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Differential responses to iron stress in blackgram (*Vigna* mungo L.) varieties

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ABSTRACT

The intention behind conducting this study was to evaluate the toxicological response of black gram (Vigna mungo) varieties exposed to iron stress at an early seedling stage. Iron is a micronutrient required to plant for various metabolic activity. However, its toxicity or deficiency can cause harmful effects to the plants. The experiment was conducted in the Department of Life Science and Bioinformatics of Assam University, Silchar during kharif season of 2019. Two blac gram varieties and three iron concentrations (control, 10µM and 2mM) were evaluated in laboratory conditions.The results clearly showed that significant difference was observed in the growth of roots and shoots and total biomass of the plants grown in three different iron conditions. An enhancement was observed in MDA, H_2O_2, and O_2^- and in IPU-073 than in Shekhar-1. At iron-deficiency, H_2O_2, MDA, O_2^- radical content of IPU-073 increased by 88.3, 79 and 50.1% whereas, in Shekhar-1, it increased by 70.5, 26.8 and 36.6% in roots respectively which was found to be higher than Fe-excess condition in both roots and shoots. The reduction rate of Chlorophyll content was lower in Shekhar-1 (9.1%) than IPU 073 (31.8%). The comparison between these two varieties showed that Shekhar-1 performed better than IPU-073 under different iron stress condition.

Key words: Iron excess, iron deficiency, blackgram, oxidative stress, ROS

INTRODUCTION

Black gram(*Vigna mungo*L.) is an important pulse legume considered as a staple food which is domestically grown and has various industrial uses and possess the ability to resist adverse climatic conditions. Undoubtedly, black gram contributes in nitrogen requirement in the soil by fixing atmospheric nitrogen as they have root nodule bacterial association. Ironis an essential nutritional element for living organisms which is involved to play pivotal roles in various cellular, metabolic processes, especially synthesis of chlorophyll, photosynthesis and respiration. It is an important element for all living organisms due to its redox properties. Iron acts as a cofactor to carryout various biological functions like photosynthesis, electron transfer, oxygen transport, heme and chlorophyll biosynthesis, regulation of protein stability. The optimum range of Fe required by the plants varied between 10^{-4} and 10^{-8} M. Any variation in this range can deleteriously affect the plant growth and development resulting various metabolic disorders. Iron is abundantly available in soil but due to its low solubility it is often a limiting nutrient for plant growth. However, when Fe is highly accumulated in plant cells it becomes highly toxic and acts catalytically via Fenton reaction, generates hydroxyl radicals which can damage lipids, DNA and protein. Therefore, plants must respond to Fe stress both excess and deficiency. A phenomenon known as stress induced morphogenic response occurs when plants comes in contact with stress responds by changing the root architecture of plants by altering its length, number as well as formation of root hair growth as roots are the primary organs which comes in contact with stress(Li *et al.* 2015). Fe excess arrests the primary root growth and inhibit the formation of lateral roots. However, in Fe deficiency plants enlarges their root surface area and it triggers the formation of root hairs. The roots can grow well in moderate Fe but drastically reduces in low or no Fe content. Bronzing is one of the major symptoms of Fe toxicity in plants which starts from the tip of the leaves and gradually spreads throughout the entire surface of the leaves and interveinal chlorosis is prominent in iron deficient plants (Dey *et al.* 2019). The objective of the study is to compare the iron stress response both in iron overload and in deficient condition in black gram and to evaluate their morphological, physiological and biochemical parameters at an early seedling stage.

MATERIALS AND METHODS

The required seeds of two black gram cultivars (IPU-073 and Shekhar-1)were surface sterilized by 0.01% of $HgCl_2$ (w/v) for about 4-5 minutes. The treated seeds were rinsed properly for 5-6 times with distilled water and were kept imbibe in water soaked cotton for 8 hours and then the seeds were transferred to petri-plates with water soaked cotton beds at 30°C for germination. After 2 days old healthy seedlings were transplanted into plastic container holding Hoagland nutrient solution at pH 6.2 and kept in growth chamber at 32°C with 16 hours light and 8 hours of dark photoperiodic condition and light illumination at 52µmol/m/s. The nutrient solution was renewed after 2 days. On the 5^{th} day, three concentrations were selected *viz*., control at pH 6.2, iron excess (2mM) at pH 4.5, and iron deficient (10µM) at pH 7.5. Five plants were randomly selected from the growth medium for the measurement of both root and shoot in centimeter scale. Fresh weight of these five plants was also scored. Both root and shoots were oven dried at 72°C for 4 days and their dry weight was measured. The extent of lipid peroxidation was quantified in terms of MDA content following Heath and Packer (1968). The total H_2O_2 content was estimated following the method described by Sagisaka (1976). The total O_2^- content was estimated following the method described by Elstner and Heupul (1976).Chlorophyll estimation was performed using Arnon *et al.* (1949) method. All data was evinced as mean (*n=3*) followed by standard error (mean± SE) format using SPSS 21 (window version) software. Along with the difference among the various treatments was determined by employing two-way ANOVA (analysis of variance) and post hoc Tukey's HSD (honest significant difference) test at 0.05 level of significance.

RESULTS AND DISCUSSION

Growth attributes

When abiotic stress hits seedling and vegetative phase of plants, it could negatively impact the growth affecting its morphological and physiological changes. During early seedling stage, stress reduces plant height as well as its total biomass (Asch *et al.* 2005). In both (2mM and 10µM) Fe-condition, the roots and shoots of the plant were significantly reduced (*p <0.001*). Plants were exposed to stress for 72 hours and they have inhibitory effect on their growth. Decline in overall growth of plants was observed at 24, 48 and 72 hours, respectively. This could be due to the shrinkage of the tissue under stress. The reductions in fresh and dry weight (DW) of IPU-073 were significantly higher than Shekhar-1. At 10µM iron stress, 23, 14.9, and 20.7% root growth inhibition and 19.7, 18.2, and 19.1% shoot growth inhibition at 24, 48 and 72 hours was observed in IPU-073, respectively as compared with control. The corresponding reductions in the root growth (18.3, 14.3, and 14.2%) and shoot growth (17.3, 16.9, and 14.9%) at 24, 48 and 72 hours respectively were recorded as compared with control (Table 1). The total growth inhibition was significantly higher in IPU-073 than Shekhar-1 with 10µM than the plant grown under 2mM iron stress. Plants in both concentrations of iron (10µM and 2mM) were affected by the amount of Fe present in their growth medium in comparison to the control plants. Similar kinds of results were obtained by Nenova, (2009) in pea plants under iron stress.

Table 1: Effect of deficient and excess iron on the root length and shoot length (cm) in black gram

Variety	Fe^{2+} Conc.	24Hrs		48Hrs		72Hrs	
		Root	Shoot	Root	Shoot	Root	Shoot
IPU-073	Control	10.56±0.11	12.47±0.14	10.90±0.37	12.86±0.10	12.50±0.07	13.26±0.83
	2mM	8.94±0.23***	11.02±0.94	9.78±0.22	11.76±0.15	11.72±0.13	11.98±0.09
	10µM	8.12±0.27***	10±0.21*	9.3±0.11	9.90±0.16***	10.90±0.06***	10.72±0.31*
Shekhar-1	Control	12.19±0.59	14. ±0.58	12.29±0.62	16.25±0.25	12.9±0.32	17.60±0.37
	2mM	11.3±0.06	12.86±0.10	11.78±0.44	14.09±0.39**	11.95±0.46	16.28±0.44
	10µM	9.96±0.27***	11.81±0.26*	10.53±0.36*	13.49±0.39***	11.06±0.24**	14.96±0.30*
Source of Variation		*F*-value					
Variety		80.57***	21.44***	26.05***	170.42***	7.76**	134.27***
Stress		17.61***	13.31***	7.63**	45.03***	36.42***	16.21***
Variety x Stress		0.85	4.16	0.48	1.93	1.79	0.07

The total biomass significantly declined after each 24 hours (24, 48 and 72 hours). The decline in FW of IPU-073 at 10µM was recorded as 33, 33.9, and 33.6% in roots at 24, 48 and 72 hours respectively. In 2mM iron concentration, the decline FW rate in IPU-073 roots was 17.5, 13.3 and 15.9%, respectively (Table 2). The decline rate was comparatively lower in the roots of Shekhar under 10µM and 2mM iron concentrations. Similar results were found in the shoots of both the blackgram varieties. Dry weight reduction was significantly higher in the roots and shoots of IPU-073 at 10µM than Shekhar-1 (Table 2). From the growth pattern it was evident that the root and shoot lengths of plant grown in 10µM were much lower than the control as well as from 2mM iron concentration. Similarly, the total biomass including fresh weight and dry weight of the plants decreased with iron toxicity and deficiency in comparison to control. Similar result was found in pea (Jelali *et al.* 2011). Not much work has been reported in iron deficiencies till now in case of pulse crops. However, according to recent reports, Fe acts a limiting factor for biomass production and yield in crops like *Oryza sativa*, *Solanumly copersicum* and *Spinaciaoleracia* (Briat and Gaymard, 2015). On the other hand, excess ferrous ion in the medium can accelerate the Fenton reaction releasing many oxidative particles which can lead to cell and tissue death (Morrisey and Guerinot, 2009). Therefore, under both fluctuating conditions of iron can affect the physiology of plants. Similar results were observed in the present study with decreased dry weight under both conditions for the genotypes.

Table 2: Effect of deficient and excess iron on fresh and dry weight (g) of roots and shoots in blackgram

Variety	Fe2+ Conc. (µM)	24Hrs		48Hrs		72Hrs	
		Root	Shoot	Root	Shoot	Root	Shoot
		Fresh weight					
IPU-073	Control	0.49±0.007	1.74±0.05	0.56±0.01	1.76±0.04	0.59±0.0005	1.77±0.04
	2mM	0.40±0.002	1.67±0.05	0.48±0.01	1.68±0.05	0.49±0.01*	1.70±0.05
	10µM	0.33±0.04*	1.56±0.05	0.37±0.02***	1.59±0.06	0.39±0.01***	1.61±0.06
Shekhar-1	Control	0.57±0.01	1.80±0.02	0.61±0.005	1.81±0.03	0.64±0.008	1.85±0.03
	2mM	0.50±0.006	1.73±0.05	0.52±0.005	1.75±0.05	0.54±0.005*	1.78±0.05
	10µM	0.39±0.04**	1.67±0.05	0.42±0.03***	1.69±0.05	0.44±0.03***	1.71±0.05
Source of Variation					*F*-value		
Variety		7.25*	13.26**	3.18	7.86*	11.55**	4.06
Stress		18.40***	20.05***	4.58*	44.61***	72.29***	4.30*
Variety x Stress		0.15	0.207	0.10	0.03	0.009	0.03
		Dry weight					
IPU-073	Control	0.07±0.001	0.18±0.01	0.07±0.0008	0.21±0.01	0.07±0.001	0.23±0.01
	2mM	0.06±0.001	0.18±0.01	0.06±0.001	0.2±0.01	0.06±0.001	0.21±0.01
	10µM	0.04±0.001***	0.17±0.01	0.04±0.001***	0.19±0.01	0.04±0.001***	0.20±0.01
Shekhar-1	Control	0.09±0.002	0.18±0.01	0.09±0.002	0.21±0.01	0.09±0.001	0.25±0.01
	2mM	0.08±0.003	0.17±0.01	0.08±0.004	0.19±0.01	0.08±0.003	0.23±0.01
	10µM	0.06±0.003*	0.17±0.01	0.07±0.003**	0.19±0.001	0.07±0.003**	0.22±0.01
Source of Variation					*F*-value		
Variety		103.52***	0.005	83.25***	0.01	119.03***	3.27
Stress		45.68***	0.12	40.75***	0.91	53.18***	1.83
Variety x Stress		1.15	0.007	1.78	0.01	0.13	0.03

Biochemical analysis

Lipid peroxidation is one of the consequences of oxidative stress and thus production of Malondialdehyde (MDA) can be used as an indicator of lipid injury caused by oxidative stress. Higher MDA content was recorded in the roots (76%) and leaves (82.2%) of IPU-073under 10 µM iron dose. Shekhar-1 reported to produce only 26.8% and 73.1% in the roots and leaves respectively at 10 µM iron dose, as compared to control (Table 3). Hydrogen peroxide (H_2O_2) production was significantly higher in the roots and leaves of

IPU-073 at 10 µM than 2mM iron concentration. In IPU-073, H_2O_2 content was recorded higher by 88.3% and 87.1% at 10µM and 87.1% and 67.9% at 2mM in the roots and leaves, respectively as compared to control. Similarly, in Shekhar-1, 70.5% and 79.4% at 10µM and 67.9% and 54.1% increase in 2mM in the roots and leaves respectively as compare with control. The data clearly indicated that excessive H_2O_2 production was found in IPU-073 than Shekhar-1 in both stress conditions (Table 3). Generation of superoxide radical (O_2^-) under iron stress condition also showed similar result like H_2O_2 which was significantly higher in the roots and shoots of IPU-073 at 10µM that at 2mM. O_2^- content of 50.1% was found in the roots of IPU-073 at 10µM which was only 36.6% recorded in the roots of shekhar-1. Similarly, 60.9% O_2^- content was found in the leaves of IPU-073 which was higher than 49.4% in the leaves of Shekhar-1 at 10 µM (Table 3). The evaluation of MDA, H_2O_2 and O_2^- concentration showed that these reactive species were strongly enhanced in iron deficient condition with highest value in IPU-073 in comparison to control and iron excess. Therefore, the result obtained from the experiment indicates that plants generate more ROS in iron starvation. Leguminous plants are great source of antioxidants like ascorbates and glutathiones in their root nodules. These provide antioxidant defense against number of oxidative damage. However, excess of transition metals like Fe^{2+} in the tissues leads to formation of peroxide and superoxide radicals (Moran et al, 2015). In the present work, excess Fe at 2 mM produced similar results with higher peroxide and MDA content. Similar kind of observation was also recorded in other crops such asrice (Kar *et al.* 2019).

Table 3: MDA content, H_2O_2 content and Superoxide radical (O_2^-) (µM g-1 FW)in blackgram roots and leaves under iron deficit and excess stress condition at 48 hours

Variety	Fe2+ Conc.	MDA		H_2O_2		O_2^-	
		Root	Leaves	Root	Leaves	Root	Leaves
IPU-073	Control	0.14±0.02	0.14±0.05	0.11±0.009	0.062±0.008	2.29±0.01	2.08±0.008
	2mM	0.35±0.01**	0.41±0.05**	0.91±0.02***	0.19±0.03**	3.70±0.20*	3.98±0.59**
	10µM	0.59±0.07***	0.83±0.03***	1.00±0.08***	0.48±0.009***	4.61±0.38***	5.33±0.33***
Shekhar-1	Control	0.15±0.27	0.14±0.001	0.15±0.002	0.07±0.002	2.44±0.09	2.10±0.05
	2mM	0.19±0.14	0.34±0.02*	0.47±0.02**	0.15±0.01	3.11±0.25	3.46±0.21
	10µM	0.21±0.005	0.54±0.03**	0.51±0.02***	0.34±0.03***	3.86±0.26*	4.16±0.11**
Source of Variation					*F*-value		
Variety		40.54***	13.79***	83.33***	12.01**	4.20	5.39*
Stress		29.36***	95.16***	145.28***	149.13***	31.29***	41.04***
Variety x Stress		17.51***	7.15***	25.93***	6.88**	2.04	2.06

Excessive ROS yield can lead to the chlorophyll breakdown. Chlorosis and bronzing are the two phenomenon which becomes visible on the surface of the leaves when plants are exposed to iron deficiency and excess respectively which indicates the onset of the oxidative stress. Chlorophyll content are regarded as the stress induced biomarker which can help in the evaluation of stress induced toxicity in field crops. Total chlorophyll and carotenoid content decreased significantly in both IPU-073 and Shekhar-1 under 10µM than 2mM of iron doses. The decline rate of total *Chl* was 46.4% in IPU-073 and only 10% decline was recorded in Shekhar-1 under 10µM of iron stress. A decline of 44.3% and 17.6% in carotenoid content was recorded in the leaves of IPU-073 and Shekhar-1 respectively under 10µM. However, there was less decline rate recorded in plants grown under 2mM iron concentration (Table 4). Total chlorophyll and carotenoid content were found higher in Shekhar-1 than IPU-073 indicating relatively tolerance against oxidative stress. Similar kind of results was also found in green gram (Verma and Pandey, 2017), groundnut (Mann *et al.* 2017).

Table 4: Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content (nM g^{-1}Fw) in blackgram roots and leaves under iron deficit and excess stress condition at 48 hours

Variety	Fe^{2+} Conc.	Chl *a*	Chl *b*	Total Chl	Carotenoid
	2mM	11.57±0.22***	6.94±0.16*	18.69±0.32***	1.06±0.01***
	10µM	9.80±0.45***	4.75±0.73***	14.70±0.70***	0.82±0.04***
	Control	16.69±0.82	10.14±0.29	21.18±0.45	1.40±0.09
Shekhar-1	2mM	14.27±0.24*	7.26±0.22**	19.76±0.47*	1.18±0.02*
	10µM	12.55±0.25***	7.01±0.27***	19.57±0.47*	1.16±0.007*
Source of Variation		*F*-value			
Variety		14.96***	17.21***	0.07	11.59**
Stress		116.03***	53.70***	222.40***	54.31***
Variety x Stress		16.32***	3.59	107.20***	10.45**

It may be concluded from the results, that iron deficit or excess conditions drastically reduced the plant growth and accumulation of biomass stimulate chlorosis and oxidative stress especially iron deficit condition. The results clearly indicated that Shekhar-1 showed minimized oxidative damage rather than IPU-073 due to the better defense mechanism against both iron deficiency and excess conditions.

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