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SHORT COMMUNICATION

INDUCTION OF CHLOROPHYLLASE ACTIVITY IN NORMAL GREEN AND CHLOROTIC LEAVES OF SUGARCANE

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Influence of supplementation of micronutrients (Fe, Cu and Zn) at the concentration in which they occur in the leaves of normal green plant on the activity of chlorophyllase enzyme was examined in normal green and chlorotic leaves of sugarcane variety CoH 92 (*Saccharum* spp. hybrid, ratoon crop) grown at the Kharika block of IISR, Lucknow. It was found that the enhancement of chlorophyllase activity was more in chlorotic leaf substrate as compared to normal green leaf substrate when iron, copper and zinc were supplemented to the chlorotic leaf enzyme preparation. In the case of normal leaf enzyme preparation, supplementation of Fe and Cu were found to enhance the activity of chlorophyllase only when the normal leaf substrate was used.

***Key words*: Chlorophyllase, chlorosis, sugarcane, micronutrient.**

Chlorosis is a widespread nutritional malady of sugarcane. Morpho-physiological and nutritional alterations in plants caused by chlorosis affect cane yield and quality (Yadav and Singh 1987, Shrivastava *et al*. 2000). The enzyme chlorophyllase is involved in biosynthesis and degradation of chlorophyll in plants. In sugarcane, normal green leaves possess higher chlorophyllase activity as compared to chlorotic leaves. The present study is an attempt to elucidate the effect of micronutrient supplementation on chlorophyllase obtained from normal green and chlorotic leaves. The substrate is the limiting factor for chlorophyllase activity in a chlorotic leaf. The chlorophyllase enzyme preparation from a chlorotic leaf showed relatively lesser activity when the substrate from chlorotic leaf was used, while relatively higher activity was observed with substrate from normal green leaf (Shrivastava *et al*. 2001).

Fresh samples of normal green and chlorotic leaves were obtained from a *Saccharum* Spp. hybrid (Var. CoH 92, ratoon crop) grown at Kharika block of Indian Institute of Sugarcane Research, Lucknow (latitude 26º56'N, longitude 80º52'E). The leaves were washed thoroughly, dried on filter paper and cut into pieces of about 2.5 × 2.5 cm (for micronutrient supplementation) and chopped in to small pieces for chlorophyll (substrate) extraction. The leaf pieces (2.5 × 2.5 cm) were dipped in to 300, 20 and 10 ppm solutions of Fe, Cu and Zn, respectively. For control, the leaf pieces were dipped in distilled water. All the solutions containing micronutrient were aerated for one hour. Leaves were again dried on filter paper sheet. The enzyme was extracted and assayed by the method of Holden (1961). Chlorophyll was extracted and estimated by the method of Arnon (1949). The enzyme activity was expressed as mg chlorophyll esterified/100 mg enzyme powder/18 h.

The activity of chlorophyllase from chlorotic leaf was more in normal leaf substrate as compared to chlorotic leaf substrate (Fig. 1). Even the activity of chlorotic leaf enzyme was higher than the normal leaf enzyme in normal leaf substrate (38.23% increase). The Fe induced normal green and chlorotic leaf enzymes enhanced the chlorophyll biosynthesis in normal leaf substrate by 23.8 and 27.94%, respectively, while in chlorotic leaf substrate, chlorotic leaf enzyme stimulated chlorophyll biosynthesis

by 600%. Similarly, in the case of copper supplementation, the normal green and chlorotic leaf enzyme enhanced the biosynthesis in normal leaf substrate by 42.86 and 16.18% respectively, while in chlorotic leaf substrate only the chlorotic leaf enzyme stimulated chlorophyll biosynthesis by 1400%. On zinc supplementation, in both normal as well as chlorotic leaf substrate, the normal leaf enzyme activity was decreased by 12.70 and 28.84% respectively as compared to control, while chlorotic leaf enzyme activity was enhanced by 7.53 and 165.2% over control in normal and chlorotic leaf substrates respectively.

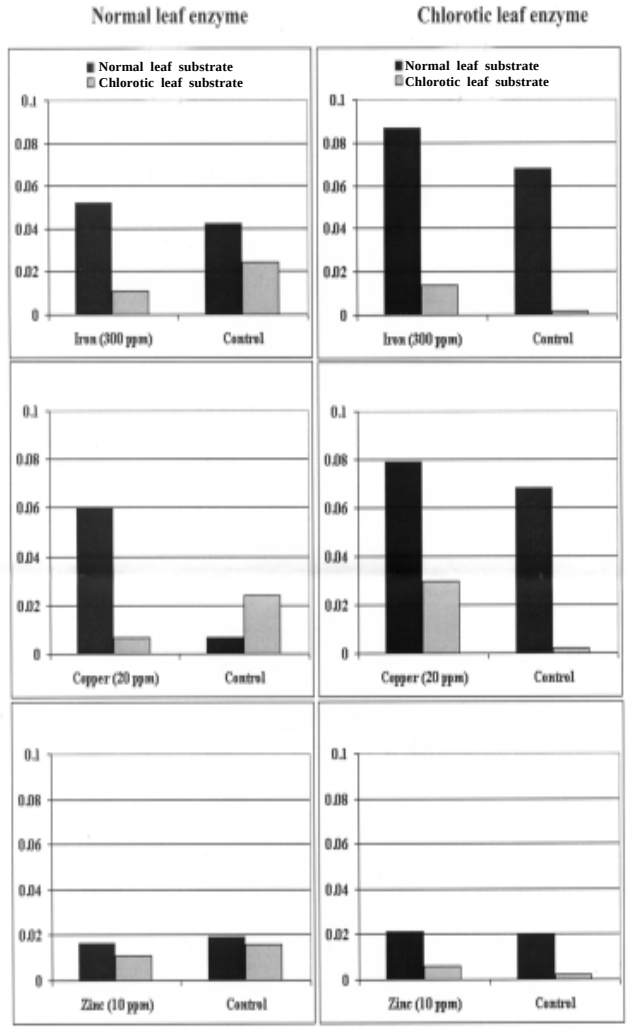

Fig. 1. Effect of iron (300 ppm), copper (20 ppm) and zinc (10 ppm) supplementation on chlorophyllase activity (mg chlorophyll/100 mg enzyme/18h) in normal green and chlorotic plants of sugarcane variety CoH 92.

Until now, there is no information available with respect to the effect of micronutrient supplementation on chlorophyllase activity in sugarcane. The results showed an enhancement in the activity of enzyme on micronutrient supplementation. Deficiency of Fe, Cu and Zn are known to cause chlorosis in sugarcane. These micronutrients play a significant role in the metabolism related to the chlorophyll biosynthesis and the related enzymes. Copper is essential to the activity of several photosynthetic oxido-reductase enzymes (Anderson *et al.* 1990). Immature leaves have varying degrees of chlorosis, in severe conditions, the entire leaf blade may become chlorotic. *Maui growth failure* in Hawaii was identified as Zn deficiency causing significant yield losses (Bowen 1968). Fe plays an important role in synthesis of chlorophyll, and serves as an electron carrier in photosynthetic phosphorylation. Certain metalloflavo proteins are active in biological oxidation-reduction reactions (Anderson *et al*. 1990). Plants deprived of an adequate supply of iron fails to synthesize sufficient amount of chlorophyll and thus becomes typically chlorotic (Tomar *et al*. 1965). Fogliata and Bustos (1980) have shown that a chlorotic leaf of sugarcane possesses only 37.67% of the chlorophyll of normal green leaves.

The enhancement of chlorophyllase activity was more in chlorotic leaf substrate as compared to normal green leaf substrate when iron, copper and zinc were supplemented to the chlorotic leaf enzyme. While in the case of normal green leaf enzyme, supplementation of Fe and Cu enhanced the activity of chlorophyllase only when the normal green leaf substrate was used. In chlorotic leaves, the maximum enhancement of chlorophyllase activity was observed on supplementation of copper (20 ppm) followed by Fe (300 ppm) and Zn (10 ppm) when chlorotic leaf substrate was used (Fig. 1).

The present study clearly demonstrated that the substrate was the limiting factor for the chlorophyllase activity and the enzyme preparation from chlorotic leaf showed many times higher activity in normal leaf substrate on micronutrient supplementation than the control.

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