

MORPHO-PHYSIOLOGICAL EVALUATION OF GROUNDNUT (*ARACHIS HYPOGAEA* L.) GENOTYPES FOR IRON DEFICIENCY TOLERANCE

SHAMIM AKHTAR¹, ARMIGHAN SHAHZAD², MUHAMMAD ARSHAD¹ AND FAYYAZ-UL-HASSAN^{1*}

¹PMAS-Arid Agriculture University, Rawalpindi, Pakistan,

²Plant Biotechnology Program (NIGAB), National Agriculture Research Centre, Park Road 45500 Islamabad, Pakistan

*Correspondence author e-mail: arshad2uaar@yahoo.com

Abstract

Iron deficiency is one of the major yield limiting factors in groundnut. The soils of Pothwar (90% of groundnut production area in Pakistan) are calcareous in nature, thus groundnut is exposed to Fe deficiency. Seeds of 20 varieties/advance breeding lines of groundnut were collected to evaluate Fe deficiency responses. Seeds were germinated in pots with 1:1 soil to sand ratio with added recommended NPK fertilizer. Fe-EDTA (0.1mmol/L) was supplemented as foliar spray to control plants, however, no additional Fe was applied to Fe deficient plants. Physiological parameters such as chlorophyll content, active and total Fe concentrations were recorded for each genotype under Fe deficient and Fe sufficient conditions. Morphological parameters including pods per plant, pod weight per plant, seeds per plant and seed weight per plant were recorded at harvesting. Genotypes were ranked by multivariate cluster analysis. Data showed that BARI-2000 and Chakori were among the Fe stress tolerant genotypes while Golden and Lisn were among the Fe deficiency intolerant genotypes. Relative values for SPAD values ranged from 60.50% in 2KCG020 to 87.8% in BARI-2000. Total Fe concentration was 48.8% in Lisn and 66.5% in BARI-2000. Relative value of biomass produced by Chakori and Golden was 85.5% and 66.3%, respectively. The genotypes ranked best on the basis of morpho-physiological parameters will be helpful for making recommendations to groundnut farmers of the Pothwar region.

Introduction

Attock and Chakwal districts in Pothwar region are major groundnut producing areas in Pakistan (Anon., 2010). The soils in these areas are alkaline in nature. These soils are conducive to the incidence of iron (Fe) chlorosis in sensitive crops including groundnut because of low solubility of Fe (Rashid *et al.*, 1997). Groundnut is an important source of edible oil and proteins (Tang *et al.*, 2007). Groundnut and groundnut oil also contains cardiovascular protective properties (Stephens *et al.*, 2010). Fatty acid composition of peanut oil significantly affects the quality and flavor of peanut and peanut products (Hassan & Ahmed, 2012). Frequent intake of groundnuts and its products helps in reducing colorectal cancer risk in women, demonstrating its anti-proliferating effect (Yeh *et al.*, 2006). Fe is essential for all living organisms and crucial for a variety of functions (Kobayashi *et al.*, 2012). It is an essential component of various proteins and plant pigments (Greenshields *et al.*, 2007).

Though Fe is abundant in most parts of the soils, yet almost insoluble in ferric form, hence unavailable to plants (Graziano & Lamattina, 2005). Fe deficiency poses a major problem in crop production among sensitive crops (Ogo *et al.*, 2008). Fe chlorosis is the third field scale disorder after Zinc and Boron in micronutrients. It has been exhibited in peanut, chickpea, cotton, citrus, ornamentals and many tree species (Imtiaz *et al.*, 2010). Plants can be classified into two groups based on Fe acquiring strategy (Ramirez *et al.*, 2008). Dicots and monocots except grasses belong to strategy I plants (Gao & Shi, 2007). In case of strategy I plants, plant roots use the tools of acidification and enzymatic reduction of Fe at the outer surface of roots (Stephan, 2002). Strategy II plants (Graminaceous species) acquire Fe through mugineic acid family phytosiderophores (Ramirez *et al.*, 2008).

Peanut plants are subjected to Fe deficiency when growing in calcareous soils, which are rich in bicarbonate ions. The severity of chlorosis increases after excessive rain fall or irrigation, but the symptoms appear after drying of

water logged soils (Zuo *et al.*, 2007). Remediation strategies for Fe chlorosis including the amending Fe to soil is an expensive practice, or using the tolerant cultivars, which is difficult to develop when not available. Fe deficiency in groundnuts resulted in an increased amount of caffeic acid, a higher rate of roots reducing capacity, and increased rates of both Fe (III) chelate splitting and Fe uptake (Romheld and Marschner, 1983). However, soil Fe can be increased by soil amendment (Khan *et al.*, 2012). Significant progress has been made in recent years in Fe-acquisition mechanisms in strategy I and strategy II plants. When grown in calcareous soil. Being strategy I plant, groundnut is susceptible to Fe deficiency. Intercropping with maize can significantly improve the Fe nutrition in groundnut on calcareous soils (Zuo & Zhang, 2008). The beneficial effects of Fe nutrition can be attributed to the rhizosphere interaction between groundnut and maize (Inal *et al.*, 2007). In Fe deficient conditions reductase activity and proton release form the roots of strategy I plants, but high pH and high bicarbonates in calcareous soils will diminish the effects of this response (Ding *et al.*, 2010). Strategy II plants, especially maize increases its access to Fe by secreting phytosiderophores. When grown together, Ferric chelate reductase activity and Fe (II) uptake across plasma membrane is enhanced. Hence uptake of Fe by groundnut is enhanced in intercropping as compared to monocropping (Ding *et al.*, 2010). Keeping in view the importance of crop and role of Fe in yield improvement, the present study was planned to screen out all available local genotypes for Fe deficiency responses under Fe sufficient and Fe deficient conditions. The genotypes with better ability to grow in calcareous soils can be selected, and the yield could be improved by other strategies like intercropping and genetic improvement of Fe sensitive crop.

Materials and Methods

Seeds of twenty groundnut genotypes including ICG2261, No. 334, 96CG005, 2KCG020, BARI-2000, Chakori, ICGS17, ICGS6, ICG2254, 02CG002, ICG641, ICG690, BARD-699, Banki, Golden, 01CG009, Lisn,

ICG485, 2KCG017 and 04CG004 were taken from BARI (Barani Agriculture Research Institute, Chakwal, and NARC (National Agriculture Research Centre, Islamabad) Pakistan.

Experimental setup: Five seeds of each of twenty genotypes were germinated directly in pots in tunnel. Earthen pots with a capacity up to 15kg were filled with soil and sand in 1:1.NPK (20:80:20) were applied after seed sowing. The experiment was run in the replica of three. In control plants seedlings were treated with the spray of 0.1 mmol/L FeEDTA, whereas in case of Fe deficient plants no treatment was applied. The treatments were applied at different plant growth stages including seedling, flowering and pegging stage.

Chlorophyll content was recorded at different time intervals with chlorophyll meter SPAD502 (Minolta, Japan) and was expressed as SPAD values. Active Fe concentration was recorded at 90 days after planting using method of Gao & Shi (2007). Total Fe concentration was recorded before harvesting by dry ashing method (Ryan *et al.*, 2001). The amounts of active and total Fe were shown in $\mu\text{g g}^{-1}$ fresh and dry weight of plant material respectively. Number of pods was recorded and number of pods per plant were calculated. After sun drying for one day pod weight was recorded along with biomass. Seed number and seed weight per plant was recorded. Data was analyzed by multivariate analysis and means were separated by Minitab13.

Ranking of genotype for Fe tolerance: In conventional methods, genotypes are compared based on few morphological parameters. However, for interval studies range of parameters are required. The process of comparing many genotypes with large number of parameters simultaneously is often inaccurate and laborious. Cluster analysis is useful to analyze genotypes on the basis of multiple parameters simultaneously. All the data were converted to relative values, i.e. Fe tolerance indexes before cluster analysis. Fe tolerance index was defined as the observations under Fe deficiency divided by the means of the controls (Fe sufficient). Cluster analysis was performed and Cluster group rankings were obtained based on Ward's minimum variance cluster analysis on the means of the Fe tolerance indexes for different morphological and physiological parameters including biomass, pod number, pod weight, seed number, seed weight, active Fe, total Fe and chlorophyll content (SPAD values). The distance between two clusters was calculated as the ANOVA sum of squares between the two clusters in all the parameters analyzed. The cluster groups were identified in dendrograms. The number of cluster groups was determined by calculating the pseudo t^2 which reached a local maximum. The cluster group rankings were obtained from the averages of means over multiple parameters in each cluster group, i.e., cluster mean, in order from highest to lowest averages. A sum was obtained by adding the numbers of cluster group ranking of each parameter in each genotype. The genotypes were finally ranked based on the sums in order that those with the largest sums were ranked as the most tolerant and those with the smallest sums were ranked as the least tolerant in terms of relative Fe tolerance (Zeng *et al.*, 2002).

Results and Discussion

There were marked differences in twenty groundnut genotypes in response to Fe deficiency when grown on calcareous soils. Some genotypes were more sensitive to Fe deficiency while others were stress tolerant. Few genotypes were of moderate type. The maximum biomass (89.06 & 79.01g plant⁻¹) was recorded in ICG690 under Fe sufficient and Fe deficient conditions, respectively. Similarly, Chakori, 96CG005, Banki and BARD-699 produced >70 g plant⁻¹ biomass under Fe sufficient conditions, while produced 51.55, 49.06, 55.27 and 53.20 g plant⁻¹ biomass, respectively under Fe deficient conditions. Chakori and Banki with higher biomass were ranked Fe deficiency stress tolerant genotypes. Similar, to our findings, Puangbut *et al.*, (2009) proved that higher SPAD values, resulted in higher biomass, hence improved yield. Golden, ICG485 and ICG2254 showed lower biomass in Fe deficient conditions nearly 40 g plant⁻¹. However, some genotypes (No. 334, ICGS17 and 01CG009) showed deviations from the trend and produced higher biomass (59.88, 53.93 and 67.09 g⁻¹plant) under Fe deficient conditions as compared to Fe sufficient conditions (Fig. 1A). When ranked with multivariate analysis, No. 334 and ICGS17 showed more than 70% average relative value of all parameters. However, except biomass these genotypes presented higher chlorophyll, active Fe and yield in Fe sufficient conditions as compared to Fe deficient conditions. We suppose this kind of behavior is related to their Fe deficiency tolerance, so these genotypes are Fe deficiency tolerant. Another genotype 01CG009 showed similar behavior, however on the basis of all parameters the genotypes was ranked as moderate.

Number of pod per plant ranged between 2-12 pods in tested genotypes. The maximum pods (12.37) plant⁻¹ was produced by BARI-2000 under Fe sufficient conditions as compared (6.73 pods plant⁻¹) to Fe deficient conditions (Fig. 1B). Similarly, ICGS17 produced 11.67 and 5.9 pods per plant under Fe sufficient and Fe deficient conditions, respectively. Less number of pods (<4 pods⁻¹ plant) were recorded from 01CGG009, 02CG002 and Golden under Fe sufficient and Fe deficient conditions. However, Banki, 96CG005, 2KCG017 and 04CG004 produced (<5 pods) under Fe sufficient and Fe deficient conditions, showing less response to Fe. Contrarily, ICG2254, 02CG002, Golden, 01CG009 and Lisen produced less than 3 pods plant⁻¹ under Fe deficient conditions (Figure. 1B). The genotypes including BARI-2000, Chakori, No.334, Banki, and ICG 690 are among the tolerant genotypes as the average relative values for all parameters was >70%. The genotypes including BARI-2000 and Chakori with high active Fe concentration and SPAD values produced higher yields. The results suggested that genotypes with higher active Fe concentration and SPAD values may produce more photosynthates, consequently higher yield.

Pod weight was considerably different among all genotypes when tested in pot culture. The pod weight varied between 2-15 g plant⁻¹. The pod weight was in direct relation to pod number. The maximum pod weight was recorded in ICGS17 in Fe sufficient condition, where 15 g plant⁻¹ pod weight was recorded. In case of BARI-2000 11.85g plant⁻¹ pod weight was recorded in Fe sufficient conditions. Among other genotypes ICG641 and Golden were among the genotypes, where pod weight was below 3 g plant⁻¹ in Fe deficient conditions. ICG2261 and 01CG009 showed lower pod weight in Fe deficient conditions i.e.,

3.96 and 3.70 g plant⁻¹. Pod weight has direct relation with Fe concentration and SPAD values. The graph shows the trending that with increasing one parameter, other also increases (Table 2). BARI-2000 and ICGS17 produce more pod weight as compared to other genotypes. In both genotypes higher active Fe concentration and SPAD values produce more pod number, hence more pod weight. This response of these genotypes showed their Fe deficiency tolerance behavior. The genotypes including Golden and Lisn with lower active Fe concentration and SPAD values showed lower pod number and pod weight (Fig. 1C). Yield is positively correlated with Active Fe Total Fe (Table. 2). The results are in agreement with Gao & Shi (2007), they concluded that the genotypes with less chlorotic symptoms produced less yield. When seed number plant⁻¹ was calculated, ICG2254 and BARI-2000 were among the highest seed number genotypes, which varied between 18.9 and 18.23 per plant, respectively in Fe sufficient conditions. Similarly, the genotypes produced 6.61 and 8.42 seed number plant⁻¹ respectively under Fe deficient conditions. Golden ranked lowest among all the genotypes, where only 4 seeds plant⁻¹ were obtained in Fe deficient conditions. Similarly, Chakori, ICG2261 and Golden produced lower number of seeds in Fe sufficient conditions. The values for these genotypes were 6.35, 6.52 and 5.15, respectively. Chakori resulted the lowest seed number⁻¹ plant in Fe deficient conditions i.e., 2.71. In general chlorosis symptoms are responsible for the yield, the genotypes with more chlorotic symptoms produced lesser yield (Fig. 1D). Chlorosis is responsible for the disturbance of all physiological parameters of the plant. Fe being an important part of electron transport chain, its deficiency disturbed the physiological function of plant.

As all physiological parameters i.e., active Fe concentration and SPAD value are related to yield. Lower SPAD values and active Fe concentration resulted in lower seed number, showing Fe deficiency sensitive behavior of the genotype. BARI-2000 was ranked as Fe deficiency tolerant as the genotype produced more seed number in response to higher SPAD values and active Fe concentration. Similar results were reported by Gao & Shi (2007) and concluded that genotypes with more active Fe and SPAD values produced higher yield. Maximum seed weight was recorded in BARI-2000, where 10.7 g plant⁻¹ was obtained. Similarly in Fe deficient condition 4.73 g plant⁻¹ seed was obtained (Fig. 1E). In other genotypes including ICG690 and 01CG009 higher seed weight was recorded in Fe sufficient conditions, where the seed weight was 7.69 and 6.33 g plant⁻¹ respectively. The seed weight among other genotypes varied from 2-6 g plant⁻¹ in Fe sufficient conditions. Lowest seed weight was recorded in ICG2254, where only 2.44 g seed was recorded in Fe sufficient conditions. However, in the same genotype, in Fe deficient conditions only 1.2g seed was recorded.

Maximum seed weight was recorded by BARI-2000 in Fe deficient conditions i.e., 4.75g. Among other genotypes including ICG2254 and ICG641 only 1.2g and 0.94g seed was obtained in Fe deficient conditions. The genotypes ICG2254 and ICG641 with lower seed number represented their Fe deficiency sensitive behavior and were placed in Fe deficiency sensitive group. BARI-2000 was among Fe deficiency tolerant genotypes. Early chlorosis caused decreased photosynthetic rate, less photosynthates production, nitrogen fixation and consequently yield losses (Singh & Sahu, 1993).

Maximum SPAD (51.1) value was given by BARI-2000 under Fe sufficient conditions, while 37.93 SPAD value in Fe deficient condition. Similarly, ICG485 gave SPAD value of 50.28 and 40.79 under Fe sufficient and Fe deficient conditions, respectively. The lowest SPAD value (39.75) was exhibited by 04CG004 under Fe sufficient conditions. However, 01CG009 and 96CG005 resulted SPAD values of 40.51 and 40.58 respectively, under Fe sufficient conditions (Fig. 1F). The genotypes with more SPAD values produced more pods and seeds showing their Fe deficiency tolerance behavior and were ranked as Fe deficiency tolerant genotypes. The genotypes with lower SPAD values resulted in lower yield as that of Golden and Lisn. These genotypes were ranked as Fe deficiency sensitive genotypes. Groundnut is one of the crops affected by Fe deficiency chlorosis specially when grown on calcareous soil. Differences in susceptibility to Fe chlorosis among groundnut cultivars was studied by Hartzook (1982). Though highest SPAD value was shown by ICG485, the yield was lower. Similar results were experienced by Costa *et al.* (2001). They proved that SPAD meter values and yield relationship varies among various maize hybrids tested. Chlorophyll content and active Fe are variable at different growth stages of plant. Pod number showed correlation with chlorophyll content under control and active Fe content under Fe deficiency. Similarly, pod weight had correlation with total Fe under control and active Fe under Fe (Table 2).

The maximum active Fe concentration (57.13, 54.43 and 53.24 μg g⁻¹ fresh weight of plant) was given by Lisn, BARI-2000 and ICGS6 under Fe sufficient conditions, at the same time these genotypes gave active Fe concentration values of 31.63, 31.20 and 37.07 μg g⁻¹ respectively, under Fe deficient conditions. However, active Fe concentration of No.334, 96CG005, Chakori, Banki and 2KCG017 resulted values of 26.14, 23.48, 22.65, 23.78 and 24.93 μg g⁻¹ active Fe concentration under Fe sufficient conditions, respectively. Lowest (10.17 and 9.37 μg g⁻¹) active Fe in case of Fe deficient conditions was obtained from ICG2261 and 01CG009 (Fig. 1G). Active Fe concentration is an important parameter in Fe deficiency. As the parameter is related with chlorophyll content, both parameters affected each other. Active Fe concentration is directly correlated with the chlorophyll content. BARI-2000 and ICGS6 with more active Fe concentration produced more yield, and were ranked as Fe deficiency tolerant genotypes. Similar results were proved by previous work (Singh *et al.*, 1990; Gao & Shi, 2007).

Total Fe was recorded from dry leaf samples. The amount of total Fe was variable between 100-350 μg g⁻¹ dry weight of plant in Fe sufficient treatment. Concentration of Fe was measured in different medicinal plants by Inductively Coupled Plasma (ICP) and the concentration varied from 90 to 590 ppm (Khattak & Khattak, 2011). However, it varied between 50-200 μg g⁻¹ dry weights of plant in Fe deficient conditions (Fig. 1H). Maximum total Fe concentration (343.33 μg g⁻¹) was recorded in BARI-2000 under Fe sufficient conditions. In same genotype, under Fe deficient conditions total Fe concentration was 133.5 μg g⁻¹ dry weight of plant. Among other genotypes ICGS17, Lisn, ICG641, 01CG009 and BARD-699 exhibited 93.1, 98, 91.54, 91.7 and 99.36 μg g⁻¹ total Fe, respectively, under Fe deficient conditions. The genotypes with more chlorotic symptoms showed less Fe concentration and reduced yield, as proved by Gao & Shi (2007).

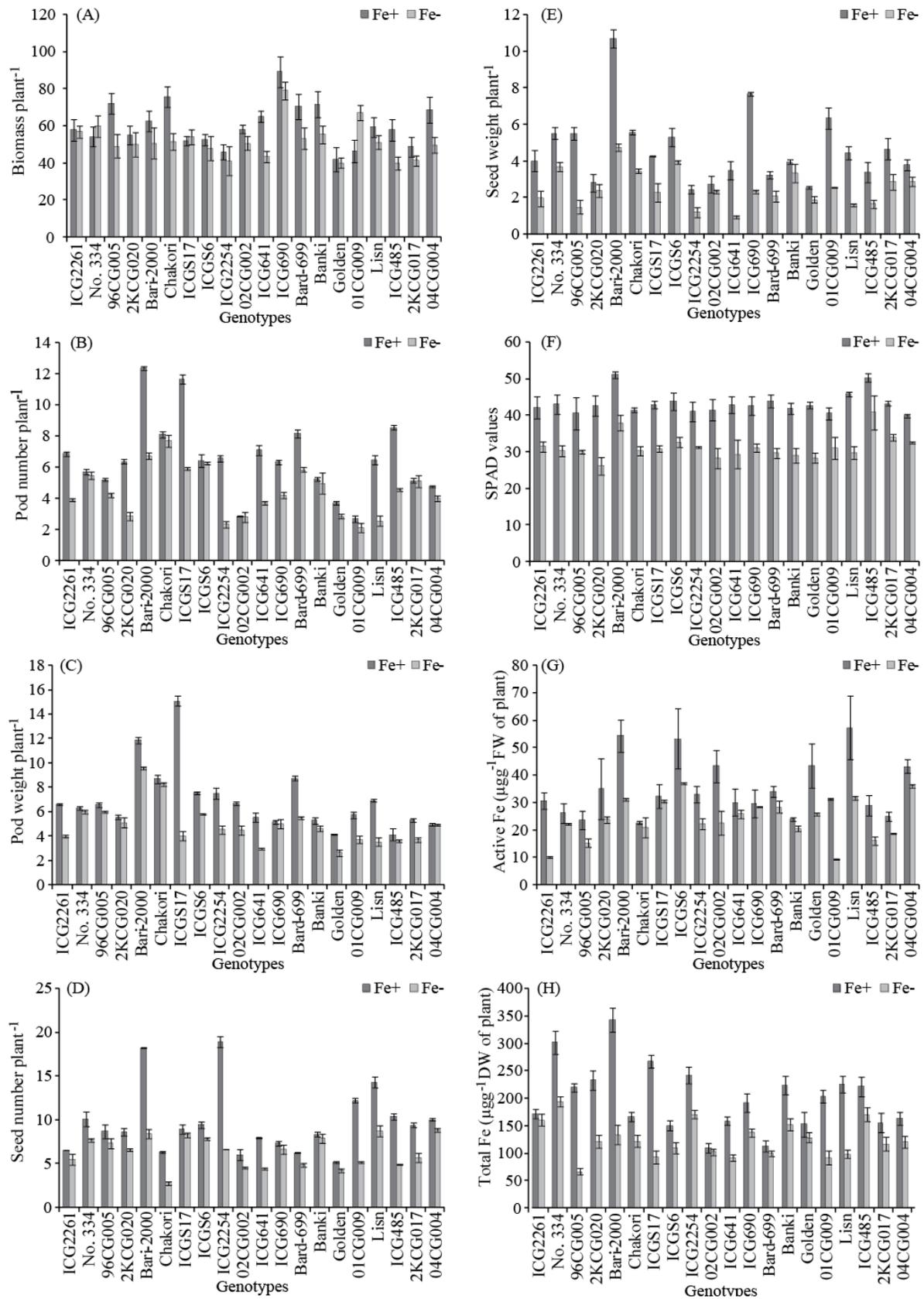


Fig. 1. Morpho-physiological data for twenty groundnut genotypes studied for Fe deficiency responses, A) Biomass, B) pod number, C) pod weight, D) seed number, E) seed weight, F) SPAD values, G) active Fe concentration and H) total Fe concentration.

Based on multivariate analysis using Fe tolerance indexes in morphological and physiological parameters using Ward's minimum variance cluster analysis, the genotypes were divided into six cluster groups (Table 1). Based on this analysis varieties, BARI-2000, Chakori and Banki ranked first falling in clusters ranked first with an averaged Fe tolerance indexes of 86 and 77.5%, whereas Golden and Lisen were among the stress sensitive genotypes ranked as 6 with averaged Fe tolerance indexes of 59%. Genotypes BARD-699 and 96CG005 produced average Fe tolerance index of 64.9%, thus declared as moderately tolerant to Fe deficiency (Table 1). Loop and Finck (1984) advocated the usefulness of total Fe, generally total Fe concentration plant tissue is not related with the occurrence of chlorosis (Rashid *et al.*, 1997).

Fe deficiency is one of the major problems in groundnut crop grown on calcareous soils of Pakistan (Imtiaz *et al.*, 2010). Typical Fe Chlorosis symptoms are characterized by inter-veinal chlorosis with veins remained green and at later stages whole leaf becomes yellow (Prasad *et al.*, 2000). The expression of Fe chlorosis may vary with soil and environmental conditions in years (Zheng *et al.*, 2003; Gao & Shi, 2007). Groundnut genotypes differ in response to Fe chlorosis (Gao & Shi, 2007; Hartzook, 1982). A widely accepted strategy to solve Fe deficiency problem is to select genotypes with high resistance to Fe deficiency response (Gao & Shi, 2007). The experiment resulted in considerable differences among different genotypes in response to Fe deficiency. The screened genotypes can be categorized as Fe sufficient and Fe deficient based on their ability to uptake Fe. From the difference of averages of all parameters we can categorize Golden, Lisen, as Fe deficiency sensitive genotypes. BARI-2000 and Chakori were among the Fe deficiency tolerant genotype. SPAD is

used to measure chlorophyll content in field and there is correlation between SPAD and Chlorophyll (Samdur *et al.*, 2000). Chlorophyll content and active Fe are variable at different growth stages of plant. Chlorophyll content and active Fe concentration are correlated to pod yield. Total Fe concentration is generally not related with the occurrence of chlorosis (Rashid *et al.*, 1997).

Pearson's correlation coefficients (Table 2) showed significant relationship recorded among different parameters studied at α 5% confidence Interval. Positive correlation was found between SPAD values and pod number under control. Pod number and pod weight were correlated under control and stress. Seed number under stress was correlated to total and active Fe concentrations under control and stress. Seed number and weight and pod weight under control were also correlated. Similarly, total Fe under control was correlated with pod number and weight, seed number and weight under control and total Fe under stress (Table 2). SPAD values under control were correlated to SPAD values under stress showing high level of genotypic control over these traits. Same was true for total Fe concentrations under control and stress.

Conclusion

Based on correlations among morpho-physiological parameters studied, it can be suggested that only morphological or physiological parameters can be used to assess Fe deficiency tolerance of groundnut genotypes. BARI-2000, Chakori and Banki are recommended as these were screened out to be Fe deficiency tolerant genotypes.

Table 1. Ranking of genotypes based on Fe tolerance indexes of morphological and physiological parameters in a cluster analysis (Ward's minimum variance analysis). All the data was presented in relative values (%age) calculated per plant.

Genotype	Biomass (g)	Pod weight(g)	Pod number	Seed weight(g)	Seed number	SPAD values	Active Fe ($\mu\text{g g}^{-1}\text{FW}$)	Total Fe ($\mu\text{g g}^{-1}\text{DW}$)	Average	Cumulative sum	Genotype ranking
BARI-2000	83.92	138.78	103.3	102.5	87.2	87.8	89.1	66.5	94.9		1
Chakori	85.53	119.81	117.9	75.0	28.1	70.1	59.5	60.8	77.1	86.0	1
No. 334	99.34	86.62	83.9	79.7	79.5	70.1	63.7	96.6	82.4		2
ICG690	131.07	72.77	64.5	50.3	68.8	72.1	81.1	68.6	76.2	77.5	2
Banki	91.69	67.21	76.2	72.4	81.5	67.4	58.6	75.8	73.9		2
ICGS17	89.47	58.59	90.5	49.5	85.8	71.5	86.8	46.4	72.3		3
ICGS6	79.77	84.08	96.2	85.4	81.0	75.6	105.9	54.3	82.8	77	3
04CG004	82.40	71.21	61.4	62.5	91.5	75.5	102.6	60	75.9		3
96CG005	81.39	86.47	65.0	32.0	75.9	69.4	43.4	32.9	60.8		4
BARD-699	88.26	79.71	89.8	44.6	50.2	68.7	81.2	49.5	69	64.9	4
ICG2261	94.41	57.43	59.9	42.2	57.0	72.8	29	79.7	61.6		5
01CG009	111.3	53.74	32.7	55.3	53.7	71.7	26.8	45.7	56.4		5
ICG485	66.38	52.36	69.9	36.1	50.5	94.5	46	84.8	62.6	61.1	5
2KCG017	68.41	53.61	78.3	61.9	59.3	78.6	53	58.1	63.9		5
2KCG020	83.15	74.01	44	51.4	67.8	60.50	67.9	60.3	63.6		6
ICG2254	67.84	65.32	35.8	26.1	68.5	72.4	63.7	85.1	60.6		6
02CG002	83.97	64.88	43.0	50.2	46.6	65.4	64.2	50.9	58.6		6
ICG641	71.98	42.61	56.8	20.4	45.8	68.0	73.7	45.6	53.1	59	6
Golden	66.26	37.96	43.5	41.0	43.3	65.7	73.8	64.1	54.4		6
Lisen	84.92	50.82	39.1	34.7	90.5	68.9	90.4	48.8	63.5		6

Table 2. Pearson correlation coefficients along with probability of significance between different parameters recorded under control (Fe sufficient) and stress (Fe deficient) conditions.

	PDNC	PDWC	BMSC	AIRC	TIRC	SDWTC	SDNC	SPAD C	PDNS	PDWS	BMSS	AIRS	TIRS	SDWTS	SDNS
PDWC	0.747														
	0.000														
BMSC	0.197	-0.026													
	0.132	0.844													
AIRC	0.088	0.191	-0.233												
	0.505	0.144	0.073												
TIRC	0.519	0.4	-0.102	0.04											
	0.000	0.002	0.44	0.763											
SDWTC	0.387	0.33	0.335	0.138	0.507										
	0.002	0.01	0.009	0.293	0.000										
SDNC	0.322	0.26	-0.272	0.352	0.658	0.359									
	0.012	0.045	0.036	0.006	0.000	0.005									
SPAD C	0.343	0.055	0.045	0.116	-0.058	-0.106	-0.018								
	0.007	0.674	0.733	0.378	0.659	0.42	0.894								
PDNS	0.583	0.49	0.303	-0.088	0.132	0.431	-0.156	0.187							
	0.000	0.000	0.018	0.504	0.314	0.001	0.234	0.153							
PDWS	0.48	0.453	0.396	0.085	0.392	0.662	0.251	-0.04	0.65						
	0.000	0.000	0.002	0.521	0.002	0.000	0.053	0.76	0.000						
BMSS	-0.056	0.059	0.48	-0.154	0.126	0.488	-0.129	-0.138	-0.015	0.168					
	0.672	0.653	0.000	0.242	0.336	0.000	0.326	0.292	0.909	0.198					
AIRS	0.298	0.314	0.154	0.62	0.016	0.098	0.122	0.101	0.282	0.21	-0.078				
	0.021	0.015	0.241	0.000	0.902	0.455	0.352	0.442	0.029	0.107	0.553				
TIRS	0.084	-0.225	-0.112	-0.182	0.361	-0.025	0.206	0.091	0.071	0.074	0.021	-0.156			
	0.522	0.084	0.396	0.163	0.005	0.851	0.114	0.49	0.591	0.576	0.872	0.234			
SDWTS	0.22	0.279	0.079	0.208	0.311	0.595	0.127	-0.112	0.621	0.682	0.212	0.263	0.207		
	0.091	0.031	0.55	0.111	0.016	0.000	0.335	0.396	0.000	0.000	0.103	0.042	0.112		
SDNS	0.235	0.273	0.03	0.373	0.563	0.303	0.486	-0.135	0.022	0.161	0.159	0.469	0.037	0.286	
	0.071	0.035	0.823	0.003	0.000	0.018	0.000	0.304	0.869	0.22	0.225	0.000	0.781	0.027	
SPADS	0.217	-0.047	0.043	-0.107	-0.174	-0.089	0.005	0.579	0.231	-0.09	-0.14	-0.041	0.151	-0.097	-0.121
	0.096	0.721	0.742	0.417	0.183	0.497	0.967	0.000	0.076	0.495	0.288	0.756	0.249	0.46	0.357

Abbreviation: PDNC Pod Number under control, PDWC Pod weight control, BMSC Biomass control, AIRC Active Fe under control, TIRC total Fe under control, SDWTC seed weight under control, SDNC seed number under control, SPADC SPAD under control and all these parameters with an S means 'under stress'.

N.B. Below each correlation coefficient there is given p value. p value of less than 0.05 is given in bold and denotes for significant correlation coefficient between two parameters

References

- Anonymous. 2010. Economic Survey of Pakistan, 2010-2011. Ministry of Finance, Government of Pakistan, Islamabad.
- Costa, C., L.M. Dwyer, P. Dutilleul, D.W. Stewart, B.L. Ma and D.L. Smith. 2001. Inter-relationship of applied nitrogen, SPAD, and yield of leafy and non-leafy maize genotypes. *J. Plant Nutr.*, 24(8): 1173-1194.
- Ding, H., L. Duan, J. Li, H. Yan, M. Zhao, F. Zhang and W.X. Li. 2010. Cloning and functional analysis of the peanut iron transporter AhIRT1 during iron deficiency stress and intercropping with maize. *J. Plant Physiol.*, 167: 996-1002.
- Gao, L. and Y. Shi. 2007. Genetic differences in resistance to iron deficiency chlorosis in peanut. *J. Plant Nutr.*, 30: 37-52.
- Graziano, M. and L. Lamattina. 2005. Nitric Oxide and iron in Plants: an emerging and converging story. *Trends Plant Sci.*, 10(1): 4-8.
- Greenshields, D.L., G. Liu and Y. Wei. 2007. Roles of Iron in Plant Defence and Fungal Virulence. *Plant Signal Behav.*, 2(4): 300-302.
- Hartzoek, A. 1982. The problem of iron deficiency in peanut (*Arachis hypogaea* L.) on basic and calcareous soils in Israel. *J. Plant Nutr.*, 5: 923-926.
- Hassan, F.U. and M. Ahmed. 2012. Oil and fatty acid composition of peanut cultivars grown in Pakistan. *Pak. J. Bot.*, 44(2): 627-630.
- Imtiaz, M., A. Rashid, P. Khan, M.Y. Memon and M. Aslam. 2010. The role of micronutrients in crop production and human health. *Pak. J. Bot.*, 42(4): 2565-2578.
- Inal, A., A. Gunes, F. Zhang and I. Cakmak. 2007. Peanut/maize intercropping induced changes in rhizosphere and nutrient concentrations in shoots. *Plant Physiol. Biochem.*, 45: 350-356.
- Khan, Z.I., K. Ahmad, S. Kashaf, M. Ashraf, F. Al-Qurainy, M. Danish, A. Fardous, S. Gondal, A. Ejaz and E.E. Valeem. 2011. Evaluation of iron content in a potential fodder crop oat (*Avena sativa* L.) grown on soil treated with sugarcane filter cake. *Pak. J. Bot.*, 43(3): 1547-1550.
- Khattak, M.I. and M.I. Khattak. 2011. Study of heavy trace metals in some medicinal herbal plants of Pakistan. *Pak. J. Bot.*, 43(4): 2003-2009.

- Kobayashi, T., R.N. Itai, M.S. Aung, T. Senoura, H. Nakanishi and N.K. Nishizawa. 2012. The rice transcription factor IDEF1 directly binds to iron and other divalent metals for sensing cellular iron status. *Plant J.*, 69: 81-91.
- Loop, E.A. and A. Finck. 1984. Total iron as a useful index of the Fe-status of crops. *Commun. Soil Sci. Plant Anal.*, 7: 69-79.
- Ogo, Y., T. Kobayashi, R.N. Itai, H. Nakanishi, Y. Takei, M. Takahashi, S. Toki, S. Mori and N.K. Nishizawa. 2008. A novel NAC transcription factor, IDEF2, that recognizes the iron deficiency-responsive element 2 regulates the genes involved in iron homeostasis in plants. *Biol. Chem.*, 283(19): 13407-13417.
- Prasad, P.V.V., V. Satyanarayana, M.V. Potdar and P.Q. Craufurd. 2000. On-farm diagnosis and management of Iron chlorosis in Groundnut. *J. Plant Nutr.*, 23(10): 1471-1483.
- Puangbut, D., S. Jogloy, N. Vorasoot, C. Akkasaeng, T. Kesmalac and A. Patanothai. 2009. Variability in yield responses of peanut (*Arachis hypogaea* L.) genotypes under early season drought. *Asian J. Plant Sci.*, 8(4): 254-264.
- Ramirez, L., M. Graziano and L. Lamattina. 2008. Decoding plant responses to iron deficiency. Is nitric oxide a central player. *Plant Signal and Behav.*, 3(10): 795-797.
- Rashid, A., E. Rafique, J. Din, S.N. Malik and M.Y. Arain. 1997. Micronutrient Deficiencies in Rainfed Calcareous Soils of Pakistan. I. Iron Chlorosis in the Peanut Plant. *Commun. Soil Sci. Plant Anal.*, 28: 135-148.
- Romheld, V. and H. Marschner. 1983. Mechanism of iron uptake by peanut plants: I. Fe (III) reduction, chelate splitting, and release of phenolics. *Plant Physiol.*, 71: 949-954.
- Ryan, J., G. Estefan and A. Rashid. 2001. *Soil and Plant Analysis Laboratory Manual*. Second Edition. Jointly published by ICARDA and NARC. Aleppo, Syria.
- Samdur, M.Y., A.L. Singh, R.K. Mathur P. Manivel, B.M. Chikani, H.K. Gor and M.A. Khan. 2000. Field evaluation of chlorophyll meter for screening groundnut (*Arachis hypogaea* L.) genotypes tolerant to iron-deficiency chlorosis. *Curr. Sci. Bangalore*, 79: 211-214.
- Singh, A.L. and M.P. Sahu. 1993. Effects of phosphate carriers, iron, and indoleacetic acid on iron nutrition and productivity of peanut on a calcareous soil. *J. Plant Nutr.*, 16: 1847-1855.
- Singh, A.L., Y.C. Joshi, V. Chaudhari and P.V. Zala. 1990. Effect of different sources of iron and sulfur on leaf chlorosis, nutrient uptake and yield of groundnut. *Fertilizer Res.*, 24: 85-96.
- Stephan, U.W. 2002. Intra- and intercellular iron trafficking and subcellular compartmentation within roots. *Plant Soil*, 241: 19-25.
- Stephens, A.M., L.L. Dean, J.P. Davis, J.A. Osborne and T.H. Sanders. 2010. Peanuts, peanut oil, and fat free peanut flour reduced cardiovascular disease risk factors and the development of atherosclerosis in Syrian golden hamsters. *J. Food Sci.*, 75: 116-122.
- Tang, R., G. Gao, L. He, Z. Han, S. Shan, R. Zhong, C. Zhou, J. Jiang, Y. Li and W. Zhuang. 2007. Genetic diversity in cultivated groundnut based on SSR markers. *J. Genet. Genomics*, 34(5): 449-459.
- Yeh, C.C., S.L. You, C.J. Chen and F.C. Sung. 2006. Peanut consumption and reduced risk of colorectal cancer in women: A prospective study in Taiwan. *World J. Gastroenterol.*, 12(2): 222-227.
- Zeng, L. M.C. Shannon and C.M. Grieve. 2002. Evaluation of salt tolerance in rice genotypes by multiple agronomic parameters. *Euphytica*, 127: 235-245.
- Zheng, Y., F.S. Zhang and L. Li. 2003. Iron availability as affected by soil moisture in intercropped peanut and maize. *J. Plant Nutr.*, 12: 2425-2437.
- Zuo, Y. and F. Zhang. 2008. Effect of peanut mixed cropping with gramineous species on micronutrient concentrations and iron chlorosis of peanut plants grown in a calcareous soil. *Plant Soil*, 306: 23-36.
- Zuo, Y., L. Ren, F. Zhang and R.F. Jiang. 2007. Bicarbonate concentration as affected by soil water content controls iron nutrition of peanut plants in a calcareous soil. *Plant Physiol. Biochem.*, 45: 357-364.

(Received for publication 5 June 2012)