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AGRO-MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF *BRASSICA RAPA* ECOTYPES TO SALT STRESS

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Abstract

Salt stress is one of the major abiotic stresses that affect normal morpho-physiological and biochemical processes of *Brassica rapa*. In present study the effect of salt stress on three important ecotypes of *B. rapa* (brown sarson, yellow sarson and Toria) was studied. The plants were regenerated at four NaCl levels (0, 50,100 and 150 mmol). The effect of salt stress on shoot length, root length, shoot fresh and dry weight, root fresh and dry weight, leaf relative water content (RWC), proline and chlorophyll a, b, a+b contents were recorded. The high salt stress significantly decreased the shoot length, root length, shoot fresh weight, shoot dry weight, and root fresh weight and root dry weight of all three ecotypes. The genotype 25007 (brown sarson) showed better morphological performance followed by Toria as compared to other genotypes. The RWC values and chlorophyll a, b and a+b contents were decreased up to several fold at high salt levels (50,100 and 150 mmol), while proline contents increased at these concentrations. Maximum RWC values and proline contents were recorded in genotype 25007 followed by Toria. The chlorophyll a, b and a+b amounts were higher in Toria followed by 25007 respectively. The brown sarson and Toria ecotypes showed better performance at high salinity levels as compared to yellow sarson. The resulted screened salt tolerant genotypes will be useful for salt affected area.

Key words: Salt stress, *Brassica rapa*, Biochemical, Morpho-Physiological, Salt tolerance.

Introduction

Salt stress affects both soil composition and quality (Nakashima *et al.*, 2000). It affect about 831 million hectare (mha) of our land area both directly and indirectly (Martinez-Beltran & Manzur, 2005). Excess amount of salt in soil retard the normal growth, development and other physiological functions of plant by excessive accumulation of Na+ and Cl- and nutrient deficiency (Ashraf & Harris, 2004; Garthwaite *et al.*, 2005; Narusaka *et al.*, 2003). Salt stress also disturbs normal oxidative and reactive oxygen species (ROS) process that leads cell injury (Cuin & Shabala, 2007; Vital *et al.*, 2008; Shinwari *et al.*, 1998; Masood *et al.*, 2005). Among all of the major crops, the yield and performance of many Brassica species such as rapeseed (*Brassica napus* L.), mustard (*Brassica juncea* L.), cabbage (*Brassica oleracea* L.) and turnip rape (*Brassica rapa* L.) are highly affected by both drought and salinity stresses. Therefore proper identification; selection and development of new salt tolerant cultivars are important against these environmental extremes (Ashraf & McNeilly, 2004; Almodares *et al.*, 2007; Islam & Karim, 2010).

Many researchers have developed efficient protocols for identification, selection and development of improved genotypes against salt stress. Quesada *et al.* (2002) reported that high salt stress affected the vegetative growth and germination frequency at early growth stage in many Arabidopsis genotypes. The germination rate and fresh, dry weight varies with different concentrations of salt used. Munir *et al.* (2013) studied that regular finding of photosynthetic rate at different salinity level is important for the selection of efficient genotypes that shows tolerance against salt stress. Study of photosynthetic rate, proline content and intracellular or intercellular ion movement at different salinity levels are useful tool for the selection of salt tolerant canola cultivars (Ulfat *et al*., 2007). Kumar *et al.* (2009) found maximum variation in all tested Brassica species by imposing different salt stress for one day at early germination stage. Munir *et al.* (2013) studied various physio-chemical responses of important winter type radish (*Raphanus sativus* L.) to low and extreme salinity levels. The chlorophyll a and b contents were decreased several fold in cultivars Deci White and Lal Pari. Khayat *et al.* (2010) described that finding of Na^+ content in shoot is a useful criterion. Variability among *Brassica rapa* ecotypes occurs for low to extreme salt levels. Therefore the present study was designed to study the effect of salt stress on the morpho-physiological and biochemical process of three important ecotypes of *Brassica rapa* (brown sarson, yellow sarson and Toria) and to screen salt tolerant genotype among these ecotypes.

Materials and Methods

The present experiment was conducted in Plant Genetic Resources Institute (PGRI), National Agriculture Research Centre (NARC), Islamabad, Pakistan. The mature seeds of *Brassica rapa* genotypes were acquired from the gene bank of PGRI, NARC, Islamabad, Pakistan. The list of *B. rapa* genotypes is mentioned in Table 1.

Seed sterilization: The seeds were surface sterilized with doubled distilled water for 10 minutes. The seeds were then treated with 10% Clorox bleach for 10-15 minutes with continuous shaking. The seeds were dried in petridesh with autoclave filter paper.

Germination of seeds and salt trials: The sterilized test tube having autoclave filter papers were used for germination of seeds at different salinity levels. Four different NaCl concentrations (0, 50, 100 and 150 mmol) were used. The 3 ml of each salt concentration was added to each test tube. The sterilized seeds were inoculated with the help of forceps into each test tube and were store in incubator at 25 ± 2ºC under light (16/8 hours photoperiod) condition. The controlled plants were regenerated with distilled water.

Table 1. List of *B. rapa* accessions

S. No.	Genotypes	Origin	Source
1.	16133	Attock, Punjab, Pakistan	NARC, Islamabad
2.	22859	Faisalabad, Punjab, Pakistan	NARC, Islamabad
3.	25007	Peshawar, Khyber Paktunkhwa (KP), Pakistan	NARC, Islamabad
4.	26815	Punjab, Pakistan	NARC, Islamabad
5.	Toria	Netherland	NARC, Islamabad

Morpho-physiological and biochemical tests: Various morphological changes such as shoot length, root length, shoot fresh and dry weight and root fresh and dry weight were studied at different salinity levels after 30 days of salt stress.

Measurement of chlorophyll a, b, a+b and proline contents: The fresh leaves were collected from both the control and salt testing plants for biochemical analysis. The leaves were treated with 80% v/v acetone and then centrifuged at 5000 rpm. The supernatant was collected and its absorbance was checked at UV-visible spectrophotometer at 645 and663 nm. The chlorophyll a, b and a+b contents in leaves were noted in both controlled and salt treated plants by following protocol of Arnon (1949). The proline test was performed by using protocol of Bates *et al.* (1973) with minor changes.

Measurement of relative water content (RWC): The percent leaf relative water content (RWC) was measured by using protocol of Barrs & Weatherly (1962). The %RWC was calculated by using following formula:

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

The fresh weight (FW) was calculated by collecting of all leaf disks from each plant and allows floating in distilled water by keeping in dark for 8 hour and weighted. Turgid weight (TW) was calculated by drying of leaves in order to remove excess of moisture/water. The dry weight (DW) was calculated by drying the leaves at 80°C for 2 days.

Data analysis: The Morpho-physiological and biochemical data was analyzed using statistical softwares, Statistic v. 8.1 (Analytical & Software, 2005) and Origin 8.1.

Results

Excess amount of salt in soil affect normal plant morphological, physiological and biochemical processes. In present study the effect of salt stress on five *Brassica rapa* genotypes i.e. 16133 (brown sarson), 22859 (brown sarson), 25007 (brown sarson), 26815 (yellow sarson) and 26167 (Toria type) was tested. All the genotypes showed different mopho-physiological and biochemical responses to different salinity levels. The following main characters were envisaged at different salinity levels (0, 50, 100 and 150 mmol).

Salinity effects on shoot and root length of *B. rapa* ecotypes: The low or high dose of NaCl affects shoot and root length of *B. rapa* ecotypes. The response for shoot and root length among these ecotypes varies with different levels of salt stress. At control condition (0 mmol) all the genotypes showed maximum shoot length as compare to other salt stress levels (Table 2). At 50 mmol salt trial the maximum shoot length 10 cm and 8.5 cm were noted from genotype 25007 followed by 16133 and Toria types respectively. While the genotypes 22859 and 26815 gave very poor shoot length at this concentration. The high salt stress further inhibited the normal shoot growth. At high salt level (100 mmol), maximum shoot length 8.5 cm and 7cm was observed in genotype 25007 (brown sarson) and Toria sarson receptively. The genotype 25007 gave high shoot length at very high salt concentration (150 mmol) followed by 16133 genotypes as compared to other genotypes (Table 2). These finding showed that brown and Toria gave better response to different salinity levels as compared to yellow sarson. The brown sarson and Toria showed more tolerance to salt stress, remain green and healthy at all salt stress levels. The yellow sarson ecotype showed poor germination rate and stunted growth at 100 and 150 mmol salt stress (Fig. 1a-e).

The salt stress adversely affects the root length of all tested genotypes. The maximum root length 3.54, 3.31 and 2.70 cm were observed in genotype 25007 at 50, 100 and 150 salt levels followed by Toria. The other genotypes showed very poor root length at these concentrations. At very high salt concentrations 100 and 150 mmol the genotype 22859 showed no root growth at all and plant death occur at these concentrations (Table 2).

Effect of salt stress on shoot fresh, dry weight and root fresh, dry weight of *B. rapa* ecotypes: The effect of salinity on shoot fresh and dry weight and root fresh and dry weight was tested among *B. rapa* ecotypes. All three ecotypes gave different response to each salt stress levels. The maximum shoot fresh weight of 8.52, 7.33 and 3.87 gm was noted in genotype 25007 (brown sarson) at all three types of salt stress (50, 100 and 150 mmol) receptively followed by Toria sarson. The lowest shoot fresh weight of 4.05 gm was obtained in accession 22859 at very low salt level 50 mmol (Table 2). Similarly the maximum shoot dry weights 1.10, 0.90 and 0.54 gm at 50, 100 and 150 mmol salt stress levels were also noted from genotypes 25007 followed by Toria and genotype 16133 respectively. From results we revealed that both brown and Toria ecotypes of *B. rapa* gave better shoot fresh and dry weight as compared to yellow sarson.

The results of root fresh and dry weight showed that the root fresh weight was the highest in genotype 25007 followed by Toria type. The maximum root fresh weight of 0.80 gm was noted in genotypes 25007 and 16133 at 50 mmol salt level as compared to control condition (0 mmol) 0.77 gm. It showed that root fresh weight increased with the increase of salt stress. A very low or no root fresh weight was observed in genotype 26815 at 50,100 and 150 mmol salt levels. Similar results were obtained for root dry weight as genotype 25007 gave better response at all salt stress levels as compared to other genotypes (Table 2).

Table 2. Effect of salinity on morpho-physiological characters of *B. rapa*.

Genotypes	Salinity level (mmol)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (gm)	Root fresh weight (gm)	Shoot dry weight (gm)	Root dry weight (gm)
16133	00	10.00	3.00	8.53	0.82	1.12	0.34
	50	8.50	3.43	7.43	0.80	0.90	0.33
	100	7.34	3.12	4.52	0.58	0.69	0.25
	150	6.13	2.76	3.96	0.43	0.46	0.16
22859	00	10.50	3.50	8.64	0.87	1.18	0.38
	50	5050	2.59	4.05	0.20	0.68	0.19
	100	0.00	0.00	0.00	0.00	0.00	0.00
	150	0.00	0.00	0.00	0.00	0.00	0.00
25007	00	8.60	3.39	7.50	0.77	0.93	0.27
	50	10.00	3.54	8.52	0.80	1.10	0.32
	100	8.50	3.31	7.33	0.73	0.92	0.15
	150	6.32	2.70	3.87	0.39	0.54	0.10
26815	00	8.12	3.00	3.90	0.56	0.60	0.21
	50	6.02	2.66	3.12	0.33	0.38	0.10
	100	5.50	2.43	3.06	0.20	0.32	0.08
	150	3.25	1.88	2.18	0.13	0.17	0.05
Toria	00	10.50	3.60	8.60	0.85	0.13	0.31
	50	8.50	3.27	7.58	0.70	0.86	0.23
	100	7.00	2.74	4.93	0.64	0.78	0.12
	150	6.02	2.62	3.65	0.33	0.48	0.09

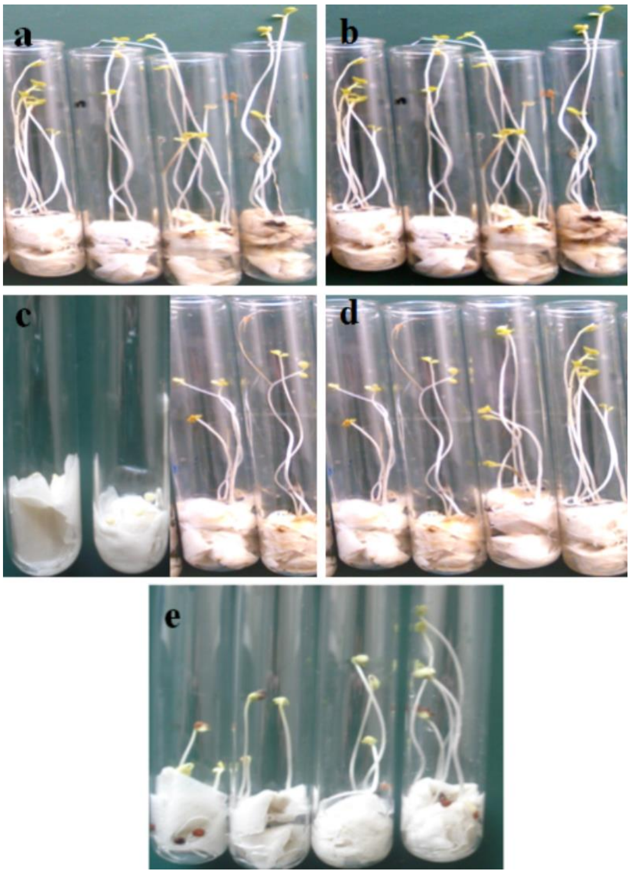

Fig. 1. The effect of salinity on *B. rapa* ecotypes (a) Better morphogenic response of genotype 16133 (brown sarson) to different salt levels (b) Excellent morphogenic response of genotype 25007 (brown sarson) to different salt events, (c) Poor morphogenic response of genotype 22859 (brown sarson) to different salt levels. No seed germination occured at 100 and 150 mmol (d) Better morphogenic response of genotype Toria (Toria sarson) to different salt levels (e) Poor morphogenic response of genotype 26815 (yellow sarson) to different salt levels.

Leaf relative water content: The leaf Relative Water Content (RWC) was significantly decreased with the increase of salt levels as compared to controlled condition in all tested genotypes. At 50 mmol salt level maximum relative water content of 87.54% was noted from genotype 25007 followed by 83.01 % in Toria. The maximum RWC values of 78.23 % and 71.61% at 100 and 150 mmol salt stress levels were calculated in Toria. The Leaf relative water content was lowest in genotype 26815 (Fig. 2).

Proline content: The low to high salt stress levels significantly increased the proline content in all three ecotypes of *B. rapa*. At 50 mmol salt stress maximum proline content of 6.24 µmol g–1was measured for genotype 25007. This amount is almost double from the controlled condition. At high salt stress levels (100 and 150 mmol) maximum proline content of 7.12 µmol g–1 and 7.61 µmol g–1was noted in genotype 25007. The genotypes 26815and 22859 gave very low amount of proline at high salt stress levels (100 and 150 mmol) (Fig. 3).

Chlorophyll content: The reduction in chlorophyll a and b were observed in all genotypes with the increased of salt levels that directly decreased the amount of total Chl (a+b) contents (Fig. 4. a-c). The maximum total chlorophyll (a+b) contents1.23, 1.05 and 0.93 mg g–1 was noted in genotype Toria at 50, 100 and 150 mmol salt concentrations respectively followed by genotypes 25007 (Fig. 4c). All other genotypes showed very low chlorophyll contents at these concentrations. As compared to controlled plants there was a sudden decreased of chlorophyll a, b and total chlorophyll (a+b) contents up to 30-40 % in all genotypes at 150 mmol salt levels (Fig. 4c)

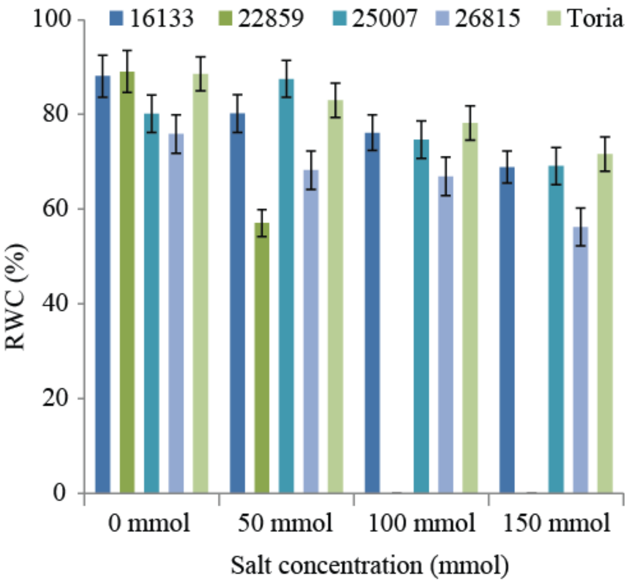

Fig. 2. Effect of salt stress on relative water content (RWC) of *B. rapa* ecotypes.

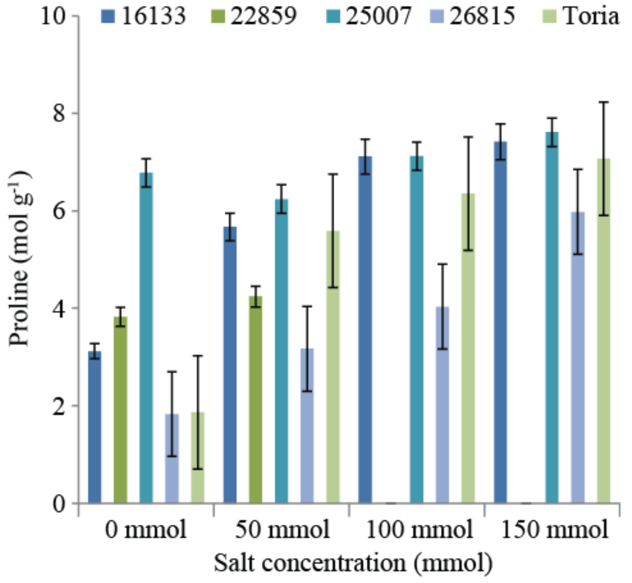

Fig. 3. Effect of salt stress on proline content of *B. rapa* ecotypes.

Discussion

Salt stress is one of the important environmental extreme that affects normal plant morpho-physiological and biochemical processes (Kidokoro *et al.,* 2009). In present study the effect of salinity on morphological, physiological and biochemical processes of three important ecotypes of *B. rapa* was studied. The brown sarson, yellow sarson and Toria ecotypes of *B .rapa* were subjected to four different salinity stress levels (0, 50, 100 and 150 mmol). All the genotypes showed different responses at different salinity levels. A sudden decrease in growth and development was observed in all genotypes at high salinity levels. The following main morphological and physiological changes were studied in three important *B. rapa* ecotypes (brown sarson, yellow sarson and Toria) at low to moderate and even high salt levels.

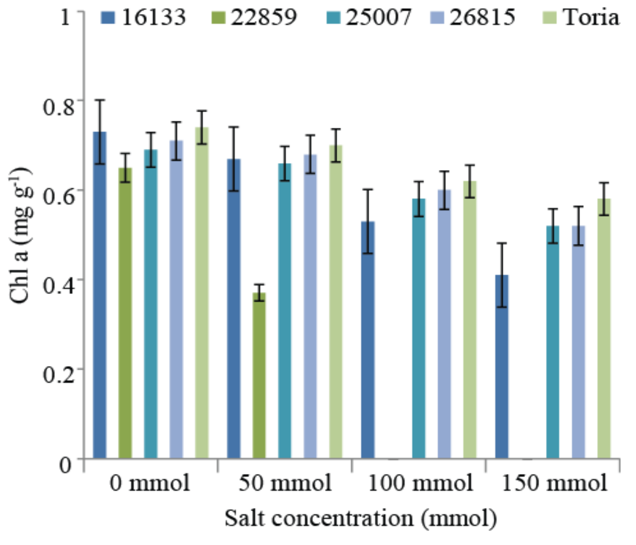

Fig. 4(a). Effect of salt stress on chlorophyll a content of *B. rapa* ecotypes.

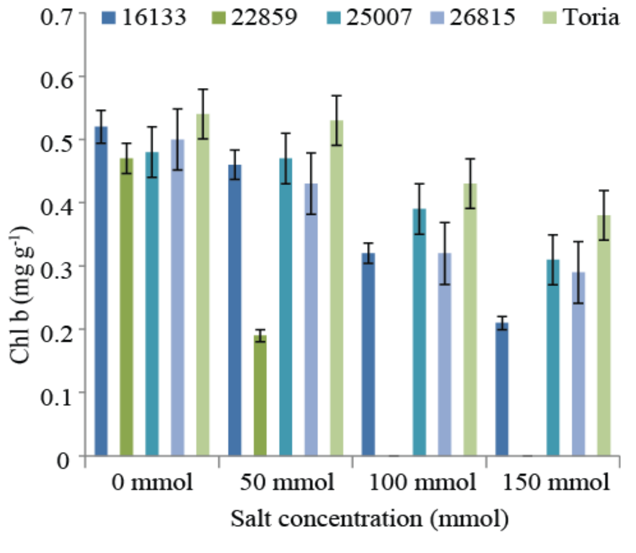

Fig. 4(b). Effect of salt stress on Chlorophyll b content of *B. rapa* ecotypes.

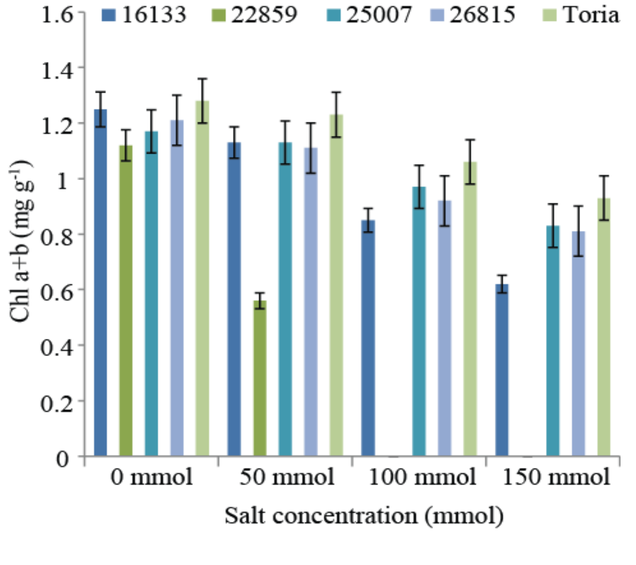

Fig. 4(c). Effect of salt stress on Chlorophyll a + b content of *B. rapa*.

The effects of salinity on shoot length and root length at early germination stage of three sub-species *B. rapa* are presented in Table 2. The results showed that maximum shoot and root length, root fresh, dry weight and root fresh, dry weight were calculated from genotype 25007 followed by Toria at 50, 100 and 150 mmol salt levels after one month from sowing. The genotype 22859 showed very poor performance at all stress level. The brown sarson and toria types of *B. rapa* are more salt tolerant genotypes as compared to yellow sarson and remain green at low to high salt stress levels. Our study is in line with Shahbazi *et al.* (2011) who reported that high salt concentrations (50, 100 and 150 mmol) inhibit the early seed germination of all tested *Brassica napus* genotypes. Our results are in accord with Ashraf & Harris, (2004) and Munns & Tester, (2008) those reported that salinity affects both normal morphological and physiological process that directly inhibit normal plant growth and development. Vital *et al.* (2008) revealed that salinity affect plant growth by various means such as imbalance normal ion exchange process; decrease the amount of essential nutrients and through accumulation of toxic substances etc. Umar *et al.* (2011) also studied the effect of salinity on *Brassica campestris*. They noted stunted growth in all tested genotypes at high salinity level of l80 mmol. Our results are in line with Munns & Tester (2008) and Arshi *et al.* (2005) who noted low quantity of leaf, shoot and total plant dry mass at high salt concentrations as compared to low salt levels. High salinity levels decrease normal photosynthetic and other biological processes (Munns *et al.*, 2006).

In present study the effect of salinity on physiological process of *B. rapa* was studied. The high salt concentration significantly decreased the percent relative water contents (%RWC) up to several folds. Alt low salt concentration (50 mmol) the genotype 25007 and Toria showed maximum %RWC as compared to other genotypes. But at high salt concentrations (50 and 100 mmol) this amount was decreased in all tested genotypes (Fig. 2). The low RWC values were recorded in *B. napus*, *B. campestris* and *B. juncea* under drought stress by Alam *et al.* (2014). Similar findings were also been reported by Gupta *et al.* (2001) and Meena *et al.* (2003). The frost stress decreased %RWC value up to several fold in tomato genotypes Riogrande, Roma and Money maker Shah *et al.* (2014).

Proline plays a vital role to maintain normal osmotic potential, signal transduction and having antioxidant properties (Bates *et al.,* 1973; Nahar *et al.,* 2013). In this study high amount of proline contents was noted in all tested *B. rapa* genotypes at high salt stress levels. At high salinity levels the proline values increased up to several folds as compare to controlled plants (Fig. 3).The high proline content in *B. campestris* under drought condition was noted by Alam *et al.* (2014). Our findings are in agreement with previous studies (Ali & Ashraf, 2011; Nounjan *et al.,* 2012). The high proline content in leaves of brassica species was also reported by Deepak *et al.* (1995).

In present study the effect of salinity on chlorophyll contents was studied. From results it is clear that salt stress significantly decreased the chlorophyll a, b and (a+b) contents in all three sub-species (brown sarson, yellow sarson and Toria types) of *B. rapa* (Fig. 4a-c). However the response of all three types varies at low to high salt stress. Among all three types Toria showed maximum total chlorophyll (a+b) content as compared to other two types. Several researchers reported that decrease in photosynthetic rate is due to damage of important photosynthetic pigments (Anjum *et al.,* 2011; Pandey *et al.,* 2012). Abiotic stresses such as drought and salinity directly decreases the chlorophyll content in many important plant species (Chaves *et al.,* 2003, Renolds *et al.,* 2005). The low amount of Chlorophyll a, b and a+b under drought stress was noted by Alam *et al.* (2014). Our findings are also in accord with those of Kauser *et al.* (2006).

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