Pak. J. Bot., 50(2): 447-455, 2018.

AN EVALUATION OF CANOLA GENOTYPES UNDER SALINITY STRESS AT VEGETATIVE STAGE VIA MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS

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Abstract

Salinity is a constraint limiting plant growth and productivity of crops throughout the world. Fourteen canola genotypes were subjected to three salinity levels 0, 150, and 350 mM Salinity effect was evaluated on the basis of biomass yield reduction and physiological attributes. Aggravated salinity stress caused significant effect in all measured parameters. Salinity stress increase reduced fresh and dry masses of shoots and roots, Chlorophyll content, RWC and K^+ content of shoots and roots. Proline content, shoot and root Na^+ content and electrolyte leakage were increased by salinity stress. A dendrogram was constructed by WARD based on fresh and dry masses of shoots and roots and physiological traits where all 14 canola genotypes were grouped into 4 clusters proving diversity among them. The 2-dimensional principal component analysis (PCA) has also confirmed the output of categorization from cluster analysis. Overall, the acquired results indicated that, among all 14 canola genotypes, salinity stressed canola genotype Safi-7 was the best salt-tolerant canola genotype considering biomass production and physiological growth and produced the highest amount of fresh and dry weight and Zafar was the most salt-sensitive genotype.

Key words: Cluster analysis, Canola, Hydroponic, Physiological attributes, Salinity.

Introduction

Soil salinity is a global problem that affects approximately 20 % of irrigated land and reduces crop yields significantly (Qadir *et al*., 2014). It is estimated that, 800 million ha of land and 32 million ha of agricultural land, in the world, are salt-affected (Anon., 2015). The impact of salt stress has been correlated with morphological and physiological traits like reduction in fresh and dry weight (Chartzoulakis & Klapaki, 2000). Soil salinization inhibits water uptake by the plants, causes ionic imbalance leading to ionic toxicity and osmotic stress (Munns & Tester, 2008). To withstand salt stress, plants accumulate compatible solutes such as proline, which decreases the cytoplasmic osmotic potential, facilitates water absorption, and scavenges reactive oxygen species (ROS) molecules (Qureshi *et al*., 2013; Pottosin *et al*., 2014; Ali & Rob, 2017). Munns & Tester (2008) stated that salt-sensitive plants reduce survival, growth, and development when exposed to even low to moderate salinities, whereas salt tolerant species are able to grow and reproduce even at oceanic salinities. The only way to control the salinization process and to maintain the sustainability of landscape and agricultural fields is to combat the salinization problems by environmentally safe and clean techniques such as using salt-tolerant species (Hamidov *et al*., 2007; Beltrao *et al*., 2009).

Canola is ranked as the third major source of edible oil, after soybean and palm oil, (Nowlin, 1991). However, its production is markedly reduced under environmental stresses such as salinity. Canola is sensitive to salinity during the early vegetative growth stage (Steppuhn *et al*., 2001), and is classified as moderately sensitive to saline conditions at the juvenile stage (Francois, 1994). However, a considerable inter-specific variation has been reported for salinity tolerance (Ashraf & Foolad, 2007). Salt-tolerant crop varieties are becoming essential in many areas of the world because of salt accumulation on soil, restrictions on groundwater use, and saltwater intrusion into groundwater (Uddin *et al*., 2011). Salt-tolerant plants have the ability to minimize these detrimental effects by producing a series of morphological, physiological, and biochemical processes (Jacoby, 1999). Studies of plant tolerance to salt stress cover many aspects on the influences of salinity on plant response, including alterations at the morphological, physiological and molecular levels. The present investigation was, therefore, undertaken to study the effect of salinity on morphological and physiological traits of canola.

Materials and Methods

Plant materials and growth conditions: Canola seeds (*Brassica napus* L. cultivars San-3, San-6, San-8, San-13, Safi-5, Safi-6, Safi-7, Zabol, Zafar, Hyola401, Amica, Goliath, Hyola308 and Sarigol) were obtained from the Seed and Plant Improvement Institute (Karaj, Iran). Seeds were sterilized (Penrose & Glick, 2003), germinated under aseptic conditions, transplanted, and cultured in a hydroponic system with sterilized Hoagland's solution (Hoagland & Arnon, 1950). The greenhouse was controlled as follows: temperature (25 ± 2 °C during the day and night), relative humidity (50% during the day and 60% at nights), light (14 h daily), and nutrient solution (pH 6.5 ± 0.5 using hydrochloric acid/potassium hydroxide). Canola cultivars were subjected to 0, 150, and 300 mM NaCl concentrations in a split plot design with three replicates. Salt treatment was initiated according to the treatments 7 days after transplanting. Three weeks after starting salt stress, whole plants were harvested from three independent biological replicates and separated

into shoots and roots. After measuring fresh masses of shoots and roots, the samples were dried at 70◦C for 48h in order to measure dry masses. The reduction of fresh and dry masses of shoots and roots, along with reduction percentage due to salinity stress were measured via the following formula:

$$\text{Reduction percentage} = \frac{\text{Control treatment value} - \text{Salinized treatment value}}{\text{Control treatment value}} \times 100$$

Determination of Na^+ and K^+ in leaves and roots: The dried samples (0.1 g) were ground and mixed with 0.2 N nitric acid. The slurry was passed through paper filter, and sodium and potassium quantities were determined by a flame photometer (Cole Parmer Instrument, IL, USA) (Ashraf & Rauf, 2001). Sodium and potassium concentrations were calculated based on standard calibration curve.

Electrolytic leakage (EL): Electrolytic leakage was calculated by (Nayyar, 2003):

$$EL = L1/L2$$

where L1 is electric conduction of leaf after putting it into the deionized water in 25°C and L2 is the electric conduction of the autoclaved samples.

Relative water content (RWC): Leaf water status was determined by measuring relative water content. RWC was obtained by the method of Weatherley (1950). Fresh leaves were taken from each genotype and weighted immediately to record fresh weight (FW). They were floated in distilled water for four hours and then weighed again to record turgid weight (TW). The leaves were dried in the oven at 60°C for 24 hours and then dry weights (DW) were obtained. Later, the fresh weight (FW), TW and DW were used to calculate RWC using the following equation:

$$RWC = [(FW–DW) / (TW–DW)] \times 100$$

Proline content: To determine free proline level, 0.5 g of leaf samples from each group were homogenized in 3% sulfosalicylic acid and then the homogenate was filtered by a filter paper (Bates *et al.*, 1973). The Mixture was heated at 100◦C for an hour in water bath after adding acid ninhydrin and glacial acetic acid. Reaction was then stopped by ice bath. The mixture was extracted with toluene and the absorbance of fraction with toluene inspired from liquid phase was read at 520 nm. Proline concentration was determined using calibration curve and expressed as mg/g FW.

Chlorophyll content: Chlorophyll measurements were carried out with a SPAD chlorophyll meter. The chlorophyll meter (or SPAD meter) is a simple portable diagnostic tool that measures the greenness or relative chlorophyll content of leaves.

Statistical analysis: All recorded data was subjected to analysis of variance using the SAS statistical software package version 9.3 (Anon., 2013). Significant differences among the mean values were calculated using Fisher's protected least significant difference (LSD) test at 5% level of significance. Cluster analysis was carried out using WARD method according to the squared Euclidean distance on standardized data (Fig. 9).

Results

Shoot fresh weight: High significant ($p<0.001$) variation was observed for shoot fresh weights in untreated control 14 canola genotypes. The highest shoot fresh weight (9.33 g) was recorded in Safi-7 and the lowest (5.15 g) was found in Amica (Table 1). Shoot fresh weights of two levels of salinity stressed canola genotypes were also affected significantly with the highest (6.03 g) in Safi-7 and the lowest (0.6 g) in Zafar at 300 mM salinity compared to control (Table 1). At 150 mM salinity levels shoot fresh weight reduction varied between 10 and 66% with the lowest reduction (10%) in Safi-7 and the highest (65.63%) reduction in Zafar. On the other hand, 35-89% shoot fresh weight reductionwas recorded in 300 mM salinity. On average over all genotypes, 40.70 and 59.61% reductions in shoot fresh weight were noted correspondingly at 150 mM and 300 mM salinity, which were statistically significant ($p<0.0001$, Table 1).

Shoot dry weight: Shoot dry weight contents in untreated control plants varied ($p<0.0001$) among the14 canola genotypes and ranged between 0.53 and 0.80 g with the highest Shoot dry weight content in Safi-7 and the lowest in Zafar (Table 1). Shoot dry weight contents were also significantly reduced by NaCl-induced salinity stress in all 14 canola genotypes. On the other hand, 20-64%, and 39-85% shoot dry weight reductions were recorded in 150 mM and 300 mM salinity, respectively. The highest reduction was found in Safi-7 and the lowest reduction was noted in Zafar in both salinity levels. The mean values of all the genotypes revealed 43.15 and 58.93% reductions in Shoot dry weight, respectively, at 150 mM and 300 mM salinity, which were statistically significant ($p<0.0001$, Table 1).

Root fresh weight: Root fresh weight contents in untreated control plants were significant ($p<0.001$) and ranged between 0.068 and 1.25 g with the highest root fresh weight content in Safi-7 and the lowest in San-6 (Table 2). Root fresh weight in salt treated 14 canola genotypes were also reduced by salinity level increase. On average, in all the genotypes, 38.82 and 60.70% reductions in root fresh weight were noted correspondingly at 150 mM and 300mM salinity, which were statistically significant ($p<0.0001$, Table 2).

Root dry weight: High significant ($p<0.001$) variation was observed in Root dry weights in untreated control 14 canola genotypes. The highest Root dry weight (0.19 g) was recorded in Safi-7 and the lowest (0.09 g) was found in Goliath (Table 2). At 300mM salinity level root dry weight reduction varied between 33 and 82% with the lowest reduction (33.16%) in Safi-7 and the highest (81.82%) reduction in Zafar. On average in all genotypes, 40.23 and 61.90% reductions in root dry weight were noted correspondingly at 150 mM and 300mM salinity, which were statistically significant ($p< 0.0001$, Table 2).

Table 1. Effect of salinity on shoot fresh weight and shoot dry weight of 14 canola genotypes.

Genotypes	Shoot fresh weight (g)			Shoot dry weight (g)		
	Salinity level (mM)					
	0	150	300	0	150	300
San-3	5.99 cd	3.06 c-f	2.52 d-f	0.597cd	0.313d-f	0.247de
		(48.91)	(57.93)		(47.57)	(58.63)
San-6	6.19 cd	3.58 c-e	2.51	0.637ac	0.307d-f	0.26de
		(42.21)	(59.45)		(51.81)	(59.18)
San-8	7.31 bc	3.86 cd	3.16 bc	0.673a-c	0.45b	0.31c
		(47.24)	(56.81)		(33.14)	(53.94)
San-13	6.42 b-d	3.40 c-f	2.21 e-g	0.577cd	0.34c-e	0.22ef
		(47.07)	(65.59)		(41.07)	(61.87)
Safi-5	7.37 bc	5.33 b	3.81 bc	0.7a-c	0.423bc	0.307c
		(27.69)	(48.32)		(39.57)	(56.14)
Safi-6	7.39 bc	4.14 c	2.85 de	0.65a-c	0.36cd	0.283cd
		(43.96)	(61.42)		(44.62)	(56.46)
Safi-7	9.33 a	8.40 a	6.03 a	0.807a	0.64a	0.487a
		(10.00)	(35.39)		(20.69)	(39.65)
Zabol	6.40 b-d	4.03 c	2.03 e-g	0.62cd	0.28d-f	0.23ef
		(36.98)	(68.23)		(54.84)	(62.90)
Zafar	5.33 d	1.83 g	0.60 i	0.533d	0.193g	0.083h
		(65.63)	(88.81)		(63.79)	(84.43)
Hyola 401	7.97 ab	5.53 b	4.00 bc	0.65ac	0.45b	0.32c
		(30.55)	(49.79)		(30.77)	(50.77)
Amica	5.15 d	2.30 fg	1.14 h	0.58 b-e	0.26 cd	0.147 c-e
		(55.34)	(77.86)		(55.17)	(74.66)
Goliath	5.95 cd	2.58 e-g	1.74 f-h	0.603 e	0.273 cd	0.18 f
		(56.57)	(70.69)		(54.73)	(70.15)
Hyola 308	7.91 ab	5.33 b	4.10 b	0.77 ab	0.583 b	0.427 b
		(32.58)	(48.13)		(24.29)	44.55
Sarigol	6.13 cd	2.86 d-g	1.60 gh	0.59 b-e	0.237 d	0.19 df
		(53.30)	(73.90)		(59.83)	67.80
Mean	2.74	4.02	2.74	0.64	0.36	0.26
		(40.70)	(59.61)		(43.15)	(58.93)

Values with different lower case letters in a row are significantly different at $p<0.05$.Values in the parentheses indicate %reduction compared to the untreated control (0 mM) plants

Na^+ content: Imposition of salt stress significantly ($p\leq0.0001$) increased Na^+ in the shoots of all 14 genotypes (Fig. 1). The lowest Na^+ increase was recorded in the shoots of San-6 and Safi-7, and the highest increase was observed in Sarigol and Zafar under saline conditions (Table 3). There was also a marked increase in root Na^+ in all genotypes under saline conditions (Fig. 3), but the highest increase (81.22%) was recorded in goliath at 300mM, whereas the lowest increase (40.34%) was found in Safi-5 at 150mM salinity (Table 3).

K^+ content: Shoot K^+ content was significantly ($p\leq0.001$) reduced in all genotypes with the imposition of salt stress (Fig. 2). On the other hand, 26-68%, and 43-75% Shoot K^+ reductions were recorded in 150 mM and 300 mM salinity, respectively (Table 3). There was also a significant reduction in root K^+ in all genotypes under saline conditions, but the highest increase (54.31%) was recorded in Sarigol and the lowest increase (33.99%) was found in Safi-7 at 300mM salinity (Table 3) (Fig. 4).

Electrolyte leakage (EL): Imposition of salt stress significantly ($p\leq0.0001$) increased the EL of all 14 genotypes (Fig. 5). On the other hand, 13-34% and 27-55% EL increase was recorded in 150mM, and 300 mM salinity, respectively. The mean values of all the genotypes revealed 24.61 and 42.63% increase in EL content, respectively, at 150 mM and 300mM salinity, which were statistically significant ($p<0.0001$, Table 3).

Relative water content (RWC): Imposition of salt stress significantly ($p\leq0.0001$) reduced RWC of all 14 genotypes, but genotypes did not differ significantly in under saline conditions (Fig. 6). On average over all genotypes, 7.53 and 14.03% reductions in RWC were recorded correspondingly at 150 mM and 300mM salinity, which were statistically significant ($p<0.0001$, Table 3).

Table 2. Effect of salinity on root fresh weight and root dry weight of 14 canola genotypes.

Genotypes	Root fresh weight (g)			Root dry weight (g)		
	Salinity level (mM)					
	0	150	300	0	150	300
San-3	0.69 fg	0.46 cd	0.28 d-g	0.117 b-e	0.057 cd	0.037 c-e
		(33.62)	59.60		(51.28)	(68.38)
San-6	0.68 g	0.41cd	0.26 d-g	0.147 bc	0.1b	0.067 b
		(39.71)	61.76		(31.97)	(54.42)
San-8	0.92 b-g	0.60 bc	0.41cd	0.113 b-e	0.06cd	0.037 c-e
		(34.57)	54.96		(46.90)	(67.26)
San-13	0.77 d-g	0.60bc	0.23	0.117 b-e	0.057 cd	0.047 c
		(21.38)	70.4 f-h		(51.28)	(59.83)
Safi-5	0.98 a-f	0.72 b	0.37 c-f	0.117 b-e	0.073 bc	0.043 cd
		(26.61)	62.44		(37.61)	(63.25)
Safi-6	0.99 a-e	0.63 bc	0.40 ce	0.113 b-e	0.073 bc	0.027 d-f
		(35.87)	59.47		(35.40)	(76.11)
Safi-7	1.25 a	1.00 a	0.74 a	0.19 a	0.167 a	0.127 a
		(19.81)	40.66		(12.11)	(33.16)
Zabol	1.07 ac	0.60 bc	0.49 bc	0.137 b-d	0.1 b	0.067 b
		(44.36)	54.33		(27.01)	(51.09)
Zafar	0.79 c-g	0.23 d	0.13 h	0.11c-e	0.04 d	0.02 ef
		(71.27)	83.16		(63.64)	(81.82)
Hyola 401	1.05 a-d	0.82 a-b	0.47 b-c	0.1d-e	0.073bc	0.033 c-f
		(21.90)	54.95		(27.00)	(67.00)
Amica	0.75 e-g	0.34 d	0.19 g-h	0.113 b-e	0.057 cd	0.037 c-e
		(54.67)	74.67		(49.56)	(67.26)
Goliath	0.87 c-g	0.31d	0.24 f-h	0.09 e	0.043 cd	0.017 f
		(63.90)	71.97		(52.22)	(81.11)
Hyola 308	1.17 ab	0.83 ab	0.6 ab	0.153 ab	0.103 b	0.077 b
		(28.88)	47.99		(32.68)	(49.67)
Sarigol	0.82 c-g	0.27 d	0.20 g-h	0.123 b-e	0.037 d	0.027 d-f
		(66.95)	75.52		(69.92)	(78.05)
Mean	0.91	0.56	0.36	0.12	0.07	0.05
		(38.82)	(60.70)		(40.23)	(61.90)

Values with different lower case letters in a row are significantly different at $p<0.05$.Values in the parentheses indicate %reduction compared to the untreated control (0 mM) plants.

Proline: Proline contents in untreated control plants were significant ($p< 0.05$) and ranged from 1.05 to 2.5 (mg/g FW) with the highest Proline content in Amica and the lowest in Safi-7 (Fig. 7). Proline content in salt treated 14 canola genotypes increased due to salinity level increase. At 300 mM salinity levels Proline increase varied between 91 and 98% with the lowest increase (91.50%) in Amica and the highest (97.30%) increase in Safi-7. On average, in all genotypes, 91.61 and 93.86% increase rates in Proline were noted correspondingly at 150 mM and 300mM salinity, which were statistically significant ($p<0.0001$, Table3).

Chlorophyll: Chlorophyll contents in untreated control plants were not significant among the 14 canola genotypes. Chlorophyll contents were significantly ($p\leq0.0001$) decreased in all genotypes with the imposition of salt stress (Fig. 8). At 300mM salinity level Chlorophyll reduction varied between 3 and 23% with the lowest reduction (3.51%) in Safi-7 and the highest (22.52%) increase in Zafar. However, the mean values of all the genotypes revealed 4.22 and 9.28% reductions in Chlorophyll content, respectively, at 150 mM and 300mM salinity, which were statistically significant ($p<0.0001$, Table 3).

Cluster and principal component analysis (PCA): In order to assess the patterns of variation, cluster analysis and PCA were done using morphological and physiological parameters where all 14 genotypes were categorized into four distinct clusters (Fig. 9). Among 4 clusters Safi-7 was totally separated from the others and formed cluster IV; cluster III included Zafar, Goliath and Sarigol; Cluster II was made up of san-13, Safi 6, Zabol and Amica; cluster I was the largest group that consisted of San-3, San-6 and San8, Safi 5, hyola401, and hyola308. The patterns of cluster analysis were also confirmed by the PCA of two-dimensional (2D) plot which was also the same as the dendrogram (Fig. 10). Principle component analysis (PCA) indicated 83% of total variation among all the genotypes studied (data not shown).

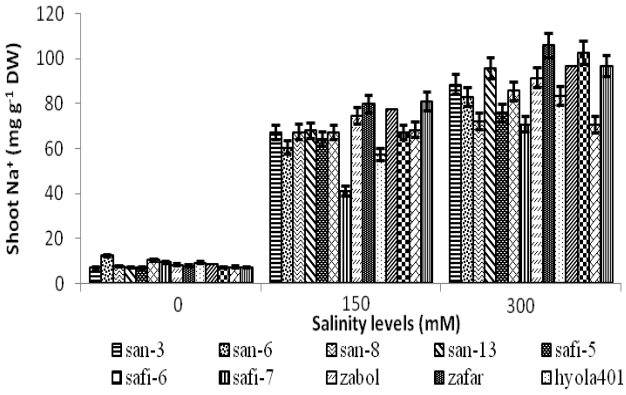

Fig. 1. Effect of salinity on shoot Na^+ content.

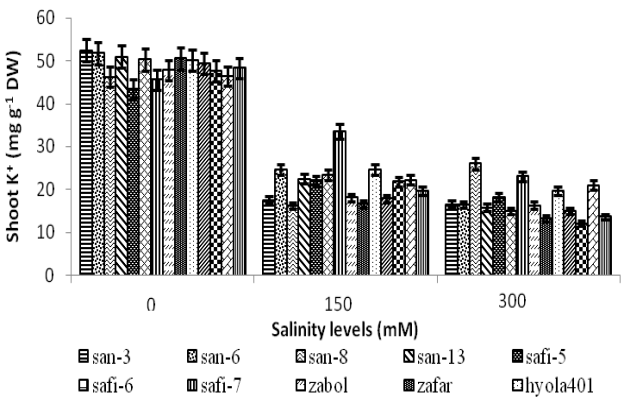

Fig. 2. Effect of salinity on shoot K^+ content of 14 canola genotypes.

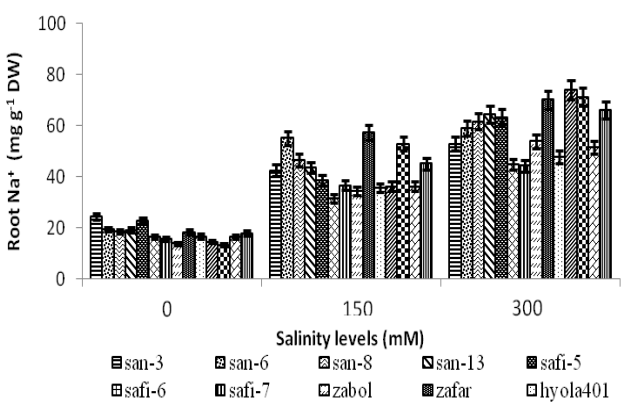

Fig. 3. Effect of salinity on root Na^+ content.

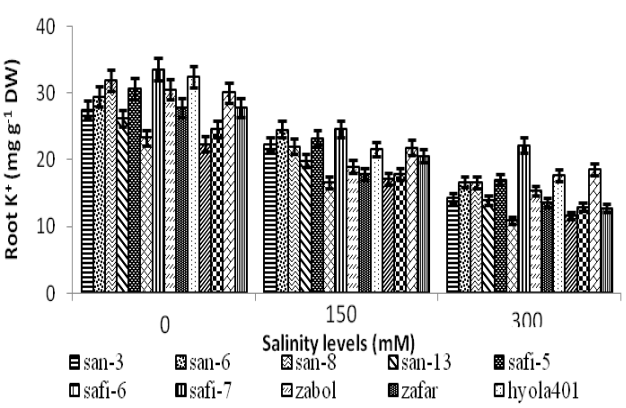

Fig. 4. Effect of salinity on root K^+ content.

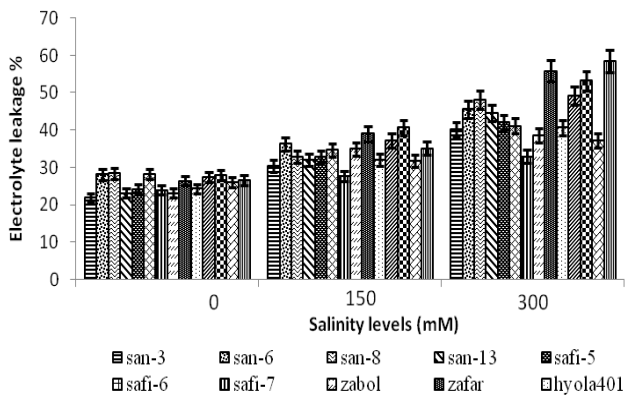

Fig. 5. Effect of salinity on electrolyte leakage.

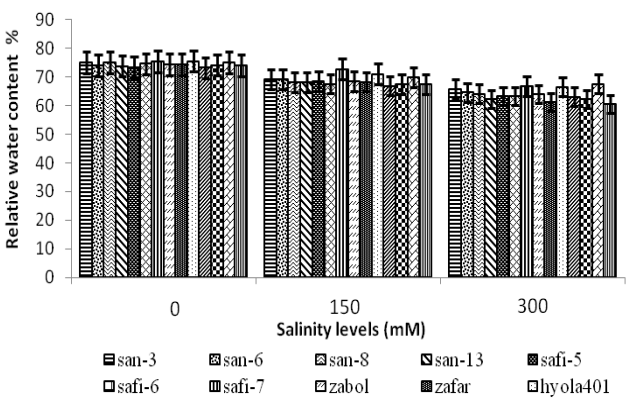

Fig. 6. Effect of salinity on RWC content.

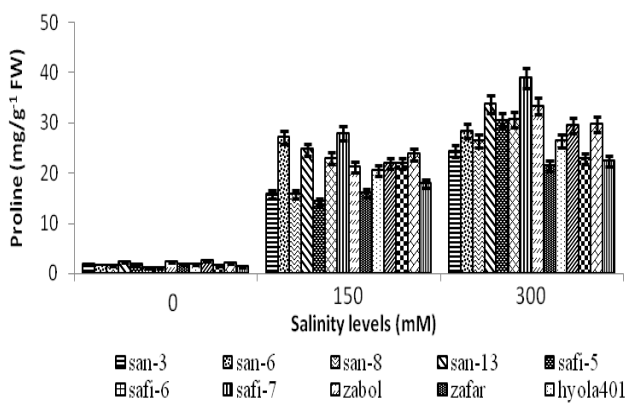

Fig. 7. Effect of salinity on proline content.

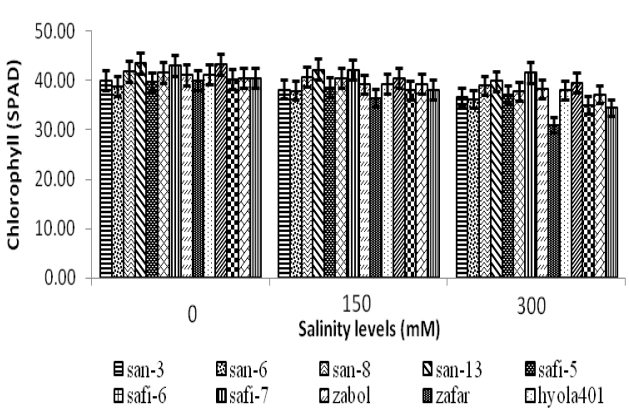

Fig. 8. Effect of salinity on chlorophyll.

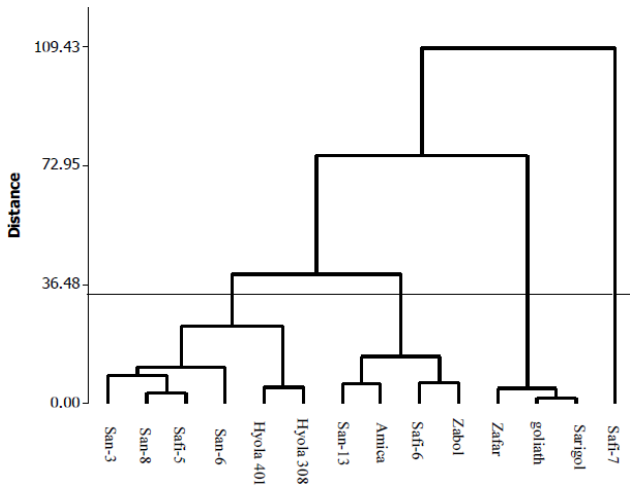

Fig. 9. A WARD dendrogram of measured traits derived from 14 canola genotypes.

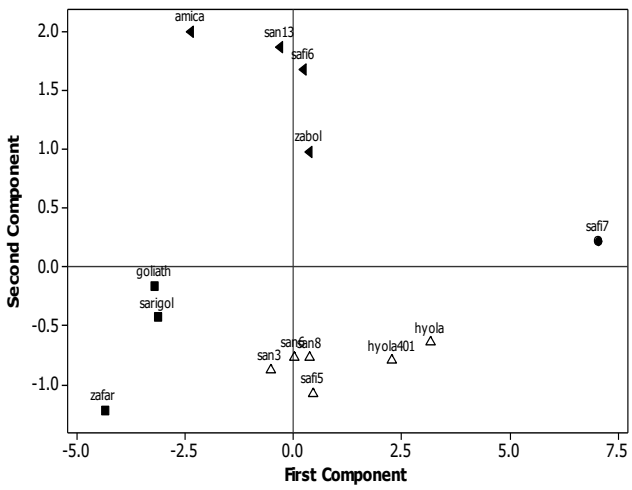

Fig. 10. A two –dimensional PCA plot indicating variations amoanola genotypesng 14 c.

Discussion

The results showed that untreated control plants greatly varied in most parameters of Shoot fresh weight, Root fresh weight, shoot dry weight, Root dry weight, and physiological traits, that is, Na^+ Shoot, K Root and proline. Salt treatment also significantly influenced all parameters investigated in this study. The responses of the 14 genotypes to salt treatment were different from each other, indicating diversity among the genotypes.

The high salinity stress caused a high reduction in the fresh and dry masses of shoots and roots of all 14 genotypes and the fresh and dry mass reduction was increased by salinity level increase. Salinity-induced fresh and dry weight reduction is a common phenomenon for most of the cultivated crop plants. Ashraf & Ali (2008) observed the high significant reduction in fresh weight of canola at 150mM salinity. The reductions of fresh weight due to salinity stress have also been investigated in tomato (Mozafariyan *et al*., 1013) and the reduction of shoot and root dry matter contents in hybrid maize varieties have also been reported by Eker *et al*., (2006). The reduction in biomass increased with the increase in salinity which is obvious because of disturbances in physiological and biochemical activities under saline conditions as shown by Craine (Craine, 2005) that may be due to the reduction in leaf area and number of leaves (Yunwei *et al*., 2007). Also a decrease in dry matter content at the highest salinity levels might be due to the inhibition in hydrolysis of reserved foods and their translocation to the growing shoots (Xu *et al*., 2008).

In this study, Na^+ content increased under salt stress, but K^+ content decreased. Once the ions in the cells exceeded a certain amount, the homeostasis of intracellular ion concentrations was disturbed and ionic stress emerged. Under salt stress, the cellular concentration of K^+ and Ca^{2+}decreases in the roots, shoots, and leaves of canola, whereas those of Na^+ and Cl^- are increased (Tunçtürk *et al*., 2011). High level of K^+ in young expanding tissues is associated with salt tolerance in many plant species (Mer *et al*., 2000; Ashaf & McNeilly, 2004; Bandeh-Hagh *et al*., 2008).

Electrolyte leakage is a hallmark of stress response in intact plant cells. This phenomenon is widely used as a test for the stress-induced injury of plant tissues and a measure of plant stress tolerance (Bajji *et al*., 2002; Lee & Zhu, 2010). Electrolytic leakage increased under salinity due to the increment of metabolites and electrolytes leakage in response to accumulation of sodium chloride together with cumulative entering of Cl^- and Na^+ and the exclusion of K+ (Iqbal *et al*., 2008). The lowest increase in electrolyte leakage was observed in Safi-7 at 300 mM salinity whereas the highest increasing was found in sarigol followed by zafar at the same salinity level (Table 3). Electrolytic leakage increases under salinity stress and tolerant genotypes usually indicate lower electrolytic leakage (Sairam *et al*., 2002).

Relative water content (RWC) is another factor in testing water balance in the planted (Gonzalez &Gonzalez-Vilar, 2001). Many scientists proposed that water balance regulation in plants favors the avoidance mechanism and diluting the effects of ionic toxicity under salt stress (Ashraf, 2004; Noreen *et al*., 2010).

Accumulation of compatible solutes in the cytoplasm is required to balance the decrease in water potential occurring in the vacuole due to ion accumulation in that compartment (Dos Reis *et al*., 2012). The accumulation of osmolytes, especially that of proline, is a common phenomenon in plants. Besides its role as an osmolyte, proline contributes to scavenging ROS and supplying energy and functioning as a signal (Kavi-Kishor *et al*., 2005; Sharma *et al*., 2011). Silva-Ortega *et al*., have reported that proline is accumulated preferentially in leaves in order to maintain chlorophyll level and cell turgor to protect photosynthetic activity under salt stress. The authors have reported stress-induced proline accumulation in *B. napus* due to the reciprocal reaction of activated biosynthesis and inhibited proline degradation. Furthermore, the response of sodium chloride stress in different spring canola cultivars has been recently studied by Toorchi *et al*., (2011) who suggested an ample genetic variability among rapeseed genotypes which could be used in breeding projects. They found a significant increase in free proline contents in canola leaves with increase in external NaCl concentration. Similarly, Nazarbeygi *et al*., (2011) also studied the response of canola to different levels of salinity and found a significant increase in proline content in leaf and root tissues.

Table 3. Effect of salinity in physiological characteristics (% reduction) of14 canola genotypes.

Genotypes	Na^+ shoot		Shoot K^+		Root Na^+		Root K^+		Electrolyte leakage		Relative water content		Proline		Chlorophyll	
	Salinity level (mM)															
	150	300	150	300	150	300	150	300	150	300	150	300	150	300	150	300
San-3	+89.49	+92.02	66.58	68.46	+42.28	+53.46	19.29	47.91	+27.38	+44.94	7.83	12.28	+88.30	+92.47	4.82	8.42
San-6	+79.24	+84.83	52.29	68.25	+64.70	+66.94	16.87	43.44	+22.54	+38.34	6.31	12.68	+93.86	+94.13	2.43	6.82
San-8	+88.46	+89.21	64.89	43.67	+59.91	+69.80	30.89	47.95	+13.63	+40.86	9.11	14.47	+90.00	+94.01	2.54	6.98
San-13	+89.14	+92.28	55.81	68.90	+55.74	+70.00	24.10	46.65	+27.59	+48.12	7.79	15.73	+90.68	+93.19	2.82	8.21
Safi-5	+88.98	+90.65	49.34	58.17	+40.34	+63.65	24.34	44.44	+26.33	+42.29	6.57	13.73	+87.74	+94.28	2.71	6.28
Safi-6	+84.68	+87.95	53.51	70.25	+47.80	+63.25	28.73	53.44	+18.70	+31.29	9.44	15.00	+95.10	+96.33	2.66	8.93
Safi-7	+76.77	+86.52	26.46	49.36	+56.86	+64.30	26.85	33.99	+13.70	+27.51	3.44	11.53	+96.23	+97.30	2.12	3.51
Zabol	+88.62	+90.73	62.03	65.83	+59.25	+73.98	37.89	49.74	+33.88	+40.12	7.73	13.89	+89.17	+93.14	4.75	6.855
Zafar	+90.01	+92.45	67.02	73.73	+67.88	+73.77	35.74	51.52	+32.24	+52.64	8.11	17.49	+89.21	+91.90	8.80	22.52
Hyola 401	+83.64	+88.74	51.07	60.56	+52.83	+64.84	33.30	45.49	+23.86	+40.23	5.99	11.84	+91.33	+93.27	4.50	7.38
Amica	+88.63	+90.88	63.67	69.67	+59.56	+80.10	23.30	47.98	+26.55	+44.42	8.59	13.85	+88.61	+91.50	659	8.58
Goliath	+89.19	+92.92	54.06	74.54	+74.75	+81.22	27.38	47.17	+31.14	+47.38	8.92	15.89	+92.99	+93.26	5.50	12.91
Hyola 308	+89.00	+89.38	52.17	54.74	+54.19	+67.71	27.36	38.45	+17.83	+30.01	6.89	9.98	+91.33	+93.07	2.97	8.12
Sarigol	+90.91	+92.38	59.26	71.69	+60.03	+72.71	26.07	54.31	+24.19	+54.39	8.79	18.24	+92.91	+94.33	5.99	15.07
Mean	+87.41	+90.27	55.80	64.42	+57.94	+69.78	27.46	46.59	+24.61	+42.63	7.53	14.03	+91.61	+93.86	4.22	9.28

Plus (+) symbol indicates % increase compared to control.

Chlorophyll is the main color agent responsible for photosynthesis. Under adverse circumstances, the chlorophyll level is a good indicator of the photosynthesis function (Xu *et al*., 2008). In the present study, aggravated salinity stress caused significant changes in chlorophyll content (SPAD value) in all 14 canola genotypes. Chlorophyll content was significantly decreased under salinity stress. Reduction of chlorophyll content due to salinity stress is very common in salt-sensitive plant species because of salt toxicity which mostly causes burning of leaves or other succulent parts and degradation of other pigments too. But those are saline tolerant species that can protect themselves from such deterioration of salinity stress (Alam *et al*., 2015). The reductions of chlorophyll content in canola (Nazarbeygi *et al*., 2011) and wheat (Iqbal *et al*., 2006) have been reported due to salinity stress. Cluster analysis is a multivariate technique that can group individuals or objects based on their characteristics. Individuals having similar descriptions are mathematically congregated into the same cluster (Ahmadikhah *et al*., 2008). Distance, similarity, and relatedness of varieties are the foundation of this method. The WARD constructed dendrogram revealed 4 clusters where Safi-7 was mostly different from all others proving the highest salt-tolerant accession compared to others. Due to the characteristics, cluster I also can be considered as tolerant to salinity stress, but compared to cluster IV (Safi-7), this group includes semi-tolerant genotypes. Considering the characteristics, cluster III includes Zafar, Goliath and Sarigol ranked as sensitive genotypes to salinity stress. Cluster II includes four genotypes that can be considered as a semi-sensitive group. For new improved variety development, the most judicious crossing combination can include Safi-7 and Zafar or Goliath or Sarigol that will bring about the greater genetic diversity (Arolu *et al*., 2012).

Conclusions

Salinity is possibly the most essential ecological restriction that causes extensive crop yield losses all over the world, and its threat is escalating day by day. Considering this urgency, in this study 14 canola genotypes have been tested for fresh and dry masses of shoots and roots and physiological characteristics on augmented salinity stress. This experiment indicated significant genetic variability among genotypes, which can be used in breeding projects. Overall, among all 14 canola genotypes, salinity stressed genotype Safi-7 proved to be the best salt-tolerant canola genotype considering biomass production and physiological growth and produced the highest amount of fresh and dry weight and Zafar was the most salt-sensitive genotype. The identified tolerant genotypes could further be used in breeding programs. Further molecular studies could be carried out to find out the molecular mechanism for salinity tolerance in tolerant genotypes.

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(Received for publication 20 June 2017)