

Short Communication

Salt tolerance classification in wheat genotypes using reducing sugar accumulation and growth characteristics

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Abstract: The aim of the present study was to assess inter and intra-specific variations in *Triticum* groups. Local hexaploid bread wheat and Synthetic Elites and Durum were characterized based on growth abilities and amount of reducing sugar accumulated under 200 mM NaCl salinity. Further, the role of reducing sugar accumulation in adaptive process of different *Triticum* groups towards salinity was also measured. Identification of inter-specific and intra-specific variation with in the gene pool is of greater importance for breeding salt tolerant varieties. Salt tolerant genotypes tend to accumulate more carbohydrates under salt stress and prevent plants from oxidative damage. Wide differences observed between *Triticum* groups under salt stress for shoot length, shoot weight and reducing sugar content. Salt stress reduced shoot length and weight in both Local hexaploids and Synthetic elites but effect was more pronounced in Local hexaploid wheat. Kharchia and LU-26 were identified as salt tolerant standard genotypes which showed significant increase in reducing sugar content where as salt stress induced reduction in amount of reducing sugars of local hexaploid genotypes. Performance of salinity tester genotypes and synthetic elite and durum genotypes was less affected by salinity and prominent increase in reducing sugar content has been observed in comparison to local hexaploid genotypes. High values for salt tolerance indices were also observed for salinity tester set and synthetic elite and durum wheat genotypes. It may be concluded from the above findings that wheat genotypes like Kharchia, LU-26, Durum 155 and SE 88 with highest accumulation of reducing sugar content under stress could be used in breeding programs to develop salt tolerant germplasm and accumulation of reducing sugars under stress conditions could be a useful bio-marker for selecting tolerant wheat genotypes.

Keywords: salinity, hexaploid wheat, durum, synthetic elites, inter-specific variation, intra-specific variation, reducing sugars.

دراسة تصنيف درجات تحمل الملوحة في أنماط جينية مختلفة في نبات القمح باستخدام نسبة السكر المنخفض وصفات النمو الظاهرية

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ملخص: تهدف الدراسة لتقييم المتغيرات الداخلية والبيئية لنبات القمح حيث تم زراعة عدة أصناف محلية ومحسنة وراثيا لدراسة مدى تحملها للملوحة ومدى تغير نسبة السكر المنخفض في أصناف محلية لخبز القمح للصنفين Synthetic Elite و Durum وذلك بناء على قدرتها على تحمل النمو بالملوحة وقد تم اخذ قياس نسبة السكر المنخفض بعين الاعتبار لملوحة تصل إلى 200 ملم NaCl ، ومن أهم الصفات لمدى تحمل الملوحة أيضا التعرف على الصفات والمتغيرات الداخلية والبيئية لأصناف القمح محل الدراسة. وقد وجد أن الأصناف المتحملة تتميز بانها أكثر تجميعا للكربوهيدرات وذلك لمساعدتها لتخفيض صدمة الملوحة العالية. وهناك اختلافات عديدة حتى بين أصناف القمح وخاصة في النمو الخضري والوزن الحيوي ونسبة السكر المنخفض. ونجد ذلك عند دراسة تأثير الملوحة على انخفاض كبير في الوزن الخضري لكافة الأصناف سواء المحلية أو المحسنة وراثيا ولكن نجد تأثيرها جليا على الأصناف المحلية لنبات القمح. وقد وجد أن Synthetic Elite و Durum مدى مقاومتها للملوحة وخاصة زيادة نسبة السكر المنخفض وأيضا انخفاض تأثير الملوحة على الأصناف المحسنة وزيادة دائمة لكمية السكر المنخفض مقارنة مع الأصناف المحلية. ويمكننا أن نستخلص أن أصناف القمح مثل Kharchia و LU-26 و Durum 155 و SE88 والتي ارتفعت بها نسبة السكر المنخفض وذلك عند زراعتها تحت الملوحة العالية يمكننا استخدامها في برامج التهجين والتعرف على الأصناف المتحملة في البيئة المحلية وذلك كأحد المؤشرات الحيوية للتعرف على أصناف القمح المتحملة للملوحة.

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Introduction

Abiotic stresses such as high temperatures, low water availability, excessive salt accumulation and mineral deficiencies or toxicities adversely affect productivity of cereal crops (Abebe et al., 2003). Extensive genetic variation for salt tolerance exists in the genetic background of tribe Triticeae (Noaman, 2000). Inter-specific, inter-varietal or intra varietal variation within the gene pool is of greater importance for breeding salt tolerant varieties (Ashraf et al., 2005). The general response of plants to increasing salt concentration includes osmotic stress, specific ion toxicity and nutrient deficits thus affecting a range of physiological processes involved in cell metabolism (Munns, 2002) and oxidative stress due to over production of reactive oxygen species (ROS's) (de Azevedo Neto et al., 2006).

During the course of stresses, active solute accumulation of compatible solutes such as amino acids, polyamines and carbohydrates is claimed to be an effective stress tolerance mechanism (Colmer et al., 2005). Water soluble carbohydrates and fructans are the sensitive markers for the selection of tolerant genotypes under salt stress (Kerepesi and Galiba, 2000). Salt tolerant wheat genotypes tend to accumulate more carbohydrates under salt stress in comparison to sensitive genotypes. High carbohydrate concentrations under salt stress prevent plants from oxidative damage and also maintain the structure of proteins (Hajihashemi et al., 2006). Increased carbohydrate concentration under salt stress has also been reported in tomato (Mizrahi, 1982), soybean (Liu and Staden, 2001) and mangrove (Parida et al. 2004). Levels of glucose and fructose however decreased when subjected to stress in potato cell cultures but total sugar content increased (Wang et al., 1999). In response to salt stress inter-specific and intra-specific variations are also evident among the salt tolerant species for accumulation of soluble sugars (Ashraf and Harris,

2004). The present study was aimed to evaluate interspecific and intraspecific variation present in *Triticum* groups: Salinity tester set, Local hexaploid bread wheat and Synthetic elites and Durum based on growth abilities and amount of reducing sugars under 200 mM NaCl and role of reducing sugar accumulation in adaptive process of different *Triticum* groups towards salinity.

Materials and methods

Experimental Material

Seeds of Salinity tester set, Local hexaploid bread wheat cultivars, Synthetic elites and Durum were used as experimental material. Seeds of Local hexaploid bread wheat were provided by National Agriculture Research Council, Islamabad, Pakistan. Seeds of Elite synthetic hexaploid wheat, Durum wheat and Salinity tester set were provided by CIMMYT, Mexico and maintained in screen house of Genetics Department, University of Karachi.

Growth Conditions

Seeds were grown in plastic cups containing sandy loam and manure (cow dung) in a ratio of 1:1. Seeds were allowed to grow normally for 2 weeks after germination. Salt stress was induced on 14 days old seedlings by applying 0 (Control) and 200 mM NaCl (Salt Stress). Seedlings were stressed for 16 days; shoot samples were harvested; length and weight were noted and used for quantitative estimation of carbohydrates.

Extraction and Estimation of Reducing Sugars (Glucose)

For quantitative estimation of soluble carbohydrates (free from combined hexoses) 1 g leave tissues were plunged in 4 mL of hot 80% Ethanol (kept over a boiling water bath) for five min. and then crushed with mortar and pestle and centrifuged. Filtrate was used for the

estimation of reducing sugars.

Carbohydrates were measured according to Nelson's modification of Somogi's method (Somogi 1937; Nelson 1944). This is very sensitive and reasonably quick method for quantitative estimation of reducing sugars. Carbohydrates with free reducing sugars undergo isomerization, oxidation and cleavage, while the oxidizing agent copper is reduced. After reduction Copper reacts with an arsenomolybdate colour forming reagent and produce blue colour.

Reducing sugars were estimated in 1 mL of plant extract in separate test tube. Each tube received 1 mL of copper reagent. After thorough mixing the tubes were placed in boiling water bath for 20 min. and quickly cooled by dipping them in cold water for five min. 1 mL of arsenomolybdate reagent was added to each tube and contents were shaken rapidly until the evolution of CO₂ was completed. The tubes were left for 15 min. for the development of blue color. The absorbance was recorded at 500 nm against reagent blank, using Photoc-100 spectrophotometer. The amount of reducing sugars was estimated as μg glucose g⁻¹ fresh weight.

Experimental Design and Statistical Analysis

Experiment was conducted in factorial CRD with 12 genotypes, two treatments (control and 200mM NaCl) where as each treatment was replicated twice. SPSS v. 11 was used to perform Analysis of Variance to evaluate the inter-specific and intra-specific variation in *Triticum* groups under salt stress. Significant differences between *Triticum*

groups and among genotypes were computed by Duncan's Multiple Range Test. Salt tolerance indices were calculated according to Zeng et al. (2002) and further used to classify genotypes using cluster analysis as described by El. Hendawy et al. (2005).

Results

Inter-specific Variation

Analysis of variance between groups revealed significant differences between *Triticum* groups with respect to shoot length and reducing sugar content however these differences were non-significant for shoot weight. Local cultivars were significantly different from Salinity tester set, Synthetic elites and Durum wheat with respect to shoot length in control and 200 mM NaCl stress. Mean comparison of the genotypes at control and 200 mM revealed that maximum shoot weight was observed in Local cultivars under control but it was drastically reduced at 200 mM stress. Overall effect of 200 mM NaCl stress was more pronounced in local cultivars than Synthetic elites and Durum.

Amount of reducing sugars was highest in Synthetic elites which were 197.88 μg glucose g⁻¹fw than Salinity tester set and Local cultivars in control. Salinity stress of 200 mM significantly increased the amount of reducing sugars in Salinity tester set and Synthetic elites where as it was reduced in local cultivars. Interaction between *Triticum* groups and treatment was also highly significant indicating that amount of reducing sugars varied between *Triticum* groups at 200 mM NaCl stress.

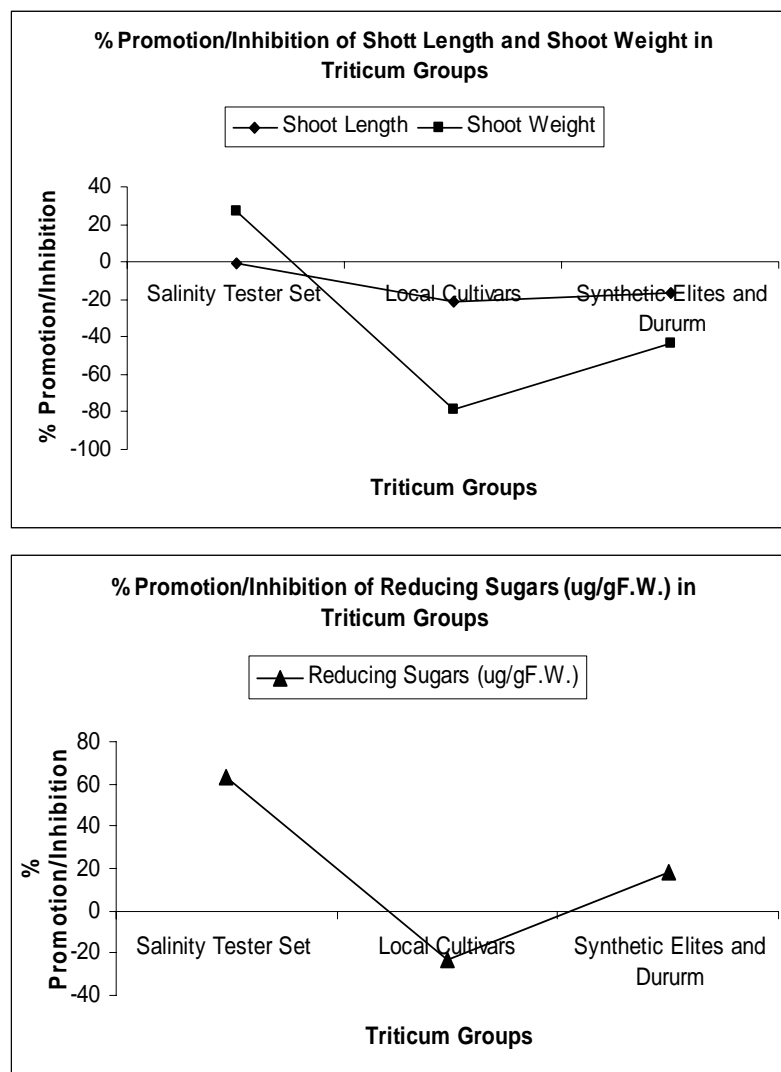


Figure 1a-1b. Percent Promotion/Inhibitions.

Intra-specific Variation Salinity Tester Set

Analysis of variance revealed non-significant differences between genotypes for shoot length but differences were significant for shoot weight. Similarly salt levels showed non-significant effect on shoot length and shoot weight of Kharchia and LU-26 but the genotypes and treatment interaction was found to be significant indicating that both the genotypes respond differently at 200 mM NaCl stress. Non-significant effect of treatment was indicating that 200 mM NaCl stress had no pronounced effect on shoot length and weight at early seedling stage.

Kharchia and LU-26 were non-significantly different with respect of to amount of reducing sugars but the effect of treatments was significant. Amount of reducing sugars was more in Kharchia than LU-26 (Table 1). Increased amount of reducing sugars under stress showed tolerance of both the varieties.

Local Hexaploid Bread Wheat

Significant differences were observed between the genotypes of Local hexaploid bread wheat for shoot length and amount of reducing sugars where as shoot weight was non-significantly different between genotypes. Effect of treatment was non-significant on shoot length and weight but

it was highly significant on reducing sugar content. Genotype and treatment interaction was also significant for shoot length and reducing sugars revealing that shoot length of all the genotypes varied significantly at 200 mM NaCl stress. Significant interaction was indicating variable response of genotypes towards salinity.

Cultivar 'Sarsabz' showed the highest shoot length in control, where as at 200

mM NaCl stress highest shoot length was observed in cv. Inqilab-91 which was 10.6 cm as compare to other genotypes (Table 1). Similarly shoot weight was not significantly different among genotypes under control conditions but it reduced drastically at 200 mM stress. Shoot weight of Inqilab-91 was highest among the genotypes at 200 mM stress which was 0.05 gm and was significantly different from the other genotypes.

Table 1. Mean Differences between Genotypes of Salinity Tester Set.

Genotypes	Shoot Length (cm)		Shoot Weight (g)		Reducing Sugars (µg glucose/g. fw)	
	Control	200mM	Control	200mM	Control	200mM
Kharchia	13.2 ^a	10.15 ^a	0.06 ^a	0.04 ^a	194 ^a	309.5 ^a
LU-26	10.55 ^a	13.4 ^a	0.07 ^a	0.125 ^b	148 ^b	250 ^b
Local Hexaploid Bread Wheat						
Kohistan-97	9.25 ^a	5.25 ^a	0.08 ^a	0.03 ^b	180 ^b	117 ^a
Inqilab-91	9.75 ^a	10.6 ^a	0.11 ^a	0.05 ^a	222.5 ^a	126 ^a
Anmol-91	4.6 ^a	6.15 ^a	0.03 ^a	0.03 ^b	96 ^c	117 ^a
Sarsabz	11.05 ^a	5.3 ^a	0.45 ^a	0.035 ^b	102 ^c	99 ^b
Synthetic Elites and Durum						
S.E. 68	9.1 ^c	7.25 ^c	0.045 ^c	0.04 ^d	193 ^a	257 ^{ab}
S.E 76	13.35 ^b	10.3 ^c	0.145 ^d	0.055 ^{cd}	218 ^a	129.5 ^c
S.E. 88	13.35 ^b	15.1 ^a	0.195 ^c	0.21 ^a	154 ^a	206 ^b
S.E. 103	15.20 ^{ab}	10 ^c	0.28 ^a	0.085 ^b	261 ^a	254 ^{ab}
S.E. 141	17.25 ^a	8.7 ^d	0.24 ^b	0.085 ^b	199 ^a	254 ^{ab}
Durum 155	10.2 ^c	14.4 ^b	0.035 ^e	0.06 ^c	162 ^a	303 ^a

Highest amount of reducing sugars was observed in genotype Inqilab-91 under control as indicated by the mean value of 222.5 µg glucose/g.fw (Table 1). This value was significantly higher than other genotypes. Genotype Anmol-91 showed lowest amount of reducing sugar under control in comparison to Inqilab-91 and Kohistan-97 but it was non-significantly different from Sarsabz. Salinity stress of 200 mM NaCl significantly reduced the amount of reducing sugars in Kohistan-97 and Inqilab-91.

Synthetic Elites and Durum

Shoot length, shoot weight and amount of reducing sugars were highly significantly different between the genotypes similarly effect of treatment was also significant. Significant interaction was indicating variable response of genotypes under control and stress conditions.

Shoot length, shoot weight and reducing sugars varied significantly among the genotypes under control and stress conditions (Table 1). S.E. 141 showed highest shoot length under control which was significantly higher than other

genotypes but it was reduced when stress was applied. Significantly higher shoot length at 200 mM was observed in Durum 155 and S.E. 88. Highest shoot weight under control was observed for S.E. 103 and S.E. 141 where as under stress condition highest shoot weight was observed in S.E. 88 which was 0.21 gm. Shoot weight was increased under stress in Durum 155 and S.E. 88 where as shoot weight was reduced in S.E. 76, S.E. 103 and S.E. 141 under stress.

Highest amount of reducing sugars was observed in S.E. 103 under control which was reduced when stress was applied (Table 1). When salt stress of 200 mM was imposed results showed variable response of genotypes. Reducing sugar content markedly increased in Durum 155 and reached 303 μg glucose/g.fw under stress this increase was about 87% of

control. Reducing sugars also increased in S.E. 68, S.E. 88, and S.E. 141 under stress.

Salt Tolerance Index

Salt tolerance indices (STI) of all genotypes have also been calculated for shoot length, weight and reducing sugars (Table 2). STI values reflected that shoot length was less affected by 200mM NaCl as for most of the genotypes STI value was ≥ 0.6 except for SE 68. Effect of salt stress was more prominent on shoot weight of the genotypes as revealed by STI values ≤ 0.5 . LU-26 and SE 88 were the only genotypes which showed increase in shoot weight under stress reflected by STI value greater than 1. Amount of reducing sugars increased under stress in Kharchia, LU-26 and Durum 155 and Synthetic elite genotypes.

Table 2. Salt Tolerant Indices for Shoot Length, Shoot Weight and Reducing Sugars in *Triticum* Groups.

Salt Tolerance Indices in Salinity Tester Set			
Genotypes	Shoot Length	Shoot Weight	Reducing Sugars
Kharchia	0.76	0.66	1.7
LU-26	1.22	1.78	1.68
Salt Tolerance Indices in Local Cultivars			
Kohistan-97	0.6	0.179	0.77
Inqilab-91	1.22	0.299	0.83
Anmol-91	0.71	0.179	0.77
Sarsabz	0.61	0.209	0.65
Salt Tolerance Indices in Synthetic Elites and Durum			
Durum 155	1.1	0.38	1.53
SE 68	0.554	0.25	1.29
SE 76	0.787	0.35	0.65
SE 88	1.13	1.34	1.04
SE 103	0.76	0.544	1.28
SE 141	0.66	0.544	1.28

STI values were then used to classify wheat genotypes as tolerant, moderately tolerant and sensitive genotypes (Figure 2). Hierarchical cluster analysis clearly classified wheat genotypes in to three distinct groups. Kharchia, LU-26, Durum 155 and 88 were classified as tolerant wheat genotypes and Kohistan, Sarsabz, Anmol

and SE 76 as sensitive genotypes at plantlet stage.

Discussion

High salt concentrations in soil water reduces the ability of plant to take up water which results slower plant growth and further reduces the plant growth by

damaging the cells of transpiring leaves (Munns, 2005). High carbohydrate content under salt stress prevents plants from the oxidative damage and maintains structure of proteins and membranes (Hajihashemi

et al., 2006). Increase in carbohydrate content depends on environmental conditions, species and genotypes within the same species (Kameli and Losel, 1993).

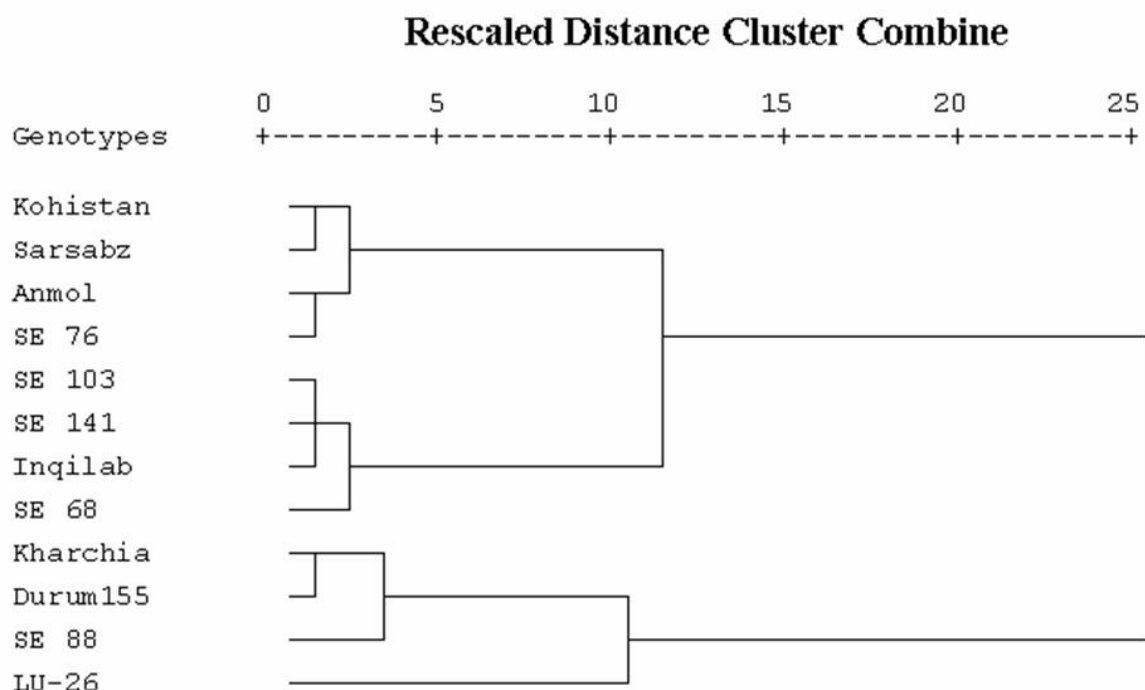


Figure 2. Hierarchical cluster analysis of wheat genotypes based on salt tolerance indices.

The study was carried out to exploit interspecific and intraspecific variation present in *Triticum* groups: Salinity tester set, Local hexaploid bread wheat, Synthetic elites and Durum under salt stress (200mM NaCl).

Assessment of Interspecific Variation

Salinity tester set was used as check to compare the salt tolerance of other *Triticum* groups. According to current findings *Triticum* group comprising Local hexaploid bread wheat was different from the Salinity tester set and Synthetic elites with respect to shoot length, shoot weight and amount of reducing sugars. Shoot length and shoot weight decreased under stress but reduction was prominent in Local hexaploid bread wheat (21% and 78%). Several studies have been done for

assessment of genotypes under salt stress at seedling stage. Under various abiotic stresses including salinity reduction has been observed in shoot length of Sorghum (Gill et al., 2001). Reduction in shoot length under salt stress was also observed in Durum wheat (Almansouri et al., 2001). Present results are significant with these findings as shoot length was reduced at 200 mM NaCl stress in both *Triticum* groups except Salinity tester set where increase in shoot length has been observed under stress.

Shoot weight was also negatively affected by salt stress (Figure 1a). Salt stress imposed negative effect on shoot weight of Local hexaploid bread wheat and Synthetic elites but shoot weight was increased in salinity tester set under 200 mM NaCl stress showing their relative

tolerance against salinity. These results are in agreement with those of Gill et al. (2001, 2002) in sorghum and Almansouri et al. (2001) in durum wheat. According to their findings shoot weight (Sorghum and Wheat) decreases under NaCl stress and other abiotic stresses.

Accumulation of carbohydrates under salinity stress and other abiotic stress has been reported by several investigators and it was also reported that amount of reducing sugars increased in tolerant cultivars. Salinity tester group used as standard and Synthetic elites showed significant increase in amount of reducing sugars under salt stress (Figure 1b). These results are in agreement with those reported earlier. Significant increase in amount of reducing sugars was observed in wheat varieties Kharchia and Ghods with increasing levels of salinity. Total soluble carbohydrates were studied under NaCl stress in wheat genotypes LU-26 and Potohar and significant increase in amount of reducing sugars was observed in tolerant wheat genotype LU-26 (Javed, 2002; Hajihashemi et al., 2006). Increased in total soluble carbohydrates and reducing sugars under salt stress were also reported by Gill et al. (2001, 2002) and Liu and Staden (2001). Kerepesi and Galiba (2000) reported significant increase in water soluble carbohydrates and reducing sugars under salinity stress in wheat seedlings.

In local hexaploid bread wheat salinity stress significantly reduced reducing sugars. Low amount of reducing sugars may be indicating their sensitivity towards NaCl of 200 mM. Hajihashemi et al. (2006) reported that amount of reducing sugars decreased with increasing concentration of salt in sensitive cultivars. Kerepesi et al. (2002) also reported that amount of fructans increased under salt stress in tolerant cultivars and decreased in sensitive cultivars under 200 mM NaCl stress.

Assessment of Intraspecific Variation Salinity Tester Set

Kharchia and LU-26 used as standard and are known salt tolerant genotypes. Decrease in shoot length was observed in Durum wheat under salinity stress (Almansouri et al., 2001). Javed (2002) described invitro technique for screening salt tolerance of wheat genotypes LU-26 and Potohar using calli tissues. Callus dry weight of LU-26 was increased by increasing concentration of NaCl. It has been reported that carbohydrate content increased under salinity and other abiotic stresses and increase was more prominent in tolerant cultivars. In Kharchia and LU-26 water soluble carbohydrates and amount of reducing sugars increased with increasing levels of salinity however in sensitive genotype Ghods amount of reducing was more in control than in stress. In genotype Potohar although carbohydrates increased under stress but the increase was prominent in tolerant genotype LU-26 (Javed, 2002, Hajihashemi et al., 2006). Results of the present study are significant with these findings as reducing sugar content in both genotypes LU-26 and Kharchia significantly increased under stress.

Local Hexaploid Bread Wheat

Among all the genotypes Anmol-91 was the only genotype with increase amount of reducing sugars under stress. Salt stress significantly reduced sugar content in all genotypes and the effect was more prominent in Inqilab-91. These results are in agreement with those of Parida et al. (2004). Biochemical components under salinity stress were studied in mangrove where 250 mM NaCl stress significantly reduced amount of total sugars content and reducing sugars however starch content increased under stress.

Synthetic Elites and Durum

Genotypes of Synthetic elites and Durum had greater variability for shoot

length, shoot weight and reducing sugars. Genotypes differ from each other under control and this variability increased when stress was applied. Increase in carbohydrate content under stress indicated that these genotypes have ability to maintain their growth under stress and are stable genotypes even at higher levels of salinity. Reducing sugar content increased in tomato species at various stages of fruit ripening when NaCl stress was applied (Mizrahi, 1982). Accumulation of carbohydrates under stress might be a sensitive marker for selecting tolerant genotypes (Kerepesi and Galiba, 2000). Results revealed that performance of all the genotypes was almost similar under control but as the stress was applied prominent differences appeared between genotypes. Increased amount of reducing sugars under stress could be the possible source of protection and provide tolerance to the genotypes.

It has been clear from salt tolerance indices (Table 2) that performance of salinity tester set genotypes and durum and synthetic elite genotypes was better under 200 mM salt stress with respect to both growth abilities and reducing sugars content in comparison to local hexaploids. Cluster analysis (Figure 2) further divided genotypes into three distinct groups with respect to salt tolerance and classified Kharchia, LU-26, Durum 155 and SE 88 as tolerant wheat genotypes where as SE 68, SE 103, SE 141 and Inqilab-91 as moderately salt tolerant at young plantlet stage.

Conclusions

Extensive variation exists among the *Triticum* groups and even between the genotypes of the same group and increased carbohydrate content under stress may provide a strong protective mechanism against oxidative damage to cope with the deleterious effects of salinity. It may be concluded from above findings that selection of wheat genotypes like

Kharchia, LU-26, Durum 155 and SE 88 with highest accumulation of reducing sugar content under stress could be used in breeding programs to develop salt tolerant germplasm. Furthermore it may be concluded that increased amount of reducing sugars under stress conditions could be a useful bio-marker for selecting tolerant wheat genotypes.

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