Assessment of drought stress and various levels of potassium on biochemical properties of two species of canola and mustard

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Abstract: In order to assess the effect drought stress and various levels of potassium on biochemical properties of two species of canola and mustard a field experiment was conducted in a factorial design based on randomized complete block design with three replications including three irrigation regimes (I1=irrigation after 50% depletion of soil water(control),I2 =irrigation after 70% water depletion and I3 =irrigation after 90% water depletion), two species (Hyola 401 hybrid of canola and landrace cultivar of mustard) and three levels of potassium fertilizer (K_1=0 ,K_2=150 and K_3= 250 kg.ha^{-1} K_2SO_4) at Agricultural and Natural Resources Research Center of Sistan in 2008-2009 cropping season. 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. Mustard landrace showed higher capability for accumulating assimilates such as proline, soluble carbohydrates and potassium than hybrid Hyola 401. Potassium application caused a decrease in proline and an increased in soluble carbohydrates concentration in the leaf under water stress condition. There were a negative correlation between grain yield and proline content and soluble carbohydrates. It was concluded that osmotic adjustment can be an important mechanism for Brassica species under water stress.
Key words: Canola, proline, Potassium, soluble carbohydrates
Introduction: Among the environmental stresses, drought is a major abiotic factor that limits agricultural crop production. Plants during exposure to drought, different mechanisms can be recruited. One of the effective mechanisms of plant under water deficit is osmotic adjustment (Ma et al., 2004 and Ma et al., 2006).During osmotic stress; plant cells accumulate solutes to prevent water loss and to reestablish cell turgor. The solutes that accumulate during the osmotic adjustment include ions such as K, Na, and Cl or organic solutes such as proline and soluble sugars. (Sharma, and Kuhad, 2006). Proline accumulation is most common reaction during water stress and salinity. Proline also as a protective osmotic it serves role as an energy source to adjust redox potential, relieving oxygen radical species, protective material of large molecules against denaturing and reduce acidity cell Sharma, and Kuhad. 2006). Accumulation of soluble sugars in response to drought stress has been documented as well as strong (Ma et al., 2004 and Ma et al., 2006).Potassium plays important physiological roles in the cell under unfavorable environmental conditions. Sharma and Kuad (2006) in species of the genus Brassica reported that in response to potassium application under stress, gradual decrease proline and increase in starch, soluble carbohydrate total and soluble protein content was observed.
Materials and Methods: A field experiment was conducted in 2007-2008 at Zahak Agricultural and Natural Resources Research Center of Sistan and Baluchestan Province (31°54′N; 61°41′E, and 483 m above sea level). Experimental design was a factorial arrangement based on randomized complete block with three replications. Three irrigation regimes including: control (irrigation after 50 %), moderate stress (irrigation after 70%), and severe stress (irrigation after 90% soil water depletion), two Brassica species (Hyola 401 Hybrid of canola and landrace cultivar of mustard) and three fertilizer K2SO4 levels (K0, (0), K1,(150) and K2,(250 kg/ha) comprised the experimental factors. Irrigation regimes time were determined by using from soil water curve with application digital hygrometer equipment of Time-Domain Reflectometry (TDR, Model Trim, FM3, and Germany). Potassium fertilizer in the form of sulphate dipotash was applied as broadcast and in front of the seeder at sowing. For measurements of biochemical traits were made at a fix time of day between 11.00 to 13.00 h at rosset, 50% flowering and 100% siliques formation stages before remove tension in each regime. The youngest fully-expanded leaf (third from the apex), intact and full sunlit leaves per plot was used for various measurements. Number 5 leaves separated from stems and inner envelope aluminum complex and placed in Coleman containing liquid nitrogen for freezing immediately. Then samples transported to the laboratory and maintained in temperature -40 ° C.
Determination of proline and total soluble sugars: For proline determination, 0.5 g of fresh leaf tissue was taken from plants, and proline content was measured according to Bates et al., (1974).Measuring carbon hydrate were performed based on the method of Dobis et al (1956). In this

way, 0/2 grams of green leaf tissue frozen along with 10 cc ethanol 70% (v/v) treated. About an hour placed in water bath with temperature 80 degrees Celsius. 1 cc of the samples taken and with 1 cc phenol 5% and 5 cc sulfuric acid 98 % treated. After 30 min, absorbance was read at 483 nm against a blank, containing water, 5% phenol, and sulfuric acid. Data were analyzed by using MSTAT-C statistical package (MSTAT-C, Version 1.41, Crop and Sciences Department, Michigan State University, USA). Duncan Multiple Range Test was used to comparing means (P≤0.05). To draw diagrams and tables were used from software Excel and Word.

Results and Discussion: Regardless of moisture stress conditions, the table Comparison of two species at different stages of growth is apparent that leaves proline in vegetative phase of low and flowering stage was maximum but in late of growing period has been reduced. Mustard in flowering and podding stages 7and 18 % had more proline than canola (Table 1). Genetic differences between two species can be reason in creating differences in leaf proline in sample stages. Results achieved based upon reduced proline with advancing age and higher proline in leaves of species mustard has been reported by other researchers such as Sharma and kuhad (2006) and Ma et al., (2004) Affected by moisture stress was observed significant increase in proline content (Table 1). This increase regardless of the severity of stress in all stages of the sampling occurred. The severity, duration of stress and plant growth stage can be affective in increase proline of rate. With increasing stress intensity at the treatment 90 % moisture depletion, synthesis proline leaf in stages 50 % flowering and full podding showed more of 3 time increase than vegetative stage. Sharma and kuhad (2006) and Ma et al., (2006) announced that more proline is produced under severe stress. Regardless of the sampling stages application potassium caused that proline accumulation in leaf reduced (Table 1). Highest of proline accumulation belonged to level zero K kg/ha. Decrease in proline accumulation due to application potassium were in vegetative stage (1.03 µm/ g. fw), at flowering stage (4/19 µm/g. fw) and podding stage (2/33 µm/g. fw) which represents more effect of potassium in the flowering stage. Morgan (1992) and Ma et al (2004) reported that genotypes of canola and mustard with high osmotic adjustment had high concentration of potassium in their tissues.

Soluble carbon hydrate: Mustard than species of rapeseed had solution carbon hydrate accumulation greater at different stages of sampling (Table 2). It seems that this attributed to differ in growth rate, occurrence phonology stages, especially beginning of reproductive growth (pollination, pod formation and translocation assimilates).

Table 1.The mean of comparison Proline content (µmol/g.fw) under affect two Brassica species, irrigation regimes and potassium fertilizer in growth stages

Species	Growth Stages Vegetative stage	50% flowering stage	100% siliques formation stage
B. napus (Hyola401)	a 4.65	15.51b	12.55b
B. juncea (Landres)	4.49a	16.65a	14.81a
	Irrigation Regime		
water depletion 50 %	3.37a	9.85c	8.44c
water depletion 70 %	4.92 b	17.66b	14.57b
water depletion 90 %	5.42a	20.72a	18.55a
	Potassium levels		
No application	5.10 a	18.23a	14.83a
150 Kg ha-1	4.61b	15.98b	13.71b
250 Kg ha-1	4/01c	14.03c	12.5c

Means followed by the same letters in each column are not significantly different at the 5%level, according to Duncan's Multiple Range Test.

In both species were obtained highest solution carbon hydrate mount (26/96 and 31/41 mg glucose/g.fresh weight) in full podding stage and highest proline levels (15/15 and 16/65 µm/g.fresh weight) in flowering stage was 50 % flowering (Table 2). The present results are comparable to those reported by Sharma and kuahad (2006). Soluble carbon hydrate content in both spices significantly increased under stressed condition. However soluble carbohydrate levels in treatment 90 % moisture depletion was more than control plants (Table 2). It seems that under drought stress increase activity of amylase enzymes led to that break down starch into smaller units and this action is occurs to stress tolerance through osmotic adjustment. Soluble sugars increased in response to drought stress, has been attributed to slow transfer and little consumption the reason of reduction in growth and changes such as hydrolysis of starch (Ingram, and Bartels. 1996). Production of osmolytes play a major role in osmotic adjustment and also protect the cells by scavenging ROS (Pinhero et al., 2001). The accumulation of soluble hydrate carbon in response to application high level potassium (250 kg/ha) showed more increase in all samling stage than control treatment . It has been shown that, provide of potassium in thought growth period of plant can be increased the concentration of soluble sugars.

Table 2.The mean of comparison carbohydrate content (mg/g.fw) under affect two Brassica species, irrigation regimes and potassium fertilizer in growth stages

Treatment Species	Growth Stages		
	Vegetative stage	50% flowering stage	100% siliques formation stage
B. napus (Hyola401)	12.46b	19.87a	26.96b
B. juncea (Landres)	14.47a	20.26a	31.42a
	Irrigation Regime		
water depletion 50 %	8.17c	16.32c	24.96c
water depletion 70 %	14.83b	20.12b	29.17b
water depletion 90 %	17.38a	23.87a	33.44a
	Potassium levels		
No application	11.17c	17.79c	27.61c
150 Kg ha-1	13.12b	20.48b	29.53b
250 Kg ha-1	16.09a	21.91a	30.44a

Means followed by the same letters in each column are not significantly different at the 5%level, according to Duncan's Multiple Range Test.

Correlation coefficients between grain yield with proline content and soluble carbon hydrate at different growth stages showed that grain yield had a significant negative correlation with leaf proline (Table 3). With increased leaf proline, grain yield has declined. Grain yield with soluble carbon hydrate solution showed, negative correlation but non-significant. In this study Such negative correlation between grain yield and organic osmolitse (proline and soluble carbon hydrate) that has an important role in the process of osmotic adjustment had indicated that the production and synthesis of these compounds, especially proline, has less energy so part of dry matter production of plant spend for production them.Such a negative correlation has been reported by Sharma and kuahd (2006) in rapeseed. Among proline and soluble carbon hydrate was observed positive and significant correlation.

Table 3. Correlation coefficient between of proline content, carbohydrate content and grain yield two Brassica species under irrigation regimes and potassium fertilizer in growth stages.

Traits	Grain yield	Proline) flowering(	Proline) podding(	Carbohydrate) flowering (	Carbohydrate) podding(
Grain yield	1				
Proline) flowering(	-0.783**	1			
Proline) podding(	-0.805**	0.912**	1		
Carbohydrate) flowering(	-0.204ns	0.566*	0.705 **	1	
Carbohydrate) podding(	0.392ns	0.589*	0.746**	0.856**	1

*, ** Significant at the 0.05 and 0.01 probability levels, respectively; n. s.: not significant (P≤0.05)

Conclusion: The results showed that proline content and soluble carbon hydrate of leaves both species canola and mustard were increased under stress condition. In the non stressed treatment and after irrigation at all sampling stages proline content was low. Probably such a reduction is caused of instability proline under moisture availability conditions. Potassium application caused proline decrease and soluble carbohydrates concentration increased in the leaf under water stress condition. Mount of organic compounds higher in mustard. This finding suggested that Landrace cultivar of mustard could be considered as more resistance cultivar against drought condition than Hyola 401 of canola. Also mechanism of osmotic adjustment can be an important reaction for Brassica species under water stress conditions.

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