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PHYSIOLOGICAL AND BIOCHEMICAL BASIS OF LIME INDUCED CHLOROSIS TOLERANCE IN SELECTED GROUNDNUT GENOTYPES (*ARACHIS HYPOGAEA* L.)

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ABSTRACT
A pot culture experiment was conducted during *rabi*-summer, 2006 to evaluate the groundnut genotypes for lime induced chlorosis tolerance. The experiment was laid out in Completely Randomized Design with nine genotypes (*viz.*, TMV2, JL-24, DERM-5-1, GPBDM 4/25, GPBD-4, DERM (VLS), GPBDM 4/6, DERM15T and DERM14T) in three replications. The physiological and biochemical parameters of groundnut genotypes differed significantly due to lime induced iron chlorosis. The genotypes DERM and GPBDM had higher values of morphological and physiological parameters with higher ferrous iron content at all the stages. The leaf peroxidase activity also differed significantly among genotypes. There was a strong and positive correlation between peroxidase activity and leaf ferrous iron content. The genotypes DERM VLS, DERM14T, DERM15T had higher leaf peroxidase activity with higher leaf ferrous content. Among the genotypes studied, DERM VLS, DERM14T, DERM15T and GPBDM were found to be relatively more chlorosis tolerant. In DERM genotypes, DERM (VLS) had higher peroxidase activity, higher ferrous iron, chlorophyll content and was more iron efficient groundnut genotype as compared to all the genotype.

KEYWORDS: Pot culture, rabi summer, genotype, chlorosis, peroxidase activity etc.

INTRODUCTION

Groundnut is a crop of global economic significance, not only in the widespread geographical areas of its production, but also in the even wider areas of its processing and consumption. It is an important protein supplement in cattle and poultry rations. It is also consumed as confectionary product. Being a legume with root nodules, it can synthesize atmospheric nitrogen and, therefore, improve soil fertility.

The application of iron to soil in the form of ferrus sulphate ($FeSO_4$) has often been recommended to alleviate the problem of iron chlorosis and concomitant loss in yield. But, this is often of little benefit to the crop as iron ionizes and gets converted into insoluble ferric compounds which are unavailable to plants. A major problem with foliar application is poor translocation of applied iron within the plant. Though, the use of iron chelates provide iron in available from, their use is not popular and not feasible from the economic point of view.

An alternate approach to combat iron chlorosis is exploitation of genetic variability observed in groundnut for iron absorption efficiency (Hartzook, 1975; Habib and Joshi, 1982). The groundnut cultivars are called 'iron efficient' if they respond to iron deficiency stress by inducing biochemical reactions that make Fe available and 'iron-inefficient' if they do not. Most of the popular groundnut bunch cultivars of Karnataka are inefficient in iron acquisition. Growing iron-efficient cultivars in irrigated black soils could be economically preferable as it does not need application of any iron compounds. An increase in 12-24 per cent of pod yield has been observed when efficient cultivars were grown in irrigated black soils (Panchaksharaiah, 1982).

MATERIALS AND METHODS

A pot culture experiment was conducted during rabi-summer 2005 to evaluate the groundnut genotypes for lime induced chlorosis tolerance in the Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. The observations on physiological and biochemical parameters of groundnut genotypes were recorded in relation to lime induced chlorosis tolerance in calcareous soils. Details of the materials used and the experimental techniques adopted during the course of investigation are presented here under

Physiological parameters

Observations on Absolute growth rate (West *et al.*, 1920), Relative growth rate (Blackman, 1919), Net assimilation rate (Gregory, 1926) and Specific leaf weight (SLW) Radford (1967) were recorded as per the standard formulas

BIOCHEMICAL PARAMETERS

Estimation of chlorophyll content

The chlorophyll content was estimated in the third leaf (fully expanded) of the plant at 45, 60 and 75 DAS by following the method of Shoaf and Lium (1976). Hundred mg of fresh leaf tissue was cut into small pieces and incubated in 7.0 ml of DMSO (dimethyl sulfoxide) at 65°C for 30 minutes. At the end of incubation period, the supernatant was decanted and leaf tissue was discarded. The volume was made up to 10 ml and absorbance was recorded at 645, 652 and 663 nm in UV-Vis spectrophotometer (ELICO, 159). The total chlorophyll, chlorophyll 'a' and chlorophyll 'b' content were calculated using the following formulae given by Arnon (1949) and expressed as mg per g fresh weight of leaf.

Preparation of plant samples for Fe^{2} + analysis

The leaf samples were collected randomly from plants in the pots. The leaves were washed once with tap water followed by 0.1 N HCl and then rinsed with double distilled water. Further, the fresh leaves were chopped with stainless steel knife. Two gram of chopped sample was extracted with 1-10 orthophenanthroline for Fe^{2} + analysis as described by Katyal and Sharma (1980).

Estimation of peroxidase activity

Peroxidase activity was estimated following the method of Mahadevan and Sridhar (1986).

Preparation of enzyme source

One gram of fresh plant tissue was extracted with 3ml of 0.1M phosphate buffer (pH 7.0) by grinding with a pre-cooled mortar and pestle. The mixture was centrifuged at 18000g at 5°C for 15 minute and the supernatant was used as enzyme source.

Measurement of peroxidase activity

Three ml of buffer solution, 0.05 ml guaicol solution, 0.1 ml enzyme extract and 0.03 ml hydrogen peroxide solution were pipetted into a cuvette and mixed well and cuvette was placed in the UV-VIS spectrophotometer (ELICO). 159) at 436 nm. And waited until the absorbance increased by 0.05, and stop watch was started and noted the time in minutes (Δt) required to increase the absorbance by 0.1 and enzyme activity was expressed units/litre of extract using the formula of Mahadevan and Sridhar (1986).

RESULTS AND DISCUSSION

The relative growth rate reveals the efficiency of dry matter production per unit dry matter already existing and it indicates the efficiency of genotype. Rate of nutrient uptake increases with an increase in RGR (Wood House *et al*., 1978; Wild *et al*., 1979 and Breeze *et al*., 1985). In the present investigation, Absolute growth rate was significantly higher in the genotype DERM 15T at 45-60 and 60-75 DAS. Net assimilation rate in general was higher in all the genotypes during early stages of crop growth and decreased towards maturity (Table 1). Such decline in this growth parameter could be attributed to decrease in the rate of dry matter production due to leaf senescence and shading in soybean (Koti, 1997

TABLE- 1. Genotypic variation in groundnut for AGR, RGR and NAR as influenced by lime induced chlorosis

Genotypes	AGR (g $plant^{-1}$ day^{-1})		RGR (g g^{-1} day^{-1})		NAR (g dm^{2} day^{-1})	
	45-60-DAS	60-75 DAS	45-60-DAS	60-75 DAS	45-60-DAS	60-75 DAS
TMV2	0.640	0.123	0.053	0.014	0.057	0.012
GPBD4	0.650	0.142	0.056	0.017	0.059	0.014
JL-24	0.663	0.132	0.055	0.016	0.058	0.013
GPBDM 4/25	0.704	0.203	0.067	0.018	0.062	0.018
DERM (VLS)	0.706	0.204	0.068	0.018	0.076	0.018
GPBDM 4/6	0.708	0.205	0.068	0.018	0.075	0.017
DERM 15T	0.893	0.218	0.068	0.018	0.074	0.019
DERM 14 T	0.704	0.206	0.078	0.031	0.077	0.019
DERM – 5-1	0.708	0.207	0.067	0.018	0.086	0.032
Mean	0.712	0.186	0.065	0.019	0.069	0.018
S.Em±	0.041	0.022	0.004	0.003	0.002	0.001
CD at 5%	0.089	0.066	0.012	0.009	0.006	0.003

DAS: Days after sowing

TABLE- 2. Genotypic variation in groundnut for specific leaf weight and specific leaf area as influenced by lime induced chlorosis

Genotypes	SLW (mg cm^{-1})			SLA (cm mg^{-1})		
	45 DAS	60 DAS	75 DAS	45 DAS	60 DAS	75 DAS
TMV2	1.55	1.84	1.23	0.097	0.103	0.07
GPBD4	1.84	1.87	1.35	0.113	0.123	0.087
JL-24	1.81	1.86	1.32	0.103	0.113	0.087
GPBDM 4/25	2.98	2.99	2.61	0.123	0.213	0.103
DERM (VLS)	2.91	2.95	2.61	0.133	0.217	0.107
GPBDM 4/6	2.97	2.98	2.76	0.123	0.243	0.117
DERM 15T	2.95	2.96	2.64	0.123	0.233	0.113
DERM 14 T	2.94	2.97	2.65	0.123	0.243	0.117
DERM – 5-1	2.95	2.96	2.67	0.143	0.253	0.187
Mean	2.64	2.76	2.21	0.120	0.194	0.110
S.Em±	0.04	0.03	0.06	0.004	0.002	0.005
CD at 5%	0.14	0.09	0.19	0.013	0.006	0.02

DAS: Days after sowing

The genotype TMV2 maintained lower NAR compared to other genotypes at 45-60 and 60-75 DAS which was attributed to higher degree of per cent chlorosis, lower dry matter production, lower iron content due to lime induced chlorosis. Mahurkar *et al*. (1995) observed in groundnut cultivars that all the chlorotic plants had lower NAR and crop growth rate (CGR) values than non-chlorotic plants. The genotypes other than TMV-2, JL-24 and GPBD-4 had higher Specific Leaf weight with lower chlorotic readings which was due to the higher leaf dry weight and maintenance of green leaves even at later stages of crop growth (Table 2). Bhatia *et al.* (1996) reported a significant positive correlation of SLW with photosynthesis and seed yield in different maturity types of soybean.

The genotype DERM (VLS) had significantly higher chlorophyll a, b and total chlorophyll at all the stages. The genotype GPBD4, TMV2, JL24 had least chlorophyll content and were very well correlated with lower iron content and peroxidase activity (Table 3).

Samdur *et al*. (2000) reported that all the tolerant groundnut genotypes (based on visual chlortic rating) had high chlorophyll content (more than 7 mg/g on dry weight basis). The chlorophyll content at 50 and 60 DAS was maximum in all the genotypes and differentiation between Fe efficient and inefficient lines were quite clear

TABLE- 3. Genotypic variation in groundnut for total chlorophyll content (mg/g) fresh weight as influenced by lime induced chlorosis

Genotypes	Total chlorophyll content (mg g^{-1} fresh weight)		
	45 DAS	60 DAS	75 DAS
TMV2	1.12	1.16	0.56
GPBD4	1.18	1.33	0.63
JL-24	1.13	1.20	0.61
GPBDM 4/25	1.67	1.91	0.78
DERM (VLS)	2.60	2.64	1.28
GPBDM 4/6	1.76	1.94	0.78
DERM 15T	1.88	1.95	0.77
DERM 14 T	1.90	2.04	0.77
DERM – 5-1	1.95	2.01	0.81
Mean	1.69	1.80	0.78
S.Em±	0.04	0.07	0.1
CD at 5%	0.12	0.21	0.3

DAS: Days after sowing

The ferrous iron content in groundnut genotypes at different growth stages indicated significant differences among the genotypes. The active iron content in the genotypes ranged from 2.40 to the maximum of 12.3 ppm. The calcareous soil in which the genotypes were grown, had less than 5 ppm DTPA extractable Fe. Most of the genotypes had active iron content lower than 12 ppm and showed chlorosis (Table 4). Singh (1994b) has reported that active iron is taken as criterion and observed less than 12 ppm active iron in chlorotic plants. The genotype

Table 4. Genotypic variation in groundnut for Fe^{2+} content (ppm) as influenced by lime induced chlorosis

Genotypes	Fe^{2+} content (ppm)		
	45 DAS	60 DAS	75 DAS
TMV2	4.15	3.35	2.40
GPBD4	5.80	6.50	5.05
JL-24	5.50	4.15	3.85
GPBDM 4/25	9.21	10.10	8.45
DERM (VLS)	9.35	12.30	12.05
GPBDM 4/6	9.22	10.35	8.55
DERM 15T	9.24	10.20	8.60
DERM 14 T	9.26	10.45	8.75
DERM – 5-1	9.16	10.57	8.90
Mean	8.10	8.68	7.42
S.Em±	0.10	0.13	0.17
CD at 5%	0.34	0.44	0.55

DAS: Days after sowing

DERM (VLS) had higher ferrous iron with the lower per cent chlorosis and higher values of SPAD as compared to other genotypes. The DERM and GPBDM genotypes had moderate iron content 9 to 10 ppm with higher peroxidase activity, whereas the genotypes JL-24. TMV-2 and GPBD-4 with the iron content 2.4 to 6.5 ppm at various stages of growth had lower peroxidase activity and SPAD values with higher per cent chlorosis.

The peroxidase enzyme in the present investigation had higher activity at 45 DAS and decreased at later stages (60 and 75 DAS) of crop growth (Table 5). A similar trend for peroxidase activity has been observed by Sanjana (2004)

in soybean, which appears to be natural phenomenon in all the crops. But, higher decrease at later stages was due to increase in iron deficiency as was evident by decrease in active iron content.

At 45 DAS, the genotype DERM (VLS) had significantly higher peroxidase activity with higher ferrous (Fe^{+}) content followed by GPBDM 4/25 and other DERM genotypes. GPBDM 4/25 and other DERM genotypes also had relatively higher concentration of iron. Least activity of peroxidase was observed in the genotypes TMV-2, GPBD-4 and JL-24, with lower iron content, higher chlorotic values and lower SPAD values.

TABLE- 5. Genotypic variation in groundnut for peroxidase enzyme activity (units/litre) as influenced by lime induced chlorosis

Genotypes	Peroxidase enzyme activity (units $litre^{-1}$)		
	45 DAS	60 DAS	75 DAS
TMV2	96.64	86.97	72.01
GPBD4	160.24	134.82	100.97
JL-24	116.42	114.16	85.81
GPBDM 4/25	301.49	232.24	163.73
DERM (VLS)	379.26	251.32	237.53
GPBDM 4/6	204.12	169.43	158.38
DERM 15T	260.43	192.95	160.94
DERM 14 T	242.14	176.83	157.44
DERM – 5-1	236.52	219.52	161.28
Mean	221.25	175.36	144.23
S.Em±	2.80	3.31	2.99
CD at 5%	8.39	9.93	8.97

DAS: Days after sowing

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