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DECIPHERING PHYSIO-BIOCHEMICAL CHARACTERISTICS OF $ZnSO_4$ PRIMED WHEAT (*TRITICUM AESTIVUM* L.) PLANTS GROWN UNDER SALT STRESS

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Abstract

Zinc dearth is an imperative soil constraint facing agriculture, particularly cereals. Zinc is a critical micronutrient involved in many physiological processes including carbohydrate metabolism and maintain high yields in plants. The study evaluated the role of Zn grain priming in alleviating the detrimental effects of salt stress. Salt stress (100 m*M*) negatively influenced plant parts that ultimately perturbed physiology and biochemistry of wheat varieties Faisalabad-2008 and Galaxy -2013. Preconditioning of *Triticum aestivum* L., wheat grains with $ZnSO_4$ (0, 0.5, 1, 2, 5 and 10 m*M*) increased growth, chlorophyll, K and Zn contents in root and shoot of both wheat varieties. Correlation analysis, dendrogram and PCA analysis clearly indicated that root and shoot Na are positively correlated with each other and negatively regulate photosynthetic pigments, ionic contents and growth of plants. Overall, results suggested that 5 m*M* $ZnSO_4$ priming was effective in attenuating salt stress in both wheat genotypes.

Key words: Salinity stress, Zn, Ionic attributes, Priming.

Introduction

Salinity is a key abiotic constraint that hampers crop development and output (Zafar *et al*., 2015). It is accountable for reduced germination, stunted growth, decreased leaf area, curtailed biomass, flower infertility and undersized seeds (Mane *et al.*, 2011; Zafar *et al*., 2018). NaCl stress affects enzymes and organelles in cytoplasm, uptake of K^+, Ca^{2+}, NH^{4+}, NO^{3-}, Mg^{2+} ultimately resulting in senescence and necrosis of leaves and they fall off (Zafar *et al*., 2015).

Wheat is the dominant crop grown in all parts of the world (Saeed *et al*., 2012). It provides 21% of calories, an important source of about 20% of protein to daily requirements of people in economically developing nations (Braun *et al*., 2010). An average yield loss of wheat reported is 65% in moderately saline areas (Shafi *et al*., 2010). The wheat crop is deficient in micronutrients especially in areas with less rainfall, zinc deficiency in particular is the most serious micronutrient disorder in saline soils with high pH values. Zinc scarcity in soil is a global problem that affects agricultural soils. About 50 % of the soil is low in zinc available to plants. Application of adequate Zn prevented Na ion accumulation in shoots and enhanced membrane integrity in cells of roots (Weisany *et al*., 2012). Nutritional disorders in food occur due to zinc deficiency, soil salinity and unbalanced fertilizer application. Wheat is particularly sensitive to Zn inadequacy or non availability (Narimani *et al*., 2010; Nadim *et al*., 2012). Foliar application of micronutrients has the potential to enhance crop yield but high cost, limits its economical use to farmers (Weisany *et al*., 2011; Nawaz *et al*., 2013). Preconditioning of wheat grains with Zn is a cost effective technique; due to the ease of the process, a practical way to increase Zn contents in grains to promote growth and yield as compared to soil application (Bench-Arnold *et al*., 2004). Zinc fertilizers are used for the biofortification of cereal grains to maintain Zn concentration in diet (Alloway, 2009). The purpose of the research work is to assess the biochemical changes in wheat genotypes in improving Zn contents by $ZnSO_4$ seed priming to evaluate the optimum level to tackle hazards caused by salinity.

Materials and Methods

Healthy seeds of wheat (*Triticum aestivum* L.) varieties Fsd-08 (Faisalabad-2008) and Galaxy-13 (Galaxy-2013) primed with $ZnSO_4$ 0.5, 1, 2, 5, and 10 m*M* for 12 hours were allowed to germinate in pots having 8 kg soil (Table 1) in the research area of Government College University, Faisalabad, Pakistan. The soil texture was analyzed by hydrometer method as described by Dewis & Freitas (1970). The physico-chemical characteristics of the experimental soil were determined by the methods of Jackson (1962) as described in Table 1. A hydro-primed control sample was also used in this experiment.

Salinity level (100 m*M*) was developed by using NaCl after one week of germination.

Table 1. Soil characteristics.

Soil texture	Sandy loam	Cl^- ($meqL^{-1}$)	2.22
Electrical conductivity (dS m^{-1})	0.51	Ca^+ Mg($meqL^{-1}$)	4.1
Ph	7.01	Na^+ (ppm)	2.61
Saturation (%)	38	Soluble K^+ (ppm)	21
Organic matter (%)	0.27	Total N (%)	0.070
CO_3^{2-} ($meqL^{-1}$)	Nil	Available P(ppm)	8.31
HCO_3^- ($meqL^{-1}$)	2.84	Zn (ppm)	1

Growth attributes were recorded and to record biochemical parameters plants from each treatment were taken out at vegetative stage.

Morphological attributes and biomass: Plant height including length of shoot and length of root was measured at vegetative stage. Biomass was recorded by using electric balance.

Pigment analysis: Chlorophyll a (chla) and chlorophyll b (Chlb) were recorded at 480, 645 and 663 nm wavelength (Arnon, 1949; Davies, 1976).

Determination of nutrients in shoot: Nutrients were determined by digesting the samples with concentrated H_2SO_4 as described by Wolf (1982). The amount of potassium (K) and sodium (Na) in digested samples was recorded by using flame photometer (Jenway, PFP). Zinc contents were recorded using atomic absorption spectrophotometer (novAA, 400, Germany).

The results were analyzed statistically by software program Statistix 8.1. Correlation, dendrogram and PCA (principle component) analysis were performed by XLSTAT VERSION 2014.05.03. software.

Results

Imposition of salt stress induced significant changes in wheat plants. The genotypes presented a clear difference to tolerate salt stress. Contrary to these injurious observations, pre-conditioning of grains with varying levels of $ZnSO_4$ presented a clear trend in terms of enhancing tolerance to salt stress.

The analyzed data indicated considerable effects of $ZnSO_4$ priming on various growth and biochemical parameters under salt stress. Regarding growth parameters shoot length exhibited 30% and 20% reduction, whereas 27.5% and 11.5% decrease in root length was observed under salinity stress in wheat varieties Fsd-08 and Galaxy- 13 genotypes respectively (Fig. 1). Grain pretreatment with $ZnSO_4$ exerted a positive influence on the attribute under both saline as well as non-saline conditions. In case of root length 2 m*M* $ZnSO_4$ grain priming in wheat variety Fsd- 08, however, in Galaxy-13, 10 m*M* $ZnSO_4$ presowing treatment proved beneficial. Comparison of means for this parameter presented more improvement in root length in Fsd-08.

Zinc priming under NaCl stress revealed an amplifying trend for wheat genotypes. Statistical analysis of the data exhibited significance of seed priming with $ZnSO_4$ under normal and salt stress condition, as far as biomass of shoot and root is concerned. Under saline conditions, Fsd-08 yielded high shoot fresh biomass than variety Galaxy-13 at 2 m*M* $ZnSO_4$ treatment. Root fresh weight increased and then decreased with increase in the level of pretreatment. An elevated increase in the attribute was noticed at 1 m*M* $ZnSO_4$ priming in variety Fsd-08 under NaCl stress (Fig. 1). The varieties exhibited a decrease in root fresh biomass under salt stress. The varieties Fsd-08 and Galaxy-13 showed reduction in shoot (34%, 10%) and (20%, 31%) root dry biomass under NaCl stress respectively.

A decline in chlorophyll a (Chl_a) contents was recorded under salt stressed environment. However, grain priming with 5 m*M* $ZnSO_4$ in both genotypes prevented damage caused by stress (Fig. 2). Regarding chlorophyll b (Chl_b) contents (Fig. 2) Fsd-08 and Galaxy-13 showed 77% and 11% decline respectively under NaCl stress. However, increasing level of $ZnSO_4$ priming showed an amplified level in Chl_b concentration. Maximum Chl_b contents in Fsd-08 appeared at 5 m*M* followed by 10 m*M* $ZnSO_4$ under stress conditions.

In accordance with data analyzed, clear relation between salinity stress and plant pigments was observed. It could be seen that total chlorophyll contents were reduced due to imposition of salinity (Fig. 3) in both the wheat genotypes. This inhibitory effect of salinity was mitigated in both genotypes by seed priming. Salinity stress non-significantly affected carotenoid contents.

Experimental results and analysis of collected information revealed that grain preconditioning with $ZnSO_4$ enhanced growth in plants of wheat genotypes. The available Na^+ level rise in different tissues of plant body i.e. root and shoot under saline stress. The toxicity caused by salt stress ultimately disturbed the growth and ionic attributes. Conversely, 10 m*M* $ZnSO_4$ application under stress conditions manifested a significant reduction of Na^+ content in studied genotypes.

In accordance with collected data and its analysis, K^+ level in root and shoot reflected a considerable variation. This variability in estimated K^+ content in control and saline conditions represent a clear difference caused by grain preconditioning with varying levels of $ZnSO_4$. The uptake of K^+ in shoot improved with $ZnSO_4$ priming levels. Under NaCl stress conditions, the K^+ in variety Galaxy-13 shoot was maximum at 10 m*M* Zn, while in Fsd-08 highest shoot K^+ content was observed at 5 m*M* Zn (Fig. 4). On the other hand, a reduction in K^+ contents was observed in shoot of Fsd-08 and Galaxy-13 (46 % and 17%) under NaCl stress.

The varieties exhibited 11% (Fsd-08) and 17% (Galaxy-13) reduction in K^+ content of roots with imposition of NaCl stress, whereas, an elevated root K^+ contents were observed at 2 m*M* and 5 m*M* $ZnSO_4$ respectively, under stress conditions. Significant results were obtained for Zn concentration in shoot and root under both saline and non-saline conditions (Fig. 4). A 53% and 38% decline in shoot Zn contents was observed in Fsd-08 and Galaxy-13 under salt stress. Maximum Zn contents were estimated at 10 m*M* followed by 5 m*M* $ZnSO_4$ under NaCl stress. An increase in root Zn content was observed with $ZnSO_4$ priming under saline conditions.

Root Na was positively correlated with shoot Na as shown in Table 2 (Figs. 5 & 6). However, root Na and shoot Na were negatively correlated with other parameters (Table 2). The principal component analysis showed that main two axis factors F1 and F2 contribute 82.43%. The major contribution was of F1 (73.55%). Sodium uptake was positively correlated showing that an imposition of NaCl increased uptake from root to shoot. Root Zn and shoot Zn have negative correlation with root and shoot Na. Shoot Zn is closely related to shoot K. Root Zn is closely related to root K.

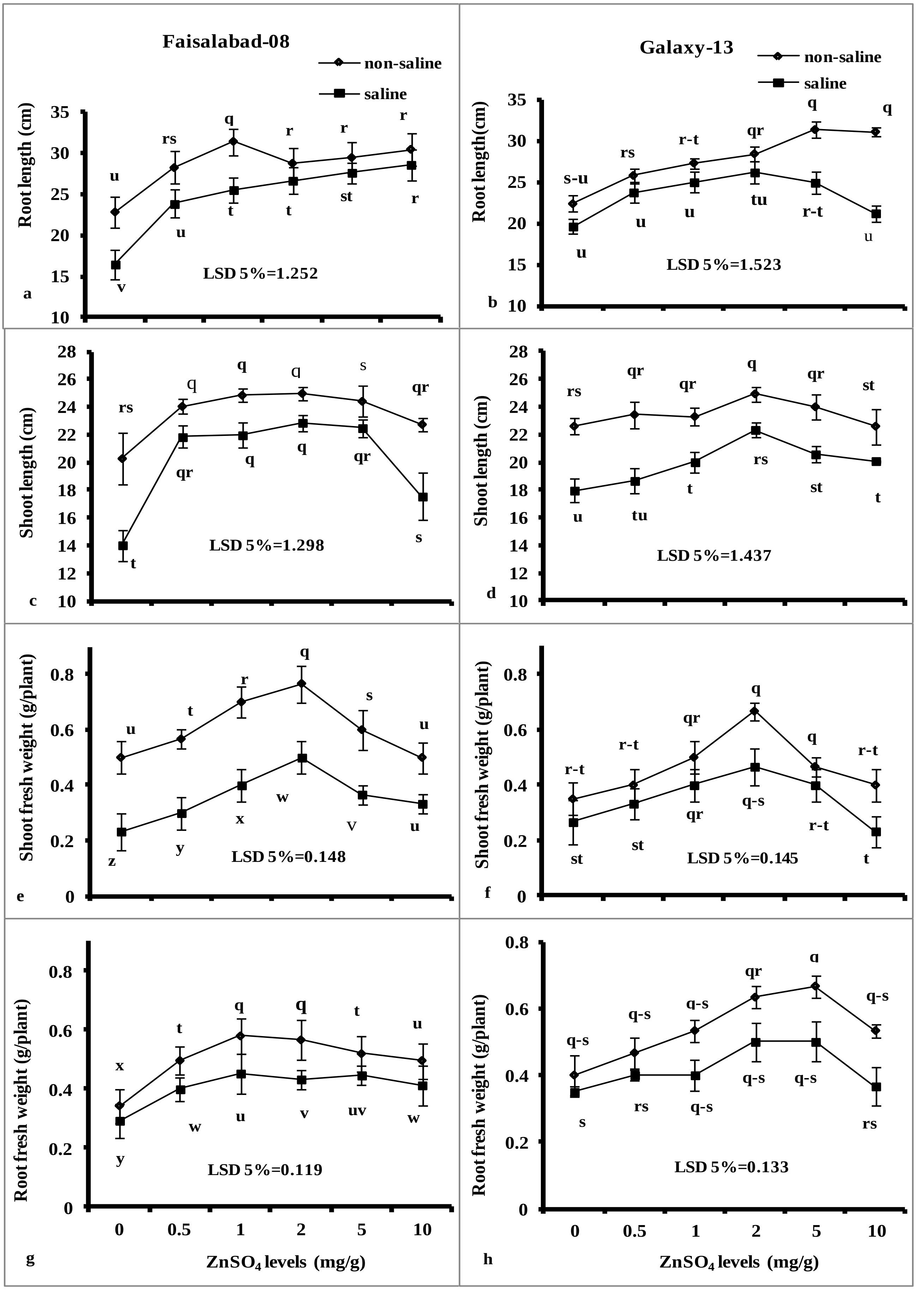

Fig. 1. Influence of $ZnSO_4$ grain priming on growth attributes of wheat varieties Faisalabad- 08 and Galaxy-13 under saline (100 m*M*) and control conditions.

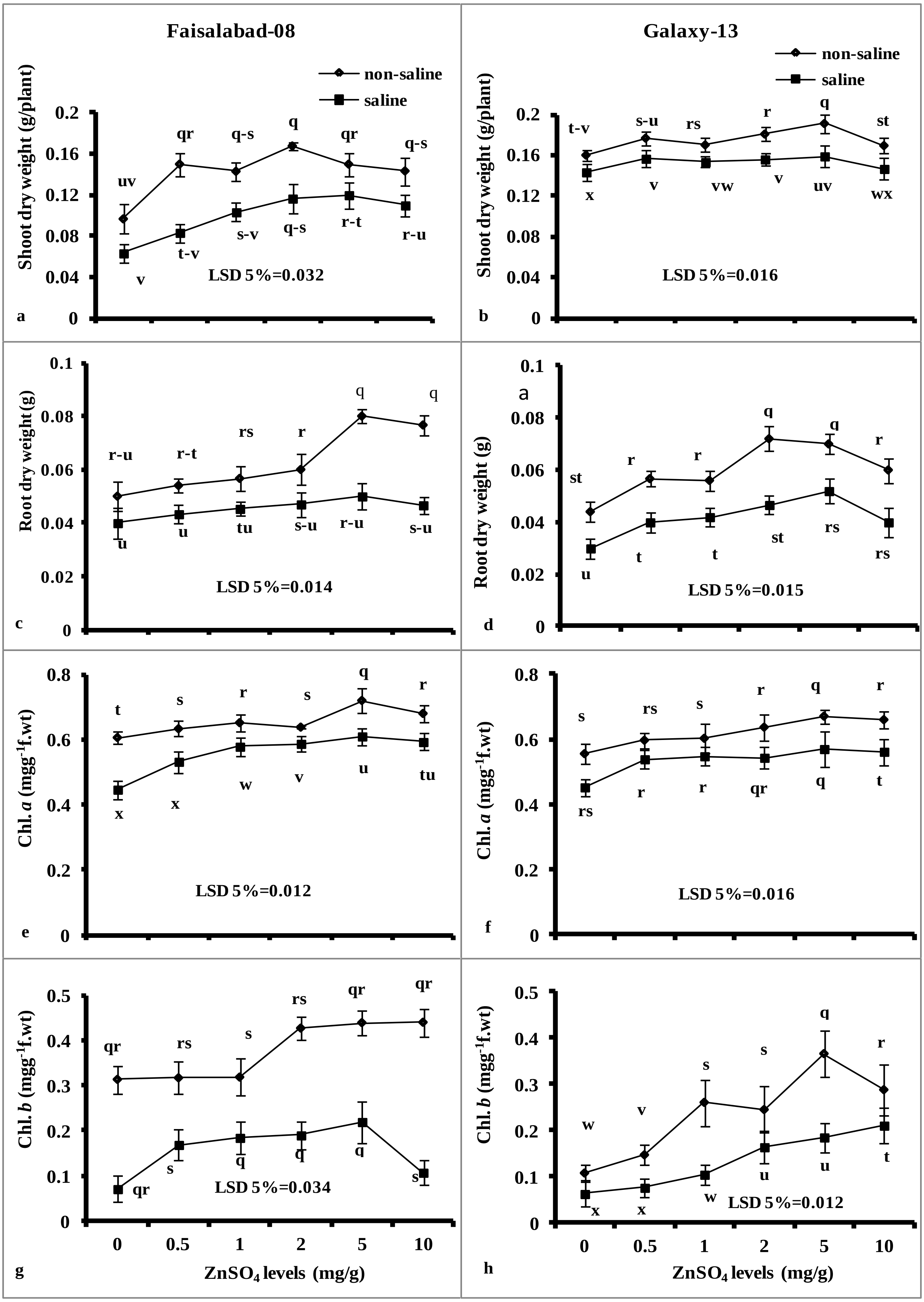

Fig. 2. Influence of $ZnSO_4$ grain priming (0, 0.5, 1, 2, 5 and 10 m*M*) on morpho-biochemical attributes of varieties Fsd- 08 and Galaxy-13 under saline (100 m*M*) and control conditions.

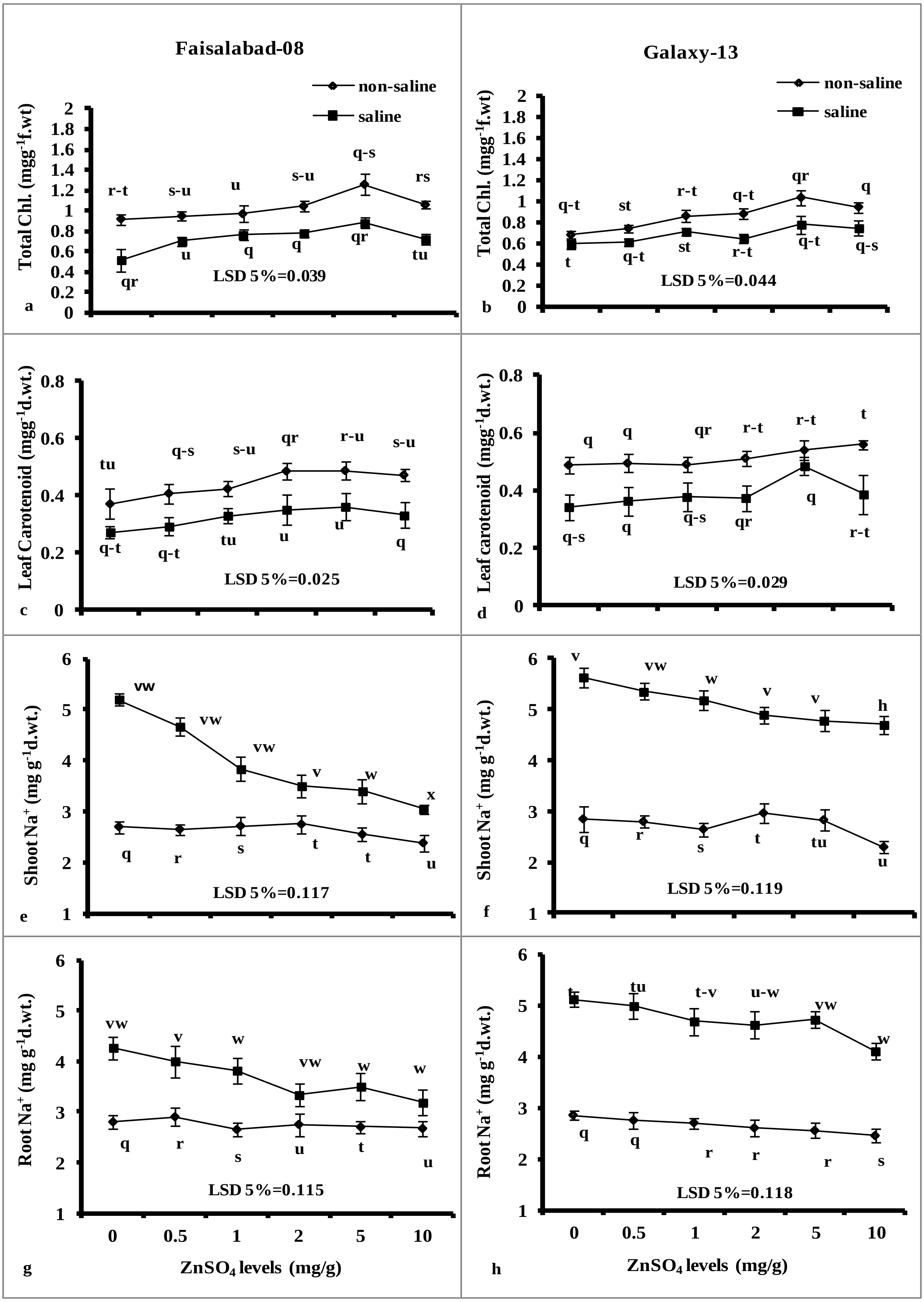

Fig 3. Influence of ZnSO$_4$ grain priming (0, 0.5, 1, 2, 5 and 10 m*M*) on pigments and ionic attributes of wheat varieties Fsd- 08 and Galaxy-13 under saline (100 m*M*) and control conditions.

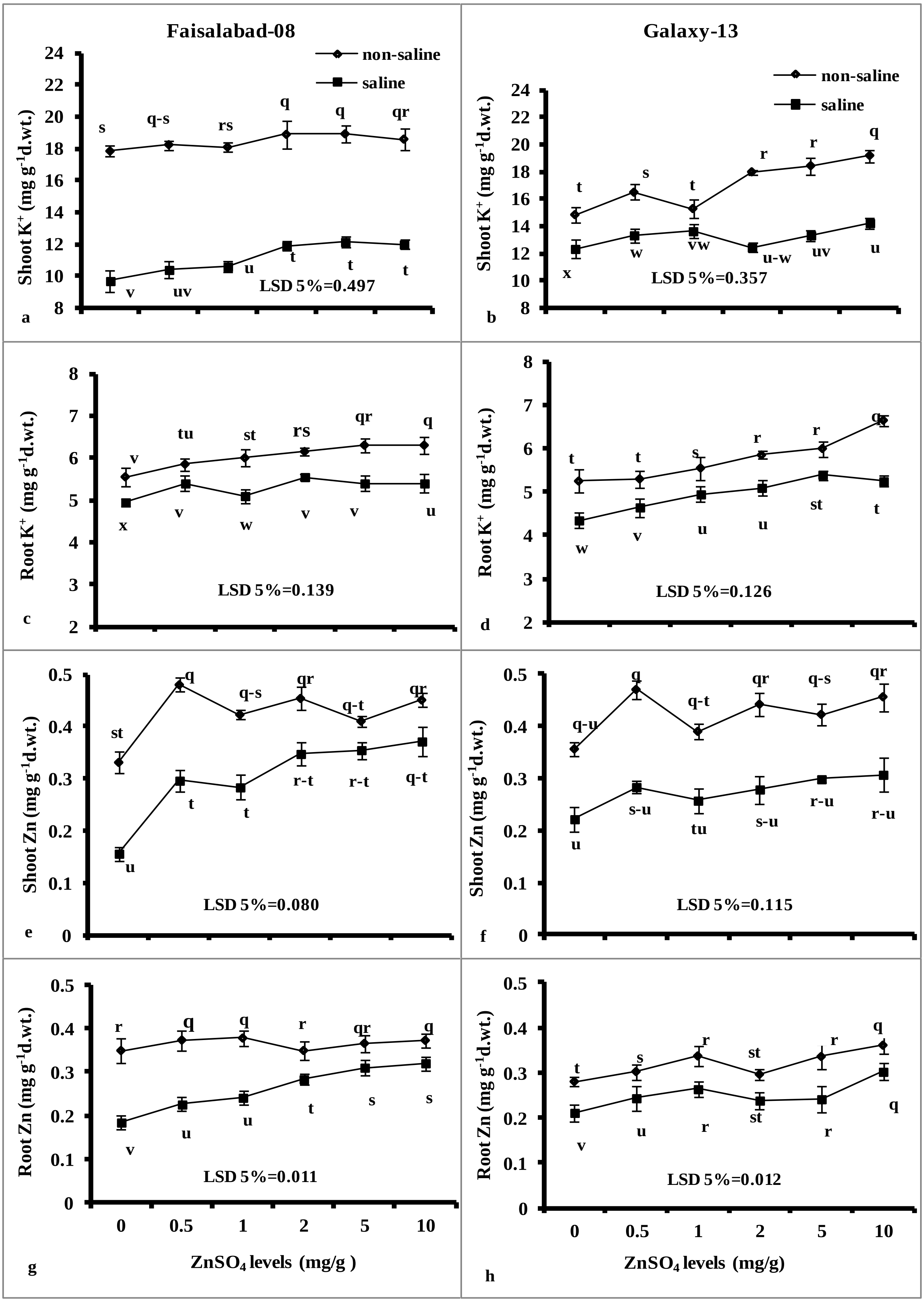

Fig. 4. Influence of $ZnSO_4$ grain priming (0, 1.5, 3, 5, 10 and 15 m*M*) on nutrient analysis of wheat varieties Fsd- 08 and Galaxy-13 under saline (100 m*M*) and control conditions.

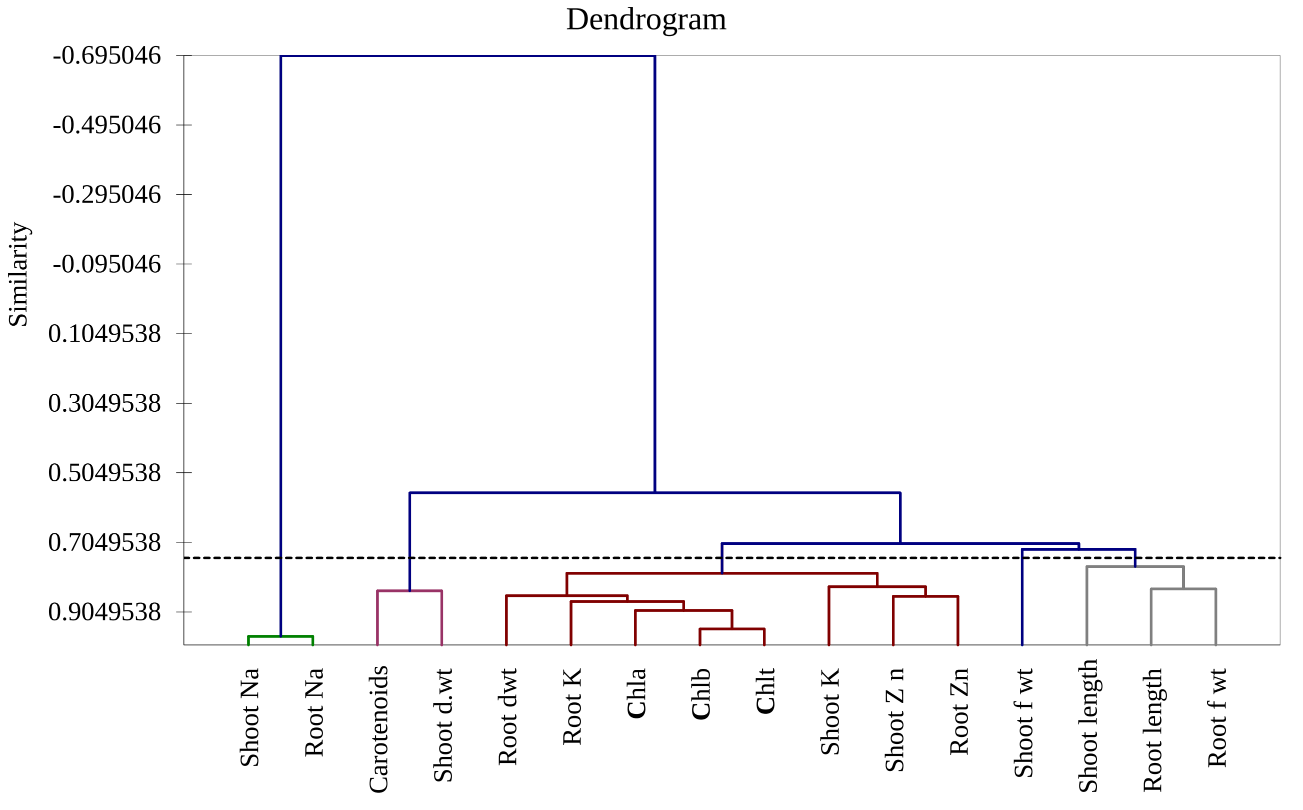

Fig. 5. Dandogram showing relationship among different physiological traits under salinity stress Principal component analysis (PCA).

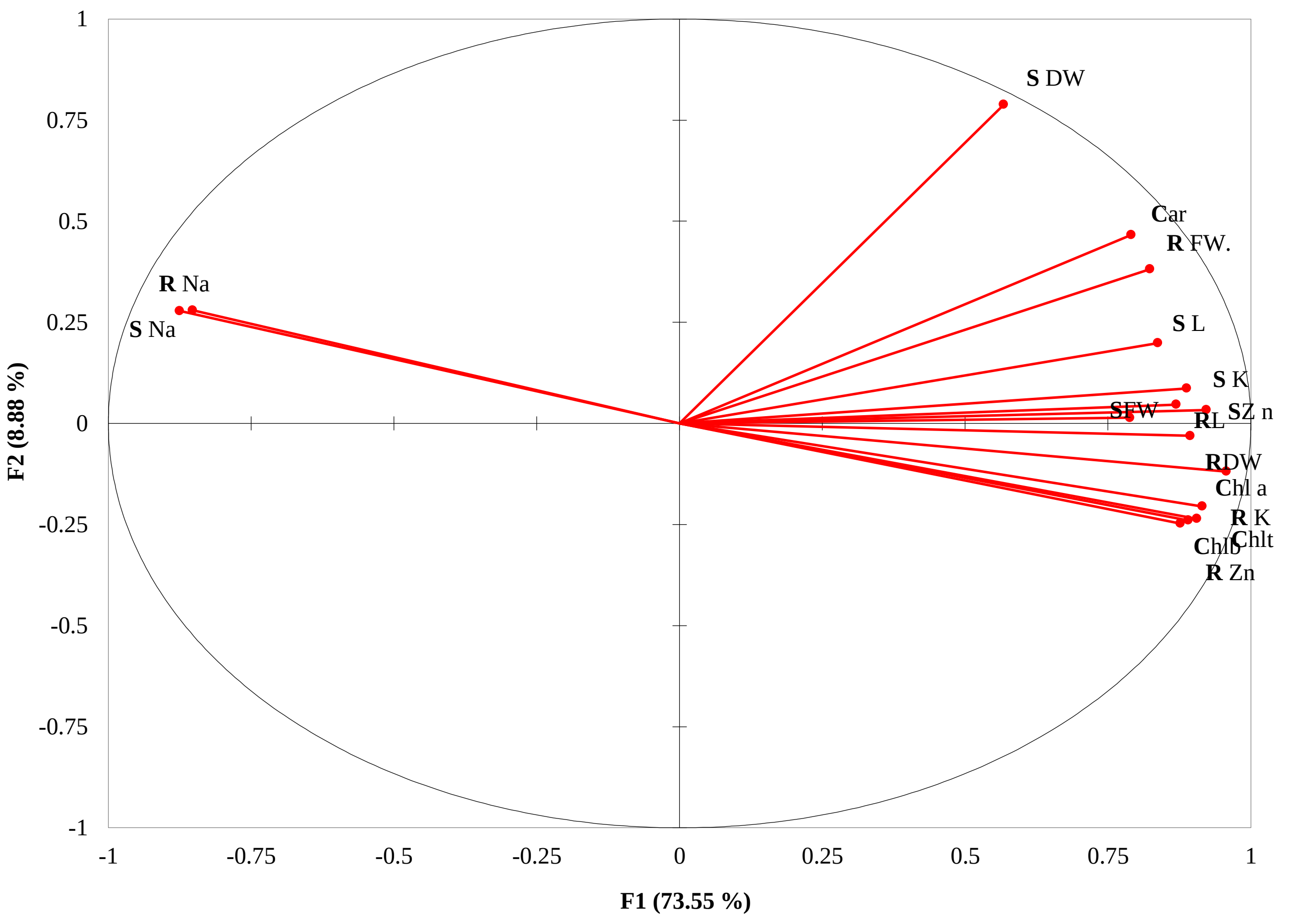

Fig. 6. Biplot analysis showing physiological traits as salt tolerance indicators.

Table 2. Correlation coefficient between physiological traits for salinity tolerance

	SL	R L	SFW	SDW	RFW	RDW	Chl. a	Chl. b	Chl.T	Car.	NaS	NaR	KS	KR	SZn	RZn
SL	1															
RL	0.794***	1														
SFW	0.715***	0.688***	1													
SDW	0.561***	0.575***	0.626***	1												
RFW	0.717***	0.778***	0.638***	0.543***	1											
RDW	0.478***	0.375**	0.399***	0.246*	0.647***	1										
Chl. a	0.615***	0.673***	0.596***	0.770***	0.588***	0.320**	1									
Chl. b	0.620***	0.675***	0.722***	0.823***	0.537***	0.186ns	0.762***	1								
Chl. T	0.657***	0.718***	0.708***	0.849***	0.589***	0.243*	0.902***	0.964***	1							
Car	0.570***	0.491***	0.486***	0.647***	0.706***	0.766***	0.608***	0.581***	0.617***	1						
NaS	-0.613***	-0.627***	-0.603***	-0.704***	-0.559***	-0.127ns	-0.749***	-0.759***	-0.802***	-0.593***	1					
NaR	-0.574***	-0.488***	-0.484***	-0.615***	-0.49***	-0.092ns	-0.653***	-0.684***	-0.711***	-0.602***	0.919***	1				
KS	0.538***	0.545***	0.676***	0.718***	0.527***	0.484***	0.727***	0.801***	0.807***	0.800***	-0.747***	-0.729***	1			
KR	0.436***	0.681***	0.489***	0.636***	0.568***	0.188ns	0.681***	0.747***	0.762***	0.613***	-0.779***	-0.737***	0.726***	1		
SZn	0.694***	0.677***	0.595***	0.657***	0.642***	0.345**	0.710***	0.687***	0.737***	0.702***	-0.903***	-0.863***	0.802***	0.721***	1	
RZn	0.164ns	0.248*	0.096ns	0.001ns	-0.018ns	-0.134ns	0.166ns	0.169ns	0.189ns	-0.092ns	-0.291*	-0.17ns	0.092ns	0.161ns	0.253*	1

Discussion

A decline in growth was noticed in both genotypes under NaCl salinity stress. The reduction in growth attributes under NaCl stress has been documented (Akbarimoghaddam *et al.*, 2011; Aytac *et al.* 2014; Zafar *et al.*, 2019). The study showed that NaCl stress supppressed growth of wheat plants. However, $ZnSO_4$ priming ameliorated the affect of stress (Gholami *et al.*, 2015). It was also noticed that the effect of Zn application may be genotype specific (Seyedi *et al.*, 2012; Aytac *et al.*, 2014).

The NaCl stress disturbed the photosynthetic attributes by decreasing chlorophyll contents and hampered K and Zn uptake as reported by Rahdari & Hoseini (2011). According to Zafar *et al.*, (2018) salinity causes imbalance of ions, disturbs plant metabolism and interferes with growth of plants (Zafar *et al.*, 2018).

As mentioned above, preconditioning of grains with $ZnSO_4$ improved growth and chlorophyll contents under salt stress in wheat. Grains preconditioning is beneficial for enhancing seed germination under abiotic stress conditions as reported by Nawaz *et al.*, (2013). It has been revealed by extensive experimentation that salinity results in reduction of growth and photosynthesis in direct relation with accumulation of Na^+ in plant parts (Ashraf & Harris, 2013). According to Khan *et al.*, (2006) priming application increase uptake of ions and yield of crops (Kaya *et al.* 2006). Similar findings by priming applications were reported by Harris *et al.*, (2008). Priming with $ZnSO_4$ under NaCl salinity stress maintained the appropriate chlorophyll contents. Our findings are in accordance with the work of Weisany *et al.*, (2011). It is reported that, the activation of carbonic anhydrase activity results in boosting up photosynthesis. In fact this rise in photosynthetic rate and pigment preservation during salinity exposure can likely be attributed to exogenous supply of Zn. Zinc being a constituent of RUBISCO enzyme contributes essentially during assimilation process and necessarily plays a role for structural constituent of membranes and regulatory co-factor for proteins in plants (Cakmak, 2000). Similar to our results, Carter & Knapp (2001) observed a positive correlation between chlorophyll contents, photosynthesis and plant productivity. Plants raised from seeds treated with Zn have shown a better pigment content. Kanwal *et al.*, (2011) and Ashraf (2009), attributed this betterment in pigments responsible for more photo-assimilation and finally to high yields. Zinc prevented plants from photo oxidative damage by helping in consumption of excess light. It is suggested that under salt stress photo oxidative damage to assimilatory machinery in plants leads to suppression of photosynthesis (Guruani *et al.*, 2015). Zinc plays very positive role in combating hazards caused by salt stress in agricultural systems. Salinity maintains the ionic homeostasis in plant tissue, when Na^+ gets accumulated and interferes with the K^+ uptake for binding site. This imbalance in ionic status of wheat genotypes can be confirmed by earlier reports El Fouly *et al*., (2010). Although root and shoot biomass is reduced under saline conditions, yet Zn application emerges beneficial by maintaining biomass of both plant parts. A reduction in dry matter is observed under NaCl stress. An increment in biomass is observed with increasing concentration of $ZnSO_4$ grain preconditioning as reported by Kumar & Qureshi (2012) in wheat. Exploring the consequences of Zn deficiency unraveled the fact that root plasma membrane permeability was increased under saline environment leading to increased Na^+ uptake as reported by Morshedi & Farahbakhsh (2010). The rise in root and shoot fresh as well as dry biomass by Zn presowing treatment was helpful in maintaining permeability of plasma membrane under high salt contents in rooting medium.

Conclusion

Preconditioning of grains with $ZnSO_4$ in wheat genotypes, especially with 5 m*M* Zn grain priming effectively enhanced the Zn and K uptake in plants and maintained growth by improving chlorophyll contents and morphological attributes. Grain priming is an important strategy for agronomic biofortification, maintenance and improvement of Zn nutritional status of crops especially in saline areas and can prove a reliable strategy for maintaining plant growth and obtaining higher yields.

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