Research Article

Comparative Study of Transgenic (DREB1A) and Non-transgenic Wheat Lines on Relative Water Content, Sugar, Proline and Chlorophyll under Drought and Salt Stresses

Sabahat Noor*[1], Shaukat Ali[1], Hafeez-ur-Rahman[1], Farhatullah[2] and Ghulam Muhammad Ali[1]

[1]*National Institute for Genomics and Advanced Biotechnology (NIGAB), Islamabad, Pakistan;* [2]*The University of Agriculture, Peshawar, KP, Pakistan*

Abstract | Wheat is the most cultivated food crop in Pakistan. Biotic and abiotic stresses severely affect the yield of this crop and require special attention to rectify the production loss. Among various abiotic stresses, drought and soil salinity have the most adversarial effect on wheat production. In this study the T2 transgenic (*At-DREB1A)* and non-transgenic line of lasani-08 from single transformation event was exposed to 15 days drought and different levels of salt concentrations separately. Four parameters i.e. sugar contents, proline contents, chlorophyll contents and relative water contents were recorded and compared in transformed and non-transformed plants. It was observed that under drought and salt stresses, proline contents, sugar contents, chlorophyll contents and relative water contents (RWC) were significantly higher in transgenic plants as compared to non-transgenic plants. From obtained results it is concluded that, increase level of RWC, proline, sugar and chlorophyll in transgenic plants contributed in enhance drought and salt tolerance.

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***Correspondence** | Sabahat Noor and Ghulam M. Ali, National Institute for Genomics and Advanced Biotechnology (NIGAB), Islamabad, Pakistan; **Email:** sabahatnoor85@yahoo.com
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Introduction

Environmental stresses like drought and salinity are the major limiting factors for plant growth and production (Zhu, 2002). Wheat cultivation is highly affected by water shortage in Pakistan especially in arid and semi arid zones of the country (Amir et al., 2013). The plants under drought and salt stresses cope with these stresses either through the amassing of secondary metabolites (El-Tayeb, M.A., 2006) or through osmotic adjustments (El-Tayeb et al., 2010). Among osmotic accumulation, plants start accumulation of various osmolytes such as proline, sugar etc. (Vendruscolo et al., 2007; Marin et al., 2010) which helps to minimize the effect of these stresses (Molinari et al., 2004; Zhu et al., 2005). Accumulation of sugar contents is observed in several plants including wheat (Chaves, M.M. et al., 2003). The sugar contents along-with other osmolytes helps to maintain integrity of plasma membrane and thus helps the plant to deal with stresses (Ramanjulu, S. and D. Bartels, 2002) whereas proline stimulates the chlorophyll synthesis while simultaneously inhibiting its degradation (Rady et al., 2015). Free Proline accumulation is also involved in osmotic adjustment of plants under water shortage (Nayyar, 2003; Zhu et al., 2005). There are reports of increase in sugar and proline contents under drought and salt stresses (Mostajeran, A. and V. Rahimi-Eichi, 2009; Rady et al., 2015).

It is reported that decrease in relative water content results in closure of stomata which ultimately lowers the photosynthetic rate (Cornic, 2000). Drought/salinity resistant plants keep their relative water content high due to more osmotic regulation (Ritchie et al., 1990). The plants having more RWC are more adaptive in water shortage environment (Gupta et al., 2012).

Chlorophyll (chl) is the most important feature of plant as it regulates the health and nutrition availability to plants (Dawood et al., 2014) and it has been studied in different plants by several researchers for both salt and water stresses (Wang et al., 2008; Shangguan et al., 2000; Pandey and A. Shukla, 2015). Water shortage significantly decrease the chlorophyll concentration in plants due to damage in PS I and PS II complexes (Shangguan et al., 2000).

The objective of this work was to compare the efficacy of drought/salt tolerant transgenic wheat plants with non-transgenic isogenics on the basis of sugar contents, proline contents, RWC and Chlorophyll content.

Materials and Methods

The research work presented was carried out in National Institute for Genomics and Advanced Biotechnology (NIGAB), National Agricultural Research Center, Islamabad, Pakistan. The efficacy of already developed transgenic wheat (*Triticum aestivum.* Cv lasani-08) harboring *DREB1A* gene under ubiquitin promoter from single transformation event (Noor, S., 2018) was compared to their isogenic non-transformed plants under drought and salt stresses separately. T1 transgenic wheat seeds were sown in pots having soil and sand in a 3:1 ratio and grown in a glass house along with control plants (non-transgenic seeds of the same cultivar). Five seeds were sown in each pot. These plants were allowed to self-pollinate and grown till seed set. The obtained seeds were harvested and again sown in the glass house to get T2 population. Molecular data was not taken at T1 generation because of less number of seeds available. The leaves of seven days seedling were taken and their DNA was isolated through CTAB method (Doyle and Doyle, 1987). The presence of *DREB1A* gene was confirmed through PCR (Noor, S., 2018) and these confirmed 20 days old transgenic seedlings were exposed to drought or salt stress. For drought stress, the water was with-held for 15 days. To impose salt stress, the NaCl concentrations of 50, 100, 150, 200 and 250mM were given until leaves turned yellow for four weeks. For both stresses, data was recorded as mean of three replications and each replication consisted of three plants. All treatments were replicated three times.

Relative water content (%)

Relative water contents were measured according to Ritchie et al. (1990).

Total soluble sugar contents (mg/g FW)

The sugar contents were measured by following the method proposed by Fales, F.W. (1951). For each treatment, average of three replicates was taken for presenting the results.

Proline estimation (µg/g FW)

Free proline was calculated by the method proposed by Bates et al. (1973).

Chlorophyll contents (mg/g FW)

Chlorophyll contents (a, b and total chlorophyll) were measured by the method proposed by Arnon (1949) and later modified by Kirk (1968).

Statistical analysis

All the experiments were performed in completely randomized design (CRD) in a factorial system. Each experiment had three replicates. The Statistix v. 8.1 (Analytical Software, 2005) was used for calculating least significant difference (LSD).

Results and Discussion

Relative water content

The results depicted that without any stress the mean relative water contents in transgenic and non-transgenic plants were non-significantly different ($p<0.01$). Drought and salt stresses caused significant ($p<0.01$) decrease in relative water contents in both transgenic and non-transgenic plants but this rate of decrease was more in non-transgenic wheat plants as compared to their isogenic plants (Table 1). Genotypic means and treatment means for relative water contents under salt and drought stresses were significantly different. Relative water contents were decreased in transgenic as well as non-transgenic wheat plants as the salt stress increases but transgenic plants showed less decrease in relative water content as compared to their non-transgenic ones (Table 2).

Total soluble sugar content

The statistical analysis revealed that genotypes and treatments were highly significant ($p<0.01$) for total soluble sugar (TSS) contents under drought stress. Without drought stress the TSS contents were non-significantly different in transgenic and non-transgenic wheat lines and were in the range of 10.74-11.79 mg/g FW. After 15 days drought stress TSS contents increased both in transgenic and non-transgenic wheat lines but in transgenic wheat lines the accumulation of TSS contents was high as compared to non-transgenic lines (Table 3). Under various salt stresses, TSS contents were enhanced both in transgenic and non-transgenic wheat lines. The highest TSS contents (69.99 mg/g FW) were recorded in transgenic plants of Lasani-08 at 150 mM NaCl stress. Further increase in salt stress (200-250 mM) resulted in gradual decrease in TSS contents both in transgenic and non-transgenic plants (Table 4).

Table 1: *Effect of drought stress on relative water content (%) of T2 transgenic (T) and non-transgenic (NT) wheat plants.*

Drought stress	Relative water content		
	Lasani-08		
	NT	T	Mean
Without stress	89.55 [a]	90.55 [a]	90.05A
15 days drought	53.40 [c]	69.26 [b]	61.33B
Mean	71.48B	79.91A	
	$LSD_{0.01}$ = 8.0		

The values are mean of three replications and each replication has three plants. Mean values followed by the different letters show significant differences (p≤0.01).

Table 2: *Effect of various salt stresses on relative water content (%) of T2 transgenic (T) and non-transgenic (NT) wheat plants.*

Salt stress (mM)	Relative water content		
	Lasani-08		
	NT	T	Mean
0	88.23 [a]	88.49 [a]	88.36A
100	66.39 [c]	74.58 [b]	70.48B
150	45.43 [d]	63.53 [c]	54.48C
200	26.03 [e]	49.14 [d]	37.58D
250	16.24 [f]	33.63 [e]	24.93E
Mean	48.46B	61.87A	
	$LSD_{0.01}$ = 7.95		

The values are mean of three replications and each replication has three plants. Mean values followed by the different letters show significant differences (p≤0.01).

Proline content

Enhanced flag leaf proline contents were recorded in transgenic and non-transgenic wheat lines under drought stress but rate of increase was significantly higher in transgenic plants as compared to non-transgenic ones (Figure 1). Proline contents were also calculated under various salt stresses both in transgenic and non-transgenic wheat lines. Under normal condition (0mM NaCl) proline contents in both transgenic and non-transgenic plants were same. As the salt stress increased (100-250 mM), enhance flag leaf proline accumulation was observed in transgenic and non-transgenic plants but rate of increment in proline contents of transgenic plants were higher as compared to non-transgenic ones (Figure 2). The maximum proline accumulation was recorded in flag leaves of transgenic lines at 250 mM salt stress.

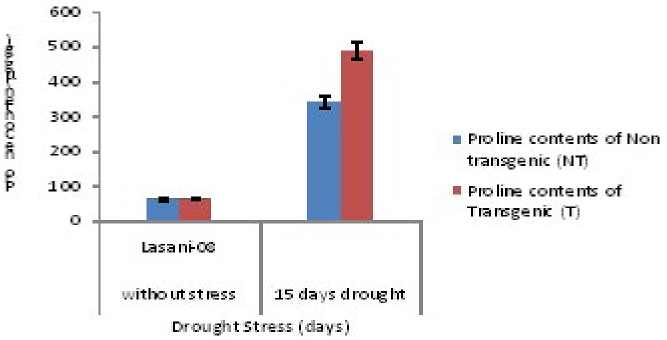

Figure 1: *Comparison of proline contents (µg/ g FW) of T2 transgenic and non-transgenic wheat plants under drought stress. Vertical bars indicate standard error of means (n=3).*

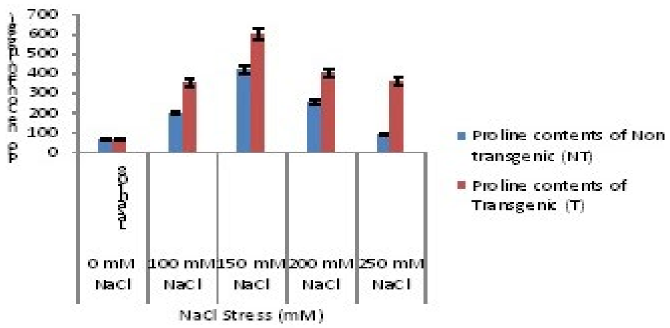

Figure 2: *Comparison of proline contents (µg/ g FW) of T2 transgenic and non-transgenic wheat plants under salt stress. Vertical bars indicate standard error of means (n=3).*

Chlorophyll content

Drought and salt stresses significantly lowers the contents of chlorophyll "a" "b" and total chlorophyll. The chlorophyll "a" and "b" contents decreased because of drought stress in both transgenic and non-transgenic plants but this decrease was lowest in transgenic plants (Table 5). Salt stress also caused significant decrease in chlorophyll "a" and "b" contents in both transgenic and non-transgenic plants. Maximum decrease was recorded in non-transgenic plants at 250mM NaCl stress (Table 6).

Table 3: *Comparison of total soluble sugar contents (mg/g FW) of T2 transgenic (T) and non-transgenic (NT) plants under drought stress in wheat.*

Drought stress	Total soluble sugar contents		
	Lasani-08		
	NT	T	Mean
Without stress	10.74 ᶜ	11.79 ᶜ	11.27B
15 days drought	33.19 ᵇ	67.99 ᵃ	50.59A
Mean	21.96B	39.89A	
	$LSD_{0.01}$ = 9.7		

The values are mean of three replications and each replication has three plants . Mean values followed by the different letters show significant differences (p≤0.01).

Drought and salt stresses significantly lowered the total chlorophyll contents (the sum of chlorophyll a and b) in non-transgenic as well as in transgenic plants. Under drought stress the highest decrease belonged to non-transgenic plants (Table 5). Similar decreasing trends were recorded under salt stresses. Increase in salt concentration lowered the total chlorophyll contents in both transgenic and non-transgenic plants but more decrease was recorded in non-transgenic plants. Lowest total chlorophyll contents were recorded in non-transgenic plants at 250 mM of NaCl stress (Table 6).

Table 4: *Comparison of total soluble sugar contents (mg/g FW) of T2 transgenic (T) and non-transgenic (NT) plants under various salt stresses in wheat.*

Salt stress (mM)	Total soluble sugar contents		
	Lasani-08		
	NT	T	Mean
0	12.09 ^{g}	14.44^{fg}	13.26 E
100	24.70^{de}	33.32 ^{c}	29.01D
150	44.69 ^{b}	69.99 ^{a}	57.34A
200	31.79^{cd}	66.46 ^{a}	49.12B
250	20.70^{ef}	65.44 ^{a}	43.07C
Mean	26.79B	49.93A	
	$LSD_{0.01}$ = 8.0		

The values are mean of three replications and each replication has three plants. Mean values followed by the different letters show significant differences (p≤0.01).

Table 5: *Comparison of chlorophyll "a" contents (mg/g FW) of T2 transgenic (T) and non-transgenic (NT) plants under drought stress in wheat.*

Drought stress	Lasani-08								
	Chlorophyll "a "			Chlorophyll "b "			Total Chlorophyll		
	NT	T	Mean	NT	T	Mean	NT	T	Mean
Without Stress	8.74 ^{a}	8.61 ^{a}	8.67 A	6.77 ^{ab}	7.89 ^{a}	7.38 A	15.52 ^{ab}	16.59 ^{a}	16.05 A
15 days drought	2.76 ^{b}	6.77 ^{a}	4.76 B	2.96 ^{c}	5.70 ^{b}	4.33 B	5.73 ^{c}	12.47 ^{b}	9.10 B
Mean	5.75 B	7.69 A		4.87 B	6.84 A		10.62 B	14.53 A	
	$LSD_{0.05}$ = 2.16			$LSD_{0.01}$ = 2.04			$LSD_{0.01}$ = 3.49		

The values are mean of three replications and each replication has three plants. Mean values followed by the different letters show significant differences (p≤0.01 and p≤0.05).

Table 6: *Comparison of chlorophyll "a" contents (mg/g FW) of T2 transgenic (T) and non-transgenic (NT) plants under different salt stresses in wheat.*

Salt stress (mM)	Lasani-08								
	Chlorophyll "a "			Chlorophyll "b "			Total Chlorophyll		
	NT	T	Mean	NT	T	Mean	NT	T	Mean
0	11.41 ^{a}	10.79 ^{a}	11.10 A	8.22 ^{a}	8.49 ^{a}	8.36 A	19.64^{a}	19.28^{ab}	19.46A
100	7.62 ^{cd}	9.98 ^{ab}	8.80 B	5.57 ^{c}	7.39 ^{b}	6.4 B	13.19^{c}	17.37^{b}	15.28B
150	6.14 ^{de}	8.59 ^{bc}	7.36 C	3.08 ^{d}	5.11 ^{c}	4.09 C	9.22^{d}	13.71^{c}	11.46C
200	2.97 ^{f}	7.30 ^{cd}	5.14 D	1.28 ^{e}	3.37 ^{d}	2.32 D	4.26^{f}	10.68^{d}	7.47D
250	1.09 ^{g}	5.02 ^{e}	3.05 E	0.20 ^{f}	1.96 ^{e}	1.08 E	1.29^{g}	6.99^{e}	4.14E
Mean	5.84 B	8.33 A		3.67 B	5.26 A		9.52B	13.60A	
	$LSD_{0.01}$ = 1.73			$LSD_{0.01}$ = 0.76			$LSD_{0.01}$ = 1.98		

The values are mean of three replications and each replication has three plants. Mean values followed by the different letters show significant differences (p≤0.01).

Links Researchers

Relative water content is an important parameter for monitoring drought tolerance in plants, since the plants having more RWC are more adaptive in water shortage environment (Gupta et al., 2012). The results of this study demonstrated that transgenic wheat plants have more RWC and hence more tolerance to water shortage as compared to their control plants under drought and salt stresses. It is also possible that this response was due to better osmotic adjustment capacity of the transgenic plant over control plants under stressful condition. Similar results were reported by Bahieldin et al., 2005 that RWC in T4 generation of wheat transformed with *HVA1* gene for drought tolerance. Their results demonstrated that expression of *HVA1* under water shortage environment resulted in higher leaf RWC which ultimately increased the drought tolerance in transgenic wheat. RWC was significantly increased in drought tolerant wheat cultivars. This tolerance might be due to better osmotic adjustment in tolerant varieties as compared to sensitive ones (Farshadfar and R. Amiri., 2016). From all of these results, it was concluded that high RWC in transgenic plants has transformed these plants to become more tolerant to water shortage as compared to NT plants.

The plants under drought and salt stresses cope with these stresses either through the amassing of secondary metabolites (El-Tayeb, M.A., 2006) or through osmotic adjustments (El-Tayeb et al., 2010). Among osmotic adjustments, accumulation of sugar contents is observed in several plants including wheat (Chaves, M.M. et al., 2003). The sugar contents along-with other osmolytes helps to maintain integrity of plasma membrane and thus helps the plant to deal with stresses (Ramanjulu, S. and D. Bartels, 2002). In the present study, sugar contents were calculated in transgenic and NT wheat cultivar under drought and salt stresses and found increase in sugar accumulation in both types However, transgenic plants showed much higher level of sugar accumulation than NT under both salt and drought conditions and this increased accumulation of sugar contents. Gao et al. (2009) observed similar results i.e. increased level of sugar accumulation in transgenic wheat as compared to NT under water stress. They transformed wheat cultivar with *DREB* gene which was isolated from cotton. They reported that increase in sugar accumulation in transgenic plants is due to enhanced expression of *DREB* gene.

It is well documented that under drought and salt stress conditions, plants start accumulation of various osmolytes such as proline, sugar etc. (Vendruscolo et al., 2007; Marin et al., 2010) which helps to minimize the effect of these stresses (Molinari et al., 2004; Zhu et al., 2005). Proline has vast range of functions under stress such as osmoprotection (Kishor et al., 1995, 2005), osmotic adjustment (Voetberg and Sharp, 1991), regulation of cytosolic acidity (Sivakumar et al., 2000), increase in various enzyme activities (Sharma and Dubey, 2005; Mishra and Dubey, 2006) and improvement in antioxidant defense system of plants (Abdelhamid et al., 2013; Dawood et al., 2014). In various studies it was reported that free proline content increases in wheat under drought and salt stress (Nayyar, 2003; Zhu et al., 2005). In this research work, the proline contents of both transgenic and NT plants were found to be increased under salt and drought stress. However, the increase in proline in case of transgenic is significantly high as compared to NT. This large accumulation of proline helps the transgenic to cope with the stress and thus performed better than NT. The underlying reason is that, proline stimulates the chlorophyll synthesis while simultaneously inhibiting its degradation (Rady et al., 2015). These results are supported by previous research work of Vendruscolo et al. (2007) who transformed P5CS gene in wheat cultivar against water stress and reported that transgenic plants have more proline accumulation as compared to NT. Their work concluded that proline helps the plants not by osmotic adjustment but through enhancing antioxidant system.

Chlorophyll (chl) is the most important feature of plant as it regulates the health and nutrition availability to plants (Dawood et al., 2014). Chl'a' is the main component of PS I plus PS II and chl b also involved in both of these photo-systems (Herppich et al., 1996). The chlorophyll contents (a, b and total) were calculated in this research work and observed that there was decrease in chlorophyll contents in both transgenic and NT under drought and salt stresses however, still the chlorophyll contents were higher in transgenics as compared to NT. Shangguan et al. (2000) concluded in their research work that this decrease in chlorophyll content is due to damage in PS I and PS II complexes. They conducted an experiment on winter wheat and calculated its chlorophyll contents under water deficiency stress. They observed decrease in chlorophyll (chl) contents in wheat cultivars. The decrease in chlorophyll contents under water shortage may also be due to dis-organization of thalakoid membrane as well as due to activation of chlorophyllase enzymes (Rady et al., 2015).

Conclusions

Transgenic wheat lines of lasani-08 performed better under drought and salt stresses. Three biochemical parameters i.e. sugar, proline and chlorophyll contents as well as relative water contents were significantly higher in transgenic plants as compared to non-transformed isogenics. These parameters revealed high tolerance for drought and salt in transgenic wheat lines as compared to non-transgenic ones.

Author's Contribution

Ghulam M. Ali provided technical inputs and scientific assistance. Sabahat Noor conducted the experiment and wrote the manuscript. Shaukat Ali did experimental designing and scientific assistance. Farhatullah helped in data collection and analysis. Hafeez-ur-Rahman helped in compiling the results. All authors read and approved the final manuscript.

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