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EVALUATION OF PHYSIOLOGICAL PARAMETERS AND ZINC CONTENT IN BREAD AND DURUM WHEAT GENOTYPES UNDER DIFFERENT ZINC DOSES

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ABSTRACT
Zinc is an essential micronutrient involved in a wide variety of physiological processes. Zinc deficiency in soils and plants is a global micronutrient deficiency problem reported in many countries. The present investigation was conducted to study the effect of zinc deficiency on zinc content and physiological traits. Two *Triticum aestivum* genotypes *viz*. WH-147 and WH-1061 and two *Triticum durum* genotypes *viz.* WH-896 and WH-912 were grown in varying levels of zinc *i.e.* 100% Zn, 50% Zn and 25% Zn. Zinc deficiency induced the reduction in relative water content, chlorophyll stability index, chlorophyll fluorescence. Less reduction was observed in WH-1061. Photosynthetic rate, transpiration rate, stomatal conductance and water use efficiency were low in zinc- deficient conditions. WH-1061 showed better performance in terms of transpiration rate and stomatal conductance at different levels of zinc as compared to other genotypes. The level of electrolyte leakage increased with the decrease of zinc level. Among all the genotypes the maximum level of electrolyte leakage was noted in WH-912 at 25% Zn level. Results indicated that with the decreasing level of zinc, zinc content decreased in grains and leaves at both stages *i.e*. vegetative and maturity. However, with decreasing level of zinc, zinc content increased in roots. Bread wheat genotype (WH-1061) was found to be more tolerant under Zn deficit conditions than durum wheat genotypes.

KEYWORDS: Bread and durum wheat, Physiological traits and Zinc dose.

INTRODUCTION

Wheat is one of the three major cereal crops worldwide and represents a main dietary source of calories, proteins and micronutrients for the majority of world's population, especially in the developing world (Shewry, 2009). Wheat is the chief source of plant based human nutrition and is a part of our daily dietary needs. Being a staple food, it is cultivated on about eight million hectares in the country with 13.7% contribution to the value addition in agriculture sector and 3% in the gross domestic products (Nawab *et al.,* 2011; Nadim *et al.,* 2013). Micronutrients help in chlorophyll formation, nucleic acid, protein synthesis and play an active role in several enzymatic activities of photosynthesis (Reddy, 2004; Rehm and Sims, 2006). They are needed in trace amounts but their adequate supply improves nutrients availability and positively affects the cell physiology that is reflected in yield as well (Taiwo *et al.,* 2001; Adediran *et al.,* 2004). Zinc (Zn) is an essential micronutrient involved in a wide variety of physiological processes (Baccio *et al.,* 2005; Broadley *et al.,* 2007). Zn is known to have an important role either as a metal component of enzymes or as a functional, structural or regulatory cofactor of a large number of enzymes (Grotz and Guerinot, 2006).

Zn deficiency in soils and plants is a global micronutrient deficiency problem reported in many countries (Alloway, 2004). Low Zn status of soils is well recognized as a nutritional constraint to crop production worldwide, particularly on calcareous soils of arid and semi-arid regions (Rashid, 2006). An estimated 50% of all soils used for cereal production throughout the world are low in available Zn, which detrimentally affects grain yield and quality (Gao *et al.,* 2006). In these high pH calcareous soils, Zn concentrations in soil solution are extremely low, with most soil Zn present in chemical forms largely inaccessible for uptake by plant roots (Cakmak, 2008; Ahmadikhah *et al.,* 2010; Narimani *et al.,* 2010; Heidarian *et al.,* 2011 and Daneshbakhsh *et al.,* 2013).

Many researchers reported that the use of micronutrients have a promising role in growth and development of crop plants which resulted in improved quality and quantity of the agricultural produce. Zn deficiency induces reduction in net photosynthesis by 50 to 70% depending on plant species and extent of deficiency (Hu and Sparks, 1991; Brown *et al.,* 1993). Chlorophyll (Chl) fluorescence yield is a sensitive indicator of changes in thylakoid membrane integrity caused by environmental stresses (Bukhov 2004; Weng *et al.,* 2008). Chl fluorescence measurements have been used to estimate, rapidly and non-invasively, the operating quantum efficiency of electron transport through photosystem II (PSII) in leaves of plants. This PSII operating efficiency is related to CO_2 assimilation (Maxwell and Johnson, 2000; Sayed, 2003; Baker and Rosenqvist, 2004; Lu and Lu, 2004). Zn nutrition caused increase in stomatal aperture as Zn is thought to be involved in stomatal regulation due to its role in maintaining membrane integrity (Khan *et al.,* 2004). Sharma *et al.* (1995) reported the involvement of Zn in

stomatal opening being the constituent of carbonic anhydrase which is required for maintaining adequate HCO_3 in the guard cells and also a factor affecting the K+ uptake by the guard cells. In addition, the accumulation of saccharides in leaves may be an important factor for the inhibition of photosynthesis under Zn- deficiency (Marschner, 1995; Cakmak 2000). This study has been conducted to study the impact of zinc deficiency on zinc uptake and physiological parameters in leaves of control and zinc deficient plants.

MATERIALS & METHODS

Investigation was carried out on two genotypes of bread wheat (*Tritium aestivum* L.) *viz*. WH-147 and WH-1061 and two genotypes of durum wheat (*Triticum durum*) *viz*. WH-896 and WH-912 in the green house. The plants were supplied with nutrient solution (Hoagland and Arnon, 1950) at regular intervals.

Treatments

Plants were supplied with following modified nutrient solution for giving varying doses of zinc.

T_{100}%	(control)	(Hoagland solution containing required concentration of zinc)
T_{50}%		(Hoagland solution containing half the concentration of zinc)
T_{25}%		(Hoagland solution containing one-fourth concentration of zinc)

Physiological Parameters

Relative water content (RWC) was measured by the method of Barrs and Weatherley (1962). Chlorophyll stability index (CSI) was estimated by the method of Murthy and Majumder (1962). The chlorophyll fluorescence was measured by using chlorophyll fluorometer (Model 0S30 p USA). Data was recorded between 10:00 to 12:00 a.m. Photosynthetic rate (mole m^{-2} sec^{-1}), transpiration rate (m mole m^{-2} sec^{-1}) and stomatal conductance (mol $m^{-2}s^{-1}$) were measured by using portable Infra Red Gas Analyser between 10.00 to 11.00 a.m. Water use efficiency (WUE) was determined by method of Rosenburg and Kriiger (1993) and it is ratio of P (photosynthetic rate) and E (transpiration rate). The relative intactness of plasma membrane was measured as the leakage percentage of electrolytes, as described by Gong *et al*. (1998). The leakage percentage of electrolytes was calculated as 1- EC1/ EC2×100%.

Estimation of Zinc content

500mg of plant material (leaves, roots and grains) was weighed in a flask. Then 20 ml of diacid mixture ($4HNO_3$: 1 $HClO_4$) was added to it and kept overnight. The sample was digested by heating gently until clear colourless solution was obtained. The obtained solution was transferred to 50ml volumetric flask and volume was made upto the mark by adding double distilled water. The solution was filtered using Whatman filter paper No. 1 and used for analysis of zinc content by Atomic Absorption Spectrophotometer (Model PERKIN-ELMER 2380).

RESULTS & DISCUSSION

In the present investigation zinc deficiency caused a strong reduction in all investigated photosynthetic parameters which have a great impact on plant growth and development. Result presented in Fig-1 exemplifies that zinc scarcity led to decline in RWC. The highest reduction was in WH-912 and lowest in WH-1061. Decline in the chlorophyll stability in terms of loss of chlorophyll content (%) was noted in all the genotypes under zinc deficiency (Fig-2). This reduction could be either due to chloroplast destruction (Fulton *et al.*, 1965; Peru and Main, 1970), inhibition of chlorophyll synthesis or increased activity of chlorophyllase. Result presented in the present study indicated that the Chl fluorescence of leaf decreased with the decreasing level of zinc in all the four wheat genotypes (Fig-3). Highest per cent reduction was noted in WH-147 and lowest per cent reduction was observed in WH-1061. The results obtained in the present study are in conformity of the results reported by previous workers Wang and Jin (2005), Shi and Cai (2009), Kummerova *et al.* (2010) and Hajiboland and Amirazad (2010). Reduction in Chl fluorescence indicates that the potential maximal quantum yield of PS2 was inhibited by Zn-deficiency (Wang and Jin 2005).

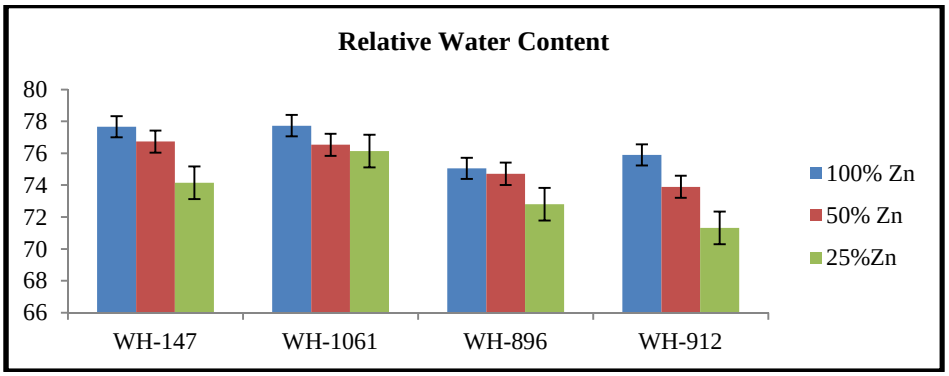

FIGURE I: The effect of different zinc doses on relative water content. Results are shown as mean ± standard error ($p<0.01$), obtained from three replicates

Gas exchange characteristics play vital role in yield stimulation. Photosynthetic rate (mol $m^{-2}s^{-1}$) showed decreasing trend as a result of decrease in Zn level (Fig-4). Highest reduction was noted in WH-1061 followed by WH-147. Similar results have been reported by Sharma *et al.* (1994), (1995) and Wang and Jin (2005) who found that Zn deficiency depressed photosynthetic capacity because of decrease in *gs*. Furthermore, the decrease in net

photosynthesis may also partly be attributed to the decrease in chlorophyll content and abnormal structure of chloroplast as a result of Zn deficiency. The decrease in transpiration rate (*E*) (Fig-5) because of decreasing Zn level substantiates the findings of Hu and Sparks (1991) and Sharma *et al.* (1994) who reported that Zn deficiency caused reduction in the instantaneous transpiration efficiency of leaves.

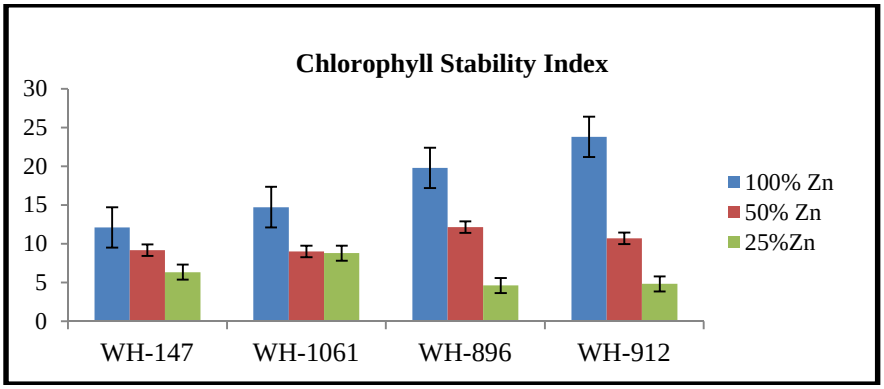

FIGURE II: The effect of different zinc doses on chlorophyll stability index. Results are shown as mean ± standard error ($p<0.01$), obtained from three replicates.

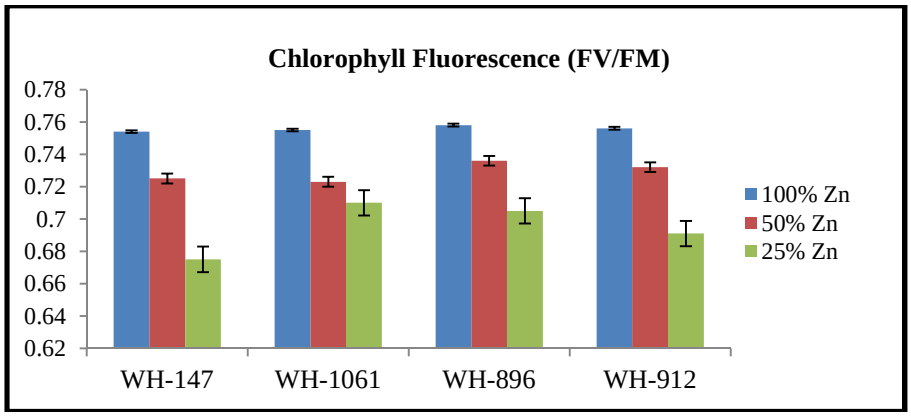

FIGURE III: The effect of different zinc doses on chlorophyll fluorescence. Results are shown as mean ± standard error ($p<0.01$), obtained from three replicates.

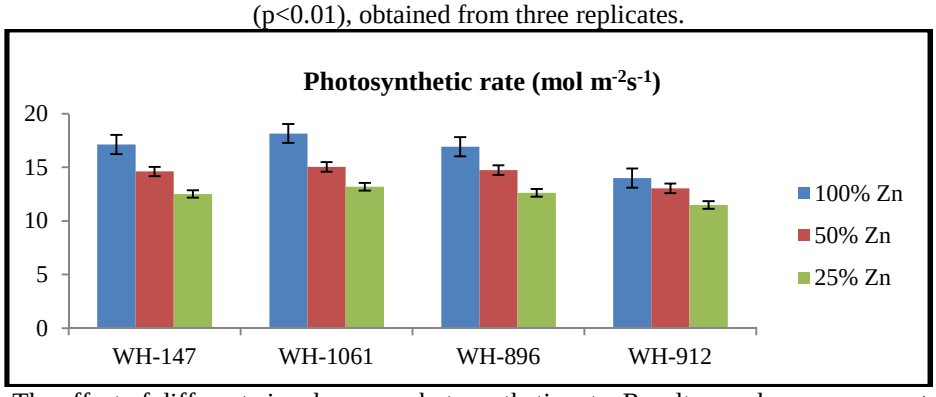

FIGURE IV: The effect of different zinc doses on photosynthetic rate. Results are shown as mean ± standard error ($p<0.01$), obtained from three replicates

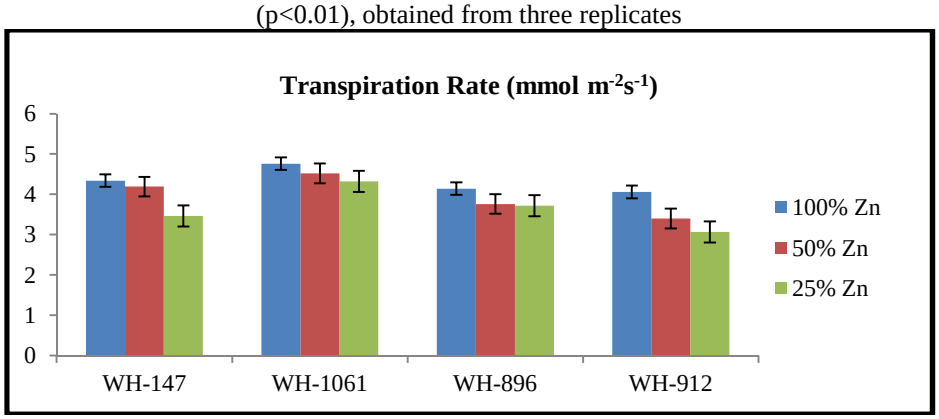

FIGURE V: The effect of different zinc doses on transpiration rate. Results are shown as mean ± standard error (p<0.01), obtained from three replicates.

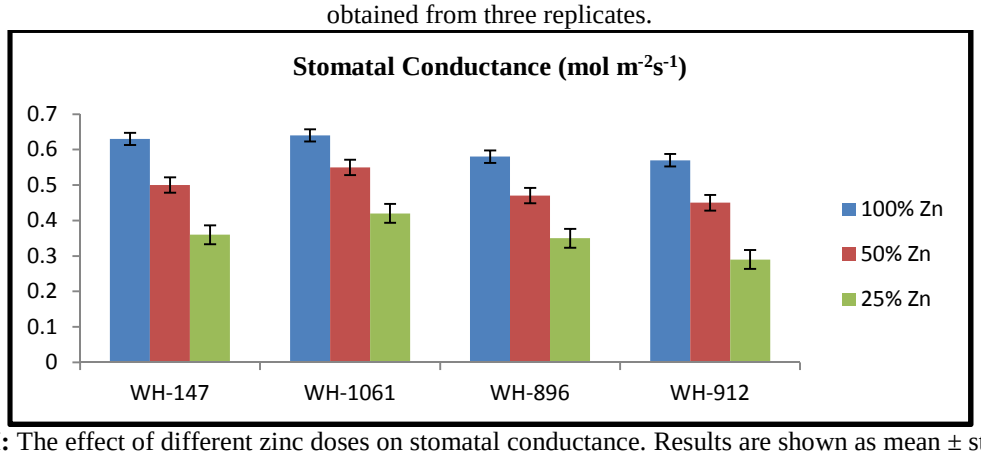

FIGURE VI: The effect of different zinc doses on stomatal conductance. Results are shown as mean ± standard error (p<0.01), obtained from three replicates.

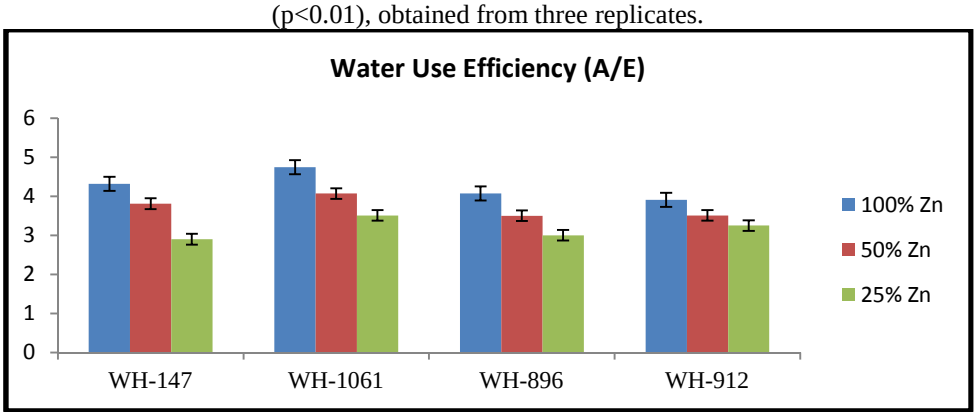

FIGURE VII: The effect of different zinc doses on water use efficiency. Results are shown as mean ± standard error (p<0.01), obtained from three replicates.

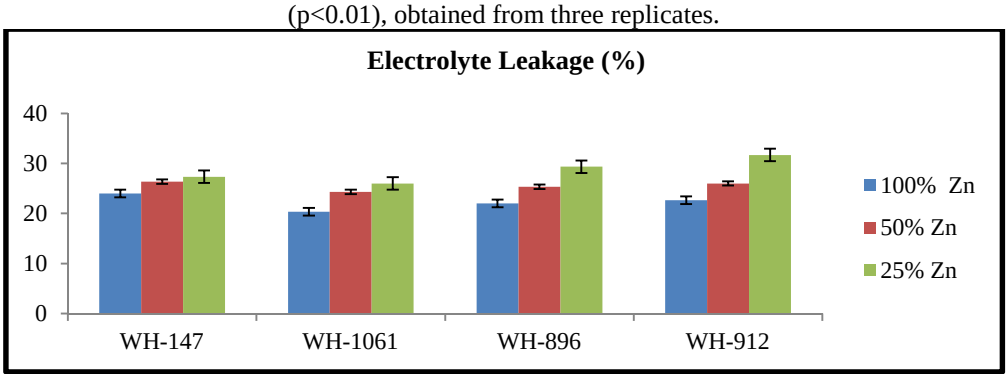

FIGURE VIII: The effect of different zinc doses on electrolyte leakage. Results are shown as mean ± standard error (p<0.01), obtained from three replicates.

WH-1061 showed better performance in terms of transpiration rate at different levels of Zn as compared to other genotypes. The stomatal conductance (mol $m^{-2}s^{-1}$) decreased with decreasing level of zinc (Fig-6). Maximum reduction was observed in WH-912 and lowest in WH-1061. The results of present investigation are in agreement with those of Khan *et al.* (2004), Wang and Jin (2005) and Hajiboland and Beiramzadeh (2008). Sharma *et al.* (1995) reported the involvement of Zn in stomatal opening being the constituent of carbonic anhydrase which is required for maintaining adequate HCO_3 in the guard cells and also a factor affecting the K+ uptake by the guard cells. Sharma *et al.* (1994) observed in cauliflower that reduction in photosynthesis induced by Zn deficiency is associated with a decrease in intercellular CO_2 concentration and stomatal conductance. Sharma *et al.* (1994, 1995) and Wang and Jin (2005) found that Zn deficiency depressed photosynthetic capacity because of decrease in *gs*. In addition, the accumulation of saccharides in leaves may be an important factor for the inhibition of photosynthesis under Zn-deficiency (Marschner 1995; Cakmak 2000). The reduction of chlorophyll content and the destruction of chloroplasts ultrastructure led to decrease in photosynthesis in Zn- deficient plants. The results of present study showed that WUE decreased with the decrease in Zn level (Fig-7). Highest per cent reduction was noted in WH-147 and lowest in WH-912. Similar results were earlier reported by Khan *et al.* (2003, 2004) and Wang and Jin (2005). Hatfield *et al.* (2001) observed that WUE could be increased from 15 to 25% by changing nutrient management practices. The level of electrolyte leakage increased with the decrease of Zn level (Fig-8). Among all the genotypes the maximum level of electrolyte leakage was noted in WH-912 at 25% Zn level. Also at the

level of 25% Zn other genotypes like WH-147, WH-1061 and WH-896 showed highest electrolyte leakage respectively.

In present studies analysis of zinc content in leaves (vegetative and maturity stage), roots (maturity) and grains (harvest stage) was done. Results indicated that with the decreasing level of zinc, zinc content decreased in leaves at both stages *i.e.* vegetative and maturity (Table 1, 2). Also, with decreasing level of zinc, zinc content was lowered in grains (Table 4). However, with decreasing level of zinc, zinc content increased in roots (Table 3). This might be due to the accumulation of zinc in roots which barred the uptake of zinc and as a result low Zn content was observed in leaves and grains. Similar response has been reported by Shi and Cai (2009) that Zn content in roots of Zn treatments were about 2.6- to 2.9-fold higher than that in the shoots.

TABLE I: Effect of different levels of zinc on zinc content (ppm) in the leaves of wheat at vegetative stage

Genotypes	Zinc content (ppm) in the leaves (vegetative stage)			
	Level of zinc			
	100%	50%	25%	Mean
WH-147	29.50	16.10	13.80	19.80
WH-1061	35.50	24.50	17.70	25.90
WH-896	27.30	12.80	7.50	15.20
WH-912	28.00	14.00	6.70	15.57
Mean	30.08	16.85	10.43	19.12
CD at 5%	Genotypes (A)=1.914; Treatments (B)=1.658; Interaction (A x B)=3.316			

TABLE II: Effect of different levels of zinc on zinc content (ppm) in the leaves of wheat at maturity

Genotypes	Zinc content (ppm) in the leaves (maturity)			
	Level of zinc			
	100%	50%	25%	Mean
WH-147	21.80	16.00	11.50	16.43
WH-1061	35.70	21.80	12.00	23.17
WH-896	20.40	11.80	8.30	13.50
WH-912	14.10	7.10	5.10	8.77
Mean	23.00	14.18	9.23	15.47
CD at 5%	Genotypes (A)= 1.471; Treatments (B)=1.274; Interaction (A x B)=2.548			

TABLE - III: Effect of different levels of zinc on zinc content (ppm) in the roots of wheat at maturity

Genotypes	Zinc content (ppm) in the roots (maturity)			
	Level of zinc			Mean
	100%	50%	25%	
WH-147	29.90	38.10	41.50	36.50
WH-1061	26.40	32.50	35.30	31.40
WH-896	34.20	44.90	46.10	41.73
WH-912	32.20	42.70	44.40	39.77
Mean	30.68	39.55	41.83	37.35
CD at 5%	Genotypes (A)=2.464; Treatments (B)=2.134; Interaction (A x B)=4.267			

TABLE IV: Effect of different levels of zinc on zinc content (ppm) in the grains of wheat

Genotypes	Zinc content (ppm) in the grains			
	Level of zinc			Mean
	100%	50%	25%	
WH-147	35	31	29	31.67
WH-1061	50	46	42	46.00
WH-896	42	38	33	37.67
WH-912	38	34	30	34.00
Mean	41.25	37.25	33.50	37.33
CD at 5%	Genotypes (A) =1.576; Treatments (B)=1.365; Interaction (A x B) = 2.729			

CONCLUSION

It is clear that differences were observed under varying zinc doses. Zinc deficiency significantly reduced the relative water content, chlorophyll stability index. Highest reduction was noted in WH-912. Photosynthetic rate (PN), transpiration rate (E), stomatal conductance (gs) and water use efficiency (WUE) decreased with decrease in level of zinc.WH-1061 showed better performance in terms of PN, E, gs at different levels of zinc as compared to other genotypes. The level of electrolyte leakage increased with the decrease of Zn level. Maximum level of electrolyte leakage was observed in WH-912 and minimum in WH-1061. Maximum mean of Zinc content in leaves (vegetative stage) was observed in WH-1061. Zinc content in roots increased with the decrease in zinc level. The highest level of Zn content was in the variety WH-896. Zinc content decreased in grains with the decrease in zinc level. Highest mean of zinc content was observed in WH-1061 and lowest in WH-912. Among bread wheat, WH-1061 was more tolerant than WH-147. Among durum wheat, WH-896 was more tolerant than WH-912.

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