Indian J. Plant Physiol., Vol. 9, No. 2, (N.S.) pp. 124-131 (April-June, 2004)

THE EFFECT OF IRON DEFICIENCY ON ROOT MORPHOLOGY, ETHYLENE CONTENT AND UPTAKE OF OTHER MICRONUTRIENTS IN *IN VITRO* GROWN CULTIVARS OF SUGARCANE

V.K. GUPTA, A.K. SINGH AND R.C. PANT

Department of Plant Physiology, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar-263145, Uttaranchal, India

Received on 8 March, 2003, Revised on 10 June, 2004

SUMMARY

A study on iron uptake in sugarcane and role of ethylene was conducted on sixty days old micropropagated plantlets of sugarcane cv. CoPant 84212 and CoPant 90223. Ethylene was estimated in sugarcane plants grown in different concentrations of iron in MS medium. The highest concentration of ethylene was produced by plantlets grown in iron free medium. Increasing concentration of iron in the medium increased the uptake of iron and manganese but decreased zinc and copper uptake. Iron content in roots was higher than the leaves. Large number of root hairs developed on the roots of plantlets grown in iron deficient medium.

***Key words*: Ethylene, iron chlorosis, micropropagation, phytosiderophores, sugarcane**

INTRODUCTION

Iron chlorosis is a widespread and persistent nutritional problem of sugarcane causing appreciable loss of cane and sugar yield in India (Singh *et al.* 1974, Somwansi *et al.*1996). The exact mechanism of iron uptake in sugarcane is not known. Soil factors such as presence of calcium carbonate and bicarbonate, high pH, nutrient ratio such as K/Ca, P/Fe and Fe/Mn affect iron uptake in sugarcane. Graminaceous species use strategy II for iron uptake, with the help of phytosiderophores. Both ethylene and phytosiderophores in higher plants have a common precursor. Ethylene is synthesized from methionine via S-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) (Yang and Hoffman 1984). Phytosiderophores in higher plants are derived from methionine via SAM and nicotianamine (Shojima *et al.* 1990, Welch *et al.* 1997). Both ethylene and nicotianamine stimulate root hair formation (Schmidt *et al.* 2000, Schmidt and Schikora 2001, Schikora and Schmidt 2002). The close link between ethylene and phytosiderophore biosynthetic pathway suggests that these compounds may be linked in some manner in controlling iron uptake by plants (Welch 1997, Romera *et al.* 1999).

Sugarcane tillers are prone to more pronounced iron chlorosis than the mother plant (Humbert and Martin 1955, Shrivastava *et al.* 2000). A heavy production of ethylene was reported at the time of tillering in sugarcane cultivar CoPant 84211 (Nailwal *et al.* 2004). Therefore, there may be some relationship between ethylene production and iron uptake in sugarcane. The present study was undertaken to study this relationship.

MATERIALS AND METHODS

Micropropagation

Apical and axillary bud explants were obtained from sugarcane cvs. CoPant 84212 and CoPant 90223.

Establishment of explants was achieved in full strength MS (Murashige and Skoog 1962) basal medium supplemented with different combinations of BAP, Kin, IAA and GA_3. Proliferation of buds was accomplished in full strength MS basal medium supplemented with different combinations of BAP, Kin and IAA. For rooting, full and half strength MS basal liquid supplemented with three concentrations each of NAA and IBA with 20 to 60 g l^{-1} sucrose used. Inoculated culture tubes/bottles were kept in culture room maintained at 25±1°C under fluorescent light (138 µmol $m^{-2} s^{-1}$) with 16 h photoperiod.

Quantification of ethylene in sugarcane

In order to ascertain the role of ethylene in iron uptake, 60 days old *in vitro* raised rooted plantlets of sugarcane cultivars CoPant 84212 and CoPant 90223 were grown in the presence of 0.0, 1.1, 2.2, 3.3, 4.4 and 5.5 ppm iron in MS medium in leak-proof bottles. The bottle caps were removed and replaced with especially made caps fitted with serum stoppers. Two layers of parafilm were wrapped around the brim of the caps to make the bottles leak-proof. Starting from 24 h to 240 h at an interval of 24 h ethylene was estimated using Hewlett Pacard (Model 5890, Series II) gas chromatograph equipped with FID detector and HP 3396 series II integrator with 1% OV-101 packed column. The operating flow rate of carrier gas (N_2) air and H_2 were 25, 400, 40 ml min^{-1}, with injector column detector temperature 100, 100, 120°C and chart speed 10 mm min^{-1}. Ethylene evolved was quantified with the help of a standard curve made with the help of 105 ppm ethylene in argon from EDT Research, London. Atmospheric temperature and pressure were recorded at the time of chromatography.

Estimation of total iron, manganese, zinc and copper

Sixty days old *in vitro* raised sugarcane plantlets of sugarcane cultivars CoPant 84212 and CoPant 90223 were further grown for 30 days in MS medium supplemented with 5.0 ppm NAA and 50 g l^{-1} sucrose with 0.0, 1.1, 2.2, 3.3, 4.4 and 5.5 ppm iron in the medium. Inoculated bottles were incubated in a culture room at 25±1°C with white fluorescent light (138 µmol $m^{-2} s^{-1}$) and 16 h photoperiod. After 30 days of incubation, plant samples were removed from the culture bottles and washed with running tap water to remove adhering medium from the roots followed by 10 sec rinse in distilled water to remove surface contamination and blotted dry to remove moisture. Roots and leaves cut from the shoots were dried at 70°C until constant weight. Dried root and leaf samples of known weight were digested in triacid mixture (HNO_3:HCl04:H_2SO4, 10:4:1, v/v/v) (Jackson 1958). Iron, zinc, manganese and copper in the digested samples were determined using GBC, 902 Atomic absorption spectrophotometer.

Microscopy

Root hair patterns were analyzed using stereozoom microscope, Olympus, Japan.

All the data were statistically analyzed by using completely randomized design (CRD). Mean, standard error of mean and critical difference at 5% were calculated.

RESULTS AND DISCUSSION

Micropropagation

The highest percentage of meristem establishment was achieved in full strength MS basal medium supplemented with 0.5 ppm each of BAP, Kin, IAA and GA_3 for both CoPant 84212 and CoPant 90223. Shoot attained a height of 5-6 cm in 12-15 days in the present study (Plate 1). Shukla *et al.* (1994) observed the highest percentage of meristem establishment in CoPant 84211 in MS medium supplemented with 0.5 ppm each of BAP, Kin and IAA. In CoJ 64, CoJ 83 and CoPant 84211, Gosal *et al.* (1998) observed this in MS medium supplemented with 0.5 ppm each of IAA, Kin and GA_3. Patel *et al.* (1999) observed that MS medium supplemented with 0.5 ppm BAP + 1.0 ppm IBA gave the highest percentage of meristem establishment in cv. CoLK 8001.

The highest percentage of bud proliferation was achieved in full strength MS basal liquid medium supplemented with 2.0 ppm BAP + 1.0 ppm Kin + 0.1

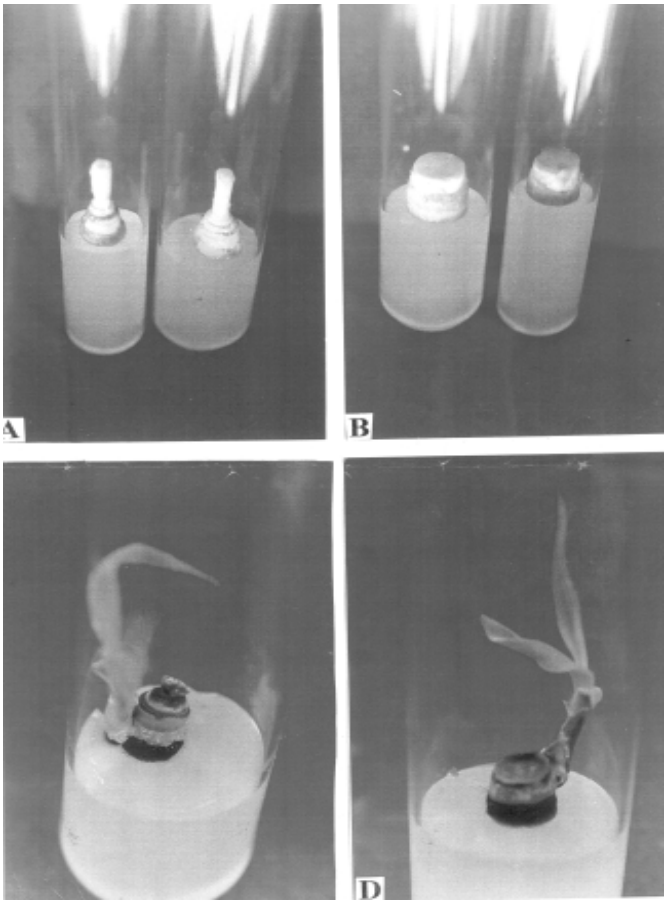

Plate 1. Apical bud and axillary bud explants of sugarcane cultivar CoPant 84212 inoculated for establishment in full strength MS medium supplemented with 0.5 ppm BAP + 0.5 ppm Kin + 0.5 ppm IAA + 0.5 ppm GA_3: (A) Apical bud, (B) Axillary bud, (C) Elongated apical bud expalnt, 10 days after inoculation, (D) Elongated axiilary bud expalnt, 10 days after inoculation

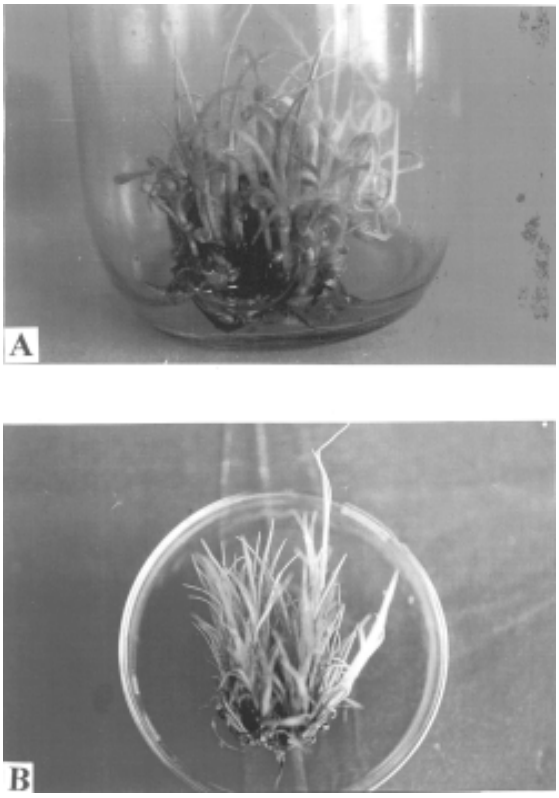

Plate 2. Proliferating bud explants of sugarcane cultivar CoPant 84212 and CoPant 90223, 30 days inoculation in full strength MS medium supplemented with 2.0 ppm BAP + 1.0 ppm Kin + 0.1 ppm IAA : (A) CoPant 84212, (B) CoPant 90223

ppm IAA (Plate 2). Alam *et al.* (1995) however, reported highest shoot proliferation in Isd-16 in full strength MS basal supplemented with 0.5 ppm BAP + 4.0 ppm NAA whereas, Gosal *et al.* (1998) observed this with 0.5 ppm each of BAP and Kin in cvs. CoJ64, CoJ83 and CoPant 84211. Patel *et al.* (1999) reported that 0.5 ppm BAP + 0.5 to 1.0 ppm IBA gave the highest number of multiple shoots in sugarcane cv. CoLK 8001 .

In the present study *in vitro* raised sugarcane plantlets produced highest number of roots in full strength MS basal liquid medium supplemented with 5.0 ppm NAA and 50 g l^{-1} sucrose (Plate 3). There are reports that regenerated shoots, rooted readily in MS medium supplemented with 5.0 ppm NAA and 60 g l^{-1} sucrose in sugarcane cv. Isd-16 (Alam *et al*. 1995) with 2.0 ppm NAA and 60 g l^{-1} sucrose in cv. CoLK 8102 (Lal *et al.* 1996) and with 5.0 ppm NAA with 70 g l^{-1} sucrose in cvs. CoJ 64, CoJ 83 and CoPant 84211 (Gosal *et al.* 1998).

Quantification of ethylene

The highest ethylene content of 30.73 nmol g^{-1} fw h^{-1} in CoPant 84212 and 24.57 nmol g^{-1} fw h^{-1} in CoPant 90223, were observed in plantlets grown in iron free medium after 48 h. A decrease in ethylene production was observed with increase in iron concentration in the medium and increase in incubation time. The lowest ethylene content in both the cvs. were observed from plantlets grown with 5.5 ppm iron in the medium after 240 h incubation in the present study (Fig.1 and 2). Iron deficiency caused a drastic stimulation of ethylene (five times higher) production in sorghum (Morghan and Hall

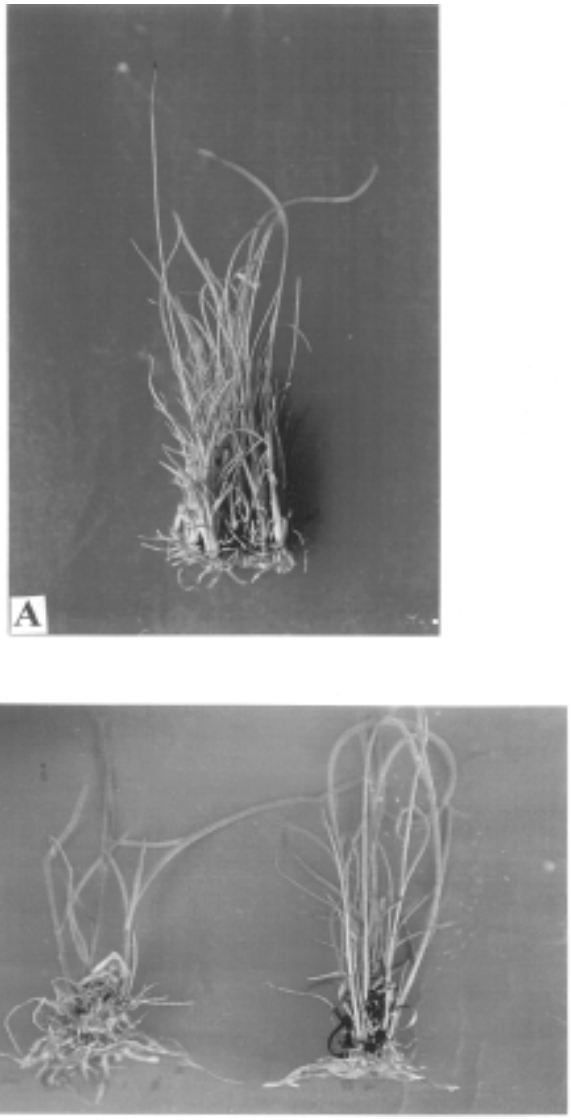

Plate 3. *In vitro* raised rooted plantlets of sugarcane cultivars CoPant 84212 and CoPant 90223, 21 days of inoculation in MS medium supplemented with 5 ppm NAA and 50 g l^{-1} sucrose : (A) CoPant 84212, (B) CoPant 90223

1962). Strategy I responses in cucumber roots can be inhibited by Aminooxyacetic acid (AOA, inhibitor of ethylene synthesis) and cobalt (action inhibitor) and accentuated by increasing ethylene synthesis by ACC (Romera and Alcantara 1994). Iron deficient cucumber, pea and tomato plants produced more ethylene than the iron sufficient plants. However, iron deficient strategy II plants (maize, wheat, barley) did not produce more ethylene than the iron sufficient ones (Romera *et al.* 1999).

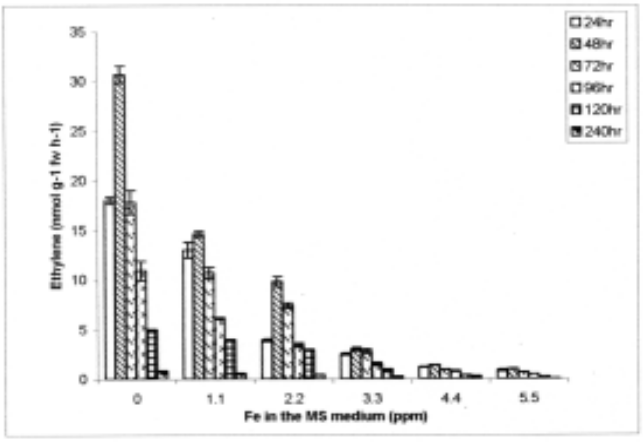

Fig. 1. Effect of different concentrations of iron in MS medium on ethylene production in *in vitro* grown plantlets of sugarcane cultivar CoPant 84212 at different incubation period. Error bars represents SEM (n=3)

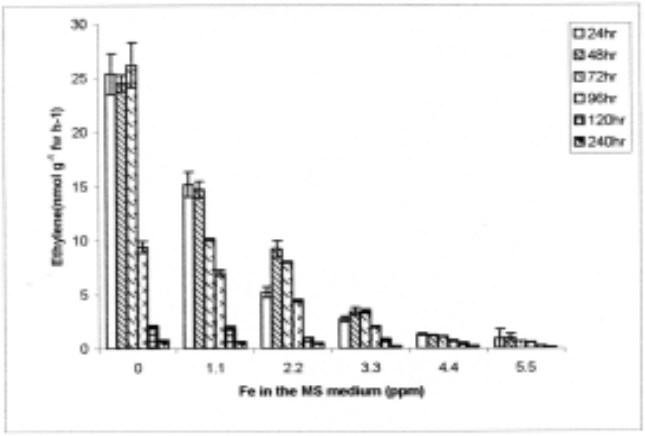

Fig. 2. Effect of different concentrations of iron in MS medium on ethylene production in *in vitro* grown plantlets of sugarcane cultivar CoPant 90223 at different incubation period. Error bars represents SEM (n=3)

Effect of iron in MS medium on zinc, manganese and copper uptake

The lowest iron content in roots and in leaves of cvs. CoPant 84212 and CoPant 90223 was found in plants grown in iron free medium. There was an increase in iron content with increasing concentration of iron in the medium up to 5.5 ppm (Table 1). The iron content in the plantlets which were grown in iron free medium is due to the fact that the plants were grown *in vitro* in full strength MS major and minor salts prior to treatment with different concentration of iron in the MS medium. Due to a close

Table 1. Effect of varying concentrations of iron in MS medium containing 5 ppm NAA and 50 g l^{-1} sucrose on iron content of root and leaf tissue of 60 days old *in vitro* raised plantlets of sugarcane cultivars CoPant 84212 and CoPant 90223 after 30 days incubation in MS medium (The experiment was repeated twice each with three replicates).

Fe in MS medium (ppm)	Iron (µg/g dry wt.)			
	CoPant 84212		CoPant 90223	
	Roots	Leaves	Roots	Leaves
0.0	351.6±11.86	278.66±2.40	413.0±5.50	375.4±5.36
1.1	405.6±3.76	376.66±4.18	507.3±10.48	424.4±5.04
2.2	435.0±7.63	351.0±4.36	519.3±4.06	418.4±4.91
3.3	453.3±8.82	452.3±10.10	551.0±4.93	441.6±2.60
4.4	586.7±7.36	578.0±10.10	607.3±6.74	503.4±3.33
5.5	655.3±2.96	504.33±3.48	626.6±0.88	569.0±3.79
CD at 5%	76.7	115.9	18.95	13.22

link between ethylene and phytosiderophore biosynthesis it is possible that the uptake of iron was suppressed due to higher ethylene production in iron deficient condition. The iron content in roots was higher as compared to leaves in both cultivars. This could be because translocation of iron from root to shoot was poor as was also reported by Singh (2001). There was a decrease in zinc content in both root and shoot with increasing concentration of iron in the medium upto 5.5 ppm (Table 2). There was an increase in manganese content with increasing concentration of iron in the medium upto 5.5 ppm. (Table 3). A decrease in copper content with increasing concentration of iron in the medium upto 5.5 ppm was observed (Table 4). A reciprocal effect of manganese deficiency in the presence of high concentration of iron has been shown in soybean (Somers and Shive 1942). In tomato, tissue content of iron, manganese and molybdenum correlated closely, iron decreased with increasing concentration of either manganese or molybdenum in the external medium (Gerloff *et al.* 1989). In tomato plants increase in manganese content in the nutrient solution increased both iron uptake and translocation upto a point, after that it decreased (Rickels and Lengle 1966). Both iron

Table 2. Effect of varying concentrations of iron in MS medium containing 5 ppm NAA and 50g l^{-1} sucrose on zinc content of root and leaf tissue of 60 days old *in vitro* raised plantlets of sugarcane cultivars CoPant 84212 and CoPant 90223 after 30 days incubation in MS medium (The experiment was repeated twice each with three replicates).

Fe in MS medium (ppm)	Zinc (µg/g dry wt.)			
	CoPant 84212		CoPant 90223	
	Roots	Leaves	Roots	Leaves
0.0	32.3±2.19	32.3±1.20	51.0±0.33	31.5±0.67
1.1	32.3±2.84	28.0±0.58	48.6±1.86	30.5±0.67
2.2	32.0±2.65	23.0±2.08	45.0±2.89	29.0±0.58
3.3	25.0±2.89	28.0±0.58	40.3±0.88	28.0±0.58
4.4	27.0±1.15	25.0±1.15	40.0±1.53	25.0±1.15
5.5	24.3±1.76	22.0±1.15	39.6±0.88	24.6±1.45
CD at 5%	6.2	5.92	4.99	2.81

Table 3. Effect of varying concentrations of iron in MS medium containing 5 ppm NAA and 50 g l^{-1} sucrose on manganese content of root and leaf tissue of 60 days old *in vitro* raised plantlets of sugarcane cultivars CoPant 84212 and CoPant 90223 after 30 days incubation in MS medium (The experiment was repeated twice each with three replicates).

Fe in MS medium (ppm)	Manganese (µg/g dry wt.)			
	CoPant 84212		CoPant 90223	
	Roots	Leaves	Roots	Leaves
0.0	40.0±1.15	33.0±1.45	34.0±2.08	31.6±0.89
1.1	40.6±2.33	36.3±1.20	40.6±0.67	40.0±0.00
2.2	44.3±2.33	35.3±1.45	40.0±1.15	41.0±0.67
3.3	49.0±0.58	42.0±1.15	50.3±0.89	43.0±1.00
4.4	57.3±1.76	43.0±1.00	57.0±1.15	46.3±0.67
5.5	63.6±1.67	44.6±0.67	63.6±2.02	48.3±0.30
CD at 5%	4.8	7.6	4.41	4.29

Table 4. Effect of varying concentrations of iron in MS medium containing 5 ppm NAA and 50 g l^{-1} sucrose on copper content of root and leaf tissue of 60 days old *in vitro* raised plantlets of sugarcane cultivars CoPant 84212 and CoPant 90223 after 30 days incubation in MS medium (The experiment was repeated twice each with three replicates).

Fe in MS medium (ppm)	Copper (µg/g dry wt.)			
	CoPant 84212		CoPant 90223	
	Roots	Leaves	Roots	Leaves
0.0	5.9±0.15	6.1±0.12	8.0±0.11	7.6±0.30
1.1	5.1±0.35	5.6±0.24	7.4±0.28	7.2±0.23
2.2	5.2±0.15	5.3±0.07	7.4±0.29	7.0±0.12
3.3	3.7±0.43	5.4±0.13	7.2±0.15	7.1±0.06
4.4	2.5±0.15	5.0±0.03	7.0±0.03	6.6±0.20
5.5	3.3±0.19	4.1±0.09	7.0±0.11	6.5±0.21
CD at 5%	1.08	0.46	0.59	0.63

and manganese had inhibitory effect on zinc absorption as well as translocation in soybean (Reddy *et al*. 1978).

In the leaves of sugarcane cv. Co760, the metabolically active iron (Fe^{2+}) decreased from 12.0 ppm to 4.3 ppm and manganese from 89 ppm to 81 ppm, respectively with increasing intensity of iron chlorosis. The total iron content in completely yellow leaves was higher (816 ppm) than the completely green leaves (760 ppm). However, completely green leaves contained more metabolically active iron. Chlorophyll a, b and total chlorophyll content decreased sharply with increasing intensity of chlorosis (Kudachikar *et al*. 1997). Malewar *et al*. (1999) cultured meristematic tissue (shoot tip) of sugarcane cvs. Co86632 and Co7219 in complete MS medium or without iron, manganese and zinc. Iron uptake was the highest in complete MS medium, while manganese uptake was greatest when zinc was omitted from MS medium, and zinc content was greatest when iron was omitted from MS medium.

Root morphology

Large number of root hairs developed in CoPant 84212 and CoPant 90223 grown in iron free medium. However, no root hairs were found on the roots of plantlets grown with 5.5 ppm iron in the medium (Plate 4). Iron deficiency has also been shown to enhance formation of root hairs in sunflower (Romheld and Marschner 1981) and in sugarbeet (Landsberg 1995). This could be because iron deficiency increased the levels of ethylene hormone, which triggers the root hair formation in *Arabidopsis* (Schmidt and Schikora 2001).

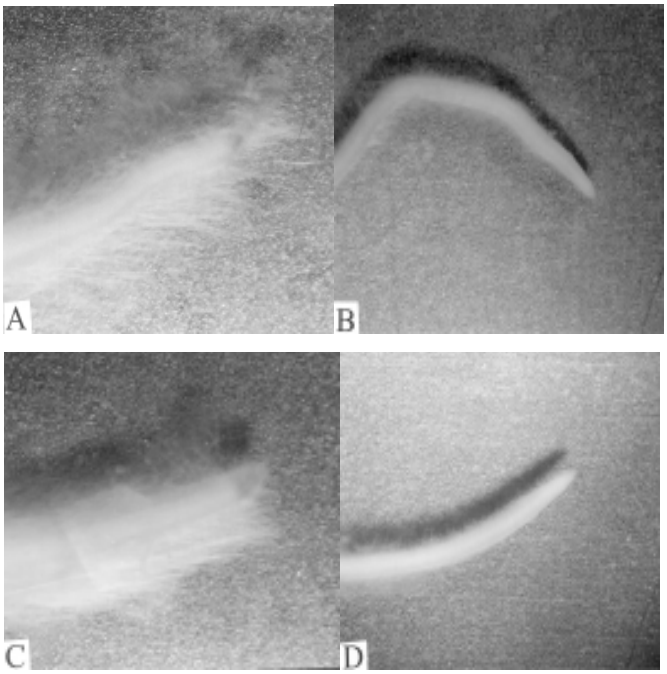

Plate 4. Root morphology of *in vitro* raised plants of sugarcane cultivars CoPant 84212 and CoPant 90223 after 30 days incubation in iron deficient and iron sufficient medium : (A) CoPant 84212 without iron in the medium, (B) CoPant 84212 with full iron in the medium, (C) CoPant 90223 without iron in the medium, (D) CoPant 90223 with full iron in the medium

ACKNOWLEDGEMENT

The authors are thankful to Mr. N.K. Sand for help in gas chromatography. This work was supported by the National Agriculture Technology Project (NATP) number PSR 44.

REFERENCES

Alam, M.Z., Hoider, S.A., Islam, R. and Joarder, O.I. (1995). High frequency *in vitro* plant regeneration in sugarcane. *Sugarcane* **6**: 20-21.

Gerloff, G.C., Stout, P.R. and Jones, L.H.P. (1959). Molybdenum-manganese-iron antagonisms in the nutrition of tomato plants. *Plant Physiol.***34:** 608-612.

Gosal, S.S. Thind, K.S. and Dhaliwal, H.S. (1998). Micropropagation of sugarcane — an efficient protocol for commercial plant propagation. *Crop Imp.* **25**: 167-171.

Humbert, R.P. and Martin, J.P. (1955). Nutritional deficiency symptoms in sugarcane. *Hawaiin Planters' Rese.* **55**: 95-102.

Jackson, M. L. (1958). Soil Chemical Analysis. Prentice Hall Inc. Englewood Cliffs, New Jersey.

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.

Lal, J., Pande, H.P. and Awasthi, S.K. (1996). A general micropropagation protocol for sugarcane varieties. *New Botanist* **23**: 13-19.

Landsberg, E.C. (1995). Transfer cell formation in sugar beet roots induced by latent Fe deficiency. In: J. Abadia (ed.), Iron Nutrition in Soil and Plants, pp. 67-75. Kluwer Academic Press, Netherlands.

Malewar, G. O., Reddy, M. R., Waikar, S. L. and Patil, V. D. (1999). Differential growth behavior, micronutrient absorption and interactions by tissue cultured sugarcane genotypes *J. Maharashtra Ag. Univ.* **24**: 183-185.

Morgan, P.W. and Hall, W. (1962). Effect of 2-4-dichlorophenoxyacetic acid on production of ethylene by cotton and grain sorghum. *Physiol Plant.* **15**: 420-427.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant* **15**: 473-497.

Nailwal, T.K., Gupta, V. K., Sand, N. K. and Pant, R. C. (2004). Role of ethylene during tillering of sugarcane (*Saccharum officinarum* L). *Physiol. Mol. Biol. Plant.* **10:** 127-130.

Patel, S.R., Patel, C.L., Patel. A.A., Prajapati, B.S. (1999). The effect of media composition on establishment and growth of *in vitro* sugarcane meristem var. CoLk 8001. *Ind. Sugar.* **49**: 25-29.

Reddy, K. R., Saxena, M.C. and Pal, U.R. (1978). Effect of iron and manganese on ^{65}Zn absorption and translocation in soybean seedlings. *Plant Soil* **49**: 409-415.

Rickels, J.W. and Lingle, J.C. (1966). Iron uptake and translocation by tomato plants as influenced by root temperature and manganese nutrition. *Plant Physiol.* **41**: 1095-1101.

Romera, F.J. and Alcantara, F. (1994). Iron deficiency stress response in cucumber (*Cucumis sativus*) roots. A possible role for ethylene. *Plant Physiol.* **105**: 1133-1138.

Romera, F.J., Alcantara, E. and Gaurdia, M.D. (1999). Ethylene production by Fe-deficient stress responses by strategy I plants. *Ann. Bot.* **83:** 51-55.

Romheld, V. and Marschner, H. (1981). Iron deficiency stress induced morphological and physiological changes in tip of sunflower. *Physiol. Plant.* **53**: 354-360.

Schikora, A. and Schmidt, W. (2002). Modulation of the root epidermal phenotype by hormones, inhibitors and iron regime. *Plant Soil.* **241**: 87-96

Schmidt, W. and Schikora, A. (2001). Different pathways are involved in phosphate and iron stress-induced alterations of root epidermal cell development. *Plant Physiol.* **125**: 2078-2084.

Schmidt, W., Schikora, A., Pich, A. and Bartels, M. (2000). Hormones induce an Fe-deficiency like root epidermal cell pattern in the Fe-inefficient tomato mutant *fer*. *Protoplasma* **213**: 67-73.

Shojima, S., Nishizawa, N.K., Tuslya, S., Nozoe, S., Irifune, T. and Mori, S. (1990). Biosynthesis of phytosiderophore. *In vitro* biosynthesis of 2-hydroxymugineie acid from methionine and nicotianamine. *Plant Physiol.* **93**: 1497-1503.

Shrivastava, A.K., Shahi, H.N. and Yadav, D.V. (2000). Chlorosis in sugarcane. *Indian J. sugarcane Tech.* **15**: 68-79.

Shukla, R., Khan, A.Q. and Garg, G.K. (1994). *In vitro* clonal propagation of sugarcane: optimization of media and hardening of plants. *Sugarcane* **4**: 21-23.

Singh, N. (2001). Studies on iron uptake and distribution in sugarcane (*Saccharum officinarum* L.) plants. M.Sc. Thesis, G.B.P.U. Agri. & Tech., Pantnagar.

Singh, R.G., Verma, H.P. and Singh, J.P. (1974). Effect of micronutrients on growth, yield and juice quality of sugarcane. In: Proc. Ann. Conv. D.S.T.A., pp. 81-89. Vasantdada Sugar Inst. Press, Pune.

Somers, I.I. and Shieve, J.W. (1942). The iron-manganese relation in plant metabolism. *Plant Physol.* **17**: 582-602.

Somwansi, R.B., Kalpe, S.S. and Yadav, A.M. (1996). Effect of chlorosis on growth and sugar content of sugarcane plants cv. Co 740 and Co 7219 under field conditions. In*:* Proc. Ann. Conv. D.S.T.A. pp. 33 –35. Vasantdada Sugar Inst. Press, Pune.

Welch, R. M., Norvell, W. A., Gesuwan, P. and Schaefer, S. (1997). Possible role of root-ethylene in Fe (III)-phytometallophore uptake in strategy II species. *Plant Soil.* **196**: 229-232.

Yang, S. F. and Hoffman, N. E. (1984). Ethylene biosynthesis and its regulation in higher plants. *Annu. Rev. Plant Physiol.* **35**: 155-189.