

Screening of Fe-Deficiency Tolerance in Okra (*Abelmoschus esculentus* L.) Through Hydroponic Culture

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Abstract

Screening for Fe deficiency tolerance in okra (*Abelmoschus esculentus*) Bangladeshi genotypes ('BARI-1', 'Local variety', 'Orca Onamica', and 'Prince') were studied based on different morphological and physiological parameters. Number of leaves, shoot height and weight were significantly reduced in 'Orca Onamica' and 'Prince', whereas 'BARI-1' and 'Local variety' did not show prominent decrease in the aforesaid growth parameters under Fe deficiency. Again, 'Orca Onamica' and 'Prince' showed significantly decreased root length and root biomass under Fe deficiency. In contrast, these parameters were unchangeable in 'BARI-1' and 'Local variety' in Fe shortage compared to controls. Furthermore, Fe deficiency caused severe decrease in chlorophyll (*a* and *b*) and Fe concentrations in leaves of 'Orca Onamica' and 'Prince' grown on hydroponic culture. In contrast, chlorophyll (*a* and *b*) and Fe concentrations were not significantly decreased in 'BARI-1' and 'Local variety' due to Fe deficiency. Based on these findings, tolerance to Fe deficiency in these okra cultivars can be categorized as: tolerant ('BARI-1' and 'Local'), and sensitive ('Orca Onamica' and 'Prince'). The ranking can be applied in plant breeding program and may have great advantage over conventional methods. This study also demonstrates the effectiveness of hydroponic culture as an efficient method to screen Fe-efficient crop plants.

Keywords: Abiotic stress, Fe concentration, Fe deficiency tolerance, hydroponic culture, okra, screening

Introduction

Iron (Fe) is the fourth most abundant element in the earth's crust and is an essential nutrient for plants (Marschner, 1995). Fe deficiency-induced chlorosis is a common disorder in dicotyledonous plants, including field peas, when grown on calcareous, high pH soil (Marschner, 1995). Chlorosis may lead to serious yield, quality losses and economic loss (Molassiotis *et al.*, 2005). Alkaline soils are regarded as potential inducers of Fe deficiency in plants even though the element might occur in high concentrations in the soil (Tangolar *et al.*, 2008). Fe is absorbed by soil particles in an insoluble form, which the plants are not capable of utilising, and the soluble portion is usually insufficient (Lindsay, 1984). A high concentration of bicarbonate contributes to the soil alkalinity (Mengel *et al.*, 1995). Fe in interaction with other nutrients may become scarcely available to the plants. Based on the World Reference Base Soil Classification System, calcareous soil is classified under the reference soil group of Calcisols covering 800 million hectares worldwide, mainly found in South Asia, Australia, West Asia and North Africa under

arid and semi-arid climates or Mediterranean climates (Srinivasarao *et al.*, 2006).

Okra (*Abelmoschus esculentus*) also known as lady's finger, is a valuable vegetable plant. Okra contains proteins, carbohydrates and vitamin C (Dilruba *et al.*, 2009), and plays a vital role in human diet (Saifullah and Rabbani, 2009). Consumption of young immature okra pods is important as fresh fruits, and it can be consumed in different forms (Ndunguru and Rajabu, 2004). However, Fe deficiency causes poor yield in many okra genotypes grown worldwide. In agriculture, Fe deficiency tolerant cultivars offer advantages in Fe deficient soil as plants require minimal fertilizer applications. Thus, the trait of Fe deficiency tolerance has long been a subject of interest in agricultural science. Hydroponic culture has often been used for screening for tolerance to mineral deficiency and toxicity. Screening in hydroponic culture allows for rapid screening, it overcomes seasonal effects and provides disease free conditions (Dragonuk *et al.*, 1989). Generally, Fe deficiency in hydroponic culture is induced by the addition bicarbonate which increases the pH of the solution making the solution Fe unavailable for plants (Zribi and Gharsalli, 2002). It has

been the most popular method used to identify tolerant genotypes in crop plants such as soybean (Dragonuk *et al.*, 1989), maize (Celik and Katkat, 2008), chickpea (Hamze *et al.*, 1987) and wheat (Chaney, 1984). Although hydroponic screening is suitable for preliminary work, field evaluation is required to confirm the results do apply in soil (Dragonuk *et al.*, 1989). However, screening of Fe deficiency tolerance in okra was not yet performed.

Different morphological parameters are used to screen for genotypes tolerant to Fe deficiency in plants (Dwyer *et al.*, 1991; Yakop, 2008). The most widely used parameter for screening Fe deficiency tolerant genotype in plants is chlorophyll score. Chlorophyll has been used for screening several species including corn (Dwyer *et al.*, 1991), wheat (Reeves *et al.*, 1993), sweet pepper (Madeira *et al.*, 2003) and field peas (Yakop, 2008). Use of SPAD-502 is reported to be more accurate than visual assessment of chlorophyll (Reeves *et al.*, 1993) and appropriate to assess Fe deficiency chlorosis in leaves (Yakop, 2008). Other morphological features, such as, root/shoot ratio, leaf and root growth have also been used to screen Fe deficiency tolerant plant species (Bertoni *et al.*, 1992; Kosegarten, 1999; Yakop, 2008). To date, no investigation was done on the screening of Fe-efficiency okra line based on morphological and physiological parameters.

Growing Fe deficiency tolerant cultivars in Fe deficient soils could be economically preferable as it does not need application of any Fe compounds. However, selection of nutrient tolerant genotype is dependent on the suitable screening method. Therefore, genotypic differences in Fe-deficient plants based on physiological and biochemical responses have long been the subjects of intensive studies. A large number of new field okra varieties with improved characteristics have been released in recent years. Nevertheless, very little is known towards the screening of okra genotypes tolerant to Fe deficiency. Thus, the present investigation was aimed at screening different okra genotypes mainly cultivated in Bangladesh. Further aim of this study was to establish the hydroponic method for screening Fe deficiency genotypes.

Materials and methods

Plant materials

Four okra genotypes ('BARI-1', 'Local variety', 'Orca Onamica' and 'Prince') were collected from local seed market.

Germination and growth conditions

Before growing, seeds were surface sterilized in 70% ethanol and 5% sodium hypochlorite for 1 and 15 min, respectively. Seeds were then rinsed five times in deionised water. Seeds were germinated on moist filter paper wetted with deionised water for 3-4 days in the dark at room temperature. Only healthy and uniform seedlings were transplanted to solution culture. A basal nutrient solution (Hoagland and Arnon, 1950) was used with the following nutrient concentrations (μM): KNO_3 (16000), $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (6000), $\text{NH}_4\text{H}_2\text{PO}_4$ (4000), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (2000), KCl (50), H_3BO_3 (25), Fe-EDTA (25), $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ (2), ZnSO_4 (2), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.5) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.5). Target pH values were obtained by

titrating the basal solution with KOH or H_2SO_4 . Plants were grown in 2 L of aerated solution and the environment was strictly maintained under 10 h light and 14 h dark (550-560 $\mu\text{mol s}^{-1}$ per μA). Fe deficiency was induced by adding NaHCO_3 (10 mM) to the treatment solutions to increase the pH up to 8.0 to initiate Fe deficiency as previously described (Kabir *et al.*, 2013; Gharsalli *et al.*, 2001). Solution was replaced every 4 days. No NaHCO_3 was added to the control solution.

Measurement of morphological features

The number of leaves on each plant was counted three weeks after Fe deficiency was imposed. Whole shoot and root lengths were measured for each plant sample using a ruler. For measurement of fresh weight of root, roots were harvested and then wiped with clean tissue paper before measuring weight in electronic balance. Fresh weight of shoot was directly measured after harvesting. For measuring dry weight, roots and shoots were quickly rinsed in deionised water and then wiped with clean tissue paper. Root and shoot samples were then dried in an oven at 70 °C for two days before dry weight was measured.

Measurement of chlorophyll concentration

A chlorophyll content of leaves was determined spectrophotometrically as described previously by Lichtentaler and Wellburn with modifications (1985). Firstly, 100 mg leaf was weighted and placed in 95% acetone in a 5 ml falcon tube. The leaf sample was then grinded using mortar-pestle. The homogenate was filtered through whatman filter and was centrifuged at 2500 rpm for 10 min. The supernatant was separated and the absorbance were read at 662 (chlorophyll *a*) and 646 (chlorophyll *b*) on spectrophotometer. The amount of these pigments was calculated according to the formula given by Lichtentaler and Wellburn (1985).

Determination of Fe content in leaves

Firstly, the leaf samples (1 g) were digested as followed by Huang *et al.* (2004). Briefly, the dried leaf sample were predigested (overnight) sample and HNO_3 mix is heated at 75 °C for 10 min, followed by 109 °C for 15 min. After cooling for 10 min, 1 ml of H_2O_2 was added to each vessel through the ventilation hole and the sample mix is heated at 109 °C for a further 15 min. The samples were then analysed for Fe concentration by Flame Atomic Absorption Spectroscopy (AAS) outfitted with ASC-6100 auto sampler and air-acetylene atomization gas mixture system (Model No. AA-6800, Shimadzu). Standard solutions of Fe were prepared from their respective concentration of 1000 ppm stock solutions (Shimadzu), from which further serial dilutions (0.1-4 ppm) were made to cover the optimum absorbance range for the standard calibration curve. For the determination, two solutions were prepared for each sample. Reagent blank determinations were used to correct the instrument readings.

Statistical analysis

Statistical analyses (t-test) were performed using Genstat software (14th Edition). Significance was set at $p \leq 0.05$. Three replications of each sample have been used for all experiments.

Results

Shoot parameters

Number of leaves was counted in all genotypes grown on both Fe sufficient and Fe deficient hydroponic conditions. The number of leaves was not significantly reduced in 'BARI-1' and 'Local' variety due to Fe deficiency compared to Fe sufficient controls (Tab. 1). In contrast, leaf number was significantly reduced in 'Orca Onamica' and 'Prince' due to shortage of Fe compared to controls. Alike leaf number, shoot height was also influenced by Fe deficiency. Shoot height in 'BARI-1' and 'Local' variety was not significantly affected by Fe deficiency (Tab. 1). However, Fe deficiency caused significant decrease in shoot height in 'Orca Onamica' and 'Prince' compared to Fe sufficient plants. Fresh and dry weight of shoots was not significantly decreased in 'BARI-1' and 'Local' variety under Fe deficiency compared to Fe sufficient plants. However, Fe deficiency caused significant decrease in shoot's fresh and dry weight in 'Orca Onamica' and 'Prince' (Tab. 1).

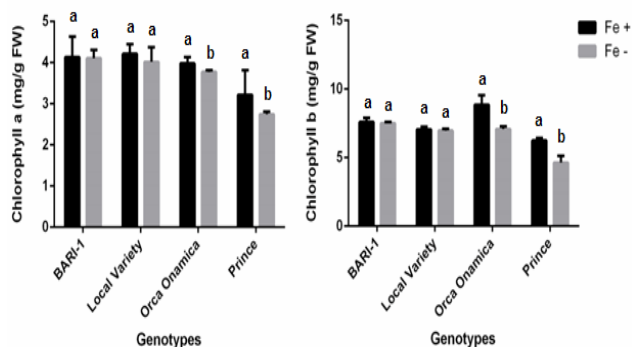


Fig. 1. Concentration of chlorophyll a and b in young leaves in a number of okra genotypes grown in Fe-sufficient (Fe+) and Fe-deficient (Fe-) hydroponic culture. Data were taken 3 weeks after Fe deficiency was imposed. Different letters indicate significant differences between means \pm SD of treatments ($n = 3$), comparisons were done for Fe + and Fe - conditions

Tab. 1. Shoot parameters in different genotypes (cultivars) of okra grown on Fe sufficient (Fe+) and Fe deficient (Fe-) hydroponic culture; there were three replications for each sample; data were taken on three weeks old plants

| Cultivar | No. of leaves (ppm) | | | Shoot height (cm) | | | Shoot fresh weight (g) | | | Shoot dry weight (g) | | |
|---------------|---------------------|---------------|----|-------------------|----------------|----|------------------------|----------------|----|----------------------|-----------------|----|
| | Fe+ | Fe- | t' | Fe+ | Fe- | t' | Fe+ | Fe- | t' | Fe+ | Fe- | t' |
| BARI-1 | 7.5 \pm 0.5 | 6.9 \pm 0.5 | * | 12.7 \pm 1.0 | 12.0 \pm 0.9 | * | 0.6 \pm 0.3 | 0.5 \pm 0.02 | * | 0.09 \pm 0.03 | 0.08 \pm 0.01 | * |
| Local Variety | 7.5 \pm 0.8 | 6.4 \pm 0.5 | * | 15.4 \pm 1.1 | 11.7 \pm 0.7 | * | 0.8 \pm 0.1 | 0.6 \pm 0.08 | * | 0.09 \pm 0.01 | 0.07 \pm 0.2 | * |
| Orca Onamica | 9.9 \pm 1.0 | 6.0 \pm 0.1 | ** | 15.4 \pm 1.2 | 10.5 \pm 0.5 | ** | 1.0 \pm 0.1 3 | 0.3 \pm 0.04 | ** | 0.12 \pm 0.02 | 0.06 \pm 0.01 | ** |
| Prince | 8.4 \pm 0.4 | 6.0 \pm 0.2 | ** | 13.9 \pm 0.5 | 10.5 \pm 0.9 | ** | 1.0 \pm 0.1 | 0.6 \pm 0.1 | ** | 0.13 \pm 0.03 | 0.07 \pm 0.30 | ** |

t' test: *Statistically non-significant; **Statistically significant

Root parameters

Length of roots was not significantly decreased in 'BARI-1' and 'Local variety' under Fe deficiency compared to the plants grown on Fe sufficient *in vitro* conditions. However, 'Orca Onamica' and 'Prince' were severely affected by Fe deficiency and their lengths of roots were significantly reduced under Fe deficiency (Tab. 2). Like length of roots, fresh and dry weights of roots were also showed similar sensitivity to Fe deficiency (Tab. 2).

Both fresh and dry weights of roots were not significantly decreased in 'BARI-1' and 'Local' variety due to Fe deficiency compared to controls. Whereas, Fe deficiency caused significant decrease in both fresh and dry weights of roots in 'Orca Onamica' and 'Prince'.

Chlorophyll concentration

The concentration of chlorophyll a was significantly reduced in 'Orca Onamica' and 'Prince' under Fe deficiency compared to Fe sufficient plants (Fig. 1). In contrast, no significant reduction in chlorophyll a concentration was observed in 'BARI-1' and 'Local variety' due to Fe deficiency. Similar pattern was also observed for chlorophyll b under Fe deficiency compared to controls (Fig. 1).

Fe content in leaves

AAS was used to determine Fe concentration in young leaves of all four genotypes of okra grown in Fe-sufficient and NaHCO₃-treated solution culture, with tissue taken 3

Tab. 2. Root parameters in different genotypes of okra grown on Fe sufficient (Fe+) and Fe deficient (Fe-) hydroponic culture. There were three replications for each sample. Data were taken on three weeks old plants

| Cultivar | Root length (cm) | | | Root fresh weight (g) | | | Root dry weight (g) | | |
|---------------|------------------|----------------|--------|-----------------------|-----------------|--------|---------------------|-----------------|--------|
| | Fe+ | Fe- | t-test | Fe+ | Fe- | t-test | Fe+ | Fe- | t-test |
| BARI-1 | 5.7 \pm 0.24 | 5.5 \pm 0.12 | * | 0.29 \pm 0.02 | 0.27 \pm 0.02 | * | 0.26 \pm 0.01 | 0.25 \pm 0.01 | * |
| Local Variety | 5.7 \pm 0.71 | 4.7 \pm 1.2 | * | 0.25 \pm 0.02 | 0.24 \pm 0.01 | * | 0.22 \pm 0.02 | 0.19 \pm 0.01 | * |
| Orca Onamica | 7.1 \pm 1.10 | 3.5 \pm 0.5 | ** | 0.29 \pm 0.01 | 0.21 \pm 0.01 | ** | 0.28 \pm 0.01 | 0.19 \pm 0.02 | ** |
| Prince | 6.3 \pm 0.31 | 4.0 \pm 0.5 | ** | 0.26 \pm 0.01 | 0.19 \pm 0.02 | ** | 0.23 \pm 0.01 | 0.18 \pm 0.01 | ** |

t' test: *Statistically non-significant; **Statistically significant

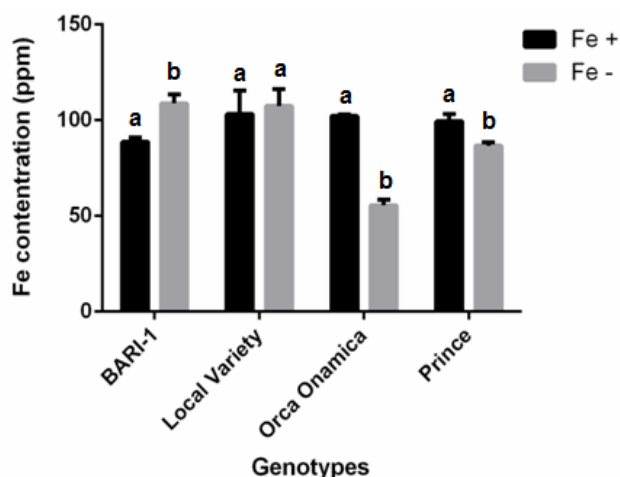


Fig. 2. Fe concentration in young leaves in a number of okra genotypes grown in Fe-sufficient (Fe+) and Fe-deficient (Fe-) hydroponic culture. Data were taken 3 weeks after Fe deficiency was imposed. Different letters indicate significant differences between means \pm SD of treatments ($n = 3$), comparisons were done for Fe + and Fe - conditions

weeks after NaHCO_3 treatment. Under Fe deficient conditions, the leaf Fe concentration significantly increased in 'BARI-1' compared to the plants grown in Fe-sufficient conditions (Fig. 2). In addition, Fe concentration was unchanged due to Fe deficiency compared to Fe sufficiency plants in 'Local variety'. In contrast, significant decline in Fe concentration was observed in leaves of 'Orca Onamica' and 'Prince' under Fe shortage compared to controls (Fig. 2).

Discussion

Screening of Fe-deficiency tolerant line has been mainly carried out *in vivo* by field tests. Moreover, screening of the okra genotypes for Fe deficiency was never studied. The present study reveals the potentiality of Fe deficiency tolerance in a number of Bangladeshi okra genotypes. Our results confirmed by different physiological parameters further pinpoint the efficiency of hydroponic culture for Fe-efficient okra germplasm.

Fe deficient plants grown hydroponically showed the typical chlorosis within few days after the beginning of the experiments. Different growth parameters were severely affected by Fe-deficiency induced hydroponic conditions. Results suggest that Orca Onamica and Prince are unable to tolerate Fe deficiency or in other words, they are not efficient to operate mechanisms conferring Fe deficiency tolerance. In general, plants survive under Fe deficiency by operating a number of Fe-efficient mechanisms in roots. 'BARI-1' and 'Local variety' were not significantly affected by Fe deficiency in their length and fresh and dry weights of roots. It suggests that Fe-efficient mechanisms are actively present in root systems that eventually let them continue normal growth and development. In contrast, these root parameters are negatively affected in 'Orca Onamica' and 'Prince' resulting stunted root and poor root biomass.

Chlorophyll (*a* and *b*) and Fe concentrations in leaves of all genotypes were studied in both Fe sufficient and Fe deficient hydroponic conditions. Results suggest that 'BARI-1' and 'Local variety' are the Fe-deficiency tolerant line showing no significant reduction in chlorophyll *a* and *b*; whereas, 'Orca Onamica' and 'Prince' were found to be Fe-sensitive. AAS data showing the Fe concentration in leaves were found to consistent with the chlorophyll concentration.

Based on these investigations, it is evident that genotypic variation exists in Bangladeshi okra analyzed cultivars for Fe deficiency tolerance. Taken as a whole, 'BARI-1' and 'Local variety' are highly tolerant to Fe deficiency, showing normal chlorophyll synthesis, Fe concentration and physiological growth. In contrast, 'Orca Onamica' and 'Prince' are highly sensitive and unable to survive or maintain normal growth and development under Fe deficiency.

This study also confirms the efficiency of hydroponic culture for screening okra genetic line for screening Fe or other mineral deficiency tolerance germplasm. This method overcomes the difficulty associated with the use of calcareous soils under field, greenhouse, and growth chamber conditions.

This paper provides the first evidences on the genotypic variations in okra plants in response to Fe deficiency. Results also enrich the knowledge for varietal characteristics of okra and can be used by farmers and plant breeders where Fe deficiency is a major obstacle for okra propagation. Efficiency of hydroponic culture for the successful screening of plant genetic lines may also be followed by future scientists.

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