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The Effect of Iron Toxicity on Seed Germination and Early Seedling Growth of Green Gram (*Vigna radiata* L. Wilczek)

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Abstract: ***The objective of this work was to determine the impact of toxic concentration of iron on green gram (Vigna radiata L. Wilczek var. SML 668) seedlings. For this seeds were sown in petridishes with varying concentration of iron (100, 200, 300, 500 µM Fe) in the form of Fe EDTA in seed germinator. Seed germination, shoot and root length and tolerance index was found to be decreased and percentage phytotoxicity and percentage difference from control was increased in seeds with increase in concentration of iron in germinating solution. Iron toxicity cause increase in tissue iron concentration and decrease in chlorophyll content at 3 and 6 days of shoot and root. High concentration of lipid peroxidation, hydrogen peroxide and proline in shoot and root were detected in toxic (200, 300, 500 µM Fe) seedlings as compared to control (100 µM Fe). The results indicate that under iron stress condition plants suffer increased oxidative damage.***

Keywords: green gram seedlings, iron stress, seed germination, phytotoxicity, oxidative damage.

1. Introduction

Iron is an essential micronutrient for all the living organisms. Iron is the sixth most abounded element in the universe (Mc Donald *et al*., 2010), and in the soil it is a fourth most common element. Iron is critical for plant life, as it is involved in plant metabolism (Guerinot and Yi, 1994). It is a limiting nutrient for plants and microorganisms due to the low solubility of the oxidized ferric form (Samaranayke *et al*., 2012). The property of iron to accept and donate electrons leads to a dual role of iron, which behaves as a cofactor for many enzymes. As a critical component of many proteins and enzymes it plays a significant role in basic biological process such as uptake mechanisms, photosynthesis, chlorophyll synthesis, respiration, nitrogen fixation and DNA biosynthesis through the action of ribonucleotide reductase (Welch, 2002; Schmidt, 2003; Briat *et al*., 2007; Hansch and Mendel, 2009; Broadley *et al*., 2012). Approximately up to 80% of iron is found in the photosynthetic cells where it is essential for the biosynthesis of cytochrome, heme molecules, chlorophyll and construction of Fe-S cluster (Balk and Schaedler, 2014; Briat *et al*., 2015). It is also stored inside the cell in the chloroplasts, mitochondria and vacuoles (Jeong and Connolly, 2009).

Iron can also be potentially toxic at high concentrations. Due to potential role of iron in induction as well as alleviation of oxidative stress, homeostasis of iron is essential for plant growth and development as both deficiency and toxicity of iron predispose the basic biological and physiological mechanism of plants (Walker and Connolly, 2008; Kuki *et al*., 2008; Briat *et al*., 2010). Excess amount of iron is harmful to living cells, which can act catalytically via the fenton reaction and catalyze the conversion of hydrogen peroxide in to harmful free radicals (Ravet *et al*., 2009). Free radical can cause damage to a wide variety of cellular structures and they can ultimately kill the cell (Crichton *et al*., 2002). So the excess of iron leads to the oxidative damage to the plants either directly or indirectly by forming ROS.

The objective of the present study was to examine the effect of toxic iron supply on seed germination, seedling development, oxidative damage in the seedlings components- shoot and root of green gram.

2. Material and Methods

To study the effect of iron toxicity on seed germination and early seedling growth in green gram (*Vigna radiata* L. Wilczek var. SML 668) seedlings, a petriculture experiment was conducted. Seeds of green gram first surface sterilized (5% v/v mercuric chloride solution) and washed properly with deionised glass distilled water (GDW) before germination. Sterile seeds were then sown in petridishes lined with three fold filter paper in Hoagland nutrition solution with varying concentrations of iron (100, 200, 300, 500 µM Fe) at 28^{o}C and relative humidity 85% in seed germinator under controlled condition of light (12 hour photoperiod). After 48 hours the effect toxic iron was evaluated by the decrease in seedling length, tolerance index, % germination, % DFC and increased phytotoxicity percentage. After 3 and 6 days of iron treatment dry matter yield, tissue iron concentration chlorophyll (chl), carotenoid and proline were determined in shoot and roots of green gram seedlings.

The percentage germination of seeds was recorded at 48 hours by using the formula of Tanveer *et al*., (2010)
GP %= [Germinated seeds / Total seeds x 100
Percentage difference from control (% DFC) for germination was calculated by using the formula of Mhatre and Chaphekar, (1982).
% DFC = [% germination of control – % germination of test solution]/
% germination of control) x 100
% phytotoxicity for shoot and root was determined by using formula Chou *et al*., (1978).

% phytotoxicity =
[shoot or root length of control – shoot or root length of test solution]/ shoot or root length of control] X 100
Tolerence index (TI) of shoot and root was calculated according to the formula of Turner and Marshal, (1972) as follows-
% TI =
[Mean length of longest shoot or root in test solution/ mean length of longest shoot or root length of control] X 100

Plants were separated into shoots and roots and total biomass was determined by oven drying (70^{o}C) the samples. The iron concentration in shoot and root was determined by the method of piper (1942). The total chlorophyll content of the fresh leaf sample was estimated by the method of Lichtenthaler (1987). Lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) formation to the protocol of Heath and Parker (1968) at 532 nm. Hydrogen peroxide was estimated by the method of Bernnan and Frenkel (1977) at 415 nm. Proline (Pro) was estimated colorimetrically as ninhydrin complex in toluene Bates *et al.*, (1973).

All measurement was made in triplicate and the data were statistically analyzed (ANOVA) for significance (LSD at P=0.05). The data are presented as mean values ± standard error (SE, n=3).

3. Results

Seeds of green gram showed maximum germination in nutrient solution containing 100 µM iron supply. At 200 and 300 µM iron concentration decrease in germination was observed as compared to control (100 µM) but above this concentration there was significant reduction in germination especially at 500 µM iron concentration where very poor germination was observed. Same results were also found by El Rasafi *et al*., (2016) in wheat and Rout *et al* 2014 in rice (Table 1). As compared to control shoot and root length was decreased as the concentration of iron increased. The decrease was found to be more pronounced in germination supplied with 500 µM iron. Same result was observed by El Rasafi *et al*., (2016), where wheat seedlings was affected significantly in the presence of high concentration of iron in the growing medium. (Nagajyoti *et al*., (2010) found that toxicity of iron may be related to high uptake of Fe^{2+} by roots and its transportation to shoots. These ions damaged membranes, DNA and proteins due to the free radicals production (Arora *et al*., 2002; Crichton *et al*., 2002; De Dorlodot *et al*., 2005; Ravet *et al*., 2009). Tolerance Index of green gram seedlings was significantly reduced in presence of high concentration of iron as compared to the control. The percentage difference from control (% DFC) for germination was increased. The phytotoxicity in green gram was less than 20% at the first two concentrations (200 and 300 µM Fe), then it increased to almost 90% in 500 µM iron supply as compared to control (Table 1). The roots of green gram were seen to be more affected by toxic iron doses than the shoots. This is in accordance with the result found by Lingua *et al*., (2008). As we know, roots are of first part of the plant that closely touch with growing medium, so they are most sensitive to toxicity by metals than shoots (Araujo and Monteiro, 2005; Shah *et al*., 2010; Yang *et al*., 2010; Yusuf *et al*., 2011).

Toxic concentration of iron caused growth inhibition and significant decline in fresh and dry matter yield in green gram seedlings, similar results were also observed by Nenova, 2006; Mehraban *et al*., 2008. Maximum decrease in yield was observed in seedlings supplied with 500 µM iron at both stages, the decrease was more in the root than shoot (Table 2). An increase in iron supply from 100 µM to 500 µM caused an increase in the iron concentration in shoot and root tissue from 21.66 to 272.1 µg Fe/g dry wt. and 25.14 to 279.6 µg Fe/g dry wt. respectively at 3 days. Maximum accumulation of iron as compared to control was found in 500 µM iron supplied seedlings at both the stages, same results were also reported by Sinha and Saxena, 2006 and Jucoski *et al* 2013 in *Eugenia uniflora* (Table 2).

The concentration of chlorophyll in shoots of green gram seedlings was found to be significantly decreased with increasing concentration of iron from 100 µM to 500 µM at both the stages. Carotenoid concentration was also decreased with increasing iron in the medium at both the stages (Table 3). Mehraban *et al*., (2008) and Arunachalam *et al*., (2009) reported a decrease in chlorophyll and carotenoid content in toxic iron concentration. Whereas ratio of chlorophyll/carotenoid was found to be decrease in increasing concentration of iron.

The level of lipid peroxidation increased in seedlings of green gram treated with excess iron at both the stages. This was evident from the accumulation of thiobarbituric acid reactive substance (TBARS) in the shoots and roots of seedlings. Enhanced lipid peroxidation under excess iron has also been observed for different species (Souza- Santos *et al*., 2001; Sinha and Saxena, 2006; Stein *et al*., 2008; Xing *et al*., 2010; Jucoski, *et al*., 2013) and has been associated with oxidative stress caused by iron toxicity. The accumulation of hydrogen peroxide was increased by increasing the concentration of iron at 3 d and 6 d, but values are higher at 6 d. Mehraban *et al*., (2008) reported increase in H_2O_2 content by iron toxicity. Hydrogen peroxide (H_2O_2) a by-product of the superoxide anion dismutation, however, is also toxic to plants and, therefore, requires to be eliminated. The increase in lipid peroxidation concentration H_2O_2 was more pronounced in root than shoot (Table 4). The accumulation of proline was increased by increasing the concentration of iron at 3 and 6 days of shoot and root, but values are lower at 3 days. The increase in proline concentration was more pronounced in roots than shoot. Accumulation of proline in plants is increased which is either due to enhanced synthesis or reduced degradation (Verbruggen and Hermans, 2008). It is an efficient scavenger of $OH^{\cdot}$ and O_2 and can prevent the damage caused by the lipid peroxidation (Table 4).

4. Conclusion

The results of this study indicated that the excess amount of iron affects the seed germination and early seedling growth of green gram. This study also showed that a high iron concentration had a significant reduction in germination parameters. Seedlings also suffer strong oxidative stress with

increase in lipid peroxidation, H_20_2 and proline content which is induced by iron toxicity.

Table 1: *Effects of iron toxicity on the % germination, length, %DFC, phytotoxicity and tolerance index (TI) of green gram (Vigna radiata L. Wilczek var. SML 668) seedlings grown in solution culture.*

		µM iron supply		
Parameters	100	200	300	500
% Germination	[a]96.17	[b]81.34	[c]54.09	[d]28.13
Shoot length (cm)	[a]3.52	[b]2.90	[c]2.34	[d]1.10
Root length (cm)	[a]3.22	[b]2.34	[c]1.24	[d]0.80
DFC	-	[d]15.90	[c]30.63	[b]50.23
% Phytotoxicity in shoot	-	[d]28.57	[c]47.38	[b]63.15
% Phytotoxicity in shoot	-	[d]38.01	[c]59.75	[b]74.00
Tolerance index in shoot	-	[b]77.16	[c]54.77	[d]33.63
Tolerance index in shoot	-	[b]61.38	[c]46.94	[d]26.11

Table 2: *Effect of iron toxicity on the fresh matter yield, dry matter yield and tissue iron concentration in green gram (Vigna radiata L. Wilczek var. SML 668) seedlings grown in solution culture.*

Days after treatment	Plant part	µM iron supply			
		10	100	200	400
Fresh matter yield: mg plant^{-1}					
3	Shoot	[a]233	[b]196	[c]168	[d]140
	Root	[a]42.5	[b]33.2	[b]37.0	[c]25.4
6	Shoot	[a]760	[b]632	[c]521	[d]301
	Root	[a]78.8	[b]67.7	[c]61.5	[d]52.1
		Dry matter yield: mg plant^{-1}			
3	Shoot	[a]14.8	[b]12.1	[b]11.8	[c]10.3
	Root	[a]3.17	[b]2.15	[b]2.09	[c]1.10
6	Shoot	[a]24.2	[b]21.2	[c]18.7	[d]10.1
	Root	[a]4.73	[b]4.11	[c]3.22	[c]2.89
Tissue iron: µg g^{-1}dry wt.					
3	Shoot	[a]21.66	[d]67.11	[c]135.2	[b]272.1
	Root	[a]25.14	[d]72.68	147.4	[b]279.6
6	Shoot	[a]22.23	[d]70.43	[c]138.9	[b]267.5
	Root	[a]28.72	[d]88.61	[c]152.1	[b]283.2

Table 3: *Effect of iron toxicity on the concentration of chlorophyll and carotenoid in green gram (Vigna radiata L. Wilczek var. SML 668) seedlings grown in solution culture.*

Days after treatment	Plant part	µM iron supply			
		10	100	200	400
Total chlorophyll: mg g^{-1} fresh wt.					
3	Shoot	[a]0.501	[b]0.448	[b]0.401	[c]0.356
6	Shoot	[a]1.001	[a]0.983	[b]0.811	[c]0.723
		Carotenoid: mg g^{-1} fresh wt.			
3	Shoot	[a]0.412	[b]0.305	[c]0.248	[c]0.221
6	Shoot	[a]0.818	[b]0.745	[b]0.713	[c]0.602
		Chl/Car: mg g^{-1} fresh wt.			
3	Shoot	[a]1.216	[c]1.468	[b]1.616	[b]1.610
6	Shoot	[a]1.224	[b]1.319	[c]1.137	[a]1.200

Table 4: *Effect of iron toxicity on the concentration of hydrogen peroxide (H_2O_2), lipid peroxidation (LPO) and proline in green gram (Vigna radiata L. Wilczek var. SML 668) seedlings grown in solution culture.*

Days after treatment	Plant part	µM iron supply			
		10	100	200	400
Hydrogen peroxide: nmol 100 mg^{-1} fresh wt.					
3	Shoot	[a]0.096	[a]0.112	[c]0.153	[b]0.201
6	Root	[a]0.121	[d]0.147	[c]0.179	[b]0.222
6	Root	[a]0.087	[c]0.176	[b]0.212	[b]0.257
6	Shoot	[a]0.096	[c]0.181	[b]0.234	[b]0.263
		TBARS: nmol gm^{-1} fresh wt.			
3	Shoot	[a]6.120	[d]13.26	[c]21.12	[b]28.67
6	Root	[a]9.325	[d]17.10	[c]24.01	[b]32.11
6	Shoot	[a]12.26	[d]16.21	[c]26.82	[b]34.00
6	Root	[a]15.54	[d]20.23	[c]29.42	[b]38.42
Proline: µmol g^{-1} fresh wt.					
3	Shoot	[a]0.063	[a]0.068	[c]0.073	[b]0.089
6	Root	[a]0.071	[a]0.077	[c]0.081	[b]0.120
6	Shoot	[a]0.072	[c]0.086	[b]0.098	[b]0.101
6	Root	[a]0.083	[d]0.134	[c]0.201	[b]0.264

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