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Differential growth response and zinc utilization efficiency of wheat genotypes in chelator buffered nutrient solution

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Abstract

Zinc (Zn) deficiency is a common micronutrient deficiency in arid and semi arid regions of the world. A solution culture study was conducted to categorize twelve wheat genotypes according to their Zn utilization efficiency. The wheat genotypes were grown in a half strength Johnson's modified nutrient solution supplied with adequate (2 μ M) and deficient (0.2 μ M) Zn level. There was a significant effect of Zn level and genotypes on biomass production and Zn concentration. Wheat genotype Sehar-06 produced maximum shoot biomass under both adequate and deficient Zn condition and it was minimum in Vatan. Sehar-06 maintained higher Zn contents at deficient Zn level compared to other genotypes. Genotypes categorized according to their Zn utilization efficiency at deficient Zn supply fell into five categories. Sehar-06 and Auqab-2000 were categorized as MDM-ME (medium dry matter with high Zn utilization efficiency) category. Genotypes with better growth performance and Zn utilization efficiency as Sehar-06 and Auqab-2000 can be recommended for Zn deficient soils and for breeding purposes.

Key words: Wheat, categorization, zinc utilization efficiency, Zn deficiency

Introduction

Zinc (Zn) deficiency is a common micronutrient disorder in arid and semi arid regions of the world (Takkar and Walker, 1993) including Pakistan (Khattak and Perveen, 1985) because of low Zn solubility and high Zn fixation under such conditions (Lindsay, 1979). According to Rahmatullah et al., (1988), a significant amount of Zn is present in soil matrix but only a small fraction of that is available to plants. Wheat a major staple cereal in the world, is the dominant winter crop of Pakisatan. Yield reduction in wheat (Triticum aestivum L.) due to Zn deficiency has been reported in several countries of the world (Graham et al., 1992). Zinc status of a plant can be improved by applying organic and inorganic fertilizers. But there are several constraints in application of fertilizers. One being the increasing cost of Zn based fertilizers. Secondly applied Zn fertilizer undergoes a number of chemical reactions which reduce its availability to plants (Rahmatullah et al., 1988).

An alternate approach to combat Zn deficiency is tailoring plants to suit soil conditions. Tailoring plants here refers to improvement of nutrient use efficiency. Scientists have identified crop genotypes that are able to grow and produce higher yield in Zn deficient soils (Graham *et al.*, 1992; Graham and Rengel, 1993). Thus exploitation of the plants' genetic capacity for efficient nutrient uptake and utilization is a promising tool to cope with nutrient deficiency stress (Irshad *et al.*, 2004). Selection and breeding for increased Zn efficiency is a promising strategy to sustain crop productivity in low input and environmental friendly agricultural production systems (Cakmak *et al.*, 2001). Genotypes that are more efficient in Zn acquisition from deficient conditions are generally considered better adaptable to Zn deficiency in soils. Sufficient genetic variability exists among several crop species and genotypes for Zn acquisition and utilization under low Zn environments (Cakmak *et al.*, 2001; Irshad *et al.*, 2004).

Screening crop genotypes in hydroponic conditions does not allow us to simulate all soil related factors important in Zn uptake; however, it is a quick and effective method for evaluating Zn deficiency tolerance. Zinc is not only an essential micronutrient but also an omnipresent contaminant in the laboratory, making it quite difficult to impose Zn deficiency using regular nutrient solution techniques (Brown, 1986). The introduction of chelator buffered nutrient solution (Parker *et al.*, 1995) is a major advancement in studying micronutrient activities (Yang *et*

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al., 1994). It can be used reliably to distinguish levels of Zn efficiency among wheat genotypes (Graham and Rengel, 1993). According to Epstien (1972), hydroponics culture satisfies many requirements of screening for breeding plant genotypes to tolerate Zn deficiency by providing a homogenous growth medium that can be easily maintained and controlled. Keeping in view the above, a screening experiment was conducted in DTPA buffered nutrient solution to select Zn efficient and responsive wheat genotypes.

Materials and Methods

The experiment was conducted in a rain protected wire house under natural conditions. The temperature of wire house varied from a minimum of 7° C to a maximum of 22° C with a mean value of 12° C.

Plant Culture

Seeds of twelve wheat genotypes were collected from Ayub Agriculture Research Institute (AARI), Faisalabad. The seeds were sown in polyethylene lined iron trays containing washed river-bed sand. Optimum moisture for germination was maintained using distilled water. Uniform seedlings were transplanted, one week after germination in foam-plugged holes of thermopol sheets floating on continuously aerated 50 L half strength modified Johnson's nutrient solution (Johnson et al., 1957) in polyethylene lined iron tubs. The solution contained 6 mM N, 2mM P, 3 mM K, 2 mM Ca, 1mM Mg, 2 mM S, 50 µM Cl, 25 µM B, $2 \mu M$ Mn, $1 \mu M$ Cu, $0.05 \mu M$ Mo and $50 \mu M$ Fe. Two Zn levels i.e., adequate $(2 \mu M)$ with ZnSO₄.7H₂O and deficient $(0.2 \mu M)$ were maintained in nutrient solution. There were 6 tubs (3 tubs per treatment) and 24 plants in each tub. Zinc deficient level in 3 tubs was induced by addition of 50 μM DTPA with additional concentration of Fe, Cu and Mn. Hydrogen ion activity (pH) of nutrient solution in tubs was monitored daily and adjusted at 6.0 ± 0.5 with 1 N NaOH or 1 N HCl.

Harvesting

Plants were harvested 32 days after transplanting. They were washed in distilled water, blotted dry and separated into shoots and roots before air drying for 2 days. The samples were then oven dried at 65° C in a forced air driven oven for 48 h to record dry matter yield (g plant⁻¹).

Zinc Concentration

Dried shoots and roots were ground in a mechanical grinder (MF 10 IKA, Werke, Germany) to pass through a 1 mm sieve. Ground samples were then mixed uniformly. A 0.5 g portion of plant sample was digested in a di-acid mixture of nitric acid and perchloric acid (3:1) at 150°C

(Miller, 1998). Zinc concentration in shoot and root digest was determined using atomic absorption spectrophotometer (Perkin Elmer Aanalyst-100). Zinc contents (mg Zn plant⁻¹) were calculated in shoots and roots by multiplying Zn concentration in the respective tissue with dry matter and on whole plant basis by adding up shoot and root Zn contents.

Zinc Utilization Efficiency (ZnUE)

Zinc utilization efficiency $(g^2 \text{ SDM mg}^{-1} \text{ Zn})$ was calculated by the following formula (Siddiqui and Glass, 1981):

Zinc Utilization Efficiency =

1 X Shoot Dry matter

Categorization of Genotypes

Wheat genotypes were grouped into nine categories on the basis of genotypic mean (μ) and standard deviation (SD) for shoot dry matter (SDM) and shoot Zn utilization efficiency (ZnUE). The genotypes were assigned as low if their mean were less than μ -SD, medium if their mean is between μ -SD to μ +SD and high if cultivar mean were higher than μ +SD (Gill *et al.*, 2004).

Statistical Analysis

The data were subjected to statistical analysis using computer based software "MS-Excel" and "MSTAT-C" (Russell and Eisensmith, 1983). Completely randomized factorial design was employed for analysis of variance (ANOVA). Least significant difference (LSD) test was used to separate the treatment means (Steel and Torrie, 1980).

Results and Discussion

Biomass Production and Partitioning

Shoot dry matter (SDM) of wheat genotypes varied significantly ($P \le 0.01$) and ranged between 1.08 g plant⁻¹ in Vatan and 4.60 g plant⁻¹ in Sehar-06 (Table 1). Sehar-06 produced the maximum SDM grown either at adequate or deficient level of Zn, while minimum SDM was produced by Vatan and Dirk. Differences in SDM among different crop genotypes with Zn application to rooting medium had also been observed by other researchers (Maqsood *et al.*, 2009; Cakmak *et al.*, 2001; Irshad *et al.*, 2004). Genotypes differed significantly (P<0.01) for their root dry matter (RDM) production and ranged from 0.18 to 0.96 g plant⁻¹ (Table 1). Similar to shoot, maximum RDM was observed for Sehar-06 when grown under Zn deficient conditions. Minimum RDM was produced by Dirk. Higher biomass production by genotypes such as Sehar-06 and Auqab-2000

under Zn deficient conditions as compared to other genotypes indicated relative tolerance of these genotypes against Zn deficiency in the growth medium (Graham *et al.*, 1992).

and cotton (Irshad *et al.*, 2004) had been reported for shoot Zn concentration under varying level of available Zn in root medium. There were significant (p<0.01) main and interactive effects of genotypes and Zn levels on shoot Zn

Table 1. Biomass production and portioning among wheat genotypes grown with adequate (2 μ M) and deficient (0.2 μ M) Zn nutrient solution

Genotype	Shoot d (g p	ry matter lant ⁻¹)	Root di (g p	ry matter lant ⁻¹)	Root: shoot ratio		
	2 µM Zn	0.2 μM Zn	2 µM Zn	0.2 µM Zn	2 μM Zn	0.2 µM Zn	
Inqalab-91	1 3.15 2.0		0.51	0.63	0.16	0.24	
Bhakar-2000	ar-2000 2.97		1.85 0.43		0.14	0.29	
Pari-73	3.19	2.07	0.39	0.58	0.12	0.29	
Yaqora	3.15	2.43	0.37	0.64	0.12	0.26	
As-2002	3.29	2.42	0.48	0.80	0.15	0.32	
Shafaq-06	3.51	2.45	0.53	0.53	0.15	0.22	
Auqab-2000	3.42	2.95	0.50	0.50 0.79		0.27	
Sehar-06	4.60	3.25	0.68	0.68 0.96		0.30	
Dirk	1.95	1.14	0.17 0.20		0.09	0.19	
Iqbal-2000	2.55	2.17	0.33	0.62	0.13	0.29	
Vatan	1.48	1.08	0.33	0.62	0.22	0.57	
SARC-1	4.01	2.30	0.67	0.99	0.16	0.43	
Mean	3.11	2.23	0.45	0.66	0.14	0.31	
LSD _{0.05} (Genotype x Zn Level)	1.63		0.46		0.17		

Significant variations were also observed in wheat genotypes for root: shoot ratio (RSR) (Table 1). Increase in root to shoot ratio was observed in all wheat genotypes when grown in Zn deficient conditions compared to adequate conditions. Root: shoot ratio ranged from 0.14 to 0.39. Maximum RSR was exhibited by Vatan and minimum by Dirk. Increased root growth at the expense of shoot is one of the possible mechanisms to cope with Zn deficiency in soil (Marschner et al., 1986). Effect of Zn deficiency was more pronounced on SDM than on RDM. This resulted in increased root:shoot ratio of all the genotypes when grown in Zn deficient nutrient solution. Since Zn is an immobile nutrient in soil and its movement is diffusion dependent, hence, increased root:shoot ratio equips plants with more root surface area for Zn absorption and exploration of root medium (Marschner et al., 1986).

Zinc Concentration and Uptake

Effects of genotypes and Zn levels on shoot Zn concentration of wheat genotypes were significant (P<0.01) (Table 2). Shoot Zn concentration was significantly lower in plants grown under Zn deficient conditions than under Zn adequate conditions. Shoot Zn concentration of wheat genotypes ranged between 18.71 μ g g⁻¹ in Shafaq-06 to 64.75 μ g g⁻¹ in Sehar-06. Significant genetic variability among wheat (Cakmak *et al.*, 2001; Maqsood *et al.*, 2009)

uptake (Table 2). Zinc uptake in shoots of wheat genotypes ranged from $24.59 \ \mu g \ plant^{-1}$ in Vatan and $298.40 \ \mu g \ plant^{-1}$ in Sehar-06.

Zinc concentration in roots reduced significantly (p<0.01) in plants grown in Zn deficient solution (Table 2). Maximum Zn concentration in roots was, 82.12 μ g g⁻¹, in Vatan and minimum was, 7.53 μ g g⁻¹, in Dirk. There were also significant (p<0.01) effects of genotypes and Zn levels on root Zn uptake (Table 2). Zinc uptake in roots of wheat genotypes ranged from 1.56 μ g plant⁻¹ to 35.41 μ g plant⁻¹.

Zinc Utilization Efficiency (ZnUE)

Genotypes differed significantly (P<0.01) for shoot ZnUE. Interaction between Zn supply and genotypes was non-significant. Shoot Zn utilization efficiency ranged between 26.93 to 141.63 g² SDM mg⁻¹ Zn in plants. There were significant (p<0.01) main and interactive effects of genotypes on root ZnUE of wheat (Table 2). It ranged from 4.10 g² SDM mg⁻¹ Zn in Vatan to 72.14 g² SDM mg⁻¹ Zn in Sehar-06. Differences among crop genotypes for Zn use efficiency had also been reported by Irshad *et al.*, (2004).

Categorization of Wheat Genotypes

Genotypes were classified on the basis of their SDM production and Zn uptake in Zn deficient conditions (Gill *et al.*, 2004). On these bases, the genotypes fell into five out

of nine categories viz i) low dry matter and low Zn utilization efficiency (LDM-NE), ii) medium dry matter with medium Zn utilization efficiency (MDM-ME), iii) medium dry matter with high Zn utilization efficiency (MDM-HE), iv) high dry matter with high Zn utilization efficiency (HDM-HE) and v) high dry matter with medium Zn utilization efficiency (HDM-HE) and v) high dry matter with medium Zn utilization efficiency (HDM-ME) (Fig. 1). Genotypes with HDM-HE were Sehar-06 and Auqab-2000. Both of these genotypes were efficient in Zn acquisition and its utilization for biomass production under low Zn availability (Table 1 & 2) hence these can be selected for soils with wide range of Zn concentrations.

Conclusion

Genotypes differed significantly for their biomass production and Zn contents. Significant interaction among genotypes and Zn application for biomass production and Zn contents indicated large genetic variations which can be exploited to select more Zn efficient wheat genotypes. Sehar-06 and Auqab-2000 were efficient in dry matter yield, and Zn contents both in shoot and root. Both Sehar-06 and Auqab-2000 can be recommended for Zn deficient soils and future breeding programs. However, field verification of results is warranted.

Table 2.	Concentration, uptake and	d utilization efficiency of Zn	in wheat genotypes g	rown in adequate (2 μM) and
	deficient (0.2 µM) Zn nutr	ient solution		

Genotype	Shoot Zn Conc. (µg g ⁻¹)		Root Zn Conc. (µg g ⁻¹)		Shoot Zn uptake (µg plant ⁻¹)		Root Zn uptake (µg plant ⁻¹)		Shoot ZnUE (g ² SDW mg Zn ⁻¹)		Root ZnUE (g ² RDW mg Zn ⁻¹)	
	2 µM Zn	0.2 μM Zn	2 µM Zn	0.2 μM Zn	2 µM Zn	0.2 μM Zn	2 µM Zn	0.2 μM Zn	2 µM Zn	0.2 μM Zn	2 µM Zn	0.2 μM Zn
Inqalab-91	55.64	19.51	48.04	14.70	175.80	52.15	24.63	9.26	57.14	136.85	10.68	43.50
Bhakar-2000	61.87	20.79	49.83	14.09	184.73	38.69	22.62	6.88	47.95	90.08	8.49	45.64
Pari-73	59.00	24.30	51.61	10.84	188.70	50.17	20.36	6.42	54.26	88.10	7.62	54.34
Yaqora	56.76	22.22	49.51	10.39	178.93	54.23	18.71	6.59	55.92	110.08	7.54	62.83
As-2002	55.80	26.86	57.51	12.15	186.30	64.50	28.67	9.35	58.41	92.88	8.26	71.32
Shafaq-06	63.31	18.71	57.13	11.79	222.48	44.85	30.50	6.28	55.58	141.63	9.32	46.34
Auqab-2000	54.04	19.83	48.31	12.15	185.81	58.58	24.43	9.61	63.35	148.74	10.30	69.24
Sehar-06	64.75	23.50	51.72	13.27	298.40	76.02	35.41	12.90	71.42	140.39	13.19	72.14
Dirk	51.16	25.26	41.57	7.53	100.50	29.72	7.24	1.56	38.27	45.51	4.32	26.49
Iqbal-2000	50.68	25.10	48.53	12.31	130.12	54.17	15.97	7.61	50.44	87.55	6.92	53.24
Vatan	55.64	22.86	82.12	9.35	81.96	24.59	26.73	5.90	26.93	47.99	4.10	66.63
SARC-1	61.07	25.10	36.62	10.55	242.28	57.97	26.48	10.10	67.00	91.68	17.96	39.98
Mean	57.47	22.83	51.87	11.59	181.33	50.46	23.48	7.70	53.89	101.78	9.06	54.30
LSD _{0.05}												
(Genotype x Zn Level)	13.23		15.50		98.56		20.20		62.06		39.25	

Genotypes of group LDM-NE (Vatan and Dirk) were least efficient in both biomass and Zn uptake. Zinc efficiency traits such as RDM and Zn use efficiency of genotypes in this group were lower than from genotypes falling in group HDM-HE. Genotype Inqalab-91 was medium in ZnUE but efficient in biomass production indicating its efficiency in Zn utilization. Shafaq-06 was medium in biomass production but accumulated more Zn, hence was less efficient in Zn utilization. All other genotypes were medium in biomass as well as Zn utilization efficiency (Figure 1).

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Figure 1. Categorization of wheat genotypes according to Zn utilization efficiency (ZnUE) and Shoot dry matter (SDM)

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