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EFFECT OF ANTI OXIDANT ENZYMES UNDER IRON DEFICIENCY STRESS CONDITIONS IN GROUNDNUT

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ABSTRACT

A pot culture experiment was conducted at Regional Agricultural Research Station (RARS) farm, S.V. Agricultural College, Tirupati campus of Acharya N .G. Ranga Agricultural University, during *kharif*, 2016 with factorial RBD design involving normal and calcareous soil. Twenty groundnut genotypes were tested for iron deficiency chlorosis tolerance at three (30, 60 and 90 DAS) different stages of growth. Enzymatic activities of certain antioxidant enzymes such as superoxide dismutase, peroxidase and catalase were altered under iron deficiency stress conditions. The results revealed that iron efficient groundnut genotypes had higher peroxidase and catalase activity and higher active iron content than inefficient groundnut genotypes. Among the antioxidant enzymes, super oxide dismutase activity was high under iron deficiency stress conditions. Significant decrease in peroxidase and catalase activity was observed at later growth stages due to increase in iron deficiency as was evident by decrease in active iron content. There was a strong and positive correlation between leaf peroxidase activity and leaf ferrous iron content.

KEYWORDS: Iron deficiency chlorosis (IDC), active iron, peroxidase, catalase and superoxide dismutase.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is the second most important oilseed crop in India, which is mainly grown in states like Gujarat, Andhra Pradesh, Karnataka, Tamil Nadu and Maharashtra. Groundnut is an important source of edible oil and proteins. Groundnut and groundnut oil also contains cardiovascular protective properties (Stephens *et al*., 2010). Fe is essential for all living organisms and crucial for a variety of functions (Kobayashi *et al.,* 2012).

In India, groundnut occupies an area of 4.8 m ha with a production of 7.4 m t and with a productivity of 1552 kg ha^{-1}. In Andhra Pradesh, groundnut occupies an area of 0.87 m ha with a production of 0.5 m t and productivity of 564 kg ha^{-1} (Anonymous, 2014).

Iron is constituent of heme compounds such as cytochromes, peroxidase, and catalase, phytoferritin and ferredoxin. Iron deficiency is commonly observed in calcareous soils, but it is major concern in groundnut as the crop is highly susceptible to deficiency which affects economic yields especially under irrigated conditions. As calcareous soils are deficient in available iron (Fe^{2+}), iron deficiency chlorosis (IDC) is more prevalent in these soils. Cultivation of Iron Deficiency Chlorosis (IDC) resistant cultivars in calcareous soils is the aim of present study, which economically feasible and sustainable approach compared to application of iron containing fertilizers through soil or foliar spray.

MATERIALS AND METHODS

In the present study, 20 advanced groundnut breeding lines were evaluated in a pot culture experiment using factorial RBD and replicated thrice in calcareous and normal soil at Regional Agricultural Research Station farm, S.V. Agricultural College, Tirupati campus of Acharya N.G. Ranga Agricultural University. Genotypes were assessed for antioxidant enzymes (peroxidase, catalase and superoxide dismutase) and active iron at different stages of crop growth (30, 60 and 90 DAS).

Estimation of Superoxide dismutase (SOD)

Superoxide dismutase was estimated using the method given by Fridovich (1975). One gram of fresh leaf sample was taken and ground with 10 ml of potassium phosphate buffer. The grounded leaf sample was centrifuged at 10,000 rpm for 10 min at 4°C. After centrifugation the supernatant was collected and refrigerated. 50 µl of enzyme extract was added to test tubes containing 600 µl of potassium phosphate buffer, 60 µl of ethylene diamine tetra acetic acid, 390 µl of methionine, 0.6 µl of riboflavin and 300 µl of nitro blue

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tetrazolium chloride. Along with the sample test tubes, blank (without nitro blue tetrazolium chloride and enzyme extract) and reference (without enzyme extract) were also maintained. The sample, reference and blank tubes were kept under fluorescent light for 15 min and absorbance was recorded at 560 nm and expressed as units g^{-1}(fr.wt) min^{-1}. One unit is defined as change in absorbance per gram fresh weight per minute.

Estimation of Catalase (CAT)

Catalase was estimated by grinding 300 mg of leaf tissue with 2.5 ml of sodium phosphate buffer and 1 ml of 1% poly vinyl pyrrolidine. The grounded sample was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was collected after centrifugation and refrigerated. 50 µl of enzyme extract was added to test tube containing 2 ml of sodium phosphate buffer and 950 µl of hydrogen peroxide solution. Besides this, blank was also run without the enzyme extract. Absorbance was recorded at 240 nm and expressed as units g^{-1}(fr.wt) min^{-1}. One unit is defined as change in absorbance per gram fresh weight per minute. The estimation was carried out as per the method given by Sadasivam and Manickam (1992).

Estimation of Peroxidase (POD)

Peroxidase was estimated as per the method given by Mahadevan and Sridhar (1986). One gram of fresh leaf sample was taken and ground with 3 ml of sodium phosphate buffer. The grounded sample was subjected to centrifugation at 18000 rpm for 15 min at 5°C. After centrifugation the supernatant was collected and 0.1 ml of this supernatant was added to test tube containing 3 ml of sodium phosphate buffer, 50 µl of guaiacol solution and 30 µl of hydrogen peroxide solution. Blank is maintained without the enzyme extract and absorbance was recorded at 436 nm and expressed as units g^{-1}(fr.wt) min^{-1}. One unit is defined as change in absorbance per gram fresh weight per minute.

Estimation of Active iron (Fe^{+2})

For extracting ortho-phenanthroline extractable iron, third fully opened leaf from top was taken. Ortho-phenanthroline extractable iron in leaf sample was estimated as per Katyal and Sharma (1980).

For this estimation two grams of fresh leaf sample from was taken, washed with diluted HCl followed by glass distilled water and the adhering moisture was removed by sandwiching between absorbent papers. Then the leaf sample was chopped into fine bits and treated with 20 ml ortho-phenanthroline extract (p^H 3.0) and was allowed to stand for 16 hours. The Fe^{+2} was determined in the filtrate by reading the transmittancy at 510 nm in spectrophotometer.

RESULTS AND DISCUSSION

The active iron content in the present investigation increased from 30 to 60 DAS and decreased at 90 DAS of crop growth (Table 1). The active iron content showed significant differences among the soil types, genotypes and their interactions.

Singh (1994) has reported that active iron is taken as criterion and observed lower active iron in chlorotic plants. The ferrous iron content in iron efficient groundnut genotypes was higher than the susceptible genotypes due to less chlorosis. There was a comparatively less reduction in active Fe from normal to Fe-deficit soil among resistant compared to susceptible groundnut genotypes confirming its direct role in IDC resistance as Fe is required for chlorophyll formation and photosynthesis (Zheng, 2010).

The activities of peroxidase, catalase and superoxide dismutase showed highly significant differences among the soil types, genotypes and their interactions. The activities of peroxidase (Table 2) and catalase (Table 3) in the present investigation was higher at 30 DAS and decreased from 60 to 90 DAS. Higher decrease of peroxidase at later stages of crop growth was due to increase in iron deficiency as was evident by decrease in active iron content. Similar results were obtained by Nagaratnamma *et al*. (2011) and Sanjana (2004).The peroxidase, catalase and among the genotypes showed significant differences evident from higher mean values. The activities of peroxidase and catalase was higher in the groundnut genotypes TCGS-1624 and TCGS-1616 and lowest in groundnut genotypes TCGS-1613 and TCGS-1609 compared to other genotypes

Reduction in peroxidase and catalase activity was observed among all genotypes in iron deficient soil (calcareous soil) compared to iron sufficient soil. However, a lower reduction was observed among resistant genotypes compared to susceptible ones probably due to comparatively higher active-Fe maintained in leaves under Fe-stress conditions. A strong and positive correlation was observed between peroxidase activity and leaf iron content. Iron deficiency has been found to reduce

Table 1. Evaluation of groundnut genotypes for Active iron content (ppm) in iron sufficient and iron deficient soil conditions

S. No.	Genotypes	30 DAS			60 DAS			90 DAS		
		T1	T2	Mean	T1	T2	Mean	T1	T2	Mean
1	TCGS-1602	8.79	7.21	8.00	10.13	9.58	9.85	8.40	6.93	7.67
2	TCGS-1603	8.64	7.17	7.90	9.83	9.26	9.55	8.34	6.85	7.60
3	TCGS-1609	8.59	6.92	7.75	9.77	8.87	9.32	8.33	6.76	7.55
4	TCGS-1611	8.82	7.44	8.13	10.26	9.76	10.01	8.44	7.12	7.78
5	TCGS-1613	8.34	6.81	7.57	9.57	8.42	8.99	8.25	6.73	7.49
6	TCGS-1616	13.01	12.27	12.64	15.73	13.46	14.59	12.38	11.73	12.06
7	TCGS-1621	10.50	9.48	9.99	15.44	11.81	13.63	9.34	8.85	9.10
8	TCGS-1622	9.87	8.98	9.43	13.81	11.36	12.59	9.23	7.88	8.55
9	TCGS-1623	10.15	9.10	9.62	14.00	11.45	12.73	9.27	7.95	8.61
10	TCGS-1624	13.52	12.71	13.12	16.07	14.74	15.40	12.85	11.99	12.42
11	DHARANI	8.89	7.64	8.27	10.43	10.13	10.28	8.57	7.26	7.91
12	K6	9.00	7.97	8.49	11.86	10.65	11.25	8.73	7.51	8.12
13	NARAYANI	9.73	8.91	9.32	13.45	11.22	12.34	9.15	7.78	8.46
14	TAG-24	9.68	8.75	9.21	13.18	11.15	12.17	9.09	7.75	8.42
15	GREESHMA	9.08	8.20	8.64	12.27	10.77	11.52	8.78	7.59	8.19
16	TCGS-1511	10.35	9.21	9.78	14.45	11.66	13.06	9.30	8.10	8.70
17	TCGS-1514	9.29	8.65	8.97	12.86	10.95	11.91	8.97	7.69	8.33
18	TCGS-1517	9.32	8.43	8.88	12.52	10.86	11.69	8.93	7.63	8.28
19	TCGS-1522	8.93	7.89	8.41	11.23	10.48	10.85	8.69	7.44	8.06
20	TCGS-1528	8.96	7.76	8.36	10.93	10.32	10.62	8.61	7.35	7.98
	Mean	9.67	8.57		12.39	10.84		9.18	7.95	
		CD(P=0.05)	**SEm±**	**CV(%)**	**CD(P=0.05)**	**SEm±**	**CV(%)**	**CD(P=0.05)**	**SEm±**	**CV(%)**
	T	0.03	0.01		0.0348	0.0123		0.0214	0.0076	
	G	0.0913	0.0324	6.6	0.11	0.039	5.9	0.0678	0.0241	6.2
	T×G	0.129	0.0459		0.155	0.0552		0.096	0.0341	

T_1: Iron Sufficient Soils; T_2: Iron Deficient Soils (Calcareous Soils); NS: Non Significant

Table 2. Evaluation of groundnut genotypes for Peroxidase activity (units g^{-1}(fresh wt) min^{-1}) in iron sufficient and iron deficient soil conditions

S. No.	Genotypes	30 DAS			60 DAS			90 DAS		
		T1	T2	Mean	T1	T2	Mean	T1	T2	Mean
1	TCGS-1602	9.45	6.56	8.01	3.15	2.86	3.01	2.75	2.13	2.44
2	TCGS-1603	8.65	6.52	7.59	3.12	2.75	2.94	2.74	2.07	2.41
3	TCGS-1609	8.64	6.37	7.51	3.07	2.65	2.86	2.65	1.92	2.29
4	TCGS-1611	9.33	6.89	8.11	3.24	2.95	3.10	2.78	2.24	2.51
5	TCGS-1613	8.46	5.35	6.91	3.03	2.53	2.78	2.30	1.85	2.08
6	TCGS-1616	15.74	11.08	13.41	4.48	4.18	4.33	3.67	3.51	3.59
7	TCGS-1621	16.00	10.69	13.35	4.01	4.05	4.03	3.35	3.31	3.33
8	TCGS-1622	13.61	9.82	11.71	3.83	3.57	3.70	3.25	2.93	3.09
9	TCGS-1623	15.48	10.28	12.88	3.88	3.67	3.78	3.29	3.11	3.20
10	TCGS-1624	19.73	11.32	15.53	4.73	4.35	4.54	3.93	3.75	3.84
11	DHARANI	10.52	7.21	8.87	3.41	3.21	3.31	2.81	2.35	2.58
12	K6	12.37	8.05	10.21	3.57	3.31	3.44	2.96	2.63	2.80
13	NARAYANI	13.29	9.56	11.43	3.76	3.55	3.65	3.23	2.90	3.07
14	TAG-24	13.19	9.28	11.23	3.68	3.48	3.58	3.17	2.83	3.00
15	GREESHMA	12.14	8.45	10.30	3.63	3.33	3.48	2.97	2.66	2.82
16	TCGS-1511	15.95	10.45	13.20	3.96	3.86	3.91	3.33	3.18	3.26
17	TCGS-1514	13.06	8.86	10.96	3.67	3.45	3.56	3.08	2.81	2.95
18	TCGS-1517	12.60	8.60	10.60	3.65	3.43	3.54	3.04	2.75	2.89
19	TCGS-1522	12.03	7.44	9.74	3.49	3.27	3.38	2.92	2.55	2.74
20	TCGS-1528	11.67	7.33	9.50	3.46	3.23	3.35	2.83	2.49	2.66
	Mean	12.60	8.51		3.64	3.38		3.05	2.70	
		CD(P=0.05)	**SEm±**	**CV(%)**	**CD(P=0.05)**	**SEm±**	**CV(%)**	**CD(P=0.05)**	**SEm±**	**CV(%)**
	T	0.1534	0.05		0.0384	0.0136		0.048	0.017	
	G	0.485	0.1722	4.5	0.1214	0.0431	3.7	0.1518	0.0539	4.5
	T × G	0.686	0.2436		0.172	0.061		0.215	0.0762	

T_1: Iron Sufficient Soils; T_2: Iron Deficient Soils (Calcareous Soils); NS: Non Significant

Table 3. Evaluation of groundnut genotypes for Catalase activity (units g^{-1}(fresh wt) min^{-1}) in iron sufficient and iron deficient soil conditions

S. No	Genotypes	30 DAS			60 DAS			90 DAS		
		T1	T2	Mean	T1	T2	Mean	T1	T2	Mean
1	TCGS-1602	1.72	1.47	1.60	1.43	1.25	1.34	1.09	0.89	0.99
2	TCGS-1603	1.68	1.44	1.56	1.42	1.21	1.32	1.04	0.88	0.96
3	TCGS-1609	1.65	1.39	1.52	1.41	1.19	1.30	1.00	0.86	0.93
4	TCGS-1611	1.75	1.50	1.62	1.45	1.27	1.36	1.10	0.91	1.01
5	TCGS-1613	1.59	1.32	1.46	1.33	1.10	1.22	0.95	0.84	0.89
6	TCGS-1616	2.36	1.91	2.14	1.84	1.62	1.73	1.59	1.35	1.47
7	TCGS-1621	2.70	1.86	2.28	1.79	1.56	1.67	1.55	1.30	1.43
8	TCGS-1622	2.19	1.75	1.97	1.70	1.48	1.59	1.44	1.24	1.34
9	TCGS-1623	2.26	1.79	2.03	1.72	1.50	1.61	1.47	1.27	1.37
10	TCGS-1624	2.53	1.95	2.24	1.92	1.70	1.81	1.65	1.42	1.54
11	DHARANI	1.77	1.51	1.64	1.46	1.29	1.38	1.13	0.94	1.04
12	K6	1.87	1.59	1.73	1.55	1.35	1.45	1.22	1.03	1.13
13	NARAYANI	2.10	1.71	1.90	1.69	1.46	1.57	1.41	1.20	1.31
14	TAG-24	1.97	1.69	1.83	1.64	1.43	1.53	1.37	1.15	1.26
15	GREESHMA	1.90	1.62	1.76	1.58	1.37	1.48	1.25	1.08	1.17
16	TCGS-1511	2.50	1.83	2.17	1.76	1.53	1.64	1.50	1.28	1.39
17	TCGS-1514	1.95	1.67	1.81	1.62	1.41	1.52	1.32	1.12	1.22
18	TCGS-1517	1.93	1.63	1.78	1.61	1.39	1.50	1.29	1.10	1.19
19	TCGS-1522	1.85	1.57	1.71	1.51	1.33	1.42	1.21	1.01	1.11
20	TCGS-1528	1.81	1.55	1.68	1.48	1.31	1.40	1.15	0.96	1.06
	Mean	2.00	1.64		1.60	1.39		1.29	1.09	
		CD(P=0.05)	**SEm±**	**CV(%)**	**CD(P=0.05)**	**SEm±**	**CV(%)**	**CD(P=0.05)**	**SEm±**	**CV(%)**
	T	0.02	0.01		0.0137	0.0049		0.018	0.0064	
	G	0.0478	0.017	2.8	0.0434	0.0154	4	0.0568	0.0202	5.9
	T×G	0.068	0.024		NS	0.0218		NS	0.0285	

T_1: Iron Sufficient Soils; T_2: Iron Deficient Soils (Calcareous Soils); NS: Non Significant

Table 4. Evaluation of groundnut genotypes for Superoxide dismutase activity (units g^{-1}(fresh wt) min^{-1}) in iron sufficient and iron deficient soil conditions

S. No	Genotypes	30 DAS			60 DAS			90 DAS		
		T1	T2	Mean	T1	T2	Mean	T1	T2	Mean
1	TCGS-1602	0.24	0.30	0.27	0.25	0.35	0.30	0.32	0.38	0.35
2	TCGS-1603	0.20	0.28	0.24	0.24	0.34	0.29	0.30	0.37	0.34
3	TCGS-1609	0.18	0.26	0.22	0.23	0.33	0.28	0.28	0.36	0.32
4	TCGS-1611	0.23	0.31	0.27	0.27	0.36	0.31	0.33	0.39	0.36
5	TCGS-1613	0.14	0.24	0.19	0.21	0.32	0.27	0.25	0.34	0.29
6	TCGS-1616	0.47	0.51	0.49	0.52	0.54	0.53	0.54	0.55	0.55
7	TCGS-1621	0.45	0.49	0.47	0.49	0.52	0.51	0.54	0.58	0.56
8	TCGS-1622	0.40	0.43	0.41	0.41	0.48	0.44	0.48	0.53	0.51
9	TCGS-1623	0.42	0.45	0.44	0.44	0.49	0.47	0.51	0.55	0.53
10	TCGS-1624	0.49	0.54	0.52	0.54	0.56	0.55	0.57	0.59	0.58
11	DHARANI	0.25	0.32	0.29	0.29	0.38	0.34	0.35	0.40	0.38
12	K6	0.29	0.35	0.32	0.33	0.41	0.37	0.38	0.43	0.40
13	NARAYANI	0.38	0.41	0.39	0.40	0.47	0.43	0.46	0.52	0.49
15	GREESHMA	0.30	0.37	0.34	0.37	0.42	0.39	0.41	0.45	0.43
16	TCGS-1511	0.43	0.47	0.45	0.48	0.51	0.49	0.53	0.57	0.55
17	TCGS-1514	0.34	0.39	0.37	0.36	0.45	0.41	0.44	0.49	0.46
18	TCGS-1517	0.32	0.38	0.35	0.34	0.43	0.39	0.43	0.47	0.45
19	TCGS-1522	0.28	0.34	0.31	0.32	0.40	0.36	0.37	0.42	0.39
20	TCGS-1528	0.26	0.33	0.30	0.30	0.39	0.35	0.36	0.41	0.39
	Mean	0.32	0.38		0.38	0.43		0.42	0.46	
		CD(P=0.05)	**SEm±**	**CV(%)**	**CD(P=0.05)**	**SEm±**	**CV(%)**	**CD(P=0.05)**	**SEm±**	**CV(%)**
	T	0.01	0.0019		0.0044	0.0015		0.013	0.0046	
	G	0.0165	0.0059	5.6	0.0138	0.0049	3.7	0.0411	0.0146	9.4
	T×G	0.023	0.0083		0.02	0.0069		NS	0.0207	

T_1: Iron Sufficient Soils; T_2: Iron Deficient Soils (Calcareous Soils); NS: Non Significant

the activity of oxidative stress-related enzymes like catalase, ascorbate peroxidase, and peroxidase in several plant species that is attributed to less Fe concentration in Fe- deficient leaves (M'sehli *et al.,* 2014 and Ishwar *et al.,* 2016). As catalase and peroxidase antioxidant enzymes are all heme-containing enzymes may not play essential roles in detoxifying reactive oxygen species under iron deficiency stress conditions.

The activity of superoxide dismutase increased throughout crop growth from 30 DAS to 90 DAS (Table 4). The super oxide dismutase among the genotypes showed significant differences and higher activity was recorded in TCGS-1624 and TCGS-1616 and lowest in TCGS-1613 and TCGS-1609 compared to other genotypes

SOD provides the first line of defense against the toxic effects of elevated levels of reactive oxygen species. Superoxide dismutase catalyses the dismutation of superoxide radicals to H_2O_2 and O_2, and constitutes the most important enzyme in cellular defense because its activation directly modulates the amounts of superoxide anion (O^{2-}) and H_2O_2 (Foyer & Noctor, 2000). Super oxide dismutase activity was high under iron deficiency stress conditions as it plays a key role in detoxifying reactive oxygen species under iron deficiency stress conditions. Some reports have shown that salt stress induces an increase in SOD activity, and this has frequently been correlated with salt tolerance (Sreenivasulu *et al*., 2000; Martinez *et al*., 2001; Sudhakar *et al*., 2001).

CONCLUSION

Under iron deficient soil conditions, the groundnut genotypes recorded significantly lower active iron content, peroxidase and catalase activities while higher super oxide dismutase across all three crop growth stages compared to iron sufficient soil conditions. In the groundnut genotypes TCGS-1624 and TCGS-1616 significantly higher values of active iron content, peroxidase, catalase and super oxide dismutase activities across all three crop growth stages compared to TCGS-1613 and TCGS-1609 in both iron sufficient and deficient soil conditions. This showed that the groundnut genotypes TCGS-1624 and TCGS-1616 were tolerant to iron deficiency chlorosis than TCGS-1613 and TCGS-1609.

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