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Effect of copper on germination, protein content and peroxidase activity of *Phaseolus vulgaris*

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ABSTRACT

An attempt was made to investigate the effect of copper on germination, protein content and peroxidase activity in *Phaseolus vulgaris.* Increasing copper concentration does not show significant effect on seed germination. An increase in total protein concentration was found with increase in copper concentration however the increase was more prominent in shoots. Peroxidase was found to enhance in shoots whereas in roots the activity increases upto 50µM after which the activity was found to decrease.

Introduction

Copper is one of the essential micronutrients for plant growth. It occurs as hydrated ionic species forming complex compounds with organic and inorganic legends (Singh *et al.* 2007). Copper is essential for various physiological functions as component of number of enzymes which are involved in electron flow, mitochondrial and chloroplastic reactions (Hansch and Mendel 2009; Manivasagaperumal et al. 2011). Copper can induce several alterations in plant cells and in higher concentrations it becomes toxic and hinders photosynthetic and respiratory process, protein synthesis and development of plant organelles (Manivasagaperumal et al. 2011; Upadhyay and Panda 2009). Excessive concentration causes chlorosis, inhibition of root growth and damage to membrane permeability leading to ion leakage (Berglund *et al.* 2002; Bouazizi *et al.* 2010; Manivasagaperumal et al. 2011) and induced mineral deficiency (Bouazizi et al. 2010; Lequeux et al. 2010). *Phaseolus vulgaris* has been reported as an excellent accumulator of heavy metals such as lead and cadmium (Garay et al. 2000). Copper causes cell damage by free radical formation such as reactive oxygen species which causes an oxidative burst (Gupta and Kalra 2006) and the damage may be alleviated by action of antioxidative enzymes such as peroxidase, catalase and superoxide dismutase (Agarwal et al. 2006; Joseph and Jini 2010). Peroxidases remove peroxide free radicals produced as a result of metal stress. They are involved in several biochemical and physiological functions such as cell growth (Fang and Kao 2000), catabolism of auxin (Passardi et al. 2004) and lignifications (Brownleader et al. 2000). In the present paper an attempt was made to study the effect of copper on seed germination, protein content and peroxidase activity in *Phaseolus vulgaris.*

Materials and Methods

Plant material and treatment with copper sulphate

Experiments were carried out at Department of Basic Science & Humanities, College of Horticulture & Forestry, Central Agricultural University, Pasighat-791102, Arunachal Pradesh, India. *Phaseolus vulgaris* seeds were surface sterilized with 2% sodium hypochlorite solution and were soaked in copper sulphate solutions of different concentrations (0, 10, 50, 100, 200, 500 and 1000µM) for 2 hours. The seeds were then transferred to petri dishes containing cotton bed soaked in respective copper sulphate solutions and were transferred to BOD incubator for germination. Growth parameters like seed germination (after 24 hours), root and shoot length (after 48 and 72 hours) were observed.

Protein Content: Root and shoot protein content after 72 hours was determined by the method of Lowry et al.(1951)

Peroxidase activity: Peroxidase activity was determined by the method of Kochhar et al. (1979)

Results and discussion

Copper is considered as an essential micronutrient for the growth and development of plants. The present study was aimed at the effect of copper on the different parameters of growth and development. Increasing concentration of copper does not show significant effect on seed germination upto 200 µM concentration but decrease in germination percentage was seen in 500 µM and 1000 µM concentration of copper as compared to the control (Table 1).

Copper showed a significant effect on the growth of shoot. A significant gradual decrease in shoot length with increasing concentration of copper was observed after 48 and 72 hrs (Fig.1). A 65% decrease in shoot length as compared to control was observed after 48 hrs at 1000 µM concentration of copper, whereas after 72 hours the decrease was 75% as compared to the control (Fig.1). The reduction in growth might be due to inhibition of mitotic division of meristematic cells (Gabbrielli *et al.* 1990; Bouazizi *et al.* 2007).

Root and shoot protein was estimated by the method of Lowry *et al.* (1951) and the results exhibited an increase in total protein content of roots and shoots with increase in copper concentration (Fig.2), however the increase was more in shoots.

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Table 1. Effect of Copper on germination of *Phaseolus vulgaris*

Concentration of Copper sulphate	% germination after 24 hours
0 µM (Control)	97
10 µM	96
50 µM	97
100 µM	98
200 µM	98
500 µM	90
1000 µM	87

The increase in root and shoot protein might be due to denovo protein synthesis as well as increase in defense related proteins (Bouazizi et al. 2007).

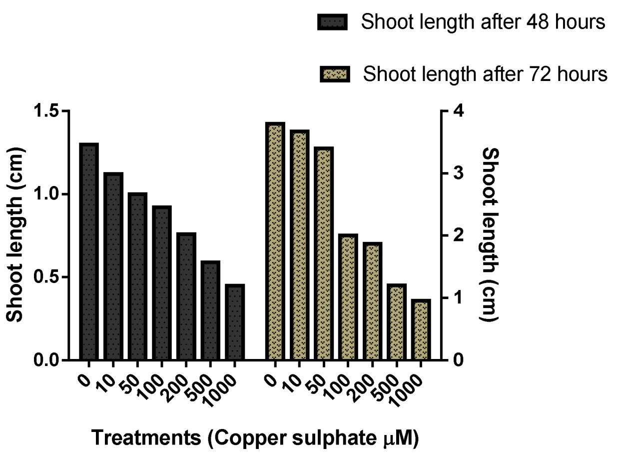

Figure 1: **Effect of copper on shoot length of *Phaseolus vulgaris.***

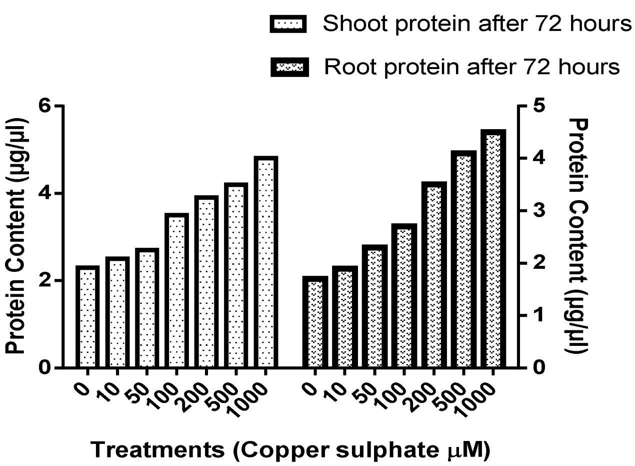

Figure 2: **Effect of copper on total proteins in roots and shoots of *Phaseolus vulgaris*.**

Study on peroxidase activity in *Phaseolus vulgaris* with increase in copper concentration showed an increase in peroxidase activity in shoots. It was interesting to note that the peroxidase activity in roots showed an increase upto 50µM concentration and then a gradual decrease in activity was observed (Fig.3).

Increase in peroxidase activity in shoots can be attributed to enhanced production of hydrogen peroxide during stress condition which in turn activates the primary defense mechanism of the plant (Tanyolac et al. 2007; Gao et al. 2008). These results are in agreement with the results of similar studies with several plant species that suggest increased peroxidase activity in response to elevated copper concentrations (Jouili and Ferjani 2003; Tanyolac et al. 2007).

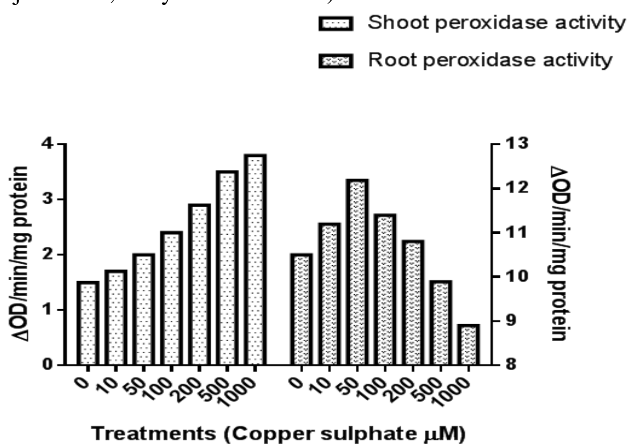

Figure 3: Effect of copper on peroxidase activity in roots and shoots of *Phaseolus vulgaris.*

Peroxidase activity in roots showed the largest increase (about 116.1%) at the copper concentration of 50 µM but further increase in copper concentration results in decline of peroxidase activity. In conclusion the peroxidase activity is more pronounced in shoot as compared to roots which might be due to enhanced hydrogen peroxide production in shoots and more cellular damage in roots.

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