



# Genetic analysis of recombinant inbred lines for iron deficiency chlorosis and productivity traits in groundnut

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## Abstract

Iron deficiency is an important abiotic constraint reducing the growth and yield of groundnut especially under calcareous soils. Foliar application of Fe-chelates can overcome iron deficiency but it is not economical. Evaluation of 318 recombinant inbred lines (RILs) along with parents for iron deficiency chlorosis (IDC) and productivity traits under iron deficient soils over three years indicated significant genotypic and genotypic x environment interaction (GE) component for both IDC and productivity traits. Among the RILs, range of variation was higher than that of the parents for visual chlorotic rating (VCR), SPAD chlorophyll meter reading (SCMR) and productivity parameters across three years indicated the presence of transgressive segregants. VCR had higher phenotypic and genotypic, variances, heritability and genetic advance as per cent of mean (GAM) in all the three years as compared to SCMR. Among productivity traits, pod yield ( $\text{g plant}^{-1}$ ) had higher PCV and GCV compared to shelling per cent and 100 seed weight. Significant negative correlation between VCR and pod yield per plant indicated effect of IDC on productivity in RILs. Nine lines were superior for both IDC tolerance and productivity traits. This extensive phenotyping of RIL population for IDC tolerance under iron deficient conditions can be used for identification of genomic regions associated with IDC tolerance by genotyping of this RIL population.

**Key words:** Groundnut, iron deficiency chlorosis, productivity traits, recombinant inbred lines

## Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop of the world, grown on 25.44 m ha with a production of 45.23 mt and productivity of  $1.77 \text{ t ha}^{-1}$  (Faostat 2014). India stands first in groundnut area

(5.25 m ha), while second in production (9.47 mt) after China (17.01 mt). The lower productivity in India is mainly attributed to various abiotic and biotic stresses affecting the crop growth and yield. Iron deficiency is an important abiotic constraint reducing the growth and yield of groundnut. One-third of the Indian soils are calcareous and spread mostly in the low rainfall areas of the western and central parts of the country which are deficient in available iron ( $\text{Fe}^{2+}$ ) because, iron forms insoluble ferric hydroxide complexes in the presence of oxygen at neutral or basic pH in calcareous soils (Guerinot and Yi 1994). Iron deficiency chlorosis (IDC) is prevalent among groundnut growing areas such as Saurashtra region of Gujarat, Marathwada region of Maharashtra, and parts of Rajasthan, Tamil Nadu and Karnataka states in India and causes significant reduction in pod yield of 16-32% (Singh et al. 1995; Singh 2001). Severity of IDC will be usually quite high after excessive rainfall and also for irrigated groundnut due to high bicarbonate ion concentration in the rhizosphere (Singh et al. 1995; Zuo et al. 2007). Further, iron deficiency in groundnut crop may also result in reduced Fe content in groundnut seed of food, triggering Fe deficiency in humans.

In plants, iron (Fe) plays an important role in various cellular processes including photosynthesis and respiration (Zheng 2010). Plants adopt two types of mechanisms (Strategy I and II) for iron acquisition from the soils. The strategy-I is found among dicots and monocots except graminaceous species, which adopts strategy-II. Groundnut adopts strategy-I and found sensitive to iron deficiency (Fageria et al. 1994)

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especially under calcareous soils resulting in iron deficiency chlorosis (IDC) with characteristic interveinal chlorosis on young rapidly expanding leaves. Under severe Fe deficiency, veins also become chlorotic, leaves become white and papery and later turn brown and necrotic; plants show stunted growth resulting in reduced yield, fodder, and seed Fe content; acute deficiency can lead to death of plants and complete crop failure.

Although application of Fe-containing fertilizers into soil or as foliar spray has been suggested (Frenkel et al. 2004; Irmak et al. 2012), however, it is often associated with problems like conversion into unavailable form ( $\text{Fe}^{3+}$ ) or poor translocation within the plant (Hüve et al. 2003). Foliar application of Fe-chelates can overcome this problem, but not economical as groundnut is predominantly grown as a rainfed subsistence crop by the resource-poor farmers in semi-arid tropics. Hence, the development of iron chlorosis tolerant genotypes would overcome the Fe deficiency in soil thereby increase the Fe content in groundnut seed and further improvement of human health (Imtiaz et al. 2010). Although many tolerant cultivars have been identified earlier in the germplasm (Samdur et al. 1999; Prasad et al. 2000; Li and Yan-Xi 2007), the studies on characterization of recombinant inbred lines for IDC response have not been done. The present study is aimed at genetic analysis of recombinant inbred line (RIL) population for iron chlorosis response and productivity traits under Fe-deficient calcareous soils which gives the scope for identification of IDC tolerant and productive lines.

## Materials and methods

### Plant materials

As per field screening at College of Agriculture, Vijayapur, during rainy season 2009, ICGV 86031 was found IDC tolerant (VCR 1.0), while TAG 24 as IDC susceptible (VCR 4.0). The RIL population of the cross TAG 24 x ICGV 86031 developed earlier at ICRISAT, Patancheru consisting of 318 lines and parents were used for the study.

### Field experiment

The field experiment was conducted for three consecutive rainy seasons (2013, 2014 and 2015) at College of Agriculture, Vijayapur, ( $16^{\circ}49' \text{ N}$ ,  $75^{\circ}43' \text{ E}$ , 593 m above mean sea level, and 597 mm average annual rainfall) on calcareous vertisol soils that are alkaline ( $\text{pH} > 8$ ) and deficient in available Fe (DTPA-

extractable Fe  $< 4$  ppm) (Table 1). Field screening for IDC response of RIL population along with parents (320 lines) was done using alpha incomplete block design in two replications with each replication having four equal sized blocks. Each genotype in a replication was planted as one row of 2 m length with an inter and intra-row spacing of 30 and 10 cm, respectively. The recommended fertilizers of major nutrients (25:50:25 kg NPK  $\text{ha}^{-1}$ ) were applied at the time of planting. Iron-containing fertilizers were not applied. However, micronutrients (Zn, Mn, Mg, S) with overlapping deficiency symptoms for Fe were taken care by applying  $\text{ZnSO}_4$ ,  $\text{MnSO}_4$ , and  $\text{MgSO}_4$ . Recommended cultivation practices were followed to raise a good crop.

**Table 1.** Soil properties of experimental sites during 2013, 2014 and 2015

Soil properties	2013	2014	2015
<b>Chemical properties</b>			
Soil pH (1: 2.5)	8.12	8.22	8.33
Electrical conductivity (1: 2.5) ( $\text{dS m}^{-1}$ )	0.28	0.16	0.23
Organic carbon (%)	0.58	0.36	0.25
Free $\text{CaCO}_3$ (%)	9.50	9.80	10.20
Exchangeable Calcium [ $\text{Cmol (+) kg}^{-1}$ ]	43.10	44.90	43.70
Exchangeable Magnesium [ $\text{Cmol (+) kg}^{-1}$ ]	8.80	9.10	9.00
<b>Available nutrients</b>			
Nitrogen ( $\text{kg ha}^{-1}$ )	348.00	307.00	269.70
Phosphorus ( $\text{kg ha}^{-1}$ )	19.50	18.80	45.60
Potassium ( $\text{kg ha}^{-1}$ )	530.00	488.00	296.22
Sulphur (ppm)	12.38	8.93	7.44
DTPA-extractable Zinc (ppm)	0.31	0.28	2.26
DTPA-extractable Iron (ppm)	3.96	3.76	3.91

### IDC tolerance and productivity traits

IDC tolerance associated traits like visual chlorotic rating (VCR) and SPAD chlorophyll meter reading (SCMR) were assessed across three stages *i.e.* 30, 60, and 90 days after sowing (d) for three consecutive years. VCR scoring was done as per the scale proposed by Singh and Chaudhari (1993) [1-5 scale where, 1 = normal green leaves with no chlorosis, 2 = green leaves but with slight chlorosis on some leaves, 3 = moderate chlorosis on several leaves, 4 = moderate chlorosis on most of the leaves and 5 = severe chlorosis on all the leaves] on overall line-basis.

The chlorophyll meter SPAD 502 (Soil Plant Analysis Development meter, Konica Minolta, Japan) was used to measure the absorbance of the leaf in the red (at 650 nm) and near infrared region (at 940 nm). Using these two transmittances, it calculated a numerical SPAD value which is proportional to the chlorophyll present in the leaf and is negatively related to chlorosis of the plants. The SCMR (SPAD values) was recorded in the standard leaf (third fully expanded leaf from tip on main stem) of five plants per genotype and mean was calculated. Higher SCMR (>25) indicates tolerance, while lower SCMR (<25) indicates susceptibility to IDC. The yield and yield components like pod yield (g plant<sup>-1</sup>), shelling per cent and 100 seed weight (g) were recorded after harvest for all the RILs.

### **Design and statistical analysis**

The data was analyzed as per alpha unbalanced design using GenStat release 15.1 statistical package. Significance of variance was tested using 'F' value at  $p < 0.05$  (Fischer 1963). Best linear unbiased predictors (BLUPs) were estimated to reduce the nuisance variables and used for comparison. To assess variability in the RIL population, genetic components like genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV) (Burton and Devane 1953), broad sense heritability ( $H_{bs}$ ) (Hanson et al. 1956) and genetic advance as per cent of mean (GAM) (Johnson et al. 1955) were estimated. Pearson correlation analysis was carried out using bivariate analysis model in SPSS Statistics version 21.0.

## **Results and discussion**

### **Variance components**

Significant genotypic variance was observed among RILs in individual years (2013, 2014 and 2015) for VCR and SCMR at 30, 60 and 90 days after sowing (d) and also for productivity traits (Table 2). In the pooled analysis over three years, significant differences were observed for genotypes, environment (year) and G x E interaction variances for all the IDC and productivity traits indicating predominance of genetic component and differential reaction of genotypes to environments due to prevalence of differential weather conditions during the different years.

### **Mean performance**

The parents of the RIL population differed in expressing IDC in terms of VCR at different stages of crop growth in all the three years. Maximum difference between

parents, TAG 24 and ICGV 86031 was observed at 60 d during 2013 and 2014 (Table 2), while at 90 d during 2015 suggesting that screening of RILs for IDC at these stages is effective. Severity was coinciding with high soil moisture due to receipt of high rainfall (data not provided) during the period which made Fe unavailable to the plants. Boodi (2014) reported higher chlorosis at 60 and 90 d, while Singh (2015) and Kulkarni et al. (1994) reported higher visual chlorosis scores at 60 d. These studies suggest that screening for IDC response at 60 days is more reliable. In highly calcareous soils, development of chlorosis though starts within 35 days after sowing, increased chlorosis occurred at 45 d in groundnut under simulated conditions through irrigating crops (Bhardwaj 2006). This suggests the necessity of screening groundnut genotypes for IDC at optimum moisture conditions to identify potential and true IDC tolerant genotypes. In all the three years, parent ICGV 86031 was IDC tolerant as evident from lower VCR and higher SCMR values, while TAG 24 was IDC susceptible as it showed higher VCR and lower SCMR values. Among the RILs, range of variation was much more than that of the parents for both VCR and SCMR at all the stages (30, 60 and 90 d) across three years indicating presence of transgressive segregants. Earlier, such a large variation was noted among parents and RIL populations for IDC tolerance related traits like visual scoring and SCMR in soybean (Lin et al. 1997; Butenhoff 2015) and mungbean (Prathet et al. 2012), and for zinc efficiency score in wheat (Genc et al. 2009). Though visual scoring is a fast and convenient method to evaluate IDC tolerance in groundnut, it is more complex as scoring differs from person to person. Hence, it is essential to confirm IDC tolerance through a quantitative means like SCMR. Earlier, Samdur et al. (2000) also established the usefulness of SPAD chlorophyll meter reading for rapid and *in situ* screening of groundnut genotypes.

Effect of IDC on productivity traits like pod yield, shelling per cent and 100 seed weight was evident from variation between parents across three years (Table 3). Wider range of variation was observed among RILs for all the three productivity traits beyond the parents in individual years as well as pooled over three years indicating scope for selection of productive and IDC tolerant lines under Fe-deficiency conditions. Earlier, Potdar and Anders (1993) also reported that iron deficiency in groundnut can cause significant reduction in pod yield, haulm yield and total dry matter production.

**Table 2.** Analysis of variance for VCR and SCMR across three stages and productivity traits for three years and pooled data

Year/source of variation	DF	VCR 30d	VCR 60d	VCR 90d	SCMR 30d	SCMR 60d	SCMR 90d	Pod yield (g plant <sup>-1</sup> )	Shelling per cent	100-seed weight (g)
<b>2013</b>										
Replication	1	0.40	0.02	0.31	2.19	157.76	13.77	0.57	4.37	3.96
Genotype	319	0.63**	0.64**	0.74**	35.13**	37.11**	47.65**	1.61**	40.06**	19.40**
Rep × Block	6	1.82	0.79	0.71	87.84	88.00	145.21	6.83	57.05	3.14
Residual	313	0.22	0.08	0.14	6.21	8.30	7.36	0.24	2.64	1.59
<b>2014</b>										
Replication	1	0.05	3.75	6.80	32.18	319.79	581.41	1.17	0.01	155.33
Genotype	319	0.55**	0.82**	0.94**	53.59**	5.05**	125.94**	1.52**	48.11**	33.17**
Rep × Block	6	0.35	2.47	3.54	68.99	265.61	609.25	6.37	53.29	17.82
Residual	313	0.14	0.14	1.65	17.41	14.49	33.21	0.13	3.18	2.09
<b>2015</b>										
Replication	1	0.18	0.15	0.00	12.37	470.93	14.40	63.33	201.68	64.90
Genotype	319	0.18**	1.01**	1.01**	21.05**	107.43**	88.75**	3.09**	13.54**	36.67**
Rep × Block	6	0.17	3.16	1.45	30.94	564.05	142.50	13.27	24.19	13.75
Residual	313	0.02	0.02	0.00	0.77	0.76	0.24	0.45	3.48	3.68
<b>Pooled</b>										
Genotype	319	0.83**	1.38**	2.01**	65.09**	113.97**	190.64**	2.88**	46.76**	48.26**
Environment (Year)	2	52.38**	114.9**	103.70**	500.99**	20959.0**	32290.3**	142.19**	5734.69**	7753.0**
Replication within environment	3	0.21	1.31	2.37	15.69	314.23	204.14	21.82	68.43	73.11
Genotype × environment	638	0.27**	0.59**	0.38**	23.29**	57.52**	41.67**	1.88**	28.25**	20.53**
Pooled error	957	0.13	0.09	0.11	8.30	9.16	14.63	0.29	3.14	2.45

\*\* Significance at  $p=0.01$ ; ns = non significant; d = days after sowing; visual chlorotic rating (VCR), SPAD chlorophyll meter reading (SCMR)

### Genetic components

Among the IDC tolerance related traits, VCR had higher PCV, GCV, heritability and GAM in all the three years compared to SCMR (Table 3) which is quite expected due to qualitative nature of VCR. The difference between PCV and GCV is minimal for both VCR and SCMR at all the three stages over three years indicating less influence of environment on the expression of these traits and reliability of VCR and SCMR in assessing IDC tolerance in case of RILs. Among the productivity traits, pod yield (g plant<sup>-1</sup>) had higher PCV and GCV (Table 3) compared to shelling per cent and 100 seed weight indicating relatively more pronounced effect of IDC on shelling per cent and 100 seed weight in the RILs. Less difference between PCV and GCV

for all the productivity traits indicated the reliability of phenotypic observation in measuring the genetic components. The heritability and GAM was high for pod yield indicating scope for selection of better productive recombinant inbred lines under iron deficient conditions.

### Association among traits

The IDC parameter, VCR had significant negative correlation with SCMR in all the three years and pooled over years (Table 4). With respect to productivity traits, VCR measured at 30, 60 and 90 d had significant negative correlation with pod yield in 2014 and pooled over years. On the contrary, SCMR at all the stages had significant positive correlation with pod yield in 2014 and pooled over years. This shows the significant

**Table 3.** Genetic components for VCR, SCMR across three stages and productivity traits for three years and pooled data

Trait	Year	TAG 24	ICGV 86031	Range in RILs	Grand mean	PCV (%)	GCV (%)	H <sub>bs</sub> (%)	GAM (%)
VCR 30d	2013	2.56	1.26	1.15 – 3.12	1.66	34.03	27.56	65.57	45.96
	2014	2.61	1.14	1.12 – 2.96	1.48	35.08	30.07	73.47	53.24
	2015	1.87	1.01	1.01 – 2.74	1.10	27.35	25.40	86.25	48.64
	Pooled	2.34	1.08	1.04 – 2.89	1.41	26.35	21.74	67.75	36.77
VCR 60d	2013	3.67	1.13	1.04 – 3.63	1.52	37.40	34.74	86.28	66.38
	2014	3.66	1.19	1.19 – 3.82	2.33	27.52	24.99	82.47	46.70
	2015	2.86	0.92	0.92 – 4.07	1.70	42.04	41.45	97.22	84.18
	Pooled	3.53	1.05	1.03 – 3.59	1.85	25.75	19.79	58.86	31.22
VCR 90d	2013	3.60	1.10	1.04 – 3.18	1.62	37.76	33.90	80.61	62.53
	2014	3.58	1.11	1.02 – 3.75	1.89	36.35	33.01	82.47	61.75
	2015	4.00	2.00	1.00 – 4.00	2.41	29.87	29.84	99.80	61.37
	Pooled	3.90	1.34	1.04 – 3.71	1.97	29.25	26.47	82.00	49.41
SCMR 30d	2013	25.03	38.44	18.32 – 42.36	34.31	12.32	11.21	82.72	20.99
	2014	25.06	38.62	22.25 – 40.29	33.25	15.56	12.78	67.47	21.63
	2015	28.14	40.07	17.98 – 40.29	34.99	9.28	9.11	96.31	18.41
	Pooled	26.07	39.48	20.70 – 40.15	34.18	9.60	7.71	64.58	12.77
SCMR 60d	2013	20.77	37.85	18.06 – 43.33	36.21	11.97	10.56	77.82	19.19
	2014	14.11	37.62	12.29 – 36.98	24.76	24.44	21.89	80.22	40.40
	2015	19.15	41.89	9.14 – 43.14	30.34	24.19	24.10	99.29	49.46
	Pooled	16.46	39.40	17.00 – 38.24	30.44	14.09	10.06	50.98	14.80
SCMR 90d	2013	19.49	41.32	21.37 – 44.15	38.18	12.81	11.77	84.57	22.31
	2014	17.36	42.19	13.59 – 42.94	31.08	25.55	21.94	73.69	38.79
	2015	7.71	27.95	8.11 – 42.02	23.96	28.11	28.07	99.74	57.76
	Pooled	13.70	38.34	15.71 – 40.45	31.07	18.00	16.12	80.16	29.72
Pod yield (g plant <sup>-1</sup> )	2013	3.31	3.67	2.54 – 6.45	4.26	21.28	19.66	85.32	37.42
	2014	1.55	3.88	1.45 – 6.67	3.37	25.86	24.72	91.36	48.69
	2015	3.86	4.93	1.69 – 8.34	4.08	30.58	28.24	85.28	53.73
	Pooled	2.88	4.14	2.13 – 5.64	3.90	16.97	9.35	30.31	10.59
Shelling per cent	2013	60.56	58.85	47.32 – 70.21	60.00	7.54	7.29	93.56	14.52
	2014	53.75	53.00	43.37 – 65.81	56.46	8.70	8.41	93.41	16.74
	2015	66.22	68.79	56.00 – 69.29	62.40	4.20	3.63	74.61	6.45
	Pooled	60.53	60.79	52.28 – 65.48	59.62	4.68	2.94	39.54	3.81
100 seed weight (g)	2013	23.62	30.99	21.12 – 37.60	26.39	11.79	11.30	91.79	22.30
	2014	28.06	30.60	18.98 – 38.94	28.39	14.35	13.89	93.70	27.69
	2015	27.53	30.72	23.85 – 45.74	33.17	12.89	12.22	89.90	23.87
	Pooled	26.26	30.79	23.32 – 38.74	29.31	9.66	7.32	57.43	11.43



**Table 4.** Correlation coefficients between IDC related and productivity traits for three years and pooled data

Variables	VCR-30d	VCR-60d	VCR-90d	SCMR-30d	SCMR-60d	SCMR-90d	PYP	SP	HSW
<b>2014 \ 2013<sup>§</sup></b>									
VCR-30d	1.000	<b>0.558</b>	<b>0.534</b>	<b>-0.681</b>	<b>-0.481</b>	<b>-0.572</b>	0.061	<b>-0.208</b>	-0.089
VCR-60d	<b>0.675</b>	1.000	<b>0.514</b>	<b>-0.522</b>	<b>-0.799</b>	<b>-0.527</b>	-0.060	<b>-0.114</b>	-0.031
VCR-90d	<b>0.648</b>	<b>0.765</b>	1.000	<b>-0.438</b>	<b>-0.417</b>	<b>-0.832</b>	<b>0.176</b>	-0.028	<b>-0.111</b>
SCMR-30d	<b>-0.904</b>	<b>-0.665</b>	<b>-0.622</b>	1.000	<b>0.523</b>	<b>0.486</b>	0.055	<b>0.215</b>	0.029
SCMR-60d	<b>-0.660</b>	<b>-0.961</b>	<b>-0.773</b>	<b>0.669</b>	1.000	<b>0.476</b>	0.077	0.078	0.028
SCMR-90d	<b>-0.595</b>	<b>-0.728</b>	<b>-0.946</b>	<b>0.588</b>	<b>0.739</b>	1.000	<b>-0.144</b>	0.031	0.064
PYP	<b>-0.189</b>	<b>-0.297</b>	<b>-0.294</b>	<b>0.183</b>	<b>0.294</b>	<b>0.256</b>	1.000	<b>0.292</b>	<b>0.133</b>
SP	<b>-0.238</b>	<b>-0.142</b>	-0.037	<b>0.249</b>	<b>0.159</b>	0.027	<b>0.211</b>	1.000	<b>0.116</b>
HSW	<b>-0.111</b>	<b>-0.115</b>	-0.048	<b>0.112</b>	0.109	0.036	<b>0.311</b>	<b>0.243</b>	1.000
<b>Pooled \ 2015<sup>#</sup></b>									
VCR-30d	1.000	<b>0.236</b>	<b>0.268</b>	<b>-0.674</b>	<b>-0.268</b>	<b>-0.249</b>	0.009	-0.047	-0.068
VCR-60d	<b>0.683</b>	1.000	<b>0.238</b>	<b>-0.227</b>	<b>-0.954</b>	<b>-0.216</b>	<b>-0.135</b>	-0.051	<b>-0.147</b>
VCR-90d	<b>0.720</b>	<b>0.795</b>	1.000	<b>-0.293</b>	<b>-0.215</b>	<b>-0.951</b>	-0.060	-0.040	-0.038
SCMR-30d	<b>-0.859</b>	<b>-0.658</b>	<b>-0.664</b>	1.000	<b>0.253</b>	<b>0.292</b>	-0.044	-0.011	0.030
SCMR-60d	<b>-0.648</b>	<b>-0.949</b>	<b>-0.750</b>	<b>0.636</b>	1.000	<b>0.202</b>	<b>0.117</b>	0.035	<b>0.161</b>
SCMR-90d	<b>-0.703</b>	<b>-0.767</b>	<b>-0.956</b>	<b>0.668</b>	<b>0.743</b>	1.000	0.057	0.020	0.045
PYP	<b>-0.117</b>	<b>-0.196</b>	<b>-0.127</b>	<b>0.142</b>	<b>0.199</b>	<b>0.112</b>	1.000	<b>-0.142</b>	<b>0.244</b>
SP	<b>-0.309</b>	<b>-0.117</b>	-0.104	<b>0.313</b>	0.107	<b>0.128</b>	<b>0.179</b>	1.000	<b>-0.137</b>
HSW	<b>-0.133</b>	<b>-0.150</b>	<b>-0.120</b>	0.083	<b>0.171</b>	0.096	<b>0.302</b>	<b>0.135</b>	1.000

PYP = Pod yield ( $\text{g plant}^{-1}$ ), SP = Shelling per cent; HSW = 100 seed weight (g); <sup>§</sup>2013 = above diagonal values; 2014 = below diagonal values; <sup>#</sup>2015 = above diagonal values; Pooled = below diagonal values; Correlation values in bold indicates significance at  $p=0.05$

**Table 5.** Superior recombinant inbred lines for IDC tolerance and productivity traits across three years

RIL / Parent	VCR-30d	VCR-60d	VCR-90d	SCMR-30d	SCMR-60d	SCMR-90d	PYP	SP	HSW
C3-52	1.04	1.09	1.35	38.42	35.92	36.77	5.30	62.72	26.52
C3-73	1.04	1.40	1.23	37.81	34.93	40.45	4.61	59.71	29.32
C3-131	1.21	1.08	1.43	38.54	37.65	38.80	4.75	61.02	32.88
C3-137	1.21	1.70	1.43	33.74	30.35	35.28	4.48	60.84	26.49
C3-138	1.49	1.08	1.39	33.93	35.87	37.31	5.54	56.70	32.15
C3-150	1.06	1.08	1.24	39.44	36.33	36.92	4.23	62.48	32.72
C3-152	1.06	1.08	1.08	36.16	38.10	38.35	4.51	63.56	28.60
C3-153	1.07	1.08	1.43	36.77	36.59	34.83	4.82	58.71	34.82
C3-187	1.21	1.77	1.19	37.15	29.23	38.67	4.52	60.89	29.81
TAG-24	2.21	3.53	3.90	28.32	16.46	13.70	2.88	60.53	26.26
ICGV-86031	1.08	1.05	1.34	39.48	39.40	38.34	4.14	60.79	30.79

PYP = Pod yield ( $\text{g plant}^{-1}$ ), SP = Shelling per cent; HSW = 100 seed weight (g)

effect of IDC in reducing pod yield among RILs. Earlier, Singh et al. (1990) also reported negative correlation of pod yield with chlorosis, while positive correlation with leaf chlorophyll and carotenoid contents when studying the effect of different sources of iron and sulphur on chlorosis in groundnut.

VCR at 30 and 60 d had significant negative correlation with shelling per cent during 2013, 2014 and pooled over years except during 2015 indicating significant effect of IDC on shelling per cent in RILs (Table 4). Hence, selecting IDC tolerant recombinant inbred line with higher shelling per cent can be effective. Significant negative correlation of 100 seed weight with VCR was observed at 90 d during 2013, 30 and 60 d during 2014, 60 d during 2015, while at 30, 60 and 90 d for pooled analysis over three years. This shows the adverse effect of IDC on seed size among RILs. Among the productivity traits, pod yield had significant positive correlation with shelling per cent and 100 seed weight during 2013, 2014 and across years, except during 2015 which indicated scope for selection of superior RILs having higher productivity and IDC tolerance.

#### **Identification of IDC tolerant RILs**

Based on the pooled analysis over three years, nine lines out of 318 RILs were superior to IDC resistant parent ICGV 86031 for VCR, SCMR and productivity traits as evident from lower VCR, higher SCMR and higher pod yield, shelling per cent and 100 seed weight (Table 5). Among the nine lines, C3-152 had consistently low VCR (1.08) and higher SCMR (> 36) at all the three stages with higher pod yield, shelling per cent but lower seed weight. On the other hand, RIL C3-138 had highest pod yield and seed weight with less VCR and higher SCMR but had lower shelling per cent. Similarly, RIL C3-52 had higher pod yield and shelling per cent with less VCR and higher SCMR, but lower seed weight. Hence, there is necessity to evaluate these identified superior IDC tolerant lines over locations under Fe-deficient conditions as well as in normal soils to test their suitability for wider adaptation in the farmer fields. These identified lines can be directly used as cultivars after thorough evaluation or they can serve as sources of IDC tolerance in the breeding programme to develop high yielding and IDC tolerant groundnut cultivars.

In conclusion, the present study has phenotyped the RIL population extensively for IDC tolerance traits under iron deficient conditions that can be used for identification of genomic regions associated with IDC

tolerance by genotyping of this RIL population. This can be of much help in marker assisted breeding program for IDC tolerance as field screening for this trait is a big challenge due to requirement of calcareous soils. Further, this study also has identified IDC tolerant lines with higher productivity that need to be tested extensively for utilization as cultivars or as sources in breeding programme.

#### **Authors' contribution**

Conceptualization of research (SKP, GKN); Designing of the experiments (SKP, GKN, BDB, MCW); Contribution of experimental materials (ICRISAT); Execution of field/lab experiments and data collection (IHB, OKS, PKV); Analysis of data and interpretation (SKP, GKN); Preparation of manuscript (GKN, SKP).

#### **Declaration**

The authors declare no conflict of interest.

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#### **References**

- Bhardwaj A. 2006. Integrated amelioration of lime induced chlorosis in peanut (*Arachis hypogaea* L.). 18<sup>th</sup> World Cong. Soil Sci., Philadelphia, Pennsylvania, USA.
- Boodi I. H. 2014. Genetic studies on iron absorption efficiency in groundnut (*Arachis hypogaea* L.). M.Sc.(Agri.) Thesis, University of Agricultural Sciences, Dharwad, India.
- Burton G. N. and Devane E. M. 1953. Estimating heritability in fall fescue (*Festuca arundinacea* L.) from replicated clonal material. *Agron. J.*, **45**: 478-481.
- Butenhoff K. J. 2015. QTL mapping and GWAS identify sources of iron deficiency chlorosis and canopy wilt tolerance in Fiskeby x mandarin (Ottawa) soybean population. M.S. Thesis, University of Minnesota, USA.
- Faostat. 2014. Food and Agricultural Organization of the United Nation, FAO Statistical Database. Accessed from <http://faostat3.fao.org/faostat.olap.ws/rest>.
- Fageria N. K., Guimarães C. M. and Portes T. A. 1994. Iron deficiency in upland rice. *Lav Arrozera*, **47**: 3-5.
- Fischer R. A. 1963. Statistical methods for research workers, 14<sup>th</sup> ed., Hafner, p. 104-117.
- Frankel C., Hadar Y. and Chen Yona. 2004. Peanut plants based bioassay for iron deficiency and its

- remediation. In: Mori S (ed), XII International Symposium on Iron Nutrition & Interactions in Plants, Tokyo, Japan, 11-15 April 2004. Soil Sci. & Plant Nutrition **50**(7): 1063-1070.
- Genc Y., Verbyla A. P., Torun A. A., Cakmak I., Willsmore K., Wallwork H. and McDonald G. K. 2009. Quantitative trait loci analysis of zinc efficiency and grain zinc concentration in wheat using whole genome average interval mapping. Plant Soil, **314**: 49-66.
- Guerinot M. L. and Yi Y. 1994. Iron: nutritious, noxious, and not readily available. Plant Physiol., **104**: 815-820.
- Hanson G. H., Robinson H. F. and Comstock R. E. 1956. Biometrical studies of yield in segregating population of Korean Lespodzoa. Agron. J., **48**: 267-282.
- Hüve K., Remus R., Lüttschwager D. and Merbach W. 2003. Transport of foliar applied iron (<sup>59</sup>Fe) in *Vicia faba*. J. Plant Nutrition, **26**(10-11): 2231-2242.
- Imtiaz M., Rashid A., Khan P., Memon M. Y. and Aslam M. 2010. The role of micronutrients in crop production and human health. Pakistan J. Botany, **42**(4): 2565-2578.
- Irmak S., C'yl A. N., Yücel H. and Kaya Z. 2012. The effects of iron application to soil and foliarly on agronomic properties and yield of peanut (*Arachis hypogaea*). J. Food Agric. & Env., **10**(3/4): 417-442.
- Johnson H. W., Robinson H. F. and Comstock H. F. 1955. Estimates of genetic and environmental variability in soybean. Agron. J., **47**: 314-318.
- Kulkarni V. N., Gowda M. V. C., Panchal Y. C. and Nadaf H. L. 1994. Evaluation of groundnut cultivars for iron absorption efficiency. Crop Res. (Hissar), **7**(1): 84-92.
- Li G. and Yan Xi S. 2007. Genetic differences in resistance to iron deficiency chlorosis in peanut. J. Plant Nutrition, **30**(1-3): 37-52.
- Lin S., Cianzio S. and Shoemaker R. 1997. Mapping genetic loci for iron deficiency chlorosis in soybean. Mol. Breeding, **3**: 219-229.
- Prasad P. V. V., Satyanarayana V., Potdar M. V. and Craufurd P. Q. 2000. On-farm diagnosis and management of iron chlorosis in groundnut. J. Plant Nutrition, **23**(10): 1471-1483.
- Prathet P., Somta P. and Srinives P. 2012. Mapping QTL conferring resistance to iron deficiency chlorosis in mungbean [*Vigna radiata* (L.) Wilczek]. Field Crops Res., **137**: 230-236.
- Potdar M. V. and Anders M. M. 1993. On-farm performance of groundnut genotypes under different land configurations and foliar iron sprays for the correction of iron chlorosis in Maharashtra state of India. In: Abstracts of 7<sup>th</sup> International Symposium on Iron Nutrition and Interactions in Plants, Zaragaza, Spain. pp. 22-26.
- Singh A. L. 2001. Yield losses in groundnut due to micronutrient deficiencies in calcareous soils of India. In: Plant nutrition: food security and sustainability of agro-ecosystems through basic and applied research. 14<sup>th</sup> International Plant Nutrition Colloquium, Hannover, Germany, pp. 838-839.
- Singh A. L. and Chaudhari V. 1993. Screening of groundnut germplasm collection and selection of genotypes tolerant to lime-induced iron chlorosis. J. Agric. Sci. (Cambridge), **121**: 205-211.
- Samdur M. Y., Mathur R. K., Manivel P., Singh A. L., Bandyopadhyay A. and Chikani B. M. 1999. Screening of some advanced breeding lines of groundnut (*Arachis hypogaea* L.) for tolerance of lime-induced iron-deficiency chlorosis. Indian J. agric. Sci., **69**(10): 722-725.
- Samdur M. Y., Singh A. L., Mathur R. K., Manivel P., Chikani B. M., Gor H. K. and Khan M. K. 2000. Field evaluation of chlorophyll meter for screening groundnut (*Arachis hypogaea* L.) genotypes tolerant to iron-deficiency chlorosis. Curr. Sci., **79**(2): 211-214.
- Singh A. L., Chaudhari V., Koradia V. G. and Zala P. V. 1995. Effect of excess irrigation and iron and sulphur fertilizers on the chlorosis, dry matter production, yield and nutrients uptake by groundnut in calcareous soil. Agrochimica, **39**(4): 184-198.
- Singh A. L., Joslli Y. C., Choudhari V. and Zala P. V. 1990. Effect of different sources of iron and sulphur on leaf chlorosis, nutrient uptake and yield of groundnut. Fertilizer Res., **24**: 81-92.
- Singh O. P. K. 2015. Minicore evaluation, inheritance and QTL mapping for iron absorption efficiency in groundnut. M.Sc. (Agri.) Thesis, Univ. Agric. Sci., Dharwad, Karnataka, India.
- Zheng S. J. 2010. Iron homeostasis and iron acquisition in plants: maintenance, functions and consequences. Ann. Bot., **105**: 799-800.
- Zuo Y., Ren L., Zhang F. and Jiang R. F. 2007. Bicarbonate concentration as affected by soil water content controls iron nutrition of peanut plants in a calcareous soil. In: Briat J. F. and Gaymard J. B. (eds.), XIII International Symposium on Iron Nutrition & Interactions in Plants, Montpellier, France, 3-7 July 2006. Plant Physiol. & Biochem., **45**(5): 357-364.