

Evaluation of Selected Indian Bread Wheat (*Triticum aestivum* L.) Genotypes for Morpho-physiological and Biochemical Characterization under Salt Stress Conditions

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Wheat is the second most important crop after rice in India and occupies approximately 28.5 million hectare area. Salinity is one of the major factors reducing plant growth and productivity worldwide, and affects about 7% of world's total land area. In India about 6.73 million hectare land area is salt affected. The aim of this study was to investigate the morpho-physiological and biochemical response of wheat to temporal salinity ($EC_{iw} = 10.0$ dSm^{-1}) exposures. Ten wheat genotypes were evaluated in two successive growing seasons (2012–2014), with complete randomized design with three replications under both salinity stress and non-stress conditions. The morpho-physiological and biochemical character measured in this investigation, inhibited under both salt stresses (S1 & S2) conditions but much more significantly inhibited under long-term salinity exposure (S2) than S1 because interrupting the metabolic process of plant, resulting in reduced growth and productivity. According to correlation result, selection of high yield genotypes can be done based on plant height (0.649*), tiller plant⁻¹ (0.808**) and leaf area (0.687*). The multivariate morpho-physiological and biochemical parameters should be further used to develop salinity tolerance in wheat breeding improvement programmes.

Keywords: abiotic stress, leaf area, sodium, salt stress, *Triticum aestivum*

Introduction

Salinity is one of the major factors reducing plant growth and productivity worldwide, and affects about 7% of world's total land area (Flowers et al. 1997). Percentage of cultivated land affected by salt is even greater, with 23% of the cultivated land being saline and 20% of the irrigated land suffering from secondary salinization. In India about 6.73 million ha land area is salt affected out of which 3.77 and 2.96 million hectare are afflicted by sodicity and salinity, respectively (Mondal et al. 2010). Excessive amounts of salts, especially sodium chloride (NaCl), in the soil induce osmotic and ionic effects, leading to changes in plant metabolism (Hasegawa et al. 2000; Qadir et al. 2008) and reduced plant growth and development in many crop species (Chaum et al. 2004; Singh et al. 2007; de Lacerda et al. 2005; Chen et al. 2007). Salt-stress defense mechanisms, in-

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cluding ion regulation and compartmentalization, antioxidant systems, plant hormones and osmoregulation, are well established (Hasegawa et al. 2000; Parida and Das 2005).

Wheat is the second most important crop after rice in India and occupies approximately 28.5 million hectare area. According to some estimates FAO (2006) and Rosegrant et al. (2001), the global wheat production must increase by at least 1.6 per cent annually to meet a projected wheat demand of 760 million tons by 2020. Biochemical and physiological parameters in higher plants cultivated in salt or water-deficit conditions have been developed as effective indices for tolerant screening in plant breeding programs (Ashraf and Harris 2004; Parida and Das 2005; Ashraf and Foolad 2007). Both water-deficit and salt-stresses detrimentally affect plant growth and developmental processes, which have been reported in terms of biochemical, physiological and morphological changes (Hasegawa et al. 2000; Wang et al. 2001; Parida and Das 2005). The objective of the present study was to identify the morpho-physiological and biochemical changes of bread wheat in response to salinity stress (S1 and S2) levels.

Materials and Methods

Plant materials and experimentation design

Pot experiment was laid out by planting ten bread wheat genotypes (Table 1), obtained from gene pool of Central Soil Salinity Research Institute (CSSRI), Karnal and Sardar Vallabhbhai Patel University of Agriculture and Technology (SVPUA&T), Meerut, India. The experiment was laid out at the experimental farm, Department of Agriculture Biotechnology, SVPUA&T: Meerut, during November, 2012–13 and 2013–14 and the experimental soil was sandy loam with initial pH 7.2 and ECe 1.13 dSm⁻¹. Desired salinity levels (EC_{iw} = 10.0 dSm⁻¹) were created by thoroughly mixed required quantity of NaCl, Na₂SO₄ and CaCl₂ (7:1:2) with irrigating water. The pot experiment was performed in complete randomized design (CRD) with three replications. Three level of soil salinity

Table 1. The detailed information of genotypes includes for study

Ser. No.	Genotypes	Parentages	Developed by	Year
1	Kharchia 65	Kharchia Local/EG 953	DURGAPURA	1970
2	HD 2009	LR 64A/NA160	N. DELHI	1975
3	PBW 343	ND/VG9144//KAL/BB/3/YCO'S'/4/VEE#5 'S'	LUDHIANA	1995
4	AKAW 4627	WH 147/SUNSTAR*/C 80.1	AKOLA	2010
5	K 9423	HP1633/KAL/UP262	KANPUR	2004
6	PBW 373	ND/VG9144//KAL/BB/3/YCO'S'/4/VEE#5 'S'	LUDHIANA	1996
7	HUW 468	CPAN 1962/TONI//LIRA'S/PRL'S	VARANASI	1999
8	K9162	K 7827/HD 2204	KANPUR	1999
9	PBW 154	HD 2160/HD 2177	LUDHIANA	1988
10	UP 1109	UP262/UP 368	PANTNAGAR	1985

i.e., control (normal irrigation water) and saline [saline irrigation at the time of sowing (S1) and pre-sowing with normal water and saline irrigation after 21 days of sowing (S2)] conditions.

Phenotypic observation

The morpho-physiological and biochemical observations of all the genotypes were recorded at the time of maturity. The investigated traits were plant height (PH), number of tillers plant⁻¹ (NT), number of productive tillers plant⁻¹ (NPT), spike length (SL), spikelet spike⁻¹ (SN), average biomass plant⁻¹ (AB), test weight (TW), average yield plant⁻¹ (AY), leaf area (LA), relative water content (RWC), potassium (K⁺) and sodium (Na⁺) were measured using standard protocols. RWC was determined for all genotypes following the procedure of Turner (1981).

$$\text{RWC} = \frac{\text{Fresh leaf weight} - \text{Dry leaf weight}}{\text{Turgid leaf weight} - \text{Dry leaf weight}} \times 100.$$

Statistical analysis

The data was analyzed by analysis of variance (ANOVA), standard deviation (SD), post-hoc for comparison of means and the significance of difference was determined according to Duncan's multiple range test (DMRT). $P < 0.05$ values are considered to be significant. All statistical analysis was done using SPSS (v 19.0 for window, SPSS Inc., USA).

Results

In the present investigation, plant height, tillers plant⁻¹, productive tillers plant⁻¹, spike length, spikelet spike⁻¹, average biomass plant⁻¹, test weight, average grain yield plant⁻¹, leaf area, relative water content, and potassium were measured. All these studied traits were decreased with increasing salinity. Plants growing under saline condition remain stunted due to reduction in cell elongation and cell division, which are under the control of different auxins, whose synthesis is retarded by the salinity (Loreto et al. 2003; Ndayiragije and Lutts 2006). The reduction in biomass increased with the increase exposure of salinity, because of disturbances in physiological and biochemical activities under saline conditions (Craine 2005; Munns et al. 2006) that may be due to the reduction in leaf area and number of leaves (Romero-Aranda et al. 1998; Dong et al. 2007). At heading salinity suppresses reproductive development, spikelet formation and ultimately spikelet number (Mans and Rawson 2004). Plant height (96.93), spikelet spike⁻¹ (17.97), biomass plant⁻¹ (28.07), SPAD value (53.64), leaf area (36.74), and RWC (71.45) in selected bread wheat genotypes as shown in Tables 2 and 3 were highest in control and non-significant and significantly ($p < 0.01$) decline under salinity stress when salinity imposed in the form of S1 and S2, respectively.

Table 2. Mean values of different studied traits grown in both, control and salinity conditions during 2012–2014

Treatment	PH	NT	NPT	SL	SN	AB
C	96.93±7.53 ^a	14.01±2.22 ^a	10.71±1.30 ^a	18.115±1.17 ^a	17.97±1.43 ^a	28.07±6.99 ^a
S1	89.75±6.37 ^a	10.835±2.21 ^b	8.22±1.33 ^b	16.605±0.86 ^b	16.63±1.56 ^{ab}	22.82±6.19 ^{ab}
S2	81.315±11.99 ^b	8.425±2.51 ^c	5.6±1.13 ^c	15.48±0.90 ^c	15.57±1.84 ^b	18.41±5.50 ^b
ANOVA	ns, **	, **, *	, **, *	, **, *	ns, **, *	ns, **, *

Within columns means (±SD) followed by the same letter are not significantly different at the 0.05 level according to Duncan's. Multiple Range Test (DMRT). PH – Plant height; NT – Number of tillers plant⁻¹; NPT – Number of productive tillers plant⁻¹; SL – Spike length; SN – Spikelet number spike⁻¹; AB – Average biomass plant⁻¹; C – Non-saline (control) water used whenever need. S1 – Salinity (EC_w = 10.0 dSm⁻¹) imposed on 21 days after sowing (Salinity level1). S2 – Salinity (EC_w = 10.0 dSm⁻¹) imposed at the time of sowing (Salinity level2). *significantly different at 0.05 level; **significantly different at 0.01 level; ns: non-significantly different.

Table 3. Mean values of different studied traits grown in both, control and salinity conditions during 2012–2014

Treatment	TW	AY	SPAD	LA	RWC	K/Na
C	33.09±3.62 ^a	9.85±2.42 ^a	53.64±3.66 ^a	36.74±4.45 ^a	71.445±8.02 ^a	15.205±4.10 ^a
S1	28.085±3.20 ^b	6.725±2.14 ^b	49.545±4.52 ^{ab}	32.645±5.12 ^a	63.15±9.69 ^a	4.23±2.42 ^b
S2	22.615±3.98 ^c	3.935±0.89 ^c	46.07±5.95 ^b	26.015±4.43 ^b	50.495±14.97 ^b	2.22±1.43 ^b
ANOVA	**, **	**, **	ns, **	ns, **	ns, **	**, **

Within columns means (±SD) followed by the same letter are not significantly different at the 0.05 level according to Duncan's. Multiple Range Test (DMRT). TW – Test weight; AY – Average yield; SPAD – Chlorophyll content; LA – Leaf area; RWC – Relative water content and K/Na ratio. C – Non-saline (control) water used whenever need. S1 – Salinity (EC_w = 10.0 dSm⁻¹) imposed on 21 days after sowing (Salinity level1). S2 – Salinity (EC_w = 10.0 dSm⁻¹) imposed at the time of sowing (Salinity level2). **significantly different at 0.01 level; ns: non-significantly different.

About 7.4% and 17.4% inhibition in plant height, about 7.5% and 14.4% inhibition in spikelet spike⁻¹, about 18.7% and 42.3% inhibition in biomass plant⁻¹, about 7.6% and 15.3% inhibition in SPAD value, about 11.1%, 32.8% inhibition in leaf area and about 11.6% and 33.2% inhibition in RWC at S1 and S2, respectively, when compared to control (Table 3). Reduction in growth parameters, plant height and number of leaves, were also decreased with salinity. Although plant height is genetically controlled but environmental factors also have strong influence in the expression of genes. The different salinity levels resulted in different plant heights under normal condition as well as under saline conditions.

Chlorophyll is membrane bound and depends upon the membrane stability thus under saline conditions it seldom remains intact (Ashraf et al. 1992; Shah et al. 2007). Decrease in chlorophyll contents due to salinity has also been reported elsewhere. However, some studies (Evain et al. 2004; Paranychianakis and Chartzoulakis 2005) have also reported an increase in chlorophyll contents in some cultivars of different plant species. Salinity may also be responsible for lower values of stomatal conductance, photosynthesis and relative water content (Naumann et al. 2008). Thus, reduction in chlorophyll may be due to variation in its synthesis between the plant species due to variation in specific enzymes

under saline conditions (Kreps et al. 2002; Keutgen and Pawelzik 2007) and our results (Table 3) also agreement with findings of previous researchers.

Tiller plant⁻¹ is most salinity sensitive trait in wheat (El-Hendawy et al. 2005). Thus to increase yield under stress condition it is necessary to maintain high plant density. Tiller formation included tiller number and tiller biomass. Salinity reduces tiller number by delaying and reducing tiller emergence at the vegetative stage. After tiller emergence, growth of tillers at all stages is inhibited by salinity due to its damage on the essential metabolic reaction in plants, resulting in low tiller biomass and small tiller size (Maas and Poss 1989). $EC_e > 7.5 \text{ dSm}^{-1}$ in soil water could eradicate most of the secondary tillers and greatly reduce formation of tertiary and lateral tillers. The yield potential of wheat is greatly dependent on the number of tillers plant⁻¹ that is affected in the early life cycle. Number of tillers regulates grain yield by its prime influence on the number of spikes in wheat (Simons and Hunt 1983). Tiller plant⁻¹ (14.01), productive tiller plant⁻¹ (10.71) and spike length (18.11) in selected bread wheat genotypes (Table 2) were highest in control and significantly decline under salinity stress when imposed in the form of S1 and S2, respectively. About 22.6% and 51.6% inhibition in tiller plant⁻¹, about 23.2% and 62.2% inhibition in productive tiller plant⁻¹ and 8.3% and 15.8% inhibition in spike length at S1 and S2 respectively when compared to control.

Discussion

Salinity of irrigation water and agricultural soils can probably be considered as the most important limiting factor of crop plant's growth in most areas of the world, adversely affecting about 7% of the world's total crop land area (Flowers et al. 1997; Flowers 2004). The reactions of various crops to salinity are different the differences being observed in various growth phases (Rehman 1996). The problem of soil salinity is further increased due to the use of poor water quality for irrigation accompanied by poor drainage (Chinnusamy et al. 2005). Adverse effects of salinity on plant growth may also be due to ion cytotoxicity (mainly due to Na, Cl and SO₄), and osmotic stress (Zhu 2002; Ali et al. 2004).

The 1000-grain weight was less affected as compare to the other yield components because it was determined at maturity which is the least salt sensitive stage in wheat (Frank et al. 1997). Previous research indicated that intracellular Na⁺ homeostasis and salt tolerance are modulated by calcium and high Na⁺ concentrations negatively affect K⁺ acquisition (Munns 2002). Sodium competes with K⁺ for uptake through common transport system and does this effectively since the Na concentration in saline environments is usually considerably greater than that of K⁺. It is also reported that sensitivity of some crops to salinity is due to the inability to keep Na and Cl out of transpiration streams (Gorham et al. 1990).

Test weight (33.09), yield plant⁻¹ (9.85) and K⁺/Na⁺ ratio (15.20) in selected bread wheat genotypes were highest in control and were significantly ($p < 0.01$) decline under salinity stress when imposed in the form of S1 and S2, respectively (Table 2). Salt tolerance in wheat is mostly related to its enhanced ability to discriminate between K and Na during transport of these ions to the shoot (Gorham et al. 1990). It has been reported that

in wheat (hexaploid), the 4D chromosome, derived from the wild grass is responsible for salt tolerance and K^+/Na^+ discrimination character (Shah et al. 1987). The K^+/Na^+ ratio has been used as an index for sodium toxicity in plant tissues, because it is assumed that activity of K^+ requires some enzymes (Cramer et al. 1994), and higher K^+/Na^+ ratio indicates less Na^+ toxicity. About 15.1% and 37.3% inhibition in test weight, 31.7% and 87.9% inhibition in yield $plant^{-1}$ while 72.1% and 306.9% inhibition in K^+/Na^+ ratio at S1 and S2, respectively, when compared to control. K^+/Na^+ ratio has been reported to decrease under salt stress (Gadallah 1999; Haroun 2002), our research finding also supported it.

Grain yield is a complex trait and govern by a number of factors especially when bread wheat is evaluated for salinity. Our result supported by previous finding, i.e., grain yield reduced via affecting spike growth (Mass and Grieve 1990), by reducing the number of fertile tillers (El-Hendawy et al. 2005), by reducing spikelet fertility (Grieve et al. 1993) and reduction of 15% in yield in stressed plants compared to control of two main Mexican dwarf wheat varieties under salinity (Grieve and Francois 1992).

Morpho-physiological and biochemical process are inhibited in wheat under salt stress when compared to control. The morpho-physiological and biochemical data were subjected to analysis using SPSS software to determine the Pearson's correlation coefficients (Table 4). In the analysis of the relationships between seed yield $plant^{-1}$ and other parameters, plant height (0.649*), tiller $plant^{-1}$ (0.808**) and leaf area (0.687*) contributed the most variation to seed yield $plant^{-1}$ when data from all genotypes were combined (Table 4). Therefore, selection of high yield genotypes can be achieved based on plant height, tiller $plant^{-1}$ and leaf area.

In conclusion, morpho-physiological and biochemical character measured in this investigation, inhibited under both salt stresses (S1 & S2) conditions but all studied traits were more significantly inhibited under long-term salinity exposure (S2) than (S1) because interrupting the metabolic process of plant, resulting in stunted growth and productivity. According to correlation result, the selection of high yield genotypes can be achieved based on plant height, tiller $plant^{-1}$ and leaf area. Finally results indicating that sufficient genetic variability for salinity among the studied genotypes were present that could be implicated in selection of tolerant wheat genotypes for salinity development in the future.

Table 4. Relationships between seed yield and others parameters studied in this investigation under control and salinity stress

Relationship to yield $plant^{-1}$											
	PH	NT	NPT	SL	SN	AB	TW	SPAD	LA	RWC	K/Na
Correlation (r)											
Control	0.490	0.283	0.337	0.193	0.360	0.520	0.154	0.407	0.371	-0.493	-0.027
S1	0.586	0.286	0.316	0.278	0.112	0.276	0.323	0.390	0.359	-0.250	0.305
S2	0.649*	0.808**	0.602	0.073	0.080	0.008	0.064	0.040	0.687*	-0.028	-0.031

PH – Plant height; NT – Number of tillers $plant^{-1}$; NPT – Number of productive tillers $plant^{-1}$; SL – Spike length; SN – Spikelet number spike $^{-1}$; AB – Average biomass $plant^{-1}$; TW – Test weight; SPAD – Chlorophyll content; LA – Leaf area; RWC – Relative water content and K/Na ratio; S1 – Salinity ($EC_{iw}=10.0\text{ dSm}^{-1}$) imposed on 21 days after sowing (Salinity level1). S2 – Salinity ($EC_{iw}=10.0\text{ dSm}^{-1}$) imposed at the time of sowing (Salinity level2). **, *Correlation is significant at the 0.01 and 0.05 level.

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