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Full Length Research Paper

Alterations in reducing sugar in *Triticum aestivum* under irrigated and non-irrigated condition

Nadia Khan* and Farzana N. Naqvi

Department of Genetics, University of Karachi, Pakistan.

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This research was conducted with the objective of observing alterations in reducing sugars, which may play a part in distinguishing tolerant and susceptible genotypes. The experimental material consisted of thirteen wheat genotypes including eleven bread wheat advanced lines, one synthetic hexaploid and its durum parent. Seeds were sown in plastic pots and allowed to grow under normal irrigation for 32 days. Stress was imposed by withholding water for a period of 12 days. Subsequently, shoots were collected from stressed and non stressed young plants and the total reducing sugars were estimated. The agronomic performance of those advanced lines that were stable, such as CIM-47, CIM-51, NR-234, NR-241 and NR-264, had more elevation in reducing sugars as compared to others. This study therefore showed that stress tolerant varieties accumulated more glucose than sensitive ones. On the contrary, CIM-48 and NR-175 showed inhibition of sugars; and from their agronomic performance, they were also unstable with respect to yield and yield components. It was concluded that tolerant genotypes depict an elevated reducing sugar, and hence they could be useful in selecting tolerant varieties against water stress.

Key words: Reducing sugars, water stress, tolerant and susceptible genotypes, *Triticum aestivum*.

INTRODUCTION

Wheat (*Triticum aestivum*), one of the important staple food crops, is grown under a broad range of environmental conditions in terms of water regimes, climatic factors, and soil types (De-Long et al., 2007). Water stress affects many physiological and biochemical processes in plants (Acevedo et al., 1979; Hanson and Hitz, 1982), thereby resulting in the alteration of some metabolic pathways. Among the major effects are those involving carbohydrate metabolisms, with the accumulation of sugars and a number of other organic solutes (Iljin, 1957; Kameli, 1990). Carbohydrate changes are of particular importance on account of their direct relationships with physiological processes such as photosynthesis, translocation and respiration (Blum et al., 1991; Kiniry, 1993; Schnyder, 1993). Accumulation of sugars in different parts of plants is enhanced in response to the variety of environmental stresses (Prado et al., 2000). Water soluble carbohydrates of leaves or stems (culm and leaf sheath) are considered as important physiological trait indicative of drought tolerance because of dual functions; they do not only act in osmotic regulation as the osmolyte under adverse environmental conditions, but also contribute to grain growth and development as the dominant carbon source for grain yield when active photosynthesis is inhibited by drought stress during grain filling (Blum, 1996; Setter et al., 1998; Diab et al., 2004; Ehdaie et al., 2006; Van Herwaarden et al., 2006).

It has been demonstrated that certain sugars may be central to the protection of a wide range of organisms against drought (Ingram and Bartels, 1996). The involvement of soluble sugars in desiccation tolerance in plants was suggested by studies in which the presence of particular soluble sugars can be correlated with the acquisition of desiccation tolerance (Leprince et al., 1993). Although, sugar accumulation is not the only way in which plant deal with desiccation (Bohnert et al., 1995), it is considered as an important factor in tolerance and soluble sugar content prove to be a better marker for selecting improvement of drought tolerance in wheat (Al

*Corresponding author. E-mail: nadiakhan@uok.edu.pk.

Table 1. Mean squares for reducing sugars (µg sugars/g fwt) in bread wheat advanced lines genotypes under irrigated and non-irrigated condition.

Sources of variation	d.f	Reducing sugar
Treatments (T)	1	1498600.538**
Genotypes (G)	12	1652943.918**
G × T	10	1424610.583**
Error	24	937.338

**Significant at 1% level of probability.

Hakimi et al., 1995; Mohammadkhani and Heidari, 2008). Recent studies also demonstrate that the remobilization of pre-anthesis-stored carbohydrate reserves in wheat stem before flowering is promoted by water deficit, which during grain filling can enhance plant senescence, accelerate grain filling and improve yield in cases where senescence is unfavorably delayed by heavy use of nitrogen (Yang et al., 2000, 2001).

A central role of sugars depend not only on direct involvement in the synthesis of other compounds, production of energy but also on stabilization of membranes (Hoekstra et al., 2001), action as regulators of gene expression (Koch, 1996) and signal molecules (Sheen et al., 1999; Smeekens, 2000). The aim of the current research was to find out the changes in the reducing sugars which may play a part in distinguishing tolerant and susceptible genotypes under water stress condition.

MATERIALS AND METHODS

The experiment was conducted in a randomized complete block design with two replications using plastic pots and filled with sandy loam soil and manure. Five wheat seeds were sown in each plastic pot. There were thirteen wheat genotypes, two irrigation levels and two replications of each. A total of 52 pots were used in this experiment. Stress was started from 32 days after sowing, and after the 12^{th} day of stress, shoots were harvested. Samples were weighed, washed and kept in sealed plastic bags with tags in the refrigerator for the estimation of reducing sugars.

For quantitative estimation of soluble carbohydrates (free from combined hexoses), 4 ml buffer solution in per gram fresh weight of shoot material were plunged in hot 80% ethanol (kept over boiling water bath) for 5 min and then crushed into pestle and mortar. The slurry thus obtained was filtered and the residue was re-extracted two or three times and the supernatant were made up to 2 ml with distilled water. Carbohydrates were measured by Nelson's modification of Somogi's method (Somogi, 1937; Nelson, 1944). This is a 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 color-forming reagent and produces blue color.

A standard curve was prepared using glucose (BDH) standard solution of 100 µg per ml. Briefly, 1 ml of the appropriately diluted sugar solution was added in separate test tube. Each tube then received 1 ml of copper reagent mixture prepared by mixing reagents in the ratio of 25:1 (v/v). After a thorough mixing, the tubes were placed in boiling water for 20 min and quickly cooled by dipping them in cold water for 5 min. Solution 1 contained 25 g each of sodium carbonate, sodium potassium tartarate and 200 g of sodium sulphate (anhydrous) dissolved in 700 ml of distilled water and volume made to 1 L. Then 5 g of copper sulphate was dissolved in 100 ml of distilled water and one drop of conc.H_2SO_4 added to it. Solution 2 had 25 g of ammonium molybdate $(NH_4)_6 MoO_{24}.H_2O$ dissolved in 450 ml of distilled water. Next, 3 g of sodium arsenate were dissolved in 25 ml of water, and the two solutions were mixed together with 21 ml of H_2SO_4 (conc.), and finally made up to 500 ml with distilled water. This reagent was stored in dark bottle and incubated at 37°C; this was found by Nelson (1944) to be necessary for the formation of the arsenomolybdate chromogenic compound. 1 ml of arsenomolybdate reagent was added to each tube and contents were shaken rapidly until the evolution of CO_2 was completed. The tubes were left for 15 min for the development of blue color. The optical densities were recorded at 500 nm against reagent blank, using spectrophotometer. The standard curve thus obtained.

Reducing sugars were estimated in 100 µL of plant extract in the manner described above. The amount of reducing sugars was estimated on fresh weight (fwt) basis as µg sugars/g fwt. Data were subjected to analysis of variance, and the differences among means were determined by Duncan's multiple range test (DMRT) at 5% level using SPSS version 11 (SPSS, Inc., Chicago, IL).

RESULTS

The analysis of variance (Table 1) exhibited that water stress treatment, genotypes and their interaction was significant for reducing sugars. Table 2 shows mean comparisons for reducing sugars under irrigated and non-irrigated condition. Genotype NR-175 had the highest (4550.13 µg sugars/g fwt) and DP-12 (260.48 µg sugars/g fwt) showed the lowest reducing sugars. NR-175, DD-4, CIM-48, CIM-50, CIM-47, NR-234 and DP-12 differ significantly with each other. Under non-irrigated condition for reducing sugars, significant differences were obtained for CIM-47, CIM-48, CIM-51, NR-230, NR-234, NR-241, NR-244, NR-264, DD-4 and DP-12. The mean reducing sugar was 1108.57 µg sugars/ g µg sugars/g fwt with an average increase of 44% over non-stressed plants. CIM-47 had the highest, while DP-12 showed the lowest reducing sugars (1922.07 and 301.25 µg sugars/ g fwt, respectively). Maximum increase in sugars was in CIM-51 followed by CIM-47 (474.51 and 403.60%, respectively), while minimum increase was in DD-4 followed by DP-12 (5.03 and 15.65%, respectively). All genotypes showed increase in reducing sugars except CIM-48 (15.01%) and NR-175 (71.18%) that showed an

Table 2. Mean values for reducing sugars (µg sugars/g fwt.) in bread wheat advanced lines under irrigated and non-irrigated condition.

Genotypes	Reducing sugar (µg sugars/g fwt)	
	Irrigated	Non-irrigated
CIM-47	381.66 ± 1.66^{ef}	1922.07 ± 0.00^{a}
CIM-48	789.41 ± 0.16^{c}	670.89 ± 0.00^{h}
CIM-49	264.80 ± 5.06^{h}	716.16 ± 0.01^{gh}
CIM-50	629.68 ± 0.00^{d}	1012.15 ± 0.15^{f}
CIM-51	317.78 ± 5.95^{g}	1825.69 ± 0.84^{b}
NR-175	4550.13 ± 0.13^{a}	1311.09 ± 0.09^{d}
NR-230	358.01 ± 0.24^{fg}	787.36 ± 0.24^{g}
NR-234	314.99 ± 0.78^{g}	1053.14 ± 0.14^{f}
NR-241	368.11 ± 0.85^{ef}	1405.88 ± 0.58^{c}
NR-244	407.37 ± 0.00^{ef}	1141.17 ± 0.00^{e}
NR-264	418.30 ± 0.30^{e}	1280.48 ± 0.00^{d}
DD-4	936.90 ± 0.00^{b}	984.11 ± 0.46^{f}
DP-12	260.48 ± 0.38^{h}	301.25 ± 0.00^{i}
Average	769.04	1108.57
Percent promotion		44.14

Means followed by the same letter within columns are non-significantly different ($P \leq 0.05$) according to DMR test. Values in last two rows indicate an average and percent promotion from control.

inhibition in sugars as compared to irrigated plants.

DISCUSSION

Like other cellular constituents, starch and sugar levels are also affected by stress (Prado et al., 2000; Abdel-Nasser and Abdel-Aal, 2002). Water stress caused a marked reduction in glucose, fructose and sucrose content of grains of sensitive cultivar (Saeedipour, 2011). An alteration has been noticed in reducing sugar content when water stress was imposed. All except two of the genotypes showed an increase over their respective control, and inhibition was also observed in CIM-48 and NR-175. It has been reported that drought tolerant varieties accumulated more sucrose than sensitive ones (Kerepesi and Galiba, 2000). A drought-induced decrease in starch contents may also be associated with inhibition of starch synthesis (Geigenberger et al., 1997).

Sugars have been long known to increase in a wide range of plants grown at low moisture level (Martin et al., 1993; Rascio et al., 1994) and under salinity (Bolarin et al., 1995). The present research confirms the fact that reducing sugars seems to be a very sensitive and genotype related marker for water tolerance improvement. There are also contradictory results on the effect of water and salt stress on sugar accumulation by many research workers. Some studies have reported an increased sugar contents (Pilon-Smits et al., 1995; Dubey and Singh, 1999; Kerepesi and Galiba 2000; Parida et al., 2007; Naureen and Naqvi 2010), while others have found sugar contents to be reduced (Hanson and Hitz, 1982) or remained constant (Morgan, 1992) during stress conditions. However, the current results indicated that water stress increased the reducing sugars in all genotypes except two. Zinselmeier et al. (1995, 1999) also found that drought stress consistently affect sugar metabolism. This observation has been supported by a number of researchers (Kameli and Losel, 1993; Al-Hakimi et al., 1995; Kerepesi and Galiba, 2000; Saeedipour, 2011). Fructans can protect membranes or other cellular component from the adverse effects of drought in a manner similar to other carbon compounds, or perhaps fructans influence growth process directly (Pilon-Smits et al., 1995). An interaction was also highly significant regarding reducing sugars indicating the variable performance of genotypes.

Conclusion

The agronomic performance of those advanced lines that were more stable, like CIM-47, CIM-51, NR-234, NR-241 and NR-264, had more elevation in reducing sugars compared to the moderate advanced lines. CIM-48 and NR-175 showed decline of sugars and their agronomic performance also indicated that they were not stable. These results led to a conclusion that tolerant genotypes showed an elevated reducing sugar, while those susceptible had decline sugar content. Reducing sugar content might therefore be a useful marker in the selection of stress tolerant genotypes under water stress.

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