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PIGMENT CONCENTRATION AND ACTIVITY OF ANTIOXIDANT ENZYMES IN ZINC TOLERANT AND SUSCEPTIBLE CHICKPEA GENOTYPES SUBJECTED TO ZINC STRESS

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SUMMARY

Physiological parameters like shoot dry weight, chlorophyll 'a', Chlorophyll 'b', total chlorophyll, carotenoids, soluble protein content, catalase, peroxidase and superoxide dismutase activities were higher in case of zinc tolerant genotypes as compared to zinc susceptible genotypes at pre and post flowering stages of plant growth. The grain yield of chickpea genotypes was positively and significantly correlated with all the physiological parameters except peroxidase and superoxide dismutase activities. At pre-flowering stage grain yield was positively correlated with catalase activity (r=0.450*) and total chlorophyll (r=0.583), while at post flowering stage grain yield was positively and significantly correlated with shoot dry weight (r=0.435*), total chlorophyll (r=0.470*), soluble protein (r=0.566**) and catalase activity (r=0.604**). From the above results, it can be inferred that total chlorophyll content, catalase, carotenoids and soluble protein are important contributing parameters towards chickpea production under zinc deficient condition. Hence, these parameters can be used as traits for screening/developing zinc stress tolerant genotypes. On the basis of per cent grain yield response, genotypes, viz. FG 897, BG 1084, CSJ 128, PBG 126 and CSG 9505 were identified as tolerant, whereas BG 372, BGM 535 and BG 256 were identified as susceptible to Zn stress.**

***Key words*: Catalase, chickpea, peroxidase, pigment, superoxide dismutase, zinc deficiency**

INTRODUCTION

Zinc has been reported to be a most limiting micronutrient for crop production. Deficiency of zinc has been established as a major cause of poor yield or even crop failure in various parts of India (Takkar and Randhawa 1978). More than 75% soils of calcareous region in Bihar have been found deficient in available zinc (Sakal 1985). In India chickpea occupies the first position in the list of pulse crops, which suffers from Zn deficiency. The severity of zinc deficiency also differs widely among chickpea genotypes (Singh and Choudhary 1987). In recent years efforts have been made for better understanding of the factors involved in genotypic variations for tolerance to Zn deficiency, which will allow development of reliable screening procedures for assessing Zn-efficient/inefficient genotypes. Development of Zn efficient chickpea cultivars is important for several reasons. Zinc efficient genotypes can contribute not only to reducing the cost of fertilizer inputs, but also reducing the problems related to Zn deficiency in food chain and consequently to consumers.

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Therefore, present study was undertaken to investigate the morpho-physiological behavior of Zn efficient/ inefficient chickpea genotypes, which will be helpful in developing tolerant chickpea genotypes for calciorthent soils.

MATERIALS AND METHODS

A preliminary field experiment was conducted at T.C.A., Dholi Research Farm with 47 chickpea genotypes in zinc deficient calcareous soil during Rabi 1999-2000. Based on the per cent grain yield response, these 47 genotypes were categorized into highly tolerant (Tolerant to zinc deficiency stress, moderately tolerant, moderately susceptible and highly susceptible group (Table-1). Out of these 47 genotypes, 8 genotypes (five highly tolerant *i.e.* Zn efficient and three from highly susceptible *i.e.* zinc inefficient genotypes) were selected for further studies.

Field experiments were conducted at T.C.A. Dholi Research Farm with these 8 selected chickpea genotypes on Zn deficient (available Zn=0.50 ppm) calcareous soils (free calcium carbonate 34.0%) during rabi 2000-2001 and 2001-2002 (in same plot but different location). The efficient genotypes were viz FG 897, BG 1084, CSJ 128, PBG 126 and CSG 9505 and 3 inefficient genotypes were BG 372, BGM 535 and BG 256. The above chickpea genotypes were grown at three zinc levels (0, 5.0 and 10.0 kg Zn ha^{-1}) replicated thrice in randomized block design (factorial). Soil was analyzed for pH (7.9), EC (0.25 dSm^{-1}), organic carbon (0.42%), available N (140.0 kg ha^{-1}), available P_2O_5 (16.2 kg/ha^{-1}) and available K_2O (114.0 kg ha^{-1}) following standard procedures.

Crop was grown till maturity to record grain yield for both the years. Plant samples of 8 genotypes from only control plots were drawn at pre-flowering (75DAS) and post-flowering (110 DAS) stages during the *rabi* season of 2001-02 and biochemical parameters like, total chlorophyll and carotenoids content (Hiscox and Irsaelstam 1979), soluble protein content (Lowry *et al.* 1951), catalase (Chance and Maehly 1955), peroxidase (Palmiano and Juliano 1973), and superoxide dismutase(SOD) activities (Giannopolitis and Ries 1977) were recorded. These plant samples were dried at 65°C in hot air oven to record a constant shoot dry weight. Zinc concentration in grain was also estimated in tri-acid mixture digest using atomic absorption spectrophotometer (Model-Perkin Elmers Analyst 100) for calculating zinc uptake by grain.

RESULTS AND DISCUSSION

The average grain yield of chickpea genotypes (mean of two years) varied from 6.89 to 19.10 q ha^{-1} (Table-2) due to zinc treatments. Different chickpea genotypes differed significantly in their yielding ability as noted in mean values 8.55 -15.40 q ha^{-1}. The overall mean grain yield was found to be significantly decreased with

Table 1. Relative susceptibility of chickpea genotypes to Zn stress based on per cent grain yield response.

Response category (%)	Susceptibility classes	Name of genotypes	Number of genotypes
(-ve)	Highly tolerant	PG 98-4, BGD-119, GNG 1320, BG 1084, CSJ 140, BG 1096, BGM 531, PG 98-5, LBCG-6, WCG 95-50, CSJD 151, PBG 126, C 235, GNG 1308, RAUG 3-1, FG 897, H-96-51, H 96-93, SBPG 99-4, Pant G-114, GCP 9605, Phu G 95126	22
0-10	Moderately tolerant	CSG 9505, H 96-151, JSC 4, CSJ 128, PBG 157, KPG 59	6
10-20	Moderately susceptible	CSJD 156, GL 94022, BG 372, RSG 963, IPC 97-34, AKG 9826, GNG 1323, GCP 105, BG 1097	9
>20	Highly susceptible	BGM 535, IG 290415, JG 975, GNG 1296, CSJD 154, KWR 108, H 96-76, CSJ 150, BDNG 788, BG 256	10
Total			47

Table 2. Grain yield (q ha^{-1}) of chickpea genotypes in Zn deficient calcareous soils (average of two years).

Genotypes	Grain yield (q ha^{-1}) Zinc treatments (kg ha^{-1})				Per cent grain yield response at 5 ppm
	0	5	10	Mean	
FG 897	12.60	7.42	8.59	9.54	-41.1
BG 1084	10.90	7.85	6.89	8.55	-28.0
PBG 126	10.90	8.52	8.30	9.24	-21.8
CSJ 128	16.10	11.10	11.10	12.80	-31.1
CSG 9505	17.60	15.20	11.10	14.60	-13.6
Mean	13.62	10.02	9.20	-	-
BG 372	10.20	15.20	11.80	12.40	49.0
BGM 535	10.40	13.90	10.70	11.70	33.7
BG 256	14.30	19.10	12.90	15.40	33.6
Mean	11.63	16.06	11.80	-	-
Overall Mean	12.88	12.29	10.17	-	-

C.D. (P=0.05): G- 0.48, T- 0.42, G x T- 1.78

increasing zinc level for zinc efficient genotypes. However, the grain yield of zinc inefficient chickpea genotypes was significantly increased due to Zn application at 5 kg ha^{-1} level. The overall optimum zinc level was found to be at 5 kg ha^{-1} as the yield was reduced at 10 kg ha^{-1} application. Tolerant genotypes produced negative response to Zn application, which confirms the findings of preliminary study, while the susceptible genotypes responded positively to Zn application.

The average data of two years with respect to zinc uptake by grain as given in table 3 indicated that zinc uptake by grains of tolerant genotypes was much higher (54.36 g ha^{-1}) as compared to susceptible genotypes (19.21 g ha^{-1}) under zinc deficient condition. This suggested that the tolerant genotypes are capable of absorbing native zinc to meet their Zn requirements under zinc deficient condition. However, zinc uptake by grain of both the categories of chickpea genotypes was comparable. This suggests that in spite of higher zinc concentration in grain at 5 kg ha $^{-1}$ zinc levels, the uptake was reduced for tolerant genotypes but it was increased for susceptible genotypes, which might be due to grain yield response of these two categories of chickpea genotypes. The overall effect of zinc levels was non significant, when both categories were combined together.

Table 3. Uptake of zinc (g ha^{-1}) in grains of chickpea genotypes as influenced by zinc.

Genotypes	Zinc uptake (g ha^{-1}) Zinc treatments (kg ha^{-1})			
	0	5	10	Mean
FG 897	63.90	47.90	49.80	53.8
BG 1084	63.70	52.10	49.10	55.0
PBG 126	37.20	28.40	27.20	30.9
CSJ 128	70.10	50.90	63.40	61.8
CSG 9505	36.90	40.20	39.30	39.7
Mean	54.36	43.90	45.76	
BG 372	7.93	29.50	33.40	23.7
BGM 535	23.10	37.50	32.10	30.9
BG 256	26.60	49.70	35.30	37.2
Mean	19.21	38.90	33.60	-
Overall mean	41.18	42.02	41.20	-

C. D.(P=0.05): G- 5.43, T- NS, G x T- 8.72

The overall Zn uptake by chickpea grain varied from 23.1 to 70.1 g ha^{-1}.

Result pertaining to shoot dry weight indicated that inefficient genotypes showed significant reduction in shoot

dry weight, which varied with genotypes. Reduction in shoot dry weight might be due to less CO_2 assimilation as a result of inactivation of ribulose bisphosphate carboxylase (Cakmak 1988). It might also be due to the fact that Zn deficiency induced increase in formation of reactive oxygen species in the chloroplasts and thus degenerative changes in the leaf cells as suggested by him. Shoot dry weight is a suitable criterion to separate genotypes for their tolerance to Zn deficiency, as efficient genotypes maintained higher shoot dry weight (0.55-0.82 g plant^{-1}), while the inefficient genotypes produced lower shoot dry weight(0.44-0.47g plant^{-1}) in untreated plots at pre-flowering stage. During post flowering stage also, efficient genotypes produced higher shoot dry weight (8.48- 10.81 g plant^{-1}), while the inefficient genotypes showed lower shoot dry weight (6.00-7.35 g plant^{-1}).

Total chlorophyll content at pre flowering stage varied from 1.18 to 1.37 mg g^{-1} fresh weight for efficient genotype, while the variation was from 0.89 to 1.01 mg g^{-1} fresh weight for inefficient genotypes. At post-flowering stage the values varied from 0.70 to 0.84 and 0.54 to 0.58 mg g^{-1} fresh weight for efficient and inefficient genotypes, respectively (Table 4). Significantly higher chlorophyll content in tolerant genotypes as compared to susceptible genotypes might be due to involvement of Zn in the biosynthesis of chlorophyll (Marschner and Cakmak 1989, Beale 1999). Zinc as a constituent of porphobilinogen synthase (Jaffe 1993-1995), catalyses the condensation of two molecules of δ-aminolevulinic acid to form porphobilinogen, involved in the biosynthesis of the chlorophyll 'a' precursor protoporphyrin IX. Based on the above results, it may be summarized that efficient genotypes had higher content of photosynthetic pigments responsible for causing efficient phototraping system leading to more photosynthate accumulation and dry matter production as compared to inefficient genotypes.

Carotenoids content at post-flowering stage was higher than pre-flowering stage and efficient genotypes had significantly higher carotenoids content as compared to inefficient genotypes. The overall variation in carotenoids conents was found to be significantly different among genotypes from 0.27 to 0.43 mg g^{-1} fresh weight at pre-flowering stage and from 0.36-0.62 mg g^{-1} fresh weight at post-flowering stage (Table 4).

The soluble protein content at pre-flowering stage significantly varied from 43.8 to 55.0 mg g^{-1} fresh weight for tolerant genotypes, while the variation was 34.7 to 41.7 mg g^{-1} fresh weight for inefficient genotypes. These variations at post-flowering stage ranged from 37.6 to 49.0 and 34.0 to 35.4 mg g^{-1} fresh weight for efficient and inefficient genotypes, respectively. Tolerant genotypes

Table 4. Dry matter and pigment concentration in chickpea genotypes at pre-and post- flowering stages under zinc stress in calciorthent soil.

Genotypes	Shoot dry weight (g plant^{-1})		Total chlorophyll (mg g^{-1} fresh weight)		Carotenoids (mg g^{-1} fresh weight)	
	Pre-flowering (75 DAS)	Post-flowering (110 DAS)	Pre-flowering (75 DAS)	Post-flowering (110 DAS)	Pre-flowering (75 DAS)	Post-flowering (110 DAS)
FG 897	0.58	8.48	1.37	0.84	0.43	0.56
BG 1084	0.56	10.29	1.36	0.83	0.38	0.44
PBG 126	0.82	9.58	1.18	0.83	0.40	0.53
CSJ 128	0.80	8.52	1.26	0.70	0.27	0.62
CSG 9505	0.55	0.45	1.20	0.79	0.43	0.50
BG 372	0.44	6.45	0.95	0.56	0.33	0.43
BGM 535	0.46	0.32	1.01	0.58	0.35	0.40
BG 256	0.47	6.00	0.89	0.54	0.28	0.36
Mean	0.59	8.44	1.15	0.71	0.36	0.48
C.D. (P=0.05)	0.040	0.040	0.082	0.050	0.033	0.040

recorded significantly higher amount of soluble protein content as compared to susceptible genotypes at both stages of crop growth (Table 5). This might be on account of increase in both synthesis and structural integrity of RNA and ribosomes (Kitagishi and Obata 1986, Kitagishi and Kondo 1987). Falchuk *et al.* (1977) has also established that zinc is an essential component of RNA polymerase.

Catalase activity at pre-flowering stage varied from 0.93 to 1.26 units mg $^{-1}$ protein for tolerant genotypes, while the variation was 0.80 to 0.93 units mg^{-1} protein for susceptible genotypes. These variations at post-flowering stage were from 0.82 to 1.03 and 0.62 to 0.80 units mg^{-1} protein for efficient and inefficient genotypes, respectively. Tolerant genotypes showed significantly higher catalase activity in comparison to susceptible genotypes at both the stages of crop growth.

Peroxidase activity at pre-flowering stage varied from 54.1 to 89.4 units mg^{-1} protein for efficient genotypes, while the variation ranged from 41.6 to 47.7 units mg^{-1} protein for inefficient genotypes. Variations at post-flowering stage varied from 52.8 to 95.4 and 38.2 to 45.5 units mg^{-1} protein for efficient and inefficient genotypes, respectively. Tolerant genotypes maintained significantly higher peroxidase activity in comparison to susceptible genotypes at both stages of crop growth. However, among tolerant genotypes, some genotypes have higher peroxidase activity at pre-flowering stage while others have higher activity at post-flowering stage.

Superoxide dismutase activity at pre-flowering stage varied from 0.26 to 0.41 units mg^{-1} protein for efficient genotypes, while the variation was 0.23 to 0.24 units mg^{-1} protein for inefficient genotypes. At post-flowering stage the value varied from 0.22 to 0.42 and 0.26 to 0.29 units mg^{-1} protein for tolerant and susceptible genotypes, respectively. It was also noticed that in tolerant genotypes the SOD activity was reduced at latter growth period, while it was significantly higher in susceptible genotypes. The lower soluble protein content in susceptible genotypes might be due to depressed protein synthesis, which suggests the decreased activities of antioxidative enzymes as a result of reduced protein biosynthesis. The lower SOD and catalase activities in susceptible genotypes were reported by Cakmak and Marschner (1988). The reduced content of enzymes like catalase, peroxidase and superoxide dismutase in inefficient genotypes may also be a consequence of either their inactivation or decreased production of their substrates *i.e.* O_2^- and H_2O_2. This impaired detoxification of toxic oxygen species could be the basis of susceptibility of inefficient genotypes to zinc (Marschner and Cakmak 1989).

Table 5. Soluble protein content and activity of antioxidant enzymes in chickpea genotypes at pre- and post-flowering stages under zinc stress in calciorthent soil.

Genotypes	Soluble protein content (mg g^{-1} fresh weight)		Catalase activity (units mg^{-1} protein)		Peroxidase activity (units mg^{-1} protein)		Superoxide dismutase activity (units mg^{-1} protein)	
	Pre-flowering (75 DAS)	Post-flowering (110 DAS)	Pre-flowering (75 DAS)	Post-flowering (110 DAS)	Pre-flowering (75 DAS)	Post-flowering (110 DAS)	Pre-flowering (75 DAS)	Post-flowering (110 DAS)
FG 897	49.6	44.9	1.26	1.03	69.5	60.6	0.30	0.22
BG 1084	49.4	42.9	1.03	0.97	89.4	95.4	0.32	0.25
PBG 126	55.0	40.0	1.12	0.92	56.7	75.0	0.37	0.42
CSJ 128	54.2	49.0	0.93	0.82	54.1	57.6	0.26	0.26
CSG 9505	43.8	37.6	1.00	0.96	63.3	52.8	0.41	0.37
BG 372	41.7	35.4	0.80	0.62	47.7	45.5	0.24	0.26
BGM 535	36.6	34.0	0.93	0.80	47.5	39.7	0.24	0.29
BG 256	34.7	34.3	0.89	0.79	41.6	38.2	0.23	0.28
Mean	45.6	39.8	1.00	0.86	58.7	58.1	0.30	0.30
C.D.(P=0.05)	3.10	1.99	0.060	0.070	6.73	5.23	0.040	0.040

The results showed that grain yield of chickpea was positively and significantly correlated with catalase activity (r=0.450*) and total chlorophyll (r=0.583**), at pre-flowering stage and shoot dry weight (r=0.435*), total chlorophyll (r=0.470*), soluble protein content (r=0.566**), carotenoids (r=0.572**), and catalase activity (r=0.604**) at post-flowering stage. From the above findings, it can be inferred that total chlorophyll content, catalase, carotenoids and soluble protein content are important determinents of chickpea growth under zinc deficient condition. Hence, these parameters can be used as traits for screening/developing zinc stress tolerant genotypes.

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