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Research Paper

PHYSIOLOGICAL AND BIOCHEMICAL INDICES IN RESPONSE TO MANGANESE DEFICIENCY IN PEA

Priyanka Singh and Nalini Pandey

Plant Nutrition and Stress physiology Laboratory,
Department of Botany,
University of Lucknow,
Lucknow (U.P.) India.

Abstract

Pea plants (*Pisum sativum* L. var. A-2001) treated with variable concentrations of viz; (0.5, 1, 5, 10 µM Mn) in form of $MnSO_4.H_2O$ under controlled glass house conditions were analyzed for different physiological parameters. At 15 and 25 days of exposure, the plants were harvested for growth, relative water content, proline and enzymes. Plants showed maximum growth at 10 µM Mn supply and this level was treated as control. At 0.5 µM Mn plants showed maximum reduction in growth. High concentration of proline content in leaves were detected in Mn deficient plants as compared to control. Under Mn deficiency the activity of catalase (CAT) was lower and peroxidase (POD) were significantly increase than in the control. The total chlorophyll and carotenoid were significantly decreased with decreasing Mn concentration. The results indicate that under Mn stress condition plant suffer from water stress and change in the activities of antioxidative enzymes.

Key words: *Antioxidative enzymes, Pisum sativum, Mn deficiency, water stress*

INTRODUCTION

Manganese (Mn^{2+}) is an essential plant micronutrient and it is the eleventh abundant element forming the earth's crust. It is required by the plants in the second greatest quantity as compared to Fe. It is absorbed mainly as Mn (II) being fairly stable and translocated as a free divalent cation in xylem from root to shoot. Divalent manganese ion (Mn II) can readily be oxidized to Mn (III) and Mn (IV). Therefore, in plant, Mn play an important role in redox process [1, 2].

Manganese play a vital role in photosynthesis, as a structural component of PS II. It also helps in electron storage and delivery to the chlorophyll reaction center [3]. Mn

acts as an activator and cofactor of various enzymes in plants [4]. It activates many enzymes which are involved in carboxylation [5], carbohydrate metabolism, phosphorous reactions and citric acid cycle and oxidative stress.

The objective of the present study was to evaluate the effect of deficient Mn supply in pea plants at varying concentration and at different growth stages.

MATERIAL AND METHODS

Pea (*Pisum sativum* L.var.A-2001) plants were grown under glass house conditions in purified silica sand in polyethylene pots with a central drainage hole covered with glass wool under an inverted watch glass that allowed free drainage. The composition of nutrient solution excluding Mn was 4 mM Ca $(NO_3)_2$, 4 mM KNO_3, 2 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 0.33 mM H_3BO_3, 1.0 µM $ZnSO_4$, 1.0 µM $CuSO_4$, 0.1µM Na_2MoO_4, 0.1 M NaCl, 0.1 µM $CoSO_4$, 0.1 µM $NiSO_4$, 100 µM Fe EDTA and Mn supplied at four level (0.5, 1.0, 5.0, 10 µM Mn) in form of $MnSO_4$.

After the germination of seedlings, nutrient solution including Mn at desired level were supplied daily. Plants were examined periodically for changes in growth parameter and visible symptoms of Mn deficiency were recorded. After 15- and 25-day plants were analyzed for physiological parameters.

Plants was sampled for determination of dry matter production and tissue Mn concentration. Total biomass was determined by oven drying the samples in electric oven at 70°C for 48 hrs. The tissue Mn concentration were estimated in oven dried leaf sample after digestion with HNO_3: $HClO_4$ (10:1).

Chlorophyll and carotenoids were estimated in comparable young leaves. The extraction and centrifugation of with 80% acetone and measured spectrophotometrically Perkin Elmer UV/VIS Lambda Bio 20 [6].

Catalase (CAT) was extracted by homogenization of fresh leaf tissue in ice cold distilled water (1:10) with a chilled mortar and pestle. The reaction mixture containing 0.005 M H_2O_2 and 0.025 mM phosphate buffer (7.0) was incubated with 1 ml of suitably diluted enzymes extract at 25°C for 5 min. The reaction was stopped with 2 ml 2N H_2SO_4 and mixture was titrated against 0.1 N $KMnO_4$ [7].

Peroxidase (POD) was extracted by homogenization of fresh leaf tissue with ice cold glass distilled water in a clean, chilled motor and pestle. The homogenate was stained through four layer of muslin cloth. The reaction mixture for POD contained 2 ml of 0.1 µPO4 buffer (6.0), 1ml of 0.01 % H_2O_2 and 1 ml of 0.5 % p-phenylenediamine. The reaction was started by adding 1 ml of enzyme extract to mixture. After 5 min incubation at 25^{o}C, the reaction was stopped with 2 ml 4 N H_2SO_4. Optical density was measured at 485 nm change in OD, was calculated [8].

For Proline assay, fresh leaves were homogenized in sulphosalicylic acid. After filtration a suitable aliquot was taken with ninhydrin reagent and glacial acetic acid and boiled for 1 hour. The color was extracted in toluene and read at 520 nm [9].

Fresh disc of equal size was weighed to determine fresh wt. and then placed in Petri dish for 4 hours at 5^{o}C.Therafter they are placed in an oven at 24 hrs. and reweighed again to dry wt. The Relative Water Content (RWC) [10] was calculated by the formula –

RWC = (F.W– D.W/ T.W.– D.W) × 100

The data have been analyzed statistically (ANOVA) for significance (LSD at P = 0.05). The data are presented as mean values ± standard error (SE, n=3).

RESULTS AND DISCUSSION

Optimum growth was observed in pea plants receiving 10 µM Mn as is evident by decrease in growth and dry matter yield of plants receiving less than 10 µM Mn. This was also evident by decrease in concentration of Mn in leaves of the pea plants. Deficient concentration of Mn resulting in growth retardation and significant decline in dry matter yield in pea plant was observed earlier [11, 12]. Maximum decrease in yield was observed in pea plants supplied with 0.5 µM Mn. The concentration of Mn in leaves was decreased as compared to control. Maximum decrease in the tissue Mn concentration. was observed in leaves with 0.5 µM at 15 days [13]. The results of dry matter yield (DMY) and tissue concentration at both the stages are presented in Table 1.

In this experiment it was observed that the concentration of chlorophyll in leaves of pea plants was significantly decreased with decreasing concentration of Mn from 10 to 0.5 µM at both the stages (Fig 1). This result indicates a critical role of manganese ion

as a cofactor in photosynthetic light dependent reactions [14]. Carotenoid concentration was also decreased with decreasing Mn concentration as compared to control (Fig 1). [15]. Lower chlorophyll and carotenoid concentration are an indicator of senescence, stress and damage to plant and the photosynthetic apparatus, expressed by faster breakdown of chlorophyll than carotenoid [16].

The relative water content (RWC) declined significantly in the leaves of Mn deficient pea plants as compared to control (Fig 2) [17]. Least RWC was reported in 0.5 um Mn supply. This result indicated that Mn deficient plants shows susceptibility to drought stress because Mn deficiency reduced the waxy content that increased transpirational water loss and lower water use efficiency [18]. The concentration of proline was increased with low concentration of Mn at 15 days and 25 days in leaves of pea plants (Fig 2). This also indicates water stress in Mn deficient plants [19,12].

During the treatment period, at 15 days and 25 days the CAT activity was decreased in Mn deficient plants and shows the inability of the plant to overcome the oxidative damage (Fig 3) [15]. The POD was increased as a result of Mn deficiency (Fig 3) but the increase was more pronounced at 15 day than at 25 days as compared to control [20].

Table 1: Effect of manganese deficiency on the dry matter yield and tissue manganese concentration in *Pisum sativum* L. var. P-2001 grown in pot culture.

Days after treatment	Plant part	µM manganese supply			
		0.5	1.0	5.0	10
		Dry matter yield: mg plant^{-1}			
15	Leaves	0.189±0.031	0.224±0.006	0.254±0.03	0.369±0.001
25	Leaves	0.215±0.01	0.353±0.02	0.428±0.001	0.687±0.01
		Tissue manganese: µg g^{-1}dry wt.			
15	Leaves	10.10±0.002	15.19±0.006	20.06±0.008	30.01±0.301
25	Leaves	12.09±0.005	18.06±0.008	24.12±0.02	35.10±0.510

CONCLUSION

This study concludes that manganese deficiency in pea plants induce changes in plant growth and metabolism. During the treatment, manganese stressed plants exhibit water stress and a complex defense mechanism in response to oxidative stress.

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