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EFFECT OF SALINITY (NaCl) AND SEED PRIMING ($CaCl_2$) ON BIOCHEMICAL PARAMETERS AND BIOLOGICAL YIELD OF WHEAT

ATTAULLAH KHAN[1], MUHAMMAD SHAFI[1], JEHAN BAKHT[2*], SHAZMA ANWAR[1] AND MUHAMMAD OWAIS KHAN[3]

[1]*Department of Agronomy, Faculty of Crop Production Sciences*
[2]*Institute of Biotechnology & Genetic EngineeringThe University of Agriculture, Peshawar, Pakistan*
[3]*Center for Agricultural Resources Research Institute of Genetics and Developmental Biology, Chinese Academy of Sciences Yanqibu Campus Beijing, Beijing 101400, China*
[*]*Corresponding author's email: jehanbakht@yahoo.co.uk*

Abstract

The experiment was conducted to validate the effect of salinity (NaCl) and seed priming ($CaCl_2$) on biological yield of wheat. The six wheat varieties (Lalma-13, Pirsabak-05, Atta-Habib-10, Pirsabak-08, Inqilab-91 and Saleem-00) were tested at two seed treatments (un-primed and Primed with 50 mM $CaCl_2$) and four salinity regimes (0, 3, 6 and 9 dSm^{-1}) in soil culture. The seed priming and salinity has significantly ($p\leq0.05$) affected physiological, biochemical parameters and biological yield of wheat varieties. Wheat variety (Pirsabak-05) was tolerant in saline environment in term of producing highest shoot chlorophyll a and b content (1.60 and1.03 mg g^{-1} fresh weight), shoot proline content (118.20 µg g^{-1} fresh weight), shoot K^+ content (1.23 mg g^{-1} dry weight), shoot and root Ca^{++} content (0.76 and 0.58 mg g^{-1} dry weight), biological yield (12.46 g $plant^{-1}$), and lowest shoot Na^+ (1.11 mg g^{-1} dry weight) & Na^+/K^+ ratio (1.03).Saleem-00 and Inqilab-91 varieties were highest accumulator of shoot Na^+ (1.26 mg g^{-1} dry weight) and Na^+/K^+ ratio(1.55) respectively. Experimental findings suggested that except shoot Na^+ and Na^+/K^+ ratio, all biochemical, physiological parameters and biological yield of wheat varieties could be enhanced with seed priming. The salt tolerant wheat varieties (i.e. Pirsabak-05 and Lalma-13**)** were more responsive to seed priming than salt sensitive varieties (i.e. Inqilab-91 and Saleem-00).

Key words: Salinity, Wheat, $CaCl_2$ seed priming, Biochemical parameters.

Introduction

Soil salinity is a foremost constraint of reduced agricultural Productivity, averting to meet feeding demand of growing population. The geological processes and human activities mainly contribute to problem of salinity (Kumar *et al*., 2017). Salinization will affect 50% of the world arable land upto year 2050 due to increasing salinity at the rate of 10% annually. The various factors contribute to problem of salinity are high surface evaporation and less rain fall, weathering of parent rock material, poor cultural practices and irrigation with saline water (Shrivastava & Kumar, 2015).

Salinity produces multiple impacts on plant growth; initially with water deficit, induced by osmotic effect of excess salts in rhizoshpere. Later on with accumulation of more and more salts in older leaves results in ionic toxicity causing premature senescence (Radi *et al*., 2013). Salinity affects balance of ion uptake and distribution of essential nutrients by over accumulation of Na^+ in plants (Srinieng *et al*., 2015), which cause growth problem (Hussain *et al*., 2013). High concentration of Na^+ in leaf cells specifically affect membrane permeability, which causes reduced supply of K^+, N and Ca^{++} ion (Badar-uz-Zaman *et al*., 2017; Plaut *et al*., 2013). Khatar *et al*., (2017) investigated during salinity and aeration stress, a strong positive correlation between chlorophyll content index with seedling dry weight and potassium content, of wheat and bean crops. The most important photosynthetic pigments, that play an indispensable role in photosynthesis, were reduced by salinity stress (Ibrahim & Al-Mishhadani, 2015). However, osmolites such as proline accumulation was increased manifold in response to salinity stress (Sarwar *et al*., 2017).

The seed priming is an affective parameter which reduces adverse impacts of salinity. The seed priming is an effective and durable technique to enhance the performance of abiotically stressed plants, in term of biochemical and physiological changes (Oliveira & Gomes-Filho, 2016). The seed priming with various types of salt solution having high osmotic potential, can be used to proceed first seed imbibition phase, but prevent radical projection phase (Mustafa *et al*., 2017).The wide variety of seed priming techniques were deployed to induce positive pre-germination changes in seed. The seed priming comprised of osmopriming, hormonal priming, hydropriming, nutrient priming, and redox priming (Paparella *et al*., 2015). The seed priming is a physiological technique recently tested by many studies and used for adaptation of glycophyte species (Dai *et al*., 2017). The seed priming with $CaCl_2$ salt has caused rapid germination, boosted growth characters and proline content in asafetida (Elyasirad *et al*., 2017), and K^+ and Ca^{++} content in wheat crop with lowered Na^+ content (Afzal *et al*., 2008).

Wheat is the fourth largest scale staple crop produced worldwide, and is basic source of human food (Anon., 2015).The increasing world population through each passing year will require 87% more food production from major crops especially wheat, rice, maze and soybean by the year 2050 (Kromdijk & Long, 2016). However, production of wheat crop is seriously threatened by increasing soil salinity (Bouthour *et al*., 2015). Most of cultivated crops are glycophyte; such as wheat crop which is sensitive to salt stress, however, varietal response varies in their sensitivity to salinity (Aflaki *et al*., 2017). The varietal selection can be made on the basis of greater salt tolerance, which is mainly due to preferable intake of K^+ uptake than Na^+ion (Rauf *et al*., 2010) and maintaining lesser Na^+/K^+ ratio (Khan *et al*., 2010). The variable ionic uptake pattern in wheat genotypes creates variations in tolerance to salinity (Shirazi *et al*., 2011). Therefore, it is necessary to consider numerous wheat varieties against different saline regime to evaluate their salinity tolerance.

The objectives of this experiment was to evaluate the response of wheat varieties to salinity stresses and seed priming technique with $CaCl_2$ salt, and to screen out suitable tolerant varieties for salt affected areas on the basis of biochemical and physiological parameters.

Materials and Methods

The current research was undertaken at Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture Peshawar, Pakistan in winter 2014-2015. Wheat varieties Pirsabak-05, Lalma-13, Atta-Habib-10, Pirsabak-08, Saleem-00 and Inqilab-91 were primed with 50 mM $CaCl_2$. The un-primed seeds were used as control. The seeds were sown in pots with sandy loam soil and salinized with four increasing salinity levels (0, 3, 6 and 9 dSm^{-1}). The completely randomized design (CRD) was used for conducting experiment with three replications. In case of seed priming, seeds of above given wheat varieties were dipped for 12 hours in a solution of 50 mM $CaCl_2$ at 25^0C, immediately followed by drying surface of seeds. Twenty seeds of primed and unprimed wheat varieties were sown in each pot (380.00 cm^2) containing 5 Kg soil. After emergence completion, thinning was carried to maintain ten plants in each treatment. The plants were up rooted at maturity and were dried in oven at 80ºC, for 48h. The oven dried samples were weighed immediately on electronic balance for biological yield. The dried samples of shoots and roots were further grounded and powdered by standard protocol of Benton *et al*., (1991) for analysis of K^+, Ca^{++} and Na^+ content (mg g^{-1} dry weight). The filtrate of wet digestion, sodium and potassium content of shoot was quantified on flame photometer (Jenway PFP-7). The calcium content was quantified with atomic absorption spectrophotometer (Perkin Elmer-2380). The ratio of Na^+/K^+ in shoot was determined when Na^+ content was divided by its respective K^+ content. The Chlorophyll "a" and chlorophyll "b" from shoot were quantified by standard procedure of Inchtenthaler (1987). The supernatant obtained after filtration was scanned at 646 and 663 nm wavelengths, with spectrophotometer (Jenway-6300). The shoot proline content of each treatment was determined on fresh weight basis by method of Bates *et al*., (1973). The proline absorption was scanned at 520 nm.

Statistical analysis

The data was statistically analyzed and presented as a mean of three replications. The experimental results were analyzed for analysis of variance (ANOVA) using completely randomized design (Gomez & Gomez, 1984). In case of significant results, treatment means were compared by Steel & Torrie, (1980) procedure of LSD test at $p<05$.

Results

Shoot Na^+ content (mg g^{-1} dry weight): The salinity levels and seed priming have significantly affected shoot Na^+ content of wheat varieties (Table 1). The interaction of salinity x priming of seed was non significant across all interaction combination. The accumulation of shoot Na^+ was highest in Saleem-00 (1.26 mg g^{-1}) variety compared with lowest from variety Pirsabak-05 (1.11 mg g^{-1}). In comparison with control, the shoot Na^+ content has suffered reduction of 3.23% compared with control due to seed priming technique. The gradual increase in salinity stress by 3, 6 and 9 dSm^{-1} has produced drastic reduction of 98.76%, 138.59 and 226.92% in shoot sodium respectively. Shoot Na^+ content reveals reduction pattern among all studied wheat varieties with treatments of salinity stress (Fig. 1). The accumulation of Na^+ content in all wheat varieties was enhanced at every increment of salinity stress, with maximum value observed in variety Saleem-00 and Inqilab-91 (Fig. 2).

Shoot K^+ content (mg g^{-1} dry weight): Table 1 indicates significant and variable influence of salinity on shoot K^+ content recorded from primed seeds of six varieties. The interaction of (salinity x seed priming) was non-significant for shoot K^+ content. Among various wheat varieties, Pirsabak-05 proved superior with highest shoot K^+ content (1.23 mg g^{-1} dry weight). The inqilab-91 variety has accumulated least shoot K^+ content (0.97 mg g^{-1}). The improvement in shoot K^+ content was 9.14% in primed seed, than control treatment. The incremental increase in salt stress with 3, 6, and 9 dSm^{-1} have consequently reduced shoot potassium content by 15.15, 31.60 and 50.85% respectively compared with control. The seed priming has indicated greater improvement in shoot potassium content of Pirsabak-05 variety and least improvement in Inqilab-91 variety (Fig. 3). Shoot K^+ content was highest from variety Pirsabak-05 with no salinity application (Fig. 4).

Shoot Na^+/K^+ ratio: The shoot Na^+/K^+ ratio was variable due to priming technique of salinity stress exposed to various wheat varieties (Table 1). Minimum ratio of Na^+/K^+in shoot was maintained by variety Pirsabak-05 (1.03) compared with highest ratio recorded from Inqilab-91 (1.55) and Saleem-00 (1.47) variety. The reduction (14.03%) in Na^+/K^+ ratio was observed due to primed seed, than control. Dramatic elevation in shoot Na^+/K^+ (136.75, 254.14 and 579.92%) was observed, when wheat crop was grown on a salinity level of 3, 6 and 9 dSm^{-1}respectively. The shoot sodium and potassium ratio shoot up with each increment of salinity, compares with lowest increase in seed priming with $CaCl_2$ (Fig. 5). The no seed priming of variety Inqlib-91 has recorded drastic increase in Na^+/K^+ ratio, while variety (Pirsabak-05) has recorded reduction in sodium and potassium ratio (Fig. 6). The elevation of salinity stress has showed many fold increase in Na^+/K^+ ratio of shoot (Fig. 7).

Table 1. Shoot Na^+, K^+, Na^+/K^+ ratio, shoot and root Ca^{++} content, chlorophyll a and b content (mg g^{-1} fresh weight), shoot proline content (µg g^{-1} fresh weight) and biological yield (g $plant^{-1}$) of wheat as affected by salinity and seed priming.

Varieties	Shoot Na^+ content (mg g^{-1} dry weight)	Shoot K^+ content (mg g^{-1} dry weight)	Shoot Na^+/K^+ ratio	Shoot Ca^{++} content (mg g^{-1} dry weight)	Root Ca^{++} content (mg g^{-1} dry weight)	chlorophyll a content (mg g^{-1} fresh weight)	chlorophyll b content (mg g^{-1} fresh weight)	Shoot proline content (µg g^{-1} fresh weight)	Biological yield (g $plant^{-1}$)
Lalma-13	1.12d	1.17b	1.23d	0.72b	0.57b	1.31b	0.84b	115.06b	11.44b
AttaHabib10	1.20c	1.13c	1.21d	0.61c	0.45c	1.28b	0.82b	100.05c	11.27bc
Pirsabak-08	1.21c	1.10d	1.27c	0.58d	0.40d	1.24c	0.80c	94.17d	11.08c
Pirsabak-05	1.11d	1.23a	1.03e	0.76a	0.58a	1.60a	1.03a	118.20a	12.46a
Saleem-00	1.26a	1.05e	1.47b	0.56de	0.30e	1.19d	0.77d	83.51e	9.73d
Inqilab-91	1.24b	0.97f	1.55a	0.57e	0.27f	0.92e	0.59e	70.51f	9.40e
Salinity dSm^{-1})									
0	0.55d	1.46a	0.38d	0.79a	0.53a	1.60a	1.03a	55.41d	14.00a
3	1.09c	1.24b	0.89c	0.69b	0.45b	1.39b	0.90b	78.81c	11.69b
6	1.31b	1.00c	1.34b	0.59c	0.39c	1.10c	0.71c	105.60b	9.95c
9	1.80a	0.72d	2.57a	0.46d	0.35d	0.92d	0.60d	147.85a	7.95d
Seed priming									
Un-primed	1.21a	1.06b	1.39a	0.61b	0.41b	1.18b	0.76b	92.78b	10.43b
Primed	1.17b	1.16a	1.20b	0.66a	0.45a	1.33a	0.86a	101.05a	11.37a
Significance for p	s	s	s	s	s	s	s	s	s
$LSD_{(0.05)}$ for V	0.010	0.015	0.037	0.012	0.009	0.032	0.021	1.602	0.248
$LSD_{(0.05)}$ for S	0.009	0.012	0.030	0.010	0.007	0.026	0.017	1.308	0.232
					Interactions				
V x P	**	**	**	**	**	*	*	**	ns
P x S	ns	ns	**	*	ns	**	**	**	**
V x S	**	**	**	**	**	**	**	**	**
P x S x V	**	**	**	**	ns	**	**	*	ns

The variable letters in the same column, indicate significant difference in means by LSD test at 0.05% level

* ,**= significant at ($p \leq 0.01$) and ($p \leq 0.05$) level of probability using LSD test

s shows significant and ns shows non-significant at probability level of 0.05%

V= Varieties, S= Salinity, P = Seed priming

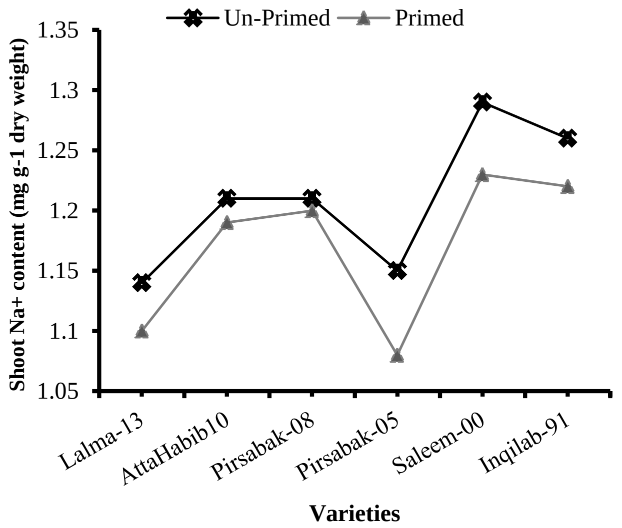

Fig. 1. Interaction of seed priming and varieties for shoot Na^+ content (mg g^{-1} dry weight).

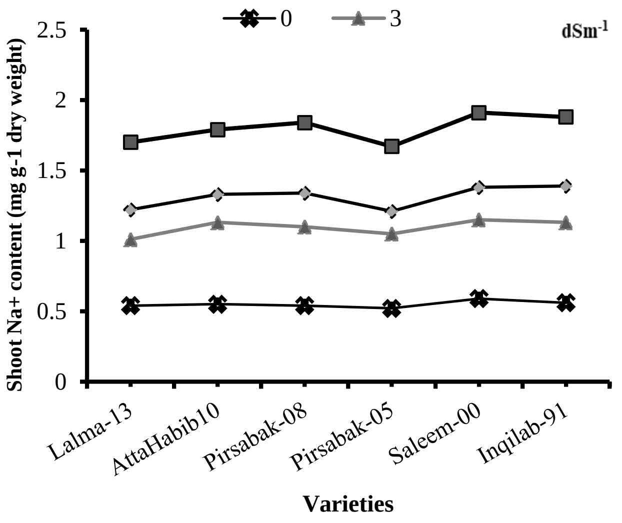

Fig. 2. Interaction of varieties and salinity for shoot Na^+ content (mg g^{-1} dry weight).

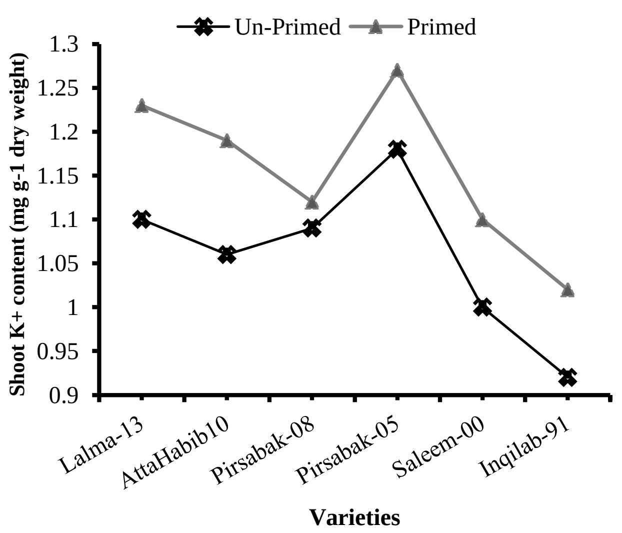

Fig. 3. Interaction of seed priming and varieties for shoot K^+ content (mg g^{-1} dry weight).

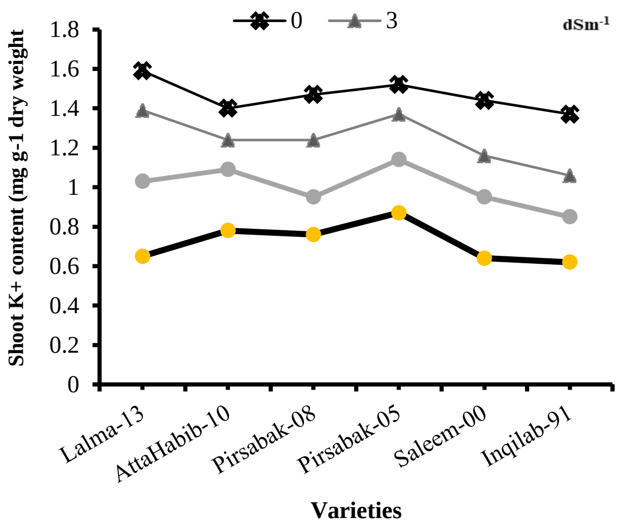

Fig. 4. Interaction of varieties and salinity for shoot K^+ content (mg g^{-1} dry weight).

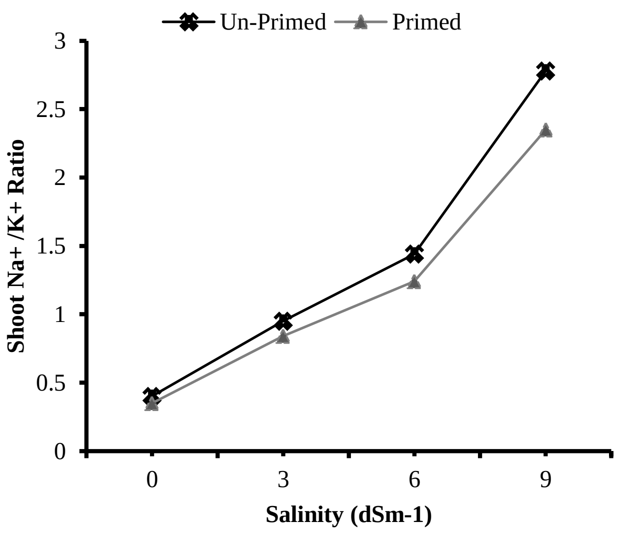

Fig. 5. Interaction of seed priming and salinity for shoot Na^+/K^+ ratio.

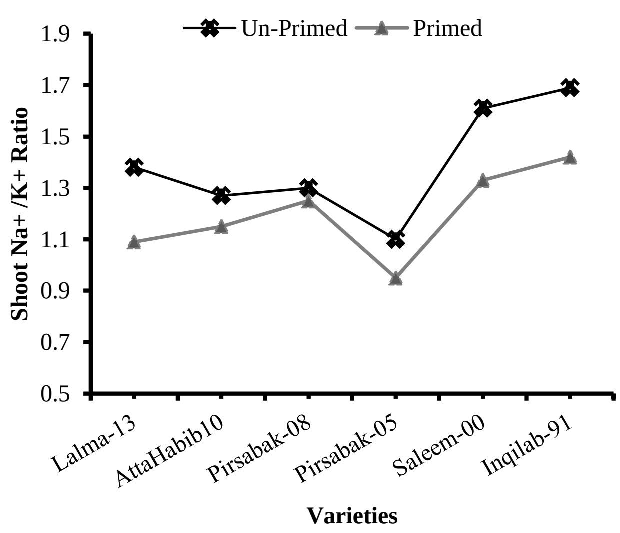

Fig. 6. Interaction of seed priming and varieties for shoot Na^+/K^+ ratio.

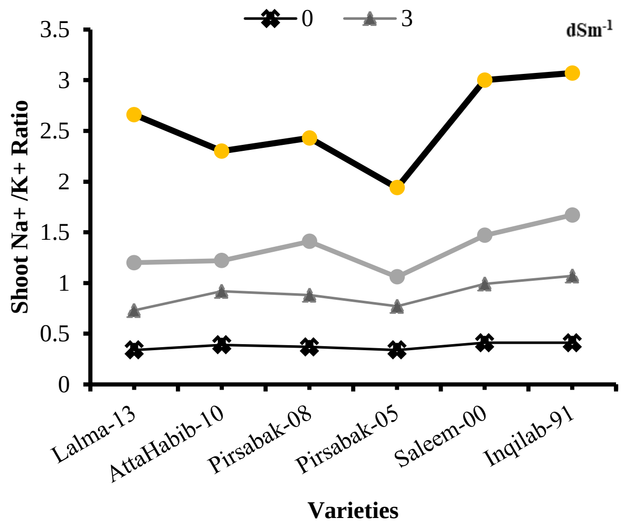

Fig. 7. Interaction of varieties and salinity for shoot Na^+/K^+ ratio.

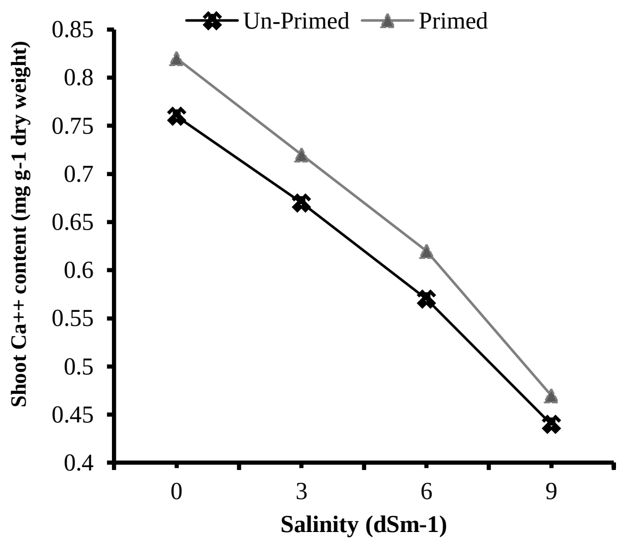

Fig. 8. Interaction of seed priming and salinity for shoot Ca^{++} content (mg g^{-1} dry weight).

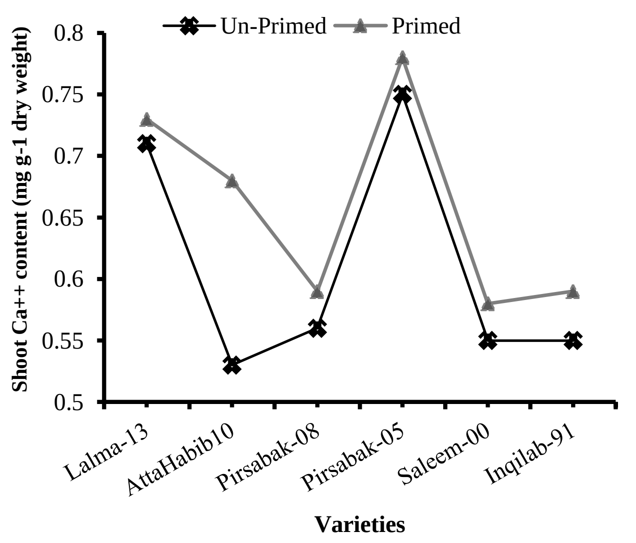

Fig. 9. Interaction of seed priming and varieties for shoot Ca^{++} content (mg g^{-1} dry weight).

Shoot Ca^{++} content (mg g^{-1} dry weight): The shoot Ca^{++} content was significantly decreased with salinity stress on studied varieties, although shoot calcium was improved by seed priming (Table 1). All possible interactions were significant for shoot calcium. In all wheat varieties, Saleem-00 has maintained minimum shoot calcium content (0.56 mg g^{-1} dry weight) and maximum shoot Ca^{++} content was recorded from variety Pirsabak-05 (0.76 mg g^{-1}) and Lalma-13 variety (0.72 mg g^{-1} dry weight). The elevation of 7.62% in shoot Ca^{++} content was observed in primed seed treatment, in comparison with control. The increment of salt stress (3, 6 and 9 dSm^{-1}), in comparison with control has reduced Ca^{++} content of shoot gradually (12.80%, 25.03% and 42.36%). The imposition of salt stress has reduced shoot Ca^{++}, and reduction pattern was minimized by priming seed treatment (Fig. 8). Tolerant variety such Pirsabak-05 has produced highest shoot Ca^{++} content with treatment of seed priming compared with control (Fig. 9). All wheat varieties have depicted reduction in shoot Ca^{++}, although minimum reduction was noticed in variety Pirsabak-05 (Fig. 10).

Root Ca^{++} content (mg g^{-1} dry weight): The seed priming has indicated significant improvement in root Ca^{++} content of studied wheat varieties to variable salt stresses (Table 1). The interaction of (variety x priming) and (variety x salinity) was significant. Inqilab-91 variety has absorbed lowest Ca^{++} content (0.27 mg g^{-1}) through roots, and highest root Ca^{++} content of 0.58 and 0.57 mg $g^{-1}$was accumulated by Pirsabak-05and Lalma-13variety, respectively. The ameliorative role of seed priming with $CaCl_2$, in comparison with no seed priming, has improved Ca^{++} content (8.23%) of roots. The root Ca^{++} content was gradually decreased by 15.26%, 26.11% and 33.78% at enhancing salinity of 3, 6 and 9 dSm^{-1} respectively compared with control. Figure 11 indicates enhancement of root Ca^{++} content across all wheat varieties with imposition of seed priming treatment, however, no seed priming of variety Inqilab-91 has recorded lowest root Ca^{++} content. The tolerant wheat variety (Pirsabak-05) compared with rest of varieties has maintained highest root calcium content at highest salinity stress (Fig. 12).

Shoot chlorophyll a content (mg g^{-1} fresh weight): Shoot chlorophyll a recorded from several wheat varieties were inversely affected by increasing salinity levels, compared with seed priming (Table 1). The varieties (i.e., Pirsabak-05 and Lalam-13) were superior in term of elevated chlorophyll content (1.60 and 1.31 mg g^{-1} fresh weight) compared with variety Inqilab-91 with accumulation of lowest chlorophyll a (0.92 mg g^{-1}). The seed priming has enhanced by12.67% shoot chlorophyll a content. The remarkable reduction of 42.21% in shoot chlorophyll a was recorded at 9 dSm^{-1} and reduction of 31.19% was observed at 6 dSm^{-1} salinity in comparison with control. The chlorophyll a was enhanced with seed priming at control level of salinity (Fig. 13). The seed priming of variety (Prisabak-05) has significantly improved shoot chlorophyll a content. The inqilab-91 variety was lowest producer of chlorophyll a content (Fig. 14). At all salinity levels, including control, chlorophyll a content was highest in variety pirsabak-05 (Fig. 15).

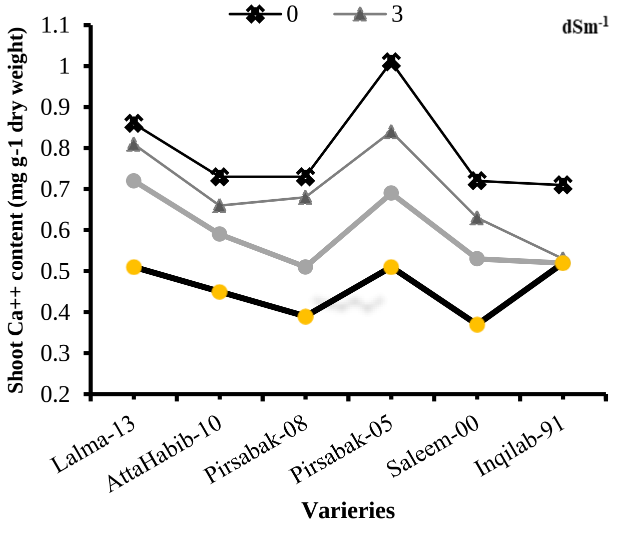

Fig. 10. Interaction of varieties and salinity for shoot Ca^{++} content (mg g^{-1} dry weight).

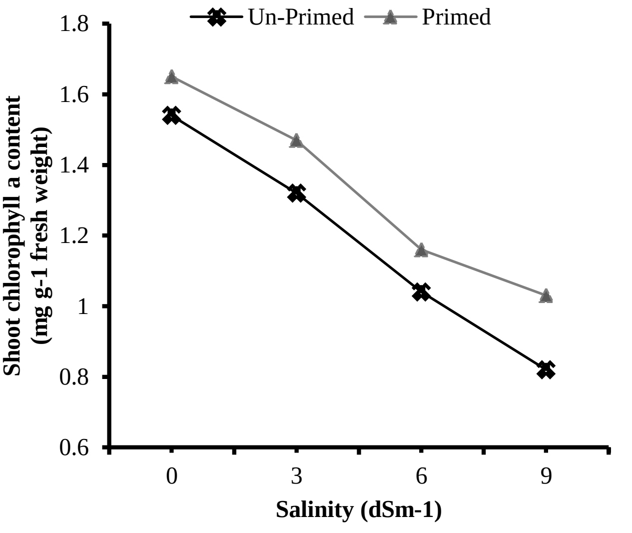

Fig. 13. Interaction of seed priming and salinity for shoot chlorophyll a content (mg g^{-1} fresh weight).

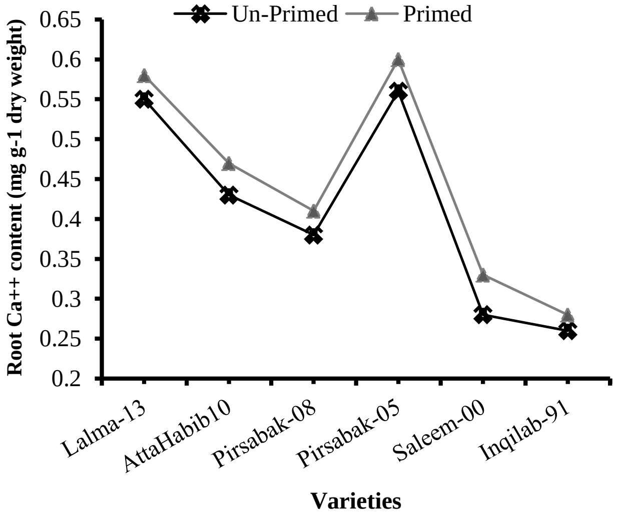

Fig. 11. Interaction of seed priming and varieties for root Ca^{++} content (mg g^{-1} dry weight).

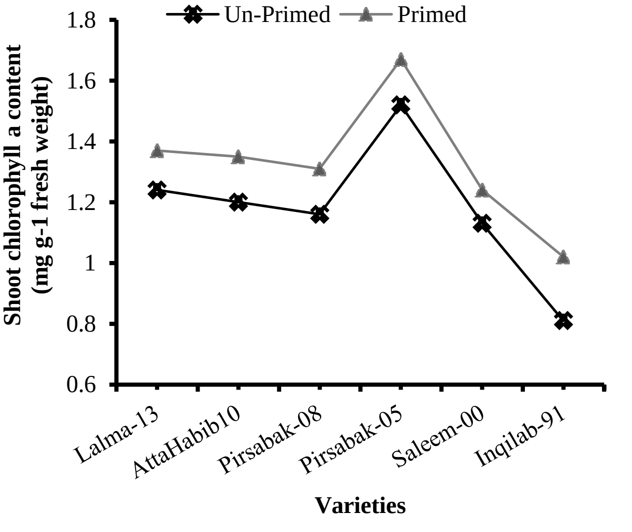

Fig. 14. Interaction of seed priming and varieties for shoot chlorophyll a content (mg g^{-1} fresh weight).

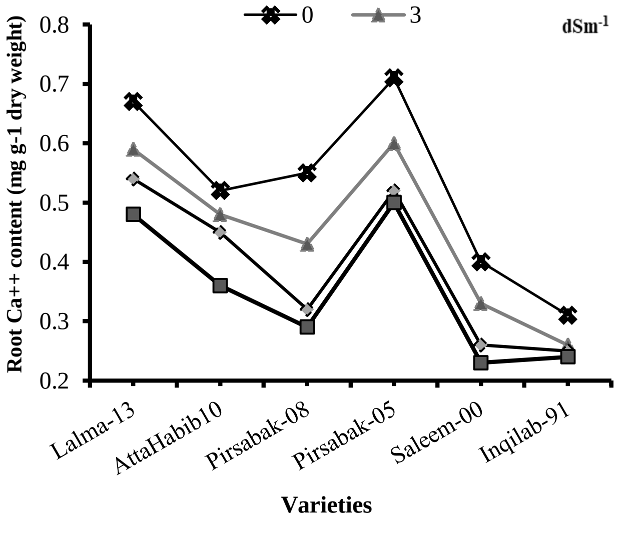

Fig. 12. Interaction of varieties and salinity for root Ca^{++} content (mg g^{-1} dry weight).

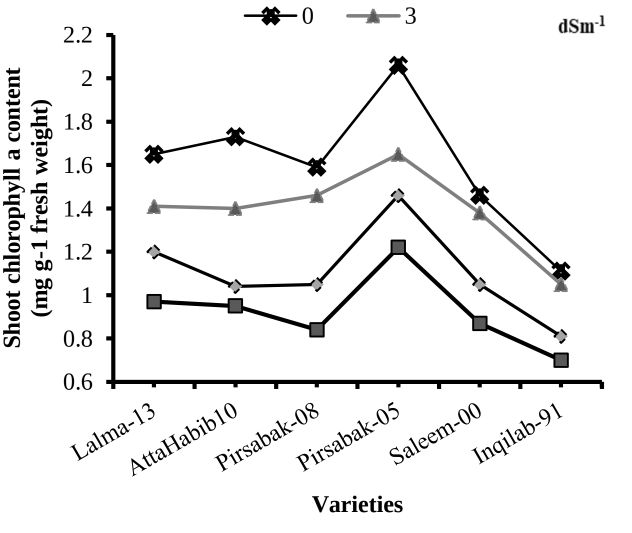

Fig. 15. Interaction of varieties and salinity for shoot chlorophyll a content (mg g^{-1} fresh weight).

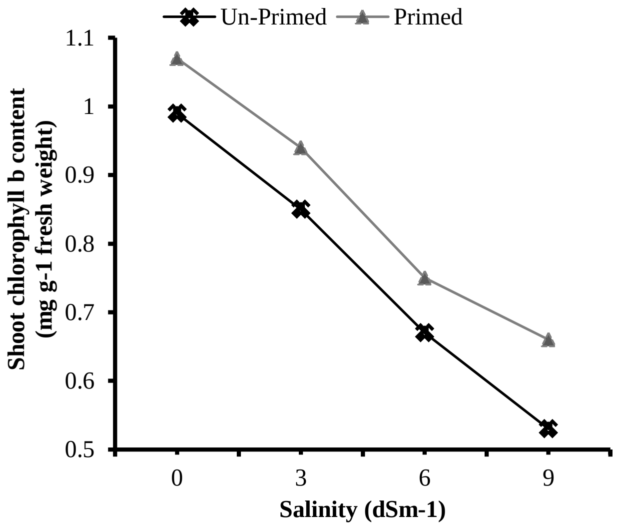

Fig. 16. Interaction of seed priming and salinity for shoot chlorophyll b content (mg g^{-1} fresh weight).

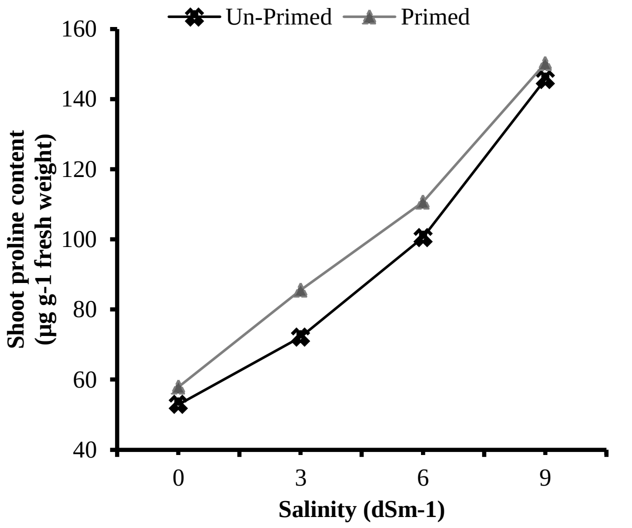

Fig. 19. Interaction of seed priming and salinity for shoot proline content (µg g^{-1} fresh weight).

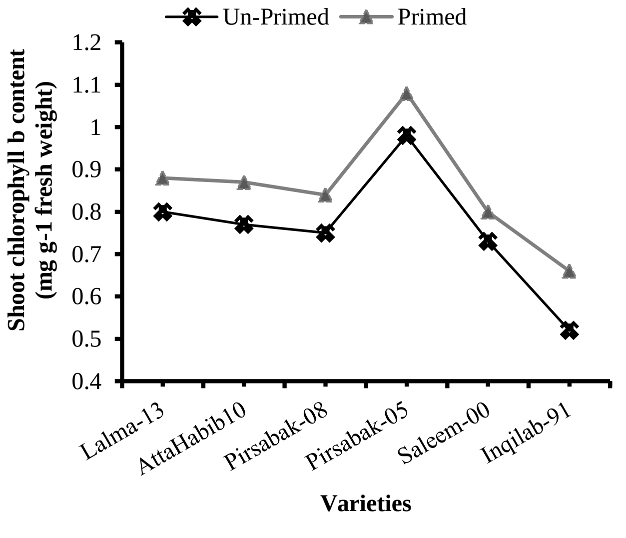

Fig. 17. Interaction of seed priming and varieties for shoot chlorophyll b content (mg g^{-1} fresh weight).

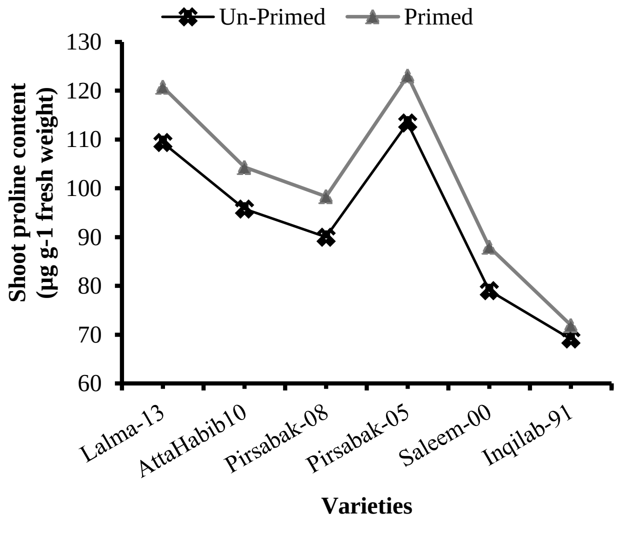

Fig. 20. Interaction of seed priming and varieties for shoot proline content (µg g^{-1} fresh weight).

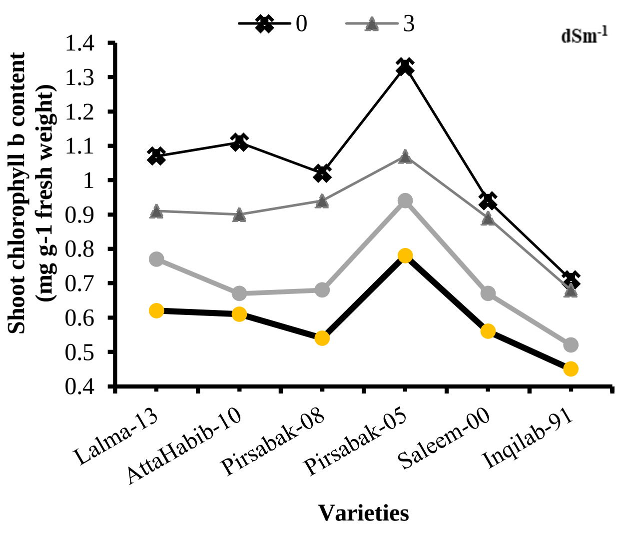

Fig. 18. Interaction of varieties and salinity for shoot chlorophyll b content (mg g^{-1} fresh weight).

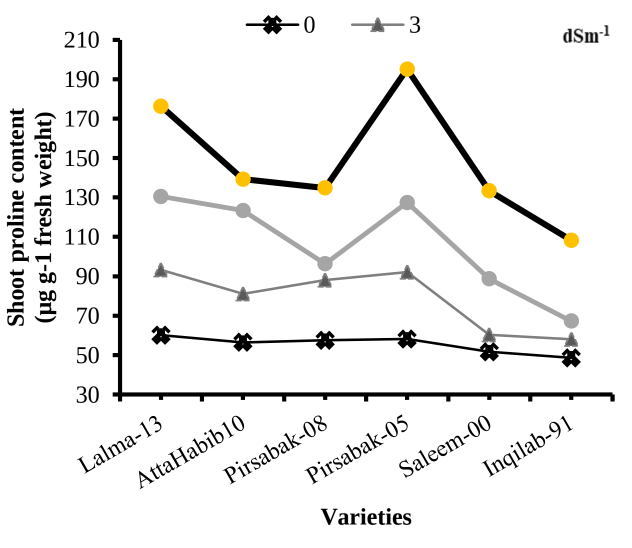

Fig. 21. Interaction of varieties and salinity for shoot proline content (µg g^{-1} fresh weight).

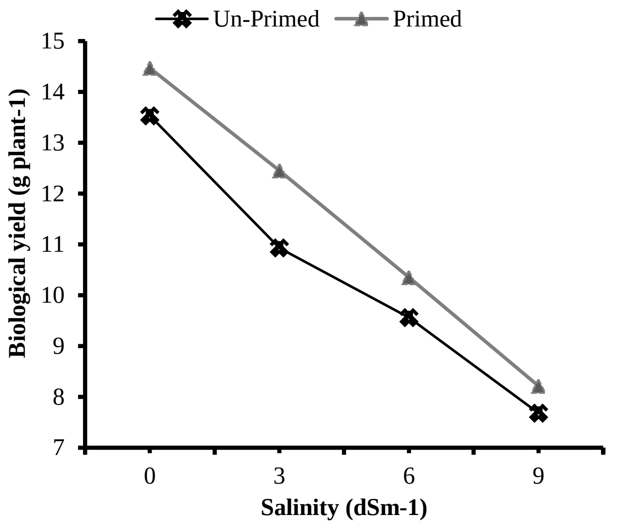

Fig. 22. Interaction of seed priming and salinity for biological yield (g plant^{-1}).

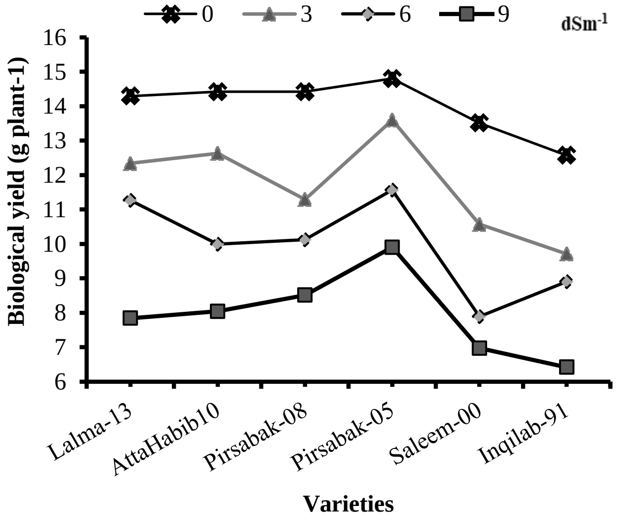

Fig. 23. Interaction of varieties and salinity for biological yield (g plant^{-1}).

Shoot chlorophyll b content (mg g^{-1} fresh weight): The evident reduction in chlorophyll b of wheat varieties was observed with elevation in salinity stress. Seed priming with $CaCl_2$ has significantly increased chlorophyll b content (Table 1). The interactions of varieties, salinity and seed priming have shown significant variation in chlorophyll b. In all wheat varieties, Pirsabak-05 and Lalma-13 has shown highest chlorophyll b content of 1.03 and 0.84 mg g^{-1} respectively, while Inqilab-91 was lowest accumulator of chlorophyll b content (0.59 mg g^{-1}). Seed priming with $CaCl_2$, in comparison with un primed seed has increased shoot chlorophyll b content from 0.76 to 0.86 mg g^{-1}. The drastically reduced chlorophyll b with elevated salinity stress was recorded with highest reduction of 42.21% from salinity (9 dSm^{-1}). The reduction of 12.93% and 31.19% in chlorophyll b content was observed in response to enhancement in salinity stress by 3 and 6 dSm^{-1}. The deleterious impact of salinity stresses on chlorophyll b was dropped down with improving effect of $CaCl_2$ priming (Fig. 16). The variety (Pirsabak-05) was more responsive to priming treatment and recorded elevated chlorophyll b pigment (Fig. 17). The varieties (Pirsabak-05 and Lalma-13) have kept maximum chlorophyll b pigments in normal and saline environment compared with other varieties (Fig. 18).

Shoot proline content (µg g^{-1} fresh weight): Shoot proline of wheat varieties has illustrated variable response to $CaCl_2$ seed priming across rising stresses of salinity (Table 1). All interactions combination of varieties, salinity and seed priming has produced significant variation in shoot proline. The proline content of shoot in variety Inqilab-91 (70.51µg g^{-1} fresh weight) was comparatively lower compared with proline content of 118.20 and 115.06 µg g^{-1} fresh weight from tolerant varieties (Pirsabak-05 and Lalma-13). The ameliorative impact of priming has caused an elevation of 8.92% in shoot proline content than control. Highest increase of 166.84% in proline content was observed at salinity (9 dSm^{-1}). Although, compared with control, the salinity level of 3 and 6 dSm^{-1} have recorded an increase of 42.24 and 90.58% in shoot proline. The interaction of salinity and seed priming has showed that proline content due to salt stress in wheat plant was further fortified by seed priming (Fig. 19). The studied varieties of wheat showed variable accumulation of proline, with highest proline accumulation was noticed in variety Pirsabak-05 when primed before sowing (Fig. 20). Highest proline in shoot of variety (Pirsabak-05) was accumulated with exposure to highest salt stress (Fig. 21).

Biological yield (g plant^{-1}): It is obvious from Table 1 that effects of priming, salinity, varieties, and interaction of variety x salinity and priming x salinity for biological yield (g plant^{-1}) were significant. Biological yield was greater in variety Pirsabak-05 (12.46 g plant^{-1}) followed by variety Lalma-13 (11.44 g plant^{-1}) comapared with biological yield of 9.40 g plant^{-1} was from Inqilab-91. Biological yield was 9.06% lower in no seed priming treatment, compared with seed priming. Highest biological yield was recorded at control salinity. The induction of 3, 6 and 9 dSm^{-1} salinity has reduced biological yield by 16.50, 28.90 and 43.24% respectively. The fall in biological yield with each incremental increase in salinity was less due to positive influence of seed priming (Fig. 22). The sensitivity of variety (Inqilab-91) to salt stress has caused greater reduction in biological yield in comparison with tolerance of variety (Pirsabak-05) which shows resistance to losses in biological yield at every level of salinity (Fig. 23).

Discussion

High salinity stress has negatively affected growth of glycophytes that include most of agronomic crop such as wheat (Almutairi & Toulibah, 2016). The osmotic effect, due to high accumulation of salts in the root area due to salinity stress causes dehydration of root cells. High amounts of Na^+an ion inside plant cells as well as in the soil solution has caused disruption of nutrients such as K^+. Photosynthetic capacity in term of chlorophyll fluorescence and gaseous exchange were adversely affected by salinity stress (Kausar & Shahbaz, 2017). The findings of our experiments revealed that seed priming and salinity have significantly affected accumulation of sodium content in shoot of wheat varieties. Salt sensitive varieties (Inqilab-91 and Saleem-00) have accumulated highest Na^+ in shoot. In comparison with salt sensitive varieties, Pirsabak-05 variety showed least magnitude of sodium in their shoot as reported by Mahmood *et al*., (2010). The retardation of growth in saline stress environment is due to presence of excess Na^+ ions, which even facilitate absorption of heavy metals (Shafi *et al*., 2011; Wu *et al*., 2015). The accumulation of Na^+ content in shoot was decreased by beneficial influence of seed priming technique. The seed priming reveals positive relationship with absorption of K^+, conversely relationship of seed priming was negative with Na^+ absorption. The use of salt water for seed priming results in high shoot K^+. In maize crop, salinity tolerance were uplifted with seed priming through lower accumulation of sodium and increased production of absicic acid and proline content (Bakht *et al*., 2011). The $CaCl_2$ seed priming was proved beneficial in crop plants which might be due to active role of Ca^{++} ion in stress sensing and signaling mechanism to abiotic stresses. The Ca^{++} ion was proved to be involved in lowering the absorption of sodium in plants (Cha-um *et al*., 2012).

The results of our experiment on wheat varieties and seed priming revealed variable impact of salinity on shoot K^+ content. The absorption of potassium was variable among varieties i.e. tolerant varieties (Pirsabak-05 and Lalma-13) in comparison with sensitive wheat variety (Inqilab-91), has absorbed maximum K^+ content in shoots. Yusuf *et al*., (2012) reported that potassium sodium ratio and potassium content in cell sap were highly accumulated in best performing genotypes (Anwar *et al*., 2011). The retention of high concentration of K^+ is necessary than restricted Na^+ entry for maintenance of electrical potential across membrane, turgor, energy transfer and photosynthesis (Kausar & Gull, 2014). In this study, seed priming with calcium chloride has induced greater concentration of K^+ in saline envronment. Anwar *et al*., (2011) reported lowest Na^+ contents, highest sugar content and K^+ content due to seed priming treatment in shoot and roots of barley genotypes. High osmotic adjustment and ionic homeostasis by upgraded accumulation of K^+ and $Ca^{++}$is an indicative of salinity tolerance (Jamal *et al*., 2011).

The Na^+/K^+ ratio of shoot showed variable response in wheat varieties due to influence of seed priming and salinity. The Varieties (Pirsabak-05 and Lalma-13) were tolerant to salinity and maintained lower Na^+/K^+ ratio in shoot. The sensitive wheat varieties viz. Inqilab-91 and Saleem-00 stored highest Na^+/K^+ ratios in shoots. The Na^+/K^+ ratio was kept low at desirable level to regulate osmotic adjustment in tolerant varieties (Keisham *et al*., 2018). Maximum potassium absorption was achieved with the initiation of Na^+/K^+ simporters due to lower K^+ in roots (Shereen *et al*., 2016). The experimental results revealed that seed priming has reduced shoot Na^+/K^+ ratio. Azooz, (2012) has confirmed role of salicylic acid seed treatment in reducing the harmful effect of salinity and Na^+ accumulation. Salicylic acid expands wheat genotypes K^+ content and lower K^+/Na^+. The possible reason could be lowest membrane leakage leading to high seed vigor, viability and improved salt stress tolerance in soybean crop with seed priming as reported by Suryaman *et al*., (2017).

The rising salinity level and seed priming with $CaCl_2$ has caused significant variation in shoot and root Ca^{++} content of wheat varieties. Highest concentration of calcium in wheat varieties (i.e.Pirsabak-05 and Lalma-13) render them more tolerant than varieties (Inqilab-91 and Saleem-00), with least calcium content. Enhancing salinity stress levels have caused drastic reduction in shoot and root calcium content as confirmed by Rahneshan *et al*., (2017). Wheat plant adoptability to abiotic stress was increased with calcium due to its influence on enhancement of proline, photosynthetic parameters, inhibition of rubisco degradation as a key enzyme of photosynthesis (Dolatabadi *et al*., 2013). The Ca^{++} content was increased due to seed priming in our experiment compare with control. The deleterious influence of Na^+ on seedling development and germination was minimized by soil applied or seed priming of K^+, Ca^{++}, Zn^+ nutrient. In seed priming the calcium ion can either be absorbed or coated on seed surface (Rahmatullah *et al*., 2012). In case of extreme salinity, high sodium causes decrease in calcium content. The calcium provides major support to maintain integrity of cell membrane. The calcium plays chief role in the formation of cell wall and central lamella of newly divided cells (Hakim *et al*., 2014).

In abiotic stresses, measurements of photosynthetic pigments are among essential parameters to assess tolerance of variety. The most important photosynthetic pigments (chlorophyll a and b) in our results were significantly increased with seed treatment of all wheat varieties. Salt tolerant varieties (Pirsabak-05 and Lalma-13) have maintained elevated chlorophyll a and b content. In our results, gradual elevation in salinity stress has markedly decreased photosynthetic pigments. The prime reason behind low photosynthesis under salt stress could be osmotic effect of salts, which creates scarcity of water, stomatal closure, limited Co_2 inflow and impaired assimilates partitioning (Anjum *et al*., 2011b). The various cellular organelles membranes were damaged with reactive oxygen species, which generated chiefly oxidative stress, which degrades chlorophyll content (Chutipaijit *et al*., 2009). Oxidative stress even disrupts photosynthetic enzymes, chlorophyll pigments and ionic contents of cell (Radi *et al*., 2013). The results of our experiment have indicated enhanced chlorophyll a and b contents with treatment of $CaCl_2$ seed priming. Abbasdokht & Edalatpisheh (2013) reported that with seed priming treatment the enhanced stomatal conductance and net photosynthetic rate, and adaptability to saline condition.

Proline content of shoot reveals significantly variable response to salt stress and seed priming. Substantially highest prolinewas recorded in wheat varieties Pirsabak-05 and Lalma-13(tolerant) than proline content of salt sensitive varieties Inqlab-1991 and Saleem-00. In salt stressed environment, cytosolic proline was increased manifold that not only bring about osmotic adjustment but also induces free radicals scavenging by proline (Jiang *et al*., 2016). Our data further revealed enhancing impact of seed priming on proline contents in shoot as compared with no seed priming. Proline content accumulation of shoot was highest in primed seeds of tolerant varieties, which were exposed to saline condition. Zare *et al*.,

(2014) and Ali *et al*., (2017) reported that plant due to salinity stress expands production and accumulation of shoot proline significantly. The production was further fortified with seeds priming treatment. The required concentration of prolinewas synthesized in response to degree of osmotic stress.

Biological yield (g plant^{-1}) was affected by seed priming and wheat varieties. The highest biological yield was produced by tolerant variety (Pirsabak-05) compared with sensitive variety (Inqilab-91). The production of biomass was least in sensitive varieties due to salt stress than salt tolerant varieties as reported by Muhammad *et al*., (2015). The dry biomass was reduced by abiotic stresses, because of reduction in germination and seedling establishment (Amudha & Balasubramani, 2011). It is clearly exhibited by the data of our experiment that priming of seed has uplifted biological yield. The $CaCl_2$ increased biological yield of those wheat varieties which showed tolerance to salinity (Yasmeen *et al*., 2013; Haider *et al*., 2016). It was proved that seed priming with in-organic salts has increased production of DNA and RNA synthesis, number and efficacy of mitochondria at germination and early growth stage, which can be positively correlated with final biomass production (Eivazi, 2012).

Conclusion

The stresses of salinity have exhibited significant influence on all biochemical parameters. The negative impact of salinity on wheat varieties was alleviated by seed priming with $CaCl_2$. The study revealed that biological yield, shoot K^+ content, root and shoot Ca^{++} content, chlorophyll a and chlorophyll b pigments in shoot and proline content were increased in primed seeds of wheat varieties. The tolerant wheat varieties (i.e. Pirsabak-05 and Lalma-13) have showed positive response to seed priming compared with salt sensitive varieties (i.e. Inqilab-91 and Saleem-00). The seed priming has more efficiently recovered the losses occurs across all salinity levels in tolerant varieties (Pirsabak-05 and Lalma-13). It is concluded that stresses induced by various salinity levels on studied biochemical parameters of wheat varieties were affectively ameliorated by seed priming with $CaCl_2$.

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