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Identification of efficient calcium absorbing groundnut genotypes under different intervals of gypsum fertilization

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Abstract

Calcium is an important secondary macronutrient which can affect the yield of groundnut. This experiment was aimed to determine the response of genotypes for different dates of gypsum application viz., no gypsum - control (T₁), 30 days DAS (T₂), 45 DAS (T₃), 60 DAS (T₄), 30+60 DAS (T₅). Analysis of variance revealed significant difference between genotypes for all the traits; significant treatment effect for the traits, single plant yield and number of pods per plant; significant genotype x treatment effect for the traits namely single plant yield, number of pods per plant, 100-pod weight and 100-kernel weight. Highest mean performance for traits single plant yield, number of pods per plant, 100-pod weight and 100-kernel weight was recorded under 30 DAS (T₂) and grey relation analysis also projected 30 DAS (T₂) as the best treatment. GT-biplot analysis and ranking of genotypes based on SPY under different treatments expressed ICGV 02266 as the best genotype. ICGV 06040 showed high concentration of calcium in kernels under 30 DAS (T₂) treatments. Results from this study clearly demonstrated that gypsum application had influence on groundnut yield and its related traits. Gypsum applied at 30 DAS (T₂) was anticipated as the best treatment to enhance yield in groundnut. The best genotype identified ICGV 02266 identified as best calcium absorbers and could be used in further breeding program.

Keywords: Calcium, groundnut, gypsum application, yield

Introduction

Groundnut or peanut (*Arachis hypogaea* L.) is an important oil, feed, food and fodder crop in many parts of the world. It is cultivated in 111 countries in an area of 31.56 million hectares with a total production of 53.63 million tonnes (FAOSTAT, 2020) [1]. Major groundnut growing countries are China followed by India, Nigeria and United States and most of the cultivated area was confined to tropical and sub-tropical regions of the world. The nutritional profile of 100 g groundnut kernels was 25 g protein, 17.86 g carbohydrates, 10.70 g fibre, 10.71 g of saturated fatty acids and 42.86 g of unsaturated fatty acids, minerals and vitamins (USDA, Food Data Central, 2019) [2]. The monounsaturated fatty acid of kernels includes oleic acid (36-81.3%) and linoleic acid (3.9-40.2%) (Janila *et al.*, 2016) [3]. The high oleic groundnuts (>70% oleic acid) have several health advantages viz., lower bad cholesterol, promote good cardiovascular health, confer anti-diabetic properties, and help to mitigate vascular inflammation (Wilson and Wilson, 2015) [4].

According to UN report (2017) [5], the population is expected to reach 8.6 million in 2030 and maintaining food production for the growing world population requires increase in production and management on the current cropland. Fertilizer is one of the essential sources for accomplishing this target of high crop production. The fertilizer usage on an average increased from 82.15 kg ha⁻¹ to 136.82 kg ha⁻¹ in last five decades (1978-2018) in the world (FAO, 2020) [6]. In this 21st century, managing soil nutrients to deliver a safe food supply and being environment friendly remains an immense challenge for the researchers (Zhang *et al.*, 2012) [7]. Groundnut is an exhaustive crop and depending upon the yield, it confiscates large amount of macro- and micro-nutrients from the soil. But farmers use a very less amount of fertilizer and

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sometimes only one or two nutrients ensuing in severe nutrient deficiencies due to insufficient and imbalance use of nutrients. In most of the developing countries where groundnut is grown, soil infertility restricts its productivity. To improve productivity, diagnosis of nutrient deficiencies and toxicities in unfertilized soils, their rectification with minimal fertilizer treatment and development of cultivars with high nutrient efficiency in deficient soils are challenges for plant nutrition researchers.

The macronutrients such as Ca, K, P and S and micronutrients such as Fe and B are playing a key role in kernel filling and oil synthesis and henceforth these minerals are required in higher extent compared to other minerals in groundnut (Singh *et al.*, 2004) [8]. The calcium ion (Ca^{2+}) is an important secondary macronutrient essential for pod filling, development of sound mature kernels and reduces the development of “pops” (unfilled pods) and “black hearts” and improves germination percentage in kernels. It is transported upwards from roots exclusively in xylem tissue with transpiration stream but the downward movement from the leaves is almost nil through phloem (Mengel and Kirkby, 1987) [9]. When peg penetrates the soil, it cannot access the root absorbed calcium and completely depends on absorbed Ca^{2+} directly from the soil which helps in the pod development. The critical levels of soil Ca are about 250 ppm in root zone and 600 ppm in pod zone (Singh *et al.*, 2004) [8].

Gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) and lime (CaCO_3) are most prominently used calcium sources of fertilizers in groundnut. Compared to lime, gypsum has more advantages *i.e.*, it contains both calcium and sulphur, increases porosity of soil, no influence on soil pH, and 200 times better soluble than lime (Norton, 2008) [10]. Hence, gypsum is considered in this experiment as calcium fertilizer for evaluating groundnut genotypes. The time of application of gypsum is crucial to provide adequate calcium to groundnut during the pod development stages. As several researchers reported genotypic differences in Ca uptake from soil, efforts are needed to exploit groundnut genotypes performing well under calcium deficient soils. Hence, this study was taken up to identify best genotypes with high calcium uptake and to analyse the best time of gypsum application for better yields in groundnut.

Materials and Methods

Genetic material

For this study, 20 diverse groundnut advanced breeding lines from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India were chosen (Table 1). We applied four treatments of gypsum at different days after sowing (DAS) along with control (without gypsum application) (Table 2).

Table 1: Details of groundnut advanced breeding lines used in this study

S. No.	Genotype	Pedigree	Growth habit
Drought tolerant			
1	ICGV 00350	ICGV 87290 x ICGV 87846	Spanish bunch
2	ICGV 00351	ICGV 87290 x ICGV 87846	Spanish bunch
3	ICGV 02266	ICGV 94143 x ICGV 94136	Spanish bunch
4	ICGV 87846	CS 9 x ICGS 5	Virginia bunch
5	ICGV 07222	[(ICGV 92069 x ICGV 93184) SIL 4 x (ICGS 44 x ICGS 76)] F2-SSD-SSD-P19-B1-B1-B1	Spanish bunch
High Fe content			
6	ICGV 06040	[(ICGV 92069 x ICGV 93184) x (NC Ac 343 x ICGV 86187) S23] F2-SSD-SSD-P5-B1-B1-B1	Spanish bunch
High oil content			
7	ICGV 05155	ICGV 99160 x ICGV 99240	Spanish bunch
8	ICGV 03043	{ICGV 99160 x [ICGV 93124 x (LI x ICGS 44)]} F2-SSD-SSD-B3-B1-B1	Spanish bunch
9	ICGV 06420	ICGV 87846 x ICGV 99240	Spanish bunch
High oleic acid content			
10	ICGV 15083	ICGV 06420 x Sun Oleic 95R	Spanish bunch
11	ICGV 15090	ICGV 06420 x Sun Oleic 95R	Spanish bunch
12	ICGV 16020	ICGV 06420 x Sun Oleic 95R	Spanish bunch
13	ICGV 16037	ICGV 06420 x Sun Oleic 95R	Spanish bunch
14	ICGV 16667	ICGV 06110 x (ICGV 06110 x Sun Oleic 95-R)	Spanish bunch
15	ICGV 181003	ICGX 120172/Sunoleic 95-R	Spanish bunch
Short duration			
16	ICGV 16593	ICGV 02022 x ICGV 97245	Spanish bunch
17	ICGV 16594	ICGV 02022 x ICGV 97245	Spanish bunch
18	ICGV 16598	ICGV 02022 x ICGV 97245	Spanish bunch
19	ICGV 16599	ICGV 02022 x ICGV 97245	Spanish bunch
Red kernels			
20	ICGV 93468	ICGV 86015 x ICGV 86155	Spanish bunch

Table 2: Description of different treatments of gypsum applied in the field

Treatment	Notation	Day of Gypsum application	Stage of the crop (Boote 1982) [11]
Treatment 1	T ₁	No gypsum (Control)	-
Treatment 2	T ₂	30 DAS	Early flowering
Treatment 3	T ₃	45 DAS	Beginning of peg
Treatment 4	T ₄	60 DAS	Beginning of pod
Treatment 5	T ₅	30 + 60 DAS	Flowering + beginning of pod

The gypsum was applied on the soil surface near to the plant base at the rate of 500 kg ha⁻¹. The field design was laid out in spilt plot alpha-lattice and genotypes were replicated twice per

each treatment and trial was conducted at ICRISAT field. The field was thoroughly characterized regarding soil properties and calcium status before the experiment.

Soil Testing

Soil samples were collected from 6 representative sites from field where experiment was taken. Samples (top 30 cm) were air-dried and sieved through a 2-mm screen, and subjected to ammonium acetate extraction (Thomas *et al.*, 1982) ^[12] and saturated paste extraction (Rhoades 1982) ^[13] and then centrifuged for 40 min at 2000 rpm. The filtered soil solutions were analysed for calcium (Ca) concentration by Inductively

Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Thermo Fisher Scientific, USA) at Charles Renard Analytical Laboratory, ICRISAT. The soil test results from the field were compared with threshold levels of calcium proposed by International Plant Nutrition Institute (IPNI), Victoria, Australia (Fig 1). The results from soil test revealed that soil is deficient in calcium content and that the conditions are suitable to conduct the experiment.

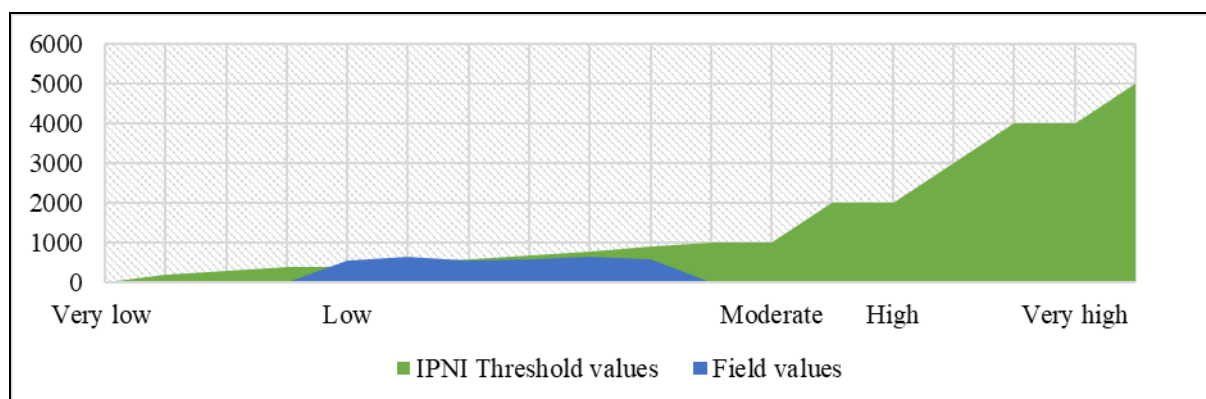


Fig 1: The results of calcium content from soil test data and comparison with threshold values from IPNI

Trait Phenotyping

During the summer 2021, the following traits were phenotyped viz., single plant yield (SPY), number of pods per plant (NOP), 100-pod weight (HPW), 100-kernel weight (HKW), pod length (PL), pod width (PW), kernel length (KL), kernel width (KW) and sound mature kernel % (SMK%). Ten plants were collected from each replication of each treatment and data was recorded on SPY and NOP and mean was calculated. The PL, PW, KL and KW of twenty pods and kernels were calculated using vernier callipers and their mean was computed. The SMK% was calculated as percentage of weight of fully matured kernels (g) by total weight of kernels (g).

The calcium content in kernels and shells were estimated by hydrogen peroxide digestion method proposed by Gilliham *et al.*, (2011) ^[14] using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Fisher Scientific, USA) at Charles Renard Analytical Laboratory, ICRISAT.

Calcium response index (CRI) was estimated separately for each treatment using the formula proposed by Furtini (2008) ^[15] and presented below.

$$CRI = \frac{(PY_A - PY_B)}{(A - B)}$$

PY_A and PY_B are corresponding pod yield under high and low calcium concentrations and (A-B) is the difference between calcium concentrations.

Statistical analysis

Two-way analysis of variance (ANOVA) was performed to assess the main and interaction effects between treatments and genotypes at 0.1, 1 and 5% level of significance. The individual variances were estimated and modelled to error distribution using residual maximum likelihood (REML) procedure using ASREML-R v4 (Butler *et al.*, 2009) ^[16]. BLUPs (Best Linear Unbiased Predictors) were estimated for genotype (G), treatment levels and genotype × treatment levels and calculated pair wise comparisons using t-statistic (LSD) for significant effects using

following equation.

$$LSD = t(5\%, Edf) \times SED$$

Where, t is the cumulative student's t distribution, 0.05 is selected α level (5%), Edf is the error degrees of freedom from the ANOVA model, and SED is standard error of differences of the mean.

The coefficient of variation (%) was calculated by following equation

$$CV(\%) = \left(\frac{\sigma}{\bar{x}} \right) \times 100$$

Where, σ is standard deviation and $\bar{x}$ is mean

Grey relation analysis (GRA) proposed by Julong (1989) ^[17] was used to identify interrelationships between multiple factors and multiple criteria decisions to identify the best treatment. Karl Pearson's correlation coefficient between traits in each treatment was calculated and corrpplots were drawn using "SemiPar" package in R version 3.6.2. The GT-biplot was framed using "ggplot" package in R version 3.6.2. Such that PC1 scores are plotted against the PC2 scores for each genotype and treatment-trait combination.

Results and Discussion

Response of traits to different treatments

The traits SPY, NOP and SMK% under control (T₁) expressed the lowest mean, whereas at 30 DAS (T₂), the same traits recorded higher mean value (Table 3, Fig 2). For the trait HPW, the control (T₁) exhibited lowest mean and 45 DAS (T₃) dominates the T₁ and other treatments. Nevertheless, the trait HKW resulted in different means across the treatments. The traits PL and PW recorded lower values in 30 + 60 DAS (T₅) compared to T₁. However, there is not much difference between means across treatments for traits KL and KW.

Our result revealed that T₂ outperforms for the traits namely SPY, NOP and SMK%; T₃ for HPW and T₅ for PL and PW. With respect to SPY, T₂ showed percentage increase of 28%,

15%, 20% and 10% over T₁, T₃, T₄ and T₅ respectively. From this study it was clear that, T₂ treatment where gypsum was applied at 30 DAS was the best to increase the yield in groundnut. All gypsum applied treatments (T₂, T₃, T₄ and T₅) showed higher yield compared to control (T₁), which is without gypsum application.

Gypsum is a source of both calcium and sulphur, it triggers the pod yield in different ways compared to control. Calcium maintains cell membrane permeability, cell integrity, protein synthesis, activates enzymes of cell division and participates in carbohydrate transfer (Thor, 2019) [18]. Whereas sulphur is a major component of S containing amino acids, acetyl CO-A, proteins, vitamins, glutathione, chlorophyll and ferredoxin (Aarabi *et al.*, 2020) [19]. The calcium and sulphur mechanisms probably activate the nutrient absorption, strong sink strength through development of reproductive structures, which cater to the production of assimilates under the influence of applied gypsum thus maintaining the balance between source-sink which might have resulted in increased yield attributes (Singh, 2007) [20].

In this study application of gypsum at pegging stage (30 DAS) at

the rate of 500 kg ha⁻¹ outperforms compared to other treatments and similar type of results were also reported by several researchers. Application of gypsum at 500 kg ha⁻¹ improved yield compared to 750 kg ha⁻¹ and 1000 kg ha⁻¹ (Sikhakhana, 2016) [21], a linear response in yield was noticed when application of gypsum was increased from 200 to 500 kg ha⁻¹ (Geethalakshmi & Lourduraj, 1998) [22], application of 500 kg ha⁻¹ of gypsum at pegging is beneficial compared to basal application and enhances yield (Kumaran *et al.*, 2000 [23]; Agasimani *et al.*, 1992 [24]) and similarly gypsum application improved pod yield and sound mature kernels in groundnut (Alva *et al.*, 1989 [25]; Rao *et al.*, 1990 [26]). Amending the soil with gypsum enhances the pod yield (Adams *et al.*, 1993 [27]; Sorenson and Butts, 2008 [28]; Bairagi *et al.*, 2017 [29]), kernel yield (Murata, 2003 [30]; Gashti *et al.*, 2012 [31]), number of pods per plant (Kabir *et al.*, 2013 [32]), sound mature kernels (Alva *et al.*, 1989 [25]; Rao *et al.*, 1990 [26]; Adams *et al.*, 1993 [27]; Murata, 2003 [30]; Sorenson and Butts, 2008 [28]), hundred pod weight (Kabir *et al.*, 2013 [32]), hundred kernel weight (Kamara *et al.*, 2011) [33].

Table 3: Descriptive statistics for nine traits among five gypsum treatments

Traits	Treatment 1			Treatment 2			Treatment 3			Treatment 4			Treatment 5		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
SPY	10.58	33.39	20.28	13.83	37.72	25.91	13.94	32.87	22.46	10.74	38.57	21.60	11.55	36.84	23.61
NOP	16.81	38.94	23.86	23.28	51.12	31.68	14.00	40.77	29.04	18.97	38.38	28.25	18.06	46.99	29.49
HPW	58.37	150.50	95.94	76.26	154.96	111.82	70.31	177.83	119.27	57.00	163.05	106.61	47.77	142.75	100.36
HKW	20.11	39.14	29.83	26.32	41.70	31.44	24.14	39.44	31.51	20.74	47.74	29.92	23.57	38.12	29.99
PL	20.51	29.00	25.91	22.26	30.51	26.25	21.99	28.74	26.36	20.50	29.50	26.30	21.25	29.99	25.61
PW	10.65	12.70	11.45	10.46	13.44	11.63	10.41	13.17	11.73	10.67	13.09	11.55	10.19	13.00	11.30
KL	9.62	12.84	11.54	10.14	13.51	11.75	9.91	13.22	11.61	9.90	13.40	11.54	9.55	12.91	11.61
KW	6.15	11.91	7.31	6.58	11.16	7.38	6.06	12.73	7.53	6.47	11.24	7.44	6.23	12.17	7.39
SMK%	54.32	81.99	74.36	64.32	89.59	80.32	64.16	87.52	77.61	61.70	89.97	76.89	62.81	87.14	76.76

SPY - Single Plant Yield (g); NOP - Number of Pods per Plant; HPW - 100 Pod Weight (g); HKW - 100 Kernel Weight (g); PL - Pod Length (mm); PW - Pod Width (mm); KL - Kernel Length (mm); KW - Kernel Width (mm); SMK - Sound Mature Kernels (%)

Analysis of variance (ANOVA)

The results from two-way ANOVA revealed that there is a significant difference between all genotypes for all the nine traits studied (Table 4). The treatment main effect was observed significant for the traits SPY and NOP. Whereas, the interaction effect of genotype x treatment was reported significant for SPY, NOP, HPW and HKW.

The ANOVA revealed significant genotypic differences for all

the traits under study suggesting good genetic variability for these traits in groundnut. Significant treatment difference was observed for SPY and NOP which indicates the differential response of genotypes to the gypsum treatments, and factor is an important component of the observed variation. Other traits such as SPY, NOP, HPW and HKW had significant genotype x treatment interaction indicating different genotypic response to different fertilizer treatments.

Table 4: Two-way ANOVA (F-value) for nine traits analyzed in this study

Characters	Genotypes	Treatments	Genotype × Treatment
Single plant yield (g)	15.85***	13.29***	3.11***
Number of pods per plant	3.21***	10.09***	1.72**
100 - Pod weight (g)	3.82***	4.68 ^{NS}	3.07***
100 - Kernel weight (g)	5.71***	0.79 ^{NS}	1.95**
Pod length (mm)	19.28***	1.85 ^{NS}	1.05 ^{NS}
Pod width (mm)	7.30***	1.06 ^{NS}	0.89 ^{NS}
Kernel length (mm)	10.35***	0.40 ^{NS}	1.25 ^{NS}
Kernel width (mm)	38.97***	0.49 ^{NS}	1.32 ^{NS}
Sound mature kernels (%)	3.67***	1.09 ^{NS}	0.64 ^{NS}

: significant at <0.01, *: significant at <0.001 probability level, ^{NS}: Non-significant

Grey relationship analysis (GRA) to identify the best treatment: To select the most effective gypsum treatment, the traits namely SPY, NOP, HPW and HKW from different treatments were used to estimate GRA. The relative correlation value which is close to 1 is considered the most optimal and the best treatment. The data from the Table 5 indicated that the best

outcome was obtained from treatment 2 followed by treatment 3, 5 and 4 and the worst comprehensively estimated treatment was the control (T₁). The value of relative correlation of the five treatments was in the range of 0.33 to 0.88, which revealed that SPY, NOP, HPW and HKW varied significantly with different gypsum treatments.

Table 5: Grey relationship analysis of groundnut under different gypsum treatments

Treatment	Relative correlation degree	Rank
T ₁	0.33	5
T ₂	0.88	1
T ₃	0.76	2
T ₄	0.44	4
T ₅	0.48	3

The traits SPY, NOP, HPW and HKW are the key indicators to improve yield in groundnut and it was considered for GRA analysis. T₂ treatment (gypsum applied at 30 DAS) was projected as the best one from the GRA analysis compared to other treatments. In our study, the optimal fertilizer treatment was confirmed through GRA.

Trait association in each treatment

The association analysis was performed for nine traits for each treatment separately and presented in Fig 3. In T₁, a significant positive association was observed between HKW with KPW (0.48*), PW with SPY (0.50*) and KL with PL (0.68***). The trait SPY showed significant positive association with NOP (0.54*), HKW (0.50*), PL (0.52*), PW (0.59**) and KL (0.59**); NOP with HKW (0.67***); HKW with PW (0.49*) and PL with KL (0.64**) under T₂. Whereas, under T₃ the trait SPY showed significant positive association with NOP (0.62**) and SMK (0.55*); KL with HKW (0.48*) and PL (0.54*) and HPW with HKW (0.70***) and in contrast KL showed negative significant association with NOP (-0.52*). Correlation in T₄ revealed that there is significant association between SPY and HKW (0.60**); KL with HKW (0.45*), PL (0.82***) and SMK (0.51*). In T₅, there is a positive association between SPY with NOP (0.52*) and HPW (0.48*); HPW with NOP (0.44*), PL (0.46*), KL (0.45*) and SMK (0.46*) and KL with PL (0.44*). In all the five treatments, PL showed significant positive association with KL and similar result was reported by Thakur *et al.*, (2013) [34] and Kotzamanidis *et al.*, (2006) [35]. Under T₁ and T₃, HKW showed significant positive association with HPW and the same was reported by Kotzamanidis *et al.*, (2006) [35]. The trait SPY showed positive association with PW under T₁ and T₂, with NOP under T₂, T₃ and T₅, with HKW under T₂ and T₄, with PL and KL under T₂, with SMK% under T₃ and with HPW under T₅. Similar results of positive correlation were reported for SPY with NOP (Patil *et al.*, 2006 [36]; Mukhtar *et al.*, 2011 [37]; Makeinde and Ariyo, 2013 [38]; Yadlapalli, 2014 [39]; Reddy *et al.*, 2017 [40]; Daudi *et al.*, 2021 [41]), with HPW (Kotzamanidis *et al.*, 2006 [35]; Vekariya *et al.*, 2011 [42]; Thakur *et al.*, 2013 [34]; John and Reddy, 2019 [43]), with HKW (Kotzamanidis *et al.*, 2006 [35]; John and Reddy, 2019 [43]; Korat *et al.*, 2010 [44]; Mukhtar *et al.*, 2011 [37]; Vekariya *et al.*, 2011 [42]; Bhargavi *et al.*, 2016 [45]; Bhakal and Lal, 2017 [46]; Reddy *et al.*, 2017 [40]; Daudi *et al.*, 2021 [41]), with SMK (Patil *et al.*, 2006 [36]; Thakur *et al.*, 2013 [34]; Reddy *et al.*, 2017 [40]; John and Reddy, 2019 [43]), with PL, PW and KL (Kotzamanidis *et al.*, 2006 [35]; Thakur *et al.*, 2013 [34]). The traits which are highly correlated with SPY in different treatments can be considered for indirect selection to target for yield improvement in breeding programs.

Ranking of genotypes based on pod yield

The mean performance of genotypes for trait SPY under each treatment was considered to rank the genotypes (Fig 4). Under T₁, the genotypes ICGVs 16593, 181003, 87846, 15083 and 02266 occupied top five positions. The genotypes ICGVs 02266, 07222, 16593, 06040 and 87846 were placed in top five under T₂. The genotypes ICGVs 15083, 16593, 02266, 06420 and

93468 are top performers under T₃. In T₄, ICGVs 02266, 16593, 87846, 15083 and 93468 are located at top five positions. Under T₅, the genotypes ICGVs 16593, 05155, 06040, 15083 and 87846 are the best performers for SPY.

Comparison of ranks of genotypes for SPY across five treatments revealed that ICGV 16593 was the best performer followed by ICGV 02266. Daudi *et al.*, (2020) [47] also identified ICGV 16593 as the top performing genotype for pod yield in his study. Another best genotype ICGV 02266 was released as Kalinga Groundnut-1 and previous studies also proved it as superior performers for various traits such as drought tolerance (Mayeux *et al.*, 2003 [48]; Upadhyaya, 2005 [49]; Nigam *et al.*, 2005 [50]; Hamidou *et al.*, 2012 [51]; Monyo and Varshney, 2016 [52]), haulm traits (Khan *et al.*, 2015 [53]), resistance to aflatoxin contamination (Salunke *et al.*, 2021 [54]), moderately resistance to collar rot (Divya Rani *et al.*, 2018 [55]), moderately resistance to groundnut bud necrosis disease (Tabassum *et al.*, 2017 [56]), tolerance to leaf minor damage close to check variety (Rahman *et al.*, 2021 [57]) and heat tolerance (Akbar *et al.*, 2017 [58]).

GT biplot analysis across treatments

The standardized data showed a total variation of 25.09% and 20.39% under T₁; 33% and 19.40% under T₂; 32.47% and 21.59% under T₃; 38.33% and 16.16% under T₄ and 31.67% and 18.24% under T₅ (Fig 5). The groundnut genotypes generated a biplot with ICGVs 16593, 06420, 15083, 02266, 07222, 87846 and 16037 at the vertex of the polygon under T₁. Among the vertex genotypes, ICGVs 15083 and 02266 showed better performance for PW, SPY and SMK; ICGVs 07222 and 87846 for HPW and HKW and ICGV 16593 for NOP and KW. The genotypes ICGV 02266, ICGV 15090 and ICGV 15083 are situated close to ideal entry and these genotypes are considered as the best genotypes for T₁. The groundnut genotypes generated a biplot with ICGVs 06420, 02266, 00350, 181003 and 16020 at the vertex of the polygon under T₂. Amidst the vertex genotypes, ICGV 00350 showed better performance for SMK; ICGV 02266 for SPY, PL, KL, PW, HKW and NOP and ICGV 06420 for KW and HPW. The genotypes ICGV 05155, ICGV 06040 and ICGV 02266 are situated close to ideal entry and these genotypes are considered as the best genotypes for T₂.

The vertex genotypes formed a polygon under T₃ are ICGVs 06420, 06040, 00350, 00351, 16599, 16594 and 15083. The vertex genotype ICGV 06040 exhibited better performance for HKW, KPW and KL; ICGV 06420 for KW and PL and ICGV 15083 for SMK, SPY, PW and NOP. The genotypes ICGV 15090, ICGV 03043 and ICGV 02266 are close to the ideal genotypes and are identified as the best genotypes under T₃. The vertex genotypes of T₄ polygon are ICGVs 06420, 02266, 87846, 16593, 16020 and 181003. The genotypes ICGV 02266 expressed better performance for NOP, SMK, KW, HKW, KL, SPY and PL and ICGV 87846 for PW and HPW. The genotype ICGV 02266 positioned close to ideal genotype and could be considered as the best performing genotype under T₄. The genotypes ICGVs 16593, 05155, 00350, 07222 and 16037 occupied vertex positions of the polygon under T₅. The genotype

ICGV 00350 showed better performance for SMK; ICGV 05155 for SPY, KW, NOP, HPW, KL and PL and ICGV 16593 for PW and HKW. The genotypes ICGV 15083, ICGV 05155, ICGV 02266 and ICGV 06040 are projected close to the ideal genotype.

One novelty of GT biplot approach is the identification of superior genotypes based on the combination of traits of interest in breeding program. Combination of important traits is meaningful compared to individual traits in selecting superior cultivar. The ideal genotype is a virtual genotype represented as

circle on average coordinate axis (ACA) that shows high mean for all the traits considered for analysis. A genotype is considered more desirable if it occupied position near to the ideal genotype. It was observed that genotype ICGV 02266 was projected close to ideal genotype in all the treatments including control under this study. The other genotypes such as ICGV 05155 and 06040 were close to the ideal genotype in at least two treatments. The genotypes ICGV 15090 and 15083 fell close to the ideal genotype under control and at least one treatment.

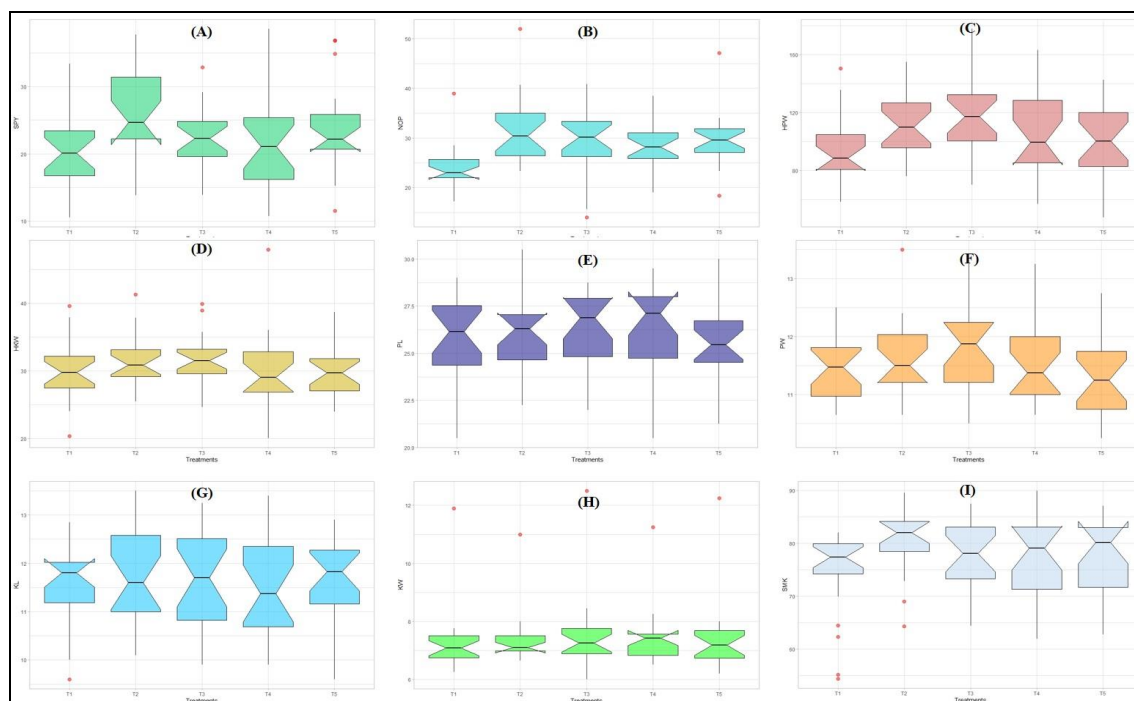


Fig 2: Boxplot showing the means distribution of nine traits among five gypsum treatments (A) SPY - Single plant yield (g) (B) NOP - Number of pods per plant (C) HPW - Hundred pod weight (g) (D) HKW - Hundred kernel weight (g) (E) PL - Pod length (mm) (F) PW - Pod width (mm) (G) KL - Kernel length (mm) (H) KW - Kernel width (mm) (I) SMK - Sound mature kernels% (T₁ - No gypsum applied; T₂ - gypsum applied 30 DAS; T₃ - gypsum applied 45 DAS; T₄ - gypsum applied 60 DAS; T₅ - gypsum applied 30+60 DAS)

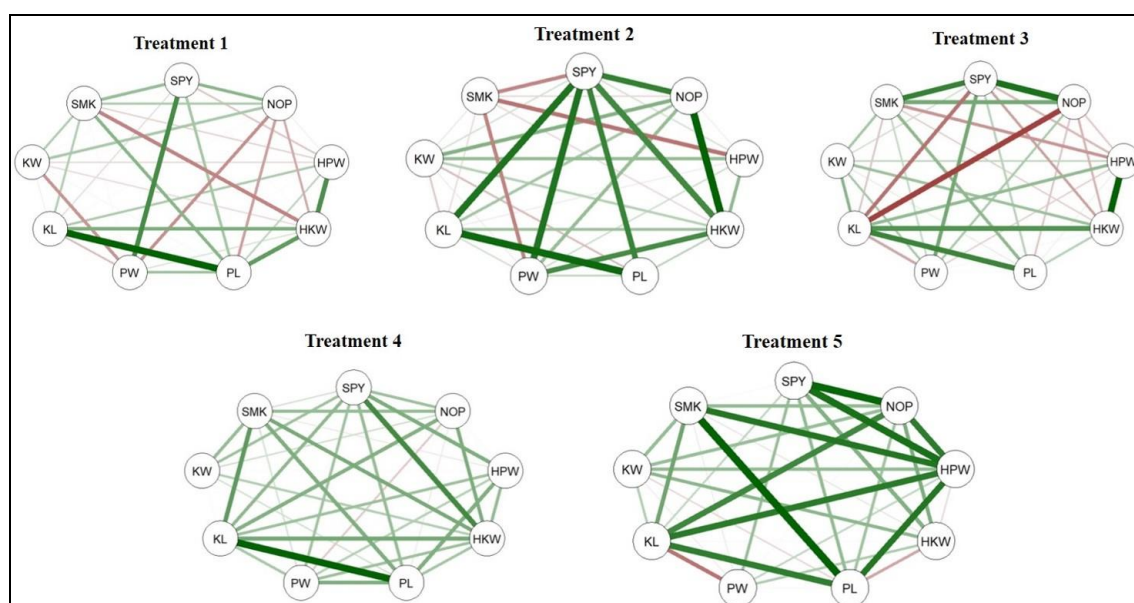


Fig 3: Associations among the nine investigated traits for different gypsum treatments. SPY - Single Plant Yield (g); NOP - Number of Pods per Plant; HPW - 100 Pod Weight (g); HKW - 100 Kernel Weight (g); PL - Pod Length (mm); PW - Pod Width (mm); KL - Kernel Length (mm); KW - Kernel Width (mm); SMK - Sound Mature Kernels (%); Green line - positive correlation; Red line - negative correlation; Thickness of the line indicates significance of the correlation; Treatment 1 - No gypsum applied; Treatment 2 - gypsum applied 30 DAS; Treatment 3 - gypsum applied 45 DAS; Treatment 4 - gypsum applied 60 DAS; Treatment 5 - gypsum applied 30+60 DAS

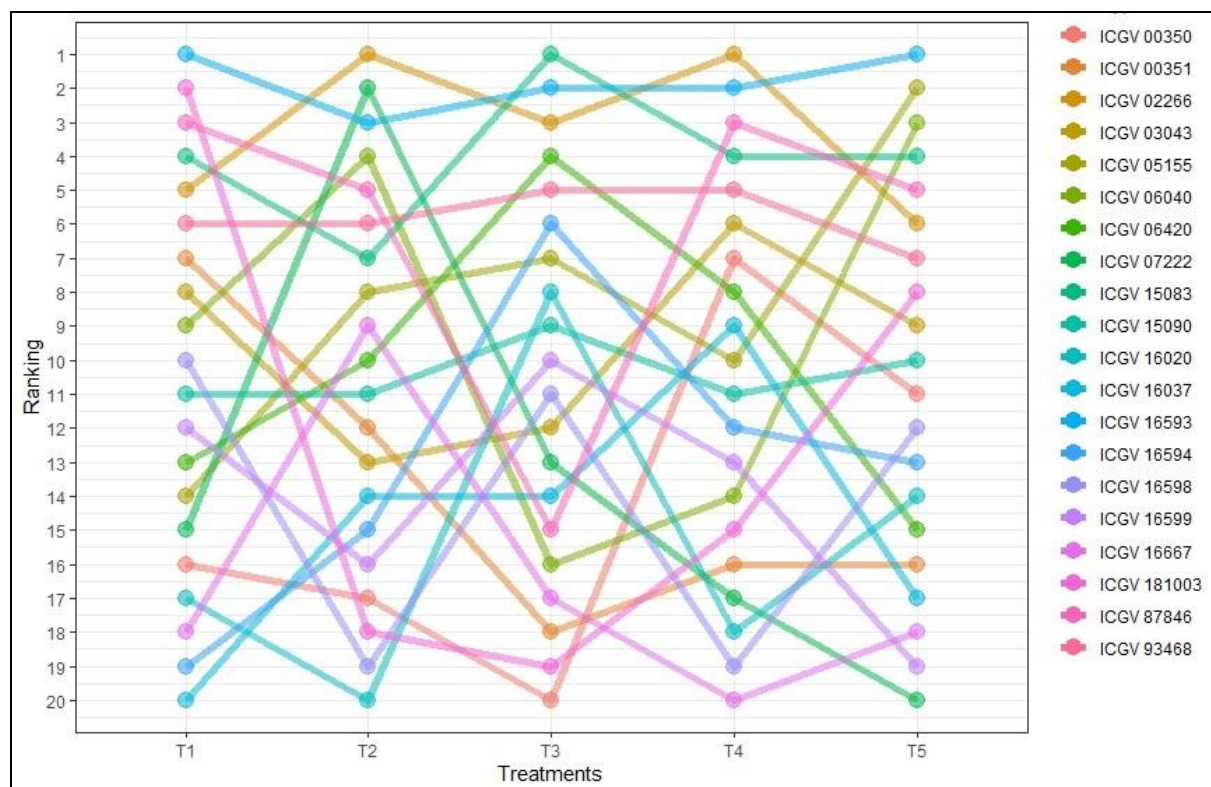


Fig 4: Ranking of genotypes based on single plant yield across five treatments. (T₁ - No gypsum applied; T₂ - gypsum applied 30 DAS; T₃ - gypsum applied 45 DAS; T₄ - gypsum applied 60 DAS; T₅ - gypsum applied 30+60 DAS)

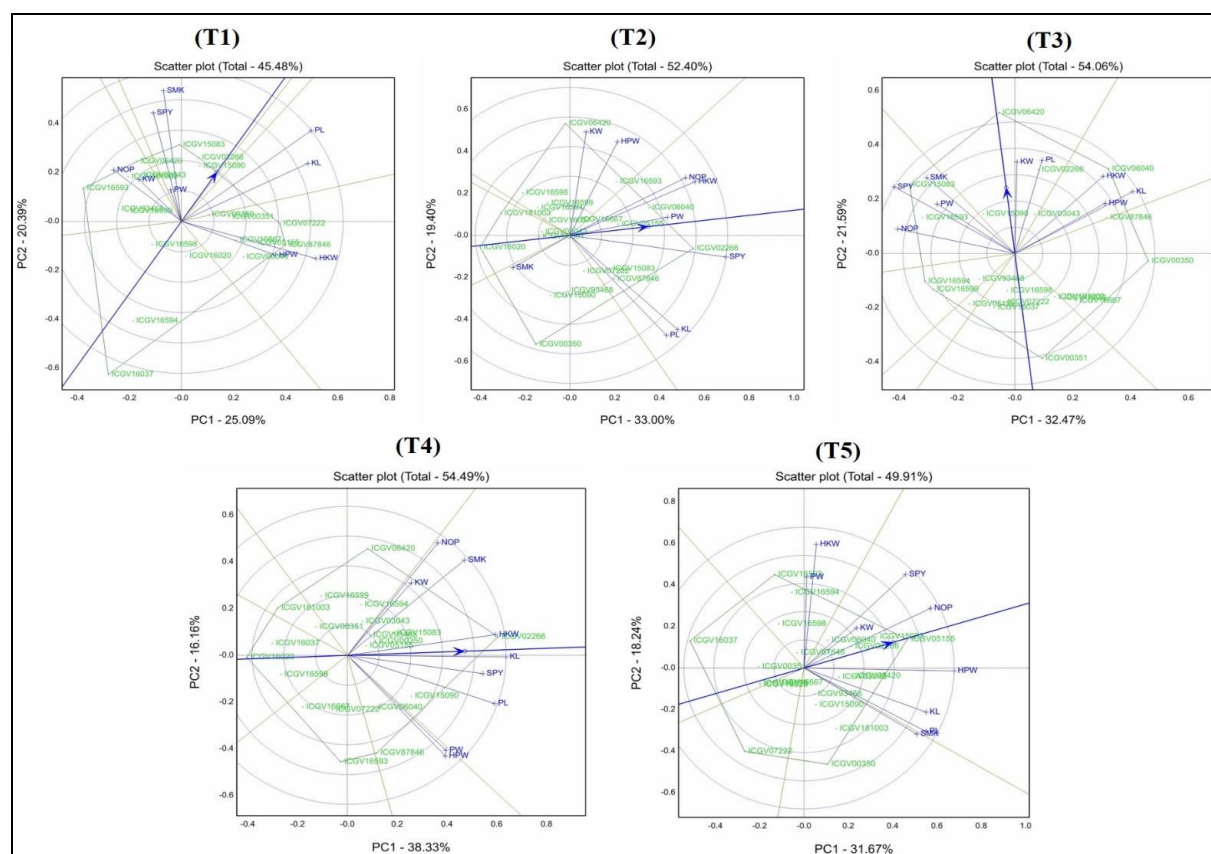


Fig 5: A genotype-by-trait bi-plot showing polygon view of genotypes and genotypes close to ideal genotype under different gypsum treatments (T₁ - No gypsum applied; T₂ - gypsum applied 30 DAS; T₃ - gypsum applied 45 DAS; T₄ - gypsum applied 60 DAS; T₅ - gypsum applied 30+60 DAS; SPY - single plant yield (g); NOP - number of pods per plant; HPW - 100 pod weight; HKW - 100 kernel weight; PL - Pod Length (mm); PW - Pod Width (mm); KL - Kernel Length (mm); KW - Kernel Width (mm))

Calcium response index

The calcium response index (CRI) was estimated using pod

yield per plant of each genotype for individual treatment by considering T₁ (no gypsum) as control. The positive values

indicate that genotype performed better under treatment compared to control. CRI calculated with T₂ against control showed all genotypes have positive values except three (ICGV 181003, ICGV 16020 and ICGV 16598), similarly CRI for T₃ against control revealed 15 genotypes with positive values and five genotypes with negative values. Whereas CRI calculated for T₄ against control represented 12 genotypes with positive values and eight genotypes with negative values. In the same way, the under T₅ CRI values are positive for sixteen genotypes and negative for four genotypes.

The genotype ICGV 07222 (0.037) followed by ICGV 06040 (0.027) and ICGV 02266 (0.026) under T₂; the genotype ICGV 16594 (0.024) followed by ICGV 16037 (0.020) under T₃; the genotypes ICGV 02266 (0.028) followed by ICGV 16037 (0.024) under T₄ and the genotypes ICGV 05155 (0.019) followed by ICGV 06040 (0.014) showed highest CRI positive values in their respective treatments. Among the twenty genotypes, nine genotypes viz., ICGVs 02266, 03043, 05155, 06420, 15083, 15090, 16037, 16594 and 93468 showed positive values of CRI across all the treatments. This represents those genotypes are performed better under gypsum treatment compared to control and responding to fertilizer application.

Calcium content in kernels and shells

The calcium content in kernels and shells was estimated from T₁ and T₂ treatments among all the genotypes. The calcium concentration in kernels varied from 0.73 g kg⁻¹ to 1.62 g kg⁻¹ in low calcium condition (T₁) and 0.78 g kg⁻¹ to 1.58 g kg⁻¹ in T₂ condition. Whereas, in shells the calcium content varied from 4.17 g kg⁻¹ to 8.25 g kg⁻¹ under T₁ and 4.26 g kg⁻¹ to 9.31 g kg⁻¹ in T₂ condition. In the present study, the higher values of calcium concentration in the kernels were obtained for ICGV 00351 and ICGV 06040 in T₁ and ICGVs 181003, 00350 and 06040 in T₂ condition. Among the cultivars, ICGV 07222 and ICGV 93468 in T₁ and ICGV 181003 and ICGV 00350 in T₂ showed higher calcium concentration in shells.

The calcium concentration in shells in T₂ was higher than T₁ except for five genotypes. Whereas calcium concentration in kernels is not in a line with gypsum application, only eleven genotypes showed higher calcium compared to control. The groundnut shells accumulated more calcium than kernels in both T₁ and T₂. From this study, it was observed that there is a difference in calcium concentration in shells and kernels among the genotypes. III JA *et al.*, (2017) ^[59] also reported significant difference in seed calcium concentration among the groundnut cultivars. In contrast, Howe *et al.*, (2012) ^[60] reported that calcium content in seed was increased with gypsum application rates. From this study it was clear that there is no improvement in calcium content in kernels and shells through gypsum application and it depends on absorption capacity of genotype and micro-environment around the pod zone during gypsum application.

Conclusion

Fertilizers like gypsum which includes both calcium and sulphur are very effective for groundnut's growth and quality. The results of the present experiment showed that applying calcium fertilizer in the form of gypsum impacted the yield in groundnut. It was demonstrated that applying calcium had significant effect on single plant yield and number of pods per plant. From this study, gypsum application at 30 DAS is identified as the best treatment and the genotype ICGV 02266 was observed to be the best. This genotype can be recommended to farmers for obtaining better yield under both calcium deficient soils and

soils amended with calcium.

Data Availability

All the collected data was mentioned in tables and no other data is available in any repository.

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