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Evaluating Pearl Millet (Hybrids) Genotypes for Zinc Use Efficiency through Fertilization Strategy Screening

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ABSTRACT

World over micronutrient deficiency is gaining an important notice that threatens human life. Selecting genotypes on the basis of higher yield and higher bioavailable zinc in grains as well as straw is required to simultaneously address Zn malnutrition in humans and animals besides satisfying the food demand. Attempts were made to screen various pearl millet (*Pennisetum glaucum*) genotypes for high zinc efficiency in potentially Zn deficient calcareous soil (zinc: 0.750 mg kg^{-1}) with mineral Zn fertilization in a greenhouse experiment during 2016-17 in the Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore, India. Eighteen pearl millet genotypes were tested at two levels of Zn (0 mg Zn kg^{-1} and 10 mg Zn kg^{-1}) in sandy clay loam soil (Vertic Ustropept). Results of the experiment revealed that the genotypes differed significantly in dry matter yield and zinc concentration. Based on the zinc use efficient indexing, genotypes were classified as efficient, inefficient, responsive and non-responsive. Among the genotypes studied, CO 9, GHB 744, Pioneer, MYS 86M84, MYS 86M86 and MYS 70428 were identified as most Zn efficient and responsive genotypes.

Introduction

High consumption of cereal-based foods with low levels and poor bioavailability of micro nutrients is thought to be a major factor for rehabilitating soil and human health today. Among the micronutrients, zinc (Zn) is affecting two third of the population worldwide. Zn deficiency in crop plants reduces not only yield, but also the nutritional quality of the crop. Zn deficiency has been reported in various parts of the world in annual crops (Cakmak *et al.,* 1998). In a global study initiated by FAO, it showed that about 30% of the cultivated soils of the world are Zn deficient. About 50% of the soils used worldwide for cereal production contain low levels of plant-available Zn (Graham *et al.,* 1992). Deficiencies in these nutrients can hamper early brain development, suppress the immune system, increase both mortality and morbidity, and reduce the capacity to do physical work (Combs *et al.,* 1996; Graham and Welch, 2012). Such deficiencies can perpetuate the cycle of poverty in developing countries (Graham and Welch, 2012).

In India, zinc is now considered the fourth most important yield-limiting nutrient after, nitrogen, phosphorus and potassium. The critical limit of effective Zn in the soil suitable for crop growth is 1.2 mg kg^{-1} (DTPA extract) (Takkar and Mann, 1975). The plant available zinc in Indian soils, extracted with DTPA constitutes a very small portion (<1%) of total zinc. The DTPA-extractable Zn in Indian soils ranges from 0.01 to 52.93 mg kg^{-1} soil. Analysis of 97,464 soils from all over India showed that 40% of the soil samples were potentially zinc-deficient. Zinc deficiency in soils of India is likely to increase from 49 to 63% by the year 2025 as most of the marginal soils brought under cultivation are showing zinc deficiency (Arunachalam *et al.,* 2013). Dobermann and Fairhurst (2000) observed that zinc deficiency occurs mostly in neutral and calcareous soils. Crop species markedly differ in their ability to adapt to Zn deficient soils (Graham, 1984). Application of zinc fertilizer is essential in keeping sufficient amount of available Zn in soil solution, maintaining adequate Zn transport to the seeds during reproductive growth stage and increasing the yield.

Living in a tropical country like India we cannot avoid cereals and cereal based foods leading to micronutrient deficiency. So enriching and enhancing the diet with nutrient rich foods agronomically is the best solution today. Millets are the best alternative. Pearl millet [*Pennisetum glaucum* (L.)] is a dual purpose crop with food, feed and fodder value. Its grain is staple food of people living in arid regions of ancient India and also has a high feed value for livestock, poultry and fish. It also provides high quality green forage in seasons of fodder scarcity. In India, the average yield of pearl millet in the country as well as in the state (Tamil Nadu) is quite low as compared to its potential yield because it is grown in the marginal areas with poor management practices. So, there is considerable scope for increasing the productivity of pearl millet by adopting better agronomic practices in the high yielding hybrids/varieties. Productivity of any crop depends on many management factors: fertilizer being the major one. The application of Zn fertilizers for alleviating Zn deficiency in animals and humans is one of the most efficient and sustainable solution in the development and use of Zn-efficient plant genotypes that can more effectively function under low soil Zn conditions, which would reduce fertilizer inputs, less time consuming process and protect the environment as well. It has been well documented that certain plant species, as well as genotypes within certain species, exhibit a significant genetic-based variation in their tolerance to Zn deficiency. The ability of a genotype to grow and yield well in a Zn deficient soil is termed as “Zn efficiency” (Hacisalihoglu and Kochian, 2003). Genotypic differences for zinc use efficiency have been reported in several crops species (Cakmak *et al.*, 2010). Keeping above points in view, the objective of the present study was to screen pearl millet genotypes in relation to Zn use efficiency in calcareous soil through fertilization strategy.

Materials and Methods

A greenhouse experiment was conducted in the Department of Soil Science and Agricultural Chemistry, Agricultural College and Research Institute, Coimbatore during 2016 -17, to evaluate Zn-use efficiency of eighteen pearl millet (*Pennisetum glauccum)* genotypes. The soil used in the experiment was sandy clay loam in texture classified taxonomically as Vertic Ustropept. The details of the initial soil analysis are furnished in table 1.

The treatments consisted of two Zn levels, i.e., without (0 mg Zn kg^{-1} of soil) and with Zn application (10 mg Zn kg^{-1} of soil) and 18 pearl millet genotypes. The details of the pearl millet genotypes used in the study are given in table 2.

The experiment was conducted in a completely randomized design with factorial arrangement, and treatments were replicated thrice. The study was conducted in plastic pots each containing 7 kg of soil with three plants. All the pots received N, P_2O_5 and K_2O at the rate of 80:40:40 respectively kg ha^{-1}. The zinc was applied in the form of zinc sulphate. The study was carried out up to pre flowering stage as per the procedure followed for selecting zinc efficient genotypes. At the end of the experiment (pre flowering stage) dry matter yield of shoot was recorded. The plant material was dried in a hot air oven at about 70°C. Zinc content of the plant was determined by triple acid extraction method (Lindsay and Norvell, 1978). Genotype classification based on nutrient use efficiency (Fageria and Baligar, 1997) otherwise can be termed as Efficient Genotype Index (EGI) was calculated with the dry matter yield and zinc concentration of each genotype given in table 4. Using the formula given below and thereafter classifying the genotypes using a scattered diagram.

Zinc Use Efficiency (each genotype) = (dry matter yield at high Zn application level/ Zn content at high Zn application level) - (dry matter yield without Zn application level / Zn content at without Zn application level)

The zinc efficient and inefficient pearl millet genotypes were identified based on the screening of cultivars by drawing a scattered diagram using ZUE of each genotype. A graph (Fig. 1) was drawn plotting dry matter yield of all the genotypes obtained for without zinc application in the X axis and corresponding Zn use efficiency in Y axis. The perpendicular and parallel line to X axis were drawn with average dry matter yield and zinc use efficiency to divide the scattered diagram and to classify the genotypes into four groups *viz.* efficient and responsive (ER), efficient and non-responsive (ENR), inefficient and responsive (IER) and inefficient and non-responsive (IENR). The ER genotypes have high yield as well as high zinc use efficiency, ENR genotypes have high yield and low efficiency, IER genotypes have low yield and high efficiency and IENR have low yield as well as low efficiency. The ER genotypes would be most suitable for cultivation under Zn stress conditions.

The data on various observations recorded during the course of investigation were analyzed statistically by adopting the procedure described by Gomez and Gomez (2010).

Results and Discussion

The results of the analysis of variance for the variables measured showed less effect of Zn treatment; however, genotypes had significant differences in relation to dry matter production and zinc concentration (Table 3).

Dry Matter Production

The expression of plant productivity in terms of the dry weight of material produced per unit area during a specified time period though had less significance of variation with the application of zinc fertilizers, significantly varies among the different genotypes studied. The maximum and minimum dry matter production (DMP) with zinc treatment was found to be 3330 kg ha^{-1} and 1920 kg ha^{-1} as against the treatments that received no zinc application which recorded between 3010 kg ha^{-1} and 1900 kg ha^{-1} for various genotypes. This variation might be due to prolonged uptake of nutrients from soil and also due to differential ability of genotypes for absorbing and accumulating micronutrients (Anandan *et al.,* 2011).

Among the genotypes, CO 9 had the highest DMP in both with zinc (3330 kg ha^{-1}) and without zinc (3010 kg ha^{-1}) treatment compared to other genotypes and this variation might be due to their differential utilization capacity of native soil Zn as well as applied zinc. Under zinc fertilization, genotypes viz., GHB 744 (3156 kg ha^{-1}) and Ankur (3120 kg ha^{-1}) were on par with each other recording the second highest DMP followed by CO 10 (3010 kg ha^{-1}), Namdari (3000 kg ha^{-1}), GHB 719 (3085 kg ha^{-1}) and MYS 86M86 (3020 kg ha^{-1}) which were on par with each other. Whereas CO 10 (2800 kg ha^{-1}) followed by MYS 86M16 (2760 kg ha^{-1}),MYS 86M86 (2728 kg ha^{-1}), Namdari (2700 kg ha^{-1}), GHB 905 (2700 kg ha^{-1}), MYS 86M84 (2700 kg ha^{-1}), GHB 744 (2680 kg ha^{-1}) and MYS 86M88 (2680 kg ha^{-1}) recorded comparable DMP in the control (0 mg kg ha^{-1} zinc) which were on par with each other. Tolerance of certain genotypes under zinc stress conditions might be due to their higher rates of organic acid excretion in the rhizosphere facilitating increased native Zn availability in soil (Kabeya and Shankar, 2013). Efficient and responsive genotypes CO 9, Pioneer, GHB 744, MYS 86M84, MYS 86M86 and MYS 70428 were found to have 9.6, 15.5, 6.6, 9.7 and 12.8 percent increase in dry matter yield over control respectively.

Zinc content in Plant

Plant Zn concentration considered as a measure of efficient Zn acquisition and its transport to shoot. The different cultivars varied greatly in respect of zinc concentration before flowering stage. The mean zinc concentration varied from 22.65 to 70.24 mg kg^{-1} in different genotypes during pre-flowering stage. Genotype CO 9 had the highest zinc concentration of 67.32 and 70.24 mg kg^{-1} while MYS 86M86 had the lowest zinc concentration of 22.65 and 24.63 mg kg^{-1} in both control and zinc applied at 10 mg kg^{-1} of soil. The different genotypes taken for study showed wide variation in total plant zinc concentration under no zinc treatment which may be due to the secretion of the phytosiderophores, a type of non-proteinogenic amino acids from the roots of efficient genotypes under zinc stress conditions and which are highly effective in complexing and mobilizing Zn from root apoplast to long distance transport of Zn within the plant (Cakmak *et al.,* 1998). The lowest zinc concentration was recorded by MYS hybrids and highest by TNAU hybrids and these differences could be due to differential ability of the genotypes in extracting zinc from the soil solution and diffusion of zinc and its redistribution (Grotz and Guerinot (2006). It was noteworthy to observe that the genotypes CO 9(T1: 67.32; T2: 70.24) followed by CO 10 (T1: 60.65; T2: 64.23) recorded significantly higher zinc concentrations (in mg kg^{-1}) in both the treatments. Pioneer (57.63) and Namdari (57.12) were on par with each other followed by Ankur (53.98), MYS 86M88 (53.19) and Mahindra(52.32) which were also on par with each other in recording higher zinc concentrations in dry matter of the plant(in mg kg^{-1}) in the control.

Zhao (2011) contemplated that increased supply of zinc favoured increased Zn accumulation in the entire plant. Crops fertilized with Zn improved the nutritional environment of rhizosphere and consequently in plant system, which might have caused higher metabolic and photosynthesis activity in plant resulted in greater accumulation of zinc by crops and higher dry matter production (Kanwal *et al.,* 2010). Among the genotypes, GHB 719 showed higher treatment variation of 19 per cent in zinc content and the lowest of 3.53 percent was observed for MYS 86M86 hybrid. The Zn x genotype interaction was significant, indicating variation in zinc concentration among genotypes with the variation in Zn fertilization.

Table.1 Initial soil characteristics of experimental soil

Parameters	**Result**	**Procedure**	**Reference**
Soil texture	Sandy clay loam	International pipette method	Piper (1966)
Soil pH	8.15	1:2.5 soil water suspension	Jackson (1973)
Soil EC	0.45dSm^{-1}		
Organic carbon	7 g kg^{-1}	Chromic acid wet digestion	Walkley and Black (1934)
Free $CaCO_3$	17 per cent	Volumetric titration	Piper (1966)
Available N	280kg ha^{-1}	Alkaline permanganate method	Subbiah and Asija (1956)
Available P	19kg ha^{-1}	0.5 M $NaHCO_3$ (pH-8.5)	Olsen *et al.,* (1954)
Available K	416kg ha^{-1}	Neutral N NH_4oAC	Stanford and English (1949)
DTPA- Zn	0.750 mg kg^{-1}	DTPA extraction and AAS method	Lindsay and Norvell (1978)
DTPA- Fe	0.465 mg kg^{-1}		
DTPA- Cu	1.499 mg kg^{-1}		
DTPA- Mn	17.05 mg kg^{-1}		

Table.2 Details of the genotypes used in the study

S.No	**Name of Genotypes**	**Source (Location)**	**Type**
1.	**CO 9**	TNAU	Hybrid
2.	**CO 10**	TNAU	Hybrid
3.	**ANKUR**	Tamil Nadu (Private)	Hybrid
4.	**MAHINDRA**	Tamil Nadu (Private)	Hybrid
5.	**NAMDARI**	Tamil Nadu (Private)	Hybrid
6.	**PIONEER**	Tamil Nadu (Private)	Hybrid
7.	**GHB 538**	Jamnagar, Gujarat	Hybrid
8.	**GHB 558**	Jamnagar, Gujarat	Hybrid
9.	**GHB 719**	Jamnagar, Gujarat	Hybrid
10	**GHB 732**	Jamnagar, Gujarat	Hybrid
11.	**GHB 744**	Jamnagar, Gujarat	Hybrid
12.	**GHB 905**	Jamnagar, Gujarat	Hybrid
13.	**MYS 86M88**	University of Mysore	Hybrid
14.	**MYS 86M16**	University of Mysore	Hybrid
15.	**MYS 86M84**	University of Mysore	Hybrid
16.	**MYS 86M86**	University of Mysore	Hybrid
17.	**MYS 86M66**	University of Mysore	Hybrid
18.	**MYS 70428**	University of Mysore	Hybrid

Table.3 Zinc content and dry matter production of eighteen pearl millet genotypes at the end of the experiment (pre flowering stage)

(Mean of three replications)

Genotypes		**Zinc Concentration (mg kg $^{-1}$)**		**Dry Matter Production (kg ha^{-1})**	
S.NO	**Details**	**T_1**	**T_2**	**T_1**	**T_2**
1.	CO 9	67.32	70.24	3010	3330
2.	CO 10	60.65	64.23	2800	3010
3.	ANKUR	53.98	63.26	2658	3120
4.	MAHINDRA	52.32	57.21	1900	2102
5.	NAMDARI	57.12	62.45	2700	3000
6.	PIONEER	57.63	60.98	2450	2900
7.	GHB 538	50.36	52.12	1920	1990
8.	GHB 558	39.23	45.32	1967	2280
9.	GHB 719	47.03	56.28	2520	3085
10.	GHB 732	30.45	32.75	2600	2800
11.	GHB 744	38.96	44.93	2680	3156
12.	GHB 905	37.82	41.35	2700	2980
13.	MYS 86M88	53.19	58.35	2680	2980
14.	MYS 86M16	25.81	27.46	2760	2995
15.	MYS 86M84	37.99	39.33	2700	2890
16.	MYS 86M86	22.65	24.63	2728	3020
17.	MYS 86M66	24.12	27.13	2423	2756
18.	MYS 70428	25.31	28.31	2590	2970
Average		**43.44**	**47.57**	**2544**	**2854**
T		**	**	**	**
G		**	**	**	**
T x G		**	**	**	**
CV		**2.91 %**		**2.38 %**	
SED		**1.09**		**52.38**	
CD (0.01)		**2.88**		**138.60**	
CD (0.05)		**2.17**		**104.43**	

*T1: 0 mg Zn kg^{-1} soil and T2:10 mg Zn kg^{-1} soil (applied as $ZnSO_4$)

Table.4 Classification of Genotypes based on dry matter yield

S.No	Genotypes	DMP @ control	ZUE	Efficiency	Responsiveness
G1	CO 9	3010	2.70	**Efficient**	**Responsive**
G2	CO 10	2800	0.70	Non Efficient	Responsive
G3	ANKUR	2658	0.08	Non Efficient	Responsive
G4	MAHINDRA	1900	0.43	Non Efficient	Non Responsive
G5	NAMDARI	2700	0.77	Non Efficient	Responsive
G6	PIONEER	2760	2.13	**Efficient**	**Responsive**
G7	GHB 538	1920	0.06	Non Efficient	Non Responsive
G8	GHB 558	1967	0.17	Non Efficient	Non Responsive
G9	GHB 719	2520	1.23	Non Efficient	Non Responsive
G10	GHB 732	2600	0.11	Non Efficient	Responsive
G11	GHB 744	2680	1.45	**Efficient**	**Responsive**
G12	GHB 905	2700	0.68	Non Efficient	Responsive
G13	MYS1 86M88	2680	0.69	Non Efficient	Responsive
G14	MYS2 86M16	2450	5.04	Efficient	Non Responsive
G15	MYS3 86M84	2700	2.41	**Efficient**	**Responsive**
G16	MYS4 86M86	2728	2.17	**Efficient**	**Responsive**
G17	MYS5 86M66	2423	1.13	Non Efficient	Non Responsive
G18	MYS 70428	2590	2.58	**Efficient**	**Responsive**
Average		**2544**	**1.4**		

Fig.1 A graph depicting classification of genotypes based on its efficiency

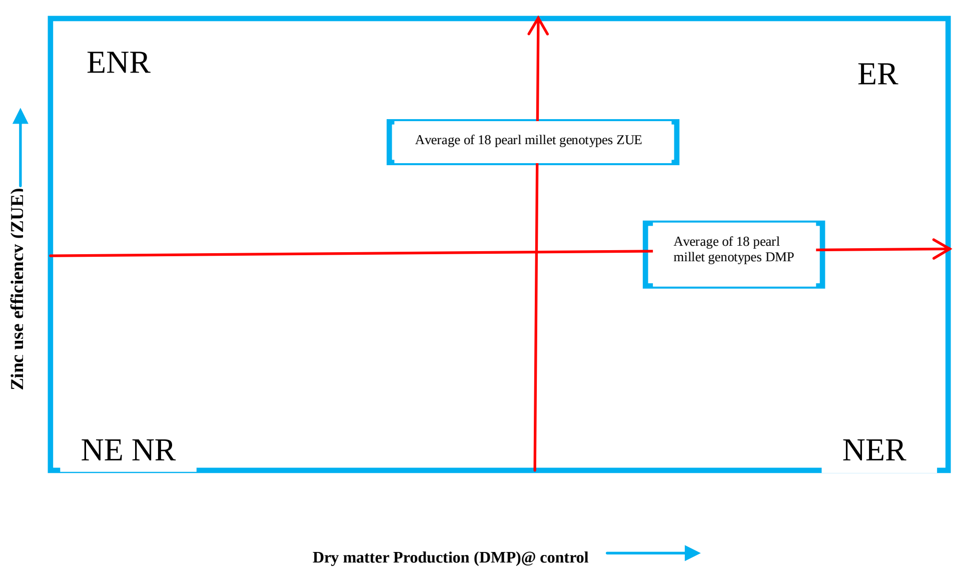
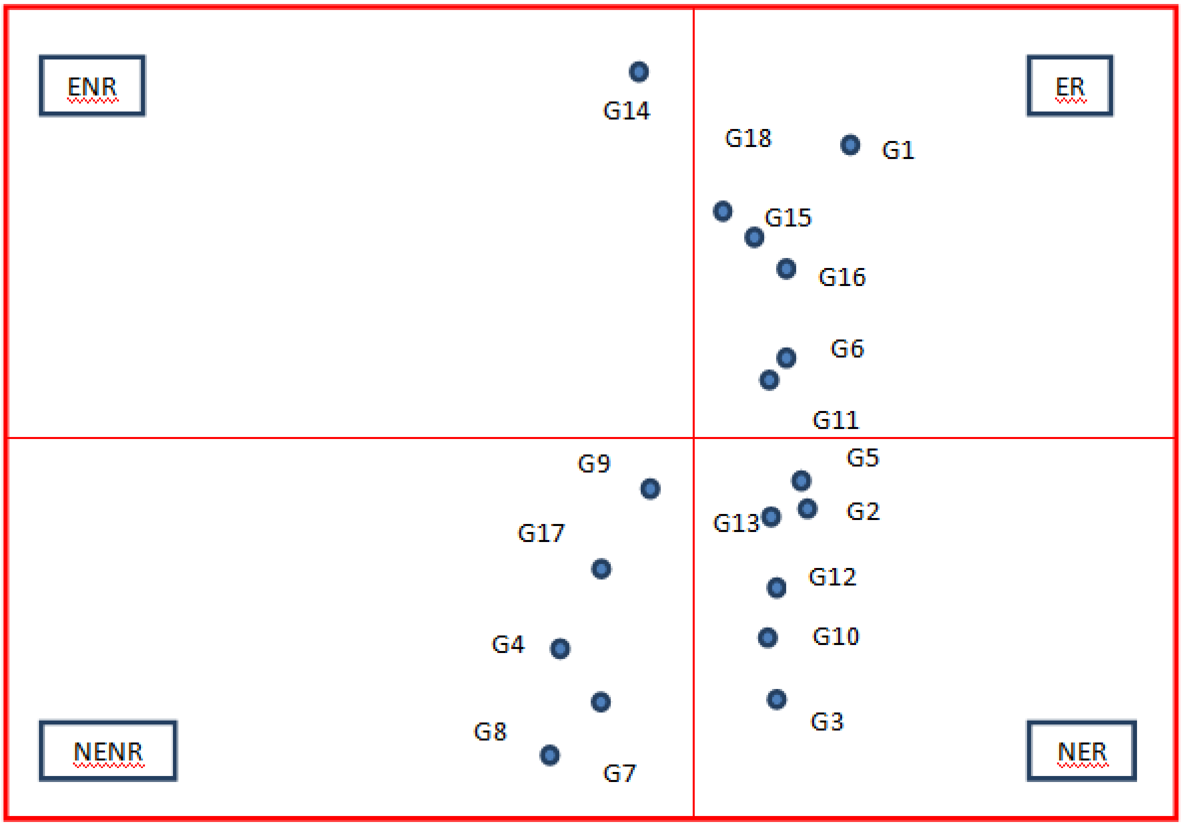


Fig.2 Screening of Zn efficient and inefficient pearl millet genotypes



Classification of genotypes to zinc-use efficiency

Genotypes of pearl millet subjected to variation in zinc fertilization were classified into efficient, inefficient, responsive and non-responsive groups using a scattered diagram. The zinc efficient genotypes were identified based on the screening of cultivars by drawing a scattered diagram using dry matter yield and zinc use efficiency as illustrated in Figure 1. According to this classification, genotypes falling under the category of Efficient Responsive (ER) would be most suitable for cultivation and improving the yield with higher zinc content under Zn stress and zinc fertilization in field conditions.

Regarding ZUE index, a value from 0.06 to 5.04 was recorded for different genotypes. Among the genotypes, CO 9 registered a ZUE value of 2.69 followed by MYS 70428 (2.58), MYS 86M84 (2.41), MYS 86M86 (2.17), Pioneer (2.13), and GHB 744 (1.45) which were found to be efficient and responsive. Whereas MYS 86M16 was found to be efficient and not responsive (ENR) though with the high value of 5.04 as its ZUE and all the other genotypes were found to be inefficient (IER, IENR) (Table 4; Fig. 2). This variation might be attributed due to the fact that the zinc efficient genotypes produce higher dry matter production under deficient and adequate Zn supply, due to their ability to absorb higher zinc from the applied Zn fertilizers and to utilize for higher DMP as stated by Chaab *et al.*, (2011). Though the Tamil Nadu private hybrids recorded higher concentration of zinc, they registered lower dry matter production than the other genotypes and thus did not fall under the category of efficient genotypes except Pioneer that recorded moderately higher zinc concentration and dry matter production. This difference in dry matter yield and varied zinc use efficiency has been used as indices to classify efficient, inefficient, responsive and non-responsive cultivars used in the study. Graham and Rengel (1993) suggested that more than one mechanism could be responsible for establishing Zn efficiency in a genotype and it is likely that different genotypes subjected to Zn deficiency under different environmental conditions will respond by, one or more, different efficiency mechanisms (Rengel and Wheal, 1997).

Zinc efficiency (ZE), mainly defined as the ability of a plant to grow and yield well under Zn deficiency. Hafeez *et al.,* (2013) reported that efficient genotypes are those with high ability to absorb nutrients from soil and fertilizer, produce high biomass yield per unit of absorbed nutrient and store relatively less nutrients which is also evident from our study (Table 3).

The difference in nutrient absorption and utilization may be associated with root geometry, ability of plants to take up sufficient nutrients from lower or subsoil concentration, plants abilities to solubilize nutrients in the rhizosphere, better transport, distribution and utilization within plants and balance source sink relationships (Fageria and Baligar, 2003). Genotypes that are efficient in utilising Zn, increased shoot weight more than genotypes responsive to Zn fertilisers. In contrast, Zn-responsive genotypes increased zinc concentration more than Zn-utilisation-efficient genotypes.

Thus screening with deficient sources could give a picture of the real efficiency of a genotype that would help in taking decisions on fertilizer application and provide required nutrition to the crop.

The identified efficient genotypes could be grown under Zn stress condition due to their differences in internal utilization or mobility of Zn that have been shown to be involved in expression of Zn efficiency. Among the genotypes studied, CO 9, Pioneer, GHB 744, MYS 86M84, MYS 86M86 and MYS 70428 were identified as most Zn efficient and responsive genotypes. Thus these efficient and responsive genotypes could be suggested to be grown under Zn stressed condition. Efficient and responsive genotypes CO 9, Pioneer, GHB 744, MYS 86M84, MYS 86M86 and MYS 70428 were found to have 4.2, 5.5, 3.4, 8.1 and 10.6 percent increase in zinc content over control respectively. Genotypes classified as responsive both as efficient and inefficient could be used under proper adaptation of zinc fertilization strategies. Zinc use efficient genotypes screened based on zinc use efficiency could be the best solution for improving zinc concentration and to avoid malnutrition in future. This approach of fertilization strategy in screening genotypes for nutrient use efficiency especially for micronutrients like zinc in a potentially nutrient deficient calcareous soil would avoid further wastage and fixation of nutrients and also maintain soil fertility to sustain crop productivity. This methodology of screening could be adopted for more time consuming genetic and breeding studies at the end to compare and evaluate the potential of different genotypes for nutrient removal from the soil as preface confirmation.

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