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Iron induce different changes at different stages of the development of *Brassica nigra*

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ABSTRACT

Heavy metals are of great importance and have a notable adverse effect leading to hazardous crop growth. Plants have a susceptibility to heavy metal toxicity and respond to avoid detrimental effects in a variety of crops. Our study is revealed that the metal toxicity after elevating on particular concentration and different stages of development. The seeds of *Brassica nigra* were used to evaluate the effect of iron (Fe) on different physiological parameters at different stage of development. The effect of Fe was studied with regard to seed germination, root length, leaf area and total chlorophyll under 50 µM, 100 µM and 200 µM of concentrations. These concentrations significantly affect the seed germination, root length, leaf area and total chlorophyll. Treatment was given from seed stage to the mustard seeds for evaluation of germination percentage in petridish, Fe exhibited a reduction in seed germination and root length but when treatment was given into pot after three leaves emerging stage, Fe was not exhibited toxic effect up to at 200 µM concentration.

Key words: *Brassica nigra,* Heavy Metals, Iron

1. INTRODUCTION

A diverse range of elements cause pollution and is made pollutant constitute called heavy metal. Heavy metals have the atomic number from 22 – 92 (Sankar Ganesh, 2008). Micro- organisms, plants and animals take micronutrient such as Mn, Cu, Zn, Mo and Ni for essential metabolism but at high concentrations, they cause atoxic effect. Crop productivity and growth are adversely affected owing to heavy metal stress (Lenntech, 2004).

Fe is an essential nutrient for plants, required in many functions such as chlorophyll biosynthesis, respiration and photosynthesis. In plant hormone synthesis, electron transport reaction, Fe takes part to make enzyme cofactor part. Deficiency of Fe in plants is caused chlorosis (Salamaet al., 2009). Excessive of Fe is caused oxidative stress which synthesize reactive oxygen species (ROS) superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical that cause biological and abiotic stress (Polle and Rennerberg, 1993). Iron storage protein ferritin, is located in chloroplast which trap iron in the soluble nontoxic form. It maintains the iron concentration between the ferritin and aqueous environment for biochemical reaction as well as minimizes radicle chemistry and reactive oxygen species (Hintze, 2006). Deoxymugenic acid (DMA) is the precursor of all phytosiderophores and it has the ability to chelate Fe^{3+} and form Fe^{3+}-complex. Fe^{3+}-DMA transporter is found in root cells which increase the Fe uptake efficiency in root cells Hossain et al.,(2018).

Brassica nigra(mustard) belongs to the family Brassicaceae, and whole seeds were used in pickles and salads as a spice and preservative."Black mustard contains about 1% sinigrin (allylglucosinolate), a thioglycoside-like compound (a so-called *glucosinolate*) of allylisothiocyanate with glucose. By action of the enzyme myrosinase, allylisothiocyanate, a pungent, lachrymatory and volatile compound, is liberated (0.7% of the dried seed)" (Katzer, 2013).

2. MATERIALS AND METHODS

Plant material and treatments

The plant material used in our study was *Brassica nigra*(var. 1021) procured from Agricultural seed Company, Belanganj, Agra. The seeds were first washed with distilled water and were then soaked in 5% bavistin (a systemic fungicide) for 15 minute followed by washing with distilled water for 4-5 times. After this seeds were surface sterilized with 0.1% $HgCl_2$ for 1 minute followed by washing with distilled water for 3-4 times.

Petridish experiment

Petridishes were sterilized by keeping them in hot air oven at 150^0c for 2 hour. Sterilized petridishes were lined with filter paper. Solution of different concentrations of Fe (50µM, 100 µM and 200 µM) were prepared. Ten surface sterilized seeds were kept in each petridish at room temperature (28 ± 2^0c). Distilled water was used as control. The seeds were allowed to germinate for studying seed germination percentage and biomass for 10-15 days. Seeds showed germination when radical emerged by about 5 mm in length (Agnihotri *et al*., 2006). Three replicates of each treatment were maintained after 12days germination percentage and root length was recorded.

Pot culture experiment

The surface sterilized seeds of *Brassica nigra* were sown in earthen pots containing 4:1 soil and farm yard manure (FYM). The plants were kept in green house. The seedlings attained 2-3 leaves stage, long Ashton nutrient solution was supplied to the plant daily. When the plants attained 3-4 leaved stage, metal treatment with three replicates in each group were supplied with 50 µM, 100 µM, 200 µM of Fe solutions supplied as $FeCl_3.6H_2O$. All the treatments groups along with control were arranged in a completely randomized design. The metal treatments at the rate of 150 ml per pot were supplied along with nutrient solution twice a week followed by irrigation with distilled water. These samples were further used to determine other physiological and biochemical parameters.

Germination percentage

Seed germination was recorded daily up to 14 days after the initial day of the experiment. Seeds were considered as germinated when the radical length attained 5mm or more (Agnihotri et al., 2006) and the germination percentage was calculated

$$\text{Germination percentage} = \frac{Number\ of\ germinated\ seed}{Total\ number\ of\ seeds} \times 100$$

Root length

Root length was measured with the help of scale (Heenan et al., 1988) at fixed time intervals from treated and controlled plants.

Leaf area

Leaf area was determined by using standard graph paper method. The leaves outlined and squares covered under the outline of leaf were measured under length and width (Taghipour and Saheli, 2008). Average of five leaves was taken per treatment from triplicate for observation.

Estimation of chlorophylls

Extraction and estimation of chlorophyll was done in leaf according to the method of Arnon (1949). Fresh leaf samples (250 mg) of treated and control plants were immersed in 20 ml of 80% acetone and stored overnight. Next day, samples were crushed by mortar and pestle and centrifuged at 5000 rpm for 10 min. The volume of the supernatant was maintained up to 25 ml with 80% acetone. Absorbance was taken at 663 and 645 nm using UV- VIS 117 Systronics spectrophotometer.

Statistical analysis

All experiments were carried out with three independent repetitions in triplicates. Values were expressed as mean ± standard error (SE).

3. RESULT AND DISCUSSION

Table 1 shows the effect of Fe on seed germination. On Comparing with control, it is clear that Fe caused a more toxic effect on plants. At 50 µM concentration Fe increased the germination up to 8.69% but at 100 µM Concentration Fe exhibited 0% increase. At 200 µM, Fe exhibited 30.43% reduction. NA (Nicotianamine) and DMA protein take part in germination of seeds (Bashir et al., 2010). Fe plays a pivotal role in the transport of NA and DMA in dorsal vascular bundle, aleurone layer and endosperm (Takahashi et al., 2009) and 5% of Fe is stored in the center of ferritin protein (Ravet et al., 2009). Ferritin protein protects cells from oxidative stress (Spertto et al., 2010). Excess of free iron is formed superoxide anions (O_2^-) which interact with oxygen and due to degrading unsaturated lipid component and it damage cell membrane (Teiz and Zeiger, 2002). This toxicity causes a sophisticated mechanism is developed to control and alter Fe transport (Bashir et al., 2010).Seed germination inhibited by Fe has also been reported by many workers (Jaja and Odoemena, 2004; Hatamzadeh, 2012).

Table 1 The effect of Fe on germination percentage, root length, leaf area and chlorophyll content

Treatment	Concentration in µM	Treatment from seed stage		Treatment after given three leaves stage	
		Germination percentage Avg	Root length In cm Avg	Leaf area In cm^2 Avg	Total chlorophyll In mg/gAvg
Control	0 µM	76.66	5.72 ± 0.22	7.31 ± 0.28	0.66 ± 0.05
Fe	50 µM	83.33	5.83 ± 0.58	8.57 ± 0.13	0.79 ± 0.05
	100 µM	76.66	4.94 ± 0.30	8.47 ± 0.13	0.84 ± 0.20
	200 µM	53.33	1.41 ± 0.27	7.49 ± 0.44	1.59 ± 0.04

Avg=Average

Root length was increased by 1.9% at 50µMrespectively over control but it started to decrease from 100 µM concentration and maximum reduction of length by 75.34% was found at 200 µM concentration. Slightly acidic pH (5.5 and 6.5) is favoured for root growth (Teiz and Zeiger, 2002). Erakhrumen (2015) reported that Fe accumulated in roots in comparison to the surrounding. Excess of iron accumulation in root is caused in acidic medium (Moraghan, 2002) and at early seedling stage, required less Fe in comparison to growing seedling (Ellsworth et al., 1997). Reduction in root length was also observed with increase $FeCl_3$ concentration (Alia, 2015).

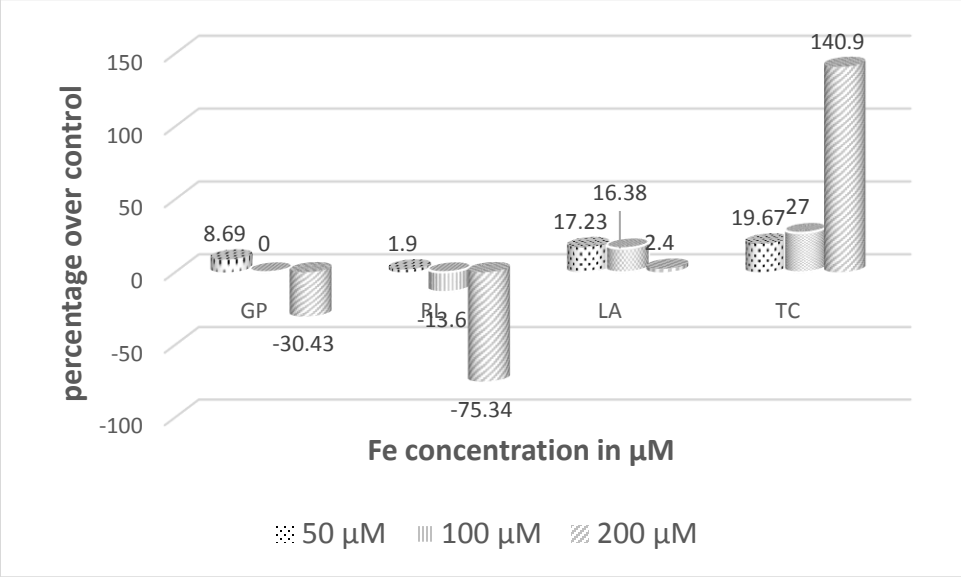

Figure 1 Depict the effect of Fe on percentage of germination (GP), length of root (LR), area of leaf (LA) and total chlorophyll (TC)

Iron exhibited 17.23%, 15.86% and 2.4% increase in leaf area at all concentrations respectively over control. Nenova (2006) reported that the leaf area of pea was increased under high Fe concentration. Total chlorophyll increased by iron, exhibited 0.79 mg/g, 0.84 mg/g and 1.59 mg/g at 50, 100 and 200 µm concentrations respectively over control (0.66 mg/g) which was promoted up to 19.67%, 27% and 140.9%. In soil, Fe is present as insoluble ferric ion but roots develop reduction mechanism of Fe^{2+} (Teiz and Zeiger, 2002). Wu (2014) reported that *Rubrivivaxgelatinosus'*s biomass and yield increased by Fe^{3+}reduction. Reduced iron Fe(II) and magnesium play a pivotal role in chlorophyll synthesis, photosynthesis and growth (Willson, 2016). Semin and Seibert (2016) reported that Fe II reduced to Mn of the oxygen evolving complex in dark and removes 2 Mn ions from water splitting complex and newly formed oxidized Fe III makes a complex with released 2 Mn ions and form hetero-nuclear clasterPSII and this complex act as water oxidation center of photosynthetic reaction. Nenova (2006) also observed that total chlorophyll content increased under iron treatment. Kumar and Chopra (2013) also reported that chlorophyll content increased by Fe at all concentrations respectively over control when treatment was given after emerging leaves. In Indian rice (*Oryzasativa* L.) Cultivar "swar and KalingaIII" chlorophyll content was increased with increased ion concentration of iron up to $5ml^{-1}$ (Panda, 2012).

4. CONCLUSION

Given metal treatment at different stages has shown a significant difference of Fe in *Brassica nigra* in regard to playing role at physiological and biochemical parameters. Fe showed a reduction in germination percentage and root length after 100µM concentration but no inhibition effect on leaf area an4d chlorophyll content. Thus this study has shown that Fe expresses negative effect on germination and seedling growth when seedling was in the cotyledonary stage but expressed positive effect on seedling, treatment was given after emerging four leaves.

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