CHAPTER - III

RESULTS AND DISCUSSION

A. EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON BUD ANALYSIS

a) Nitrate Reductase

Effect of presowing soaking treatment with distilled water, CCC and ethephon on enzyme nitrate reductase is depicted in Fig.1. It is clear from the figure that in the eye buds of sugarcane setts the NR activity is increased due to all the three presowing soaking treatments. This increase is more significant in case of CCC pretreated eyebuds of sugarcane setts.

Nitrate is principle source of nitrogen available to most of plant species. Nitrate reductase (EC 1.6.6.1) is a metalloflavoprotein that catalyzes the reduction of nitrate to nitrite with the help of NADH in cytoplasm. This enzyme is important in the first stage of protein synthesis. The enzyme system includes a reduced pyridine nucleotide (NADH or NADPH) as an electron donor, flavinadenine dinucleotide (FAD) and molybdenum. It is considered that during the reduction electron are directly transferred from molybdenum to nitrate (Gurrero et al., 1981). This enzyme is regarded as a 'rate limiting step' in nitrogen assimilation process in higher plants (Srivastava, 1980). A positive correlation between leaf NR activity and growth rate has been noticed by some workers (Reillay, 1976; Austin et al.,

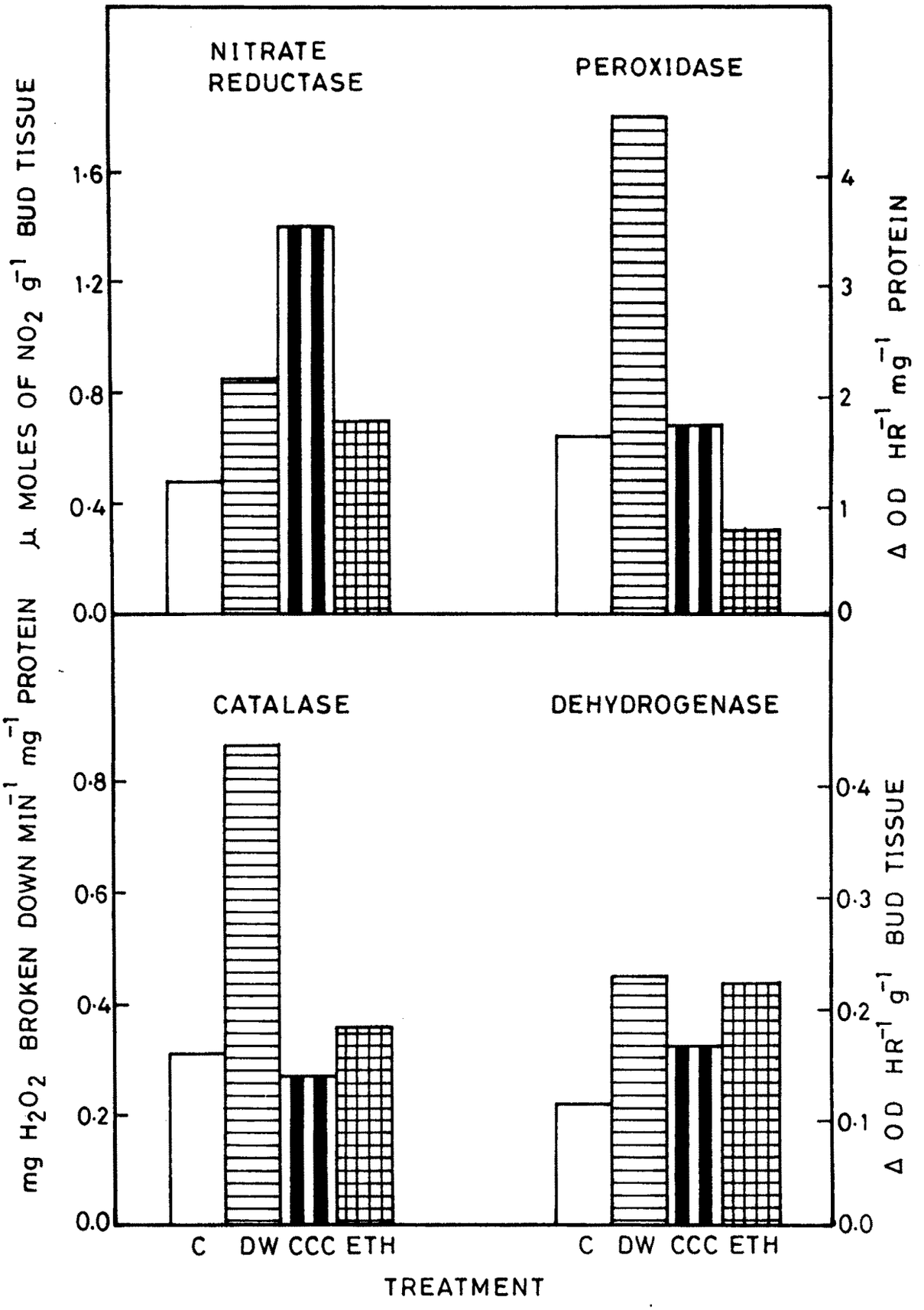

FIG. 1 - EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON ENZYME ACTIVITIES IN THE BUDS OF SUGARCANE VAR - CO - 671 .

1978). In the group of C_4 species to which sugarcane belongs, mesophyll and bundle sheath cells differ in their function not only in CO_2 assimilation but also in their nitrate assimilation (Marschner, 1986). Both NiR and NR are localized in the mesophyll cells and are vertually absent in the bundle sheath cells. This had lead to interesting speculation that the higher nitrogen use efficiency (% of nitrogen in dry matter production) in C_4 plants is due to division of labour, where by mesophyll cells utilize light energy for CO_2 reduction (Moore and Black, 1979). Nitrate reductase activity is generally inhibited by extremes of environmental conditions such as extreme water and heat stress (Sinha and Nicholas, 1981). The activity of NR is stimulated by CO_2, light and nitrate. The half life of NR is short. It's synthesis is stimulated by light. Hageman et al. (1961) reported that light intensity and nitrate availability affect the activity of nitrate reductase in corn.

Sinha and Nicholas (1981) speculated that activity of NR may be regulated through (i) the availability of nitrate, (ii) an inhibition of protein synthesis consequent upon the reduction in the polyribosome level during stress and the inherent high turnover rate of the enzyme or (iii)a reduction in availability of NADH through effect on

photosynthesis and respiration. The NR is an inducible enzyme and the inducer for the formation of NR seems to be nitrate. Dias and Costa (1983) have indicated that activity of NR is dependent upon the concentration and rate of NO_3 supply to the tissue. They noticed that maximum NR activity in sugarbeet leaves is related to increased NO_3 content at that time. Results of Huber et al. (1992) have shown that NR is indeed a phosphoprotein and that at least a crude correlation exists between the degree of phosphorylation of some seryl residues and the catalytic activity. Kaiser et al. (1993) have further proposed that modulation of NR is achieved by reversible phosphorylation/dephosphorylation of NR and regulation of NR is through protein turnover.

Several attempts have been made to understand the influence of growth regulations on NR activity. In view of Knypl (1979) in some special times cytokinin especially in combination with ethylene can possibly act directly on NR synthesizing genetical system. Prakash et al. (1984) observed that cytokinin induced the appearance of NR activity in leaves of cow pea seedlings. Gzik and Guenther (1984) noticed that cytokinin caused an increase in NR activity in leaf discs of both *Beta vulgaris* and *Chenopodium album* independent of presence of nitrate and light Borris (1967) noticed a marked enhancement of NR activity in

Agrostemma embryos in the absence of exogenous nitrate by benzyl adenine and kinetin. In maize leaf tissue, a remark-able synergium between cytokinin and ethylene has been noticed by these workers in this respect. Knypl (1978) tried to investigate the effect of ethrel (5 mg/l) on activity of NR in light sensitive seeds lettuce imbibed in solution of 50 mM KNO_3 under darkness and in light. He observed a slight enhancement of NR activity by ethrel treatment under conditions of darkness. Under continuous light however, a significant increase in NR activity has been noticed. Chavan (1987) observed that the foliar spray of kinetin, GA and CCC caused stimulatory effect on activity of enzyme NR in leaves of both groundnut cultivar's JL-24 and TMV-10. Among these three growth regulators GA appeared most effective while CCC was least effective. Knypl (1973) found that nitrate induced activity of NR in cucumber cotyledons by CCC. At the same time he found no effect of CCC on NR activity in lettuce cotyledons. Another growth retardant succinic acid-2, 2-Dimethylhydrazide (B-9) has been also reported to induce NR activity in cucumber cotyledons (Knypl, 1974). According to Knypl (1974) B-9 can be possibly inhibits production or accelerate inactivation of repressor for NR synthesis in cucumber cotyledons and/or facilitate cytoplasmic enzyme synthesis by inhibition of protein synthesis. However, still no experimental evidence

has been put forth to support the above hypothesis. Dev (1970) noticed that presowing soaking with CCC solution led to increase in the activity of NR in the leaves, at the three leaf stage and decrease of the same in the young cotton roots. Prakash (1970) found that soaking cotton seeds in solution of CCC leads to increase in activity of NR in leaves and decrease of same in the roots of young cotton plants. Bhadre (1983) observed an increase in the NR activity in leaves of cotton varieties Varlaxmi and NCT-9 when seeds were pretreated with CCC. The favourable effect of growth regulators on the nitrate reductase may be due to either increased availability of substrate or increased synthesis of enzyme protein or increased activation of enzyme protein through dephosphorylation. Whatever may be the reason, the increase in NR activity will be certainly advantageous for the sprout during early stages of establishment.

b. Peroxidase

Influence of presowing soaking treatment with distilled water, CCC and ethephon on enzyme peroxidase activity in bud tissue of sugarcane is depicted in Fig.1. It is clear from the figure that the peroxidase activity is considerably increased in buds due to DW pretreatment. On the other hand it is considerably decreased in ethephon pretreatment during germination of sugarcane setts.

Peroxidase is an oxidative enzyme whose primary reaction is to oxidize molecules at the expense of hydrogen peroxide. Hydrogen peroxide (H_2O_2) serves as an electron acceptor and a redox reaction is catalyzed by peroxidase utilizing different types of substrates (Phenolic substances, aromatic amines, ascorbic acid, ferrocytochrome and $NADH_2$ etc.)

$$H_2O_2 \xrightarrow{\text{Peroxidase}} H_2O + (O)$$

Peroxidase shows oxidase activity besides the peroxidase activity i.e. it catalyzes the oxidation of different substances by atmospheric oxygen under aerobic conditions without exogenous peroxide i.e. $HADH_2$, indole acetic acid (Chinoy, 1984), phenyl pyrurate (Fric, 1976). Mazelis and Ingraham (1962) reported that oxidative decarboxylation of amino acids like serine, alanine, phenylalanine, methionine and tryptophan can take place through action of peroxidase. Staham and Demorest (1972) have suggested the possibility that peroxidase may alter the repressor properties of the histone influencing the DNA dependent RNA synthesis. These workers further showed that ribosomal peroxidase may catalyze the synthesis of new ribosomes by depression of donor envolved in ribosome synthesis. In view of Rao (1973) some basic isoenzymes of peroxidase can have a histone like function.

Peroxidases and isoperoxidases have been purified from different plant material and it appears that they do not significantly differ in size (MW from 40,000 to 50,000 daltons). They are formed from a colourless glycoproteins combined to a brown red ferriporphyrin (Gasper et al.,1982). Peroxidase is found to be present in various subcellular components e.g. nucleus, cell walls, mitochondria, ribosomes, cell membranes etc. It has been suggested that the peroxidase localized in the nucleus might be envolved in the structural organization of chromosomes. In plant cells, peroxidase may also have the function of IAA oxidase. The increased peroxidase activity has often been studied in relation with the oxidation of phenols in diseased plants and resistance in the host was attributed to the toxicity of these oxidation products. According to Gasper et al. (1982), besides the possible but not specific involvement of peroxidase in many reactions, on the basis of available evidence isoperoxidases play four major roles in growth and development through their control and/or participation in -

i) auxin catabolism and consequently the endogenous free auxin level.

ii) lignin formation and cell wall biogenesis

iii) defence mechanism against pathogens

iv) some respiratory processes

An increase in peroxidase during seed germination has been evident in several experiments. Gopalchari (1963) observed a regular steep increase in peroxidase activity in all parts of *Sorghum vulgare* and in roots and cotyledons of *Phaseolus mungo* during seed germination and further seedling growth. In several legume seeds an intensification of peroxidase was noticed by Ioana (1969). Similar observations have been also made in several studies (Palmiano and Juliana, 1972; Kruger and Laberge, 1974 and Singh and Singh, 1975). Kamboj and Nainawatee (1978) reported that in four varieties of soyabean, peroxodase activity increased between 2 and 6 days of germination, but activity remained constant in seed which failed to germinate during 6 days of soaking. These workers further carried out peroxidase isozyme analysis. The number of isozymes in matured seeds ranged from 6 to 3 depending on variety. In all varieties the number of isozymes increased at 48 hours of germination Hong et al. (1983) also noticed an increase in peroxidase isozymes during germination of soyabean red bean and mung bean seeds. Zairov et al. (1983) investigated behaviour of peroxidase during wheat seed germination. He observed that peroxidase activity of wheat seeds increased slowly and intermittently soon after soaking and sharply increase beginning 48-72 h after soaking or in the early stages of germination. Peroxidase activity in the embryo was much

higher than in the endosperm through out the process. however, after 48 h of germination the total increase in peroxidase activity was mainly caused by fast migrating isoperoxidases of the embryo. These observations indicate that both quantitative and qualitative changes takesplace in peroxidase activity during germination and active site may differ in the germinating seeds. An evidence for *denova* synthesis of peroxidase during seed germination has been also presented by Dhindsay and Sachar (1978). They found that actinomycin and cyclohexamide severely reduced peroxidase activity both in auxine treated and untreated mung bean cotyledons during seed germination. Based on these observations these workers concluded that transcription and translation are required for peroxidase enhancement in germinating cotyledons.

Poul and Mukherji (1972) studied the changes in respiration rate of rice seedling as affected by storage, viability and its possible relation with catalase and peroxidase activities during seed germination. These workers observed a strong correlation between catalase and peroxidase activity and respiration which according to them was due to the possibility that these enzymes are acting as part of respiratory mechanism of rice seedlings. Hendricks and Taylorsen (1975) speculated possible participation of

peroxidase in NADPH oxidase and electron transport during dormancy breaking in *Amaranthus albus* and *Lactuca sativa*. Zhang et al. (1983) observed that crude extract of dormant seeds of *Pinus koraiensis* strongly inhibited peroxidase activity, respiration rate and seed germination. Sircar (1967) in summarizing various publications concluded that dormancy in rice results from super-optimal auxin concentrations in the endosperm. It is suggested by John and Amen (1977) that excess auxin accumulation can be checked by IAA oxidase like activity of peroxidase which in turn can result in breaking of seed germination. These workers further admitted that the role of peroxidase in seed germination can be clearly defined only after finding the location of different isozymes within the seed or within the subcellular fractions. According to Radriquez and Tames (1983) peroxidase activity changes from cytoplasm to cell wall during chickpea seed germination and seedling development indicating its participation in the control of not only IAA content but also in elongation and differentiation process. It is quite probable that like germinating seeds, peroxidase might be playing similar roles during sprouting of sugarcane nodal buds.

A stimulation of enzyme peroxidase activity by the treatment of GA in sugarcane has been shown by Alexander

(1968). According to Vora et al. (1976), peroxidase activity of pearl millet seedlings rose upto 72 hr. of germination and then declined. Stimulation in the enzyme activity by GA pretreatment was seen under both adequate and restricted moisture levels. This stimulation was so much that the enzyme activity of GA treated seedlings under restricted moisture level is more or less equal to that of untreated 'adequately' watered seedlings. Distilled water treatment stimulated the enzyme activity only under adequate or optimum moisture level. Ram et al. (1976) reported that seed treatment with GA, IAA and NAA inhibited the activity of peroxidase which was accompanied by increase in growth of *Phaseolus* seedlings. Sengupta (1977) also found that the treatment with GA_3, kinetin and ABA decreased activity of enzyme peroxidase during germination of rice (*Oryza sativa* L.) Sangeeta and Varshney (1991) reported that GA_3 (1,10 and 100 ppm) treatment supressed the activity of peroxidase in *Avena sativa* L. Recently, Kaul and Farooq (1992) found that the treatment with kinetin decreased the activity of enzyme peroxidase in *Ipomea purpurea* seedlings.

In case of sugarcane the distilled water pretreatment has caused increase in peroxidase activity in the bud region. A slight increase is also seen in CCC pretreated buds. On the other hand ethephon has caused a

decline in peroxidase activity. It is difficult to interpret the exact significance of these alterations since different isozymes of peroxidase are envolved in different metabolic processes (Gasper et al., 1972). But a possible link may be there between increased rooting at nodal region in ethephon pretreated sugarcane buds and a decline in peroxidase activity.

c. Catalase

Effect of presowing soaking treatment with distilled water, CCC and ethephon on enzyme catalase activity in bud tissue of sugarcane is depicted in Fig.1. It is clear from the Figure that the catalase activity is considerably enhanced due to distilled water pretreatment and decreased in case of CCC while it is slightly increased in ethephon pretreated buds during germination.

Catalase is an oxidative enzyme and it differs from peroxidase by its higher efficiency in catalysing the peroxidative oxidation of certain substances. According to Brill (1966) catalase and peroxidase differ mutually mainly in their affinity to H_2O_2 as an electron donor. Catalase is able to catalyze even the oxidation of other substrates besides H_2O_2. But Grinberg (1971) has suggested since catalase has got more affinity for H_2O_2, it is mainly involved H_2O_2 breakdown in plant tissue. This is highly

desirable as H_2O_2 is shown to be capable of oxidizing sulphahydryl groups, thereby denaturing various enzymes (Patham, 1953). Cytochemical and biochemical findings suggest that catalase in plant cell is located only in microbodies (Peroxisomes, glyoxysomes etc). It is presumed that catalase decreases the H_2O_2 level in the cells, thus limiting the peroxidative reaction catalyzed by peroxidase. A variety of toxic oxygen species (e.g. oxygen superoxide anion, hydroxyl radical, single oxygen and/or hydrogen peroxide) and produced in plants exposed to environmental stress and may lead to serve damage of cell molecules, membranes and other structures (Asada, 1992). Generally, the protective enzymatic systems includes GPx (Guicol peroxidase) and CAT (catalase) that react directly with H_2O_2 and decompose it (Franck et al., 1995).

According to Tolbert (1971,a,b) this enzyme should be regarded as a photorespiratory enzyme as catalase breaks down the H_2O_2 liberated during the reaction of glycolate oxidation. Regulation of the level of H_2O_2 produced during photorespiration and other metabolic processes is done by catalase. This enzyme catalyzes the following reaction.

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Mihalyfi (1968) studied catalase activity during germination

of almond seeds. He observed that after initial decrease, catalase showed a continuous increase upto 11th day. Scandalics (1969) recorded that during seed germination the quantity of catalase increased with time and new isozymes developed in shoots. According to Nanda (1950), the activity of catalase in seeds is very well correlated with germination capacity of seeds. Mukharji and Paul (1971) noticed that during rice seed germination the rate of increase in catalase was maximum between 24 and 48 hrs and then declined in the next 24 hrs. On the other hand a continuous increase in catalase upto first 6 days of germination of rice seeds was evident in the experiments Palmiano and Juliano (1972). De Olivieva et al. (1976) noticed that catalase activity was more than doubled during germination of *Coffea arabica* L. seeds.

Hendricks and Taylorson (1974) suggested that catalase may be involved in the process of dormancy. They observed stimulatory effect of nitrates, nitrites, hydroxylamine, ammonium chloride, potassium cynide and acids on the germination of dormant seeds of *Amaranthus albus, Lactuca sativa, Setaria glauca, Phleum pratense* and *Echinocoia crusgalli*. In *Amaranthus* and *Lactuca* these workers observed that the dormancy breaking was due to inhibition of catalase activity by these compounds which

made H_2O_2 available for peroxidase by action involved in NADPH oxidase system. However, the increase in catalase activity during germination of several nondormant seeds can put several questions in accepting this hypothesis as applicable to all cases of germination. Mukharji and Paul (1971) have indicated involvement of catalase activity in the respiratory machinary of germinating rice seeds as they noticed a positive correlation between catalase activity and respiratory rate. Tregubenko et al. (1973) stated that the activity of catalase is related with respiration rate.

Hormonal regulation of catalase has been also demonstrated by some workers. Mehta et al. (1974) reported that GA and AA have reversed the morphactin induced inhibitory effect on the catalase activity in *Cicer arientinum*. Mehta and Subhadradevi (1974) investigated the effect of ascorbic acid (25 mg/l), GA (10 mg/l), maleic hydrazide (50 mg/l) and combination of GA na MH on enzyme catalase in seeds of soyabean upto 10 days of germination at 27-30°C. They noticed that ascorbic acid and GA had promote effect while MH had inhibitory effect on catalase activity. However, the inhibition caused by MH is overcome by the addition of GA in seeds of soyabean. Mehta et al. (1974) reported that catalase activity is enhanced by GA and AA as well as their combination with morphactins in *Cicer*

arientinum cotyledons. Vora et al. (1976) GA pretreatment stimulated the activity of enzyme catalase while distilled water pretreatment lowered the catalase activity in sesamum seedlings. They indicated that stimulation of the enzyme activity by GA treatment results the enhanced seedling length through increased available energy. Sangeeta and Varshney (1991) also noticed that the treatment with GA_3 (1,10 and 100 ppm) enhanced the activity of enzyme catalase while MH and CCC (10 and 100 ppm) reduced the catalase activity. Kaul and Farooq (1992) recently found that the treatment with kinetin lowered the activity of enzyme catalase in *Ipomea purpurea* seedlings.

In the present investigation also a decrease in catalase activity of CCC pretreated sugarcane buds is noticeable as and these observations conquer with the findings of Sangeeta and Varshney (1991). At the same time distilled water and ethephon pretreatments appear to be effective in stimulating the catalase activity in bud tissue. This may be helpful in checking the level of harmful metabolite hydrogen peroxide.

d. Dehydrogenase

Influence of presowing soaking treatment with distilled water, CCC and ethphon on activity of enzyme deydrogenase in bad tissue of sugarcane is shown in Fig. 1. It is evident from the figure that the activity of enzyme dehydrogenase is increased due to all above pretreatments.

Dehydrogenases play a vital role in cellular metabolism. These are oxidising enzymes catalyzing electron transfer from the donor to an acceptor other than molecular oxygen. Dehydrogenases are the key enzymes of Kerb's cycle, glycolysis and pentose phosphate pathway in seeds (Chakravarty and Burma, 1959). There is an increasing amount of evidences to indicate that the hexose monophosphate shunt is perticulary important during the early stages of germination and may be involved in dormancy breaking phenomenon (Roberts, 1972). It seems possible that important function of pentose cycle is to provide adequate amount of NADPH for various synthetic processes (Mayer and Poljakoff Mayber, 1975).

The reduction of 2-3-5 triphenyl tetrazolium chloride (TTC) to Formazan is taken as a measure of broad spectrum dehydrogenase activity in the present study. Mostly the reduction of tetrazolium molecules with hydrogen atoms

released by the dehydrogenase enzymes which are involved in the respiration processes of living tissues, results in the production of a water insoluble, oil soluble red pigment formazan. In case of seeds TTC reduction generally sheads light on the respiratory capacity and overall viability of seeds. (Moore, 1973). A relationship between dehydrogenase activity and respiratory rate has been established as early as 1954 by Price and Thimann (1954). Although there are no attention has been paid to the effect of growth regulators on dehydrogenase activity, some attempts are made to study influence of these compounds on respiration.

In general terms, it has been found that tissues whose growth is augmented by gibberllin treatment also manifests an increase in respiratory rate (Halevey, 1964; Adams, 1969). On the other hand Fang et al. (1960) and Norris and Foulds (1961) found that where gibberllic acid did not cause growth response, no enhancement in respiration rate could be detected. Adams (1969) in studies on *Avena* stem segments concluded that growth enhanced by GA_3 was not mediated directly through a change in rate of respiration, rather the magnitude of respiration was directly proportional to the amount of growth. Adams (1969) further concluded that the increased rate of respiration due to GA_3 treatment could simply due to synthetic reaction of growth

metabolism drawing off high energy phosphate as ATP, and that a minimal level of respiration is required for GA_3 to induce a growth response.

The ability of ethylene to increase respiration was first described by Denny (1924a, 1924b). Later Harvey (1968) found that ethylene enhanced the respiration of bannana. Nicholas (1968) also showed that ethylene promotes or increases respiration from a variety of fruits and other plant tissues. Starrett and Latties (1991) reported that when fruits of *Avocado* were pulsed 24 hrs after picking the treatment with both ethylene or propylene pulse induced a transient increase in respiration rate. Mahajan and Chopra (1992) recently reported that silver nitrate a well known inhibitor of ethylene action malic dehydrogenase activity in apple fruits after harvest.

In the present investigation an increment in broad spectrum dehydrogenease is clearly noticeable due to preasowing soaking treatments of distilled water, CCC and ethephon. This certainly indicates an activation of metabolism in the bud tissues which would definitely contribute to increased vigour of the resulted seedlings.

B. GERMINATION STUDY

Influence of presowing soaking treatment of DW, CCC and ethephon on germination of eye buds of sugarcane is

depicted in Fig. 2. It is evident from the figure that the germination of sugarcane buds is accelerated due to all the three presowing soaking treatments and in this aspect CCC pretreatment appears more effective.

In sugarcane sprouting of eye buds determines the establishment of crop stand. High rate and extent of germination which indicates the initial density of crop which is one of the major factor affecting both cane and sugar yields. For healthy stand of sugarcane crop earliness and rapidity of germination is of prime importance. Several internal and external factors influences germination of sugarcane. In the view of Bendigery et al. (1986) the potentiality for germination in sugarcane is not the same form base to apex, as each eye bud is a different entity in respect of availability of its energy for germination. The sucrose content goes on increasing from apex to base while the vice versa is the case of glucose content. Van Dillewijn (1952) stated that maximum germination and shoot vigour will result when both internal and external factors are optimum. The germination can be influenced by sett moisture and temperature. Since sugarcane is propagated by the cane stalks, the intrinsic stalk characteristic and soil characteristic at the time of planting and after planting are of much significance. Of these the soil moisture,

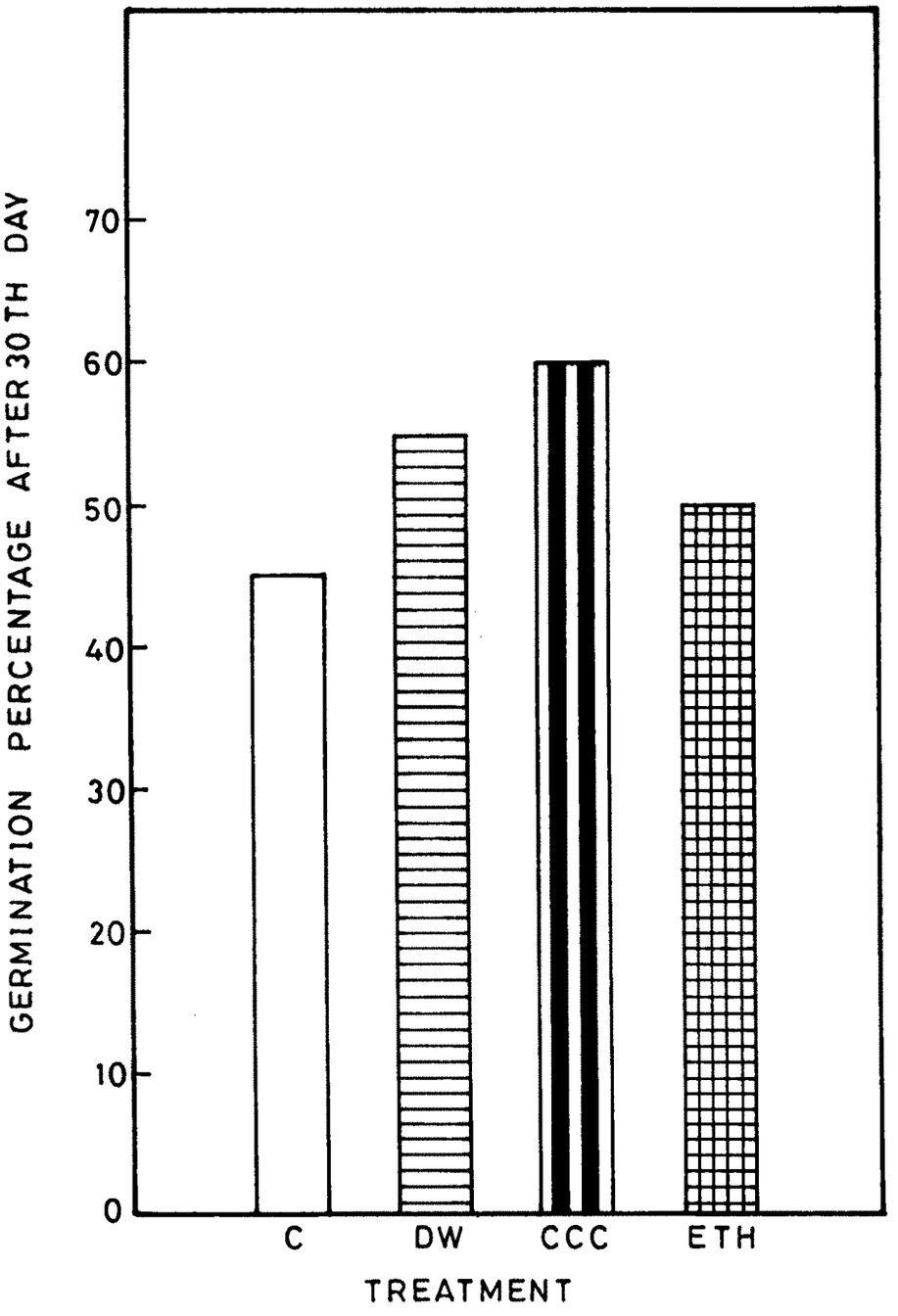

FIG. 2 - EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON GERMINATION PERCENTAGE OF SUGARCANE VAR - CO - 671 .

plating season healthy and immature conditions of buds and various preplanting treatments are more important factors. Different methods are used for better seed germination and the seed treatment is one of the method to overcome the problem of germination. Various chemicals, hormones are used for seed treatment. According to Bendigery et al. (1980), the pretreatment of setts by chemical have definitely influence on the velocity and percentage of germination. Singh and coworkers (1955) reported that soaking of setts in water for about 24-48 hours and in various chemicals like sanitory solutions, Agronone etc. for different durations had caused improvements of germination. Bendigery (1980) observed that water soaking of scooped buds or single sett from bottom portion of cane for two minute have given better germination (35.8%) as against control (29.8%).

The positive effects of presowing soaking treatment of sugarcane setts with growth regulators and other chemicals have been well documented. Shamugasundaram et al. (1974) noticed that four hours of soaking in 100 ppm Etherel, 2000 ppm ammonium sulphate, 100 ppm naphthyl acetic acid, 100 ppm superphosphate or 100 ppm potassium sulphate has induced early germination as did even water as compared with untreated control in variety CO-419. Sherra et al. (1974) studied the effect of pretreatment of NAA and IAA

(100 ppm solution) and a commercial product Exuherone (2% solution) on cane setts of variety CO-740 and come to conclusion that germination was suppressed by above treatments. Joshi and Naik (1980) reported that ascorbic acid pretreatment reduced germination percentage in sugarcane. On the other hand Mohandas et al. (1984) found that presowing soaking treatment with ascorbic acid (at 100 ppm concentration) increased germination percentage by 16-52% in sugarcane. Chinoy (1967) and Chinoy and Saxena (1971) explained the effects of ascorbic acid pretreatment on seed germination and seedling growth of wheat. On the basis of ascorbic acid-nucleic acid - protein metabolism concept, according to them ascorbic acid influences the production of m-RNA modifies the genetic coding and thereby controls the biosynthesis as well as reaction velocities of different enzymes. The change in various enzymes may lead to positive effect on overall physiology thus promoting germination.

Ethylene is known to stimulate germination and break dormancy in many kinds of seeds including groundnut (Esashi, 1991). Ethylene has been shown to enhance the germination of several species at non optimal temperatures (Saini et al., 1986; Schonbeck and Egley, 1981). While increasing temperature (13°C-33°C) are suggested to

stimulate ethylene production in cocklebur seeds due to enhanced enzyme activation and high temperature (30°C and 37°C) has been shown to be inhibition of germination and ethylene production in seeds of *Cicer arientinum*. (Gallardo et al., 1991). According to Bisaria and Paliwal (1982) pretreatment with ethephon enhanced the germination of triticale seeds. Presowing seed treatment with GA_4 and GA_7 in combination with ethephon stimulated germination of celery and celeriac seeds at high temperature in dark. Both treatments increased final germination percentage and reduced the time to 50% germination as compared to control (Thomas, 1983). Chattergi et al. (1971) reported that etherel strongly enhanced both the rate and final percentage of germination. They further observed that etherel not only stimulated seed germination but also was able to reverse the germination, inhibition caused by caumarin and maleic hydrazide, Yadav (1971) observed that presowing setts in 10,000 and 100 ppm of CCC did not have stimulatory effect on germination. Mengal et al.(1988) suggested that quick and maximum okra seed germination with cycocel treatment might be due to increase in diameter of hypocotyl which gives better thrust power. As cycocel is growth retardant, it inhibits the elongation of hypocotyl accompanied by increase in diameter. Perhaps as a result seedling from CCC treated seeds could emerge earlier. Similarly cycocel might have

promoted quick and better seed germination (Goeschl et al., 1966) in okra. Another possible explanation for maximum seed germination of okra with cycocel treatment may be due to increase in the water holding capacity of seeds which help in quick germination (Arora and Dhankhar, 1992).

In the present study we noticed that the eye bud sprouting of sugarcane is significantly improved due to CCC while ethephon is less effective in this respect. Thus these growth regulators might be playing an important role in cell division and water holding capacity of eye buds which may improve the quality of crop stand which further leads to vigrous growth of cane.

C. GROWTH AND YIELD STUDY

The pattern of initial root growth during germination of sugarcane setts under the influence of presowing soaking treatment is depicted in Fig. 3 and Fig. 4. It is clear that significant adventitious rooting is promoted by presowing soaking treatment especially the pretreatment of CCC. Effect of presowing soaking treatment of DW, CCC and ethephon on average plant height, root length, internodal length, average internode number, cane diamter, fresh weight, leaf area and tiller number are depicted in figures 5,6 and 7. It is evident from the figures that all the above

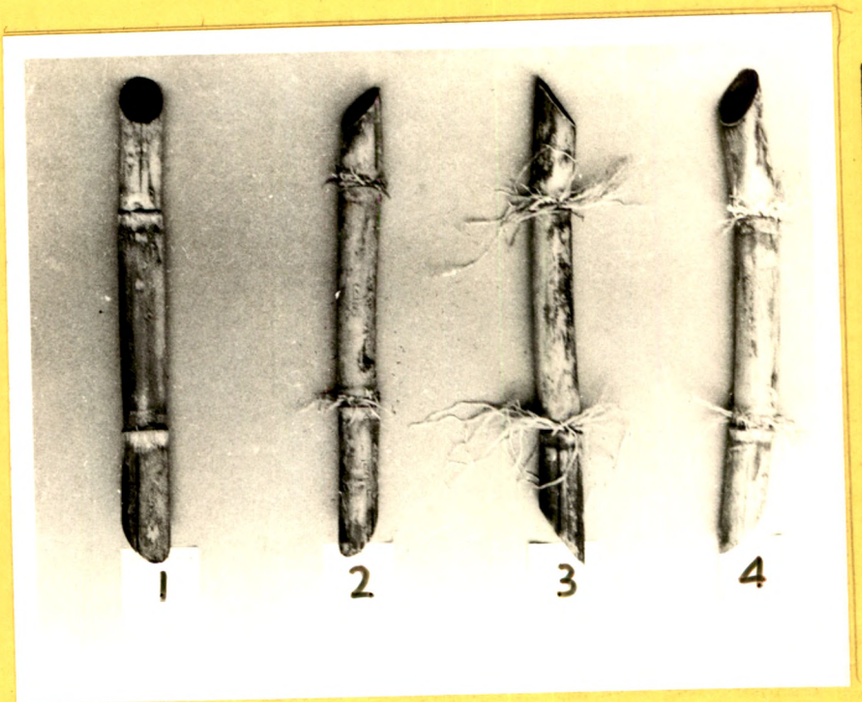

Plate No.2 : Effect of presowing soaking treatment of DW,CCC and ethephon on growth of nodal roots in sugarcane variety CO.671

1) Control (2) Distilled water

3) CCC (4) Ethephon

Plate No.3 : Effect of presowing soaking treatment of DW,CCC and ethephon on vegeative growth of sugarcane variety CO.671

1) Control (2) Distilled water

3) CCC (4) Ethephon

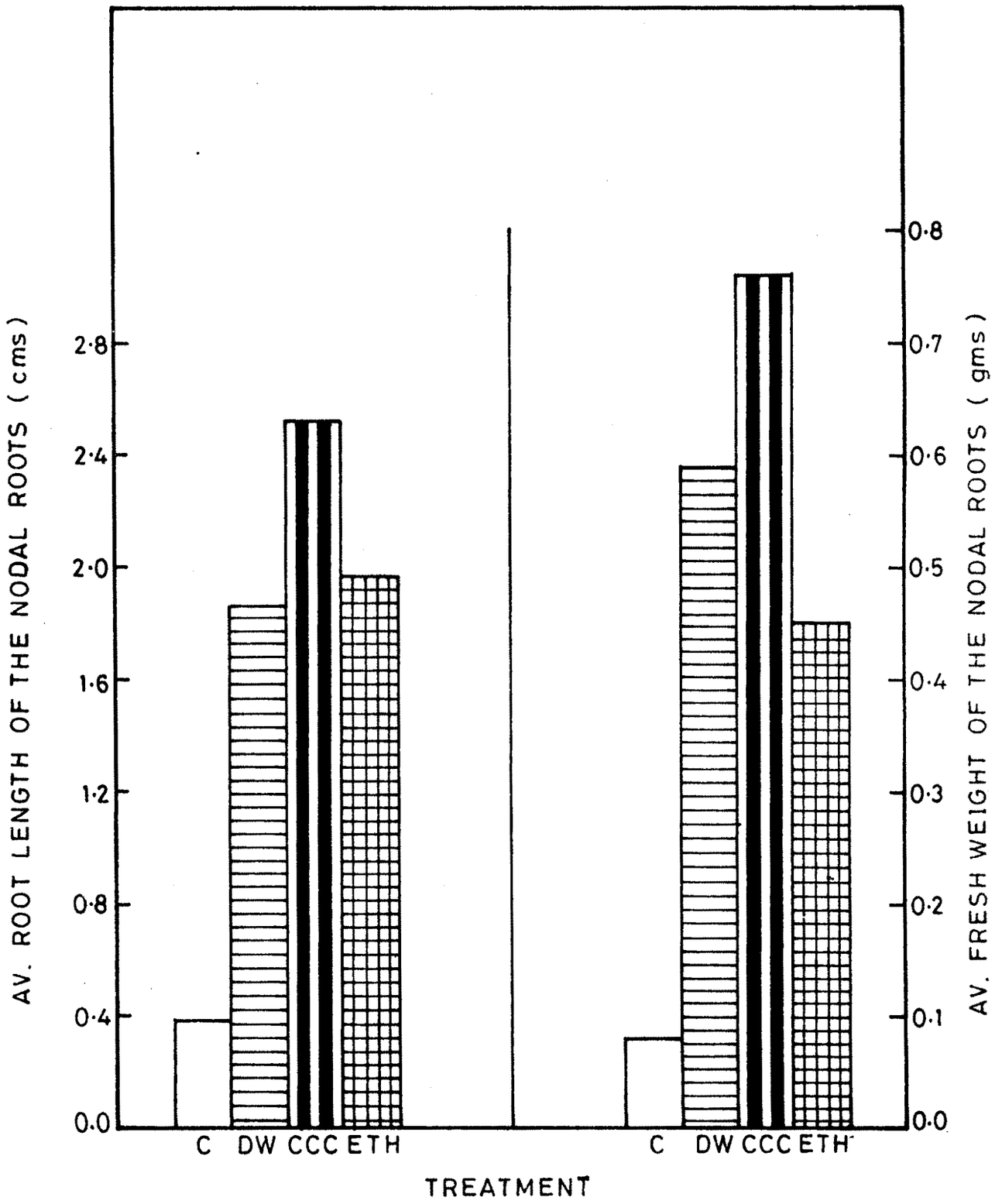

FIG. 3 – EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON THE AV. ROOT LENGTH AND AV. FRESH WEIGHT OF NODAL ROOTS IN SUGARCANE VAR - CO - 671 .

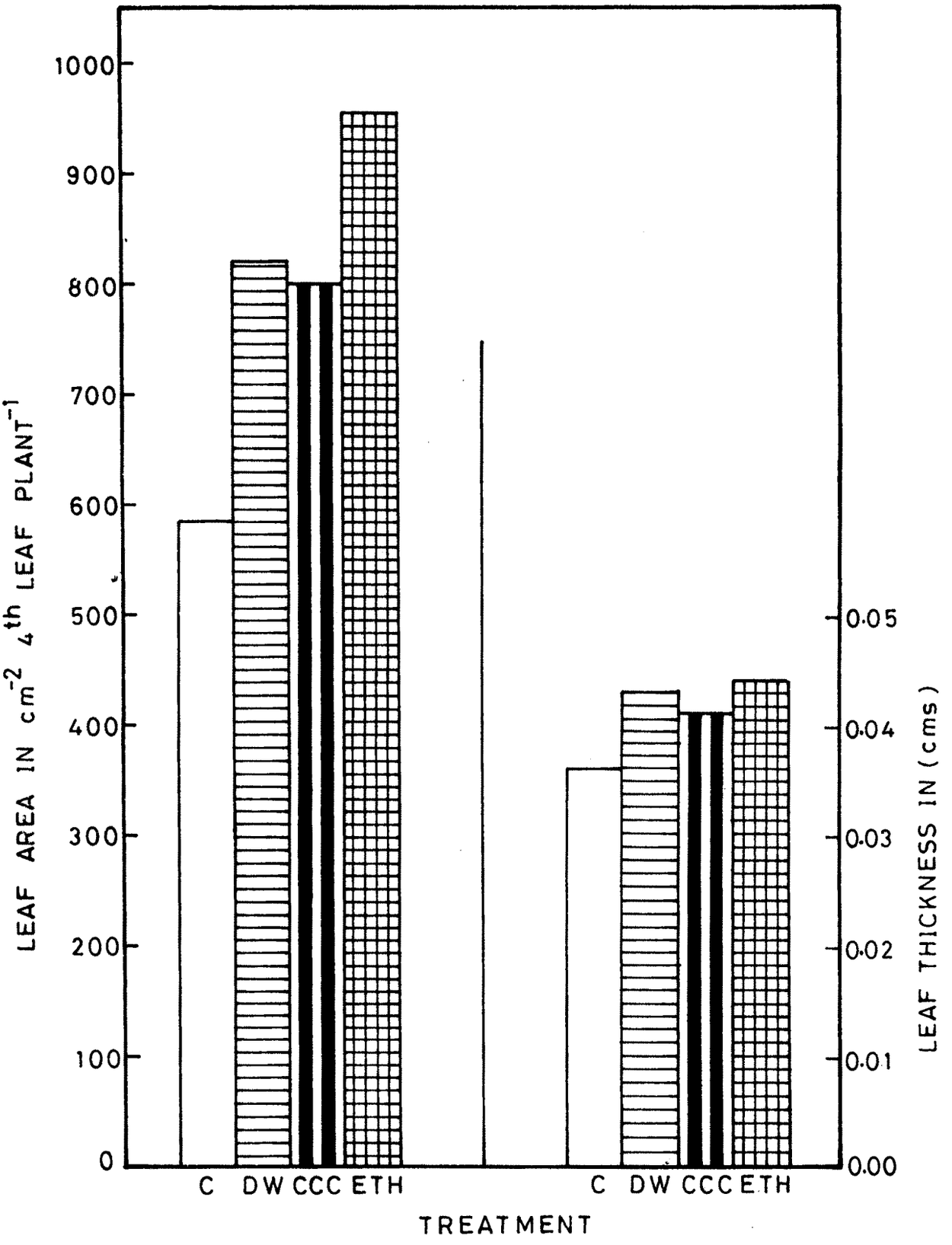

FIG. 4 - EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON LEAF AREA OF 4th LEAF AND LEAF THICKNESS IN SUGARCANE VAR - CO - 671

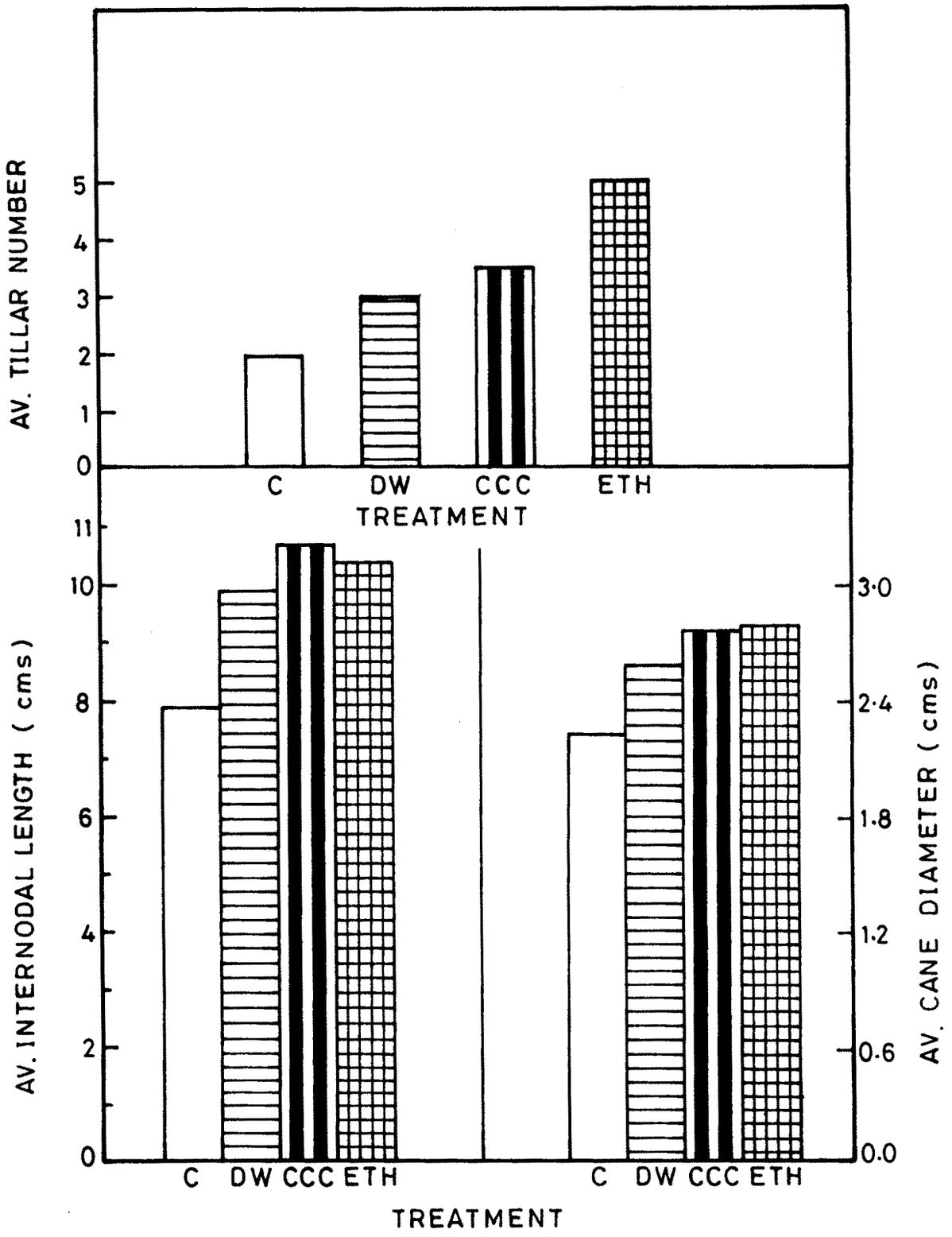

FIG. 5 – EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON AV. TILLER NUMBER, AV. INTERNODAL LENGTH AND AV. CANE DIAMETER OF SUGARCANE VAR – CO – 671.

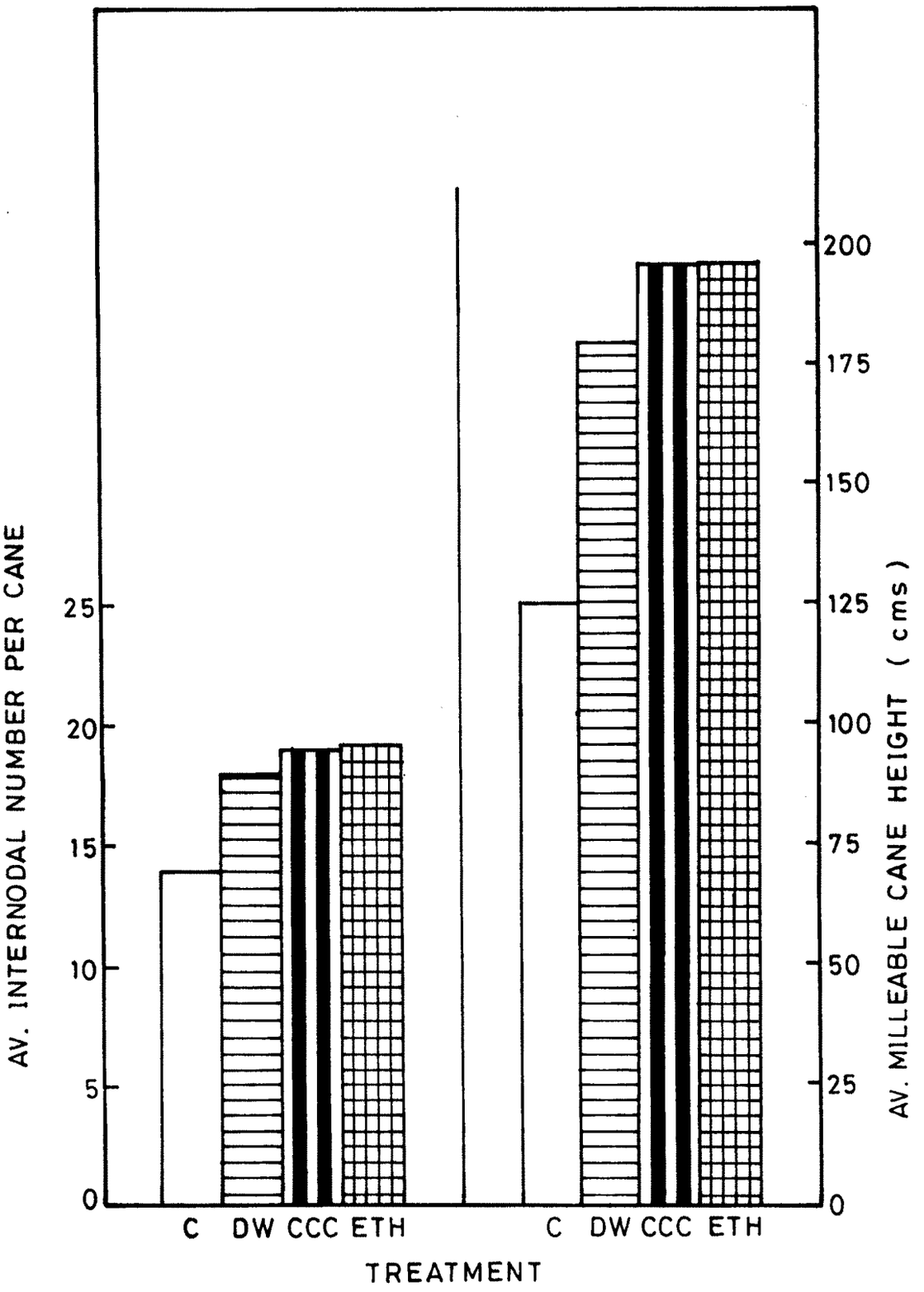

FIG. 6 - EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON AV. INTERNODAL NUMBER PER CANE AND AV. MILLEABLE CANE HEIGHT OF SUGARCANE VAR - CO - 671 .

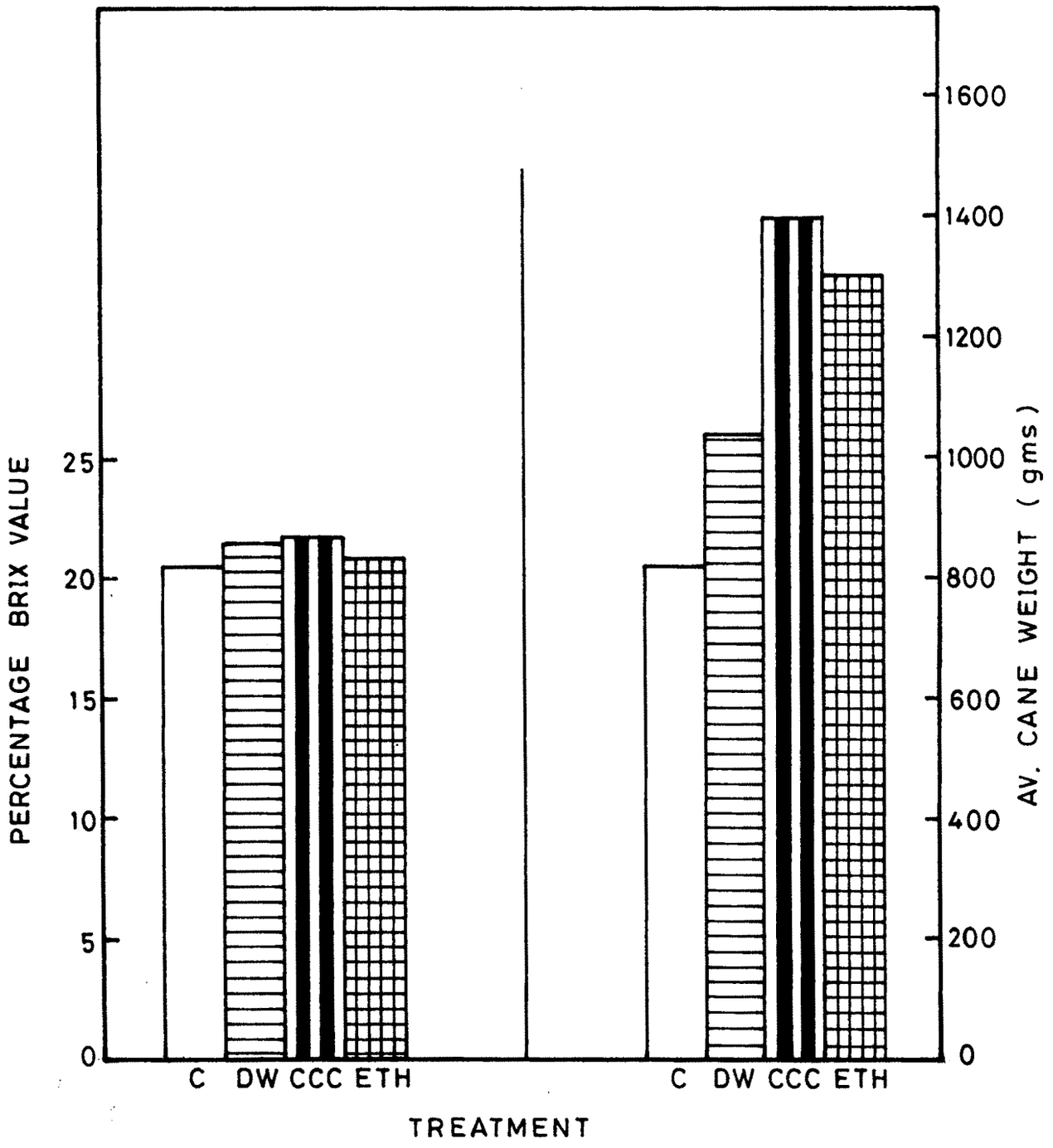

FIG.7 – EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON PERCENTAGE BRIX VALUE AND AV. CANE WEIGHT OF SUGARCANE VAR-CO-671 .

growth and yield parameters are considerably increased due to presowing soaking treatments. This is perticularly significant in case of CCC pretreated canes. The tiller number is markedly increased due to ethephon pretreatment. The juice quality is also improved by pretreatments.

The roots perform two major function in plant namely absorption of water and absorption of minerals. They also provide lot of support to plants. Hence root growth during initial phase of plant growth is very important from the point of view of overall performance of the plants. The auxin especially indole butyric acid are highly reported for induction of rooting in cuttings of vegetatively propagating plants. Sugarcane also shows vegetatively mode of propagation and our observations indicate that presowing soaking treatment favour the development of adventitious roots. The growth analysis in crop species involves non destructive measurements of height, number of leaves and leaf area. The height of the plants usually a very good indicator of their conditions which indicates successful establishment of species in various environmental conditions. Height is also a very easily detectable growth characteristic of the plants growing in field conditions. In sugarcane height of cane has special significance with respect to productivity. For the photosynthetic producers, light interception and photosynthetic rate depends to a

large extent upon the available leaf area. Besides photosynthesis the leaves also control water loss and also act as a center of number of metabolic activities. Thus measurement of leaf area determines the plants "Productive investment" (Causton and Venus, 1987). Also leaf area is more related to crop yield as leaf is a major 'Source' and leaf development is a major determinant of plant growth and productivity. According to Chow et al. (1990), total leaf area is important determinant of shoot dry weight than photosynthetic capacity per unit leaf area. The overall growth of plant is indicated by increment of dry weight of plant over a period of time. The rate of growth is linearly proportional to the product of substrate and plant weight. According to Charles (1982), the proportion of new dry matter partitioned to leaf and stem tissues did not change, implying that the leaf and stem densities increased with the time and ontogenetic changes in partitioning of dry matter patterns may also be attributable to ontogenetic changes in the specific activities of roots and shoots with respect to uptake or assimilation of perticular elements. The total dry matter production gives an idea about carbon budget and productivity capacity of plant which depends upon total area of photosynthetic organ and its efficiency to harvest solar energy.

A favourable effect of presowing soaking treatment

on crop growth is evident in many studies. Dowson (1965) noted highly significant increase due to pretreatment of GA, NAA and IAA in tiller number, plant hight, shoot height and grain weight in different crops. Darra and Saxena (1973) noticed that presowing soaking treatments of growth regulators for about 24 hours caused significant improvement in growth and development of crop plants. Effect of presowing soaking of wheat seeds for 24 hours in 10, 100 ppm 2,4-D, IAA and NAA on growth and yield has been studied by Bhardwaj and Rao (1955). He concluded that in all treatments growth is inhibited first and recovered afterwards but yield is decreased by 2,4-D and NAA and increased by IAA. According to Dave and Gaur (1970), presowing soaking treatment with GA_3 and L ascorbic acid caused stimulation in vegetative growth and grain yield was increased up to 30% by AA in barley, while presowing soaking treatment with ascorbic acid (25 mg/lit) and sucrose (1%) enhanced height of main stem, dry weight of roots, stem, leaves, spikes and total grain weight of barley. Presowing soaking treatment with L ascorbic acid and gibberellic acid enhanced height of main stem, fresh weight, dry matter accumulation and increased grain yield (Patil and Lall, 1973). In case of wheat Singh et al. (1974) reported that pretreatment with GA and IAA enhanced plant height while IBA pretreatment increased root length. IBA and NAA pretreatments increased

dry shoot production and grain yield. Ogbonna James et al. (1989) noticed that pretreatment of seeds of cowpeas with GA increased plant height while the plants raised from seeds treated with DW were same as untreated on the other hand MH has inhibitory effect on dry matter production and yield. In pot experiments with wheat variety Raj - 911. Chippa and Pal (1988) noticed that presowing soaking treatment with distilled water, IAA (200 ppm), IBA (200 ppm), Na_2SO_4 (3%), Ca $(No_3)_2$ (3%) plus pyridoxin (0.3%) increased the plant height, tillering and straw yield. According to Arora and Dhankhar (1992), treatment with cycocel was effective in suppressing apical dominance, thereby promoting growth of axillary buds into new shoots. Similar results were recorded by Marisiddaiah and Gowada (1977). Reduction in plant height with foliar CCC application was ascribed to its effect in reducing cell expansion and synthesis of diffusible endogenous substances (Cathey, 1964). Singh and Kumar (1988) reported increased fruit weight with foliar spray of ethrel at 150 ppm in okra Cv. *Pusa sawani*. Several workers noticed that sugarcane growth is also considerably promoted due to presowing soaking treatments. Singh and Singh (1964) used five different concentrations of IAA (5 to 80 ppm) and noted that the treatment enhanced tillering and growth in sugarcane. Nimbalkar (1973) reported that preplanting treatment with GA, IAA, kinetin and ascorbic acid enhanced

overall growth of sugarcane crop. Also Singh and Singh and Nimbalkar (1973) used IAA for preplanting treatment of sugarcane setts and stated that the treatment can be utilized for improving the production along with its quality. Nickell (1987, 88) noticed that the pretreatment of CCC is effective in enhancing tillering in sugarcane thus causing an increase in the number of plants per unit area. Bischoff et al. (1967) observed that pretreatment of sugarcane setts with ethephon increased shoot production but had no effect on overall growth. Doi (1983) showed that sparying the seed cane in the field with ethephon at 1 b/acre three weeks before cutting the setts and planting or dipping the setts into ethephon at 5000 ppm stimulated tillering in sugarcane. In 1987 Nickell noticed that pretreatment of ethephon enhanced stalk fresh weight between 8-10 weeks and was active in inducing tillering at Hawaii. Also recent results from Jamaica confirmed the Hawaiian results in response to ethephon results from Brazil showed no effect of ethephon on plant crop but significant increase in number of milliable stalks in ratoon pretreatment of ethephon was found to promote stalk elongation in sugarcane (Nickell, 1988). In the present investigation also similar response is evident. Generally ethephon increases sugarcane yield by inhibiting the process of flowering (Nickell, 1976). But even before flowering we can notice increase in

various yield parameters due to ethephon pretreatment in the present investigation. Kumar and Pande (1988) observed that cycocel at 2.5 lit/ha applied in early November did not show any change in sugarane juice. On the other hand the foliar application of CCC treatment for 36 days at concentration of 5 kg/ha was effective in causing increasing brix value and sucrose content in cane juice. Our observation indicate favourable effect of pretreatment of CCC on juice quality. The quality of cane treated with GA_3 is at least equal to and generally better than that of untreated cane provided that sufficient time is allowed between treatment and harvest, it also shows the increased cane tonnage at a quality level where nine tone of cane produce one tone of sugar would result in calculated yield increase of 1.46 to 2.17 metric tone/hact. (Moore, 1978). According to Naik and Joshi (1981) the preplanting treatments are useful to a certain extent to improve cane yield. The juice quality is significantly improved. This can also be attributed to inhibited invertase activity in pretreated cane leaves and NADP maleic enzyme activity leading towards more sucrose accumulation with high photosynthetic rate.

Since sugarcane is an important crop for sugarcane industry and its yields potential is determined by the quality of juice, weight of cane and internodal length while

crop productivity is directly depends on its foliage leaf area for maximum photosynthesis and sucrose production thus all these yield parameters show appreciable increase due to presowing soaking treatments in the present study. Thus this technique appears to be beneficial for sugarcane growers to increase the yield potential of this crop.

D. EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON INORGANIC CONSTITUENTS

a. Potassium

The effect of presowing soaking treatment with distilled water, CCC and ethephon on potassium status in sugarcane leaf tissue is shown in the Fig. 8. It is clear from the figure that due to DW and CCC pretreatments potassium content in sugarcane leaves is decreased while the presowing soaking treatment with ethephon has caused increase in the potassium content.

Potassium is regarded as most essential macronutrient and it is required by sugarcane in greater amounts than any other nutrient. Its uptake is highly selective and closely coupled with metabolic activities. Potash fertilizers plays an important role in increasing yield in Hawaiian Sugar Industry (Humbert, 1963). Potassium is also concerned in the formation and neutralization and organic acids and by developing a balance of sugar

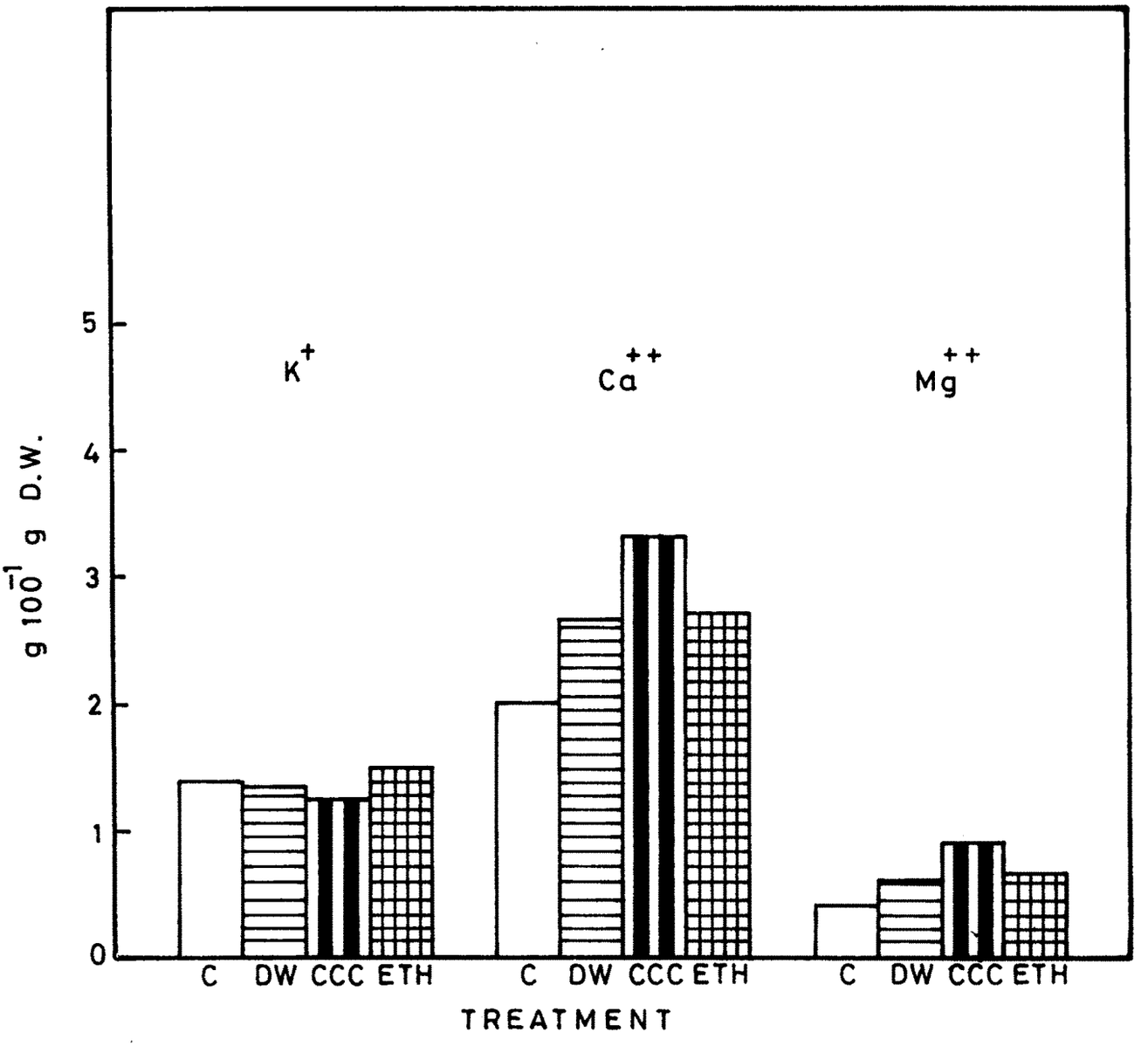

FIG.8 - EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON POTASSIUM, CALCIUM AND MAGNESIUM CONTENTS IN LEAVES OF SUGARCANE VAR - CO - 671.

(Humbert, 1963). K is major plant nutrient which plays biophysical roles in cellular water relations and biochemical role in variety of metabolic processes. The functions of K are many it is known to be required for cell structure, carbon assimilation, photosynthesis, protein synthesis, starch formation, translocation of proteins and sugars, the entry and water into plants, normal root development and other life processes like stomatal movements and osmoregulation (Bayley and Kushner, 1964). It is also involved in enzyme activities (Evans and Sorger, 1966), photoreduction and photophosphorylation (Pfiuger and Mengel, 1972) and nitrogen turnover (Helal et al., 1975). It is required for the stability of ribosomes (Bayley and Kushner, 1964). Although K is not involved specifically in photosynthetic metabolism, it is required in relatively high concentrations for other biophysical and biochemical processes which influences photosynthesis (Huber, 1985). The requirement of external K may be related to maintenance of alkaline pH in the stroma by Mg activated K-H antiporter in the chloroplast envelope (Huber and Maury, 1980). Hartt (1929) found a definite effect of K on the activity of certain enzymes in sugarcane. Potassium seems to be special activator of invertase in sugarcane, while amylase activity is reduced by K in both blades and stalks. The activity of peptase and catalase in the above ground parts of the

sugarcane plant is stimulated by K fertilization. According to Mengel and Kirkby (1980), the mechanism of water uptake by roots and the ability of plants to exhaust soil water depends upon the potassium nutritional status of the plant. Low concentration of K results in the production of plants with high transpiration rate could be regulated by varied potassium concentrations. Reduction in the rate of transpiration is one of the criteria used to designate drought resistance in plants. According to Weimberg et al. (1982), there exists positive correlation between K content and proline accumulation in sorghum. Hubac et al. (1986) proposed positive correlation between the ability of a plant to resists water stress and the K content. Thus it is obvious that potassium status of leaf tissue is of great importance in evaluating drought resistance capacity. According to Mengel and Krikby (1982), K in plant is very mobile and the bulk of K is mainly taken up during the vegetative growth stage.

Potassium is unevenly distributed between the vaccuole and cytoplasm in plant cells. A critical concentration of K can be determined which is defined as the concentration at which 90% of maximum yield is obtained (Ulrich and Hills, 1967). Above this concentration (1% K in dry matter) growth shows little response to increased tissue

K but at lower concentrations it declines rapidly (Leigh and Wyn Jones, 1984). According to Asher and Ozanne (1967), for many plants the critical K concentration is in the range of 0.5 to 2% in dry matter, which is well below the concentrations found in plants that are well supplied with K. However, the critical K concentration is decreased if the rate of supply of other cations such as Na, Mg is increased (Leigh and Wyn Jones, 1984). According to Humbert (1963), during the first six months the amount of K in the sugarcane leaves increases until a maximum canopy is obtained, after which the quantity in the leaves remains practically constant until the number of leaves per stalk decreases in the stalks and dry leaves parallels their development, reaching a maximum in the ripening period. Borden's (1944) data indicted that K may move out of the above ground portion of the cane plant during maturing period. When sugarcane is grown in complete nutrient solutions a high concentration of K occur in the youngest organs, the elongating cane and meristem. The next highest K composition is found in the elongating cane sheaths and the spindle clusters, followed by older leaves and the green leaf cane. The internodes decreases rapidly from 5.7% in the top of the stalk to 0.75% in the basal, internodes. Nimbalkar (1973) noticed that there is decline in potassium status during sugarcane leaf senescence. Clements et al. (1947) found that

the concentration of K in sugarcane leaves varies from 0.17 to 5-10%. The content of K in sugarcane leaves recorded in the present study is in the above range.

The deficiency of K results in stunted growth, leaf damage, decrease in photosynthetic activity and translocation of photosynthates. Sugarcane plants suffering from a lack of potassium show depressed growth, yellowing and spotting of older leaves and the development of slender stalks. The older leaves develop an orange - yellow colour with numerous chlorotic spots which later become brown with necrotic centers (Humbert, 1963). As early as 1929 Hartt reported that K deficiencies results in abnormal distribution of vessels in the pith of roots, small size of vessels and the parenchyma cells in the stalk, large cavities in the cortex of the roots and the under development of the root hairs. She also observed that under certain conditions, the development of root system is stimulated by K deficiency, resulting in drastic changes in the shoot - root ratios. Hartt (1929, 1934) studied the effect of K storage on protein synthesis and translocation. She found an accumulation of amino nitrogen in the blades and stalks associated with a decrease of protein nitrogen indicating that K deficient sugarcane plants are unable to synthesize protein as usual. These early but extensive

investigations clearly threw a light on the important role in sugarcane metabolism.

There are few attempts to study the effect of growth regulators on the potassium uptake. The experiments of Nimbalkar (1973) showed that the preplating treatment with GA, IAA kinetin and ascorbic acid increased the leaf potassium content. Saimbhi et al. (1975) observed that pretreatment with 20 ppm GA increased K content but 10 ppm ascorbic acid decreased the potassium content of pea seedlings and came to conclusion that GA caused higher K/Ca and K/Na ratio and AA caused lower K/Ca and K/Na ratio. Naphade et al. (1986) reported that seed pretreatment with ascorbic acid increased potassium status in sunflower seeds and stalks. Shanshidhar (1981) investigated influence of calcium chloride pretreatment on groundnut variety RS - 218 and they noticed that the plants raised from pretreated seeds had higher level of potassium. According to them the better performance of this cultivar under stress conditions in fields following calcium chloride treatment is due to higher potassium content and higher degree of proline accumulation. Kutwal (1989) shown that the presowing soaking treatment of groundnut seeds with CCC and kinetin caused a rise in the potassium content of all parts viz. leaf, stem and roots. He further suggested that the increase in

potassium content may have induced drought resistance in groundnut plants.

In contrast to above observations very slight alterations are evident in the leaf potassium status in the present investigation. The pretreatment with distilled water and CCC have slightly lowered the potassium status while the ethephon pretreatment has caused elevation of potassium level. However, the extent of these alterations in the level of this macroelement is very small and hence does not indicate any major shifts in the normal metabolism of sugarcane plants.

b. Calcium

Influence of presowing soaking treatment with distilled water, CCC and ethephon on the calcium content of sugarcane leaves is depicted in the Fig. 8. It is evident from the figure that all pretreatments records an increase over control.

Calcium is an important divalent cation found in highest concentration in meristamatic tissues and in younger leaves. In contrast to other micronutrients a high proportion of total calcium is found in the cell walls. Calcium plays an important role to make alkali soil fit for agriculture. Gypsum or calcium sulphate is often applied to

alkali soils to counteract the harmful effects of magnesium because high magnesium concentrations are made nontoxic by addition of calcium. In plants calcium exists in different forms in plant tissues, it may occur as free calcium or in the form of calcium adsorbed to indiffusible ions like carboxylic phosphorylic and phenolic hydroxyl groups. The compounds of calcium usually occurs as deposit in cell vaccuoles. The seeds contains calcium mainly in the form of salt of phytic acid while in many plant families calcium oxalate crystals are found.

The importance of calcium in the functioning of membrane and maintenance of cell integrity and synthesis of pectin in middle lamella of the cell wall is very well documented. It is helpful to minimise iron diffusion to stabilize membrane which maintain selective ion transport mechanism and decrease permeability (Hanson, 1984). According to Marme (1989), changes in the concentration of cytosolic calcium triggers the chain of events that results in turning a phosphorylation process on and off thus ultimately affecting large number of biochemical reactions. This is became of the fact that the regulation of activity of many enzymes takes place through phosphorylation dephosphorylation process.

According to Clarkson and Hanson (1980), a major

role of calcium appears to be its binding with proteins, nucleic acids and lipids to affect cell adhesion, membrane chromatin organization and enzyme conformation. One of the roles calcium is to activate and stabilize the α-amylase molecule which is Ca containing metalloenzyme that binds at least one atom of calcium per molecule (Jones et al., 1993). Calcium has recently been recognised as transducer of hormonal and environmental signals to the responsive elements of cell metabolism (Evans et al., 1991). Swamy (1991) attributed the importance of calcium in plant growth and development to the calmodulin. Calmodulin is a calcium binding protein which binds the calcium in such a way that calcium protein complex stimulates many enzymes and physiological process at very low concentrations. Besides calmodulin, the occourance of other Ca binding proteins have been reported in plants. There is now ample evidences which indicate that calcium ions help in regulating phytohormone responses (Raux et al., 1986).

Evans (1942) found that the calcium content of leaves of sugarcane gradually increases from 0.25 to 0.5% of dry weight as the crop develops. Ayres (1936) data also showed that calcium concentrations increases as the leaves grows older. Nimbalkar (1973) found accumulation of calcium in senescent leaves of sugarcane.

The symptoms of calcium deficiency in sugarcane

have been described by Martin (1955) and Evans (1959). The first symptom of calcium deficiency on leaves are minute chlorotic spots with dead centers that lateron turns dark reddish brown. The intensity of chlorotic spots increases with age of leaves. Retardation of growth, weakening of plant and rind becomes soft. Eventually growth is completely stopped and plant die.

Calcium uptake is influenced by several environmental and endogenous factors. Darra and Saxena (1973) concluded that the presowing soaking treatment of maize seeds with IAA caused increase in the calcium uptake. Nimbalkar (1973) reported that preplanting treatment with GA, IAA and ascorbic acid increased calcium content of sugarcane leaves while kinetin pretreatment reduced calcium content. On the other hand Saimbhi et al. (1975) noticed that seed treatment with 20 ppm GA lowered Ca content and at 10 ppm AA has no effect on calcium but GA caused higher K/Ca ratio and AA caused lower K/Ca ratio. Chippa and Pal (1988) observed that presoaking treatment with IAA (20 ppm), IBA (200 ppm) and distilled water increased calcium in straw and grain of wheat. Kutwal (1989) found that the presowing soaking treatment with CCC and kinetin (in acetone) are effective in increasing calcium status of groundnut leaf tissues under the drought condition. We noticed in our

experiments that the calcium status of leaf tissues is increased considerably due to presowing soaking treatment with distilled water, CCC and ethephon. This can exert favourable influence on calcium mediated physiological processes in sugarcane.

C. Magnesium

The influence of presowing soaking treatment with distilled water, CCC and ethephon on magnesium status in sugarcane leaves is recorded in Fig. 8. It is obvious from the figure that there is a definite increase in the magnesium content of leaf tissue due to the three pretreatments. The increase is highest in case of CCC pretreatment.

Magnesium occupies a very significant place in plant mineral nutrition. It is small and strongly electropositive divalent cation. Magnesium functions as bridging element for joining the subunits of ribosomes (Cammarano et al., 1972). Under magnesium deficiency conditions or in presence of excessive levels of potassium, the subunits of ribosomes dissociates and protein synthesis ceases (Sperrazza and Spermalli, 1983). Mg plays a crucial role in various physiological processes in plants. The most well known role of Mg is its contribution to the center of the chlorophyll molecule, although 'chlorophyll - Mg' is

relatively in small fraction of the total Mg content of plant. Mg is required for the synthesis and assembly of solar energy harvesting molecules - chlorophylls. Magnesium is required for photosynthesis. High concentration of Mg^{2+} and K^{+} are required in the chloroplasts and cytoplasm to maintain a high pH between 6.5 and 7.5 compared to much lower vaccuolar pH of 5 to 6. Smith and Ravan (1979) reported that the enzyme activity is regulated by pH which is maintained by Mg^{2+}, K^{+} and up to certain extent by Ca^{+2}. Mg is also required for the RNA polymerase and hence for the formation of RNA in the nucleus. This role of magnesium might be related to both joining between individual DNA strains and neutralization of the acid proteins of the nuclear matrix (Wunderlich, 1978). Mg is highly essential in many enzyme reactions (Clark, 1984). Mengel and Kirkby (1982) explained that the substrate for ATP ases is Mg - ATP. Mg works as a co-factor in almost all enzymes activating phosphorylation processes and it forms bridge between the phosphate structure of ATP, ADP and the enzyme molecule. A key reaction of Mg is activation of RuBP case (Clarkson and Hanson, 1980). In general terms, enzyme reactions that requires Mg include nucleotide transfer (i.e. phosphatase, kinase, ATP ases, synthatases, nucleotide transferases) and enzymes such as dehydrogenases, mutases and lyases. According to Jacob (1958), magnesium is highly

essential for the activity of sucrose phosphate synthatase, one of the key enzyme responsible for biosynthesis of sucrose which is major sugar in sugarcane. Magnesium also promotes the formation of vitamins especially carotene. Under some circumstances Mg may contribute to the electrical neutrality of organic compounds such as sugar phosphate, sugar nucleotides and organic amino acids (Clark, 1984). Kirkby and Mengel (1967) noticed that Mg is often associated with such organic anions as malate, citrate, pectate and oxalate as well as with inorganic anions.

Magnesium deficiency in chlorotic leaf condition fallowed by depressed growth. In advanced cases of Mg deficiency the sugarcane leaves are chlorotic and severely spotted, the stalks are smaller in diameter with shortened internodes and show an internal browning. Root systems are restricted in growth (Humbert, 1963).

Ayres (1937) found the accumulation of magenesium in various parts during growth of sugarcane. The amount of magnesium accumulated in the dry leaves is considerably smaller than in case or calcium. Nimbalkar (1973) noticed that there is accumulation of Mg during sugarcane leaf senescence. In higher plants average value of magnesium ranges form 50 mg to 100 mg per 700 g dry tissue. Epstein (1972) recorded that magnesium is one of the abundant divalent cations in the plants. Although 2% Mg on a dry

weight basis regarded as critical value. Evans (1936) noticed that leaf blade magnesium ranges from 0.08 to 0.35% and is associated with good growth of cane. Nimbalkar (1973) studied effect of preplanting treatment with GA, IAA, kinetin and ascorbic acid on leaf magnesium status of sugarcane. He found a decline in Mg level in pretreated plants. Saimbhi et al. (1975) also reported that seed treatment at 20 ppm GA lowered the Mg content of pea seedlings. Kutwal (1989) observed in case of groundnut that presowing soaking treatment with CCC and kinetin caused elevation in Mg content of water stressed groundnut leaf, stem and root tissue. In the present study a marked increase in the Mg content of sugarcane leaves pretreated with distilled water, CCC and ethephon is evident. Thus the magnesium nutrition of sugarcane is markedly improved due to presowing soaking treatments with distilled water, CCC and ethephon and this will certainly cause beneficial effects on sugarcane metabolism in view of paramount significance of magnesium in cellular biochemistry.

d. Phosphorus

Effect of presowing soaking treatment with distilled water, CCC and ethephon on phosphorus content in leaves of sugarcane is depicted in Fig. 9. It is clear from the figure that due to above pretreatments there is decrease

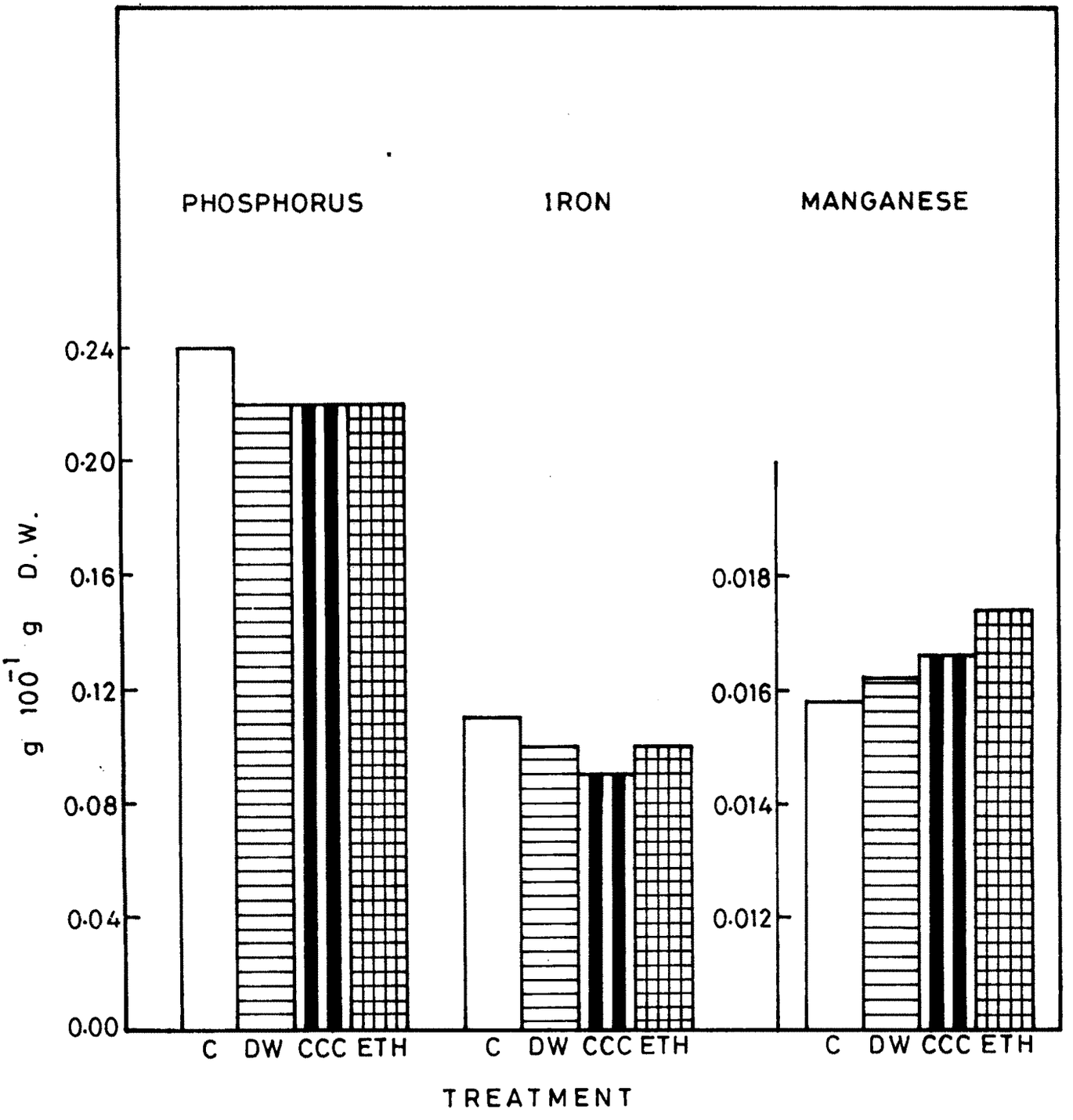

FIG. 9 – EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON PHOSPHORUS, IRON AND MANGANESE CONTENTS IN LEAVES OF SUGARCANE VAR – CO – 671.

in phosphorus content of sugarcane leaf but these decrease is not so significant.

Phosphorus is one of the indispensible element in all living organisms. It is essential constituent of large number of metabolites which plays a key role in various life processes. It is the structural component of DNA and RNA. It plays major role in energy transfer during plant metabolism like glycolysis, respiration, photosynthesis etc. in the form of ATP, pyrophosphate and also in cell division and expansion. 'P' is also incorporated in the phospholipids which form backbone of cellular membrane RuBP and PEP the two main CO_2 acceptors in the plants containing phosphorus. P is taken by plants mainly as $H_2PO_4^-$ and it remains as inorganic phosphate (Pi) or it is esterified through a hydroxyl group to a carbon chain (C-O-P) as a simple phosphate ester (e.g. sugar phosphate) or attached to another phosphate by the energy rich pyrophosphate bond (P)~(P) (e.g. in ATP). In vacuolated cells of higher plants two major phosphate pools exists. In the "metabolic pool" represented by the cytoplasm and including chloroplast; phosphate ester dominate where as in the "nonmetabolic pool" or the vaccuole, Pi is the dominant fraction. In addition to its role in the non metabolic pool, Pi is absolutely necessary in the metabolic pool. It is either a substrate or

an end product in number of enzyme reactions (e.g. ATP ————> ADP + Pi) and it also controls some key enzyme reactions . Therefore, the compartmentation of Pi is important for the regulation of metabolic pathway in the cytoplasm and chloroplasts. It has been very clearly established that the partitioning of photosynthates in cytoplsm and starch in controlled by iP (Marschner, 1986). In-organic phosphates in the form of phytate plays significant role during seed germination and early stages of seedling growth as it serves storage form of phosphorus in seeds. The regulatory function of Pi in photosynthesis and carbohydrate metabolism of leaves may be considered to be one of the major factor limiting growth perticularly during the reproductive stage (Marschner, 1986).

For the optimal growth of plants the requirement of phosphorus is in the range of 0.3 to 0.5% of the plant dry weight during the vegetative stage of growth (Marschner, 1986). Stout (1961) has given 0.2% as an adequate level of P for plant growth. Clements (1948) used the phosphorus level of sheaths 3,4,5 and 6 expressed on the sugar free dry matter basis during the early years in the use of crop logging. It became evident that the sheath phosphorus was not sensitive to the phosphorus need of the cane plant and he also modified it with the development of the Standard

Phosphorus Index (SPI). This index was also discarded it called for more phosphorus than needed, perticularly on ratoon crops. Burr (1955) has resulted in the use of amplified index which shows promise as a realistic guide to the phosphorus requirement. On the basis of studies in 23 replicates field experiments the critical level for 8-10 P was tentatively established at 0.031% (Alexander, 1973). Our observation indicates good capacity of sugarcane for phosphorus uptake as phosphorus levels are considerably more than this optimum level.

According to Humbert (1963), phosphorus concentrates in the sugarcane plant in the centers of greatest activity. High concentration of P occours in the meristamatic tissues and elongating cane. As soon as phosphorus becomes limiting, the P in older stalk tissues declines much more rapidly than in the immature sections of stalk, inducting migration towards the tissues of maximum activity. Ayres (1937) showed that more than 50% of P absorbed by the plant is found in the green leaves. During the next 8 months the quantity of P in leaves does not increases.

Phosphorus deficiency symptoms in sugarcane have been described by many investigators (Martin, 1934, 1938; Saito and Kenjo, 1939, Clements et al., 1941; Humbert and

Martin, 1955). The length and diameter of stalk are reduced resulting in stout, slender stalks, shortening of internodes etc. Also P deficiency affects the chemical composition of cane plant to a considerable degree. Anon (1960) reported that low P markedly reduced the chlorophyll content, depressed N content and severely curtailed phothosynthesis. With respect to P deficiency Hartt and Burr (1967) found that at 2 months of age plant deprived of P experienced a greater loss of photosynthesis than those deficient in N or K. However, no consistent effect of P deficiency on photosynthesis was found in older plants. In the latter instance the P effects appeared to be confined to primary stalk while photosynthesis remained unaffected by low P supply in secondary stalks. According to Alexander (1967) sucrose accumulation has also been observed in sugarcane leaves grown with inadequate P, K and Ca.

There are very few attempts regarding the effect of growth regulators on uptake of phosphorus Darra and Saxena (1973) concluded that due to pretreatment of IAA there is increase in the phosphorus uptake in Ragi, wheat and Bajara. In 1973 Nimbalkar also reported that the preplanting treatment of sugarcane setts with GA, IAA kinetin and ascorbic acid increased the P content of sugarcane leaves. Abdou et al. (1975) found that seed

pretreatment with 150 ppm IAA increased the total P uptake in alfa-alfa. Naphade et al. (1983) concluded that the pretreatment with IAA + NAA increased the P uptake in sunflower plant. All the above reports indicate that pretreatment with growth promoters like IAA, GA and kinetin are effective in enhancing P level in plants. However, it is evident from the present investigation that CCC and ethephon are effective in this respect. According to Martinez and Lauchli (1991) total phosphorus in cotton plants was diluted over time by growth. Whether similar situation has been created in sugarcane due to pretreatment with CCC and ethephon, is a point of worth considering since leaf growth is considerably enhanced due to presowing soaking treatment.

e. Iron

Effect of presowing soaking treatment with distilled water, CCC and ethephon on iron content in leaves of sugarcane is depicted in Fig. 9. It is clear from the figure that due to three pretreatments iron content of leaf tissue is decreased. This decrease is more prominent in leaves of CCC pretreated plants.

Eventhough iron is a micronutrient, it plays vital role in plant metabolism due to its association with proteins and formation of prosthetic group of many enzyme systems. According to Sandmann and Boger (1983), there are two groups of well defined iron containing proteins : hemoproteins and iron sulphur proteins. The most intensively studied iron containing prosthetic groups are iron porphyrins (hemes). Such well known heme proteins are the cytochromes which contain a heme - iron porphyrin complex as prosthetic group. Cytochromes are constituents of redox system in mitochondria and chloroplast which participates in electron transport in cyclic and non cyclic reactions of light reactions as well as in respiratory chain. The iron sulphur proteins also play important role in respiratory electron transport. The other heme enzymes are catalase and peroxidase. In non heme iron sulphur proteins the iron is co-ordinated to the thiol group of cystein and/or to

inorganic sulphur. The most important one is ferredoxin and it act as an electron transmitter in a number of metabolic processes such as light reaction, sulphate reduction and nitrate reduction. It is well established that Fe (II) is a component of the enzyme aconitase, where it is required for both stability and activity of enzyme (Hsu and Miller, 1968). Machold and Stephan (1969) reported the role of iron in the biosynthesis of chlorophyll as well as for the formation of protochlorophyllid from Mg - protoporphyrin. The enzyme corpoporphyrinogen oxidase is an iron protein that catalyzes the oxidative decarboxylation of Mg - protoporphyrin (Vlcek and Gassman, 1979). The remaining iron is stored in the form of ferric phosphoprotein known as phytoferretin. The reserve stock of phytoferretin in leaf tissue might be used for photosynthetic needs by developing plastids (Hyde et al., 1963). Marschner (1986) reported that in green leaves about 80% of iron is localized in the chloroplast regardless of the iron nutritional status. He also stated that iron is stored in stroma of plastids as phytoferritin, which is a hallow shell and can stored up 5000 atoms of iron as Fe (III) (Fe content 12-23% dry weight) with the proposed formula $(FeO\text{-}OH)_8 . (Fe\text{-}OPO_3H_2)$. Ferrous iron [Fe (II)] is the physiologically available form of iron and the fraction which undergoes reversible Fe (III) / Fe - II oxidoreduction (Marschner, 1986). Though iron

plays important role in plant metabolism, the heme pigments constitute only about 0.1% of the total iron in plant leaves.

According to Humbert (1963), the first symptom of iron deficiency in sugarcane appears as a pale coloured youngest leaves fallowed by the development of alternating green and chlorotic stripes extending the full length of the leaves. He further noticed that if the deficiency continues, the striping becomes less conspicuous and the leaves appear more uniformaly chlorotic. Plant deficient in iron are depressed in growth and exhibit a restricted root system. Limited secondary root development results in markedly stubby appearance. Hewitt (1948) has shown that an excess of zinc, copper, cobalt and other elements induce a deficiency of iron in plants and that some of these elements are more active than manganese in this respect. Iron deficiency may be due to lack of mobility in the tissues (Humbert, 1963).

Iron toxicity in case of sugarcane has been also noticed by several workers MC George (1932) reported accumulation of iron at the nodes without adequate potassium. Evans (1959) confirmed that there are other causes than potassium deficiency for the accumulation of large deposits of iron in the nodal regions. He observed

that the available iron in the soil is very high, that heavy deposition of iron at the nodes takesplace even in the presence of adequate potassium. According to Humbert (1963), on the saline frontland clays, ferrous iron concentrations are very high, perticularly when the clays are waterlogged and the iron is reduced from the ferric to the ferrous state. Under these conditions gray node symptoms appears. Evans (1959) given distribution of iron in sugarcane. According to him the concentration is higher at the nodes with a distinct tendency for the concentration to be less in the root band than in the adjucent growth ring and wax band regions. The concentration of iron in the sugarcane internode region shows a decreasing gradient downwards (Humbert, 1963). Nimbalkar (1973) observed that there is accumulation of iron content during sugarcane leaf senescence. Smith et al. (1984) studied iron nutrition in some C_3 and C_4 species (*Agrostris capillaris, Medicago sativa, Trifolium pratense, T. rapens, Zea mays, Sorghum bicolor, Pennisetum clandestimum and Paspalum dialatatum*). They found that plants having the C_4 photosynthetic pathway required higher concentration of iron in the nutrient solution for maximum growth when grown in sand culture than those with C_3 pathway. The concentration of iron in the leaves and in the nutrient solution, when the dry matter yield was 90% of the maximum was estimated by them for each

species. The results indicated little difference in the concentration of iron in the leaves among the species, but the C_4 plants required more iron in the nutrient solution than the C_3 plants to achieve these leaf concentration.

According to Stout (1961), the adequate value of iron for optimal growth of plant is 0.01%. In sugarcane leaves 0.04 and 0.16 g/ 100 g dry weight has been recorded by Nimbalkar (1973) and Ghorpade (1982) respectively. The leaf iron content recorded in the present investigation is quite high indicating a 'luxury consumption' of iron in this variety. Influence of presowing soaking treatment of growth regulators on iron content of plant has been investigated by some workers. Darra and Sexena (1973) noticed that pretreatment of maize seeds with IAA reduced the iron content of maize leaves. Chavan (1978) also observed that the pretreatment of ragi seeds with 50 ppm solution of GA, IAA and ascorbic acid decreased the iron content of leaves. On the other hand Kutwal (1989) come to conclusion that the presowing soaking treatment of aqueous solutions of CCC and kinetin (in acetone) caused increase in iron content of groundnut leaves. In the present study we noticed that presowing soaking treatment with distilled water, CCC and ethephon has resulted decrease in iron content of leaf tissue of sugarcane. However, in the pretreated plants also

the iron content is above the adequate iron level required for normal growth and metabolism. Hence a decline in iron level in the leaves of pretreated plants may not prove fatal for the plants.

f. Manganese

Effect of presowing soaking treatment with distilled water, CCC and ethephon on manganese content in leaves of sugarcane is depicted in Fig. 9. It is evident from the figure that Mn content shows an increase with all these pretreatments.

Manganese is one of essential micronutrient which is translocated as free divalent cation in xylem from the roots to the shoots (Graham, 1979). It is now very well established that manganese is highly essential in both lower and higher plants for the operation of Hill reaction (Cheniae and Martin, 1968). Photosystem II is believed to contain manganese protein which catalyses the early stages of evolution (Basiounty and Biggs, 1976). There are about 5-8 atoms and this ion per 400 molecules of chlorophyll. In chloroplasts large fraction of Mn is held in less tightly combined state and seems to be most closely involved in oxygen evolution, where as the smaller fraction may be more directly involved in thylakoid structure of stability

(Takahashi and Asada, 1977). In act as a activator of many enzymes and also plays on important role in plant metabolism. Mn is essential for the activities of enzymes like superoxide dismutase, acid phosphatase, decarboxylase and dehydrogenase of TCA cycle. Mn brings about oxidation of IAA by activating IAA oxidase (Mumford, et al., 1962). Thus with respect to auxin metabolism this element is important. It is directly involved as a component of the biotin enzyme in the biosynthesis of fatty acids (Marschner, 1968). This element is also involved in regulating the decarboxylating enzymes of C_4 pathway, especially NADP - malic enzyme, NAD - malic enzyme and PEP carboxykinase which is highly Mn^{2+} specific. In its biochemical functions Mn resembles Mg and both ion species bridge ATP with the enzyme complex. (Phosphokinase and phosphotransferases). In some cases Mn activates decarboxylases and dehydrogenases of TCA cycle (Mengel and Kirkby, 1982). Manganese is found to be highly essential during cell division and expansion.

Mn deficiency has been recognised in different crop species growing under field conditions. (Bar - Akiva 1977; Bable et al., 1984). The symptoms of manganese deficiency are characterised by fading of the normal green colour between the leaf bundles followed by development of definite pale yellow green to white longitudinal strips,

(Humbert, 1963). A specific requirement of photosystem II for manganese (Kessler, 1937; Kessler, 1957; Kelly and Avery, 1949) and its extreme sensitivity to the poison DCMU (Kelly and Avery, 1949; Wessels and Van der Veen, 1956). Humbert (1955) reported that high levels of Mn reduces the absorption of iron. Mn is a prominent component of chloroplast and participates in the reactions leading to the evolution of oxygen (Heath and Hind, 1969). It also act as a cofactor at the time of sucrose synthesis. Mn deficiency also reduce photosythetic electron transport capacity, photosynthesis, net assimilation rate (NAR). An increase in the peroxidase activity is a typical feature of Mn deficiency. Also Mn deficient leaves exhibits expantially high IAA oxidase activity (Morgan et al., 1976), which leads enhanced auxin (IAA) degradation in tissues.

The manganese concentration for optimal growth of plant is 0.003 to 0.005% on the dry weight basis (Stout, 1961). Humbert (1963) noticed that the Mn content of dry leaf cane is approximately 0.01% on the dry weight basis. Thus our values of Mn content in control plants fallow the similar pattern but the value are some what higher than the concentrations for optimal growth of crop plants as indicated by Stout (1961) and thus sugarcane shows 'Luxury' consumption of Mn.

Not much attention has been paid to the influence of growth regulators on Mn content. Chavan (1978) observed that there is reduction in the Mn content of Ragi (*Eleucine coracona*) leaves due to pretreatment with IAA, AA, kinetin and NaCl. In the present investigation it is observed that the presowing soaking treatment with DW, CCC and ethephon causes marked enhancement in manganese content of sugarcane leaf. Increase in Mn content of sugarcane leaves due to pretreatment with growth regulators would undoubtedly leads to some metabolic changes or may cause stimulation of some enzyme systems and improves the growth rates of sugarcane asindicated by Morgan et al. (1976).

g. Zinc

Effect of presowing soaking treatment with distilled water, CCC and ethephon on zinc content of leaf tissue is depicted in Fig. 10. It is evident from the figure that there is decrease in the zinc content due to distilled water and CCC pretreatment while it is considerably increased due to pretreatment with ethephon.

Zinc is regarded as one of the important trace element in plants and it is taken up predominately as a divalent cation and at high pH as a monovalent cation. It plays role either as a metal component of enzyme or as a

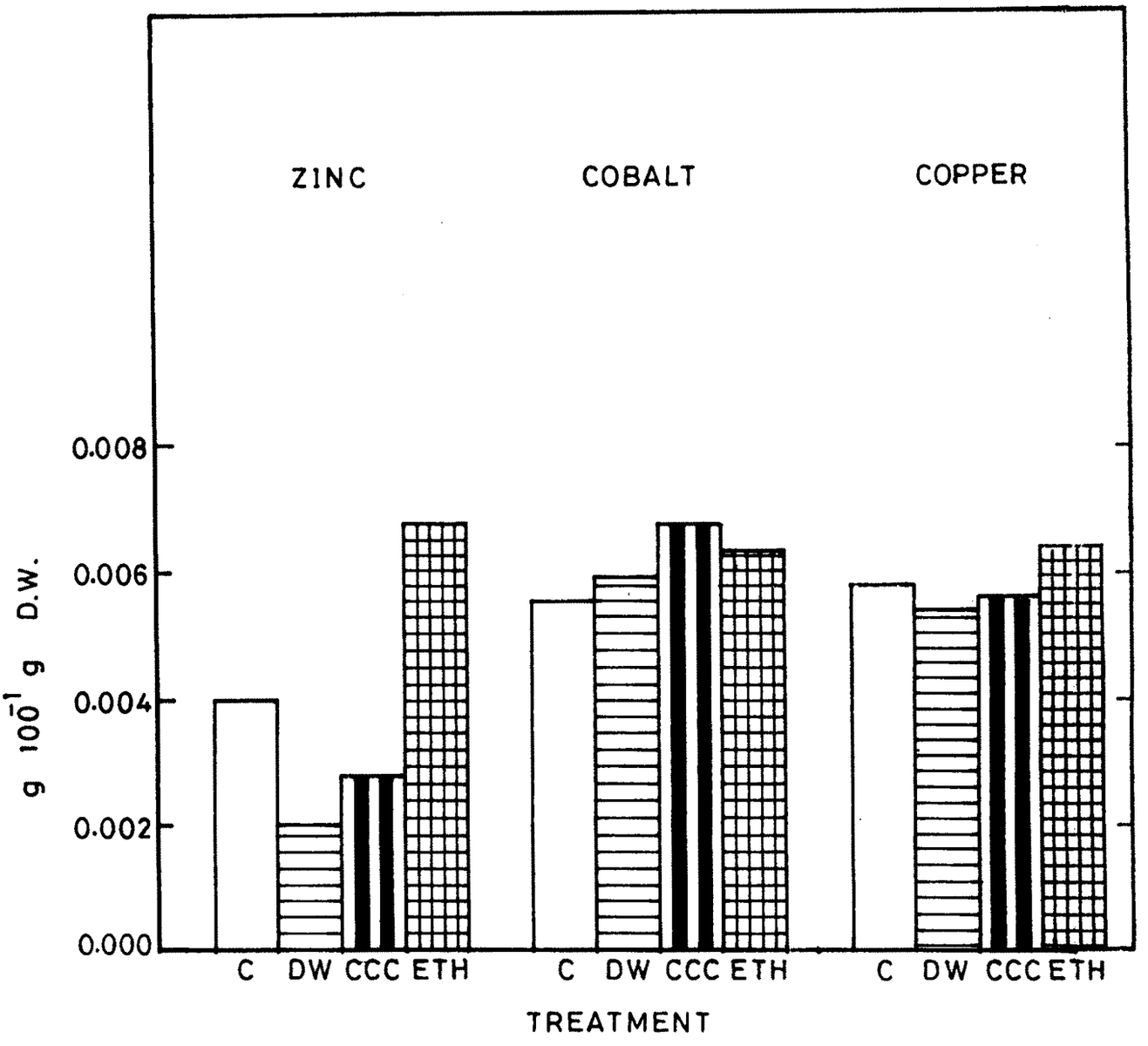

FIG. 10 - EFFECT OF PRESOWING TREATMENT OF GROWTH REGULATORS ON ZINC, COBALT AND COPPER CONTENTS IN LEAVES OF SUGARCANE VAR - CO - 671.

functional, structural or regulatory cofactor of a large number of enzymes. Zn metalloenzyme have been identified in plants, these includes glutamic acid dehydrogenase, Cu - Zn superoxide dismutase, carbonic anhydrose as well as proteinases and peptidases. Besides these enzymes Zn is required for the activity of various types of enzymes, including aldolases, isomerases, transphosphorylases and RNA and DNA polymerases (Marschner, 1986).

The high content of zinc is seen in the meristamatic region of the growing point in sugarcane and it is understandable in the view of the important part that zinc plays role in production of growth substances (Van Overbeek, 1943). Bonner (1950) reported that in the absence of zinc the plant is unable to synthesize tryptophan, the amino acid from which indole acetic acid the normally occurring auxin of sugarcane is produced. Zinc plays an important role in carbohydrate metabolism, protein synthesis and also sexual fertilization and development of reproductive parts. Ghildiyal et al. (1986) have observed a decrease in protein free amino acid content of linseed varieties under deficiency which indicates that Zn is playing important role in nitrogen metabolism under conditions of Zn deficiency. Several metabolic processes are impaired such as control mechanism of generation and detoxification of the nitrogen derived radicals (Cakmak and

Manschner, 1988), structural and functional integrity of cell membrane (Cakmak and Manschner, 1988). Also uptake of nutrient is altered due to Zn deficiency in roots (Cakmak and Marschner, 1990) Aftab Hussian et al. (1993) indicated that increase in the ribonuclease activity may be used to identify Zn deficiency in french bean leaves. Due to Zn deficiency the concentration of sulphydryl groups at the surface of plasma membrane vesicle is depressed (Pinton et al., 1993). Pinton et al. (1994) recently documented a role of Zn in membranes stabilization by controlling the level of oxidizing O_2 species. According to Marschner (1986), the critical deficiency level of Zn are below 15-20 mg/kg dry weight of leaves and the critical toxicity level of zinc in leaves is more than 400-500 mg/kg dry weight. Epstein (1965) has given average zinc concentration required for adequate growth of sugarcane plant is 20 µg/g.

In sugarcane zinc deficiency causes characteristic pronounced lightening of green colour along with major veins (Evans, 1959). According to Humbert (1963), there is inter veinal chlorosis, formation of stripes due to loss of chlorophyll along with veins, necrosis and finally ceasation of growing point in zinc deficient sugarcane plants.

Eventhough zinc plays such an important role in higher plants not much attention has been paid to various factors influencing uptake of this microelement.

h. Copper

Effect of presowing soaking treatment with distilled water, CCC and ethephon on copper content in leaves of sugarcane is depicted in Fig. 10. It is evident from the figure that distilled water and CCC pretreatments have caused a slight decrease in the copper content and it is slightly increased due to pretreatment with ethephon.

Copper is an important micronutrient which mainly participate in enzymatic redox reactions. More than 50% of leaf Cu is localized in chloroplast. It is bound with plastocyanin which is the component of PS I. The synthesis or stability of chlorophyll are possibly dependent on copper (Boardman, 1975). It is also a main component of many enzymes, viz. cytochrome oxidase, superoxide dismutase, ascorbate oxidase, phenolase, laccase, amino oxidase etc. Cu plays an important role in carbohydrate and nitrogen metabolism (Marschner, 1986). It is also required for synthesis of quinones. Cu plays major role in the pollen formation and fertilization. It is believed that deficiency of Cu affect the polyphenol oxidase activity and hence the synthesis of lignins. According to Rahimi and Bussler (1973) lignification is inhibited in Cu deficient tissue and this is associated with inadequate development of xylem vessel. Evans (1959) reported the high accumulation of Cu in the

region of the growing point and is correlated with the intensity of polyphenol oxidase activity in the sugarcane. Schutte and Schendel (1958) have shown that Cu influences the protein composition of cane plants. Even though copper plays an irreplaceable role in a large number of enzymes vital to cell metabolism, it is taken up by the plants in very small quantities. The critical deficiency level of Cu is generally in range of 3 to 5 µg/g dry tissue (Marschner, 1986). While Robson and Reuter (1981) reported that for most crop species it is in range of 20 to 30 µg/g dry tissue. The Cu content of most plants is generally between 2 to 20 ppm in the dry material. The normal range of Cu content in agricultural crops is reported to be 5 to 30 mg/kg dry weight (Gupta, 1979). According to Morrison et al. (1981), in some tolerant species the Cu content of the leaves can be as high as 0.1% of dry weight.

Copper deficiency affect the formation of grains and seeds formation much more than vegetative growth (Rahimi, 1970). Deficiency symptoms of Cu are poor development of the stool, droopy top, chlorosis of leaves and the failure of the spindle to un-roll. At the same time copper inexcess causes the toxicity and chlorosis is commonly observed symptoms of Cu toxicity (Daniels, et al., 1972).

There are hardly any report on the influence of phytohormones on the fate of this micronutrient. Our observations with sugarcane indicate that the changes in the level of this element brought about by the three pretreatments are too insignificant to account for any major changes in the metabolism.

i. Cobalt

Effect of presowing soaking treatment with distilled water, CCC and ethephon on cobalt content in leaves of sugarcane is depicted in the Fig. 10. It is obvious from the figure that the cobalt content is increased in the leaf tissue of plants raised from pretreated setts. This increase is more significant in CCC and ethephon pretreated plants.

On an average the 'cobalt content of plant varies between 0.05 and 0.30 mg/kg (Kuboto and Allaway, 1972). The cobalt content in hay and pastures of graminae members were noticed in the range of 0.5 to 3 mg/kg (Young, 1979). Datta and Datta Biswas (1951) found that all grasses had high cobalt content in young stage but with increasing age there is a gradual decrease in the content of cobalt. Wilson and Nicolas (1967) reported cobalt deficiency symptoms in both legumes and nonlegumes as shown by chlorosis in younger

leaves. The fact that cobalt is an essential micronutrient for plant growth and metabolism has been realized from several studies. The addition of cobalt to the soil, or the presowing soaking of seeds in a dilute solution have been found to increase the amount of chlorophyll in barley (Lipskays and Zelenaya, 1975) buckwheat (Semina, 1967; Elagin, 1970) and oats (Shkol'nik, 1961). The requirement of cobalt for nitrogen fixation in legumes and in root nodules of nonlegumes has been very well established by Ahmed and Evans (1960). Among cereals, cobalt addition to soil or seed treatment with cobalt solution increased the protein content in buckwheat (Ivanou et al., 1975; Minima, 1973) oats (Asmus, 1967) and wheat (Minima, 1973). Cobalt increased the starch content of wheat (Minima, 1973) and of corn (Ashkhbabyan, 1968) and also the sugar content of corn (Beveznitskaya, 1966), cobalt enhanced sugar formation in timothy (Potakhina, 1965).

In Barley, cobalt was found to depress the development of glutamate oxaloacetate transaminase, but had no influence on the activity of either urease or amylase (Pavel and Zakova, 1967). Cobalt was found to decrease the activity of dehydrogenase in barley (Satsukevich, 1974). Catalase activity was elevated by adding cobalt to cotton plants (Mamedov, 1960). Cobalt additions to grapevines

increased the activity of catalase, ascrobic oxidase, polyphenoloxidase and peroxidase (Dobrolyubskii et al., 1962). In sunflowers cobalt treatment depressed catalase in young leaves but not generally in older leaves (Agarwala and Kumar, 1962). Cobalt increased the activities of both catalase and peroxidase in the meadow foxtail (Potakhina, 1965).

It has been reported that applications of lime changed soluble cobalt compounds in to less available forms and addition of cobalt significantly increased both the yield and quality of crops in this limed areas (Semina, 1970). Also there are number of reports describing positive effect of cobalt on various aspects of plant growth. In Mulberry, addition of cobalt increased leaf area of seedlings, stimulated enlargement of root necks and improved the frost resistance of seedlings (Khakimov and Alizhanov, 1974). Calcium absorption in beans was increased by cobalt additions (Petersburgskii and Yang, 1963). Also in Mulberry, addition of cobalt sulphate improved the absorption of phosphorus (Guseinov and Guseinov, 1961). The application of 0.1 - 0.2% cobalt nitrate solution caused differentiation of the endoplasmic reticulum complexes in horsebeans (Herich and Bobak, 1976). With cucumbers, it was found that cobalt promotes hypocotyl elongation of seedlings by inhibiting ethylene production (Grover and Purves, 1976).

Cobalt increased the drought resistance of corn and soyabean (Pirozhnikov, 1962), oats (Shkol'nik, 1961), oats and barley (Bozhenko and Shkol'nik, 1963) and barley (Bozhenko et al., 1963). It has been reported that the effect of cobalt is related to its capacity to increase the content of bound water, maintain a high protein content and to increase the rates of synthesis and migration of carbohydrates from the leaves to fruit bearing organs (Shkol'nik et al., 1960). The another publication stated that, among trace elements, cobalt had the most effective action on drought resistance and increased the content of adinosine triphosphate (Bozhenko et al., 1963). The increase of drought resistance induced by cobalt has been ascribed to its direct or indirect participation in nucleic acid metabolism (Bozhenko, 1968). Thus extensive work of Russian plant physiologists has very clearly highlighted the key role of cobalt in plant metabolism and stress tolerance. Although cobalt plays such an important role very little attention has been provide to the uptake of this element as well as influence of various factors on its level in plant tissue. In the present study we noticed that the cobalt content is considerably increased due to CCC than ethephon pretreatment. This can cause favourable effects of various growth processes in general and also induce drought resistance as indicated by Bozhenko and coworkers (1963).

E. EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON ORGANIC CONSTITUENTS

a. Carbohydrates

The influence of presowing soaking treatment with distilled water, CCC and ethephon on the level of various carbohydrate fractions (total sugar, reducing sugars and starch) in the leaves of sugarcane is depicted in the Fig.11. It is evident from the figure that pretreatment with above growth regulators has caused a marked enhancement in total sugars as compared to control. This is mainly due to increase in non reducing sugars. Due to pretreatment the starch content is decreased and more decrease is observed in case of ethephon pretreatment.

Carbohydrates are among the most important organic compounds in plants as they form a connection between photosynthesis and respiration and thus a basis of dry matter production. The starch and sucrose are major terminal product of photosynthetic carbon metabolism while glucose is the substrate of respiration. Sugars also serves an osmoregulatory role because of their osmotically active nature. The four carbon sugar erythrose is a precursor of aromatic pathway. The pentose sugar ribose is an important part of nucleic acid. Sucrose is the central carbon metabolite in plants. It is the principle end product of

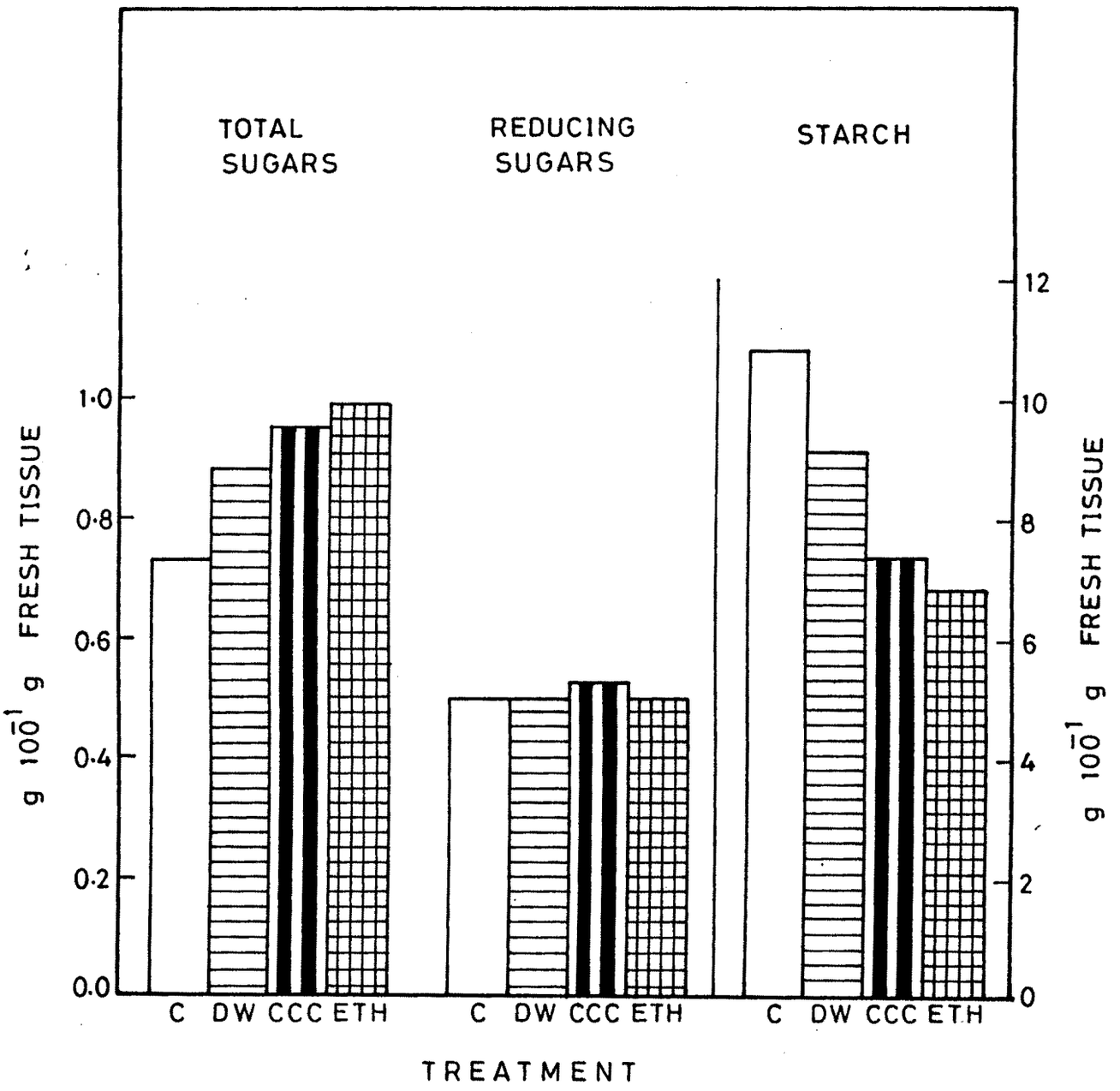

FIG. 11 - EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON CARBOHYDRATE CONTENTS IN LEAVES OF SUGARCANE VAR - CO - 671.

photosynthesis. Carbon assimilation and in assimilate partitioning, it is the predominate form of reduced carbon transported to the heterotropic cells. In recent times the discovery that sucrose is the principle form of transient carbohydrate plus the fact that it is not the form most readily accumulated in cellular storage compartments has shown that a sucrose molecule might be synthesized and inverted several times before fulfilling its physiological density. In the view of Alexander (1963) the sugarcane problem is one of the starch conversion to sucrose rather than of sucrose conversion to starch. Sucrose is synthesized in the cytosol of source organ; transiently stored in vaccuole and translocated via the phloem to sink organs. In sink organs sucrose is cleaved via, sucrose synthatase or invertase to yield glucose and fructose and metabolised or deposited in the form of reserve compounds such as starch or lipids. In addition carbon storage is frequently associated with sucrose assimilation in the vacuole. This is a usual fate of sucrose in case of mature sugarcane plants where it is predominantly stored in vacuoles of parenchymatus cells in the stalk. In view of Mooney (1972) the level of soluble sugars can be elucidated as a parameter of plant energy status. Besides their key role in cellular carbon.

The physiological role of starch and other

polysaccharides in the cane stalk is quite uncertain. Sucrose is only carbohydrate conducted in appreciable amount of storage region in sugarcane (Hartt, 1963; Hatch and Stack, 1969), it must be assumed that starch biosynthesis fallows an outer space sucrose inversion and subsequent conversion of fructose to glucose in sugarcane stalk. Haddon (1926) found starch distributed along the entire length of 8 months old stalks of sugarcane but attributed the starch to low pH soil conditions. Weller (1930) observed that starch accumulated in all parenchyma cells of sugarcane. The findings of Alexander (1963) have shown that starch is present in *S. officinarum* but in relatively small quantities. Hartt (1936) recorded seasonal as well as diurenal variation in carbohydrate fractions namely sucrose, starch and hexose constituents in sugarcane. It was evident that starch literally increased all day and decreased all night. Sucrose is formed as a result of photosynthetic carbon assimilation in virtually all higher plants.

The significance of sugar nucleotides such as UDPG, ADPG and GDPG in carbohydrate metabolism is now very well established and this aspect has been extensively investigated in sugarcane among various enzymes, enzyme invertase accupies an important place in sugarcane metabolism as it controls the level of sucrose in sugarcane

stalk tissue. Several studies have indicated that various plant growth regulators have marked influence on the activity of this enzyme. Sachar and Glasziou (1962) reported that NAA at 1.42 x 10^{-5} M concentration caused three fold increase in the activity of enzyme invertase in sugarcane. Sacher et al. (1963) indicated that IAA functions in regulation of enzyme invertase. Sacher et al. (1963) and Alexander (1965) indicated that similar to auxin GA is also involved in regulation of invertase activity in sugarcane. Glasziou and Bull (1965) reported that treatment of GA caused rapid stimulation of acid invertase level in immature sugarcane. Cobas and Gonalez (1981) also found that GA treatment caused increase in activity of soluble invertase in sugarcane. Alexander and Montolvo (1971) noticed that foliar spary of cycocel at concentration of 0.3% significantly lowered the acid invertase of immature storage tissue of sugarcane cultivar C.P. 52-43. Kishan Singh et al. (1986) observed that setts of cultivar Co. J. 64 and Co.1148 treated with 5 ppm tricantanol for 12 h showed rapid mobilization of sucrose with a corresponding increase in acid invertase activity. An appreciable increase in amylase activity was also noted in cane setts and buds.

There are several reports regarding the influence of growth regulators on carbohydrate status in different

plant species. According to Stoddart (1964), treatment of CCC was fallowed by rapid accumulation of ethanol soluble free sugars and fructosans Berridge and Ralph (1971) studied the effect of kinetin on carbohydrate metabolism. It was evident that kinetin mobilized starch reserves and increased the flow of sugars, required for the synthesis of lipid and structural materials in the floated discs of chinese cabbage. According to Stoddart (1964), treatment of CCC was fallowed by raid accumulation of ethanol soluble free sugars and fructosans especially under conditions of nitrogen stress in *Phleum pratense*. Agakishiev and Nikitina (1972) reported that the foliar applications of CCC increased the carbohydrate level. At the same time contradictory observations in this respect have been also made. Litvinova and Yuldasher (1971) reported that CCC was effective in causing decrease in total carbohydrate content in stem and leaves of cotton variety G-460. Bhandari and Sen (1975) found that CCC treatment caused decrease in sugar content of citrus seedlings. Asmaeva and Avundzhyan (1973) noticed that wheat seed pretreatment with CCC increased the carbohydrate content. 14 C feeding experiments of Nimbalkar (1973) indicated that preplanting treatment with growth promoters caused reduction, the total sugar synthesis of glucose was stimulated. He also noticed that IAA treatment caused higher content of reducing sugar and lowered starch content level.

Chavan (1978) noticed that presowing soaking treatment of IAA, AA, kinetin and NaCl caused increase in sugar content in leaves of *Eleucine coracona*. Mustafa et al. (1982) observed that seed treatment with cycocel and Alar caused higher content of sugars and amino acids in *Vicia faba*. Bisaria and Paliwal (1982) found that pretreatment with ethephon increased sugar content on 4th and 5th day of triticale variety JNK - 6T.039. Bhadre (1983) reported that pretreatment with CCC increased carbohydrate production in leaves of cotton variety Laxmi and H-4. In this respect he observed more increase in sugar content than starch. Sushma Rani et al. (1985) observed that presowing seed treatment with B-9 and CCC caused higher accumulation of total soluble carbohydrates under stress conditions. Upretty et al. (1985) reported that CCC treatment caused increased amount of total sugars, non reducing sugars and starch content in *Avena sativa* cv. kent. Saha and Gupta (1992) reported that salinity induced decrease in carbohydrate level and it was partially negated by pretreatment of seeds with LAB 150978 a triazole type plant growth retardant perticularly in sensitive varieties of mung beans.

In the present investigation a marked enhancement in leaf total sugar content in sugar cane leaf tissue due to presowing soaking treatment is noticed while apposite trend

is seen in case of starch. This may be possibly due to positive influence of pretreatments on photosynthetic carbon metabolism since the sugars are main product of CO_2 fixation. Further translocation of assimilates is also possibly altered by pretreatment. Chang and Ryan (1987) reported that starch decrease may be due to increase in α-amylase activity and an inhibition of an enzyme sucrose synthatase which cause accumulation of sucrose and orthophosphate in the chloroplasts. They suggested that orthophosphate inhibits ADP - glucose pyrophosphorylase activity which is responsible for starch synthesis in chloroplast. Similar observation may also prevail in leaves of sugarcane plants raised from pretreated setts.

b. Total Nitrogen

It is clear from the Fig.12 that due to pretreatment of distilled water, CCC and ethephon total nitrogen content is increased in leaf tissue of sugarcane cultivar CO. 671. This increase is more marked in case of ethephon pretreatment.

The indespensibility of nitrogen for all living organisms is unquestionable. Nitrogen is one of the most essential component of all fertilizers. It is building blocks of amino acids and protein molecules. Nitrogen

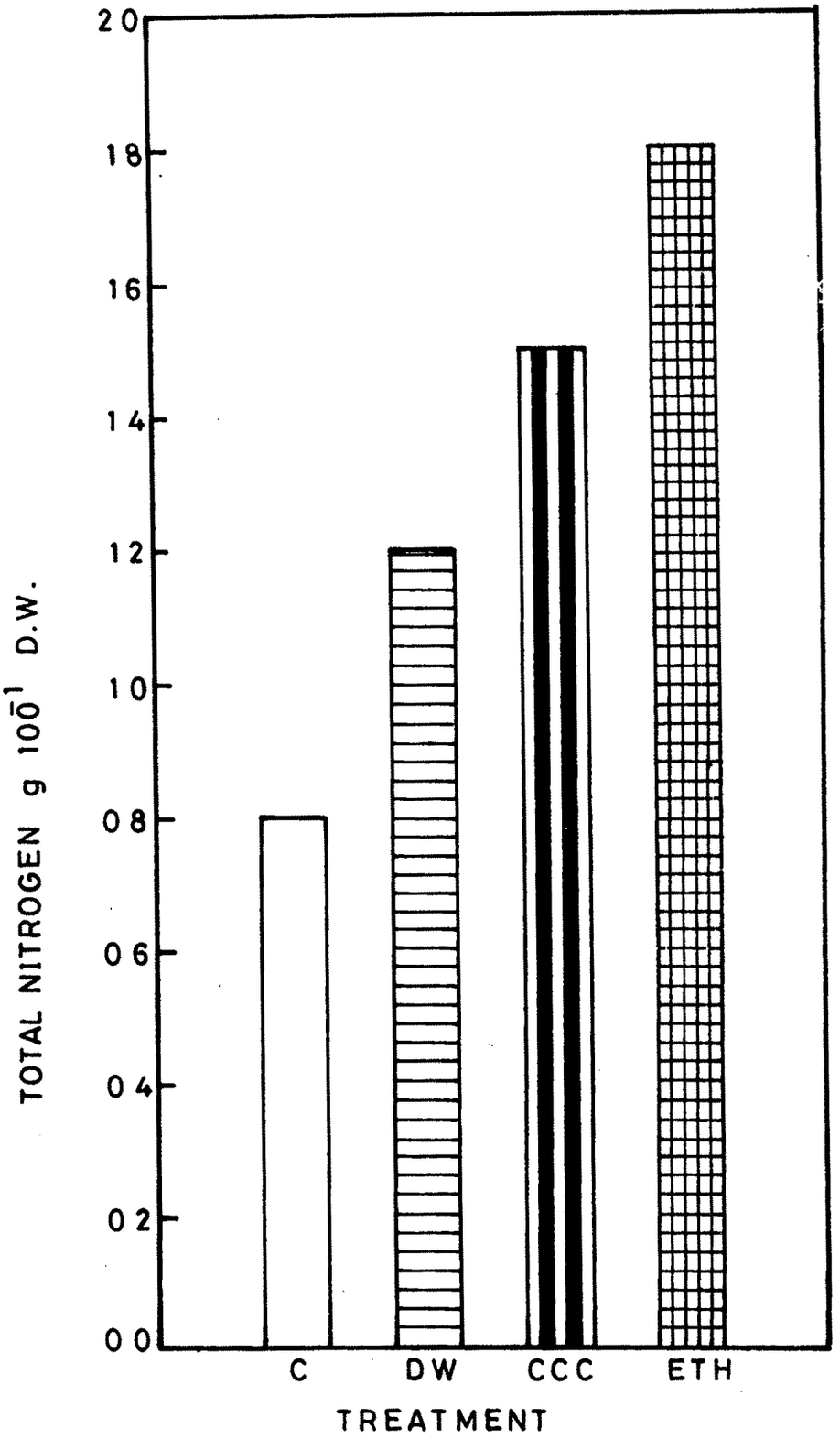

FIG. 12 - EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON TOTAL NITROGEN CONTENTS IN LEAVES OF SUGARCANE VAR-CO-6[illegible].

occurs in chlorophyll molecule. Nitrogen is fundamental component of essential compounds such as nucleic acids and in some of growth regulators viz. IAA and cytokinins and in number of vitamins. The insufficiency of nitrogen is mostly a single major factor limiting crop growth and yield. All our fertilizer practices are ultimately based on proper, wise and reasonable application of this element in various forms. The nitrogen content required for optimal growth varies between 2 to 5% of the dry weight and it depends upon the plant species, developmental stage and organ of plant (Marschner, 1986). Clements et al. (1942) observed that highest percentage of nitrogen occurs in meristamatic tissues in sugarcane. The elongating cane associated with leaves 3, 4, 5 and 6 contains the next highest nitrogen concentration indicating close relationship between growth and nitrogen content. Cane plant absorbs more nitrogen during early period of growth and during maturation nitrogen migrates to the growing parts from the mature one (Takahasi, 1959). According to Ayres (1952) the bulk of nitrogen is stored in the green leaves during the first 6 months of growth in sugarcane under field condition and it is about 0.7 to 1 gm.

Nitrogen deficiency exhibit a yellow green colour all leaves and a retardation of growth. Cane stalks are

smaller in diameter and premature drying and dying of old leaves takes place. Nitrogen has greatest effect on sugarcane ripening and juice quality (Humbert, 1963). Singh (1941) reported the decline of growth parameters, carbohydrates,chlorophyll content and CO_2 assimilation efficiency in N - deficient sugarcane. Lal and De (1953) correlated subnormal photosynthesis in N - deficient sugarcane with a marked decline in pigment content. Both chlorophyll a and b were lowered by N - deficiency (Alexander, 1963).

There are few attempts to study the effect of growth regulators on nitrogen content. Sytnik and Mastenko (1967) noticed that 0.1% GA pretreatment increased the total nitrogen in wheat. The experiments of Nimbalkar (1973) shows that preplanting treatment with Aretan, GA + Aretan, IAA and kinetin caused enhancement in the total nitrogen content in leaves of sugarcane variety CO.740 but other growth regulators GA, aspartic acid and combination of above all resulted in decreased amount of nitrogen. Asthana and Srivastava (1977) reported that presowing treatment of maize seeds with ascorbic acid and salicylic acid and combination of these two acids enhanced the ethanol soluble nitrogen and soluble nitrogen of primary leaf. Chavan (1978) observed that presowing soaking treatment with IAA, ascorbic acid, kinetin and NaCl caused increase in total nitrogen content

in the leaves of Ragi (*Eleusine coracona*. Guertn). Moustafa Seham et al. (1982) also observed that seed treatment with cycocel and alar showed higher content of protein nitrogen in *Vicia faba* leaves. Naphade et al. (1986) found there was maximum uptake of nitrogen in sunflower plants raised from seeds pretreated with IAA + NAA. Chippa and Pal (1988) also showed that presowing soaking treatments increased the absorption of nitrogen in wheat. Maximum nitrogen content was noted under IAA fallowed by IBA and distilled water. Sahay and Verma (1992) reported that pretreating the seeds of linseed with growth hormones viz. IAA, GA_3, NAA and 2, 4, 5-T increased soluble nitrogen protein nitrogen and total nitrogen.

In the present investigation presowing soaking treatment appear to be beneficial in terms of improved nitrogen economy of plants, which might have caused improvement in the growth and yield of sugarcane (as noticed in earlier chapter, Fig.No.5,6, and 7).

c. Titratable acidity status or Titratable acid number (TAN)

The influence of presowing soaking treatment with distilled water, CCC and ethephon on titratabe acidity status in leaves of sugarcane is depicted in Fig. 13. It is evident from the figure that presowing soaking treatment with above growth regulators decrease the acidity status of sugarcane leaves.

Organic acids are considered as an important metabolites in living cells because many of these compounds are intermediates of a central metabolic pathway. The Kreb's cycle is metabolic pathway of prime importance in both photosynthetic and non photosynthetic tissues and represents a major source of mejority of organic acids found in higher plants. These includes malate, citrate, cisaconitate, isocitrate, fumarate and succinte. Besides above acids, organic acids like tartarte, oxalate, glycolate are heavily synthesized and extensively accumulated in some plant tissues. It has been considered that accumulation of organic acids in plant is relevant to the adjustment of cation and anion balance in plant sap and to facilitate the transport of metabolic cations in plant (Popp and Kinzel, 1971; Triplett et al., 1980). Organic acids also provide carbon skeletons for synthesis of number of important role in stomatal movement. Malic acid also plays a key role in CAM

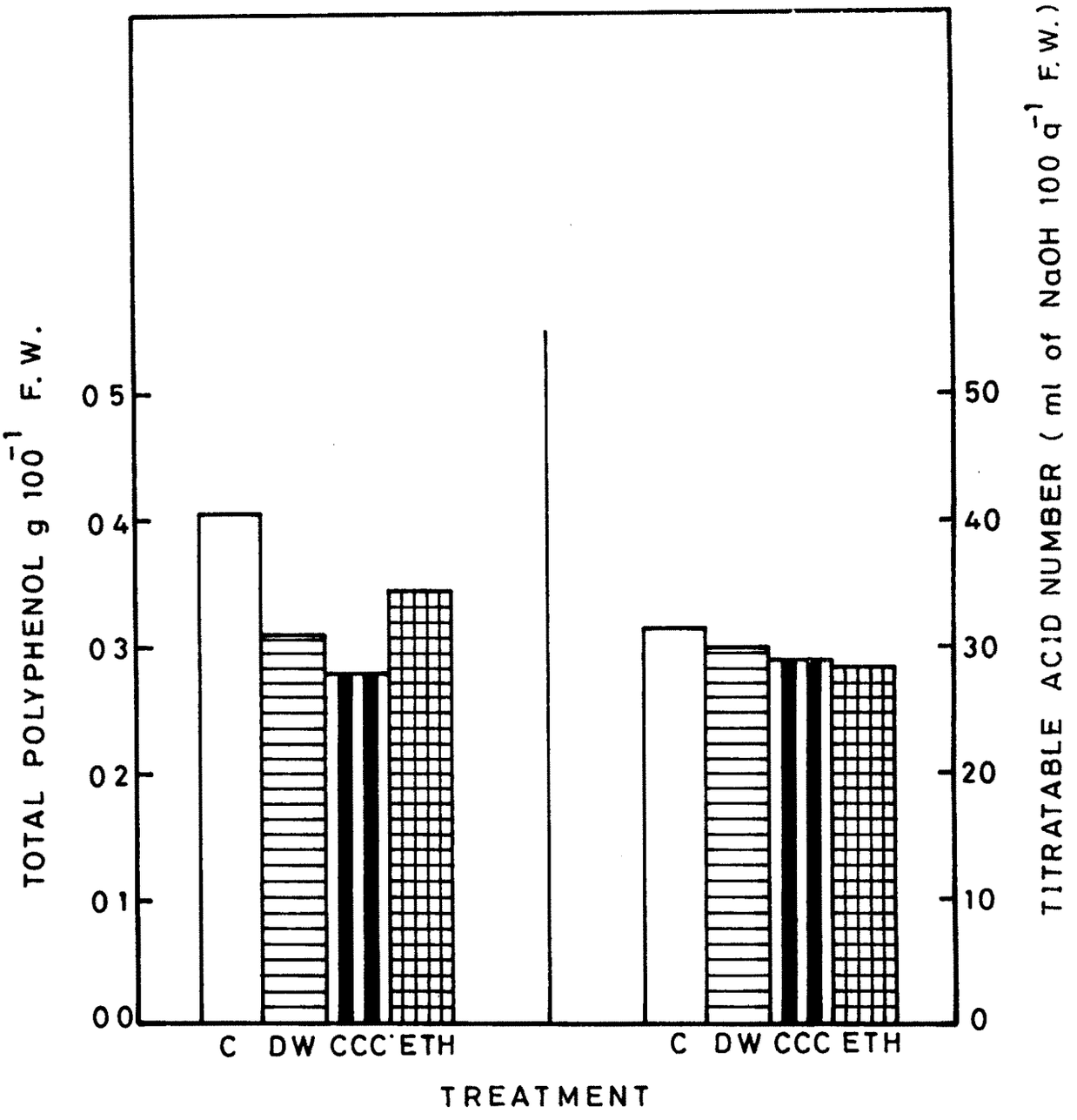

FIG.13 - EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON TOTAL POLYPHENOLS AND TITRATABLE ACID NUMBER (TAN) IN LEAVES OF SUGARCANE VAR - CO - 671 .

species and C_4 species like sugrcane. Oxalic acid is reported to play an osmoregulatory role in halophyte *Atriplex*. According to Jones et al. (1979), organic acids play an important role during stress conditions. Burke et al. (1990) have expressed the opinion that organic acids are the pivotal like as metabolic intermediates in the formation of ATP from carbohydrates, in nitrogen metabolism and in ionic balance.

Influence of growth regulators on organic acid content of plant tissue has been studied by some workers. Zeml and Zvyagnistsv (1966) noticed that IAA (10 ppm) treatment caused definite decrease in oxalic and citric acid and less definate drop in succinic acid, perticularly in roots of sunflower. Shetty (1971) noticed a marked increase in TAN in *Acrosticham aureum* leaves treated with 100 ppm GA. Nimbalkar (1973) observed that in sugarcane leaves there is slight but insignificant increase in TAN plants treated with IAA. On the other hand under drought condition Gill and Singh (1978) reported that sparying of CCC (500 ppm) on wheat caused increase in acidity status of wheat leaves. Kutwal (1989) reported that there is slight decrease in acidity status of groundnut leaves due to presowing soaking treatment of CCC and kinetin.

In the present investigation the presawing soaking treatment with distilled water, CCC and ethephon have resulted lowering of the acidity status of leaf tissue. The increase in organic acid contain is many times correlated with increase in the respiration rates and hydrolytic activities perticularly during leaf senescence (Rane, 1991). Hence lowering of acidity status as indicated by TAN values may reflect the opposite situation which may prove beneficial for the plant. The lowering of organic acid level can also be attributed to the contribution of carbon skeleton of these metabolites to other compounds in the plant cell.

d. Ascorbic acid

Effect of presowing soaking treatment of sugarcane setts with distilled water, CCC and ethephon on ascorbic acid content in leaves is depicted in Table 1. It is clear from the table that these pretreatments considerably enhanced the ascorbic acid content. This increase is more prominent in case of CCC pretreated plants.

According to Jones and Hughes (1983), the status of ascorbic acid in plants is somewhat contraversial. Thus the apparently obiquious presence of ascorbic acid in angiosperm tissues would appear to point to an essential role in the

Table 1. EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON ASCORBIC ACID, CHLOROPHYLL STABILITY INDEX (CSI) IN LEAVES OF SUGARCANE VAR. CO. 671.

No.	Treatment	Ascorbic acid mg 100^{-1} g fresh tissue	Chlorophyll Stability Index (CSI) mg 100^{-1} g fresh tissue
1.	Control	41.25	0.432
2.	Distilled water	55.0	0.484
3.	CCC	56.25	0.493
4.	Ethephon	55.0	0.562

Values are expressed as mg 100^{-1} g fresh tissue.

"survial and well being of organism"-a characteristic of primary metabolities (Mann, 1978). On the other hand, the failure to ascribe to ascorbic acid a specific and universal role in plant metabolism would be more in keeping with the features of secondary metabolite. Although this is the case important role of ascorbic acid in several metabolic processes is well documented. Excellent work of Chinoy and coworkers has demonstrated that this metabolite plays on important role as the plant growth regulator in number of physiological processes such as germination, flowering and stress tolerance. Ascorbic acid stimulates amylase, protease and RNA ase activities and RNA content in various plants (Chinoy et al., 1969; Chinoy and Saxena, 1972). Chlorophyll bearing tissues (including leaves, green tomatoes etc.) are responsible for the greater accumulation of AA in light grown plants compared with that in the dark grown ones (Haffman and Albrecht, 1966). The chlorphyll-a destruction was inhibited by iron, hydroquinone and ascrobate (Shimazaki et al., 1980). Ascorbic acid is reported to delay leaf senescence (Garg and Kapoor, 1972). Sechenska et al. (1968) have reported the localization of ascorbate oxidase in the grana of spinach chloroplasts which may indicate the possible close relationship of this enzyme to the photosynthetic transport of electrons and also form one of the possible enzymatic pathway for transport of electrons to

melecular oxygen in its light induced consumption by isolated chloroplasts. Bohme and Trebst (1969) also suggested that AA donates electron to the primary oxidant (Z) of system II trapping center thus by passing a phosphorylation site located between the O_2 yielding reaction of Z of the system II trapping center. It has been also shown that ascorbate serves as on electron donar at site which is prior to photosystem II (Gozal and Avron, 1970). Ascorbic acid plays an important role in oxidative phosphorylation (Abraham et al., 1968). Arnon et al. (1954, 56, 57) reported that ascorbic acid serves an important role as electron transporting agent during photosynthetic phosphotylation in illuminated chloroplast or as a factor stabilizing the activity of chloroplast. According to Chinoy (1984) there is close correlation between photosynthesis and AA formation as well as between AA content and surface area of leaves. The difference in AA content depends chiefly on the physiological conditions and on the stage of growth and development. Early and more rapid biosynthesis of AA by wheat embryo axis from sucrose in the presence of IAA at low temperature throws some light on the mechanism of vernalization (Chinoy, 1984) Chinoy (1984) also postulated that the formation of charge transfer complex (CTC) between AA and DNA in the shoot apex helps in establishing a direct flow of electron energy for biosynthesis of cell

constituents. This charge transfer paves the way for the production of different types of RNA and subsequently of structural proteins, enzymes and other cell constituents at greatly accelerated rates.

In view of Chinoy et al. (1984) rapid turnover of ascorbic acid helps in maintaining normal metabolic activity in the growing parts under water stress conditions. The accumulation and binding of ascorbic acid ans ascorbigen to macromolecules protect them against oxidation (Vora et al., 1975). Further the decrease in the free water content of the cell due to wilting brought about the molecules of ascorbic acid and macromulecules like DNA and RNA closer to each other thus facilitating the formation of charge transfer complex (Chinoy, 1969). Nimbalkar (1973) found that pretreatment of sugarcane setts with ascorbic acid caused the improvement of tillering, height of plant and total leaf area. There was also enhancement in the rate of photasynthesis and diversion of more carbon to the amino acid fraction. Pretreatment with ascorbic acid increased the yield of sugarcane and also improved the quality of juice (Nimbalkar, 1973). Joshi and Hegade (1976) showed that ascorbic acid stimulated chlorophyll synthesis and rate of carbon utilization in rice and sugarcane. They also observed that ascorbic acid induced resistance to salt stresses,

Joshi and Naik (1981) noticed increase in the leaf area and tiller number in sugarcane due to treatment with ascorbic acid.

Jones and Hughes (1983) have estimated foliar ascorbic acid in 223 species of angiosperm. They found that the ascorbic acid ranged from 25 mg/100 g fresh weight (*Nymphoides peltata*) to 804.8 mg/100 g fresh weight (*Primula vulgaris*). These workers noticed that foliar ascorbic acid in four members of gramineae namely *Avena fatua, Dactylis glomerata, Holcus lanatus* and *Phleum pratense* was 247.7, 87.5, 160.7 and 154.7 mg/100 g fresh tissue respectively. The ascorbic acid level in sugarcane appears somewhat lower than the above values. Thus sugarcane leaves obtained from plants subjected to different treatments the AA content varies from 41 mg to 56 mg/100 g fresh tissue. There are some reports describing influence of seed pretreatment on ascorbic acid content in plants. Startseva (1963) reported that presowing soaking treatment of seeds with micro elements increased ascorbic acid content in plant tissues under the conditions of drought. Kamynina (1965) noticed increase in ascorbic acid content of pea plants at the time of blossoming when seeds were pretreated with aqueous solution of salts of microelements such as Mn, Cu, Co, Mo and Vn. Vyas et al. (1965) reported that prewawing soaking

seed treatment with maleic hydrazide caused increased ascorbic acid content. Dogra and Sinha (1983) reported that application of 50 ppm cycocel as foliar sparys were more effective in increasing ascorbic acid content in *Phyllanthus urinaria* than higher concentrations (250-500 ppm). In the present investigation also an increase in AA content due to pretreatment of sugarcane setts with DW, CCC and ethephon is seen. This is certainly a beneficial change for the plants in view of key role of ascorbic acid in various metabolic processes in general and stress tolerance in perticular.

e. Total polyphenols

Effect of presowing soaking treatment with distilled water CCC and ethephon on the total polyphenols in leaves of sugarcane is depicted in Fig. 13. It is clear from the figure that due to all above pretreatment the polyphenol content is decreased and more decrease is observed in case of CCC pretreatment.

Polyphenols are substances having more than one hydroxyl group in the nuceleus. They encompas a large variety of substances including catechol having two hydroxyl groups, various tannins and betallins, anthocyanins, leucoanthocyanins and anthoxanthins, hydroxy benxoic acids, glycosides, sugar esters of quinane and skikmic acid esters

and caumarine derivatives. The lignification of cellwall and pigmentation of flower due to lignin and anthocyanins are well established functions of these compounds. In oxidation reduction reaction phenolics functions as H^+ donors or acceptors. Phenolics also interfere with growth and other enregy dependant activities by uncoupling oxidative phosphorylation. The phenolic compounds, in some instances, affect fundamental plant processes such as photosynthesis, chlorophyll production, plant water relations, (Rice, 1979), protein synthesis (Dank et al., 1975), respiration and membrane permiability (Glass and Dunlap, 1974).

The formation of highly reactive quinones due to oxidation of phenols inhibits enzymes by coupling with metal ions reaching with sulphahydryl gropus or binding nonspecifically to proteins. While number of processes which are essential for normal plant growth and development may disturbed due to small changes in phenol metabolism. According to Baldry et al. (1970), O-phenols inhibits both corboxylaltion and oxygen evolution reactions from distrupted leaf tissues of sugarcane and spinach. Further they proposed that the most active inhibitors were chlorogenic and caffeic acid, which are converted to quinones through the metabolism of phenol oxidase which inhibits photosynthetic enzyme-S via SH- group reactions.

Chiranjivi Rao et al. (1968) studied phenols in relation to red rot disease and have reported higher phenolic content in cane juice of resistant varities than susceptible varities. They also observed an increase in polyphenol oxidase activity in the resistant varities.

The phenolic compounds are generally regarded as product of secondary metabolism. It is obivious that the secondary matabolism is stimulated due to pathogenesis and various kinds of stress conditions in many plant species. There are few attempts to study effect of growth regulators on polyphenols. Kogel and Elema (1960) have found that in peas the content of polyhydroxy cinnamic acids (ferulic acid, caffeic and chlorogenic acids) increased due to foliar treatment of GA. Biswas (1965) observed that GA treated tea plants contain about 28 times more tannin than the control. When tea plants sparyed with 100 ppm IAA the tannin content shoots upto 25 times more than control one. Reid and Marsh (1969) have shown that GA increased the activity of phenylalanine ammonia layse, which is the key enzyme in the polyphenol synthesis. Shetty (1971) reported that GA and IAA (10 and 100 ppm) treatment increased the polyphenol content in *Acrostichum aureum*. In the present study also presowing soaking treatment with distilled water, CCC and ethephon increased the polyphenol content in leaves of sugarcane

which is beneficial for the sugarcane crop as stress tolerance.

F. EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON PHOTOSYNTHETIC PIGMENTS

a. Photosynthetic pigments

Influence of presowing soaking treatment with distilled water, CCC and ethephon on leaf chlorophyll content is as shown in Fig. 14. It is evident from the figure that presowing soaking treatment with DW has caused a slight decrease in the levels of both chlorophylls a and b and total chlorophylls while due to CCC and ethephon the levels of both total cholorophylls and chlorophyll a and b were increased and this increase in more marked in case of CCC pretreatment. The level of carotenoids is increased due to all the above pretreatment and this increase is more significant in CCC pretreated leaves of sugarcane.

Chlorophyll are important pigments in higher plants because they play a vital role in the process of photosyntheisis. In higher plants chlorophylls a and b are present. Chlorophylls belongs to a class of lipids and they are structurally a tetrapyrrole ring with magnesium at the center. Higher plants are characterised by the presence of chlorophyll a and chlorophyll b which are constituents of

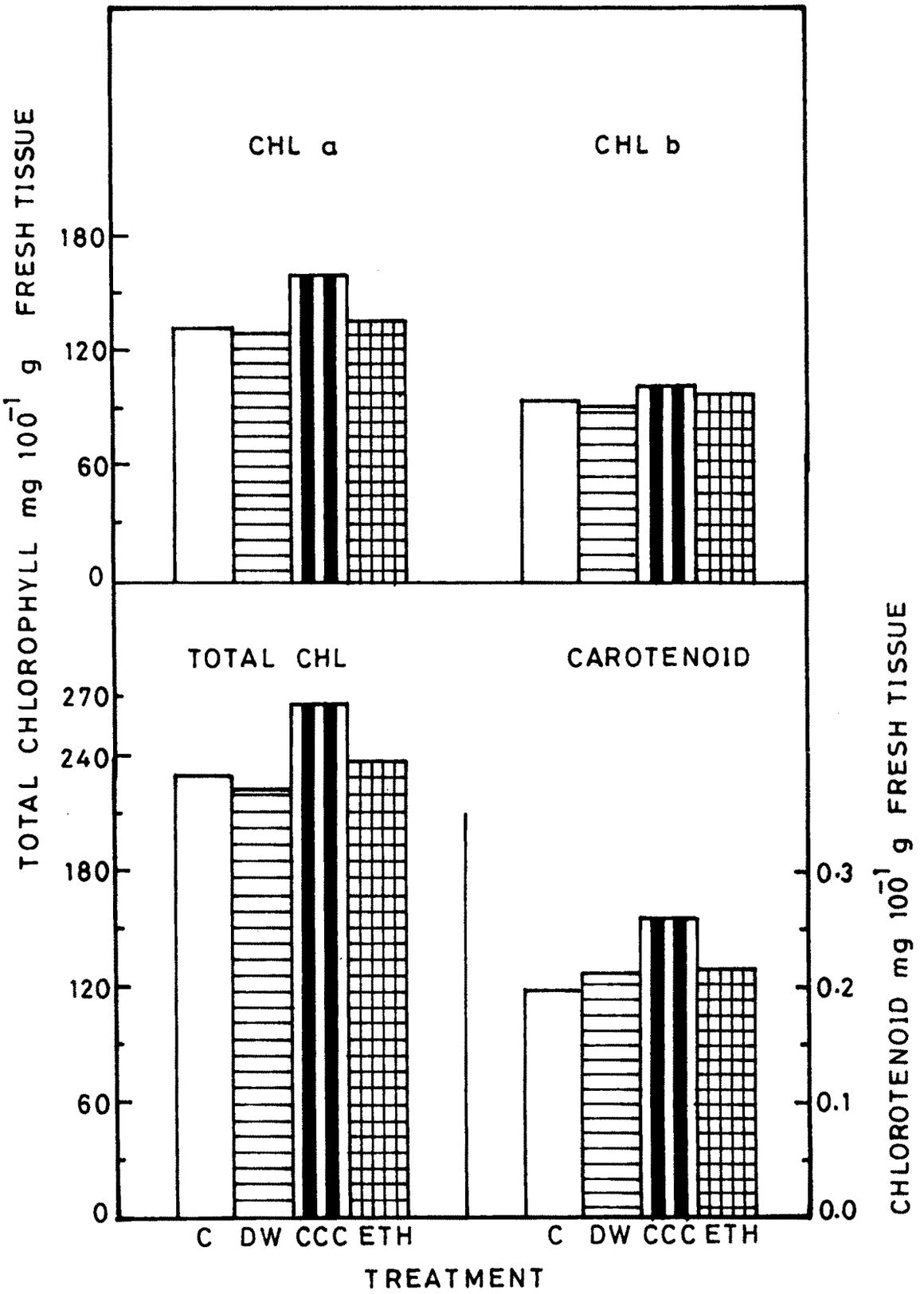

FIG. 14 - EFFECT OF PRESOWING SOAKING TREATMENT OF GROWTH REGULATORS ON CHLOROPHYLLS AND CHLOROTENOID CONTENTS IN THE LEAVES OF SUGARCANE VAR - CO - 671.

photosynthetic appartus. Among these two pigments chlorophyll a plays a major role during the process of photosynthesis. Chlorophylls take part in the conversion of solar energy into chemical energy and act as a 'electron gun' of green plants. Since chlorophylls play a key role in light reactions of photosynthesis,its state and content have direct influence on the photosynthetic efficiency of plant. Induced chlorophyll a fluoroscence has been widely used as nondestructive photosynthetic probe. Changes in chlorophyll a fluoroscence *in vivo* reflects underlying changes in pigment composition and the electron transport through ps II (Papageorgiou, 1975). Chlorophyll fluorescence yield is also used as measure of photosynthetic efficiency.

There are few reports regarding the influence of pretreatment of grwoth regulators on chlorophyll content in plant leaves. Nimbalkar (1973) found that preplanting treatment with GA, kinetin and ascrobic acid, there was reduction in chlorophyll content in all above pretreatments but more reduction was observed due to GA pretreated leaves of sugarcane variety CO 740. Naik and Joshi (1981) reported that the chlorophyll content both on fresh weight and leaf area basis was maintained at higher levels in leaves of NAA pretreated sugarcane plants. Appebly (1966) found that there is increase in chlorophyll content in leaves of wheat plants

pretreated with CCC. Bhadre (1983) from our laboratory also noticed that seed pretreatment with CCC in cotton varities Varalaxmi and H_4 caused an increase in chlorophyll content. Saran (1988) observed that seed soaking in 50 ppm IAA for 24 hrs. increased chlorophyll content but lowered chlorophyll a/b ratio in mustard. Kutwal (1989) also found that presowing soaking treatment with CCC and kinetin caused increase in the both chlorophylls a and b in the leaves of waterstressed plants. In the present study also increase in the chlorophyll a and b content in sugarcane leaves due to CCC and ethephon presowing soaking treatment is evident. This can have a positive effect on the phtosynthetic efficiency in sugarcane.

Besides chlorophylls the chloroplasts contain another important pigment system carotenoids. These accessory pigments are located in the two photosystems in the thylakoid membrane, where they are non covalently linked to membrane assoicated proteins. The role of carotenoids in the photosynthetic system of higher plants is two folds (Demmig-Adams, 1990). Firstly, corotenoid act as accessory hight harvesting pigment, trapping light energy and passing this into chlorophyll molecules. Thus it functions as accessory light harvesting pigment in the photosynthetic apparatus by absorbing light energy in the 400 - 500 nm

region, which is not accessible to the chlorophyll molecules. Secondly and more importantly carotenoids protect the photosynthetic apparatus from light mediated stress. According to Demming-Adams (1990) carotenoids are involved in interconversion of three xanthophylls (Violoxanthin, antheraxanthin and zeaxanthin) and is related to the dissipation of energy under adreses conditions. Production of zeaxanthin is the fundamental importance in establishing stress tolerance in plants.

There are very few attempts to study fate of these accessory pigment. Mercer and Pughe (1969) noticed 30% decline in carotenoid formation due to ABA application in maize. Yadav et al. (1978) found an increase in carotenoid content in leaves of *Trifolium alexandrium* L. due to CCC treatment. Zayed et al. (1985) observed an increase in carotenoid content of leaves of okra plants when they were sparyed with 500, 750 mg/l CCC solutions. Lischchuck et al. (1985) reported that under water stress there is accumulation of carotenoids when treated with CCC. Kutwal (1989) noticed that preasowing soaking treatment with CCC and kinetin raised the level of carotenes in the leaves of stressed plants of groundnut but there is no marked difference in the level of carotenes in the leaves of water stressed plants raised from seeds subjected to above pretreatments. The carotenoid content in the sugarcane

leaves showed an increasing trend with the presowing soaking treatments of DW, CCC and ethephon and this effect was more pronounced in case of CCC. This is certainly advantageous for the sugarcane crop in view of protective role of these pigments under stress conditions.

b. Chlorphyll Stability Index (CSI)

Effect of presowing soaking treatment with distilled water, CCC and ethephon on chlorophyll stability index (CSI) is shown in the Table. 1. It is evident from the table that due to pretreatment there is increase in the chlorophyll stability index in all above pretreatments but this increase is more marked in case of ethephon pretreatment.

Since the chlorophylls play a key role in the light reaction, the status of these pigments in the chloroplasts has great influence on the overall photo-synthetic efficiency of the plants. This is perticularly true when the plants are expressed to variety of enviornmental constituents. According to Strogonov et al. (1970), the stability of pigment - protein - lipid complex in chloroplast is very important in salt tolerance process. Tolerant plants have greater stability and they loose chlorophylls less slowly than salt sensitive plants.

Kaloyereas (1958) obtained correlations between drought resistance and heat stability of chorophyll "bound water" and -SH content. Chlorophyll stability index is the ratio of chlorophyll content of stress leaf material to that of the normal leaf material. In several instances the CSI has been correlated with drought resistance of certain crop plants (Murty and Majumdar, 1962; Sahadevan, 1961). Observation on three rice varieties namely MTU 18, MTU 17 and MXW 49 have indicated the correlation between CSI value and drought resistance (Bhiravmurty and Prasad, 1979). Similar trend has been noticed by Yadav et al. (1975) and Chabra et al. (1980) for oat and *Brassica* cultivars respectively. Thus it is obivous that higher the chlorophyll stability index (CSI) higher will be the capacity to resists drought and heat stress.

It is evident from the present investigation that presowing soaking treatment with distilled water, CCC and ethephon are helpful in increasing the stability of chlorophylls in sugarcane leaves. The increase in chloropyll content caused by presowing soaking treatment would undoubtedly help the sugarcane plants in maintaining photosynthetic activity which leads to develop the stress tolerance mechanism in sugarcane and in this respect ethephon may play an important role in developing stress tolerance of sugarcane.