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Omesh Kumar
Department of Genetics and
Plant Breeding, College of
Agriculture, Vijayapur,
Karnataka, India

O Sridevi
Department of Genetics and
Plant Breeding, College of
Agriculture, Vijayapur,
Karnataka, India

GK Naidu
Department of Genetics and
Plant Breeding, College of
Agriculture, Vijayapur,
Karnataka, India

BC Patil
Department of Genetics and
Plant Breeding, College of
Agriculture, Vijayapur,
Karnataka, India

Corresponding Author:
Omesh Kumar
Department of Genetics and
Plant Breeding, College of
Agriculture, Vijayapur,
Karnataka, India

Evaluation of groundnut mini core for resistance to iron deficiency chlorosis under calcareous soils

Omesh Kumar, O Sridevi, GK Naidu and BC Patil

Abstract

The groundnut mini core set consisting of 184 accessions were evaluated under iron deficient calcareous soil. Genotypes were assessed for iron absorption efficiency related traits like VCR and SCMR at different stages of crop growth and also impact of iron chlorosis on yield and yield components. The maximum severity of iron deficiency chlorosis was recorded at 60 day after sowing coincided with high soil moisture due to receipt of rainfall during the period which made Fe unavailable to the plants. Out of 184 accessions evaluated, five accessions (ICG # 11322, 6888, 9961, 14475 and 4389) were found resistant to calcium induced iron deficiency chlorosis by recording significantly lower VCR and higher SCMR at severe stage (60 DAS) of iron chlorosis expression.

Keywords: Calcareous, IDC, mini core, SCMR, VCR

Introduction

Groundnut (*Arachis hypogaea* L.) is a most important oilseed crop which is grown in arid and semi-arid regions of the world. Iron plays an important role in photosynthesis, respiration, nitrogen fixation, DNA synthesis, hormone production, chlorophyll formation and is also a component of various redox and iron-sulphur enzymes (Zheng, 2010) [23]. Iron deficiency chlorosis (IDC) is common world-wide among crops grown in calcareous, alkaline, coarse textured, eroded and low organic matter containing and cold region soils as iron is less available for uptake in these soils. High pH and bicarbonate ion concentration in calcareous soils leads to IDC by suppressing iron uptake and/or translocation in plants (Li-Xuan *et al.*, 2005) [10]. Iron deficiency symptoms appear on younger leaves, indicating yellowish interveinal areas of leaves commonly referred as 'iron chlorosis'. In case of severe deficiency, leaves become almost pale white due to loss of chlorophyll.

In India, more than one third of the soils are calcareous and spread mostly in the low rainfall areas of the western (Gujarat, Maharashtra, Rajasthan and Karnataka) and central (Madhya Pradesh, Uttar Pradesh) parts of the country where groundnut is a major crop. The chlorosis is mainly observed in the soils with high calcium carbonate content immediately after irrigation or high rainfall. High bicarbonate levels especially in moist calcareous soils appear to affect the plants ability to absorb iron. Iron deficiency in groundnut causes considerable reduction in pod yield (16-32%) (Tandon, 1998; Potdar and Anderes, 1995; Singh *et al.*, 1995; Singh, 2001) [21, 11, 18, 16] and in extreme cases may lead to complete crop failure. The soil application of iron in the form of ferrous sulphate (FeSO₄) has often been recommended to alleviate the problem. But 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 form, their use is not popular and not feasible from the economic point of view.

The feasible approach to combat iron chlorosis is development of iron deficiency chlorosis resistant cultivars by exploiting the genetic variability observed for resistance to IDC (Reddy *et al.*, 1993; Kulkarni *et al.*, 1994; Samdur *et al.*, 1999, 2000) [13, 8, 14, 20]. The largest collection of groundnut genotypes is conserved at ICRISAT gene bank. A representative sub-set of the entire collection, mini core comprising 184 accessions (Upadhyaya *et al.*, 2002) [22] is an ideal set for identifying iron deficiency chlorosis resistant genotypes of diverse origin for utilization in the breeding programmes.

Material and method

One hundred ninety six groundnut genotypes which include 184 groundnut genotypes from ICRISAT mini core collection, 4 ICRISAT control genotypes and 8 local checks were evaluated for their reaction to calcium induced iron chlorosis.

Field screening of 196 genotypes was carried during *Kharif* 2016 at Regional Agricultural Research Station (RARS), Vijayapur located in the northern dry zone of Karnataka, India in iron-deficient calcareous soil under unbalanced alpha lattice design with two replications. Each genotype was planted as one row of 2 m length with a spacing of 30 x 10 cm for bunch types and 60 x 10 cm for spreading types. The recommended cultivation practices were followed to maintain healthy plant population. However, iron containing fertilizers were not applied.

The iron absorption efficiency was recorded based on severity of calcium induced interveinal chlorosis on the five randomly selected plants in each genotype at different stages *viz.*, 15, 30, 45, 60, 75 and 90 days after sowing (DAS) using Visual chlorotic rating (VCR) [Singh and Chaudhari, 1993]^[17] and SPAD chlorophyll meter reading (SCMR).

The yield and yield components *viz.*, main stem height (cm), number of primary branches, number of pods per plant, pod yield per plant (g), shelling per cent and hundred seed weight (g) were recorded on the five randomly selected plants in each genotype at harvest or after harvest for all the genotypes.

The data generated were analyzed as per alpha lattice incomplete block design using GenStat statistical package. Significance of variance was tested using 'F' value at $p=0.01$ (Fischer 1963)^[7]. Significance in 'F' and 't' tests were done at 5% and 1% level of probability.

Result and discussion

Diverse set of germplasm with less number of genotypes provides good opportunity for identification of resistant sources. 196 groundnut genotypes (184 mini core genotypes +4 control and 8 checks) analysis of variance indicated highly significant variation (among the mini core suggesting scope for selection of resistant genotypes for iron deficiency chlorosis. Visual scores ranged from 1 to 4.9 across different stages in groundnut mini core. Among the different stages, there was high incidence of iron chlorosis at 45 and 60 DAS as indicated by high VCR and low SCMR value. Similarly for yield and yield related traits like plant height (cm), number of primary branches per plant, number of pods per plant, yield per plant (g), hundred seed weight (g), shelling per cent, and haulm weight per plant (g), significant variation was observed among the groundnut mini core.

Components of genetic variation

The range of variation was maximum at 60 DAS for VCR (1.2 to 4.9) and SCMR (5.1 to 43.52) (Table 1). The genotypic coefficient of variation was high for VCR at 90 (28.368) DAS whereas for SCMR at 45 (25.112) and 60 (37.346) DAS. Phenotypic coefficient of variation for VCR was high during 60 and 90 (20.854 to 32.829) DAS. It indicates high degree of variability and better scope for selection. Phenotypic coefficient of variation (PCV) for SCMR was high during 45 to 90 (20.071 to 37.346). In general the phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for both character (VCR and SCMR). The close correspondence between the estimates of GCV and PCV indicated that the environment influence is low and hence selection for the character would be made based on their phenotypic performance. High heritability was observed for VCR at 60 (68.000) and 90 (74.700) DAS whereas for SCMR at 45 to 90 (59.900 to 80.400) DAS. Genetic advance over mean was high during 45 to 90 for VCR (23.014 to 50.498) and SCMR (26.600 to 52.037). The high heritability with high genetic

advance as per cent over mean (GAM) indicated that there was lower environment influence on the expression of these character and governed by additive gene action hence better scope for selection.

There was high GCV was recorded for plant height (24.063), number of pods per plant (23.805) and yield per plant (32.065) (Table 2). Phenotypic coefficient of variation was high for plant height (26.944), number of primary branches per plant (21.160), number of pods per plant (33.368), yield per plant (39.463) and haulm weight per plant (24.663). Higher magnitude of GVC and PCV indicates the presence of high degree of variability and better scope for improvement.

High heritability along with the high genetic advance over mean were observed for traits like plant height, yield per plant and hundred seed weight indicate that there was lower environment influence on the expression of the character and governed by additive gene action and hence better scope for selection (Table 2).

The availability of variability in the genetic material requires for the effective selection. The importance of heritable variation in the breeding material for realizing gain from selection needs no emphasis (Falconer, 1967). In the present study variability existed in the genotypes for iron absorption efficiency as indicated by higher phenotypic and genotypic coefficients of variation. The high range provides a preliminary idea about the variability existed in the genotypes.

Mean performance and frequency of lines showing resistance to IDC

Among all the six stages, most severe iron deficiency chlorosis symptoms were observed at 60 DAS indicating higher metabolic activity at 60 DAS, higher requirement of iron at peak growth stages. Also, iron taken up by the plants was metabolized for other functions of plant decreasing the chlorophyll synthesis, thereby leading to iron deficiency chlorosis in groundnut. Severity was also coincided with high soil moisture due to receipt of high rainfall during the period which made Fe unavailable to the plants. Boodi (2014)^[3] reported higher chlorosis at 60 and 90 days after sowing, Singh (2015)^[20] reported severe expression of chlorosis at 60 days after sowing, while Prakhyat (2016)^[12] reported severe expression at 90 DAS. But, development of chlorosis starts within 35 days after sowing and increased chlorosis occurred at 45 DAS in groundnut under simulated conditions through irrigating crops in highly calcareous soils (Bhardwaj, 2006)^[2]. Similarly, Kulkarni *et al.* (1994)^[8] found higher visual chlorosis scores at 60 DAS and suggested screening at 60 days is more reliable. Deotale *et al.* (2007)^[4] reported iron deficiency symptoms were observed mainly at 45 days after transplanting in Hoagland solution. Li *et al.* (2009)^[9] reported highest chlorosis scores for susceptible cultivars occurred 50 – 65 days after emergence.

Based on actual VCR score, genotypes were grouped as 'resistant' (1 to 2), 'moderately resistant' (>2 to 3) and 'susceptible' (>3 to 5). Genotypes ICG #11322 (hypogaea), 6888 (fastigiata), 9961, 14475 and 4389 (hypogaea) recorded lowest VCR at 60 DAS and were 'resistant' to IDC (Table 5). Sixty eight genotypes which recorded VCR score were grouped as 'moderately resistant', while 115 genotypes were 'susceptible' to IDC at 60 day after sowing (Table 3). Among the checks, ICGV 86031, A-30b, GPBD 5 and ICGV 06146 were resistant, while GPBD 4, TMV 2 and DSG-1 were moderately resistant, but R9227 was susceptible (Table 5). Several efficient groundnut genotypes were also identified by

earlier researchers (Boodi, 2014; Singh, 2015; Parakyat, 2016; Dwivedi *et al.*, 1993; Singh *et al.*, 2005) [3, 20, 12, 5, 19]. In the present study, genotypes identified at most severe stage can be utilized for developing cultivar with high iron absorption efficiency in different botanical groups.

Frequency of mini core genotypes for iron deficiency chlorosis based on VCR indicated that resistant genotypes were more at 15 DAS (1.00) but went on decreasing up to 45 (0.02) and 60 DAS (0.03) (Table 3). In the mini core, at most severe stage of iron chlorosis (60 DAS), only few genotypes were found resistant (0.03), many of them were moderately resistant (0.36), but susceptible types (0.61) were highest in frequency. After 60 DAS, the severity started reducing due to recovery in many of the genotypes which was evident from increase in resistant and moderately resistant types at 75 DAS and 90 DAS (Fig. 1). This suggests that the requirement of iron in plant increases with growth of plant. During the later part of plant growth, increased activity of peroxidases and ferrous reductase enzymes in the resistant/moderately resistant plants helps in their recovery from iron deficiency stress and reduced VCR scores, while the plants with lesser activity remains as susceptible.

Based on extent of iron deficiency chlorosis, wide variation was observed among the mini core genotypes at different stages for SCMR. Among all the five stages, most severe iron deficiency chlorosis symptoms were observed at 60 DAS. At this stage, highest SCMR (>25) was recorded by five genotypes of mini core *viz.*, ICG #11322 (hypogaea), 6888 (fastigiata), 9961, 14475 and 4389 (hypogaea) and are considered as 'resistant' to IDC (Table 5). Among the checks, those which recorded higher SCMR (>25) like ICGV 06146 (43.52), ICGV 86031 (42.30), A-30B (31.33), GPBD 5 (29.39) were 'resistant', while those with moderate SCMR like GPBD 4 (25.15), DSG-1 (20.40) and TMV 2 (19.48) were moderately resistant, but those with lower SCMR like R9227 (18.83) was found 'susceptible'. Among the different botanical genotypes the maximum resistant and moderate resistant lines were reported in Hypogaea type at 45 and 60 DAS (Table 4). Under iron-deficient calcareous soils, SCMR values clearly indicated that reduction in chlorophyll content was much lesser in iron deficiency chlorosis resistant genotypes compared to susceptible genotypes due to higher severity of iron deficiency chlorosis.

Main stem height (cm) among different genotypes ranged from 3.415 to 32.200 cm (table 2). Among the checks, the

lowest plant height was recorded for ICGV 06146 (12.10 cm), while the highest for GPBD 4 (25.15 cm) (Table 5). The number of primary branches per plant among different genotypes ranged from 1.335 to 6.300 (Table 2). Among checks, R 9227 showed the lowest number of primary branches per plant (3.60), while highest in DSG-1 (4.95). The number of pods per plant differed significantly among the mini core genotypes and ranged from 2.200 to 11.900. Among the checks, ICGV 06146 (4.80) had the lowest, while GPBD 4 (11.80) and GPBD 5 (11.80) had highest number of pods per plant.

Pod yield per plant differed significantly among the mini core genotypes and ranged from 1.575 to 12.415 g (Table 2). Among the checks, highest pod yield per plant was recorded for TMV 2 (8.89 g), while lowest by A-30b (2.50 g). Hundred seed weight differed significantly among the mini core genotypes and ranged from 19.56 to 45.87 g. Among the checks, highest hundred seed weight was recorded by GPBD 4 (36.93 g), while lowest in A-30b (23.37 g). Shelling per cent differed significantly among the mini core genotypes and ranged from 35.860 to 71.710 per cent. Among checks, shelling per cent was found lowest in ICGV 06146 (52.51%), while highest in R 9227 (69.06 %).

Haulm weight per plant differed significantly among the mini core genotypes and ranged from 6.400 to 19.045 g. Among the checks, highest haulm weight per plant was recorded for TMV 2 (14.86 g), while lowest by GPBD 5 (8.60 g).

Groundnut being sensitive to iron deficiency, iron chlorosis is most commonly seen in areas of groundnut cultivation particularly in calcareous, alkaline and black soils. Iron chlorosis causes reduction chlorophyll in leaves which in turn results in reduction in groundnut yield. In order to obtain optimum yield from these soils, identification of iron absorption efficient genotypes with higher productivity is essential. For this purpose the genotypes were assessed for iron absorption efficiency related traits like VCR and SCMR at different stages of crop growth and also impact of iron chlorosis on yield and yield components. Out of 184 genotypes tested for iron absorption efficiency, five were found resistant to calcium induced iron deficiency chlorosis *viz.*, ICG # 11322, 6888, 9961, 14475 and 4389 by recording significantly lower VCR and higher SCMR at severe stage of iron chlorosis expression (60 DAS) under iron deficient calcareous soils.

Table 1: Components of variation for VCR and SCMR at different stages in groundnut mini core

Components	15 DAS		30 DAS		45 DAS		60 DAS		75 DAS		90 DAS	
	VCR	SCMR										
Minimum	1.000	30.160	1.000	12.285	1.500	7.145	1.200	5.100	1.000	13.545	1.000	18.080
Maximum	1.600	47.095	3.500	36.550	4.335	33.680	4.900	43.520	3.365	42.130	2.900	50.800
Mean	1.059	40.151	1.995	27.822	3.049	18.014	3.122	18.121	2.089	27.627	1.445	35.963
GCV (%)	7.595	4.641	10.891	8.117	14.603	25.112	17.195	30.715	18.716	16.685	28.368	17.996
PCV (%)	11.438	8.169	17.563	12.532	19.087	31.273	20.854	37.346	26.090	21.558	32.829	20.071
h ² (%)	44.100	32.300	38.500	42.000	58.500	64.500	68.000	67.600	51.500	59.900	74.700	80.400
GA	0.110	2.181	0.278	3.013	0.702	7.483	0.912	9.430	0.578	7.349	0.729	11.953
GAM	10.390	5.431	13.913	10.831	23.014	41.540	29.207	52.037	27.659	26.600	50.498	33.237

VCR: Visual chlorotic rating ; GCV: Genotypic coefficient of variance; PCV: Phenotypic coefficient of variance; h² : Heritability in broad sense; GA- Genetic advance; GAM- Genetic advance over mean

Table 2: Components of variation for yield and yield related traits in groundnut mini core

Components	PH	NPB	NPP	YPP	HSW	SP	HWP
Minimum	3.415	1.335	2.200	1.575	19.560	35.860	6.400
Maximum	32.200	6.300	11.900	12.415	45.870	71.710	19.045
Mean	16.606	3.808	6.813	4.930	29.802	57.825	11.452
GCV (%)	24.063	13.417	23.805	32.065	15.538	9.374	17.755
PCV (%)	26.944	21.160	33.368	39.463	18.093	12.739	24.663
h ² (%)	79.800	40.200	50.900	66.000	73.800	54.200	51.800
GA	7.352	0.667	2.384	2.646	8.192	8.217	3.015
GAM	44.271	17.524	34.983	53.673	27.489	14.210	26.330

GCV: Genotypic coefficient of variance; PCV: Phenotypic coefficient of variance; h²: Heritability broad sense; GA- Genetic advance; GAM- Genetic advance over mean; PH: Plant height; NPB: Number of primary branches per plant; NPP: Numbers pods per plant; YPP: Yield per plant; HSW: Hundred seed weight; SP: Shelling per cent; HWP: Haulm weight per plant

Table 3: Frequency of mini core genotypes for response to iron deficiency chlorosis based on VCR

Resistance*	15 DAS		30 DAS		45 DAS		60 DAS		75 DAS		90 DAS	
	Number of lines	Frequency										
Resistant	188	1.00	113	0.60	4	0.02	5	0.03	78	0.41	164	0.87
Moderately resistant	0	0.00	73	0.39	72	0.38	68	0.36	104	0.55	24	0.13
Susceptible	0	0.00	2	0.01	112	0.60	115	0.61	6	0.03	0	0.00

*Based on mean VCR: Resistant (1.0 to 2.0); Moderately Resistant (>2 to 3); Susceptible (>3 to 5); DAS - Days after sowing

Table 4: Frequency of lines showing resistance to iron chlorosis in different botanical types of groundnut mini core based on visual chlorotic rating

Botanical type	Total number of lines	Efficiency*	15 DAS		30 DAS		45 DAS		60 DAS		75 DAS		90 DAS	
			number of lines	Frequency										
Fastigiata	38	R	38	1.00	28	0.74	2	0.05	1	0.03	19	0.50	36	0.95
		MR	0	0.00	10	0.26	23	0.61	20	0.53	18	0.47	2	0.05
		S	0	0.00	0	0.00	13	0.34	17	0.45	1	0.03	0	0.00
hypogaea	87	R	87	1.00	51	0.59	2	0.02	4	0.05	36	0.41	73	0.84
		MR	0	0.00	34	0.39	36	0.41	28	0.32	48	0.55	14	0.16
		S	0	0.00	2	0.02	49	0.56	55	0.63	3	0.03	0	0.00
Vulgaris	59	R	59	1.00	32	0.54	0	0.00	0	0.00	22	0.37	53	0.90
		MR	0	0.00	27	0.46	11	0.19	18	0.31	35	0.59	6	0.10
		S	0	0.00	0	0.00	48	0.81	41	0.69	2	0.03	0	0.00
Aequatoriana	1	R	1	1.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
		MR	0	0.00	1	1.00	0	0.00	0	0.00	1	1.00	1	1.00
		S	0	0.00	0	0.00	1	1.00	1	1.00	0	0.00	0	0.00
Hirsuta	1	R	1	1.00	1	1.00	0	0.00	0	0.00	0	0.00	0	0.00
		MR	0	0.00	0	0.00	1	1.00	0	0.00	1	1.00	1	1.00
		S	0	0.00	0	0.00	0	0.00	1	1.00	0	0.00	0	0.00
Peruviana	2	R	2	1.00	1	0.50	0	0.00	0	0.00	1	0.50	2	1.00
		MR	0	0.00	1	0.50	1	0.50	2	1.00	1	0.50	0	0.00
		S	0	0.00	0	0.00	1	0.50	0	0.00	0	0.00	0	0.00

*Based on mean visual chlorotic rating; R: Resistant (1 to 2); MR: Moderately Resistant (> 2 to 3); S: Susceptible (> 3 to 5); DAS: Days after sowing

Table 5: Performance of selected genotypes for iron absorption efficiency related traits and yield parameters in each botanical type of groundnut mini core

Sl. No.	Genotypes (ICG#)	Botanical Type	Efficiency	IAE related traits @ 60 DAS		Yield and yield related parameters						
				VCR	SCMR	PH	NPB	NPP	YPP	HSW	SP	HWP
1	11322	hypogaea	R	1.20	42.10	16.60	3.50	4.20	3.70	30.23	66.00	17.26
2	9961	hypogaea	R	1.70	31.97	22.40	3.50	4.10	3.19	29.21	49.53	11.52
3	14475	hypogaea	R	1.90	29.17	18.60	4.60	6.50	5.29	35.48	46.55	16.92
4	4389	hypogaea	R	1.90	29.56	6.60	2.90	7.30	3.80	30.63	59.91	6.40
5	6888	fastigiata	R	1.40	34.21	16.50	3.90	11.20	6.67	19.56	63.87	15.17
Checks												
1	ICGV 86031	vulgaris	R	1.25	42.30	12.20	4.50	6.70	3.38	34.44	54.38	11.58
2	A-30b	vulgaris	R	1.40	31.33	16.40	4.20	5.00	2.50	23.37	57.54	12.75
3	ICGV 06146	vulgaris	R	1.40	43.52	12.10	4.50	4.80	3.92	28.81	52.51	14.83
4	GPBD 5	vulgaris	R	1.70	29.39	14.50	3.70	11.80	5.85	28.37	58.83	8.60
5	GPBD 4	vulgaris	MR	2.50	25.15	18.40	4.10	11.80	5.48	36.93	59.35	11.41
6	TMV 2	vulgaris	MR	2.90	19.48	13.60	3.80	11.50	8.89	30.80	50.60	14.86
7	R 9227	vulgaris	S	3.20	18.83	17.30	3.60	10.30	4.53	29.40	69.06	9.28
8	DSG-1	hypogaea	MR	2.95	20.40	16.63	4.95	7.48	7.86	29.93	59.90	9.06

R: Resistance; MR: Moderately resistance; S: Susceptible; VCR: Visual chlorotic rating; SCMR: SPAD chlorophyll meter reading; PH: Plant height; NPB: Number of primary branches per plant; NPP: Numbers pods per plant; YPP: Yield per plant; HSW: Hundred seed weight; SP: Shelling per cent; HWP: Haulm weight per plant

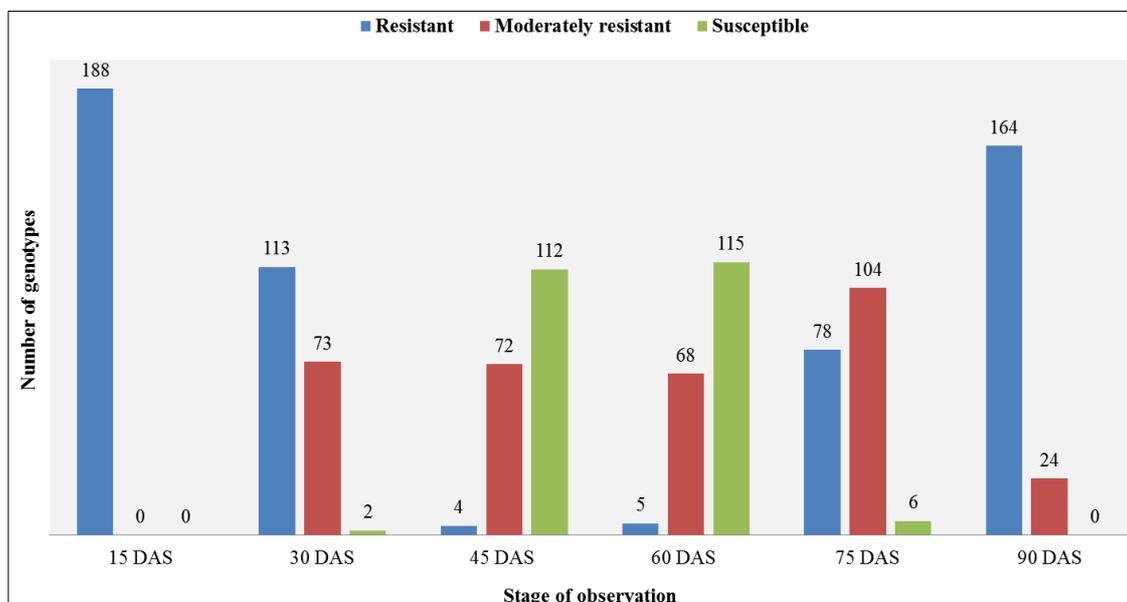


Fig 1: Frequency of groundnut mini core genotypes for response to iron deficiency chlorosis at different stages

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