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Full Length Research Paper

Influence of lanthanum on biochemical constituents and peroxidase activity of cowpea (*Vigna unguiculata* (L.) Walp.)

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The effect of various concentrations (0.1, 0.5, 2.5, 12.5 and 62.5 µg g^{-1}) of Lanthanum as Lanthanum nitrate added to soil was studied on the content of foliar chlorophyll (Chl. 'a', Chl. 'b', Total chl.), soluble leaf protein, total phenol contents and peroxidase activity of cowpea [*Vigna unguiculata* (L.) Walp.]. Lower applications (0.1 to 2.5 µg g^{-1}) of La resulted in enhancement in foliar chlorophyll contents (Chl. 'a', Chl. 'b', total chl.) and a decrement in soluble leaf protein and total phenol contents. A significant (at p=0.05 level of significance) increase in the activity of peroxidase enzyme at higher concentrations (12.5 to 62.5 µg g^{-1}) of lanthanum was observed.

Key words: Lanthanum nitrate, foliar chlorophyll, total phenols, soluble leaf protein, POD activity, cowpea.

INTRODUCTION

REEs include 17 elements of the 6^{th} period of the III rd group in the Periodic system with Scandium (21), Yttrium (39) and lanthanides (57-71). These REEs frequently occur together in rare earth minerals and have similarities in ionic radii and physical /chemical activities (Henderson, 1984). Lanthanum (La) and Cerium (Ce) are the main components of commercial REEs micro fertilizer and are widely used in China since 1970s. Lanthanum and Cerium belong to the group of light rare earth elements because of their atomic mass lower than 153 amu. Numerous studies suggest that REEs as micro fertilizer promote seed germination, stimulate growth of roots, increase crop yields and at low concentrations REEs result in an increase in O_2 evolution, chlorophyll and chlorophyllase synthesis and increases the activity of PS-I and PS-II (Xie et al., 2003).

Chen et al. (2000) reported positive effects of REEs on the crop production such as faster development, greener/dark foliage, larger roots, and better fruit color and quality in different species.

POD has been considered as a defensive enzyme and is able to protect the cells from active oxygen damage. It has been observed that after applying REEs the POD activity in plants increases which resulted in an increase in the environmental stability of the crop vis-à-vis the yield (Wang, 1996).

Keeping the above facts in view, experiments were undertaken to study the effects of Lanthanum (La) on different biochemical constituents and POD activity of cowpea, an important leguminous agricultural crop of this region.

MATERIALS AND METHODS

Certified seeds of cowpea [*Vigna unguiculata* (L.) Walp. (Var. Sephali Sikha- 313)] were obtained from Udaipur Krishi Kendra, Udaipur. Three kilograms of soil were filled into earthen pots of 30 cm height and 25 cm diameter. The soil was silty sand. Five concentrations (0.1, 0.5, 2.5, 12.5, 62.5 µg g^{-1}) of La were applied as lanthanum nitrate ($LaN_3O_9.6H_2O$) which were prepared separately by taking corresponding amounts (Calculated on the basis of molecular weight) of the chemical/ kg of air dried soil. Pots without added La constituted the control.

Experiments were set up during the month of July under natural

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Abbreviations: POD, Peroxidase **; ROS,** reactive oxygen species; **REEs,** rare earth elements; **UV,** ultraviolet.

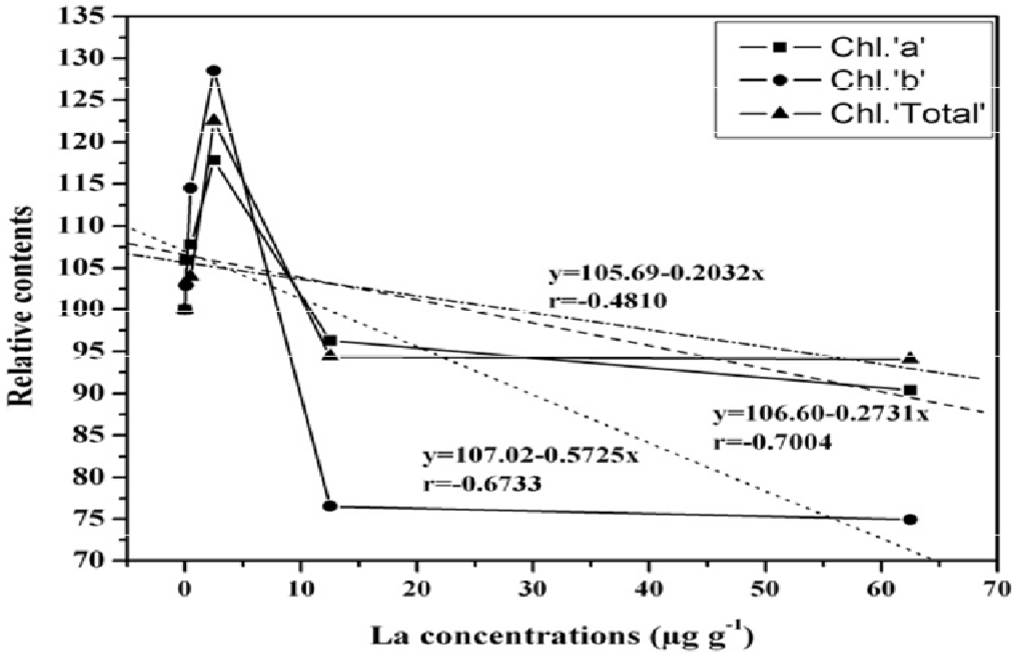

Figure 1. Showing the effect of various concentrations of Lanthanum on chlorophyll contents of *Vigna unguiculata* (L.) Walp.

conditions. Maximum and minimum temperature during the study period was 31.16^{0}C and 24.54^{0}C, respectively and photoperiod was 12 h.

Fifteen seeds of *Vigna unguiculata* (L.) Walp. were sown equi-distantly at 2 cm depth in each pot. Watering was done on alternate days. 200 ml water was added in each pot. After establishment, seedlings were thinned to 10 in each pot. Sets in triplicate were prepared to record observations during the life stage.

Fresh leaves of seedling were used for biochemical analysis. Foliar chlorophyll was determined according to Arnon (1949) and soluble leaf proteins and total phenol contents and POD activity were estimated respectively, after Bradford (1976) and Mahadevan and Sridhar (1982).

Enzyme assay

Leaf tissues (0.5 g) were homogenized in 10 ml ice-cold 0.1 M Phosphate buffer (pH 6.0).The homogenate was centrifuged for 30 min at 2000 g at 4^{0}C. The supernatant (enzyme source) was used for assaying the enzyme activities. 3 ml of 0.05 M pyrogallol and 0.1 ml of supernatant were taken in a clean dry cuvette which was transferred to a spectrophotometer. In order to start the reaction 0.5 ml of 1% H_2O_2 was added to the cuvette. Initial absorbance and then change in absorbance was noted after an interval of 30 s for 3 min at 420 nm in a UV-visible spectrophotometer (PHARMASPEC UV 1700, SHIMADZU).

RESULTS AND DISCUSSION

Lanthanum affects the biochemical constituents of *Vigna unguiculata* (L.) Walp. The effects of various concentrations of lanthanum on foliar chlorophyll contents of cowpea are presented in Figure 1.

REEs mainly La, results in an increase in the chlorophyll contents (both Chl. a and b), which was 4.7 and 31.8%, respectively in sugar beets (Xie and Chen, 1984) when seeds were mixed with rare earths. In the present study the maximum enhancement in foliar chlorophyll (Chl. 'a', Chl. 'b', Total Chl.) contents was observed at 2.5 µg g^{-1} concentration of lanthanum. The increase in chlorophyll 'a' (Chl. 'a'), chlorophyll 'b', (Chl. 'b') and total chlorophyll were 17.8, 28.4 and 22.5% respectively, over the controls. Chen et al. (2001) reported that in tobacco seedlings La^{3+} promoted the content of chlorophyll at low (5 to 20 mg/L) concen-trations, but inhibited it at higher concentrations. Fashui et al. (2003) reported that rice plant treated with 20 to 1500 µg/ml La $(NO_3)_3$ showed a significant increment in chlorophyll contents. Shi et al. (2005) reported that La^{3+} at 0.002 to 0.2 mM concentration promote plant growth and showed an enhancement in chlorophyll a and b in cucumber seedlings (*Cucumis sativus* L.).

In soybean plant, REEs (5 to 10 µg/g) increased the content of photosynthetic pigments and chloroplast in aquaculture experiments. Increase in chlorophyll a and b contents were observed in wheat leaves after the plants were treated with individual rare earth elements and a mixture of light rare earths (Jie et al., 1985). Liao et al. (1994) indicated that the effect of REEs on the chlorophyll contents of spinach was to increase the

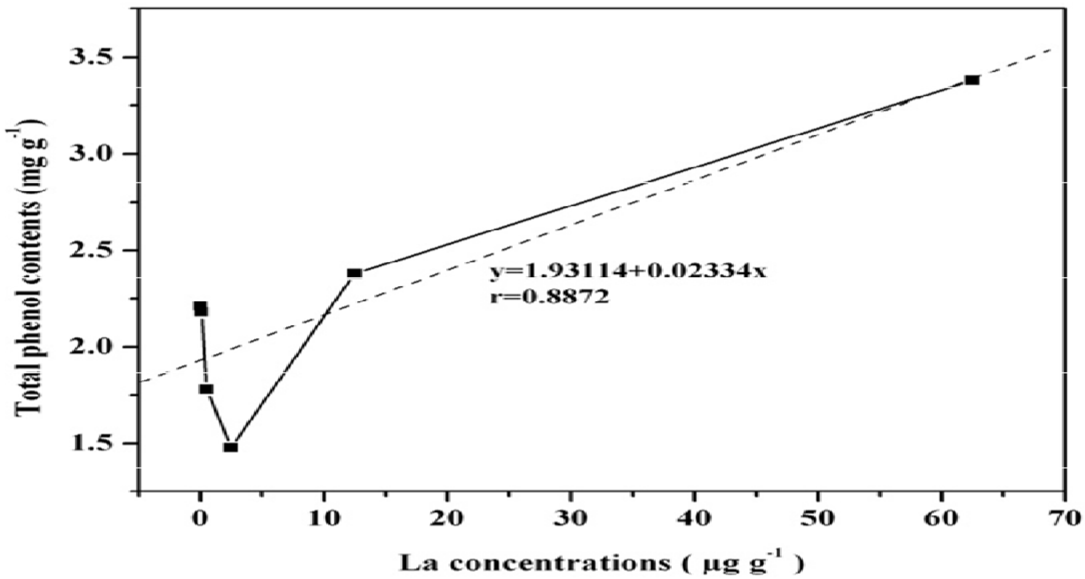

Figure 2. Showing the effect of various concentrations of Lanthanum on total phenol contents of *Vigna unguiculata* (L.) Walp.

absorption of nitrogen and phosphorus and induce greatly the synthesis of a precompound of chlorophyll and reported that some REEs are catalyst and play an indirect role in chlorophyll formation. Further, La^{3+} may substitute Mg^{2+} for chlorophyll formation under Mg^{2+} starvation (Hong et al., 2002).

In the present studies higher concentrations of La (12.5 µg g^{-1} and 62.5 µg g^{-1}) resulted in a decrease in chlorophyll (Chl. 'a', Chl. 'b', Total Chl.) contents, over the control. Wang et al. (2007) have also observed that La and Ce reduce chlorophyll content in *Hydrilla verticillata.* Reduction in chlorophyll content (Figure 1) may be due to the formation of chlorophyllase, which is responsible for chlorophyll degradation (Sabater et al., 1978; Mali and Aery, 2009), as well as damage to the photosynthetic apparatus.

Correlation coefficients (r) and regression equations (y) were computed for applied La concentrations and Chl. 'a', Chl. 'b', total Chl., soluble leaf protein contents and total phenol contents. The negative correlation coefficient (r) value indicated the degree of toxic effects of La on the chlorophyll contents. Positive values for correlation exist between La concentration and soluble leaf protein and total phenol contents.

Phenols are known to provide resistance to plants in various fungal, bacterial and viral infections and also under other stressed conditions. Phenols are the known fungal toxic substances which inhibit the growth of the pathogens and retard the pectin and cellulolytic enzyme production by the pathogens (Lyr, 1965).

A decrement in total phenol contents, over the control, was observed up to 2.5 µg g^{-1} La level, which was 14.5%, where the maximum enhancement was observed at 62.5 µg g^{-1} La level (Figure 2). This enhancement was significant at $p = 0.01$ level of significance.

Proteins are the structural components of cells and many of them are functionally active as enzymes. La^{3+} toxicity affect the soluble leaf protein contents. In the present study, higher dose of La^{3+} resulted in an increment in soluble leaf protein contents. Maximum enhancement (Figure 3) in soluble leaf protein contents was observed at 62.5 µg g^{-1} dose of La. This enhancement was significant at $p=0.05$ level of significance. Wang et al. (2007) observed a decrease in soluble proteins at higher levels (30 to 100 µM) of REEs (La and Ce) and attributed the reduction in soluble proteins content to toxic effects of ROS, which are especially prone to attack protein, resulting in protein degradation (Davies, 1987).

Minimum soluble leaf protein content was observed at 2.5 µg g^{-1} level of La which was 7.2% lower, over the control in cowpea. The amounts of proteins in the leaf at any time reflect the net results of its rate of synthesis and breakdown of structural or insoluble proteins (Mukhopadhyay and Aery, 2000).

Peroxidase is an antioxidant enzyme and belongs to a large family of enzymes. It is related to lignin and suberin synthesis, which increase the hardness of tissues and to the production of quinones and active oxygen (Bowles, 1990). The results of various experiments have proved that REEs can result in an increase in POD activity in crop plants. An increase in the POD activity has been

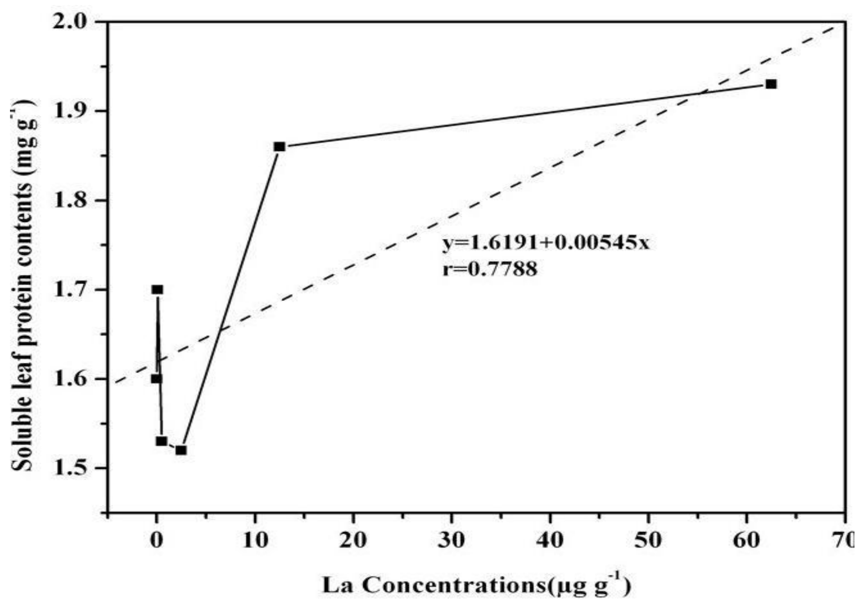

Figure 3. Showing the effect of various concentrations of Lanthanum on soluble leaf protein contents of *Vigna unguiculata* (L.) Walp.

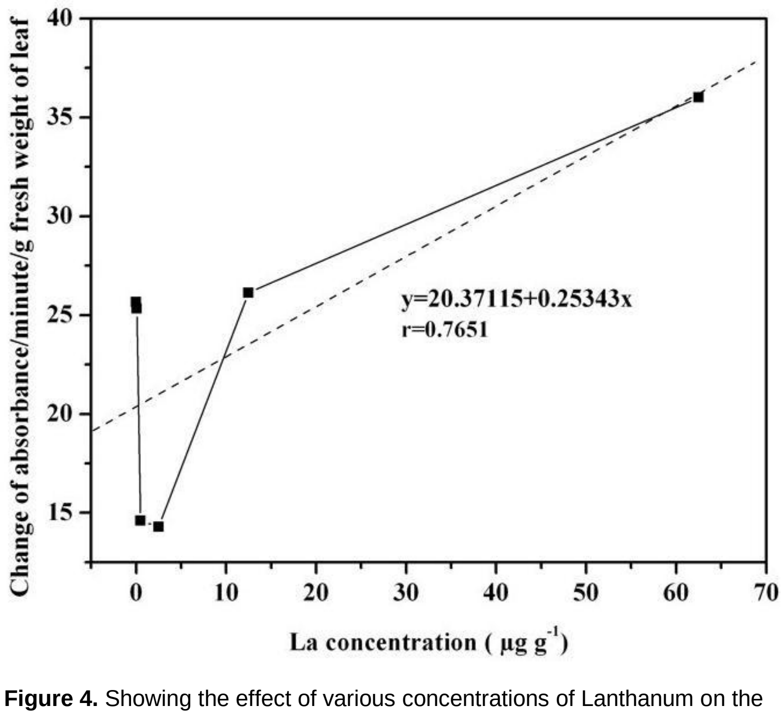

Figure 4. Showing the effect of various concentrations of Lanthanum on the activity of peroxidase enzyme in *Vigna unguiculata* (L.) Walp.

observed at 0.4 µmol l^{-1} level of Lanthanum, in the tea shoots (Wang et al., 2003) and at 0.02 mM La^{3+} level in cucumber seedlings (Shi et al., 2005).

POD activity was found to be minimum at 2.5 µg g^{-1} concentration of lanthanum which was 7.2% lower, over the control. Higher doses of lanthanum (12.5 and 62.5 µg g^{-1}) showed an increment in the activity of peroxidase enzyme (Figure 4). The maximum enhancement in POD activity was observed at 62.5 µg g^{-1} level of lanthanum which was 5.4%, over the control. This enhancement was significant at $p = 0.05$ level of significance. Wang et al. (2007) reported an increment in POD activity at 30 µM REEs level and indicated that POD play a decisive role in eliminating poisonous H_2O_2 in *H. verticillata* under imposed stress.

Under stressed condition the increase in the amount of phenols is accompanied by an enhancement in the peroxidase activity (Figure 4) causing the destruction of auxins (Stenlid, 1976). The increase in POD activity may be used as an indicator of stress (Pandolfini et al., 1992). Lanthanum (La^{3+}) is similar to calcium (Ca^{2+}) in chemical properties. It can substitute bound Ca^{2+} (Ni, 2002) and like Ca^{2+} it may be involved in signal transduction (Buchanan et al., 2002; Wu, 2003) . Replacement of Cerium for calcium and consequent improvement and growth of spinach plant under calcium deficient condition has been reported (Chao et al., 2008).

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