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Characterizing the expression of genes involved in iron transport in Pakistani peanut varieties under iron deficiency stress

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Abstract: Iron (Fe) deficiency is one of the major yield-limiting factors in peanut (*Arachis hypogaea* L.), especially when grown in calcareous soils. The soils of the Pothwar region in Pakistan are calcareous in nature, exposing peanut to Fe deficiency. The molecular mechanisms governing Fe deficiency response in peanut have not been fully revealed. We compared 4 locally important varieties of peanut to evaluate Fe deficiency responses at the molecular level. The expression profiles of 7 important genes including *AhIRT1*, *AhFRO1*, *AhNRAMP1*, *AhYSL1*, *AhYSL3.1*, *AhYSL4*, and *AhYSL6* in roots and young and old leaves of local peanut varieties were investigated 19 days after Fe deficiency treatment. Significant differences were observed for gene expression patterns among these varieties. The expression of *AhIRT1* was upregulated only in Banki and BARD-699. However, some of the genes, like *AhFRO1*, *AhNRAMP1*, and other genes of the YSL family, were upregulated in BARI 2000 roots and young and old leaves, which has been reported for uptake and transport of Fe. BARI 2000 and Chakori had more accumulation of Fe in young leaves and roots in Fe deficiency conditions as compared to other varieties. This suggests that other mechanisms of Fe uptake and transport may be more important than *AhIRT1* to mitigate Fe deficiency in BARI 2000 and Chakori.

Key words: *Arachis hypogaea*, iron, iron deficiency, iron uptake gene

1. Introduction

Iron (Fe) is an important micronutrient that is essential for most living organisms and it severely limits crop production in calcareous soils by formation of Fe(III) oxides (Bashir et al., 2010; Kobayashi et al., 2012; Xiong et al., 2014). In addition to the reduction in crop productivity, Fe deficiency may also result in reduced Fe content of food, triggering Fe deficiency in humans. Fe deficiency affects approximately one-third of the human population and severely affects the health of females and children (Bashir et al., 2010). In order to cope with Fe deficiency, plants have evolved two response mechanisms, termed as strategy I and strategy II (Marschner et al., 1986). Strategy I is used by all dicots and monocots with the exception of graminaceous plants. In Fe-deficient conditions this strategy is characterized by enhanced excretion of protons and increased activity of Fe(III) reductase to solubilize Fe(III) oxides to Fe(II) chelates. In most cases, strategy I plants secrete chelating/reducing compounds, mainly phenolics (Marschner, 1995; Tomasi et al., 2009; Cesco et al., 2010). After solubilization, Fe^{3+} is reduced to Fe^{2+} by a membrane-bound Fe(III) reductase oxidase (FRO) (Jeong and Guerinot, 2009). Then Fe^{2+} is taken up through an iron-regulated transporter (IRT1) (Eide et al., 1996; Xiong et al., 2014). Strategy II plants (the graminaceous monocots) increase their access to Fe by release of mugineic acid family phytosiderophores, which mobilize sparingly soluble Fe(III) (Takagi, 1976; Marschner, 1995).

The soils of Pakistan under peanut cultivation (Attock and Chakwal districts, major peanut-producing areas) are calcareous in nature (Manaf and Ejaz, 2005; Akhtar et al., 2013). These soils are conducive to the incidence of Fe chlorosis in sensitive crops including peanut because of the low solubility of Fe (Rashid et al., 1997). Fe chlorosis is one of the major abiotic stresses affecting crops in calcareous soils, resulting in reduced growth and yield. Remediation

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strategies have shortcomings, e.g., soil amendments are expensive, and tolerant cultivars are difficult to develop. Significant progress has been made in recent years in Fe-acquisition mechanisms in strategy I and strategy II plants, but still there is a lack of knowledge as to how Fe is taken up by peanut in Fe-deficient conditions. *AhFRO1* (ferric chelate reductase-oxidase enzyme), *AhIRT1* (iron-regulated transporter), and *AhNRAMP1* (natural resistance-associated macrophage protein) genes are reported to play a critical role in Fe uptake from soil (Ding et al., 2009, 2010; Xiong et al., 2012). AhFRO1 reduces Fe^{3+} to Fe^{2+}, which is then taken up by roots through *AhIRT1*. Moreover, *AhNRAMP1* is also reported to play a role in Fe uptake from soil (Xiong et al., 2012). The yellow stripe-like (YSL) genes can transport Fe-nicotianamine complexes across plasma membranes (Bashir et al., 2010) and long-distance transport (Ishimaru et al., 2010); however, the complete mechanisms of Fe deficiency tolerance have not been characterized yet. The present study aims at the comparison of 4 Pakistani peanut varieties under Fe deficiency stress by studying the expression of 7 genes related to Fe uptake and transport at the vegetative growth stage.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of four Pakistani peanut varieties (BARI-2000, Chakori, Banki, and BARD-699) were selected from 20 varieties on the basis of various morphophysiological parameters in pot (Akhtar et al., 2013) and hydroponics (Akhtar et al., 2014) experiments. The morphophysiological parameters studied in peanut genotypes were shoot root fresh and dry weights, total and active iron contents, photosynthetic rate, chlorophyll contents, transpiration rate, number of pods and seeds per plant, and pod and seed weight per plant. Genetic diversity among these genotypes was estimated based on DNA markers analysis. Based on morphophysiological evaluation in soil and hydroponics media, BARI-2000 and Chakori were declared as Fe deficiency-tolerant varieties and BARD-699 was found to be Fe deficiency-sensitive among the 20 groundnut genotypes studied.

Seeds were surface-sterilized with 10% H_2O_2 for 30 min. The seeds were washed thoroughly with tap water and then with Milli-Q water three times. Seeds were soaked in tissue papers for 3 days at 25 °C. After 3 days, germinated seeds were transferred to moistened filter papers in beakers containing water. Beakers were kept at 30 °C with 14 h of light and 10 h of dark. Filter papers were changed daily. One week later, seedlings of similar size were transferred to nutrient solution (Figure 1a). The composition of the nutrient solution was as follows: 0.70 mM K_2SO_4, 0.10 mM KCl, 0.10 mM KH_2PO_4, 2.0 mM $Ca(NO_3)_2$, 0.50 mM $MgSO_4$, 10 μM H_3BO_3, 0.50 μM $MnSO_4$, 0.50 μM $ZnSO_4$, 0.20 μM $CuSO_4$, 0.01 μM $(NH_4)_6Mo_7O_{24}$, and 100 μM Fe(III)-EDTA. After 4 days, the plants were grown on Fe-free medium. However, for control plants, Fe was provided. The solution was replaced weekly. The pH of the nutrient solution was adjusted to 6 every day. The experiment was run in three replicas.

2.2. Physiological parameters

The plants were harvested 19 days after Fe deficiency treatment (Figure 1b). Single-photon avalanche diode (SPAD) values for both young and old leaves were recorded with a SPAD-502 (Konica-Minolta, http/www.konicaminolta.com) (Akhtar et al., 2013).

2.3. Quantitative RT-PCR analysis

The expression of 7 candidate genes for mobilization, uptake, and transport of Fe was analyzed in 4 varieties. Samples were analyzed in triplicate for each genotype. For each replication in the hydroponics experiment, young and old leaves and roots of each plant of a variety were sampled separately and ground in an appropriate buffer, and RNA was extracted using the RNeasy Kit (QIAGEN). The genes (*AhIRT1*, *AhFRO1*, *AhNRAMP1*, *AhYSL1*, *AhYSL3.1*, *AhYSL4*, and *AhYSL6)* were selected based on their positions in corresponding pathways in groundnut/maize intercropping and Arabidopsis (Petit et al., 2001; Ding et al., 2009, 2010; Xiong et al., 2012, 2013, 2014). YSL genes are important in cross-membrane and long-distance transport of Fe. First-strand cDNA was synthesized from 600 ng of total RNA by reverse transcriptase (Toyobo, Tokyo, Japan, http://www.toyobo.co.jp/e/). Quantitative real-time PCR was performed using the Cepheid Smart Cycler and SYBR Green Premix Ex Taq (Takara). Conditions for PCR were set according to Xiong et al. (2014). Gene-specific primers (Table 1) were used for PCR and PCR products were confirmed by DNA sequencing. Each gene expression was normalized to peanut ubiquitin (Xiong et al., 2014).

2.4. Measurement of Fe concentration in roots

Root samples were collected from four varieties growing under Fe-deficient and Fe-sufficient conditions and were digested with 3 mL of 13 M nitric acid (HNO_3) at 220 °C for 30 min using a MARS XPRESS microwave reaction system (CEM, Matthews, NC, USA), in triplicate. The samples were collected and the final volume was adjusted to 5 mL with Milli-Q water. The samples were filtered and Fe concentration was measured by ICP-OES as described previously (Bashir et al., 2011).

2.5. Statistical analysis

One-way ANOVA was used to analyze varieties separately under both Fe-sufficient and Fe-deficient conditions at the 5% level of significance using CoStat. Means were separated by least significant differences (LSDs).

Table 1. Primers used for real-time PCR.

Primer	Sequence	Annealing temperature
AhIRT1 (F)	ATGGTAAGAAG AGTAGTGATGCAG	58 °C
AhIRT1 (R)	GCCTATCACTACTGAATGAACAATA	
AhFRO1 (F)	GAAACTGGAGGACGCAGGACTAA	60 °C
AhFRO1 (R)	ATGGGCAGTGAAGAAAGTGAGAA	
AhNRAMP1 (F)	CCTCATCACTGCCTTCGT	60 °C
AhNRAMP1 (R)	ATTGCTGTGTTATCCTTGGTC	
AhYSL1 (F)	CAAGAAGGCAAAGTTGATGGTT	60 °C
AhYSL1 (R)	AATTCCTATGGCACTAGAAAGC	
AhYSL3.1 (F)	AGCCGCAGATTGAGGGTGAG	60 °C
AhYSL3.1 (R)	CCGAGTGAAAGGTGTTGAAATAATA	
AhYSL4 (F)	GGAGCCGCTGGTTGTGGAG	60 °C
AhYSL4 (R)	AGAACCCTAACAATCCCGCAGA	
AhYSL6 (F)	GGAGCCGCTGGTTGTGGAG	60 °C
AhYSL6 (R)	AGAACCCTAACAATCCCGCAGA	

3. Results

3.1. SPAD values

SPAD is used to measure chlorophyll content in a nondestructive manner. SPAD values were significantly different in young leaves of all genotypes. Significantly higher SPAD values were recorded in BARI-2000 than other varieties under Fe-sufficient and Fe-deficient conditions (Figure 2). In old leaves, significantly higher SPAD values were recorded in Banki and Chakori. However, under Fe-deficient conditions, nonsignificant differences were recorded (Figure 2).

3.2. Gene expression in roots and young and old leaves

We analyzed the expression of 7 different genes including *AhIRT1*, *AhFRO1*, *AhNRAMP1*, *AhYSL1*, *AhYSL3.1*, *AhYSL4*, and *AhYSL6*. RT-PCR was performed to investigate the differences in transcription levels of these genes in peanut varieties in roots and young and old leaves under iron deficiency stress.

We observed significant differences among the 4 peanut varieties in terms of gene expression in roots and young and old leaves. As peanut belongs to strategy I plants, *AhIRT1*, *AhFRO1*, and *AhNRAMP1* are important in the roots.

a b

Figure 1. Comparison of 4 peanut varieties under Fe-sufficient and Fe-deficient conditions: (a) plants on the day of treatment, (b) 19 days after Fe deficiency treatment.

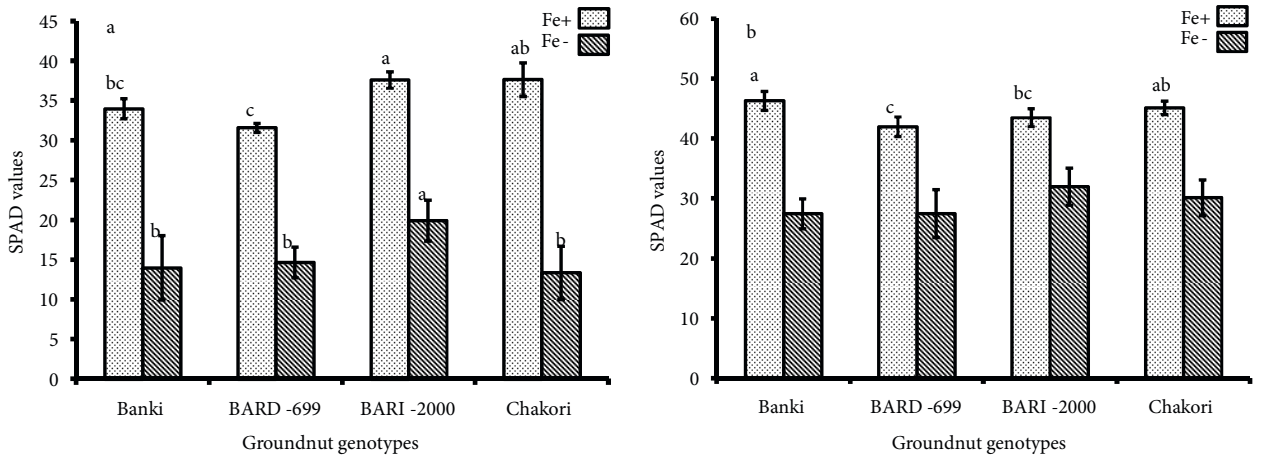

Figure 2. Comparison of SPAD values of 4 varieties 19 days after Fe deficiency treatment: (a) SPAD values of young leaves, (b) SPAD values of old leaves. Error bars represent standard deviations. Values with the same letters are not significantly different.

3.2.1. Expression of *AhIRT1*

The expression of *AhIRT1* was upregulated in the roots of all varieties. BARI-2000 and Chakori showed similar responses under Fe-deficient conditions in roots. BARD-699 showed significantly higher expression than BARI-2000 and Chakori; however, the genotype showed lower expression as compared to Banki (Figure 3a). The expression of *AhIRT1* was very low in young leaves of all varieties under Fe-sufficient as well as Fe-deficient conditions. The genotypes showed nonsignificant differences (Figure 3b). In old leaves, the expression was nonsignificantly different in all varieties and no change in expression pattern was detected (Figure 3c). The expression of *AhIRT1* was very low under Fe-deficient conditions in Banki in old leaves. The expression was not detected in BARD-699, BARI-2000, and Chakori under both Fe-sufficient and Fe-deficient conditions (Figure 3c).

3.2.2. Expression of *AhFRO1*

The expression of *AhFRO1* was nonsignificantly upregulated up to one-fold as compared to ubiquitin in roots of all varieties under Fe deficient conditions (Figure 4a). However, in young leaves, *AhFRO1* showed significantly different expression under Fe-sufficient and Fe-deficient conditions (Figure 4b). Banki showed lower upregulation as compared to Chakori. BARI-2000 showed the highest level of upregulation under Fe-limited conditions. *AhFRO1* showed nonsignificant differences among all varieties under Fe-sufficient as well as Fe-deficient conditions in old leaves; however, the expression was higher under Fe-deficient conditions among all varieties (Figure 4c).

3.2.3. Expression of *AhNRAMP1*

The expression of *AhNRAMP1* was upregulated in all varieties in roots under Fe-limited conditions; however, there were nonsignificant differences among the varieties (Figure 5a). In young leaves, the expression of *AhNRAMP1* was significantly different among varieties under Fe-sufficient and Fe-deficient conditions. In Banki, significantly lower expression was recorded under Fe-deficient conditions than in BARD-699 and Chakori. BARI-2000 showed the highest upregulation under Fe-deficient conditions (Figure 5b). Under Fe-sufficient conditions in old leaves, significantly higher expression was recorded in BARD-699. However, significantly higher expression was recorded in BARI-2000 under Fe-deficient conditions. There was upregulation of *AhNRAMP1* in BARI-2000 and Chakori and downregulation in Banki and BARD-699 (Figure 5c).

3.2.4. Expression of *AhYSL1*

In roots higher expression of *AhYSL1* was recorded in BARI-2000 under Fe-sufficient and Fe-deficient conditions. In Banki lower expression was recorded under Fe-sufficient and Fe-deficient conditions (Figure 6a). BARI-2000 and Chakori showed nonsignificant differences under Fe-deficient and Fe-sufficient conditions when compared in terms of *AhYSL1*. Banki and BARD-699 showed similar trends under Fe-deficient conditions. Banki was significantly lower in expression than BARD-699, BARI-2000, and Chakori under Fe-sufficient conditions when expression of *AhYSL1* was observed in young leaves. Nonsignificant differences were observed under Fe-deficient conditions (Figure 6b). In old leaves *AhYSL1* showed nonsignificant differences among all varieties under Fe-deficient and Fe-sufficient conditions (Figure 6c).

3.2.5. Expression of *AhYSL3.1*

Expression of *AhYSL3.1* was nonsignificantly different under Fe-sufficient and Fe-deficient conditions in roots and young and old leaves of all varieties (Figures 7a–7c). However, slightly higher expression was observed in

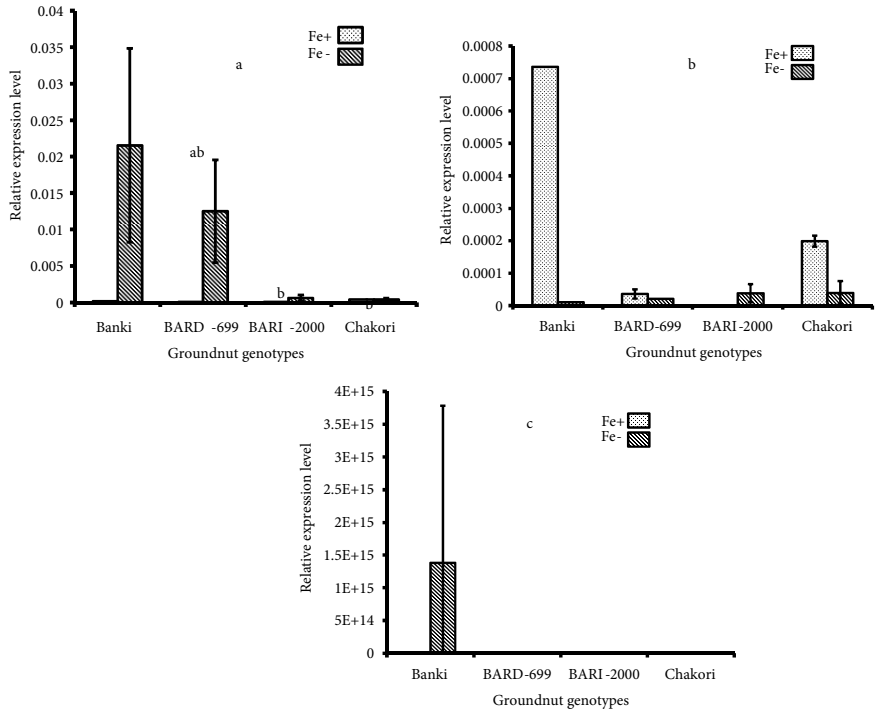

Figure 3. Gene expression of *AhIRT1* in a) roots, b) young leaves, and c) old leaves of 4 varieties under Fe-sufficient and Fe-deficient conditions (one-way ANOVA was used and means were separated using LSD at 5% level of significance separately under iron-sufficient and iron-deficient conditions). Error bars represent standard deviations. Values with the same letters are not significantly different.

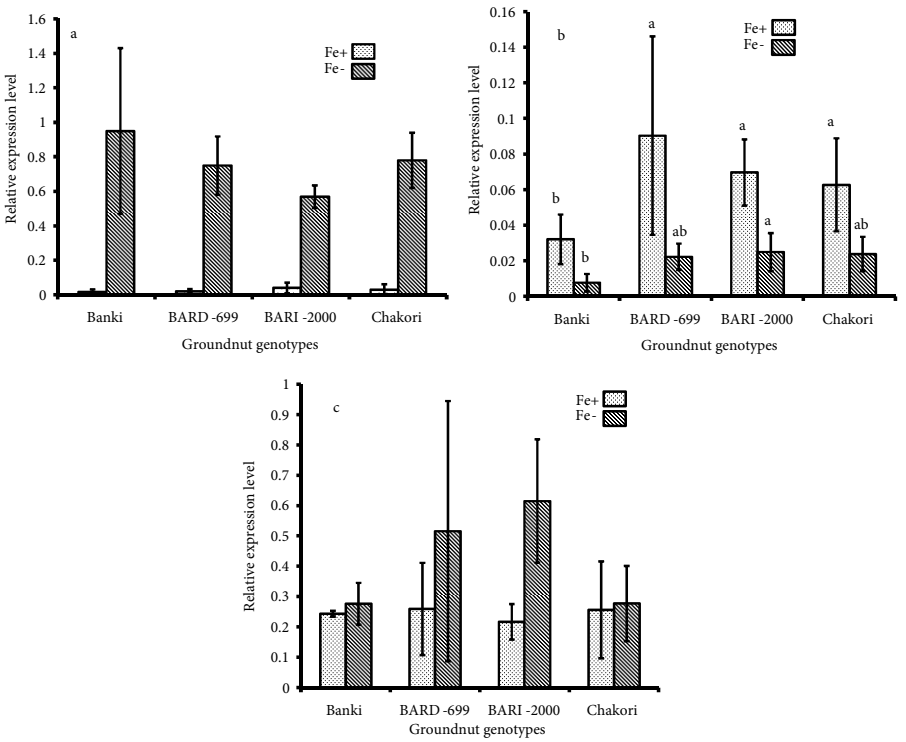

Figure 4. Gene expression of *AhFRO1* in a) roots, b) young leaves, and c) old leaves of 4 varieties under Fe-sufficient and Fe-deficient conditions (one-way ANOVA was used and means were separated using LSD at 5% level of significance separately under iron-sufficient and iron-deficient conditions). Error bars represent standard deviations. Values with the same letters are not significantly different.

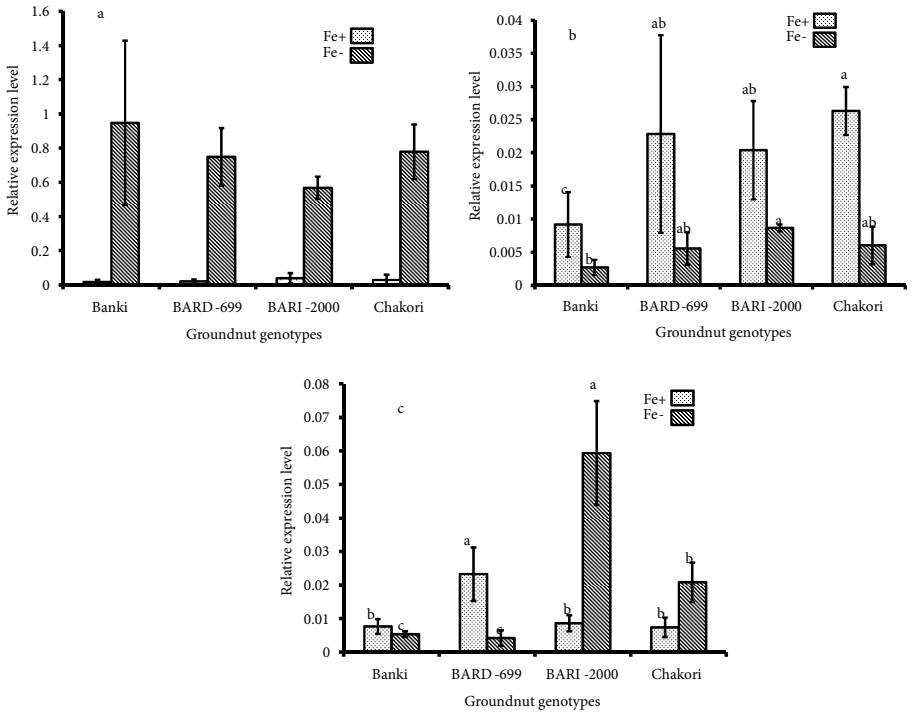

Figure 5. Gene expression of *AhNRAMP1* in a) roots, b) young leaves, and c) old leaves of 4 varieties under Fe-sufficient and Fe-deficient conditions (one-way ANOVA was used and means were separated using LSD at 5% level of significance separately under iron-sufficient and iron-deficient conditions). Error bars represent standard deviations. Values with the same letters are not significantly different.

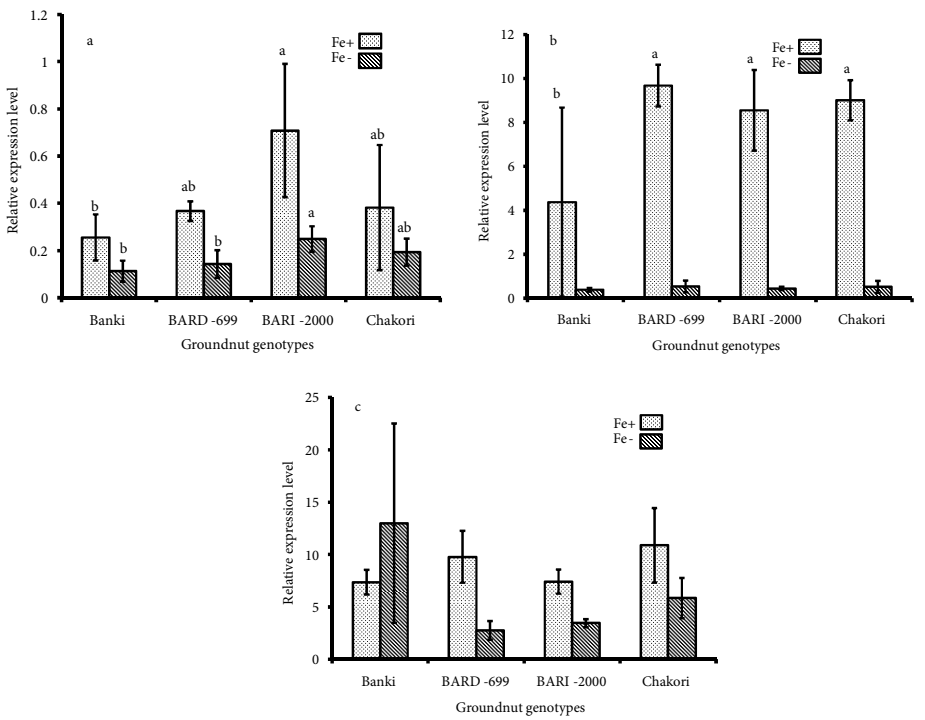

Figure 6. Gene expression of *AhYSL1* in a) roots, b) young leaves, and c) old leaves of 4 varieties under Fe-sufficient and Fe-deficient conditions (one-way ANOVA was used and means were separated using LSD at 5% level of significance separately under iron-sufficient and iron-deficient conditions). Error bars represent standard deviations. Values with the same letters are not significantly different.

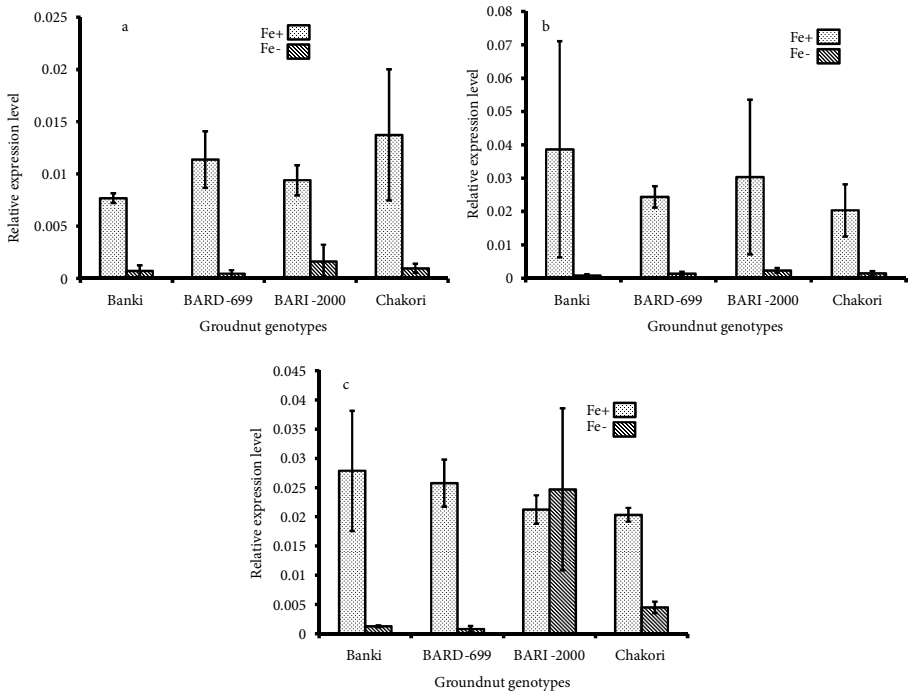

Figure 7. Gene expression of *AhYSL3.1* in a) roots, b) young leaves, and c) old leaves of 4 varieties under Fe-sufficient and Fe-deficient conditions (one-way ANOVA was used and means were separated using LSD at 5% level of significance separately under iron-sufficient and iron-deficient conditions). Error bars represent standard deviations. Values with the same letters are not significantly different.

old leaves of BARI-2000 under Fe-deficient conditions compared to other varieties (Figure 7c).

3.2.6. Expression of *AhYSL4*

Under Fe-deficient conditions BARI-2000 showed higher expression of *AhYSL4* than other varieties. Banki showed significantly lower expression than BARD-699 and Chakori under Fe-deficient conditions when expression of *AhYSL4* was observed in roots (Figure 8a). Nonsignificant differences were observed under Fe-sufficient conditions.

The expression of *AhYSL4* was higher under Fe-deficient conditions in young leaves of BARD-699 as compared to others (Figure 8b). However, the expression of *AhYSL4* was upregulated in BARI-2000 as compared with others in old leaves under Fe-deficient conditions (Figure 8c).

The varieties showed significant differences under Fe-sufficient and Fe-deficient conditions when expression of *AhYSL4* was observed in old leaves. BARI-2000 showed significantly higher expression under Fe-deficient conditions. However, Banki, BARD-699, and Chakori were nonsignificantly different under Fe-deficient conditions. BARD-699 showed higher expression under Fe-sufficient conditions as compared to Banki, BARI-2000, and Chakori (Figure 8c).

3.2.7. Expression of *AhYSL6*

In roots *AhYSL6* showed significant differences among varieties under Fe-deficient conditions (Figure 9a). Higher expression was recorded in roots of BARD-699 under Fe-deficient conditions. Lower expression was recorded in Banki and Chakori, whereas the expression was upregulated in BARI-2000 as compared with others under Fe-deficient conditions (Figure 9a).

Expression of *AhYSL6* was significantly higher in young leaves of BARD-699 and BARI-2000 as compared to Banki and Chakori under Fe-sufficient conditions. Young leaves of Chakori showed nonsignificant differences with young leaves of Banki and BARI-2000 under Fe-sufficient conditions. Nonsignificant differences were recorded among the young leaves of all varieties under Fe-deficient conditions (Figure 9b).

Significantly high expression of *AhYSL6* was recorded in old leaves of BARI-2000 under Fe-deficient conditions as compared to Banki, BARD-699, and Chakori. The genotypes Banki, BARD-699, and Chakori were nonsignificantly different from each other (Figure 9c).

3.3. Root Fe concentration

Nonsignificant differences were recorded among genotypes when Fe concentration was measured in Fe-sufficient conditions among all genotypes. However, in Fe-limited conditions Banki showed significantly lower Fe concentration as compared to BARD-699, BARI-2000, and Chakori (Table 2).

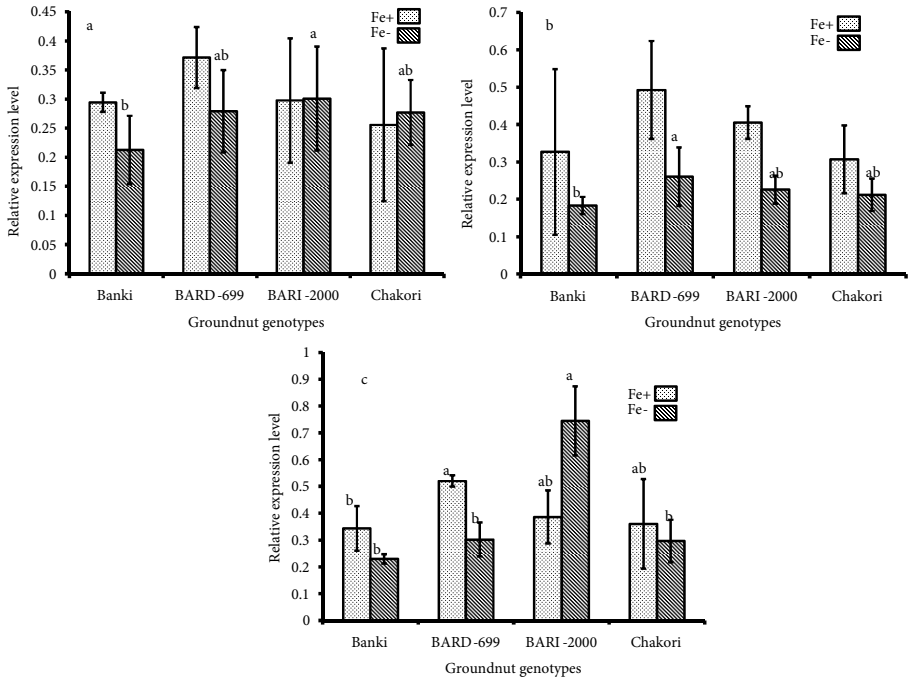

Figure 8. Gene expression of *AhYSL4* in a) roots, b) young leaves, and c) old leaves of 4 varieties under Fe-sufficient and Fe-deficient conditions (one-way ANOVA was used and means were separated using LSD at 5% level of significance separately under iron-sufficient and iron-deficient conditions). Error bars represent standard deviations. Values with the same letters are not significantly different.

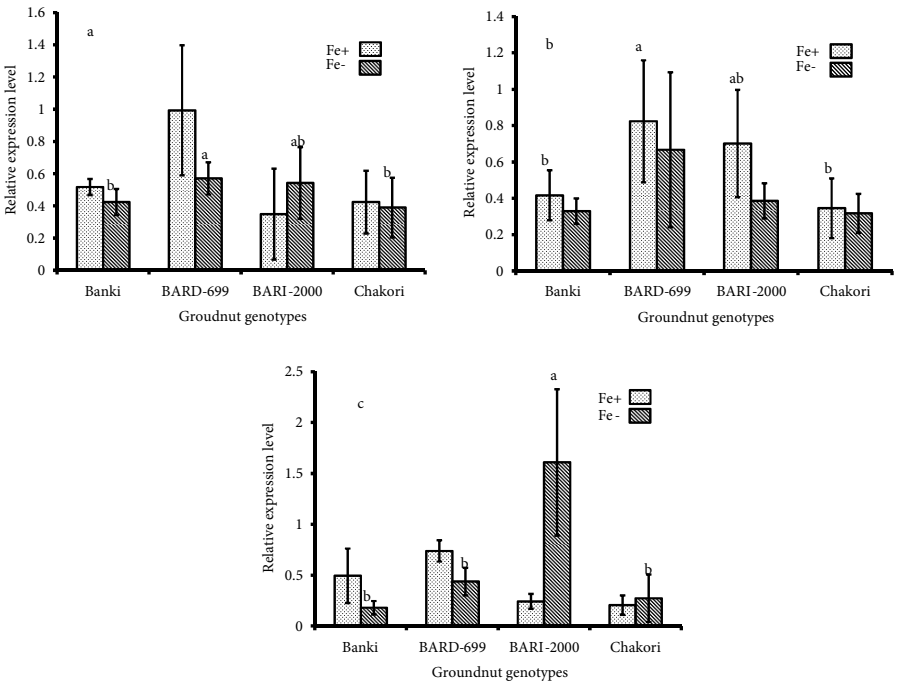

Figure 9. Gene expression of *AhYSL6* in a) roots, b) young leaves, and c) old leaves of 4 varieties under Fe-sufficient and Fe-deficient conditions (one-way ANOVA was used and means were separated using LSD at 5% level of significance separately under iron-sufficient and iron-deficient conditions). Error bars represent standard deviations. Values with the same letters are not significantly different.

Table 2. Iron concentration (µg/g) in roots of four peanut varieties. Triplicated samples were analyzed and means were separated by LSD at 5% level of significance. Values with the same letter are not significantly different. Mean ± SD.

Variety	Fe^{+}	Fe^{-}
Banki	1037.12 ± 247.12	176.37 ± 23.03 b
BARD-699	698.05 ± 60.27	331.95 ± 94.47 a
BARI-2000	1228.01 ± 670.96	371.01 ± 46.23 a
Chakori	1298.23 ± 979.76	416.88 ± 29.05 a

4. Discussion

Fe deficiency is a widespread problem of crops grown in calcareous soils (Bashir et al., 2010; Xiong et al., 2014). Peanut is a strategy I plant and its molecular mechanisms abating Fe deficiency are not well known. The Pothwar region is a major peanut-producing area of Pakistan. However, the calcareous nature of soil in this area results in Fe chlorosis and limited yields (Akhtar et al., 2013).

In the present study, we compared 4 peanut varieties locally grown in the Pothwar region of Pakistan. These varieties were selected from 20 genotypes on the basis of various morphophysiological parameters studied under Fe-deficient conditions in pot (Akhtar et al., 2013) and hydroponics (Akhtar et al., 2014) experiments. SPAD values were higher in BARI-2000 as compared to Banki, BARD-699, and Chakori. These three varieties were not different in terms of SPAD values in young leaves under Fe-deficient conditions (Figure 2a). It was reported that SPAD values were not different under Fe-deficient and Fe-sufficient conditions before anthesis; however, the SPAD values of Fe-deficient peanuts decreased suddenly after anthesis in fully expanded leaves, showing Fe deficiency behavior (Waters et al., 2002). However, the four Pakistani varieties studied here showed differences under Fe-deficient and Fe-sufficient conditions as well as differences among themselves at the vegetative growth stage before anthesis, showing their differences in Fe uptake and homeostasis mechanisms. SPAD is a useful tool to measure chlorophyll contents of expanded leaves in a nondestructive way, which is an indirect measure of Fe contents of leaves. This method determines chlorophyll contents in relative terms, which could be converted to quantifiable terms using conversion models (Coste et al., 2010).

Expression of *AhIRT1* was statistically nonsignificant in the roots of BARI-2000 and Chakori and was significantly lower than in Banki and BARD-699 (Figure 3a). IRT1 belongs to the ZIP family of transporters that are also involved in uptake of Zn and Fe, and it is highly expressed under Zn- and Fe-limited conditions (Eide et al., 1996; Bashir et al., 2012). IRT1 is also involved in uptake of Mn from soil (Korshunova et al., 1999). SPAD values were higher in BARI-2000 as compared to Banki, BARD-699, and Chakori, depicting higher iron concentrations in young leaves (Figure 2a). Higher concentration of Fe was also recorded in roots of Chakori and BARI-2000 as compared to BARD-699 and Banki under Fe-deficient and Fe-sufficient conditions (Table 2) despite the fact that *AhIRT1* expression was many-fold higher in BARD-699 and Banki. This suggests that there are other mechanisms involved in Fe uptake along with the well-known mechanism of *AhIRT1*.

AhIRT1 mRNA level increased approximately 70-fold with 6 days of Fe deficiency treatment in Fe-deficient peanuts roots (Ding et al., 2010). As the expression of *AhIRT1* was upregulated under Fe-deficient conditions, the expression of *AhFRO1* and *AhNRAMP1* was also upregulated in roots under Fe-deficient conditions though all the varieties were nonsignificantly different. The upregulation of *AhIRT1*, *AhFRO1*, and *AhNRAMP1* was observed under Fe-limited conditions in previous studies (Ding et al., 2009, 2010; Xiong et al., 2012). This may suggest that *AhFRO1* (Figure 4a) and *AhNRAMP1* (Figure 5a) along with *AhYSL4* (Figure 8a) and *AhYSL6* (Figure 9a) were involved in higher root Fe concentrations and higher SPAD values under Fe-deficient conditions in BARI-2000 and Chakori (Figure 2a; Table 2), where gene expressions were upregulated in roots of BARI-2000 and Chakori in Fe-deficient conditions.

Interestingly, the expression of *AhFRO1* (ferric chelate reductase gene localized to roots) was higher in Fe-deficient old leaves of all varieties (Figure 4c). Similarly, *PsFRO1* mRNA levels were higher in Fe-deficient shoots of pea (Waters et al., 2002). Further investigations may suggest its role in ferric reductase activity in shoots. The expression of *AhFRO1* was higher in young leaves of BARI-2000 as compared with the other varieties (Figure 4b), implicating higher Fe chelate reductase activity that may be involved in higher SPAD values.

In roots of all varieties expression of *AhNRAMP1* was higher under Fe-deficient conditions. Similar results were reported by Xiong et al. (2012), where *AhNRAMP1* was

induced by Fe deficiency in roots at 4, 7, and 11 days after Fe deficiency treatment. The expression of *LeNRAMP1*, *AtNRAMP1*, *MbNRAMP1*, and *OsNRAMP1* was also significantly higher under Fe limitations as compared to Fe-sufficient conditions (Curie et al., 2000; Bereczky et al., 2003; Xiao et al., 2008; Takahashi et al., 2011). We observed higher expression of *AhNRAMP1* in Fe-sufficient conditions in young leaves than in Fe-deficient conditions; however, the expression was higher for BARI-2000 than other varieties under deficiency conditions (Figure 5b). In old leaves, expression of *AhNRAMP1* was upregulated in BARI-2000 and Chakori and downregulated in Banki and BARD-699. This may be indicative of better homeostasis of Fe in these varieties. The expression of *AhNRAMP1* was delayed in leaves and at day 11, where higher expression was obtained under Fe-limited conditions (Xiong et al., 2012).

BARI-2000 showed significantly higher expression of *AhYSL1* in roots under Fe-sufficient conditions as compared to Banki and BARD-699. The expression of *AhYSL1* increased in Fe-deficient roots 11 days after Fe-deficient treatment (Xiong et al., 2012). The expression of *AhYSL1* was higher in Fe-sufficient conditions in young and old leaves. The YSL protein family is known for uptake of Fe-nicotianamine complexes and long-term transport (Bashir et al., 2010; Ishimaru et al., 2010), which may be indicative of an alternate pathway for Fe uptake and homeostasis in peanut. In our results, higher expression of *AhYSL1* in roots of BARI-2000 may be implicative of higher SPAD values and Fe contents of roots in BARI-2000. Along with *AhYSL1*, upregulation of *AhYSL3.1*, *AhYSL4*, and *AhYSL6* in old leaves of BARI-2000 supports this notion. The expression of *AhYSL4* and *AhYSL6* was observed in only Fe-starved conditions in young and old leaves, while no upregulation was observed in roots (Xiong et al, 2013).

Our data indicated upregulation of *AhYSL6* in roots in BARI-2000 (Figure 9a), suggesting its role in Fe-starved roots. *AhYSL3.1* showed nonsignificant differences among all varieties with respect to gene expression in roots and young leaves. The Fe concentration of young leaves of BARI-2000 and Chakori was nonsignificantly different. The expression of *AhYSL3.1* was higher in roots and young and old leaves under Fe-sufficient conditions (Xiong et al., 2013).

Peanut/maize intercropping was more focused on the improvement of Fe nutrition of peanut in calcareous soils (Zuo et al., 2000; Zhang et al., 2004; Ding et al., 2009, 2010; Xiong et al., 2012). The Fe concentration in the roots and young leaves of intercropped peanuts (Fe-sufficient) was higher as compared to monocropped peanuts (Fe-deficient). In intercropped peanuts, DMA promotes Fe acquisition and Fe nutrition in peanut plants (Xiong et al., 2012). The increased expression of genes involved directly or indirectly in Fe uptake and transport under Fe deficiency was investigated. *AhFRO1*, *AhIRT1*, and *AhNRAMP1* are key genes for strategy I-based Fe acquisition in plants (Ding et al., 2009, 2010; Xiong et al., 2012). FRO and FRO homologues show expression both in roots and shoots (Li et al., 2004; Wu et al., 2005; Jeong et al., 2008). *AhFRO1* and *AhIRT1* mRNA levels were increased in response to Fe deficiency and nonsignificant results were recorded for BARI-2000 and Chakori. The expression patterns of both the genes are similar in response to Fe deficiency, suggesting coordination between the two genes (Ding et al., 2009, 2010). The responses of BARI-2000 and Chakori are similar in response to Fe-starved conditions, showing common mechanisms involved in the uptake of Fe. This behavior could be linked to the common parentage of BARI-2000 and Chakori, as Chakori is a selection of BARI-2000.

AhIRT1 is the key gene for uptake of Fe from growth medium and the expression of this gene was very low under Fe-deficient conditions in BARI-2000 and Chakori. However, the higher expression and upregulation of *AhNRAMP1*, *AhFRO1*, *AhYSL1*, *AhYSL3.1*, *AhYSL4*, and *AhYSL6* in different tissues of BARI-2000 and Chakori as compared to Banki and BARD-699 suggest that these genes may be involved one way or another in mitigating Fe deficiency in BARI-2000 and Chakori, more so than in other two varieties. *AhIRT1* remained the sole mechanism for Fe uptake in Banki and Chakori; however, it is recommended that the role of other genes be further investigated to elucidate their key functions in iron uptake and transport in these varieties.

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