 6% saturated fat and is high in mono-unsaturated fat. It has 50% less saturated fat than Corn oil (Weiss, 1983). Canola (Canadian oil, low in acid) is now the third largest source of edible oil after soybean (*Glycine max*) and palm (*Elaeis oleifera*) oil (Nowlin, 1991). Canola is recent introduction in Pakistan and area under this crop is expanding rapidly especially under moderate climatic conditions. However yield is less than potential of existing

cultivars due to many reasons, shortage of water being the most important one. Canola is relatively poorly adapted to drought condition (Wright *et al.*, 1997). Many aspects of its production package technology need to be unveiled.

Water is one of the main abiotic factors limiting crop production in several regions of the world (Araus *et al.*, 2002). Drought stress (water deficit or low water availability) is a major problem, widely distributed world wide over 1.2 billion ha in rain fed agricultural land (Kijni, 2006; Passioura, 2007). In Pakistan, heavy crop losses occur due to low and irregular rainfall (less than 100 mm) resulting in shortage of water (Anon., 2003). All physiological processes like photosynthesis, cell turgidity, growth of cells and tissue in plant are directly affected by water (Reddi & Reddi, 1995). Yield losses up to 60-100% are reported due to long spell of water shortage (drought) in different crop species including canola type brassica (Singh *et al.*, 2002)

Due to great economic importance of canola for farmers and shortage of water in the country, the evaluation of canola genotypes/mutants with high yield and stable seed under low water availability is an important need of the day as drought tolerant genotypes may be the only reasonable alternative to many small-scale farmers (Tabassum, 2004). For high yielding mutants under low water environment, recommendation can be given to breeder to pursue these mutants for their release as a variety. Furthermore, the evaluated genotypes can be used in hybridization programme to develop superior genotypes for drought tolerance and high yield.

Keeping in view above facts present study was therefore undertaken to evaluate drought tolerant genotypes and locally developed mutants of Rainbow under various water regimes using physiological indices as evaluating tools.

Materials and Methods

The experiment was conducted at the farm of NIA, Tandojam in the cemented tanks measuring 2.25m x 2.25m x 0.45 m (depth) during 2007-08 on clay loam soil containing 1.08% O.M, 40 mg kg⁻¹ available nitrogen, 8.5 mg kg⁻¹ AB-DTPA extractable-P, 214 mg kg⁻¹ extractable-K. Three canola type varieties (Con-III, Hyola-42 and Shiralee (Check)), and two canola type mutants of Rainbow (Rainbow-1 (R-75/1), Rainbow-2 (R-100/6), along with parent (Rainbow-P) were sown as per randomized complete block design (RCBD) with three replications. The sowing was done by single coulter hand driven drill and row to row and plant to plant distance was 30 cm. Recommended doses of fertilizers were applied @ 120-60-0 Kg NPK ha⁻¹ at the time of 1st and 2nd irrigation (split doses). The soil moisture contents were taken at an interval of 15 days from 0-30 cm depth (Fig. 1). Three plants were randomly selected from each row or replicate at maturity for recording height, branches, siliquae and grain yield per plant.

Physiological indices: Leaf relative water content (LRWC) was determined following the method as described by Turner (1986). Fully expanded 2nd leaf was excised from each plant and fresh weight was recorded. After taking fresh weight, all the leaves were immersed in distilled water for 10 h then saturated weight of each leaf was recorded. Samples were then dried in an oven at 70°C for 48 h and dry weight was determined. Leaf relative water content (LRWC) was estimated according to the equation:

$$\text{LRWC} = (M_f - M_d) / M_s - M_d) \times 100$$

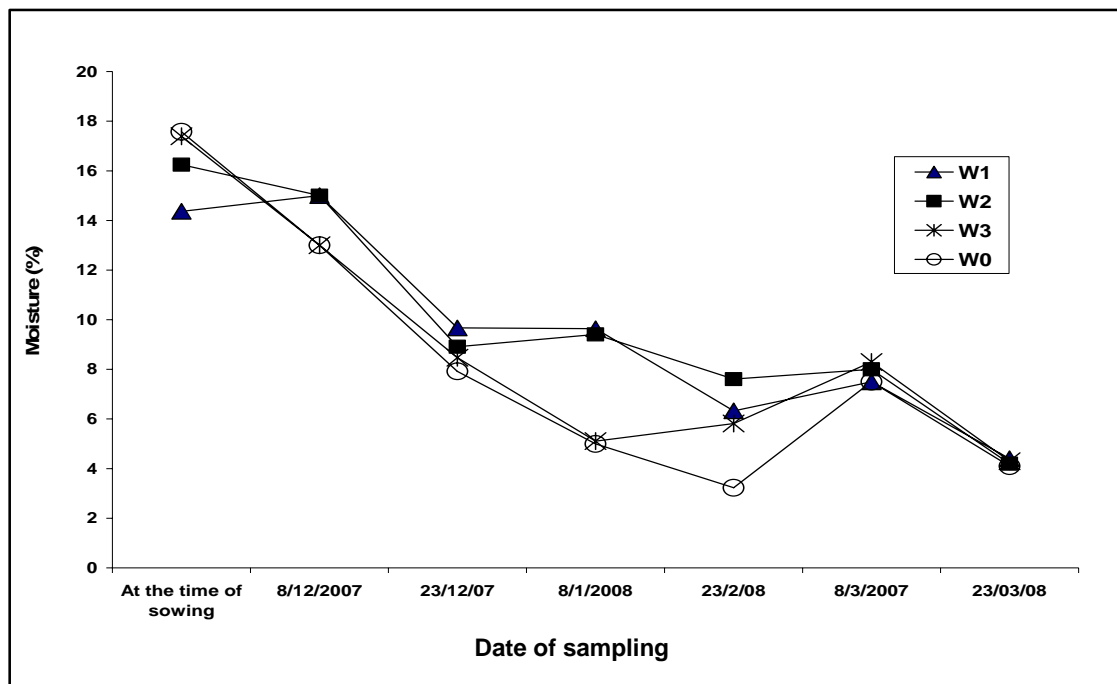


Fig. 1. Soil moisture contents at the interval of 15 days (0-30 cm).

where M_f , M_d and M_s are the fresh, oven dried and water saturated weight of the leaves, respectively. Chlorophyll content was measured after 70 days of sowing by chlorophyll meter (Minolta, SPAD-502, Japan). The fully expanded 2nd leaf was measured at six positions and the average is being presented as Total greenness (Spad value). Proline content was determined after extraction in 3% sulfosalicylic acid as described by Bates, (1973). Leaf osmotic potential was measured by measuring osmolality of extracted leaf sap using a calibrated Osmomat 030 (Khan *et al.*, 1992). Leaf potassium (K^+) contents were determined after extraction in 0.1 M acetic acid (CH_3CO_2H) following the method as described by Ansari & Flower, (1986). Seed oil contents were determined following the official methods of analysis (Anon., 1990).

The measured quantity of water was applied with the help of water gauge. The experiment comprised of four water regimes.

- W₀ (300 mm) = Three irrigations of 100 mm each at flowering, siliquae formation and at maturity stage (control)
 W₁ (200 mm) = Two irrigations of 100 mm each at flowering and siliquae formation stage
 W₂ (100 mm) = Single irrigation of 100 mm at flowering stage
 W₃ = No irrigation except soaking one.

The data were subjected to statistical analysis using computer software MSTAT-C and DMRT test was applied to compare the treatment means at 5% level of significance.

Results and Discussion

Growth parameters presented in Table 1 show that generally there was a marked reduction in plant height in all the genotypes/mutants under water limited environment. Non-significant ($p \leq 0.05$) reduction in plant height was observed under the water regime W₂ in all the genotypes/mutants as compared to control water regime W₁. Under the water regimes W₃ and W₀, Hyola-42, Shiralee, Rainbow-1(R-75/1) and Rainbow-P exhibited non-significant reduction, however Con-III and Rainbow-2 (R-100/6) differed significantly

compared to control regime (W_1). Maximum plant height was observed in Rainbow-2 (R-100/6) and minimum in Con-III under the water regime W_1 and W_2 . General reduction in plant height under water deficit environment has been confirmed by many workers (Francois, 1994; Ashraf & Sarwar, 2002). Growth of plant depends on cell expansion and enlargement which is probably the most sensitive physiological aspect of a plant with regard to water deficit leading to reducing plant productivity (Larson, 1992) which ultimately affect plant height. Phenolic compounds produced in plants during water stress conditions also respond to reduce plant growth (Einhelling & Souza, 1992; Blum *et al.*, 1991). Number of branches plant⁻¹ also differed non-significantly under the water regime W_2 in the genotypes Con-III, Hyola-42 and Shiralee over control regime (W_1), however under the regime W_3 and W_0 , significant reduction as compared to W_1 was observed in the genotypes Con-III, Hyola-42, Shiralee and Rainbow-1(R-75/1). Rainbow-2 (R-100/6) and Rainbow-P exhibited non-significant reduction under all the water regime. Maximum branches plant⁻¹ was observed by Con-III as compared to all other genotypes/mutants under the water regime W_1 and W_2 ($W_1= 5.80$ and $W_2= 5.20$). Also maximum number of siliquae plant⁻¹ were produced by Con-III under W_1 (130.5), W_2 (100.7) and W_3 (41.0) as compared to all genotypes/mutants followed by Shiralee under W_1 (96.20) and W_2 (67.55). Minimum siliquae plant⁻¹ was produced by Hyola-42 under W_1 (60.6). Maximum number of branches and number of siliquae plant⁻¹ in Con-III might be due to dwarfness of the variety. This fact was in line with Olenjniczak & Adamaska, (1999), who reported that reduction in plant height causes an increase in vegetative growth and grain yield because of tolerance to lodging under unfavorable condition.

Table 1. Yield contributing characters as influenced by various water regimes.

Genotypes/ mutants	Water regimes	Plant height (cm)		Branches plant ⁻¹		Siliquae plant ⁻¹	
Con-III	W_1	73.33	a	5.80	a	130.50	a
	W_2	69.44	a	5.20	a	100.70	a
	W_3	60.22	b	3.00	b	41.00	b
	W_0	49.67	c	1.50	c	21.39	b
Hyola-42	W_1	86.44	a	4.30	a	60.60	a
	W_2	84.00	a	4.40	a	64.60	a
	W_3	52.78	b	2.40	b	23.44	ab
	W_0	62.50	b	2.50	b	17.70	b
Shiralee	W_1	89.67	a	4.30	a	96.20	a
	W_2	86.05	a	3.40	a	67.55	b
	W_3	60.00	b	2.30	b	21.40	c
	W_0	60.00	b	2.50	b	23.60	bc
Rainbow-1 (R-75/1)	W_1	93.33	a	5.00	a	71.80	a
	W_2	90.55	a	4.00	ab	54.80	b
	W_3	68.89	b	3.00	bc	26.70	c
	W_0	56.66	b	2.20	b	31.80	c
Rainbow-2 (R-100/6)	W_1	94.88	a	3.50	a	79.08	a
	W_2	91.11	a	2.80	a	52.10	b
	W_3	65.11	b	3.30	a	34.43	c
	W_0	38.33	c	1.80	a	27.70	c
Rainbow-P	W_1	80.77	a	3.50	a	67.90	a
	W_2	78.89	a	3.80	a	66.10	a
	W_3	50.61	b	3.30	a	34.70	a
	W_0	44.72	b	2.90	a	28.30	a

Means in a column followed by same letter (s) do not differ significantly at 5% probability level according to DMRT.

Relative water contents (RWC) (%) measurement characterizes the internal water status of plant tissues and is also a convenient method for following changes in tissue water content without errors caused by continually changing tissue dry weight (Erickson *et al.*, 1991). Under all the water regime and in all the genotypes/mutants RWC were generally lower in water stressed plant than in plants grown under control water regime (W_1). However, data showed non-significant ($p \leq 0.05$) reduction under the water regime W_2 as compared to control regime (W_1) in all the genotypes/mutants except in Rainbow-P where significant reduction was observed. Within the water regime W_3 and W_0 , non-significant reductions were observed in the genotypes/mutants Con-III, Shiralee and Rainbow-2 (R-100/6) whereas Hyola-42, Rainbow-1 (R-75/1) and Rainbow-P showed significant reduction. These two water regime i.e., W_3 and W_0 also showed significant decrease in Con-III, Shiralee and Rainbow-2 (R-100/6) as compared to control regime (W_1). Non-significant difference between W_1 and W_2 might be due to the fact that irrigation difference of 100 mm ($W_1 = 300$ mm, $W_2 = 200$ mm, $W_2 - W_1 = 100$ mm) could not make any significant reduction in the RWC in all the genotypes/mutants. These results are in line with those of Yordanov *et al.*, (2003), who reported that mild drought induces no significant changes in RWC. Omae *et al.*, (2007) reported that maintenance of RWC in some cultivars might relate with their water absorbing ability and contribute to less reduction in seed yield. Significantly higher reduction in W_3 (100 mm) and W_0 (no irrigation) as compared to control regime (W_1) in the Con-III, Shiralee and Rainbow-2 (R-100/6) is in accordance with the results obtained by Begum & Paul (1993). They reported that under severe drought conditions, only tightly bound water inside the cell is preserved whereas loosely bound water is lost or transpired. Hyola-42 ($W_3 = 80.6\%$, $W_0 = 75.3\%$) and Rainbow-1 (R-75/1) ($W_3=80\%$, $W_0 = 75\%$) maintained high RWC even at W_3 and W_0 (severe drought condition). Maintenance of high RWC, even at severe drought conditions, has been considered to be a drought resistance rather than drought escape mechanism and it is a consequence of adaptive characteristics such as osmotic adjustment (Grashoff & Ververke, 1991). Osmotic potential (-MPa) was generally reduced (more -ve values) under all the water regime and in all the genotypes/mutants as compared to control regime (W_1). Hyola-42 exhibited non-significant ($p \leq 0.05$) reduction under W_2 as compared to W_1 (control regime). All other genotypes/mutants formed a pattern in which osmotic potential was more affected under the water regimes W_3 and W_0 as compared to control regime (W_1). It has now been well established that severe stress reduces the osmotic potential (more -ve values) due to more accumulation of solutes in all the crops (Santos-Diaz & Ochoa, 1994). In brassica crop, similar decrease in osmotic potential due to soil moisture stress has already been reported by Kumar & Singh, (1998). Proline (μ mole g^{-1} fresh wt) accumulation was increased in all the genotypes/mutants under all water regimes. Significantly ($p \leq 0.05$) higher proline accumulation as compared to control (W_1) was observed under water regimes W_0 followed by W_3 and W_2 in all the genotypes/mutants. The genotype Con-III and mutant Rainbow-1 (R-75/1) had similar values of proline accumulation under W_0 and W_3 . Maximum proline accumulation as compared to control regime (W_1) was observed in Hyola-42 under water regime W_0 (38.90μ mol g^{-1} fresh wt, 10 fold increase) followed by W_3 (30.6μ mol g^{-1} fresh wt, 8 fold) and W_2 (17.30μ mol g^{-1} fresh wt, 4.5 fold). Con-III also showed significantly ($p \leq 0.05$) higher proline accumulation under W_3 (40.7μ mol g^{-1} fresh wt, 9.6 fold) and W_0 (42.80μ mol g^{-1} fresh wt, 10 fold). Minimum proline increase compared to control regime (W_1) was observed in Rainbow-1(R-75/1) under W_0 (41.70μ mol g^{-1} fresh wt, 4 fold increase) followed by W_3 (35.0μ mol g^{-1} fresh wt, 3 fold increase) and W_2 (22.40μ mol g^{-1} fresh wt, 2 fold). Rainbow-P also showed significantly ($p < 0.05$) higher proline accumulation under W_0 (47.90μ mol g^{-1} fresh wt, 8 fold increase) as compared to W_1 (5.70μ mol g^{-1} fresh wt). Proline accumulation is well documented as

an osmoregulatory solute in plants subjected to hyper osmotic stress. Its accumulation in drought stressed plants is one of the vital compatible solutes to function in cellular osmotic adjustment and scavenge detoxify oxidants (Delauney & Verma, 1993; Seki *et al.*, 2007; Yamada *et al.*, 2005).

The comparatively higher accumulation of proline under W_3 and W_0 in the genotype Hyola-42, Con-III and the mutant Rainbow-P might be due to higher osmotic adjustment in these genotypes/ mutant (Serraj & Sinclair, 2002). Leaf K^+ contents were generally decreased in all the six genotypes/mutants under all the water regimes except Hyola-42, which exhibited non-significant ($p \leq 0.05$) increase under the water regimes W_2 (4.90%) and W_3 (4.10%) as compared to control regime W_1 (3.90%). The generally low uptake of K^+ under water deficit environment in all the genotypes might be due to excessive leakage of ions from the cell (Umar, 2006). In addition under water stress, older roots surrounded by dry soil apparently lost their ability to function and nutrients (K^+ in this case) were supplied exclusively by more active root tips. This leads to a low uptake of K^+ under water deficit environment (Pessarakli, 1993). Umar (2006) reported that under water deficit condition, the nutrient film around the soil particle becomes thin, therefore, the distance for movement of ions increases resulting poor diffusion of ions into the plant roots, thus causing low K^+ contents in the plant. The genotype Con-III exhibited non-significant ($p \leq 0.05$) decrease under all the three water regimes i.e., W_2 (3.7%), W_3 (3.30%) and W_0 (3.40%) as compared to control water regime W_1 (3.90%), however Shiralee and Rainbow-1 (R-75/1) showed significant reduction under W_2 , W_3 and W_0 as compared to W_1 . The genotypes Shiralee and mutant Rainbow-1 (R-75/1) had significantly lowest value of K^+ contents under W_2 (2.70%) and W_0 (2.80%), respectively. The mutant Rainbow-2 (R-100/6) had significantly similar values of K^+ contents under W_2 (4.0 %) and W_3 (3.90%) except W_0 (3.0%) which had significantly lower value of K^+ contents as compared to W_1 (4.10 %). Rainbow-P had similar values under the water regimes W_2 (3.10%), W_3 (3.50%) and W_0 (3.20%) whereas all these values are significantly different than that of control regime W_1 (3.70 %). The non-significant ($p \leq 0.05$) decrease of K^+ contents in the genotype Con-III under all the water regime might be due to higher osmotic adjustment in this genotype. Similar (non-significant) values of K^+ contents in the mutant Rainbow-1 (R-75/1) (under W_2 , W_3 and W_0) and Rainbow-P (under W_2 , W_3 and W_0) might also be due to higher osmotic adjustment in these mutants. These results are in agreement with Patakas *et al.*, (2002), who suggested that K^+ accumulation is a component of osmotic adjustment in water stressed plants. Significantly higher value of K^+ contents higher than that of control regime W_1 (3.90%) in the genotype Hyola-42, under W_3 (4.10%) and W_0 (4.0%) could be ascribed due to high accumulation of proline (8 fold and 10 fold increase, respectively, Table 2) under these water regimes. Cuin & Shabalah, (2007) reported that solutes like proline reduced K^+ efflux from the cell and maintains cyto-solic K^+ homeostasis possibly through enhanced activity of H^+ - ATPase.

Total greenness (Spad chlorophyll) values were higher under all the water regimes i.e., W_2 , W_3 and W_0 in all the genotypes/ mutants as compared to control water regime W_1 (Table 2). Significantly ($p \leq 0.05$) higher Total greenness values (Spad chlorophyll) were found in the water regimes W_0 in the genotypes Hyola-42, Rainbow-1, Rainbow-2 and Rainbow-P. Plants under water stress conditions have evolved mechanism to protect against photodamage. One such mechanism for protection entails changes in chlorophyll contents in order to reduce the extent of absorbed light. (Murchie & Horton, 1997). So the increase in chlorophyll content (Spad chlorophyll) under water deficit environment is a common observation (Estill *et al.*, 1991; Hamda, 1996) (Table 2). Similar results for spad value were reported by Singh *et al.*, (2002) in brassica.

Table 2. Physiological indices as influenced by various water regimes.

Genotypes/ mutants	Water regimes	Relative water content (%)	Osmotic potential (-MPa)	Proline ($\mu\text{mol g}^{-1}$ fresh wt.)	K ⁺ contents (%)	Total greenness (Spad value)					
Con-III	W ₁	90.00	a	0.94	c	6.50	c	3.90	a	44.60	b
	W ₂	79.50	a	0.96	c	19.00	b	3.70	a	46.90	b
	W ₃	59.70	b	1.20	b	40.70	a	3.30	a	59.30	a
	W ₀	65.60	b	1.60	a	42.80	a	3.40	a	48.30	b
Hyola-42	W ₁	87.40	a	0.96	bc	3.80	d	3.90	ab	45.80	c
	W ₂	83.50	a	0.87	c	17.30	c	4.90	a	53.00	b
	W ₃	80.60	ab	1.30	ab	30.60	b	4.10	b	55.60	ab
	W ₀	75.30	b	1.40	a	38.90	a	3.00	b	59.60	a
Shiralee	W ₁	88.00	a	0.89	c	8.00	d	4.40	a	47.40	b
	W ₂	85.80	a	0.95	c	21.30	d	2.70	c	58.90	a
	W ₃	64.00	b	1.20	b	32.00	b	3.70	b	60.80	a
	W ₀	68.40	b	1.50	a	41.80	a	3.80	b	58.30	b
Rainbow-1 (R-75/1)	W ₁	88.60	a	0.85	b	10.80	c	4.70	a	51.60	bc
	W ₂	88.50	a	0.84	b	22.40	b	3.50	b	50.50	c
	W ₃	80.00	ab	1.40	a	35.00	a	3.20	b	56.80	ab
	W ₀	74.90	b	1.20	b	41.70	a	2.80	b	61.90	a
Rainbow-2(R2100/6)	W ₁	88.00	a	0.95	b	7.00	d	4.10	a	47.50	c
	W ₂	85.70	a	0.95	b	22.00	d	4.00	ab	52.50	b
	W ₃	64.80	b	1.30	b	33.80	b	3.90	ab	48.60	c
	W ₀	63.00	b	1.60	a	40.00	a	3.00	b	65.90	a
Rainbow-P	W ₁	87.20	a	0.88	c	5.70	d	3.70	a	49.70	b
	W ₂	85.00	ab	0.87	c	16.70	c	3.10	ab	53.80	b
	W ₃	78.00	bc	1.20	b	32.00	b	3.50	ab	59.00	ab
	W ₀	70.67	c	1.40	a	47.90	b	3.20	ab	66.00	a

Means in a column followed by same letter (s) do no differ significantly at 5% probability level according to DMRT

Grain yield plant⁻¹ was significantly ($p < 0.05$) affected due to different water regimes in all the genotypes/mutants except Con-III which exhibited non-significant decrease under all the water régimes. Comparatively low reduction as compared to W₁ was observed under the regime W₂ (5.8 g) in the mutant Rainbow-2 (R-100/6). Shiralee as a check variety showed comparatively less reduction under all the water regimes as compared to control regime (W₁). Maximum grain yield plant⁻¹ and non-significant reduction compared to control (W₁) in the genotype Con-III can be attributed to high number of branches plant⁻¹ and siliquae plant⁻¹. Panda *et al.*, (2004) demonstrated that increase in number of siliquae plant⁻¹ and number of branches plant⁻¹ directly influenced the seed yield in mustard. Higher osmotic adjustment in the genotype Con-III could also be the probable reason for higher grain yield under all the water regimes. Comparatively low reduction in the mutant Rainbow-2 under W₂ compared to W₁ might also be due to higher osmotic adjustment in this mutant. High seed oil content is an important selection criterion in the *Brassica* genotypes/ mutants. Oil contents were affected significantly ($p < 0.05$) and non-significantly due to different water regimes as compared to control regime (W₁). Similar values were observed by Hyola-42 (40.0 & 39.0 %) under W₁ and W₀ by shiralee (38.5 & 38.0 %) and Rainbow-1 (R-75/1) (40.0 & 40.0 %) under W₂ and W₀. Rainbow-2 (R-100/6) (40.7 & 41.5) exhibited significantly similar values under W₃ and W₀ and Rainbow-P (40.0, 40.5 & 40.5) under W₁, W₂ & W₀. Of all the genotypes/mutants under study, Rain-2 (R-100/6) produced significantly higher oil contents under W₁ (43.50 %) followed by significantly decreased oil contents under W₂ (42.0 %), W₃ (40.7 %) and W₀ (41.5 %) (Table 3).

Table 3. Grain yield and oil contents as influenced by various water regimes.

Genotypes/mutants	Water regimes	Grain yield plant ⁻¹ (g)		Oil contents (%)	
Con-III	W ₁	7.43	a	41.30	a
	W ₂	5.53	a	40.00	c
	W ₃	4.23	a	40.50	b
	W ₀	2.56	a	37.50	d
Hyola-42	W ₁	5.60	a	40.00	ab
	W ₂	3.20	b	39.00	b
	W ₃	1.38	c	40.70	a
	W ₀	1.07	d	39.50	ab
Shiralee	W ₁	5.49	a	42.00	a
	W ₂	4.56	b	38.50	c
	W ₃	3.21	c	40.50	b
	W ₀	1.02	d	38.00	c
Rainbow-1 (R-75/1)	W ₁	5.00	a	40.70	b
	W ₂	3.60	b	40.00	c
	W ₃	1.10	c	41.50	a
	W ₀	0.32	d	40.00	c
Rainbow-2 (R-100/6)	W ₁	6.96	a	43.50	a
	W ₂	5.80	b	42.00	ab
	W ₃	1.83	c	40.70	b
	W ₀	0.87	d	41.50	b
Rainbow-P	W ₁	2.80	a	40.00	a
	W ₂	2.60	a	40.50	a
	W ₃	2.40	b	38.50	b
	W ₀	2.10	c	40.50	a

Means in a column followed by same letter (s) do not differ significantly at 5% probability level according to DMRT.

Conclusion and recommendations: In conclusion, leaf relative water contents (LRWC), Osmotic potential (O.P) and potassium (K⁺) contents were decreased under water deficit environment in all the genotypes/ mutants. However proline contents and total greenness (spad value) were increased. Of all brassica genotypes/mutants tested in the present study, the genotype Con-III and mutant Rainbow-2 (R-100/6) were found relatively tolerant to drought stress. The genotype Con-III can be used in hybridization programme to develop superior genotypes with drought tolerance. For mutant Rainbow-2 (R-100/6), the breeders can be advised to pursue this mutant for release as a variety.

Acknowledgement

The authors highly acknowledge Mr. Saqib Ali Memon for typing and composing this manuscript and Mr. Tarique Mehmood for providing technical assistance during this study.

References

- Anonymous. 1990. *Official Methods of Analysis, Association of Official Agricultural Chemists*. 14th Edn. Arlington, Virginia, USA.
- Anonymous. 2003. Government of Pakistan, Ministry of Economic Affairs and Statistics, Islamabad.
- Anonymous. 2006. *Economic survey of Pakistan*. 2005-06. Ministry of Finance, Government of Pakistan, pp. 11-16.

- Ansari, R. and T.J. Flower. 1986. Leaf to leaf distribution of ions in some monocotyledonous plants grown under saline conditions. In: *Prospects for biosaline research* (Eds): R. Ahmed and A. San Pietro. University of Karachi, p. 167-181.
- Araus, J.L., G.A. Slafer, M.P. Reynolds and C. Royo. 2002. Plant breeding and drought in C₃ cereals: what to breed for? *Ann. Bot.*, 89: 925-940.
- Ashraf, M.Y. and G. Sarwar. 2002. Salt tolerance potential in members of Brassicaceae. Physiological studies on water relations and mineral contents. In: *Prospects for saline Agriculture* (Eds): R Ahmad and K.A. Malik. Kluwer Academic Publishers, Netherlands, pp. 237-245.
- Bates, L.S. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39: 205-7.
- Begum, F.A. and N.K. Paul. 1993. Influence of soil moisture on growth, water use and yield of mustard (*Brassica juncea* L.). *J. Agron. & Crop Sci.*, 170: 136-141.
- Blum, A., J.W.E. Janson, L. Ramseur and E.W. Tollner. 1991. The effect of drying top soil and a possible non-hydraulic root signals on wheat growth and yield. *J. Exp. Bot.*, 42: 1225-1231.
- Chopra, V.L. and S. Prakash. 1996. Biotechnology boosts utilization of wild relatives for crop improvement. *Indian Brassica Crop Diversity*, 12: 87-88.
- Cuin, T.A. and S. Shabalah. 2007. Compatible solutes reduce ROS-induced potassium efflux in *Arabidopsis* roots. *Plant Cell Environ.*, 30(7): 875-885.
- Delauney, A.J. and D.P. Verma. 1993. Proline biosynthesis and osmoregulation in plants. *Plant J.*, 4: 215-23.
- Einhelling, F.A. and I.F. Souza. 1992. Phytotoxicity of sorgoleone found in grain sorghum root exudates. *J. Chem. Ecol.*, 18: 1-11.
- Erickson, I.J., D.L. Ketring and J.F. Stone. 1991. Response of internal tissue water balance of peanut to soil water. *Agron. J.*, 72: 73-80.
- Estill, K., R.H. Delaney, W.K. Smith and R.L. Ditterline. 1991. Water relations and productivity of alfalfa leaf chlorophyll variants. *Crop Sci.*, 31: 1229-1233.
- Francois, L.E. 1994. Growth, seed yield and oil content of canola grown under saline conditions. *Agron. J.*, 86: 233-237.
- Grashoff, C. and D.R. Ververke. 1991. Effect of pattern of water supply on *Vicia faba* L., plant water relations, expansive growth and stomatal reactions. *Netherland J. Agric Sci.*, 39, 247-262.
- Hamda, A.M. 1996. Effect of NaCl, water stress or both on gas exchange and growth of wheat. *Biol. Plant.*, 38: 405-415.
- Khan, A.H., M.Y. Ashraf and A.R. Azmi. 1992. Osmotic adjustment in wheat. A response to water stress. *Pak. J. Sci. Ind. Res.*, 36: 151-155.
- Khatri A., I.A. Khan, M.A. Siddique, G.S. Nizamani and S. Raza. 2004. Performance of Oil seed Brassica in different water regimes. *Pak. J. Bot.*, 36(2): 351-357.
- Kijni, J.W. 2006. Abiotic stress and water scarcity: Identifying and resolving conflicts from plant level to global level. *Field Crops Res.*, 97:3-18
- Kumar, A. and D.P. Singh. 1998. Use of physiological indices as a screening technique for drought tolerance in oilseed brassica species. *Ann. Bot.*, 81: 413-420.
- Larson, K.L. 1992. Drought injury and resistance of crop plants. In: *Physiological aspects of dry land farming* (Ed.): S.U. Gupta. pp. 147-162. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.
- Murchie, E.H. and P. Horton. 1997. Acclimation of photosynthesis to irradiance and spectral quality in British plant species: chlorophyll content, photosynthetic capacity and habitat preference. *Plant cell Environ.*, 20: 438-448.
- Nowlin, D. 1991. Winter canola. *Agric. Consultant*, 47(4): 8.
- Olenjniczak, J. and E. Adamaska. 1999. Achievement of mutation breeding of cereal and oil seed crops in Poland. *Proc. Int. Symp. New Genetical Approaches to Crop Improvement-III*, Nuclear Institute of Agriculture, Tandojam, Pakistan, pp. 55-63.

- Omae, H., A. Kumar, K. Kashiwaba and M. Shono. 2007. Assessing drought tolerance in snap bean (*Phaseolus vulgaris*) from genotypic differences in leaf water relations, shoot growth and photosynthetic parameters. *Plant Prod. Sci.*, 10(1): 28-35.
- Panda, B.B., S.K. Bandyopadhyay and Y.S. Shivay. 2004. Effect of irrigation level, sowing dates and varieties on yield attributes, yield, consumptive water use and water-use efficiency of Indian mustard (*Brassica juncea*). *Indian J. Agric. Sci.*, 74(6): 339-342.
- Passioura, J. 2007. The drought environment: Physical, biological and agricultural perspectives. *J. Exp. Bot.*, 58:113-117
- Patakas, A., N. Nikolaou, E. Zioziou, K.S. Radoglou and B. Noitsakis. 2002. The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines. *Plant Sci.*, 163(2): 361.
- Pessaraki, M. 1993. *Hand book of plant and crop stress*. 1st edn. University of Arizona, Tucson Arizona, USA.
- Reddi, G.H.S. and T.Y. Reddi. 1995. Irrigation of principal crops, In: *Efficient use of irrigation water*, 2nd edn, Kalyani Publishers, New Delhi, India, pp. 229-259.
- Santosh-Diaz, M.S. and N.ocha-Alejo. 1994. Effect of water stress on growth, osmotic potential and solute accumulation in cell culture from chilli pepper (amesophyte) and creosol bush (a xerophyte). *Plant Sci.*, 96: 21-29.
- Seki, M., T. Umezawa, K. Urano and K. Shinozaki. 2007. Regularory metabolic networks in drought stress responses. *Curr. Opin. Plant Biol.*, 10: 296-302.
- Serraj, R. and T.R. Sinclair. 2002. Osmolyte accumulation: can it really increase crop yield under drought condition? *Plant Cell Env.*, 25 : 333-341
- Shao, H.B., L.Y. Chu, C.A. Jaleel and C.X. Zhao. 2008. Water deficit stress-induced anatomical changes in higher plants. *Comp. Ren. Biol.*, 331:215-225.
- Singh, M.P., U.N. Pandey, R.K. Lal and G.S. Chaturvedi. 2002. Response of *Brassica* species to different irrigation regimes. *Indian J. Plant Physiol.*, 1(7): 66-69
- Tabassum, M.I. 2004. Development of maize under water stress areas. Published in DAWN, Internet Edn., November 14, 2004.
- Turner, N.C. 1986. Crop water deficits, a decade of progress. *Adv. Agron.*, 39:1-51.
- Umer, S. 2006. Alleviating adverse effects of water stress on yield of sorghum, mustard and ground nut by potassium application. *Pak. J. Bot.*, 38(5): 1373-1380.
- Vermorel, M., R.K. Heaney and G.R. Fenwick. 1986. Nutritive value of rapeseed; Effect of individual glucosinolates. *J. Sci. Food Agric.*, 37: 1197-1202.
- Weiss, E.A.. 1983. *Oil seed crops*. pp. 161-215. Longman Group Limited.
- Wright, P.R., J.M. Morgan and R.S. Jessop. 1997. Drought stressed mustard yields more than canola due to greater leaf turgor. *Ann. Bot.*, 80: 313-319.
- Yamada, M., H. Morishita, K. Urano, N. Shiozaki, K. Yamaguchi-Shinozaki, K. Shinozaki and Y. Yoshiba. 2005. Effects of free proline accumulation in petunias under drought stress. *J. Exp. Bot.*, 56: 1975-1981.
- Yordanov, I., V. Velikova and T. Tsoney. 2003. Plant response to drought and stress tolerance. *Bung. J. Plant Physiol.*, Special Issue, pp. 187-208.

(Received for publication 11 January 2010)