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SHORT COMMUNICATION

EFFECT OF BORON STRESS ON GROWTH, SOLUBLE PROTEIN AND ENZYME ACTIVITIES IN FLUE-CURED TOBACCO

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Sand culture experiment was conducted using Hoagland solution to study the effect of deficient and excess levels of boron (B) on the growth, protein pattern and enzyme activities in flue cured tobacco (*Nicotiana tabacum* L). Boron deficiency resulted in stunted growth, drastic reduction in internodal length and progressive death of shoot apex followed by the growth of lateral shoots. The lateral shoots were also deformed and died subsequently. The leaves of boron deficient plants were thick and brittle. The boron content of the leaf lamina of the deficient plants ranged from 6 to 16 ppm. The plants supplied with excess of boron showed yellowing of leaf tips followed by progressive necrosis. Necrotic symptoms spread progressively towards the leaf margins and midrib. When the symptoms were severe, the bottom leaves dropped prematurely. The boron content of the leaves showing severe symptoms of toxicity was 1950 ppm. Leaf soluble protein content decreased in both deficient and excess levels of boron. The activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase increased by 29, 18 and 14% respectively, in B deficiency whereas, the activity of acid phosphatase decreased by 33%. In excess boron, the activity of peroxidase and acid phosphatase increased.

Key words: **Boron, flue-cured tobacco, oxidative enzymes**

Boron is an essential micronutrient for growth and development of vascular plants and it is of interest in crop production as it is required in optimum levels for high quality crop. Tobacco is an important commercial crop, which has more value for its quality. In recent years the symptoms similar to boron deficiency were observed in tobacco fields and there was positive response to boron application (Anonymous 2006). An attempt was made to document the visual symptoms of boron stress in tobacco and to study its effect on growth and development, leaf protein pattern, enzyme activities and boron content in flue cured tobacco (*Nicotiana tobacum* L.) variety Kanchan.

Tobacco seedlings (var. Kanchan) were grown in 5 kg ceramic pots filled with acid washed quartz sand using modified Hoagland solution (Jhonson *et al*. 1957) with three treatments *viz* control, no boron (0 mM) and excess boron (100 mM).

The plants were carefully observed for the development of visual deficiency symptoms. The green leaf samples collected at 45 days after planting were analysed for total soluble protein (Lowry *et al*. 1951), protein pattern (Laemmli 1970) using sodium dodocyl sulphate ployacrylamide gel electrophoresis (SDS-PAGE), activities of enzymes, viz. peroxidase, polyphenol oxidase (Kar and Mishra 1976), acid phosphatase and phenylalanine ammonia lyase (Sadasivam and Manikam 1991). The plant samples were dried in hot air oven and these were processed and analysed for boron (Gaines and Mitchell 1979). The data

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was analysed statistically to determine ± SEM (Panse and Sukhatme, 1954).

Boron deficiency resulted in stunted growth and death of the growing tip followed by growth of lateral shoots with deformed tips (Fig. 1). The leaves of B deficient plants were thick with a coppery texture, curled and brittle. The leaf blades developed a pronounced crinkling and crackling of the petioles. The young leaves wilted even under the adequate water supply. This may be due to disruption of water transporting tissue caused by boron deficiency. Loomis and Durst (1992) observed that boron deficiency produces plants with weak cell walls. The boron concentration of the leaves showing deficiency symptoms ranged from 6 to 16 ppm. Excess boron resulted in yellowing of leaf tip followed by progressive necrosis. These symptoms first appeared at margins and spread between the lateral veins towards the midrib (Fig. 1) and leaves dropped prematurely. The leaves showing severe symptoms of toxicity contained 1950 ppm boron. As the growth advances in the excess boron, the necrotic spots developed all over the leaf. The leaf margins were desiccated and thin band of dead tissue appeared. At very high concentration even before the chlorosis became quite apparent, necrotic spots developed very rapidly which enlarged and coalesced into bands of dead tissue involving the entire lamina leaving only the mid rib and veins. The dead tissue subsequently dropped out leaving the leaves in rugged appearance. Bangarayya and Narasimhamurthy (1970) reported that the boron toxicity symptoms started with mild interveinal chlorosis followed by stray appearance of necrotic spots at the base and margins of the leaves.

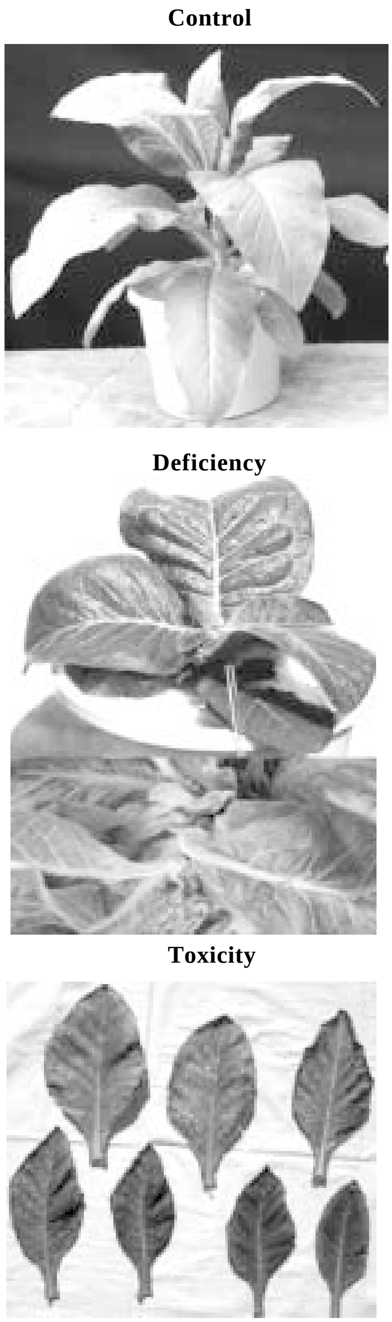

Fig. 1. Effect of deficient and excess levels of boron on flue cured tobacco

Boron deficient leaves recorded more specific leaf weight (5.8 mg cm^{-2}) as compared to control (4.83 mg cm^{-2}), which indicated that the boron deficient leaves were thicker than control (Fig. 3a). Soluble protein content decreased both in B deficient (19 mg g^{-1} fw) and in leaves with excess B (22.45 mg g^{-1} fw) as compared to control (24.66 mg g^{-1} fw). The reduction in protein content may be due to reduced protein synthesis. The electrophoretic protein pattern in both boron deficiency and excess are shown in Fig. 2. The protein content declined in boron deficiency when compared to control and excess, especially low molecular weight protein bands (29 – 14.2kD) were less prominent in boron deficient leaves followed by excess boron and control. Intensity of high molecular weight proteins was more in boron deficient leaves followed by excess boron and control plants.

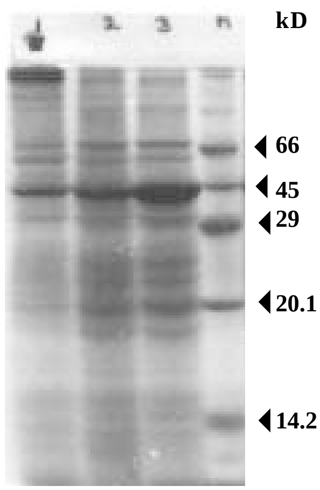

Fig. 2. SDS-PAGE of soluble protein leaf of flue-cured tobacco Lane (1) B deficient, (2) B excess, (3) Control and (M) protein marker

The activity of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase was increased by 29, 18 and 14%, respectively, in response to B deficiency (Fig. 3 c, d, e). The boron deficiency led to the accumulation of phenolics and increase in polyphenol oxidase in sunflower (Pfeffer *et al*. 1998). Polyphenol oxidase catalyses the oxidation of phenolic compounds into quinones, which can react with oxygen leading to the formation of toxic oxygen species which can impair cellular functions (Cakmak and Romheld 1997). With the

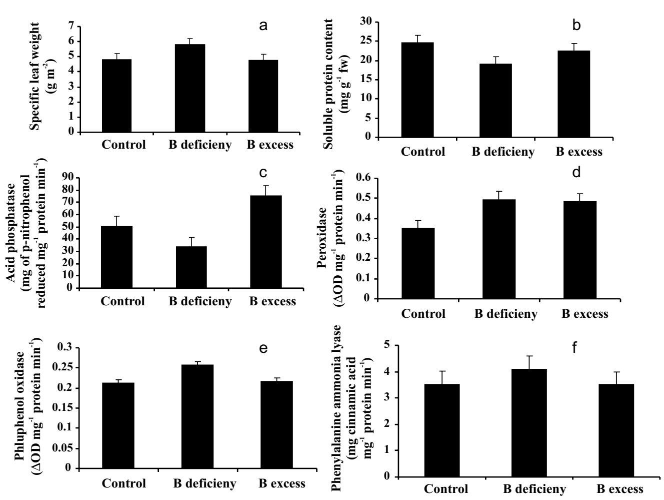

Fig. 3. Effect of boron stress on specific leaf weight (a), soluble protein content (b), activity of acid phosphatase (c), peroxidase (d), polyphenol oxidase (e) and phenylalanine ammonia lyase (f) in flue cured tobacco

increase in reactive oxygen species, cells express more peroxidase to counter the toxic oxygen species. Even though we have not correlated the increase in PPO activity with phenolic concentration it has been reported that increase in phenolics is the consequence of increased phenylalanine ammonia lyase activity (Korth *et al*. 2001). Although boron deficient leaves had high activity of antioxidant enzymes, the increase is generally not sufficient for protection against oxidative damage (Han *et al*. 2008). In excess boron, the activity of peroxidase and acid phophotase increased by 30 and 33%, respectively, whereas differences were not found in the polyphenol oxidase and phenylalanine ammonia lyase activities.

The results indicate that the boron deficiency causes drastic reduction in growth and increase in phenylalanine ammonia lyase, polyphenol oxidase and peroxidase activities. The boron deficient/excess symptoms documented were of a great help in the identification of boron deficiency/toxicity in the tobacco field crop.

REFERENCES

Anonymous (2006). Annual Report (2005-2006) Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh, India.

Bangarayya, M. and Narasimhamurthy, Y. Ch. (1970). Boron toxicity in flue cured tobacco. *Sci. Cult.* **36:** 118-120.

Cakmak, I. and Romheld, V. (1997). Boron deficiency induced impairments of cellular functions in plants. *Plant Soil* **193:** 71-83.

Gaines, T.P. and Mitchell, G.A. (1979). Boron determination in plant tissue by the azomethine H method. *Commun. Soil Sci. Plant Anal.* **10:** 1099-1108.

Han, S., Chen, L.S., Jiang, H.X., Smith, B.R., Yang, L.T. and Yie, C.Y. (2008). Boron deficiency decreases growth and photosynthesis and increases starch and hexoses in leaves of citrus seedlings. *J. Plant Physiol.* **165:** 1331-1341.

Jhonson, C.M, Stout, P.R., Broyer, T.C. and Carlton, A.B. (1957). Comparative chlorine requirements of different plant species. *Plant Soil* **8:** 337-357.

Kar, M. and Mishra, D. (1976). Catalase, peroxidase and polyphenol oxidase during rice leaf senescence. *Plant Physiol*. **57:** 315-319.

Korth, K., Blount, J.W., Chen, F., Rasmussen, S., Lamb, C. and Dixon, R.A. (2001). Changes in phenylpropanoid metabolites associated with homology-dependent silencing of phenylalanine ammonia lyase and its reversion in tobacco. *Physiol. Plant.* **111:** 137-143.

Laemmli, U.K. (1970). Clevage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature* **227:** 685-689.

Loomis, W.D. and Durst, R.W. (1992). Chemistry and biology of boron. *Biofactors* **3:** 229-239.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin - Phenol reagents. *J. Biol. Chem*. **193:** 265-275.

Panse, V.G. and Sukhatme, P.V. (1954). Statistical Methods for Agricultural Workers. ICAR Publication, New Delhi.

Pfeffer, H., Dennel, F. and Romheld, V. (1998). Are there connections between phenol metabolism, ascorbate metabolism and membrane integrity in leaves of boron-deficient sunflower plant*? Physiol. Plant*. **108:** 478-485.

Sadasivam, S. and Manikam, A. (1991). Biochemical Methods for Agricultural Sciences. (II Edition). New age International Publishers, New Delhi.