

SHORT COMMUNICATION

CHLOROSIS-INDUCED ALTERATIONS IN LEAF LAMINAE OF SUGARCANE

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Morpho-physiological characteristics were studied in the leaf laminae displaying progressive symptoms of chlorosis at tillering stage in a ratoon crop of sugarcane var. CoLk 8102. With progressive symptoms of chlorosis, average fresh and dry weights, total carbohydrates, reducing sugars, N and P content gradually declined. Na and Ca contents were marginally higher in the chlorotic leaves. K content initially increased, but decreased with progressing symptoms. Among the micronutrients, Fe content was relatively lesser in chlorotic leaf laminae but no trend was associated with progressing chlorotic symptoms. Mn content increased initially but at peak symptom expression, was numerically lesser as compared to the normal green leaf lamina. Zn and Cu contents gradually declined with progressing symptoms of chlorosis. With appearance of initial symptoms of chlorosis, protein content declined, but, with further progression of symptoms, it degraded faster.

Key words: Carbohydrates, chlorosis, macronutrients, micronutrients, reducing sugars, sugarcane.

Chlorosis is a widespread nutritional malady of sugarcane (Yadav and Singh 1988, Shrivastava *et al.* 2000). Chlorosis has been known to influence root development (Sharma *et al.* 1960, Singh, 1973), nutrient composition (Dey *et al.* 1999, Naidu *et al.* 1980, Shrivastava *et al.* 2000), absorption spectrum of chlorophylls (Dey and Yadav 1996), organic and amino acid contents (Singh and Singh 1964), rate of photosynthesis and activities of enzymes associated with it (Naik *et al.* 1985, 1986) and alterations in dry matter production and carbohydrate contents (Anonymous 2001). All these alterations ultimately influence growth, development and sugar productivity. The present study is an effort to work out the morpho-physiological characteristics of the normal green leaf laminae and the leaf laminae displaying progressive symptoms of chlorosis in the ratoon crop of sugarcane var. CoLk 8102 at the tillering stage.

A ratoon crop of sugarcane (*Saccharum* spp. Hybrid) var. CoLk 8102 was grown at the Kharika block of the Indian Institute of sugarcane Research, Lucknow (latitude 26°56'N, longitude 80°52'E). Normal green and chlorotic last transverse mark (LTM) leaves displaying symptoms

of varying levels of chlorosis (ranging from normal green to nearly white leaves. Fig. 1) were obtained from the

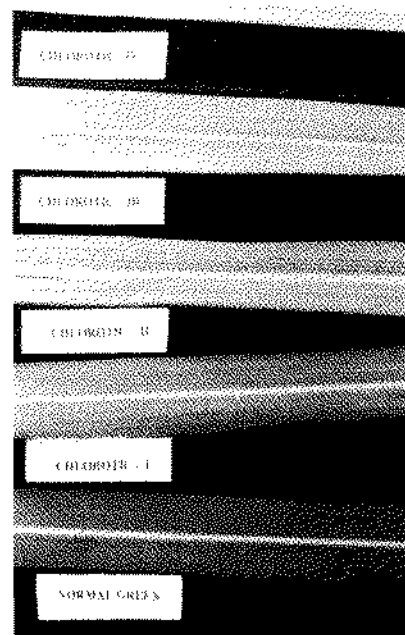


Fig. 1. Leaves displaying progressive symptoms of chlorosis in sugarcane var. CoLK 8012

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field during the tillering phase. The ratings of different degree of chlorotic symptoms were made as chlorotic I, II, III and IV. The leaf laminae, displaying gradually increasing symptoms of chlorosis, were washed gently in distilled water and dried in hot air oven at 102°C for 2 h and then at 85°C to a constant weight. They were finely powdered in a Wiley mill.

Structural dry matter was determined as acid (0.1 N HCl) insoluble dry matter. Total carbohydrates and the reducing sugars were determined by the method as described by Ashwell (1957). Nitrogen content was determined by microkjeldahl's method using Tecator Kjeltex analyzer (Page *et al.* 1982). Ca and K were estimated by flame photometry and P content as described by Jackson (1973). For the determination of micronutrients (Fe, Cu, Mn and Zn), dried leaf samples were digested in di-acid mixture (3 : 1, HNO₃ : HClO₄) and assayed in atomic absorption spectrophotometer (Hitachi). NR activity *in vivo* in fresh leaves was assayed as described by Jayorski (1971). The protein was determined by Lowry *et al.* (1951). The chlorophyll was estimated by the method of Arnon (1949). The data was statistically analysed by taking standard deviation of mean values (Represented as error bars in the figures).

Morpho-physiological alterations in the LTM (last transverse mark) leaf laminae displaying progressive symptoms of chlorosis in the ratoon crop of variety CoLk 8102 were studied. With increasing severity of chlorotic symptoms, the chlorophyll content gradually declined. Initially on development of chlorotic symptoms, a significant decrease of 53.74% was observed which reached up to 95% in completely chlorotic leaves (Fig. 2A). Kudachikar *et al.* (1997) also reported a decrease in chlorophyll content with increasing intensity of chlorosis. Nautiyal *et al.* (2000) observed decrease in chlorophyll content in chlorotic plants of sugarcane variety CoS 767 due to micronutrient deficiencies.

Structural components of dry matter (insoluble in 0.1 N HCl) in the leaves with early symptoms of chlorosis were at par with the normal green leaves, but declined with increasing symptoms (Fig. 2B). Total carbohydrates and reducing sugars, declined gradually and significantly with progressing symptoms of chlorosis (Fig. 2C & D). As mentioned earlier that with progressing symptoms of

chlorosis, chlorophylls content decreased. This might have caused a reduction in photosynthesis consequently total carbohydrates and reducing sugar contents. Rai (1997) also observed decreased carbohydrate levels in newly-sprouting chlorotic plants of sugarcane. Initially there was an increase in nitrate reductase activity up to second stage of chlorosis, but as the symptoms proceeded the nitrate reductase activity was significantly decreased by 52.51% in severely chlorotic leaf lamina (Fig. 2E). This may be due to occurrence of certain compensatory ability in the plant system to utilize the more nitrogen but as the symptom progressed the plant could not keep pace with this. With early expression of chlorotic symptoms, protein content decreased by 22.7 per cent. On further progression of chlorotic symptoms, it increased (with contents being more than the normal green leaf). However, with the expression of peak symptoms, protein contents suffered a substantial decrease (Fig. 2F). The data indicated that with appearance of initial symptoms of chlorosis, when protein content declined, plant exhibited a compensatory ability to sustain its metabolic system by increasing its protein content. However, with further progression of chlorotic symptoms, plant was unable to cope up with and protein content declined at a faster rate.

With increasing symptoms of chlorosis, nitrogen content declined but at peak symptom stage, it increased. However, it still remained significantly below that of the normal green leaf (Fig. 3A). P content also declined with development of chlorotic symptoms and at peak expression of symptom, leaf contained 32.35 per cent less P as compared to the normal green leaf (Fig. 3B). Dubey *et al.* (2001) also observed decreased P content at higher deficiency levels in *Capsicum annum*. Potassium content initially increased but with progressing symptoms, it decreased. With further progression of symptoms, it again increased (Fig. 3C). Singhvi *et al.* (2002) found higher K content in chlorotic leaves of mulberry plants at advanced stage of chlorosis. Sodium content was higher in the chlorotic leaf lamina as compared to normal green leaf lamina but no definite trend was associated with progressing symptoms of chlorosis (Fig. 3D). Ca content was also marginally higher in the leaves with chlorotic symptoms (Fig. 3E).

Iron content was relatively lesser in chlorotic leaf laminae but no trend was associated with different levels

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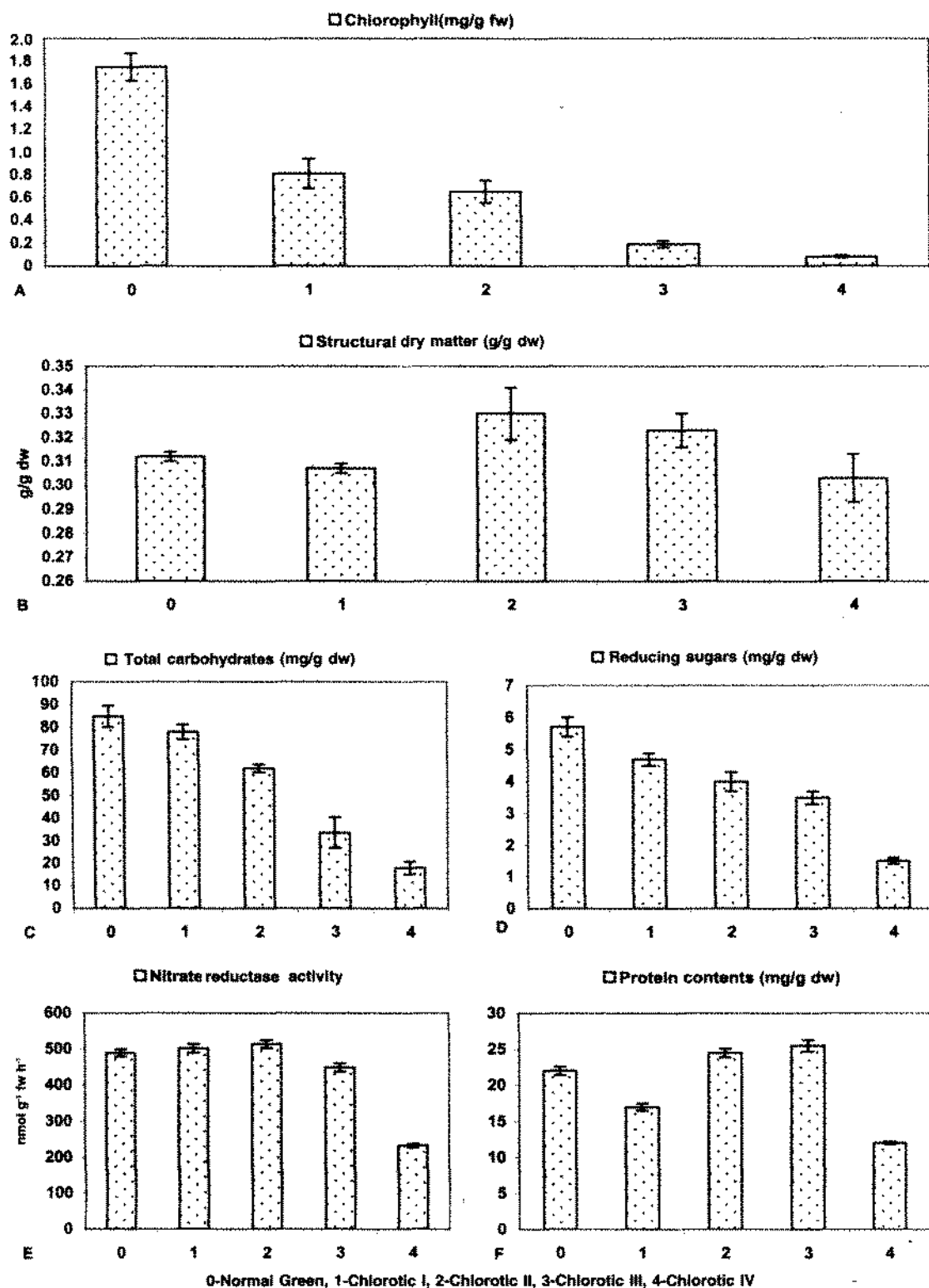
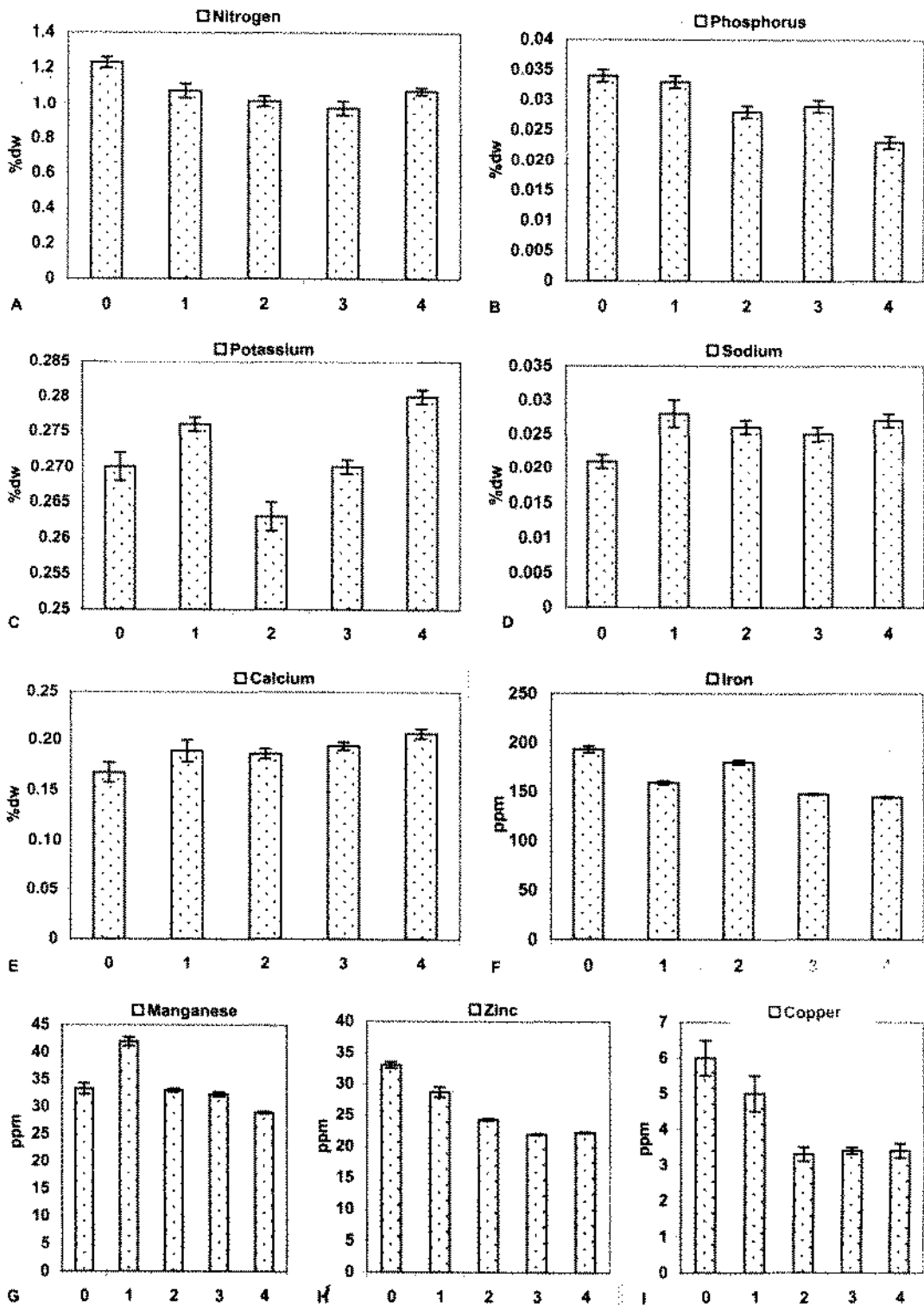


Fig. 2. Influence of increasing severity of chlorosis on total carbohydrate, reducing sugars, NR activity (*in vivo*), protein, chlorophyll and structural dry matter content in leaf lamina of sugarcane (Bars showing standard deviations from mean values)



0-Normal Green, 1-Chlorotic I, 2-Chlorotic II, 3-Chlorotic III, 4-Chlorotic IV

Fig. 3. Influence of increasing severity of chlorosis on N,P,K,Na,Ca,Fe,Mn,Cu and Zn contents in leaf lamina of sugarcane (Bars showing standard deviations from mean values)

of chlorotic symptoms (Fig. 3F). Raj (1997) and Dametie (1995) observed the same or even greater quantity of total Fe in chlorotic plants as compared to healthy green plants but concentration of active Fe was low in chlorotic plants. It was suggested that this could be due to physiological inactivation of Fe either in the soil or in the plant. Mn content (Fig. 3G) initially increased but decreased afterwards with increase in symptoms. However, Kudachikar *et al.* (1997) observed a gradual decrease in Fe and Mn content with increasing intensity of chlorosis. Zn and Cu content gradually declined with progressive symptoms of chlorosis (Fig. 3H and I).

The progressive symptoms of chlorosis are, therefore, associated with alterations in the levels of proteins chlorophyll, total carbohydrates, reducing sugars, macro and micronutrients and nitrate reductase activity.

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REFERENCES

Anonymous (2001). Half yearly report (July 2000-December 2000) of the NATP Project "Elucidation of Causes and Mechanism of Iron Chlorosis in Sugarcane" (PSR-44), IISR, Lucknow.

Arnon, D.J. (1949). Copper enzymes in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* **24**: 1-15.

Ashwell, G. (1957). Colorimetric analysis of sugars. *Method in Enzymology* **III**: 73-107.

Dametie, A., Mamo, T. and Zelleke, A. (1995). Studies on iron chlorosis of sugarcane (*Saccharum officinarum* L.) at Metahara, Ethiopia: soil and plant characterization and efficiency of different iron sources. *J. Agron. Crop Sci.* **175**: 317-324.

Dey, P. and Yadav, D.V. (1996). Chlorophyll scan, yield and juice quality of chlorotic sugarcane ratoon as influenced by application of ferrous sulphate. *Indian J. Sugarcane Technol.* **11**: 131-135.

Dey, P., Yadav, D.V. and Rai, R.K. (1999). Distribution pattern of sulphur and micronutrients in chlorotic and non-chlorotic sugarcane ratoon. *Indian J. Sugarcane Technol.* **14**: 65-67.

Dube, B.K., Sinha, P. and Chatterjee, C. (2001). Changes in yield, metabolism and fruit quality of chilli by low manganese. *Haryana J. Hort. Sci.* **30**: 262-264.

Jackson, M.L. (1973). *Soil Chemical Analysis*, Prentice Hall of India Pvt. Ltd., New Delhi.

Jaworski, E.G. (1971). Nitrate reductase assay in intact plant tissue. *Biochem. Biophys. Res. Commun.* **43**: 1272-1279.

Kudachikar, V.B., Chetti, M.B. and Basoarkar, P.W. (1997). Change in the mineral constituents and chlorophyll content during chlorosis in sugarcane. *Ann. Plant Physiol* **11**: 111-116.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin-phenol reagent. *J. Biol. Chem.* **193**: 265-275.

Naidu, K.M., Ramakrishnan, S. and Bhagyalakshmi, K.V. (1980). Causative factors for the occurrence of chlorosis in sugarcane growth in red loamy soils. *Proc. Inter. Soc. Sugar. Cane Technol.* **17**: 225-226.

Naik, G.R., Patil, T.M. and Hegde, B.A. (1985). Shift in the pattern by iron chlorosis in sugarcane leaves. *Photosynthetica* **19**: 561-565.

Naik, G.R., Patil, T.M. and Hegde, B.A. (1986). Shift in the carboxylation pattern by iron chlorosis in sugarcane. *Iron in Agri.* **7**: 3-9.

Nautiyal, N., Dube, B.K. and Chatterjee, C. (2000). Symptoms of nutrient deficiencies (N, P, K, Ca, Mg and S) in sugarcane grown in refined sand. *Sugarcane International*, March **18**: 12-17.

Page, A.L., Miller, R.R.H. and Keeny, D.R. (1982). *Methods of Soil Analysis: Chemical and Microbiological Properties*. Pt II. American Soc. Agronomy, Inc., Soil Sci. Soc. America Inc. Madison, USA.

Rai, R.K. (1997). Differences in water balance, stomatal characteristics and other physiological parameters in chlorotic and green plants of ratoon cane of variety Co.Lk 8102. *Indian Sugar* **47**: 17-21.

Sharma, S.L., Singh, R.K. and Shrivastava, B.B. (1960). Chlorosis disease of sugarcane in Bihar. *Indian J. Sugarcane Res. & Dev.* **4**: 112-113.

Shrivastava, A.K., Shahi, H.N. and Yadav, D.V. (2000). Chlorosis in sugarcane. *Indian J. Sugarcane Technol.* **15**: 90-101.

Singh, U.S. (1973). Iron chlorosis-A devastating disease of sugarcane. *Indian Sugar* **23**: 755-756.

Singh, A. and Singh, O.S. (1964). Studied into the metabolism of albino and healthy leaves of sugarcane. *Proc. All India Conf. Sugarcane Res. Dev. Workers.* **5**: 337-339.

Singhvi, N.R., Kodandaramaiah, J., Reddy, M.M., Katiyar, R.S. and Sarkar, A. (2002). Symptomatological study of nutrient

deficiency in mulberry variety under field conditions. *Indian J. Sericulture.* **41**: 66-69.

Yadav, D.V. and Singh, K. (1988). Lime-induced iron chlorosis in sugarcane. *Fert. Res.* **16**: 119-136.