FOLIAR APPLICATIONS OF $FeSO_4$ ALONE AND IN COMBINATION WITH CITRIC ACID CAN REDUCE IRON DEFICIENCY INDUCED CHLOROSIS IN TWO PAKISTANI PEANUT (*ARACHIS HYPOGAEA* L.) VARIETIES

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Abstract. Present study was conducted to check the ameliorative effect of foliar applications on iron (Fe) deficiency in peanut. The hydroponics experiments were performed at National Agriculture Research Centre, Islamabad, Pakistan. Various foliar treatments were used to check $FeSO_4$ alone and its combined effect with citric acid and surfactants. $FeSO_4$ alone and in combination with citric acid showed ameliorative effect on Fe deficiency in peanut. Active Fe concentration of BARI-2000 and BARD-699 increased up to 52% and 25% as compared to control, when foliar treatment of $FeSO_4$ was applied. Combined effect of $FeSO_4$ and citric acid resulted 55% and 56% increase of active Fe concentrations compared to control. However, foliar application of surfactant (sodium dodecyl sulphate) suppressed the growth of peanut. Various morpho physiological parameters showed that BARD-699 was more responsive to foliar applications, while citric acid alone and in combination can be used in correcting Fe deficiency in both genotypes.
Keywords: *BARI-2000, BARD-699, Calcareous soils, Pothwar region, hydroponics experiments*

Introduction

Iron (Fe) deficiency chlorosis is a common problem in calcareous soils reducing yield significantly (Inal et al., 2007; Boodi et al., 2016). Peanut is susceptible to Fe deficiency while grown on calcareous soils with high pH and high bicarbonate levels.

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The problem is common in Pothwar tract of Pakistan (Rashid et al., 1997). Various strategies are in practice to combat Fe deficiency. These strategies include either improving the mechanism of Fe uptake or enhancing the Fe in soil solution. Among various fertilizers in practice, EDDHA and analogues (the most stable chelates) proved to be effective in maintaining Fe in soil and transporting it to the roots of plants (Lucena, 2003).

Breeding (Cianzio, 1995) and genetic modifications (Robinson et al., 1999) are commonly used strategies for uptake of Fe from soil. Due to high cost of these strategies, other solutions should also be focused. Other methods to provide Fe to the plant are foliar applications and trunk injection. Foliar applications has been advocated by various researchers (Tagliavini and Rombola, 2001; Reed et al., 1988). Foliar applications produced good results and can be practically used (Song et al., 2017a,b). Being an expensive strategy, trunk injection is used for garden trees only (Fernández-Escobar et al., 1993).

Foliar applications are useful in mitigation of Fe deficiency. Foliar applications of $FeSO_4$ increased leaf Fe concentration, however, basal treatments are more effective for long results (Mann et al., 2017). Foliar applications of salicylic acid and sodium nitroprusside in combination can increase chlorophyll, active Fe and increased Fe accumulation in cell organelles by decreasing interveinal Chlorosis (Kong et al., 2014). In *Prunus persica* L. foliar applications of Fe(III)-EDTA, Fe(III)-citrate, $FeSO_4.7H_2O$ and Fe(III)-DTPA, Fe(III)-IDHA always resulted in leaf chlorophyll increase. Re-greening varies with application of different compounds (Fernández et al., 2006).

Fe-sulphate, Fe-citrate and Fe-EDTA were very effective in re-greening of effected leaves of peach (*Prunus persica* L.). After one week of spraying, Fe sources induced physiological and biochemical responses in Fe deficient low-chill peach plants in the following order Fe-sulphate>Fe-citrate>Fe-EDTA. Different peach cultivars showed different responses towards different Fe sources (Chakraborty et al., 2012). Foliar applications are cheap source to recover Fe deficiency symptoms (Song et al., 2017a). Present study was aimed to explore the effect of different foliar application alone and in combination in correcting Fe deficiency in BARI-2000 and BARD-699.

Materials and Methods

Purpose of Experiment

The purpose of this experiment was to check the effect of $FeSO_4$ alone and with different treatments on the morpho-physiological parameters of peanut. As $FeSO_4$ is cheaper than other chemicals so it can be used alone or in combination with other chemicals for correction of Fe deficiency induced chlorosis.

Selection of Genotypes

The experiments were performed at National Agriculture Research Centre, Islamabad, Pakistan. Two genotypes were selected as Fe deficiency tolerant (BARI-2000) and Fe deficiency sensitive (BARD-699) genotypes based on previous hydroponics and pot experiments (Akhtar et al., 2014; Akhtar et al., 2013).

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Seed Germination

After surface sterilization with H_2O_2, seeds of two genotypes were washed with distilled water and germinated on wet sterile sand at 25°C in the darkness.

Hydroponics Setup

After germination uniform seedlings were transferred to aerated Hoagland's nutrient solution (Epstein, 1972). The seedlings were aerated for 24 h. The setup was kept in controlled conditions of 14/10 light /dark period with 30/20°C ± 2°C temperature and 800 cd of light. The pH of nutrient solution was maintained at 6.2. The experiment was performed in four replications.

Foliar Treatments

Following treatments were made to two weeks old seedlings;(a) Fe limited (without any treatment) (b) 0.1% citric acid (c) 0.5%$FeSO_4$ (d) 0.5%$FeSO_4$+0.1%citric acid (e) 0.5% $FeSO_4$+0.1%citric acid+1%surfactants and (f) 1%surfactant. SDS (sodium dodecyl sulphate) was used as surfactant.

SPAD Values

Young fully expanded leaves from three plants were used to record chlorophyll content by SPAD-502 (Minolta, Japan). The average of three leaves of one plant was considered as one replication and data was recorded for four replications. During the experiment, after every week data was recorded for SPAD values and average values were calculated.

Active Fe Content

Active Fe content was measured from young fully expanded leaves (Gao and Shi, 2007). Each plant was considered a single replication.

Photosynthetic and Transpiration Rates

At first detection of chlorotic symptoms, Photosynthetic rate (A) and transpiration rate (E) were recorded using Infra-Red Gas Analyzer (LCA4 Bioscientific Ltd.).

Morphological Parameters

Seedlings were harvested before the anthesis stage (Three weeks after emergence) and number of leaves was counted. Root lengths, plant height and root and shoot fresh weights were recorded. The samples were dried at 60°C for three days to record dry weights of root and shoots.

Total Fe Content

Total Fe content was measured by dry ash method by considering single plant a replication and data was recorded for four replications (Rashid et al., 2001).

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Data Analysis

Pearson's correlation coefficients were recorded by Minitab 13 at 5% confidence interval. Mean ± S.E was calculated by MS Excel. Percentage increase was calculated by formula:

$$\frac{(100 - \text{Values in control(-Fe)} \times 100)}{(\text{Values in treatment})} \quad \text{(Eq.1)}$$

Replications were compared by using SPSS. Pairwise comparison was done by applying t-test using SPSS.

Results

Photosynthetic Rate

Fe deficiency is a widespread problem in calcareous soils. Significantly higher photosynthetic rate was recorded for BARD-699 with foliar application of $FeSO_4$, 53% increase was recorded as compared to Fe deficient treatment. With same treatment, BARI-2000 showed 44% increase in photosynthetic rate as compared to its Fe limited condition. Surfactant significantly lowered the photosynthetic rate of BARI-2000 (56%) as well as BARD-699 (20%). With foliar application of citric acid, $FeSO_4$+citric acid and combination of $FeSO_4$, citric acid and surfactant resulted increased photosynthetic rate of both genotypes. In case of BARI-2000, 3, 27 and 23% increase in photosynthetic rate was recorded as compared to Fe deficient treatment. With same treatments BARD-699 showed 35, 39 and 30% increase in photosynthetic rate as compared to Fe deficient treatment (*Figure 1*).

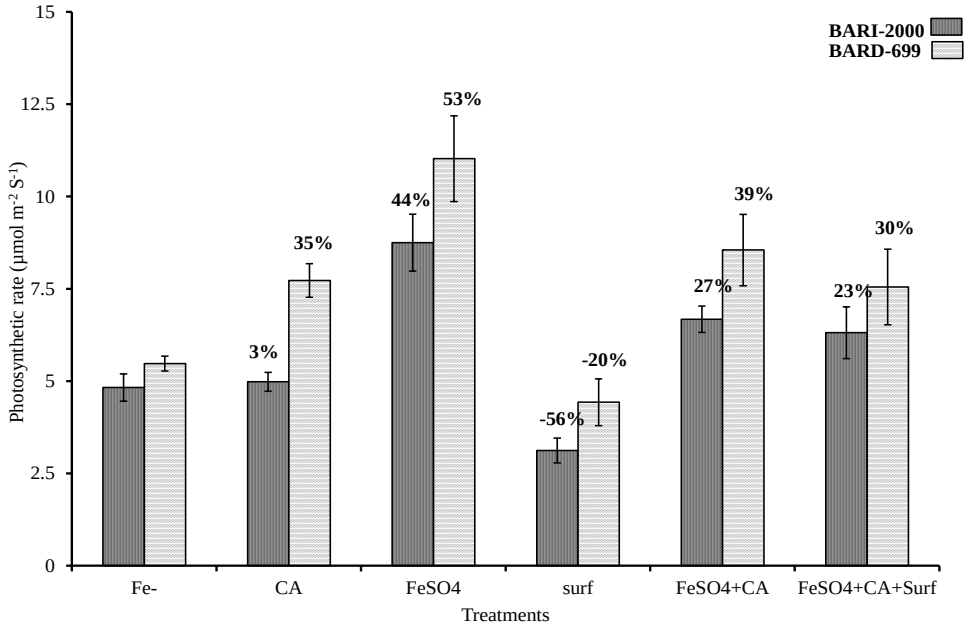

Figure 1. *Photosynthetic rates of Peanut genotypes in response to different foliar applications of equal concentration of Fe (CA: Citric acid; Surf: Surfactant)*

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Transpiration Rate

Foliar application of $FeSO_4$ along with citric acid significantly increased the transpiration rate of BARI-2000 (28%) and BARD-699 (35%) as compared to the respective Fe limited conditions. Transpiration rate with foliar application of citric acid, $FeSO_4$ and citric acid+$FeSO_4$+surfactant resulted increase of transpiration rate for both genotypes as compared to their respective Fe limited treatments. BARI-2000 resulted 20, 21 and 1% increase in transpiration rate, whereas BARD-699 showed 11,13 and 9% increase as compared to their respective Fe limited treatments. Surfactants resulted decreased transpiration rate of both genotypes *(Figure 2*).

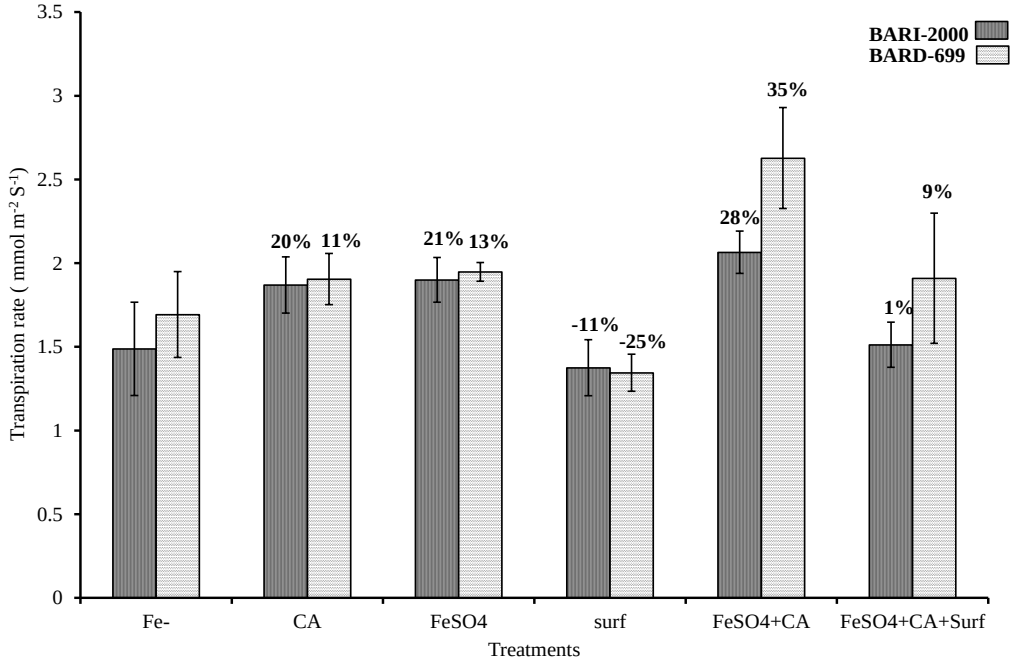

Figure 2. *Transpiration rate of Peanut genotypes in response to different foliar applications of equal concentration of Fe (CA: Citric acid; Surf: Surfactant)*

SPAD Values

More chlorotic symptoms will result in lower SPAD values. SPAD values decreased significantly with foliar application of surfactant. The decrease was 61% in BARI-2000 and 15% in case of BARD-699 as compared to their respective Fe limited conditions. This showed that surfactant disturbed plant functions resulting in decreased SPAD values. Other treatments including citric acid, $FeSO_4$, combination of citric acid and $FeSO_4$ and citric acid+$FeSO_4$+surfactant resulted increase in SPAD values of both genotypes. With these treatments BARI-2000 showed 24, 24, 25 and 12% increase in SPAD values as compared to Fe limited treatments. BARD-699 showed 28, 24, 28 and 17% increase in SPAD values in respective treatments as compared to Fe limited treatments (*Figure 3*).

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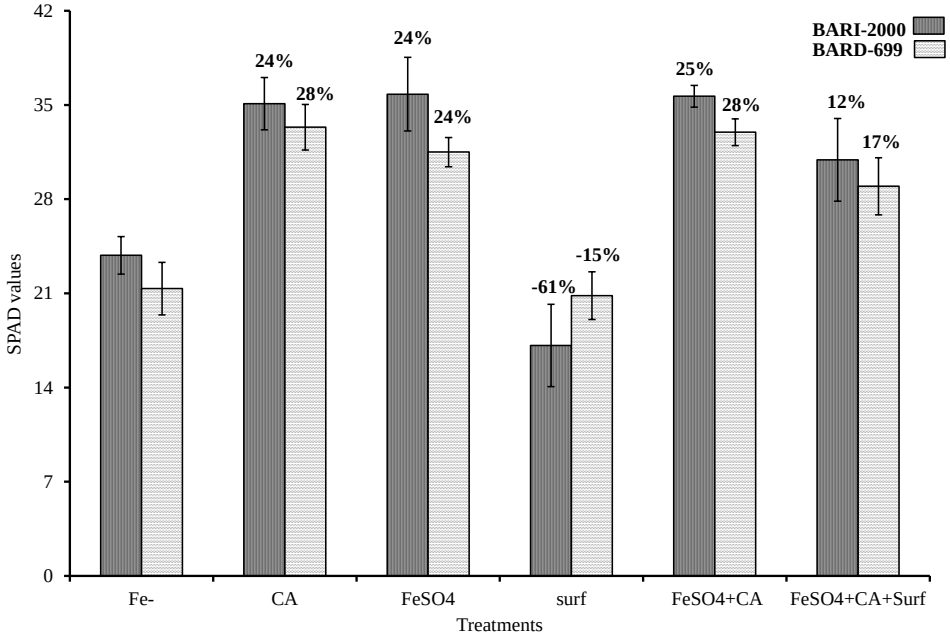

Figure 3. *SPAD values of Peanut genotypes in response to different foliar applications of equal concentration of Fe (CA: Citric acid; Surf: Surfactant)*

Correlation

SPAD values were positively correlated with active (R^2=0.697) and total Fe concentrations (R^2=0.587). Photosynthetic (R^2=0.625) and transpiration rate (R^2=0.620) was also positively correlated with SPAD values (*Table 1*).

Table 1. *Pearson correlation coefficients along with probability of significance between different parameters recorded under Fe deficient conditions and different foliar applications (Citric acid, $FeSO_4$, citric acid+$FeSO_4$, surfactant, citric acid+$FeSO_4$+surfactant)*

	NL	RL	PH	RFW	RDW	SFW	SDW	A	E	SPAD	Actv.Fe
RL	0.613**										
PH	0.454**	0.514**									
RFW	0.497**	0.432**	0.589**								
RDW	0.400**	0.512**	0.518**	0.420**							
SFW	0.583**	0.491**	0.712**	0.583**	0.448**						
SDW	0.585**	0.493**	0.570**	0.588**	0.499**	0.772**					
A	0.558**	0.798**	0.552**	0.451**	0.687**	0.529**	0.535**				
E	0.371**	0.510**	0.585**	0.434**	0.561**	0.475**	0.557**	0.552**			
SPAD	0.484**	0.495**	0.643**	0.599**	0.719**	0.512**	0.697**	0.625**	0.620**		
Actv.Fe	0.520**	0.724**	0.592**	0.467**	0.819**	0.551**	0.627**	0.850**	0.697**	0.740**	
Total Fe	0.459**	0.760**	0.527**	0.397**	0.657**	0.562**	0.658**	0.836**	0.738**	0.587**	0.853**

NL; number of leaves, RL; root length, PH; plant height, RFW; root fresh weight, RDW; root dry weight, SFW; shoot fresh weight, SDW; shoot dry weight, A; photosynthetic rate, E; transpiration rate, SPAD, active Fe and total Fe content

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Active Fe concentration was significantly higher with foliar application of $FeSO_4$ alone and in combination with citric acid.

Active Fe Content

In case of $FeSO_4$ increase in active Fe for BARI-2000 was 53% and BARD-699 gave value 58%. Similarly with combination of citric acid and $FeSO_4$, BARI-2000 showed 56% while BARD-699 showed 55% increase as compared to their respective Fe limited treatments. Citric acid showed 25% increase of active Fe of BARI-2000 and 52% increase in case of BARD-699 as compared to Fe limited treatment. $FeSO_4$ in combination with citric acid and surfactant showed 25% increase in BARI-2000 while 34% increase in BARD-699 as compared to Fe limited treatments. Surfactant resulted decrease in active Fe concentration of both genotypes as compared to Fe limited treatments (*Figure 4*).

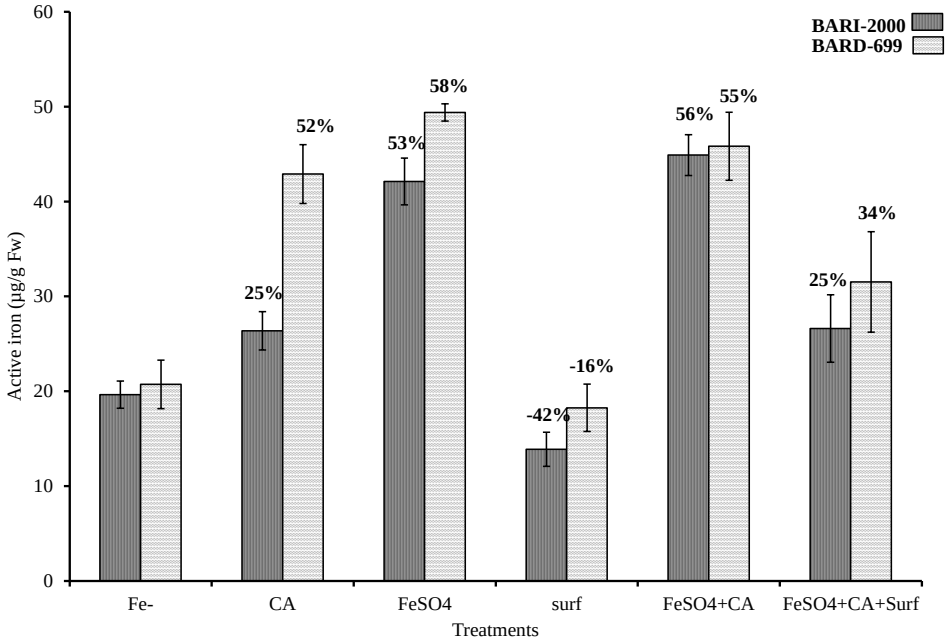

Figure 4. *Active Fe content of Peanut genotypes in response to different foliar applications of equal concentration of Fe (CA: Citric acid; Surf: Surfactant)*

Total Fe Content

Total Fe content increased significantly in BARD-699 with foliar application of $FeSO_4$ (66%) and citric acid +$FeSO_4$ (68%) as compared to Fe limited treatments. With same treatments, BARI-2000 showed 43% and 35% increase in total Fe respectively as compared to Fe deficient conditions. Citric acid resulted 6% increase in BARI-2000 while 45% increase in total Fe of BARD-699 as compared to their respective Fe deficient treatments. $FeSO_4$ in combination with citric acid and surfactant increased the total Fe concentration of BARI-2000 up to 11% while that of BARD-699 up to 44% as compared to Fe limited conditions. Foliar application of surfactant decreased the concentration of total Fe of both genotypes when compared to total Fe in Fe limited conditions (*Figure 5*). Total Fe concentration was positively correlated with active Fe concentrations (R^2=0.853) (*Table 1*).

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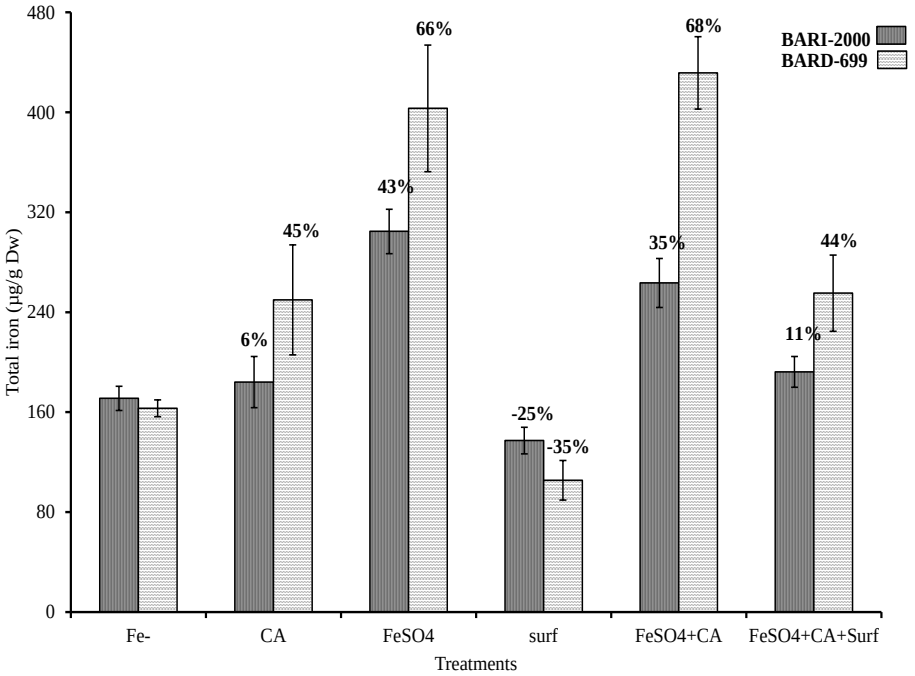

Figure 5. *Total Fe content of Peanut genotypes in response to different foliar applications of equal concentration of iron (CA: Citric acid; Surf: Surfactant)*

Comparison of Replications

The replications in both genotypes were non-significant (*Table 2*).

Table 2. *Comparison of replications in two genotypes*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Genotypes	7237.538	1	7237.538	9.169	0.003
Parameter	2448495.072	11	222590.461	281.993	0
Treatment	59821.011	5	11964.202	15.157	0
Rep	324.295	3	108.098	0.137	0.938

Pairwise Comparison of Genotypes

Pairwise comparison showed that both the genotypes were highly significant in few parameters when different foliar applications were made. The genotypes were non-significant in –Fe conditions except for shoot dry weight and transpiration rate. No. of leaves and root lengths were significantly different, when foliar applications of $FeSO_4$ were made. Shoot fresh weights and SPAD values were significantly different with foliar applications $FeSO_4$ were made in combination with citric acid. Foliar applications of Citric acid and $FeSO_4$ showed significant differences for Active Fe and total Fe content in both genotypes. SPAD values and total Fe content were significantly different with foliar applications of $FeSO_4$ and Citric acid when applied in combination. Genotypes were significantly different with respect to total Fe when $FeSO_4$+Citric acid +Surfactants were applied in combination (*Table 3*).

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Table 3. *Pairwise comparison of BARI-2000 and BARD-699*

	-Fe	Citric Acid	$FeSO_4$	Surfactant	$FeSO_4$+Citic acid	$FeSO_4$+CA+Surfactant
NL	-1.273	-0.217	-4.264**	-1.112	1.754	-0.891
RL	-1.163	-1.973	-4.013**	-2.898*	-2.472*	-0.107
PH	1.362	-0.993	0.116	-2.774*	-1.022	-3.516*
RFW	0.42	-0.194	2.316	-1.001	-2.112	-0.41
RDW	0.745	-2.165	2.959*	-0.299	1.855	-1.378
SFW	3.386*	-3.374*	-1.192	-1.828	-3.769**	-2.335
SDW	-25.927***	-24.026***	-16.172***	-10.235***	-14.161***	-10.235***
A	-3.081*	-10.473***	-3.262*	-3.634*	-3.632*	-1.995
E	-1.083	-0.308	-0.656	0.299	-3.438*	-1.931
SPAD	2.067	1.355	2.919	-2.094*	4.179**	1.056
Active Fe	-0.738	-8.926***	-5.551 ***	-2.842*	-0.445	-1.539
Total Fe	1.352	-2.709*	-3.668**	3.334*	-9.609***	-3.831**

CA; Citric acid, NL; number of leaves, RL; root length, PH; plant height, RFW; root fresh weight, RDW; root dry weight, SFW; shoot fresh weight, SDW; shoot dry weight, A; photosynthetic rate, E; transpiration rate, SPAD, active Fe and total Fe content

Discussion

Being an important oil seed crop, peanut ranks fifth in vegetable oil seed production throughout the world (Song et al., 2017a). The Fe deficiency is the yield limiting factor in dicot plants with main symptoms of interveinal chlorosis due to inhibition in chlorophyll synthesis (Song et al., 2017b). The problem is more pronounced in the Pothwar tract of Pakistan with calcareous soils resulting in reduced peanut yield. The study was planned to check the effect of foliar applications on the Fe deficiency induced chlorosis in peanut. When nutrients are applied in the form of foliar application, leaf cuticle is the first obstacle to absorb nutrients (Kannan, 1990). In our results foliar applications increased the SPAD values, Photosynthetic rate, transpiration rate, active and total Fe content of peanut. Similarly foliar applications of Salicyclic acid and Sodium Nitroprusside alleviated the Fe deficiency of peanut by increasing the photosynthetic rate and carbohydrate increase (Kong et al., 2014). Similar results were also reported by Song et al. (2017b), where lower SPAD values were recorded in Fe deficient plants as compared to the plants treated with foliar applications.

Active Fe content is an important parameter that shows the concentration of physiologically active Fe in plant tissue (Rashid et al., 1997). Our results showed an increase in active and total Fe content when foliar applications were applied. Similar results were reported by Kong et al. (2014). In peanut plants, foliar applications of SNP and NA enhanced the active Fe content. As compared to total Fe, physiologically active Fe is considered a better nutritional Fe indicator (Hakan and Vahap, 2007). Active Fe content was increased after foliar application of SNP and SA (Song et al., 2017b). In another study, exogenous application of Nitric oxide improved Fe availability in plants (Graziano et al., 2002), that could improve Fe storage by ferritin protein (Briat et al., 2010). Active Fe content was significantly different by foliar applications as described

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by Pairwise comparison of BARD-699 and BARI-2000. This suggests the different responses of genotypes towards foliar applications. BARD-699 showed earlier response towards foliar applications, that may be related to its sensitive nature. In order to cope with Fe deficiency, the genotypes increased the uptake of Fe.

SPAD values were positively correlated with active Fe and total Fe content. Photosynthetic and transpiration rates were also positively correlated. This shows that by applying Fe exogenously, Fe deficiency induced chlorosis can be recovered and hence yield can be improved. SPAD values were significantly different in both genotypes when citric acid was applied in the form of foliar application. Foliar application of Fe is a common mean to correct Fe deficiency in agricultural crops (Salahi et al., 2017; Álvarez-Fernández et al., 2005). These applications included the foliar sprays of sequestrene (FeEDDHA, $FeSO_4$, FeEDTA) and other treatments (Lucena, 2003; Rashid et al., 1997). The treatments are used either alone or in combination with acids to lower down the pH of leaf tissues (Kumawat et al., 2006). With all foliar treatments, total Fe was significantly different among two genotypes. Citric acid is a good source to recover Fe deficiency induced chlorosis by reducing the pH as indicated by our study. FeEDDHA is the most common in Fe deficiency areas for correction of Fe chlorosis in fruit trees (Lucena, 2003). After foliar Fe treatment to chlorotic plants, various effects have been reported due to many factors involved in the process of leaf penetration, plant translocation and cell Fe uptake (Curie and Briat, 2003). When subjected to Fe deficiency, plants responded in different ways (Rombolà et al., 2002), such as chlorophyll synthesis inhibition and disturbed enzyme activities, which requires Fe as cofactor (Bisht et al., 2002). Surfactants are frequently added to the different foliar sprays, as they can increase the penetration of these substances to the leaf cuticle (Stock and Holloway, 2006). However, few surfactants are considered less toxic or less effective, as Agarol-600 resulted negative effect on wheat shoot growth when combined with $ZnSO_4$ (Haslett et al., 2001), while other surfactants are not effective at all (Kannan, 1984). Our results suggested that Sodium Dodicyl Sulphate cannot be used as surfactant as SDS alone resulted decreased growth of both genotypes. It may be concluded that citric acid and $FeSO_4$ alone and in combination can increase the growth of both genotypes particularly of BARD-699.

Conclusion

It may be concluded that citric acid and $FeSO_4$ alone or in combination can increase the growth of both genotypes particularly of BARD-699. Citric acid and $FeSO_4$ together was most effective among all treatments tested. Both the treatments can be successfully used to ameliorate Fe deficiency induced chlorosis. SDS can not be recommended as a surfactant.

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