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IDENTIFICATION OF SOME WHEAT (*TRITICUM AESTIVUM* L.)LINES FOR SALT TOLERANCE ON THE BASIS OF GROWTH AND PHYSIOLOGICAL CHARACTERS

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Abstract

To evaluate salt tolerance in some newly developed wheat lines (*Triticum aestivum* L.) on morphological and physiological basis, a net house study was conducted at Plant Physiology Division, Nuclear Institute of Agriculture (NIA), Tandojam. The tested genotypes were WSP-1, WSP-2, WSP-3 WSP-4 and S-24 along with LU-26s as salt tolerant check. The study was conducted in cemented beds, filled with coarse gravel (up to 30 cm depth). The experiment was laid out using randomized complete block design (RCBD), with three replicates. Two treatments were imposed i.e. non-saline control (1.56 dS/m) and saline (12.0 dS/m). Salinity was imposed gradually through irrigation after two weeks of germination, using sodium chloride (NaCl) salt. The beds were irrigated with modified Hoagland solution of respective salinity, weekly or when ever required. Growth performance (plant height, productive tillers, spike length, number of grains/ spike, grain weight/ plant and 1000 grain weight) were recorded at the time of maturity. Studies on solute contents i.e. organic (Proline and total soluble sugars) and inorganic (Na, K and K/Na ratio) were carried out at the time of flowering. The physiological parameters were also correlated with morphological characters. The results indicate that there was decrease in growth under salinity stress. Among the tested genotypes, the performance of WSP-1 was comparatively better at 12.0 dS/m salinity level, with maximum growth variables having < 50% relative decrease. The data indicates that wheat lines are maintaining their osmotic potential (O.P) through the accumulation of organic solutes especially proline. Comparatively higher accumulation of organic solutes (proline & total soluble sugars) and less reduction in K/Na ratio in genotypes WSP-1 was mainly responsible for its better response to NaCl stress.

Key words: Wheat (*Triticum aestivium* L), Morphological and physiological parameters.

Introduction

Wheat is the staple food crop for more than one third of the world population, used mainly as a human feed. It supplies 20% of the food calories. Spring wheat (*Triticum aestivum* L.) is cultivated all over the Pakistan. It is cultivated on more than 9.0 Mha area in Pakistan. Wheat production during 2013-14 was reported as 23.4 M tones with an average yield of about 2584 Kg/ ha (Anon., 2013). It has been observed that apart from its increased production due to increased in area of cultivation, its average yield per unit area could not be increased. There are several factors (biotic and a-biotic) which may cause its low productivity. Among them soil salinity is the major factor in Pakistan. The problem of soil salinity is more sever in the province of Sindh, where almost half of the cultivated area is affected by varying degree of salinity. Wheat is categorized as medium tolerant to salinity (Khan *et al.*, 2004). The threshold values are about 7.0 dS/m, where its yield decline gradually and remain below the economic limits (>50%) at 12 dS/m. It has been reported that there exist variation in salt tolerance among the genotypes (Farooq *et al.*, 2008). The variability in salt tolerance within the genotypes might be due to variation in selective uptake of Na^+ ions or limited transportation of these ions to photo-synthetically active parts of the plant (leaves). Efficient uptake of K^+ over $Na^+$is also an important strategy to with stand these harsh environments successfully (Schachtman & Munns, 1992; Aurangzeb *et al.*, 2013). Screening of salt tolerant wheat genotypes on the basis of ions selectivity is important physiological trait and may provide salt tolerant wheat genotypes for cultivation on salt affected soils. In the present studies some newly developed wheat genotypes were evaluated for their salt tolerance on the basis some morphological and physiological basis. It is hoped that the studies may provide suitable salt tolerant wheat genotypes and also helps the wheat breeders for producing high yielding salt tolerant wheat genotypes for salt prone areas of Pakistan.

Material and Methods:

The studies were conducted in gravel culture in glass house at Nuclear Institute of Agriculture (NIA), Tandojam, Pakistan. Five wheat genotypes were tested along with salt tolerant check (LU-26s). The experiment was laid out using completely randomized design (CRD) and was replicated thrice. Wheat genotypes were sown manually by dibbler at the distance of 20 x 10 cm (row to row and plant to plant, respectively).There were two treatments (1) Non-saline (control) and(2) Saline (12dS/m). Salinity treatment was imposed gradually after two weeks of germination through irrigation using sodium chloride (NaCl) salt. Physiological parameters (organic and inorganic solutes) were determined in leaves samples at the time of flowering. The samples were washed thoroughly with distilled water, dried for 72 hours in hot air oven (±70°C). Solute extraction was done in (0.5%) toulene water, according to Weimberg *et al.* (1981). Sodium (Na+) and Potassium (K+) were determined by flame Photometer (Jenway PFP-7) against the standards after making suitable dilutions. Proline and total soluble sugars were also determined in 0.5% toulene extract. Proline was determined as described by Bates *et al.* (1973). Total soluble sugers (TSS) were determined according to Riazi *et al.* (1985) by UV spectrophotometer (Hitachi 150-20). The crop was harvested at maturity and morphological characters (Plant height, plant biomass, and spike length, number of grains / spike, grain weight / plant and 1000 grain weight) were recorded. The data was subjected to analyze statistically for significance by Analysis of variance (ANOVA) and Duncan multiple range test (DMRT). Correlation studies among the morphological and physiological were also carried out using Statistix 8.1, computer package (Table 1).

Results and Discussions

Morphological performance: The results with respect to morphological performance of different wheat genotypes revealed that there was a significant effect of salinity. The differences among the individual genotypes were also significant except in 1000 grain weight, where the values were statistically non significant within the genotypes.

Effect of salinity was more pronounced in case of plant biomass, where the relative decrease in plant biomass under saline condition was 53% (Table 2). The only genotypes which had < 50% rel. dec. were WSP-1 and LU-26s, showing 47 and 21% decrease, respectively. Reduction in plant biomass due to salinity has also been reported in early studies (Pessarakli & Huber, 1991; Al-Rawahy *et al*., 1992; Ashraf *et al*., 2002). They have the opinion that a negative response of salinity to plant biomass might be attributed to decreased rate of photosynthesis. Plant exposed to saline environments seriously face shortage of water due to differences in osmotic potential (OP) in and outside the plant, which ultimately results in stomatal closing and thus the rate of photosynthesis decreases.

Tillering capacity in wheat genotypes was also affected significantly due to presence of salts in the medium. Mean decrease under salinity was 48% in grain bearing tillers (productive tillers). The relative decrease in WSP-2, WSP-3 and WSP-4 was recorded above the economic limits (i.e.> 50%), where as minimum decrease in productive tillers was observed in LU-26s followed by S-24 and WSP-1 (i.e. 37.4, 44.8 and 47.2%, respectively). According to Hendawy *et al*. (2005) salt stress during tiller emergence can inhibit their formation and can cause their abortion at later growth stages. Furthermore, when salinity levels are greater than 7.5–10.0 dSm^{-1} or 75-100 mM NaCl, most of the secondary tillers of moderately tolerant genotypes eliminate, and their numbers greatly reduce (Hendawy *et al*., 2005, Ahmed *et al*., 2005, Francois *et al*., 1986).

Wheat genotypes also exhibited higher decrease due to salinity in grain weight/ plant. However all the tested genotypes had < 50% relative decrease, except S-24, showed almost 50% reduction under salinity. Mean values for relative reduction in grain weight was 33%. Ali *et al*. (2008) reported that variation in grain yield not only depends on diverse genetic makeup of wheat genotypes but also on their differential response to prevalent environmental conditions at grain filling stage. Among the tested genotypes least decrease in grain weight was observed in WSP-1 followed by LU-26s. Kamkar *et al*. (2004) also reported less reduction in grain size and seed index in tolerant genotypes, which consequently lead to produced higher yield. Kirby, (1974); Wardlaw *et al*. (1980).

The data with respect to 1000 grain weight (Seed index) also showed less decrease in LU-26s and WSP-1 (i.e. 20 and 21% rel. dec., respectively). Like in grain weight/ spike the relative reduction in S-24 was also higher (i.e 35%). Mass & Grieve, (1990) found that salinity affects adversely at grain filling stage which results in decreased 1000-grain weight. Adverse effects of salinity on yield due to water and nutritional imbalance in plant were also reported by Muhammad (1983).

The effect of salinity on plant height, spike length, number of spiklets/ spike and number of grains/ spike was comparatively less, where the relative decrease under saline conditions was 10.4, 6.7, 4.4 and 10.8%, respectively.

The response of wheat genotypes with respect to relative decrease in different morphological attributes was summarized in table 3. The data showed that the relative decrease in all the growth parameters was within the economic limits (< 50% relative decrease) in genotypes WSP-1 and LU-26s and hence can be categorized as better performing genotypes under medium to high salinity levels (i.e. 8-12 dS/m).

Physiological performance

Inorganic solutes: There was higher accumulation of sodium (Na^+) in plant (leaves) under salinity. The increased Na^+ contents in leaf samples could be due to high concentration sodium salts in the growing medium (Shafqat *et al*., 1998). Maximum accumulation was observed in WSP-2 WSP-3, WSP-4 and S-24 having about 67, 60, 50 and 52% rel. inc., respectively). This might have created toxicity in plant tissues and this toxic concentration of Na^+may have disturbed the various metabolic activities in plant (Akram *et al*., 2007),resulting in higher decrease in plant biomass (i.e. > 50%).On the other hand comparatively less accumulation of Na^+ was observed in WSP-1 and LU-26s. Our results are agreement with the findings of Ashraf *et al*. (2005), who also reported maximum dry matter yield in wheat genotypes with the low Na^+ accumulation.

In the present study all wheat genotypes displayed decreasing trend in potassium K^+ content due to salinity stress. The reduction in K^+ might be due to the presence of excessive Na^+ in the growth medium is known to have an antagonistic effect on K^+ uptake in plant (Ashraf.*et al*, 2013, Sarwar & Ashraf, 2003). It is also believed that sufficient supply of potassium (K^+) is the strategy to overcome the toxic effects of Na^+. Relative reduction in K^+ uptake was also less in WSP-1 and LU-26s i.e. 21 and 23%, respectively. The increased K^+ contents in these genotypes under salinity stress could be due to efficient K^+ absorption by selective inclusion of Na^+ by cortical cells (Schachtman & Munns, 1992).

The K^+/Na^+ ratio is the trait which is supposed to be the main criteria for salt tolerance in plant. It is well reported that increasing soil salinity, positively increases concentrations of Na^+ and decreases K^+ in wheat genotypes, which led to a decreased K+/ Na+ ratio (Aurangzeb *et al*., 2013). Increased sodium contents and decreased K^+ concentration in expressed leaf tissues under salinity were also reported by Qureshi *et al*. (1991). Higher reduction of K^+ in sensitive genotypes also showed decreased K/Na ratio under salinity. There was almost above 60 to 70% decrease in sensitive genotypes. On the other hand the genotypes WSP-1 maintained K^+/Na^+ ratio quite satisfactorily (i.e.41%), followed by LU-26s (50%).Correlation studies between biomass and grain weight with Na showed significantly –ve (R^2 = -0.68, -0.64, respectively) and significantly +ve with K (R^2 = -0.76) and K/ Na ratio (R^2 = -0.78) (Table 4). Significantly negative relations between grain yield and Na contents were also reported by Khan *et al*. (2009).

Table 1. Mean squares from analysis of variance (ANOVA) of growth parameters under saline conditions.

Source	Degree of freedom	Plant height	Plant biomass	Productive tillers	Spike length	No. spiklets/ spike	No of grains/ spike	Grain weight/ spike	1000 grain weight
Salinity	1	517 ***	295.3***	26.92**	2.72**	3.42*	155.0**	1.9***	889.0***
Genotypes	5	269 ***	11.9***	1.16**	4.41**	13.18**	288.32**	0.12***	28.4 NS
Salinity x genotype	5	56.9 ***	10.3***	0.161	0.095	0.389	16.04 NS	0.11***	9.12
Error	22	11.15	1.33	0.176	0.274	0.735	13.13	0.029	20.35

** = Significant @1% prob., * = Significant @ 5% prob., NS= Non-significant

Table 2. Agronomical performance of wheat genotypes under salinity (12 dS/m) stress.

Genotypes	Plant height (cm)		Plant biomass (g)		Productive tillers		Spike length (cm)		Number of spiklets / spike		Numbers of grains/ spike		Grain weight / spike (g)		1000 grain weight (g)	
	Cont.	12 dS/m	Cont.	12 dS/m	Cont.	12 dS/m	Cont.	12 dS/m	Cont.	12 dS/m	Cont.	12 dS/m	Cont.	12 dS/m	Cont.	12 dS/m
WSP-1	63.0	62.8 (0.2)	7.8	4.1 (47.4)	3.2	1.7 (47.2)	7.7	7.3 (4.8)	11.8	11.6 (2.0)	32.9	32.1 (2.4)	1.12	0.86 (1.0)	34.4	27.2 (21.2)
WSP-2	66.2	56.5 (14.7)	10.5	4.8 (54.1)	4.6	2.3 (50.0)	7.0	6.8 (2.8)	12.8	12.5 (2.4)	36.2	31.2 (13.7)	1.22	0.88 (28.1)	33.8	24.8 (26.8)
WSP-3	77.3	69.8 (9.8)	14.7	4.8 (67.1)	4.6	2.1 (54.1)	9.0	8.3 (7.4)	16.3	15.4 (5.9)	47.2	43.3 (8.3)	1.58	0.98 (38.3)	33.5	22.6 (32.5)
WSP-4	73.7	66.8 (9.4)	10.5	4.1 (60.8)	3.6	1.7 (53.1)	8.7	7.9 (10.1)	14.9	14.7 (1.5)	42.6	39.2 (8.0)	1.56	1.00 (35.8)	36.5	25.4 (30.4)
S-24	68.3	65.3 (4.5)	9.5	3.9 (59.2)	3.2	1.8 (44.8)	8.2	7.7 (5.7)	14.4	13.8 (4.4)	43.4	33.4 (23.2)	1.72	0.86 (50.0)	39.7	26.0 (34.7)
LU-26s	88.1	70.1 (20.4)	9.2	7.3 (20.7)	4.1	2.6 (37.4)	9.7	8.9 (8.0)	14.1	12.6 (10.4)	29.2	27.4 (6.2)	1.10	0.98 (10.9)	37.7	30.2 (20.0)
Mean	72.8	65.2 (10.4)	10.4	4.8 (53.3)	3.9	2.0 (47.9)	8.4	7.8 (6.7)	14.06	13.42 (4.4)	39	34 (10.8)	1.38	0.93 (33.1)	34.4	27.2 (27.7)
LSD (0.05) (Treat. x Var.)	**5.65**		**1.96**		**0.71**		**0.89**		**1.45**		**6.14**		**0.92**		**7.64**	

Values in parenthesis are relative decrease over control

Table 3. Summarized results showing < 50% relative decrease due to salinity in different morphological parameters.

Variety	Plant height	Plant Biomass	Productive tillers	Spike length	Spiklets/ spike	No. of grains / spike	Grain wt/ spike	100 grain wt.	No of morphological parameters showing <50% relative decrease
WSP-1	+	+	+	+	+	+	+	+	**8**
WSP-2	+	-	+	+	+	+	+	+	**7**
WSP-3	+	-	-	+	+	+	+	+	**6**
WSP-4	+	-	-	+	+	+	+	+	**6**
S-24	+	-	+	+	+	+	-	+	**6**
LU-26s	+	+	+	+	+	+	+	+	**8**

Table 4. Correlation between growth and physiological parameters.

Parameters	Plant height	Plant Biomass	Grain wt.	1000G.wt	Na^+	K^+	K^+/ Na^+ ratio	Proline
Plant biomass	0.56NS	---	---	---	---	---	---	---
Grain wt.	0.44 NS	0.82**	---	---	---	---	---	---
1000G.wt	0.50 NS	0.75**	0.78 **	---	---	---	---	---
Na	-0.42 NS	-0.68**	-0.64 **	-0.90**	---	---	---	---
K	0.44 NS	0.759**	0.84 **	0.86 **	-0.82**	---	---	---
K/ Na ratio	0.43NS	0.784**	0.77 **	0.96**	-0.93**	0.92**	---	---
Proline	-0.61*	-0.576*	-0.51NS	-0.73**	0.72**	-0.56 NS	-0.65 *	---
Total soluble sugars	-0.61*	-0.28NS	-0.04NS	-0.09 NS	-0.06 NS	0.05 NS	0.04 NS	0.42NS

** = Significant @1% prob., * = Significant @ 5% prob., NS= Non-significant,

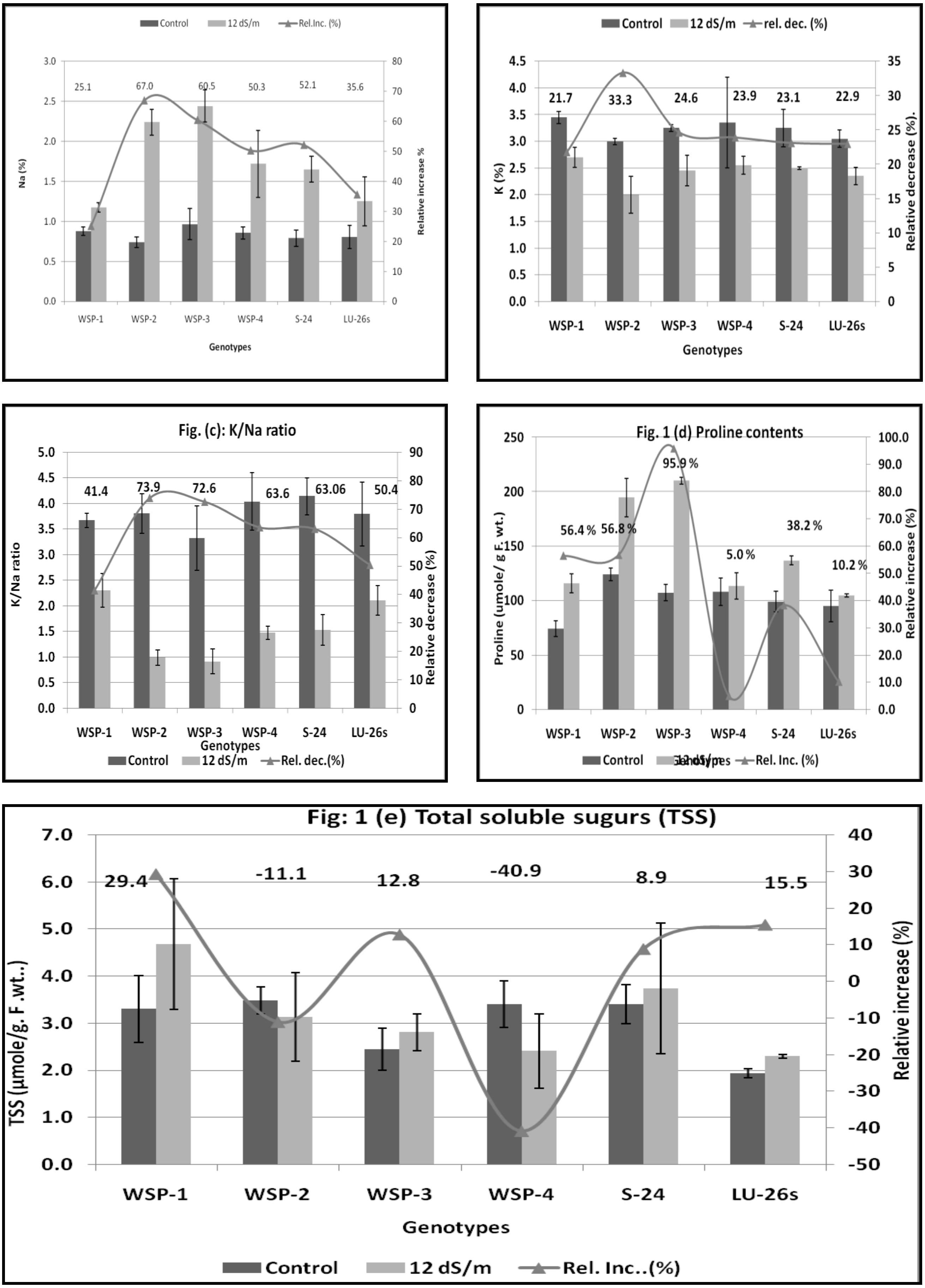

Fig. 1. Physiological performance (a. Sodium, b. Potassium, c. K/ Na ratio, d. Proline ande. Total soluble sugars) in different wheat genotypes under salinity.

Organic solutes: Accumulation of organic solutes to maintain the internal osmotic potential (OP) is an important strategy for smooth uptake of water and essential minerals by plant under stress. Among them proline is an important osmolyte to adjust the plant under drought/saline conditions (Ashraf *et al*., 1998), Almost all the genotypes showed an increased proline accumulation under salinity. Proline accumulation in WSP-2 and WSP-3 was found maximum (i.e. 210 and 196 µmole / F. wt., respectively) showing relative increased of about 95.6%. The genotypes WSP-1 also showed high proline accumulation, showing about 56% relative increase. Proline accumulation in LU-26s was found minimum (105 µmole / F. wt). Proline contents had significantly negative correlations with biomass/ plant (R^2 = -0.57) and non-significant with grain weight/ plant (R^2 = -0.51).

Sugar plays an important role in osmotic adjustment under salinity stress in grasses (Akhtar *et al*., 2004). There was higher accumulation of total soluble sugars (TSS) in wheat genotypes under salinity stress except WSP-2 and WSP-4. Comparatively higher accumulation of TSS was observed in WSP-1 (4.68 µmole/g. dry wt.), showed 29% relative increase under salinity. Relative increase in TSS was also high in LU-26s (15.5%) as compared to other tested genotypes. Ahmed *et al*., 1979 reported comparatively more increase in sugar in stress adapted plants than proline and betaine and contributed more towards osmotic adjustment under saline conditions (Fig. 1).

Higher accumulation of total soluble sugars and high K/Na ratio in genotypes WSP-1 and LU-26s indicates that the genotypes utilizing both (organic and inorganic solutes) for their osmotic adjustment. It is therefore concluded that lower accumulation of Na and less reduction in K and comparatively higher increase total soluble sugars in genotype WSP-1and LU-26s is mainly responsible for their better response under salinity (NaCl) stress.

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