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Research Note

Biochemical basis of iron deficiency chlorosis resistance in groundnut (*Arachis hypogaea* L.)

Ishwar H. Boodi*, S.K. Pattanashetti, B.D. Biradar and C.D. Soregoan
College of agriculture Vijayapura, Karnataka-586101, India.
E-mail: ishwarhb.uasdagri@gmail.com

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Abstract
A pot experiment with factorial design involving normal and calcareous soil and five genotypes with differential response to iron deficiency chlorosis (IDC) viz., ICGV 86031 and A30b (Resistant), TG 26 (moderately Resistant), TAG 24 and TMV 2 (susceptibe) were tested for various traits like VCR and SCMR, chlorophyll a, b and total chlorophyll, active iron content, specific activity of peroxidase at five different stages and also know the effect of IDC on yield and yield components. Iron deficiency chlorosis resistant genotypes recorded significantly lower VCR, higher SCMR, higher active iron content, chlorophyll a, b and total chlorophyll and peroxidase activity in leaf across all stages compared to susceptible genotypes. A strong and positive correlation was observed between peroxidase activity and leaf iron content. Yield and yield components were significantly reduced in susceptible genotypes compared to resistant genotypes.

Key words
Iron deficiency chlorosis (IDC)**,** chlorophyll, Active iron (Ferrous, Fe^{2+}), resistance and peroxidase activity.

Groundnut being sensitive to iron deficiency, iron deficiency chlorosis is most commonly seen in areas of groundnut cultivation particularly in calcareous, alkaline and black soils. Iron chlorosis causes reduction in groundnut yield. The application of iron to soil in the form of ferrous sulphate (Fe_2SO_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 IDC is exploitation of genetic variability observed in groundnut for iron absorption efficiency (Hartzook, 1975; Habib and Joshi, 1982). The IDC resistant lines could also be used further in groundnut crop improvement programme. The groundnut cultivars are called 'IDC resistant' if they respond to iron deficiency stress by inducing biochemical reactions that make Fe^{2+} available and 'IDC-Susceptible' if they do not. Growing iron-resistant 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).

Pot experiment was conducted as per factorial design with soil type (normal black soil and calcareous soil) as factor 'A' and above listed genotypes (five) as factor 'B' to know their individual effects and interaction. The recommended cultivation practices were followed to maintain healthy plants. Iron containing fertilizers were not applied.

Visual chlorotic rating (1 to 5 scale proposed by Singh and Chaudhari, 1993) and SPAD chlorophyll meter reading (SCMR) values were recorded and mean was calculated.

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).

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Preparation of sample: One gram of fresh leaf tissue was extracted with 3 ml of 0.1 M phosphate buffer (pH 6.0) by grinding with a pre-cooled mortar and pestle. The mixture was centrifuged at 3000 rpm at 5°C for 15 minutes and the supernatant was used as enzyme source.

Estimation of activity: Peroxidase activity was estimated as per the method of Mahadevan and Sridhar (1986). 3ml 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. The change in absorbance was noted at an interval of 20 seconds after adding 0.5 ml of 2 percent H_2O_2 and inverting the cuvette. The protein content of enzyme extract was determined by Lowry's method (Lowry et al. 1951). The peroxidase activity was expressed as change in optical density per minute (Δ OD / min).

Yield and yield parameters: All the readings were recorded on standard leaf (third fully opened leaf from top of the main stem) of the five plants for every treatment in four replications of calcareous and normal soils at five different stages viz., 20, 40, 60, 80 and 100 DAS. Yield and yield components like main stem height (cm), number of primary branches, pod yield per plant (g), haulm yield per plant (g), shelling percentage and 100 seed weight (g) were recorded at the before or after harvest for all the genotypes.

Plantlet regeneration from *in vitro* root (intercalary expanded portion of root) and in vitro conservation of *C. orchioides* is reported in this publication for the first time. A reproducible protocol for *in vitro* conservation of this endangered medicinal plant is reported.

Mean squares based on ANOVA for IDC related traits like visual chlorotic ratings (VCR), SPAD chlorophyll meter readings (SCMR), active iron (Ferrous, Fe^{2+}) content, specific activity of peroxidase and chlorophyll 'a', 'b' and total chlorophyll content at all the five stages viz., 20, 40, 60, 80 and 100 days after sowing (DAS) showed highly significant differences among treatments, factor A (soil types) and factor B (genotypes) (Table 1, 2 and 3). Whereas, factor A (soil types) x factor B (genotypes) interaction variances showed significant differences for VCR at all the five stages, SPAD values at 60 and 80 and for specific activity of peroxidase at 100 DAS.

Similarly for yield and yield components like main stem height (cm), number of primaries per plant, number of pods per plant, pod yield per plant (g), shelling percentage and test weight, highly significant differences were observed among the treatments and factor B (genotypes). Among factor A (soil type), significant differences observed for main stem height (cm), number of primaries per plant, number of pods per plant, pod yield per plant (g) (Table 4).

Iron deficiency chlorosis resistant genotypes ICGV 86031 and A30b had lower VCR followed by TG 26 across all the growth stages viz., 20, 40, 60, 80 and 100 DAS under normal soil than calcareous soil, exhibiting higher uptake of Fe^{2+} and utilization efficiency and susceptible genotypes TMV 2 and TAG 24 had higher VCR score compare to resistant genotypes (Table 5). Visual scores on 1-5 scale in general ranged from 1.00 to 3.00 during the crop growth. The values of visual scores were higher between 40 to 60 DAS than initial or later stages of crop growth, indicating higher metabolic activity at these stages and higher requirement of iron at peak growth stages, however, iron taken up by the plants was metabolized into other functions of plant. Bhardwaj (2006) reported development of chlorosis within 35 days after sowing but increased chlorosis occurred at 45 DAS in peanut under simulated conditions through irrigating crops in highly calcareous soils. Whereas, Kulkarni *et al.* (1994) found visual chlorosis scores at 60 DAS were more reliable than scores of other stages in groundnut.

The mean SCMR values, active iron content, chlorophyll 'a', chlorophyll 'b' and total chlorophyll content and also peroxidase activity of genotypes grown in different soil types showed highly significant differences evident from higher mean values of the traits in normal soil compared to calcareous soil (Table 5, 6 and 7). The genotypes showed significant differences for all traits evident from higher values in IDC resistant/moderately resistant genotypes like ICGV 86031, A30b and TG 26 compared to susceptible genotypes like TMV-2 and TAG 24. Iron deficiency chlorosis appears 10-15 days after emergence in the field and remains throughout the cropping season, but its maximum intensity was observed between 30-70 days after emergence (Singh and Chaudhari, 1993).

There is also self-recovery of chlorosis as leaves become older, but the newly emerging leaves further show chlorosis (Singh, 1994a). Iron deficiency first appears as chlorosis on young rapidly expanding leaves which is characterized by interveinal chlorosis. During severe deficiency, the veins also become chlorotic and leaves become white and papery (Singh *et al*., 1991a, b) and later becomes brown and necrotic. The acute deficiency leads to death of plant in the field and crop failure. The sufficiency level of Fe in groundnut leaves is 50-300 ppm and the critical limit is 40 ppm, but Fe deficiency in groundnut is visible when tissue

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concentration falls below 30 ppm in leaves (Singh, 1994b).

The ferrous iron content in groundnut genotypes at different growth stages indicated significant differences among the genotypes. The mean active iron content in the genotypes ranged from 10.1 to the maximum of 6.7 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 8 ppm and showed chlorosis (Table 6). Singh (1994b) has reported that active iron is taken as criterion and observed lower active iron in chlorotic plants. The genotypes ICGV 86031, A30b and TG 26 had higher ferrous iron with the lower VCR score and higher values of SCMR with higher peroxidase activity, whereas the genotypes TMV-2 and TAG 24 with the mean iron content 6.73 to 6.85 ppm at various stages of growth had lower peroxidase activity and SPAD values with higher VCR values.

The peroxidase enzyme in the present investigation had higher activity at 60 DAS and decreased at later stages (80 and 100 DAS) of crop growth (Table 6). 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 60 DAS, the genotype ICGV 86031, A30b and TG 26 had significantly higher peroxidase activity with higher ferrous (Fe^{2+}) content. Least activity of peroxidase was observed in the genotypes TAG 24 and TMV 2 with lower iron content, higher VCR score and lower SCMR values.

The genotypes ICGV 86031, A30b and TG 26 had significantly higher chlorophyll a, b and total chlorophyll at all the stages. The genotypes TMV-2 and TAG 24 had least chlorophyll content and were very well correlated with lower iron content and peroxidase activity (Table 7). 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 40 and 60 DAS was maximum and differentiation between Fe resistant and susceptible lines was quite clear.

The yield and yield components like main stem height, number of primary branches, pod yield per plant, haulm yield per plant, shelling percentage and 100 seed weight among the soil types showed highly significant differences as evident from higher mean values in normal soil compared to calcareous soil (Table 8). All yield and yield components among the genotypes showed significant differences evident from higher mean values in IDC resistant/ moderately resistant genotypes like ICGV 86031, A30b and TG 26 compared to iron susceptible genotype like TMV 2 and TAG 24. Soil types (factor A) x genotypes (factor B) interaction showed non-significant differences for all yield and yield parameters. In normal soil, treatments A_1 B_1 and A_1 B_2 recorded numerically higher mean values compared to A_1 B_3 and A_1 B_4 for all the parameters. Similarly in calcareous soil, A_2 B_1 and A_2 B_2 recorded numerically higher mean values for all parameters compared to A_2 B_3 and A_2 B_4 due to their tolerance to iron deficiency chlorosis. Yield reduction to the extent of 13-50 per cent has been reported earlier due to iron deficiency chlorosis (Kulkarni, 1989).

Association studies in normal and calcareous soil revealed that VCR is significantly negative correlation with SCMR, active iron content, chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll content and also peroxidase activity (Nagarathnamma, 2006). There is a negative correlation between VCR and various yield and yield parameters like main stem height, number of primaries per plant, number of pods per plant, pod yield per plant and test weight whereas, positive correlation between SCMR and various yield and yield parameters like main stem height, number of primaries per plant, number of pods per plant, pod yield per plant and test weight and test weight (Table 9 and 10). A strong and positive correlation was observed between peroxidase activity and leaf iron content. Hence, higher active iron content, chlorophyll a, b and total chlorophyll and peroxidase activity are the probable factors responsible for iron absorption efficiency in efficient genotypes.

References

Bhardwaj, A. 2006. Integrated amelioration of lime induced chlorosis in peanut (*Arachis hypogaea* L.). 18th World Congress of Soil Science, Philadelphia, Pennsylvania, USA.

Habib, A.F. and Joshi, M.S. 1982. Combating iron chlorosis in black soils of Malaprabha and Ghataprabha Project Area. *Current Sci.,* **11**: 51-54.

Hartzook, A. 1975. Lime induced iron chlorosis in groundnut treatment and prevention. Plant Protection Bulletin FAO. 23, 40-42.

Katyal, J.C. and Sharma, B.D. 1980. New technique to resolve iron chlorosis. *Plant and Soil*, **55**(1), 105-109.

Kulkarni, V.N. 1989. An assessment of genetic potential of some iron-efficient cultures for the improvement of bunch cultivars of groundnut (*Arachis hypogaea* L.). M.Sc. (Agri.) Thesis, Uni. Agric. Sci. Dharwad. 203.

Kulkarni, V.N., Gowda, M.V.C., Panchal, Y.C. and Nadaf, H.L. 1994. Evaluation of groundnut cultivars for iron absorption efficiency. *Crop Research*, **7**(1), 84-92.

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Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with folin phenol reagent. *Journal of Biological Chemistry*, **193**: 265- 275.

Mahadevan, A. and Sridhar, R. 1986. Methods in Physiological Plant Pathology. Sivakami publishers, Madras. 103-104.

Nagarathnamma. 2006. Evaluation of groundnut genotypes for lime induced chlorosis tolerance. M.Sc. (Agri.) Thesis, submitted to the Uni. Agric. Sci. Dharwad. 96-99.

Panchaksharaiah, S. 1982. Investigations on the effect of nitrogen, iron, gypsum, sulphur and calcium on growth and yield of groundnut (*Arachis hypogaea* L.) and iron efficiency of genotypes on black soil under irrigation. Ph.D. Thesis, Uni. Agric. Sci. Bangalore.

Samdur, M.Y., Singh, A.L., Mathur, R.K., Manivel, P., Chikani, B.M., Gor, H.K. and Khan, M.A. 2000. Field evaluation of chlorophyll meter for screening groundnut (*Arachis hypogaea* L.) genotypes tolerant to iron deficiency chlorosis. *Indian Journal of Agricultural Sciences*, **79**(2): 211-214.

Sanjana, K. and Koti, R.V. 2004. Effect of manganese and boron on oxidative enzymes and yield in rust infected soybean *Glycine max* (L.) Merrill. International Conference on Biotechnology Approaches for Alleviating Malnutrition and Human Health, 9th - 11th January 2006, held at GKVK, Uni. Agric. Sci. Bangalore.

Shoaf, T.W. and Lium, B.W. 1976. Improved extraction of chlorophyll 'a' and 'b' from algae using dimethyl sulfoxide. *Limnoloceanor*, **21**: 926-928.

Singh, A.L., Chaudhari, V. and Koradia, V.G. 1991a. Foliar nutrition of nitrogen and phosphorus in groundnut. *In:* Tyagi, D.N. *et al*. (ed.) Physiological strategies for crop improvement. Proceedings of the International Conference of Plant Physiology, BHU Varanasi, India. 129-133.

Singh, A.L., Joshi, Y.C., Devi Dayal and Misra, J. B. 1991b. Application of different sources of sulphur in groundnut. *In:* Proceedings of "The National Seminar on Sulphur in Agriculture". In Siddaramappa R., Vijaya, P.K. Chaudhary and Susheela Devi, L., (eds.), Uni. Agric. Sci., Bangalore and fertilizer and chemicals Travancore Limited, Cochin India, p.76-81.

Singh, A.L. and Chaudhari, V. 1993. Screening of groundnut germplasm collection and selection of genotypes tolerant to lime-induced iron chlorosis. *Journal of Agricultural Science*, **121**: 205-111.

Singh, A.L. 1994a. Screening of groundnut cultivars for tolerance to lime-induced iron chlorosis. In plant productivity under environment stress, Karan Singh and S.S. Purohit (edt.). Agrobotanical publishers, Bikaner, India. 289-294.

Singh, A.L. 1994b. Micronutrients nutrition and crop productivity in groundnut. In plant productivity under environment stress, Karan Singh and S.S. Purohit (Edt.), Agrobotanical Publishers, Bikaner, India. 67-72.

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Table 1. Mean squares for visual chlorotic rating (VCR) and SPAD chlorophyll meter reading (SCMR) of groundnut genotypes in normal and calcareous soil

Source of variation	df	Visual chlorotic rating (VCR)					SPAD chlorophyll meter reading (SCMR)				
		20 DAS	40 DAS	60 DAS	80 DAS	100 DAS	20 DAS	40 DAS	60 DAS	80 DAS	100 DAS
Replications	3	0.07	0.09	0.4	4.36	0.00	5.18	5.21	1.04	0.61	1.47
Treatments	9	3.96**	3.36**	3.04**	0.91**	5.54**	149.47**	137.30**	141.18**	212.73**	86.24**
Factor A (Soil types)	1	6.40**	4.23**	2.50**	0.59**	2.50**	250.00**	425.10**	499.14**	622.52**	315.84**
Factor B (Genotypes)	4	6.21**	5.28**	5.79**	1.08**	11.35**	272.61**	196.57**	154.20**	271.93**	102.69**
Factor A x Factor B (Soil types x Genotypes)	4	1.09**	1.23**	0.44*	0.36*	0.50*	1.2	6.07	38.68**	51.09**	12.4
Error	18	0.21	0.14	0.1	0.73	0.14	9.95	4.09	2.38	3.62	5.3

*, ** Significant at 5 and 1 per cent level, respectively.

Table 2. Mean squares for active iron (Ferrous, Fe^{2+}) content and specific activity of peroxidase of groundnut genotypes in normal and calcareous soil

Source of variation	df	Active iron (Ferrous, Fe^{2+}) content					Specific activity of peroxidase (ΔOD/mg of protein)				
		20 DAS	40 DAS	60 DAS	80 DAS	100 DAS	20 DAS	40 DAS	60 DAS	80 DAS	100 DAS
Replications	3	4.00	10.25	5.95	3.75	2.57	0.008	0.02	0.002	0.039	0.03
Treatments	9	11.25*	15.46*	15.32*	8.75**	8.55**	0.120**	0.78**	1.030**	0.750**	0.67**
Factor A (Soil types)	1	21.15*	24.38*	1.77*	22.85**	37.19**	0.167**	4.40**	6.935**	5.359**	3.84**
Factor B (Genotypes)	4	19.61**	27.74*	33.33**	13.46**	9.34**	0.225**	0.61**	0.573**	0.342**	0.42**
Factor A x Factor B (Soil types x Genotypes)	4	0.41	0.95	0.70	0.50	0.59	0.003	0.04	0.010	0.005	0.13**
Error	18	3.82	8.96	6.13	2.15	1.60	0.008	0.02	0.021	0.023	0.03

*, ** Significant at 5 and 1 per cent level, respectively.

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Table 3. Mean squares for chlorophyll content of groundnut genotypes in normal and calcareous soil

Source of variation	df	Chlorophyll 'a'					Chlorophyll 'b'					Total Chlorophyll				
		20 DAS	40 DAS	60 DAS	80 DAS	100 DAS	20 DAS	40 DAS	60 DAS	80 DAS	100 DAS	20 DAS	40 DAS	60 DAS	80 DAS	100 DAS
Replications	3	0.014	0.037	0.029	0.018	0.021	0.010	0.025	0.111	0.008	0.011	0.039	0.116	0.314	0.005	0.025
Treatments	9	0.248**	0.452**	0.496**	0.393**	0.226**	0.094**	0.220**	0.253**	0.150**	0.503**	0.637**	1.266**	1.526**	0.990**	1.311**
Factor A (Soil types)	1	0.769**	0.295**	0.322**	0.175*	0.427**	0.179**	0.479**	0.962**	0.608**	3.189**	1.657**	1.558**	1.498**	1.247**	5.998**
Factor B (Genotypes)	4	0.361**	0.928**	0.995**	0.821**	0.395**	0.164**	0.375**	0.325**	0.186**	0.311**	1.001**	2.440**	3.032**	1.893**	1.398**
Factor A x Factor B (Soil types x genotypes)	4	0.004	0.015	0.041	0.020	0.007	0.004	0.001	0.003	0.001	0.023	0.018	0.020	0.027	0.024	0.054
Error	18	0.033	0.029	0.035	0.024	0.019	0.008	0.021	0.023	0.016	0.022	0.035	0.056	0.178	0.035	0.058

Note: Factor A (Soil types) (2): Normal soil, Calcareous soil, Factor B (Genotypes) (5): ICGV 86031, A30b, TG 26, TAG 24, TMV 2, Factor A x Factor B interaction (Soil type x Genotypes) df – Degrees of freedom; DAS - Days after sowing, *, ** Significant at 5% and 1% level of probability, respectively.

Table 4. Mean squares for yield and yield components of groundnut genotypes in normal and calcareous soil

Source of variation	Df	Main stem height (cm)	No. of primaries / plant	No. of pods / plant	Pod yield / plant (g)	Haulm yield / plant (g)	Shelling Percentage	Test weight (g)
Replications	3	5.77	3.52	1.56	0.96	0.12	1.23	10.70
Treatments	9	126.00**	5.22**	47.68**	25.79**	4.59	297.94**	64.98**
Factor A (Soil types)	1	26.72	14.04**	157.61**	30.12*	0.06	78.64	1292.63
Factor B (Genotypes)	4	269.29**	7.91**	64.58**	48.91**	10.19	649.14**	63.68**
Factor A x Factor B (Soil types x Genotypes)	4	7.55	0.32	3.30	1.59	0.11	1.57	14.60
Error	18	9.84	0.90	5.43	5.25	0.63	3.76	5.96

Note: Factor A (Soil types) (2): Normal soil, Calcareous soil; Factor B (Genotypes) (5): ICGV 86031, A30b, TG 26, TAG 24, TMV 2; Factor A x Factor B interaction (Soil type x Genotypes); df – Degrees of freedom; DAS - Days after sowing ; *, ** Significant at 5% and 1% level of probability, respective.

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Table 5. Visual chlorotic rating (VCR) and SPAD chlorophyll meter reading (SCMR) of groundnut genotypes in normal and calcareous soil

Factors	Treatments	VCR						SCMR					
		20 DAS	40 DAS	60 DAS	80 DAS	100 DAS	Mean	20 DAS	40 DAS	60 DAS	80 DAS	100 DAS	Mean
Factor-A	A1 (Normal)	1.50	1.45	1.55	1.80	1.95	1.65	32.29	35.06	37.57	35.16	28.73	33.76
(Soil types)	A2 (Calcareous)	2.30	2.10	2.05	2.30	2.45	2.24	27.29	28.54	30.51	27.27	23.11	27.34
	SEm±	0.10	0.08	0.07	0.10	0.08		0.70	0.45	0.35	0.43	0.52	
	CD (5%)	0.31	0.25	0.21	0.31	0.25		2.10	1.34	1.03	1.26	1.53	
	CD (1%)	0.42	0.34	0.29	0.42	0.34		2.87	1.84	1.41	1.73	2.09	
Factor-B	B1 (ICGV 86031)	1.00	1.00	1.00	1.00	1.00	1.00	36.09	37.35	38.91	37.55	30.10	36.00
(Genotypes)	B2 (A30b)	1.00	1.00	1.00	1.00	1.00	1.00	35.78	36.65	38.59	37.15	29.00	35.43
	B3 (TG 26)	2.00	1.75	1.63	2.13	2.25	1.95	28.31	30.63	32.31	29.73	25.59	29.31
	B4 (TAG 24)	2.63	2.25	2.75	2.88	3.25	2.75	24.39	27.31	30.26	25.90	22.68	26.11
	B5 (TMV 2)	2.88	2.88	2.63	3.25	3.50	3.03	24.36	27.06	30.11	25.73	22.21	25.90
	SEm±	0.16	0.13	0.11	0.17	0.13		1.12	0.72	0.55	0.67	0.81	
	CD (5%)	0.48	0.39	0.33	0.49	0.39		3.31	2.13	1.62	2.00	2.42	
	CD (1%)	0.66	0.53	0.46	0.67	0.54		4.54	2.91	2.22	2.74	3.31	
Factor A x	A1 B1	1.00	1.00	1.00	1.00	1.00	1.00	39.13	39.37	40.00	38.30	32.03	37.77
Factor B	A1 B2	1.00	1.00	1.00	1.00	1.00	1.00	38.40	39.00	39.67	38.03	30.73	37.17
(Soil types x	A1 B3	1.33	1.00	1.33	2.00	2.00	1.53	33.03	34.27	37.37	35.00	28.97	33.73
Genotypes)	A1 B4	2.00	1.67	2.33	2.33	2.67	2.20	26.57	29.90	35.37	31.60	26.20	29.93
	A1 B5	2.33	2.67	2.33	3.00	3.00	2.67	27.27	31.40	35.33	31.97	27.17	30.63
	A2 B1	1.00	1.00	1.00	1.00	1.00	1.00	33.70	35.10	37.10	36.23	28.23	34.07
	A2 B2	1.00	1.00	1.00	1.00	1.00	1.00	32.67	34.40	38.13	36.13	27.93	33.85
	A2 B3	2.67	2.67	2.00	2.33	2.33	2.40	25.67	27.20	27.17	24.00	22.50	25.31
	A2 B4	3.33	3.00	3.33	3.33	4.00	3.40	20.50	24.07	25.13	20.10	18.43	21.65
	A2 B5	3.67	3.00	3.00	3.67	4.00	3.47	21.20	23.30	24.50	19.63	17.40	21.21
	SEm±	0.23	0.19	0.16	0.23	0.19		1.58	1.01	0.77	0.95	1.15	
	CD (5%)	0.68	0.55	0.47	0.69	0.55		4.68	3.01	2.29	2.82	3.42	
	CD (1%)	0.94	0.76	0.64	0.95	0.76		6.42	4.12	3.14	3.87	4.68	
	CV (%)	23.77	20.60	17.25	22.52	16.94		10.58	6.36	4.54	6.11	8.86	

DAS - Days after sowing;

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Table 6. Active iron (Fe^{2+}) (ppm) content and specific activity of peroxidase enzyme of groundnut genotypes in normal and calcareous soil

Factors	Treatments	Active iron (Fe^{2+})						Peroxidase activity					
		20 DAS	**40 DAS**	**60 DAS**	**80 DAS**	**100 DAS**	**Mean**	**20 DAS**	**40 DAS**	**60 DAS**	**80 DAS**	**100 DAS**	**Mean**
Factor-A	A1 (Normal)	8.37	9.72	11.23	8.49	7.31	9.02	0.32	0.56	0.69	0.44	0.27	0.45
(Soil types)	A2 (Calcareous)	6.92	8.15	10.81	6.98	5.38	7.65	0.45	1.22	1.52	1.17	0.89	1.05
	SEm±	0.44	0.67	0.55	0.33	0.28		0.02	0.03	0.03	0.03	0.04	
	CD (5%)	1.3	1.99	1.65	0.97	0.84		0.06	0.09	0.1	0.1	0.1	
	CD (1%)	1.78	2.72	2.25	1.33	1.15		0.08	0.12	0.13	0.14	0.14	
Factor-B	B1 (ICGV 86031)	9.3	11.28	13.31	9.11	7.52	10.1	0.56	1.16	1.39	1.02	0.83	0.99
(Genotypes)	B2 (A30b)	9.16	10.27	12.95	8.9	7.18	9.69	0.54	1.15	1.37	1.01	0.8	0.97
	B3 (TG 26)	7.56	8.88	10.68	7.83	6.57	8.3	0.41	0.91	1.07	0.77	0.54	0.74
	B4 (TAG 24)	6.18	7.28	9.15	6.33	5.28	6.85	0.23	0.61	0.87	0.63	0.36	0.54
	B5 (TMV 2)	6.02	6.97	8.99	6.52	5.16	6.73	0.2	0.59	0.83	0.58	0.35	0.51
	SEm±	0.69	1.06	0.88	0.52	0.45		0.03	0.05	0.05	0.05	0.06	
	CD (5%)	2.05	3.14	2.6	1.54	1.33		0.09	0.14	0.15	0.16	0.17	
	CD (1%)	2.81	4.31	3.56	2.11	1.82		0.13	0.2	0.21	0.22	0.23	
Factor A x	A1 B1	10.16	10.52	14.05	10.08	9.28	10.82	0.48	0.8	0.92	0.62	0.44	0.65
Factor B	A1 B2	10.37	12.09	12.05	10.26	8.21	10.6	0.46	0.73	0.9	0.62	0.39	0.62
(Soil types x	A1 B3	9.1	9.62	11.08	9.05	6.91	9.15	0.36	0.53	0.75	0.43	0.26	0.47
Genotypes)	A1 B4	6.35	7.6	9.81	7.93	6.06	7.55	0.18	0.36	0.45	0.29	0.19	0.3
	A1 B5	6.55	6.8	9.29	8.06	5.78	7.3	0.13	0.35	0.42	0.23	0.17	0.26
	A2 B1	8.3	11.28	13.01	8.28	6.13	9.4	0.63	1.55	1.82	1.39	1.29	1.34
	A2 B2	8.12	8.23	12.72	8.2	5.47	8.55	0.65	1.53	1.87	1.39	1.26	1.34
	A2 B3	7.24	8.01	10.4	7.08	5.98	7.74	0.47	1.26	1.47	1.12	0.92	1.05
	A2 B4	5.37	7.01	9.43	5.54	4.22	6.31	0.27	0.92	1.26	0.85	0.55	0.77
	A2 B5	5.67	6.97	9.61	5.51	4.62	6.48	0.28	0.83	1.23	0.99	0.56	0.78
	SEm±	0.98	1.5	1.24	0.73	0.63		0.04	0.07	0.07	0.08	0.08	
	CD (5%)	2.9	4.45	3.68	2.18	1.88		0.13	0.2	0.21	0.23	0.23	
	CD (1%)	3.98	6.09	5.04	2.98	2.57		0.18	0.28	0.29	0.31	0.32	
	CV (%)	20.29	23.96	22.22	18.32	20.18		22.37	15.37	12.98	19.25	19.07	

DAS - Days after sowing;

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Table 7. Chlorophyll 'a', Chlorophyll 'b' and total chlorophyll content of groundnut genotypes in normal and calcareous soil

Factors	Treatments	Chlorphyll a						Chlorophyll b						Total chlorophyll					
		20 DAS	40 DAS	60 DAS	80 DAS	100 DAS	Mean	20 DAS	40 DAS	60 DAS	80 DAS	100 DAS	Mea n	20 DAS	40 DAS	60 DAS	80 DAS	100 DAS	Mean
Factor-A	A1 (Normal)	1.025	1.285	1.406	1.185	0.859	1.152	0.468	0.795	0.959	0.727	0.859	0.762	0.468	0.795	0.959	0.727	0.859	0.762
(Soil types)	A2 (Calcareous)	0.748	1.114	1.227	1.053	0.652	0.959	0.335	0.576	0.649	0.48	0.294	0.467	0.335	0.576	0.649	0.48	0.294	0.467
	SEm±	0.04	0.038	0.042	0.034	0.031		0.02	0.032	0.034	0.028	0.034		0.02	0.032	0.034	0.028	0.034	
	CD (5%)	0.12	0.112	0.125	0.102	0.092		0.059	0.095	0.101	0.084	0.1		0.059	0.095	0.101	0.084	0.1	
	CD (1%)	0.164	0.154	0.171	0.14	0.126		0.081	0.131	0.138	0.114	0.136		0.081	0.131	0.138	0.114	0.136	
Factor-B	B1 (ICGV 86031)	1.131	1.575	1.67	1.462	1.001	1.368	0.552	0.919	0.992	0.78	0.791	0.807	0.552	0.919	0.992	0.78	0.791	0.807
(Genotypes)	B2 (A30b)	1.085	1.555	1.646	1.409	0.983	1.336	0.531	0.883	0.981	0.748	0.781	0.785	0.531	0.883	0.981	0.748	0.781	0.785
	B3 (TG 26)	0.84	1.068	1.359	1.13	0.684	1.016	0.411	0.686	0.868	0.56	0.514	0.608	0.411	0.686	0.868	0.56	0.514	0.608
	B4 (TAG 24)	0.694	0.901	0.96	0.808	0.566	0.786	0.263	0.488	0.595	0.473	0.404	0.444	0.263	0.488	0.595	0.473	0.404	0.444
	B5 (TMV 2)	0.681	0.899	0.949	0.785	0.544	0.772	0.25	0.451	0.585	0.456	0.393	0.427	0.25	0.451	0.585	0.456	0.393	0.427
	SEm±	0.064	0.06	0.067	0.054	0.049		0.032	0.051	0.054	0.044	0.053		0.032	0.051	0.054	0.044	0.053	
	CD (5%)	0.189	0.177	0.198	0.162	0.145		0.094	0.151	0.159	0.132	0.157		0.094	0.151	0.159	0.132	0.157	
	CD (1%)	0.259	0.243	0.271	0.222	0.199		0.128	0.207	0.218	0.181	0.216		0.128	0.207	0.218	0.181	0.216	
Factor A x	A1 B1	1.264	1.654	1.822	1.586	1.093	1.484	0.646	1.04	1.127	0.926	1.093	0.966	0.646	1.04	1.127	0.926	1.093	0.966
Factor B	A1 B2	1.25	1.617	1.8	1.54	1.123	1.466	0.57	0.997	1.18	0.887	1.123	0.951	0.57	0.997	1.18	0.887	1.123	0.951
(Soil types x	A1 B3	0.957	1.21	1.36	1.097	0.783	1.081	0.42	0.75	0.96	0.693	0.783	0.721	0.42	0.75	0.96	0.693	0.783	0.721
Genotypes)	A1 B4	0.827	0.953	1.01	0.877	0.633	0.86	0.34	0.603	0.76	0.613	0.633	0.59	0.34	0.603	0.76	0.613	0.633	0.59
	A1 B5	0.87	0.867	0.993	0.813	0.627	0.834	0.343	0.54	0.777	0.587	0.627	0.575	0.343	0.54	0.777	0.587	0.627	0.575
	A2 B1	0.977	1.497	1.457	1.363	0.827	1.224	0.483	0.85	0.883	0.623	0.497	0.667	0.483	0.85	0.883	0.623	0.497	0.667
	A2 B2	0.927	1.477	1.433	1.337	0.807	1.196	0.477	0.75	0.787	0.61	0.52	0.629	0.477	0.75	0.787	0.61	0.52	0.629
	A2 B3	0.7	0.92	1.31	1.1	0.593	0.925	0.393	0.587	0.707	0.37	0.303	0.472	0.393	0.587	0.707	0.37	0.303	0.472
	A2 B4	0.493	0.843	0.923	0.837	0.463	0.712	0.19	0.393	0.463	0.307	0.15	0.301	0.19	0.393	0.463	0.307	0.15	0.301
	A2 B5	0.51	0.863	0.92	0.78	0.44	0.703	0.19	0.36	0.337	0.353	0.163	0.281	0.19	0.36	0.337	0.353	0.163	0.281
	SEm±	0.09	0.084	0.094	0.077	0.069		0.045	0.072	0.076	0.063	0.075		0.045	0.072	0.076	0.063	0.075	
	CD (5%)	0.268	0.251	0.28	0.229	0.206		0.133	0.213	0.225	0.187	0.223		0.133	0.213	0.225	0.187	0.223	
	CD (1%)	0.367	0.344	0.383	0.314	0.282		0.182	0.292	0.309	0.256	0.305		0.182	0.292	0.309	0.256	0.305	
	CV (%)	20.55	14.19	14.45	13.60	18.73		22.01	20.90	18.99	21.06	20.44		22.01	20.90	18.99	21.06	20.44	

DAS - Days after sowing

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Table 8. Yield and yield components of groundnut genotypes in normal and calcareous soil

Factors	Treatments	Main stem height (cm)	No. of primaries / plant	No. of pods / plant	Pod yield / plant (g)	Haulm yield / plant (g)	Shelling Percentage	Test weight (g)
Factor-A	A1 (Normal)	17.07	5.01	16.32	11.14	2.35	54.10	36.91
(Soil types)	A2 (Calcareous)	15.44	3.83	12.35	9.40	2.28	51.30	31.55
	SEm±	0.70	0.21	0.52	0.51	0.18	0.43	0.82
	CD (5%)	2.08	0.63	1.55	1.52	0.53	1.29	2.45
	CD (1%)	2.86	0.86	2.12	2.09	0.72	1.76	3.35
Factor-B	B1 (ICGV 86031)	23.15	5.71	17.78	13.12	3.39	59.35	40.47
(Genotypes)	B2 (A30b)	21.39	5.13	16.73	12.27	2.76	47.53	38.26
	B3 (TG 26)	12.03	4.29	13.90	10.37	1.25	50.26	33.82
	B4 (TAG 24)	9.86	3.54	11.68	7.90	0.97	42.06	29.49
	B5 (TMV 2)	14.86	3.43	11.60	7.67	3.21	64.30	29.10
	SEm±	1.11	0.34	0.82	0.81	0.28	0.69	1.30
	CD (5%)	3.30	1.00	2.45	2.41	0.83	2.04	3.87
	CD (1%)	4.51	1.37	3.35	3.30	1.14	2.79	5.30
Factor A x	A1 B1	26.90	6.13	19.13	13.31	3.76	60.92	40.02
Factor B	A1 B2	23.88	5.33	18.60	13.11	2.84	48.61	39.85
(Soil types x	A1 B3	12.02	4.40	15.47	12.08	1.23	50.39	35.85
Genotypes)	A1 B4	10.65	3.47	14.33	8.56	1.23	44.28	34.28
	A1 B5	14.37	3.27	14.07	7.46	3.07	65.87	33.80
	A2 B1	21.66	5.27	17.93	13.96	3.19	57.70	41.19
	A2 B2	19.38	4.43	13.87	11.67	2.78	46.14	37.66
	A2 B3	12.13	3.67	11.60	8.26	1.32	49.92	32.08
	A2 B4	9.19	3.03	9.07	7.24	0.87	39.84	26.10
	A2 B5	14.37	3.00	9.53	7.07	2.53	62.38	25.23
	SEm±	1.57	0.47	1.17	1.15	0.40	0.97	1.84
	CD (5%)	4.66	1.41	3.46	3.40	1.18	2.88	5.47
	CD (1%)	6.38	1.93	4.74	4.66	1.62	3.94	7.50
	CV (%)	19.07	22.59	16.22	22.30	34.79	3.68	10.65

Days after sowing

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Table 9. Phenotypic correlation (r) among different characters of groundnut genotypes in calcareous soil

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.000	0.564**	0.630**	0.542**	-0.566**	-0.463**	-0.526**	-0.429**	0.115	0.090	-0.346*	-0.069	-0.428**	0.032	-0.043	-0.402**
2		1.000	0.746**	0.577**	-0.616**	-0.699**	-0.599**	-0.603**	-0.176	0.138	-0.601**	-0.284**	-0.632**	0.090	0.072	-0.304*
3			1.000	0.717**	-0.569**	-0.559**	-0.840**	-0.756**	0.026	-0.019	-0.521**	-0.239*	-0.657**	0.093	-0.149	-0.182
4				1.000	-0.294**	-0.448**	-0.556**	-0.900**	-0.200	-0.093	-0.473**	-0.387**	-0.590**	0.236*	-0.112	-0.262**
5					1.000	0.338*	0.550**	0.320*	-0.196	-0.205	0.343*	0.036	0.479**	-0.027	-0.003	0.261**
6						1.000	0.469**	0.513**	-0.044	-0.170	0.533**	0.189	0.430**	-0.087	-0.100	0.048
7							1.000	0.612**	-0.231*	0.140	0.429**	0.186	0.614**	-0.083	0.341*	-0.007
8								1.000	0.101	0.074	0.454**	0.391**	0.559**	-0.201	0.217*	0.131
9									1.000	-0.019	0.400**	0.493**	0.304*	-0.172	-0.183	0.281**
10										1.000	0.234*	0.266**	0.241*	-0.011	0.209	0.276**
11											1.000	0.482**	0.853**	-0.132	-0.128	-0.141
12												1.000	0.490**	0.307**	0.032	0.078
13													1.000	-0.100	0.170	-0.076
14														1.000	0.012	0.063
15															1.000	-0.169
16																1.000

*, ** Significant at 5% and 1% level of probability

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Table 10. Phenotypic correlation (r) among different characters of groundnut genotypes in normal soil

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1.000	-0.933*	-0.942*	-0.923*	-0.934*	-0.930*	-0.954*	-0.555**	-0.911*	-0.888*	-0.962**	-0.145	0.305	-0.911**
2		1.000	0.990**	0.999**	1.000**	0.992**	0.997**	0.817**	0.984**	0.992**	0.965**	0.487*	-0.012	0.986**
3			1.000	0.991**	0.993**	0.999**	0.995**	0.774**	0.982**	0.976**	0.990**	0.442	0.007	0.976**
4				1.000	0.999**	0.994**	0.996**	0.832**	0.982**	0.992**	0.964**	0.509*	0.014	0.983**
5					1.000	0.994**	0.998**	0.813**	0.988**	0.992**	0.970**	0.487*	0.003	0.988**
6						1.000	0.994**	0.798**	0.978**	0.979**	0.984**	0.470*	0.023	0.972**
7							1.000	0.776**	0.981**	0.982**	0.980**	0.429	-0.055	0.981**
8								1.000	0.808**	0.868**	0.682**	0.890**	0.433*	0.815**
9									1.000	0.989**	0.959**	0.519*	0.092	0.998**
10										1.000	0.937**	0.583**	0.096	0.993**
11											1.000	0.327	-0.062	0.947**
12												1.000	0.758**	0.523*
13													1.000	0.066
14														1.000

1.VCR 3.Active iron (Fe^{2+}) 5. Chlorophyll b 7. Peroxidase enzyme 9. No. of primaries 11. Pod yield/ plant 13. Shelling percentage
2.SCMR 4.Chlorophyll a 6. Total chlorophyll 8. Main stem height 10. No. of pods/ plant 12. Haulm yield / Plant 14. Test weight

Table 'r' value at df (N-2), where N=20: 0.444 (5%) and 0.561 (1 %);
*, * * Significant at 5 % and 1 % level of probability, respectively.