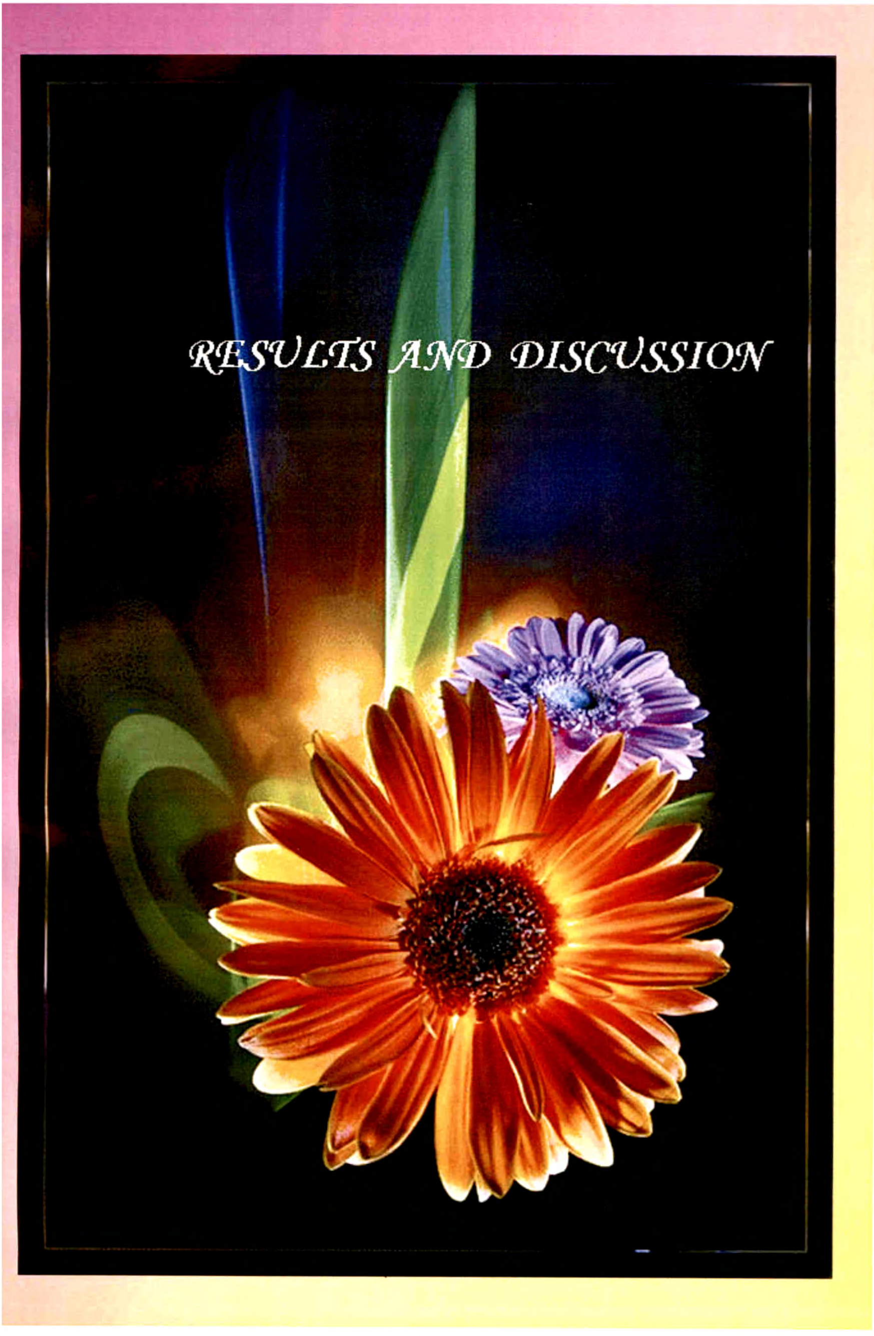

RESULTS AND DISCUSSION

Growth parameters

The effect of three plant growth regulators on the different growth parameters such as number of tillers and dry weight of roots and shoots of the two Vetiver varieties-local and KS1 is shown in Fig no.1. It is clear from the table that local Vetiver variety has given better response to foliar application of all the three PGRs with respect to various growth parameters. Among different PGRs studied 200ppm Vipul treatment appears highly effective with respect to dry matter partition in roots. Except 100ppm CCC treatment, other treatments of the three PGRs have caused promotion of growth in cultivar KS1 as revealed by tiller number and dry matter accumulation in roots and shoots. Salicylic acid is more effective in case of this culture than treatments of other two PGRs.

Among different growth parameters which determine crop productivity, tillering efficiency and rate and extent of dry matter production are perhaps the most important one. In case of aromatic plant like Vetiver, where roots yield an economically important and fragrant essential oil, the dry matter production of the root tissue is more important than that of the shoot.

Several attempts have been made to study chemical manipulation of growth, development and dry matter production in crop plants. Effect of CCC treatment on different growth parameters of plants was studied by some workers. Radwan *et al.,* (1971), observed the effect of CCC on dry matter production and yield character of potato. They observed that as the concentration of CCC increases, the shoot height decreased, while the dry matter content of leaves, of total foliage and tubers get increased by some treatments. Physiological effect of CCC on growth of grape wine was investigated by Niimi (1979). He observed that shoot elongation and dry weight of shoot remain unchanged, while dry weight of root increased. Gliozeris *et al.,* (2007) studied the effect of some PGRs on *Viola* X *wittrockiana* 'Wesel ice'. They found that B9+CCC imparted negative effect on the fresh weight of leaves. The higher concentration (1000ppm) of CCC was found to increase in dry weight of source leaf and pods in mung bean- *Vigna* *radiate* (L.) Wilczek var Guj-2 (Shah and Prathapasenan, 1991). In case of Vetiver, the CCC treatment have caused increase in total tiller numbers in case of local variety while increase in total tiller number was only found in response to higher concentration (200ppm) of CCC in case of KS1 variety. The dry weight of root and shoot is significantly increased in local variety in response to CCC while in case of KS1 it gets least affected or sometimes decreased. Hence the CCC showed more effective results in case of local variety of Vetiver which is more effective in contrast to cultivar KS1.

Coronado *et al.*, (1998) studied the effect of SA on root and shoot growth of soybean. They found that root growth was increased significantly in response to SA. Khan *et al.*, (2003) studied effect of foliar spray of SA on corn and soybean and observed that in some treatments, it caused increase in leaf area and plant dry mass but plant height and root length remained unchanged. Effect of SA on wheat seedling under stress was studied by Singh and Usha, (2003). These workers noticed increase in dry mass in response to SA treatments in seedlings subjected to water stress. Fariduddin *et al.*, (2003) observed increase in dry mass and number of pods and seeds of mustard in response to SA treatment. In Vetiver grass, SA application has caused increase in tiller number in both (KS1 and local) varieties. The dry weight of roots and shoots is considerably increased in response to SA in local variety. The root dry weight is considerably increased in contrast to shoot due to treatment in case of cultivar KS1. Thus for good production of root system SA application is beneficial in case of both Vetiver cultivars.

The effect of triacontanol on growth characters of some plants was studied by few workers. The effect of triacontanol formulation 'miraculan' on different aspects of lemongrass was studied by Misra and Srivastava, (1991). They observed increase in plant height, tiller per plant and biomass yield in response to miraculan. In tissue culture studies, triacontanol caused increase in number and length of roots; shoot growth, fresh weight at 5μg/l concentration in balm (*Mellisa officinalis* L.) (Tantos *et al.*, 1999). Significant increase of plant height, fresh mass

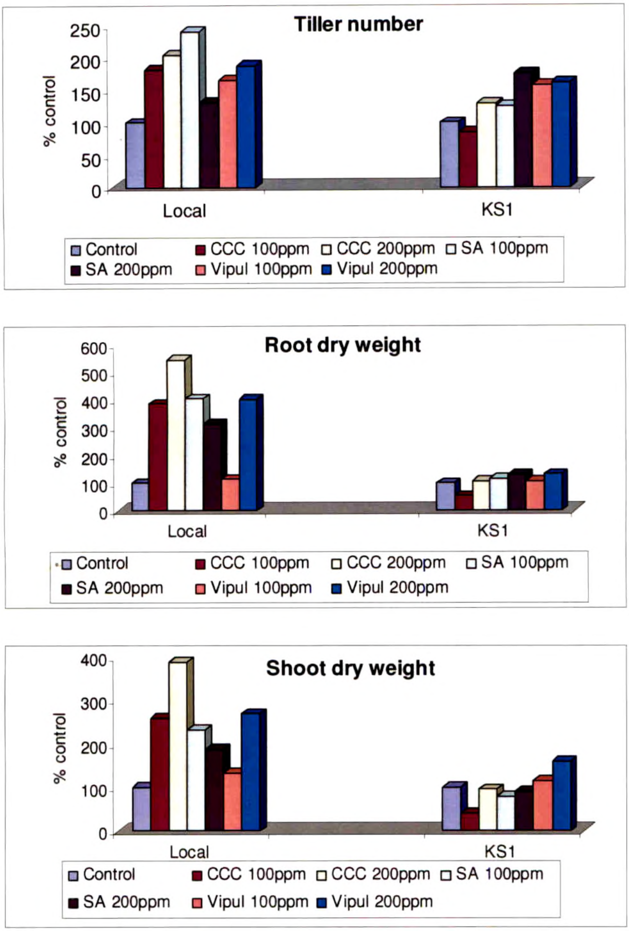


Fig. No.1. Effect of PGRs (CCC, Salicylic acid and Vipul) on some growth parameters in Vetiver cultivars (Local and KS 1).

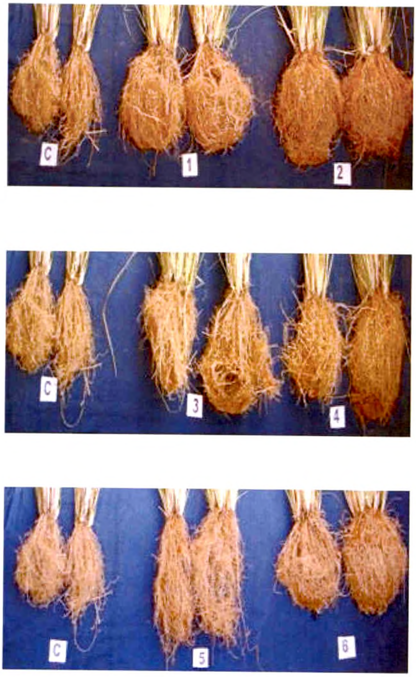


Plate No.2. Effect of PGRs (CCC, Salicylic acid and Vipul) on root system in plants of Local Vetiver cultivar

C- Control, 1- CCC 100 ppm, 2- CCC 200 ppm, 3- SA 100 ppm, 4- SA 200 ppm, 5- Vipul 100 ppm, 6- Vipul 200 ppm.

is observed in gram in response to TRIA at different concentrations (Kumaravelu *et al.*, 2000). In case of Vetiver grass, foliar treatments with both the concentrations of Vipul have caused increase in tiller number in both the varieties (KS1 and local). The dry weight of both roots and shoots also markedly increased in response to Vipul treatment.

Two among the three PGRs studied in the present investigation foliar application of Vipul may prove beneficial for promotion of growth as well as dry matter production in roots and shoot in the two Vetiver cultivars (local and KS1).

A. Photosynthetic pigments

a. Chlorophyll

Effect of three different plant growth regulators like CCC (2-chloroethyl, trimethyl ammonium chloride), SA (salicylic acid) and Vipul (triacontanol) of two concentrations-100ppm and 200ppm was sprayed on leaves of Vetiver variety KS1 and local variety of Vetiver. After spraying on KS1 variety, as compared to control, the chlorophyll content was reduced in all used PGRs except 100ppm CCC while vice versa results was found in local variety (Fig. no. 2).

The life of higher plants on this planet depends on the action of green pigments chlorophylls. These pigments play a fundamental role in light reaction of photosynthesis. They are localized in the thylakoids of photosynthetic apparatus chloroplast. The structure of chlorophyll contains a bivalent cation as magnesium, which has an aliphatic alcohol; phytol esterified to a propionic acid residue on one of the tetrapyrrole rings. Two main types of chlorophylls namely chlorophyll a and chlorophyll b are found in the higher plants among which chlorophyll a plays important role in photosynthetic act while a secondary supporting function is played by chlorophyll b. One of the 200 antenna chlorophyll a molecules associated with each PS-1 reaction centre absorbs a photon to initiate the photochemical charge separation (Golbeck, 1992). The PS 1 system is situated in the thylakoid membranes of higher plants while its reaction centre is light driven membrane bound pigment protein complex plastocyanin, ferrodoxin oxidoreductase. Limited number of chlorophyll containing reaction centres trap this absorbed excitation energy which is migrated towards it and energy conservation is initiated by charge separation (Zinth and Kaisar, 1993). The water is oxidized at the reaction centre of photosystem II which is composed of two proteins such as D1 and D2 and it contains six chlorophylls, two pheophytins, two ß carotenes, two quinines and one or two haems of cytochrome-b559 (is a precursor of chlorophyll pigments are degraded by the action of enzyme chlorophyllase. Light exerts a major control on chlorophyll biosynthesis through a phytochrome pigment system). The chlorophyll content in leaf tissue is important factor which determines the efficiency of photosynthetic process of the plants. This content is influenced by both endogenous factors, environmental factors and leaf applied chemicals such as plant growth regulators. It is evident from our findings that although both cultivars local and KS 1 belong to the same species *Chrysopogon,* the chlorophyll content in improved cultivar KS1 is considerably higher than the local cultivar. This is particularly significant in case of photosynthetically important chlorophyll- chlorophyll a. thus judging from chlorophyll it appears that the improved Vetiver cultivar has higher efficiency than the local cultivar.

Chlorophyll content was found to be increased after application of CCC (Tezuka *et al.,* 1980 and 1989; Niimi., 1979; Stoddert, 1965; Sharma *et al.,* 1998). Stoddert (1965) recorded increase in chlorophyll content due to application of 0.01-0.5M concentration of CCC in *Lolium temulentum* L. Niimi (1979) found that chlorophyll content of CCC treated grapewine leaves was higher as compared to control. Tezuka *et al.,* (1980); noticed that the chlorophyll content was increased after treatment of CCC in Kyoho grapes. Similar results were found by Sharma *et al.,* (1998) in case of potato (*Solanum tuberosum* L.). Tezuka *et al* (1989) noticed that photosynthetic activity was stimulated by CCC and improved content of chlorophyll in CCC treated hollyhock plant. In the leaves of local cultivar

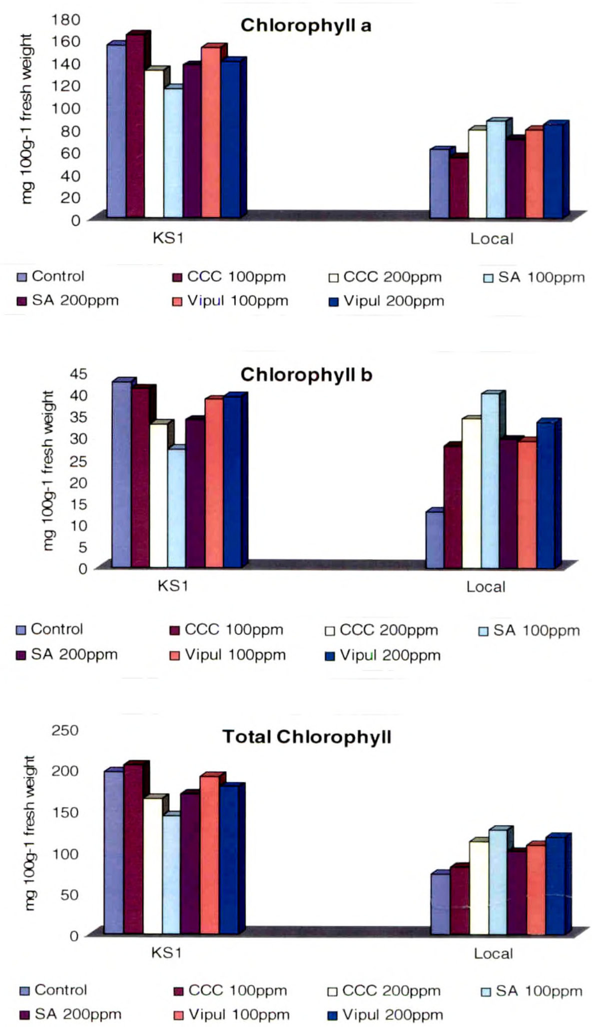


Fig. No. 2. Effect of PGRs (CCC, Salicylic acid and Vipul) on chlorophyll content in Vetiver cultivars (KS 1 and Local).

application of CCC is effective in causing increase in content of both chlorophyll a and chlorophyll b. In improved cultivar KS 1 such increase is noticed only in plants treated with 100ppm CCC. In case of Vetiver variety KS 1 at lower concentration of CCC (100ppm), there was found on increase in chlorophyll content while at higher concentration (200ppm) there was decrease and exactly apposite trend was observed in variety- local of Vetiver.

Very little change in leaf chlorophyll content in response to SA treatment have been noticed in case of *Wolfia arrhiza* and corn, soybean by Czerpak *et al.*,(2002) and Khan *et al.*, (2003) respectively. Moharekar *et al.*, (2003) found that with increasing concentration of SA, there was decrease in chlorophyll content in wheat and moong seedlings. Similar trend has been noticed in case of Vetiver cultivars KS 1 in the present investigation. However, exactly apposite response is shown by local cultivar as chlorophyll content is markedly increased by SA treatment.

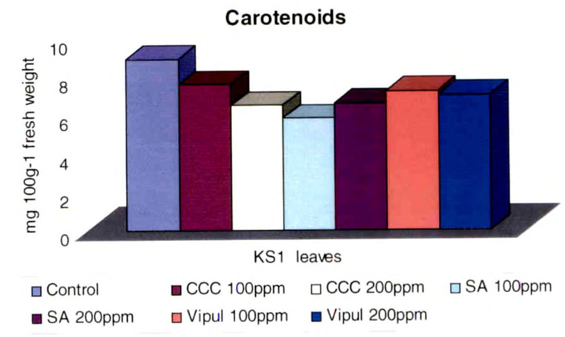
The Vipul (triacontanol) is one of the plant growth regulators which is reputed to cause increase in chlorophyll level in some plants. The leaf chlorophyll was found to be increased in apple cv Red Delicious due to triacontanol treatment (Sharma and Joolka, 1997). In micro propagated *Melisa officinalis* (Balm) also Vipul application caused elevation in chlorophyll content (Tantos *et al.*, 1999). Vipul treatment has brought about increase in chlorophyll content in the leaves of local Vetiver cultivar. But in case of cultivar KS 1 such response is not noticed. It is clear from the foregoing account that there is a clear varietal difference in Vetiver with respect to response of chlorophyll accumulation to foliar application of PGRs.

b. Carotenoid

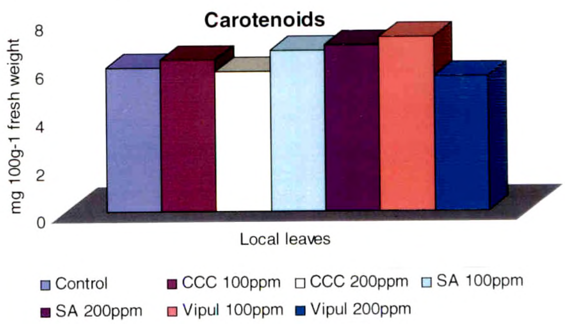
Effect of foliar application of three plant growth regulators- CCC, SA and Vipul on carotenoids in *C. zizanioides* (L.) Roberty is depicted in Fig.no. 3. From the figure it is clear that in Vetiver variety KS 1, all the three PGRs has caused decrease in carotenoid content while in case of local variety the carotenoid content increased after application of all three PGRs.

Carotenoids are isoprenoid molecules, these molecules are categorized into hydrocarbon carotenes like lycopene and β carotene or xanthophylls, typified by leutin. In some fruits, flowers and roots, coloured carotenoids are present which are useful to attract pollinaters and in seed dispersal (Cunningham and Gantt, 1998). In past, the carotenoid synthesis is assumed to be mediated through mevalonic pathway but now it is proved that synthesis of carotenoids takes place via 1-deoxy-D xylose-5 phosphate pathway (DOXP). From this pathway, plastidic isoprenoids such as carotenoids, phytol, plastoquinone 9 and diterpenes are formed (Lichtenthaler, 1999). Various photoprotective functions of carotenoids have been characterized by different workers. Williams *et al.,* (2003) mentioned its potential role as passive light filters that would reduce light intercepted by chlorophylls while Steyn *et al.,* (2002) mentioned that these pigments offer protection from reactive oxygen species. Koiwa *et al.,* (1986) assumed the location of red (retro) carotenoids in outer cell layer of the chromoplast, which may play a protective role like reducing light interception by chloroplasts which are situated below and chlorophyll over excitation Hormaetxe *et al.,* (2005). Cunningham and Gantt (1998) suggested that the carotenoid efficiency quench triplet chlorophyll, single oxygen and superoxide anion radicals therefore these are essential and integral component of photosynthetic apparatus. According to Rock and Zeevart (1991), epoxy carotenoids, 9-cis-volaxanthin (9c-voc) and 9-cis-neoxanthin (9c-neo) are metabolized into abscisic acid, which is very important stress hormone.

The increased accumulation of carotenoids was found after application of CCC in the experiments of Gamburg (1978) and Wang and Xiao, (2009). The effect of CCC on carotenoid in tobacco tissue in suspension culture was studied by Gamburg in 1978. They observed four fold increase in carotenoids accumulation per one cultural flask by CCC. Foliar spray of CCC on potato (*Solanum tuberosum* L.) also caused increase in carotenoid content (Wang and Xiao, 2009). It is evident



KS1



LOCAL

Fig. No. 3. Effect of PGRs (CCC, Salicylic acid and Vipul) on carotenoid contents in Vetiver cultivars (KS 1 and Local).

from our observations that level of this photo protective pigment is almost double in control plants of improved cultivar KS1 in comparison to local cultivar. The carotenoid content was increased by the action of CCC in case of local variety, but in case of KS1 variety the level of this photo protective pigment is found to be reduced due to CCC application.

The effect of auxin and SA on carotenoid content in *Wolfia Arrhiza* (L.) Wimm was studied by Czerpak *et al.,* (2002). They found slight increase in carotenoid in response to SA while SA+IAA caused significant increase in carotenoid especially β carotene and leutin + zeaxanthin. Moharekar *et al.,* (2003) observed increase in total carotenoid content due to SA treatment in wheat and moong seedling. They suggested that SA may create oxidative stress and in order to cope with such stress, carotenoid synthesis is stimulated. SA treatment is favourable for local Vetiver cultivar with respect to accumulation of these protective pigments. However same cannot be stated about improved variety KS1.

Effect of triacontanol on hyacinth bean (*Lablab purpureus* L.) was investigated by Naeem *et al.,* (2009). They observed that 10^{-6} M concentration of TRIA caused increase in most of the attributes like plant fresh weight, dry weight, chlorophyll, carotenoids etc. The effect of triacontanol in combination with potassium was studied by Khan *et al.,* (2009). This combination caused increase in carotenoid content in tomato. The present investigation has shown that Vipul alone is effective in bringing about elevation in carotenoid status in local cultivar. But the same PGR causes a negative effect on carotenoid accumulation in improved cultivar KS1.

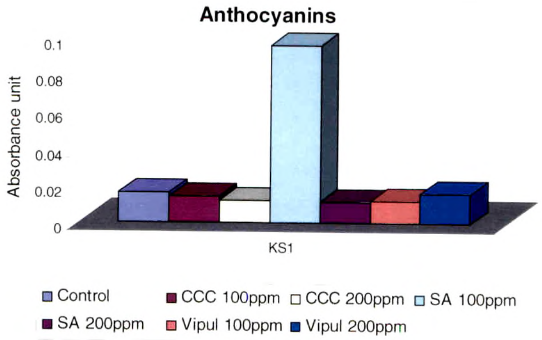
B. Anthocyanin

The foliar application of the three plant growth regulators CCC, SA and Vipul on the anthocyanin content from leaves of two Vetiver varieties- KS1 and local variety is presented in Fig.no. 4. It is evident from the fig. that local cultivar has greater leaf anthocyanin level than the improved cultivar KS1. Application of

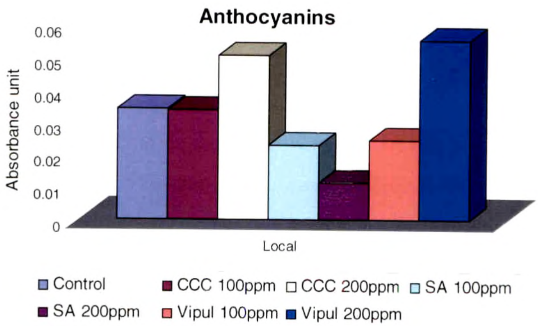
Vipul and higher dose of CCC (200ppm) have caused elevation of anthocyanin content in local cultivar only. But the anthocyanin content is reduced due to other PGRs of treatments in local as well as improved cultivar.

Anthocyanin is the pigments belonging to the flavonoid class. These impart red, pink, purple and blue colour to the different plant parts, which help in attracting animals and insects for pollination and seed dispersal. Anthocyanin is glucoside in form, which bear sugar compound. Anthocyanins without sugar molecule termed as anthocynidins (Taiz and Zeiger, 2006). Anthocyanins may contain chelated metal ions and flavone co-pigment and thus form super molecular complexes. Hahlbrock and Scheel (1989) demonstrated that the anthocyanins are synthesized from photosynthetically produced carbohydrates and the reactions are catalyzed by the enzyme of shikimic acid, phenyl propanoid and flavonoid biosynthetic pathways. The addition of sucrose of 0.015M concentration promoted the anthocyanin synthesis but other sugars like glucose or mannitol did not show any stimulatory effect on anthocyanin production. The important role of leaf anthocyanins in protection against ultraviolet radiation is very well documented.

The effect of benzyl adenine (BA), kinetin (K) and fusicoccin (FC) on anthocyanin content of excised sunflower cotyledon was studied by Servettaz *et al.,* (1975). They observed that BA and K increased the accumulation of anthocyanin while FC caused reduction of anthocyanin content. Addition of GA_3 and ABA as well as 2, 4 D in carrot suspension culture causes inhibition of anthocyanin content (Ozeki and Komamine, 1986). Different PGRs along with sucrose effect in which kinetn increased the anthocyanin in *Comptotheca acuminate* cell culture, as compared to benzyl adenine while 2,4D and NAA did not caused any change in anthocyanin content except 2 μM 2,4D caused greater production of anthocyanin (Pasqua, 2005). The PGRs like ABA, ethephon and uniconazol stimulated anthocyanin synthesis in chicory (*Cichorium intybus* L.) (Boo *et al.,* 2006). Alar is one PGR which not caused increased in the anthocyanin content in Rome apple fruit (Gianfagna *et al.,* 1986). Triazole treatment caused



KS1



LOCAL

Fig. No. 4. Effect of PGRs (CCC, Salicylic acid and Vipul) on anthocyanin contents in Vetiver cultivars (KS1 and Local) increase in anthocyanin content of *Plectranthus aromaticus, P. vettiveroides, Catharanthus,* potato, *Solenostemon* while paclobutrazol treatment increased the anthocyanin content of *Catharanthus roseus* (Rajalekshmi *et al.,* 2009).

Effect of CCC along with B9 on anthocyanin content of *Primula obenica* was studied by Abou-zied and Bakry, (1978). They observed that CCC and B9 both caused increase in anthocyanin content. CCC also showed stimulatory effect on anthocyanin biosynthesis while GA_3 displayed inhibitory effect in reddish seedlings (Jain and Guruprasad, 1989).CCC also counteracted the inhibitory action of GA_3. In case of Vetiver grass, the higher concentration of CCC (200ppm) have caused increase in anthocyanin content in leaves of local variety while all other treatments of CCC caused inhibition of anthocyanin accumulation in local variety and KS1 variety. Hence the higher concentration is only effective for local variety while it is non effective for KS-1.

Effect of 0.01 and 5mM SA with other phenolic acids on anthocyanin content of maize roots was investigated by Jain and Srivastava (1984). They observed an increase in anthocyanin content due to all treatments. SA also ameliorated and then increased in anthocyanin content in carrot plant which was subjected to salinity and boron stress (Eraslan *et al.,* 2007). In case of Vetiver grass having both the doses of SA has caused low entity of anthocyanin content in KS1 and local variety.

Jindal *et al.,* (2004) noticed positive effect of promalin (GA+CK) and mixtalol (TRIA) on anthocyanin content in apple. Thakur *et al.,* (1991) also found increase in anthocyanin content by the action of triacontanol along with activol, NM and ergotism, in berries of strawberry fruit. In case of Vetiver grass, the higher concentration of Vipul (200ppm) has caused increase in anthocyanin content in leaves of local variety.

Thus foliar treatments of all the PGRs are not effective in causing any major positive effect on accumulation of these pigments in both cultivars of Vetiver grass.

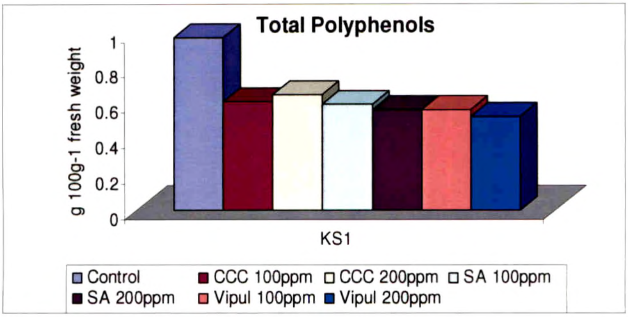
Polyphenols

The effect of foliar application of three PGRs CCC, SA and Vipul on the polyphenol contents from leaves of Vetiver local variety and KS1 variety is shown in Fig. no. 5. From figure it is observed that polyphenol content is increased slightly due to treatment in local variety while a marked apposite trend is noticeable in KS1 variety.

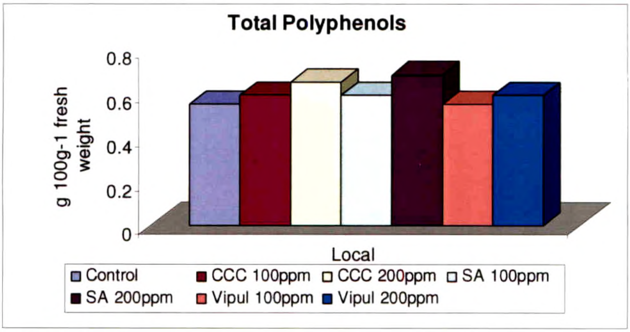
Phenolic compounds are those which contain a phenol group- a hydroxyl functional group on an aromatic ring which belongs to secondary metabolic products. There are about 10,000 individual compounds which forms a chemically heterogeneous group. Most of the phanerogamic species contains such polyphenolic compounds. Compounds like phenolic acids, flavonoids, flavonols, anthocyanidins and other related substances are included in this group. Some phenols are soluble in organic solvents while others are soluble in water. Carboxylic acid, glycoside etc are large insoluble polymers example of phenolic compounds (Taiz and Zeigar, 2006).

In pharmalogical studies, polyphenols are considered a secondary product of vegetable metabolism without specific physiological function (Adzet, 2002). Polyphenols have a natural antioxidant properties, therefore impart a tonifying action (Thompson and Williams, 1976), which is also demonstrated *in vitro* (Letan, 1967). Stimulation of protein synthesis and promotion of ammonia elimination are two functions carried out by polyphenols (Greppin and Horn, 1969 and Letan, 1967).

There are different pathways for the biosynthesis of aromatic compounds. Two basic pathways such as shikimic acid pathway and mevalonic acid pathway are prominent, but in biosynthesis of most of the phenolics, shikimic acid pathway is involved (Taiz and Zeigar, 2006). In shoots as well as in roots of legumes, phenolics are synthesized via the phenyl propanoid/ shikimic acid pathways (Heller and Forkmann, 1988). In these metabolites, the flavonoids which are polyphenolic compounds are formed through the activity of two enzymes- phenyl



KS1



LOCAL

Fig. No 5. Effect of PGRs (CCC, Salicylic acid and Vipul) on total polyphenls in leaves of Vetiver cultivars (KS1 and Local) alanine ammonia lyase and chalcone synthase. Flavonoids are categorized into subgroups as chalcones, flavones, flavanones, aurones and isoflavonoids, occur in most legume tissues (Wollenweber and Jay, 1988) and with other aromatic compounds can be exuded into the rhizosphere (Cooper and Rao, 1992). The very important role of polyphenols is in passive and active defense mechanism against variety of pathogens. Some flavonoids acts as powerful electron scavengers of free radicals (Rice-Evans *et al.,* 1997) and also acts as electron doners to the H_2O_2 scavenging peroxidases of plant cells. Most of the phenolic compounds posses antioxidant activity and hence may help to protect cells against the oxidative damage which is due to free radicàls, it is proved in *Crataegus laevigata* and *C. monogyna* (Howthorn) (Kirakosyan *et al.,* 2003). Zhang *et al.,*(2001) showed that various phenolic compounds from Howthorn (*C. monogyna*) caused increase in myocardial contractibility, reduced reperfusion arrythmias, dilated peripheral arteries and mildly decreased blood pressure. At the same time Butler (1992) highlighted antinutritional effects of condensed and hydrolyzed tannins.

The polyphenol content was found to be increased when cell cultures of *Cornus kouse* grown on MS medium supplemented with 2,4D+BA or IBA+BA in combination, while MS with BA and 2,4D caused decrease in polyphenol contents. The deletion of NH_4NO_3 from MS medium and addition of 2,4D and BA caused significantly increase in polyphenol content (Ishimaru *et al.,* 1993). While studying the effect of different PGRs on rooting on *Pinus* cutting, it was observed that there was absence of relation between phenol content and rooting process and also observed that NAA causes noticeable increase in phenol contents. Auxins also caused increase in phenolic content especially orthodehydroxi phenolics (Henrique *et al.,* 2006). Li *et al.,* (1970) observed that high concentration of GA3 caused increase in accumulation or phenols like scopolin and chlorogenic acids while low concentration of GA_3 causes decrease in accumulation of that phenols. Jeong *et al.,* (2007) found that PGRs like BAP and kinetin causes increased phenol in genetically transformed hairy roots of *Panax ginseng* C.A.Meyer while the auxin caused change in phenolic contents. The PGRs like GA, IBA and BA additionally with vitamins when applied as presowing soaking treatment on *Thymus vulgaris* L. caused increase in phenol content significantly (Reda *et al.,* 2007). Gopi *et al.,* (2009) observed the increase in phenol content in response to PBZ and ABA in Holy basil (*Ocimum sanctum*).

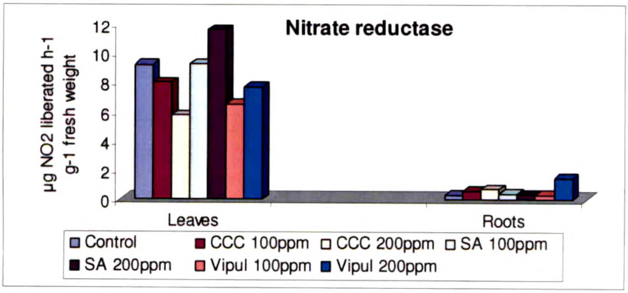
A varietal difference is clearly evident with respect to influence of PGRs on aromatic metabolism and level of phenolic compounds. The level of leaf polyphenols in control plants of variety KS1 is considerably higher than that of local cultivar. Such a high accumulation of phenols will definitely contribute to disease resistance as well as free radical scavenging in improved cultivar. However foliar application of all the three PGRs has caused reduction in total polyphenol content in this cultivar. An exactly apposite trend is evident in case of local cultivar. In this cultivar the biosynthesis and accumulation of phenolic compounds in the leaves is slightly stimulated by application of three PGRs. In this respect higher doses of SA and CCC are highly effective.

Enzymatic studies

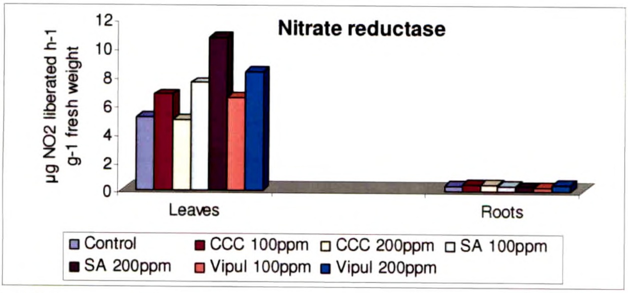
a. Nitrate reductase

Effect of three plant growth regulators like CCC, SA and Vipul on enzyme nitrate reductase from Vetiver roots and leaves of KS1 variety and local variety is shown in Fig. no. 6. From figure it is evident that in both Vetiver cultivars NR activity more predominant in the leaf tissue than the root tissue. Comparison of NR activity in control plant of the two varities reveal that the leaf NR activity in improved cultivar KS1 is almost double the leaf NR activity of local cultivar. However there is not much difference in the root NR activity of the two cultivars.

Foliar spray of PGRs has caused alteration in NR activity in both leaves and roots in case of both the cultivars. 200ppm salicylic acid treatment has caused marked increase in leaf NR activity in both the cultivars. The NR activity in the



KS1



LOCAL

Fig. No.6. Effect of PGRs (CCC, Salicylic acid and Vipul) on activity of enzyme nitrate reductase in leaves and roots of Vetiver cultivars (KS1 and Local) root tissue in cultivar KS1 is increased due to foliar application of all the three PGRs, 200ppm Vipul being most effective. In case of local cultivar such increase in root NR activity is noticed in case of Vipul and CCC treatment only. Treatment of all the three PGRs (Vipul, SA and CCC) has caused a decrease in leaf NR activity in case of KS1. But in case of local cultivar foliar treatment of all the three PGRs (except 200ppm CCC) have caused an increase in the foliar NR activity.

Nitrate reductase is one of most important enzymes of nitrogen assimilation process in plants. It converts nitrate to nitrite and it is complex metalloenzyme which is substrate inducible (Beevers and Hageman, 1969; Hewitt, 1975). It is considered to be a rate limiting factor because nitrate reduction is an initial step in nitrogen assimilation process (Beever and Hageman, 1969). According to Ward *et al.,* (1989 and 1988) nitrate reductase is associated with the plasma membrane of corn roots and barley seedlings. Wide variation is seen in molecular masses of assimilatory NR from different sources which ranges from 200kD in spinach (Hewitt, 1975) to 500kD in *Ankisdrodesmus braunii* (blue green algae) (de la Rosa *et al.,* 1981). Amino acid sequence of NR protein was studied by different workers from different sources e.g. from tobacco by Vauchert *et al.* (1989), in *Arabidopsis* by Crawford *et al.,*(1988). The single polypeptide of NR enzyme from all studied plants showed 70-80% amino acid sequence similarity with 904-917 similar amino acid residues. Kaisar *et al.,* (1993) showed that the NR activity is regulated by protein phosphorylation and dephosphorylation. According to the catalytic activity of NR is depend on- 1)substrate availability in the cytoplasm (steady state concentration of NAD(P)H and nitrate) 2)functional NR level (amount of NR polypeptide, availability of cofactor and metal ions- FAD, heme Fe, Mo-MPTand Mo) and 3)the activity level of functional NR. The distribution of the NR activity within the plant has been studied by some workers. In wheat (Beevers and Hageman, 1969) and maize (Reilly, 1976) 2/3rd of the nitrate was taken by the plants is reduced in leaf. Wallace and Pate, 1965 observed that major part of total nitrate reduction occurs in the root tissue in pea. Kapoor and Li (1982) and

Marwaha (1998) found that leaves are the major site of nitrate reduction while in roots and tubers very low NR activity was observed in potato crop.

Since nitrate reductase enzyme is a key enzyme of nitrogen assimilation process, several experiments have been performed to study responses of this enzyme to various environmental features as well as to application of various plant growth regulators. In case of lettuce *Lactuca sativa* L. the NR activity is not affected by CCC (Knypl and Janas, 1979), on the other hand NR activity is found to be increased due to CCC (1000ppm) in case of wheat under water stress condition (Singh *et al.,* 2007). Farooqi *et al.,* (2010) reported that CCC application caused increase in NR in drought stressed Vetiver plants. At the same time they observed decrease in NR activity due to CCC in unstressed plants.

The effect of SA on NR activity is studied by Fariduddin *et al.,* (2003) and Jain *et al.,* (1981). The NR activity was found to be increased as SA concentration increased from 10^{-5}M to 10^{-3} M in case of mustard (*Brassica juncea* Czern and Coss cv Veruna) (Fariduddin *et al.,* 2003). Jain and Srivasrtava, (1981) observed that the NR activity in the maize (*Zea mays* L.) seedlings was increased at low concentration of SA.

Effect of triacontanol on the NR acticity investigated by few workers. In seedling of green gram (*Vigna radiate* (L.) Wilezek) cultivar KM-2, NR activity increased at concentration 0.5 and 1.0mg dm^{-3} of triacontanol (Kumaravelu *et al.,* 2000). Naeem *et al.,* (2009) studied effect of the 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} concentrations of triacontanol on NR activity. These workers reported that at 10^{-6} M concentration NR activity was increased.

It is evident from the present investigation that the growth retardant CCC has a negative influence on NR activity in cultivar KS1 and in local cultivar also the effect is not very encouraging. But in the roots of both cultivars the enzyme activity is promoted by CCC treatment and this may improve N metabolism in root tissue. Foliar application of triacontanol (Vipul) has caused increase in NR activity in the leaf tissue in case of local cultivar while such increase is seen in case of root tissue in case of improved cultivar. Amongst the three PGRs salicylic acid is perhaps the most effective in enhancing NR activity and this treatment may promote over all nitrogen metabolism in Vetiver grass.

b. Peroxidase

Changes of peroxidase activity in response to treatment of three plant growth regulaters-CCC, SA and Vipul in two Vetiver cultivars are recorded in Fig.no. 7. Among the two cultivars, the improved cultivar KS1 has considerably more peroxidase activity than local cultivar. In both the varieties, there is decrease in activity of peroxidase enzyme in case of all the three treatments of both varieties (except 200ppm CCC treatment in case of local cultivar).

Enzyme peroxidase belongs to class oxidoreductase and is ubiquitous (Vamos- Vigyazo, 1981). These enzymes are glycoprotein containing heme and it takes part in biosynthesis of lignin and ethylene, in metabolism of auxin and protecting against pathogen and wounding. (Halbrock and Gricebach, 1979; Kim *et al.,* 1999). These enzymes protect the cells from harmful effect of H_2O_2 and oxygen radicals and dehydrogenation of structurally diverse phenolic and endiolic substrates of H_2O_2 and therefore these enzymes are called as antioxidant enzymes (Prasad *et al.,* 1994 and Vainello, 1997). Ingham *et al.,* (1998) studied that the cell wall formation is catalyzed by peroxidase. This enzyme also plays an important role in regulation of lignifications of vascular tissue in *Coleus.* Cross linking of cinnamic acid ex. Ferulic acid in case of non-lignified cell walls is done by peroxidase. Alkaline pH and presence of reductant H_2O_2 are suitable for the peroxidase activity (Vranova *et al.,* 2002)

The effect of CCC on behavior of peroxidase in different plants was studied by some workers. Caspar and Lacoppe, (1968) studied effect of CCC along with Amo 1618 on activity of peroxidase in young barley seedlings. They found that increasing concentration of CCC stimulated peroxidase activity. Gudrupa *et al.,* (2002) studied the effect of CCC and pH on *Sedum rubrotinctum* RT clausen.

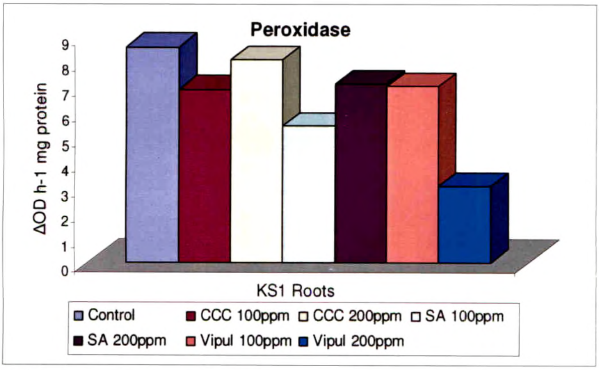
They found concomitant changes in peroxidase activity with ethylene production in CCC treated plants. Farooqi *et al.,* (2010) studied effect of CCC on peroxidase activity in *Chrysopogon zizanioides* Roberty. They found that peroxidase activity increased by application of CCC. In present investigation such response is noted only in case of local Vetiver cultivar treated with 200ppm CCC. But in other cultivar the CCC treatment has brought about decrease in peroxidase activity.

Mahdavian *et al.,* (2007) recently studied effect of different concentrations of SA on peroxidase activity of pepper (*Capsicum annum* L.). The treatment increased activity of peroxidase enzyme. Ali *et al.,* (2007) studied effect of methyl jasmonate and SA on root suspension cultures of *Panax ginseng.* Chlorogenic acid activities were found more in SA treated roots than methyl jasmonate treated once. Inhibition of ascorbate peroxidase by SA and 2, 6-dichloroisonicotinic acid was observed by Durner and Klessig, (1995). They observed that SA caused decrease in ascorbate peroxidase activity. Our findings with Vetiver cultivar also indicate a similar trend to that of ascorbate peroxidase.

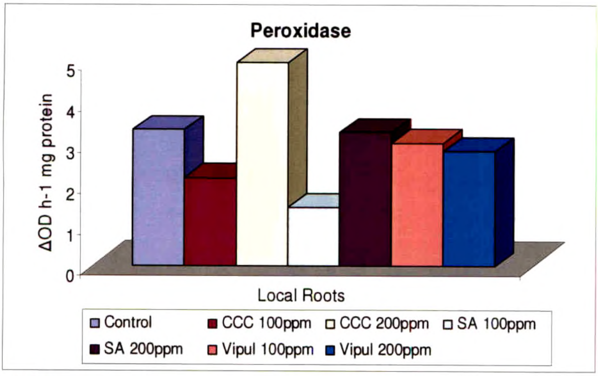
Effect of triacontanol on peroxidase activity in treated plants was investigated by Lesniak and Ries, (1983). They found that peroxidase activity in treated plants was relatively constant on a per plant basis and there was slight decrease in peroxidase activity on a per mg protein basis due to triacontanol treatment. In the present investigation peroxidase activity is analyzed on a protein basis and in both the Vetiver cultivar there is decrease in peroxidase activity due to triacontanol treatment.

c. Acid Phosphatase

Effect of three plant regulators like CCC, SA and Vipul on the activity of acid phosphatase enzyme in root of KS1 and local variety of Vetiver is shown in Fig. no. 8. It is evident from the figure that application of Vipul (triacontanol) has caused increase in acid phosphatase in roots of both cultivars. Application of CCC is also effective in causing increase in enzyme activity in case of cultivar KS1 treated with 200ppm CCC. Salicylic acid treatment has lowered the enzyme

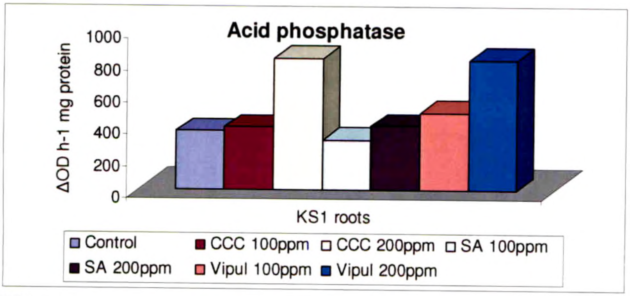


KS1

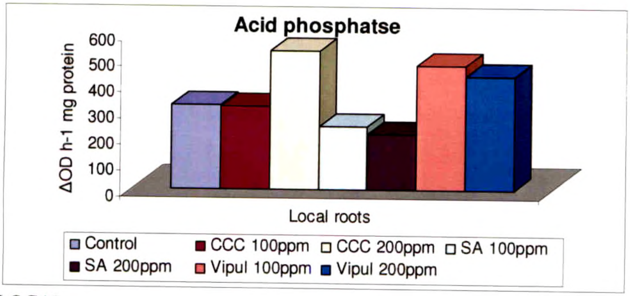


LOCAL

Fig. No.7. Effect of PGRs (CCC, Salicylic acid and vipul) on enzyme peroxidase in roots of KS1 and local Vetiver cultivars



KS1



LOCAL

Fig. No.8. Effect of PGRs (CCC, Salicylic acid and vipul) on enzyme acid phosphatase in roots of KS1 and local Vetiver cultivars activity in local cultivar. Similar trend is noticed in cultivar KS1 treated with 100ppm SA.

Phosphorus assimilation, storage and metabolism are of immense importance in plant growth and development as the element plays a key role in all processes of life. The ubiquitous class of enzyme- phosphatases which hydrolyzes organic phosphate is very important in utilization of phosphorus (Vincent *et al.*, 1992). Plant acid phosphatases (ACP) posses great variability in respect to their native molecular mass, subunit structure and pH. Majority of the ACPs are monomeric or dimeric glycoprotein having subunit molecular masses of approximately 50-60kDa. Phosphatase catalyses hydrolysis of organic phosphates above or below pH 7.0 (Barret-Lennard *et al.*, 1982).

Extra cellular ACP appear everywhere in roots and plant cell cultures they found to be localized within the cell wall or secreted by the root or suspension cells into the surrounding root zone (culture) media (Miernyk , 1992). Hydrolysis and mobilization of Pi from organic phosphates from soil is catalyzed by the extra cellular ACP of roots, which is located in apical meristem and outer surface cells (Lee, 1988 and Lefebore *et al.*, 1990) which is important step in plant reaction and the root surface of many plants is found to be acidic, so the activity of acid phosphatase is well suited for this region (Duff *et al.*, 1994). Intracellular ACP is ubiquitous in nature which is found in different plant parts including roots (Panara *et al.*, 1990) and storage tubers (Sugiura *et al.*, 1981) etc. Three isoforms of ACP was noticed in yam tubers, in which two were membrane bound while one was localized in cytoplasm (Kamenan and Diopoh, 1983). There is strong evidence that all plant vacuoles posses the ACP activity, which is supported by histochemical and subcellular fractionation studies (Nishimura and Beevers, 1978, Duff *et al.*, 1991). The intracellular phosphatases which are localized in cytosol, plastid and vacuoles are responsible for the release of Pi from organic compounds and thus help on Pi mobilization and translocation. Duff *et al.*, 1994 demonstrated that some special types of intracellular plant ACP have clear but non absolute substrate specificity such as phytases, phosphoglycolate phoshatase, 3-PGA phosphatase, phosphoenol pyruvate and phosphoprotein phoshatase.

High activity and specificity for amino acids and/ or phosphopeptides from diverse sources such as beet leaves (Polya and Hunziker, 1987) and potato tubers (Gellate *et al.*, 1993) studied ~~that of plant ACP~~. Plants are able to remobilize Pi from less active site such as old leaves and vacuoles by increasing ACP activity (Scachtman *et al.*, 1998). A positive relationship was noticed between leaf ACP activity and phosphorus concentration in some plants, therefore it is suggested that leaf ACP activity could be used as a diagnostic criterion for phosphorus deficiency (Besford, 1980). However such studies are not carried out in case of roots. But it obvious that lowering of the enzyme activity can pose limitation of Pi availability.

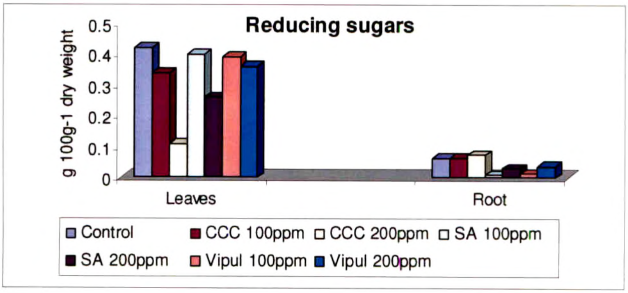
There are very few reports which explain the effect of PGR on acid phosphatase activity. Deleo and Sacher, (1970) reported that the ABA caused increase in activity in acid phosphatase which accompanied fruit ripening of banana. Chidrawar, (1996) noticed the increase activity of acid phosphatase enzyme in response to pretreatment to SA, ABA, CCC and ethephon in soybean.

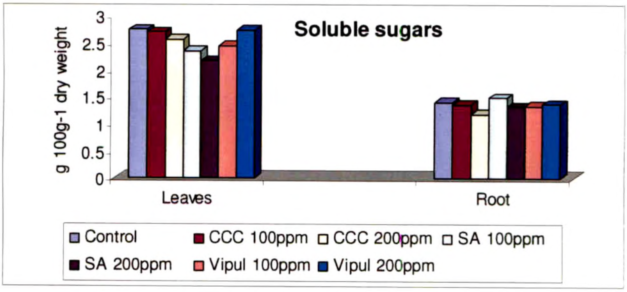
Our observations indicate that application of triacontanol (Vipul) and higher dose of CCC (200ppm) bring about considerable increase in acid phosphatase activity in roots of both cultivars. Such an increase leads to greater availability of Pi for various metabolic processes in the root. Further if the excess enzyme is released from the roots in the rhizosphere it can cause improvement in P uptake process through breakdown of phosphates in the soil. At the same time it is evident that SA application has a slight negative influence on the enzyme activity.

Carbohydrates

(Reducing sugars, Soluble sugars and Starch)

Effect of three plant growth regulators-CCC, SA and Vipul on carbohydrate contents from leaves and roots of Vetiver grass of variety- KS1 and local, which recorded in Fig. no. 9 and 10. From the figure it is clear that the leaves of control





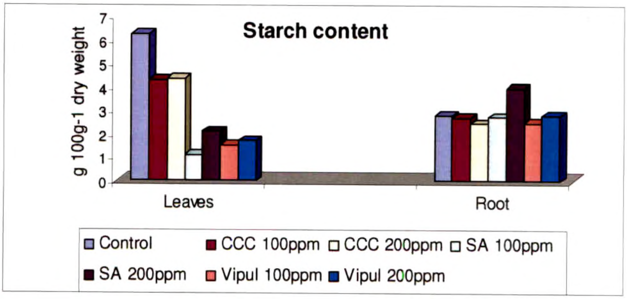
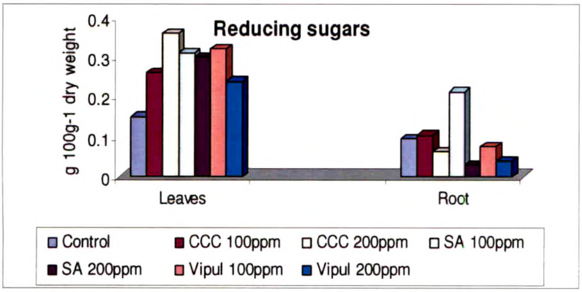
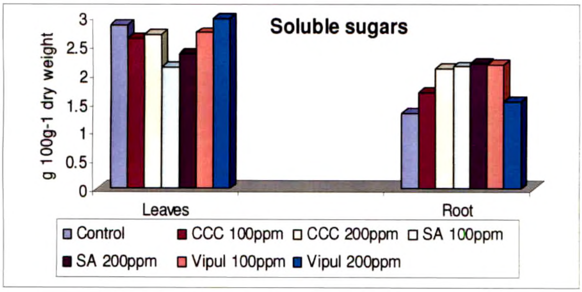


Fig. No.9. Effect of PGRs (CCC, Salicylic acid and Vipul) on carbohydrate content in leaves and roots of KS 1 Vetiver cultivar





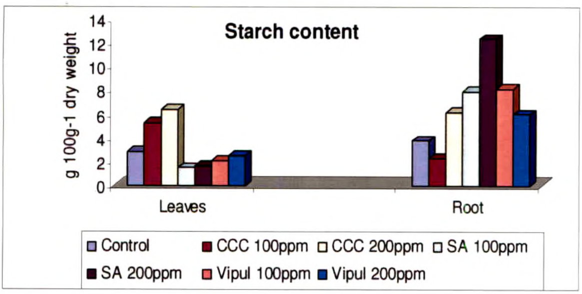


Fig. No.10. Effect of PGRs (CCC, Salicylic acid and Vipul) carbohydrate content in leaves and roots of Local Vetiver cultivar plants of improved cultivar KS1 have higher amount of starch and reducing sugars than that of the local cultivar. On the other hand, the contents of reducing sugar and starch are higher in case of roots of local cultivar. Reducing sugar as well as starch in leaves of KS1 variety is reduced by all PGR treatments. While in case of local variety apposite trend is seen regarding the effect of different PGR treatments on reducing sugar level. The level of starch in leaves is elevated due to CCC treatment. But the other two PGRs (SA and Vipul) have caused decline in leaf starch content. There is general reduction in leaf soluble sugars due to PGR treatment in both cultevars.The reducing suger content in the roots of cultivar KS1 is decreased due to treatment of SA and Vipul. Both concentrations of Vipul and higher doses of CCC and SA have caused similar effect in case of local cultivar also. Except 200ppm SA, all other treatments have caused a slight reduction in root starch content in case of cultivar KS1. On the other hand treatment of Vipul and SA and a higher (200ppm) of CCC has resulted in increase in starch content in the root tissue. The level of soluble sugars in the roots of local cultivar is also increased by treatment of all the PGRs.

Photosynthetic carbon metabolism is perhaps the very fundamental metabolic pathway which is responsible for biosynthesis of all organic compounds present on this planet. Among these organic compounds carbohydrates are the most important compounds, which are regarded as primary metabolites. These are the major products of photosynthetic carbon assimilation. They also serve as the major substrates for the respiration; therefore they occupy a very important place in primary metabolism. Polysaccharides like cellulose and pectin are the main constituents of cell wall in cells of higher plants. Carbohydrates provide a skeleton of carbon for wide range of carbon compounds present in the plant tissue, including secondary metabolites of which many posses medicinal value. Starch is found in seeds and various tubers therefore they are referred as storage carbohydrates ex. Hemicellulose amyloids with some raffinose series oligosaccharides are main carbohydrate reserve in potato (Bewley and Black, 1994).

The major form of carbohydrates which is transported within the different organs of plant through phloem tissue is sucrose. Sucrose is one of the oligosaccharide, non-reducing sugar. It is major product of photosynthetic carbon assimilation in higher plants and energy for growth of plant tissues (George, 1993). Sucrose is not directly used in metabolic processes, it is cleaved into hexoses by enzyme invertase (β- fructocidase E.C. -3.2.1.26 or sucrose synthase UDP glucose: D- fructose 2-α-D glucosyl transeferase E.C.- 2.4.1.13) before entering into carbohydrate metabolism. The sucrose is broken into two sugars-fructose and glucose which are two major reducing sugars in plants. They served as substrate for respiration and as substrate for starch synthesis in storage organs such as in seeds and tubers. Sugars serve as osmolytes and play a role in osmoregulation. The ribose is pentose sugar which is constituent of nucleic acid while tetroses like erythrose serves as precursor for biosynthesis of aromatic compounds. It is now very well established that starch synthesis occurs in plastids while sucrose biosynthesis takes place in cytosol. Therefore these all carbohydrates play a very important role in plant cell metabolism. Several experiments have demonstrated that the level of different carbohydrate fractions in plant tissue is greatly influenced by both endogenous and environmental factors.

Effect of CCC with other two PGRs GA3 and B9 on carbohydrate status of *Primula obonica* was studied by Abou-zied and Bakry, (1978). The carbohydrate content in stalk of inflorescence was increased by the action of CCC. The starch content in potato tuber was found to be increased by 11% due to treatment of CCC (Sharma *et al.*, 1998). Shah and Prathapasenan , (1991) studied the effect of CCC on the changes in the level of starch in mung bean (*Vigna radiate* (L.)). They observed increase in contents of starch in source leaf and pods of plants treated with 1000ppm. The radiotracer studies of Wang *et al.*, (2009) revealed that soluble sugar and starch accumulated in roots and stolon rather than leaves and stem in

CCC treated potato. Wang *et al.,* (2009) further reported that due to treatment of CCC, the starch and sucrose content in potato tubers were increased. There is an intervarietal difference in Vetiver with respect to influence of CCC in carbohydrate metabolism. Thus accumulation of different carbohydrate fractions in both leaves and roots is lowered by CCC in cultivar KS1. On the other hand in leaves of local cultivar accumulation of reducing sugars starch is followed by CCC treatment. Similar situation also prevails in roots with respect to soluble sugar and starch content in 200 ppm CCC foliar treatment.

The effect of SA on cucumber cotyledons was investigated by Singh and Singh, (2008). They observed that due to application of low concentration of SA (50μM) there was increase in total structural carbohydrates while higher concentration of SA caused inhibitory effect on the same parameter. SA application favored starch accumulation in root tissue of both Vetiver cultivars however in the leaf tissue an apposite trend is clearly noticeable. The leaf soluble sugar content is also affected by SA treatment. Thus the activities of enzymes of carbohydrate metabolism are probably greatly altered due to SA treatment in both leaf and root tissue.

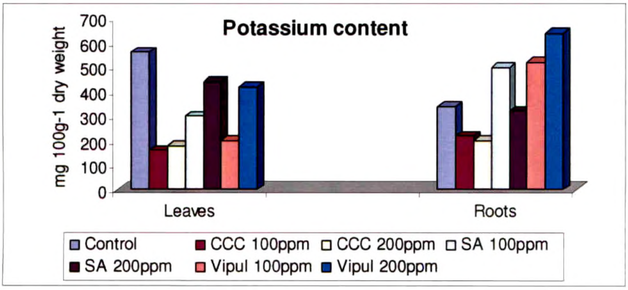
The effect of triacontanol on the rice seedlings (*Oryza sativa*) was studied by Bittenbender *et al.,* (1978). They found increase in contents of soluble carbohydrates. Sharma and Joolka, (1997) studied effect of different plant growth regulators on carbohydrate and other contents of apple cv. Red Delicious. They found that as compared to biozyme, protozyme, miraculan, paras photosynth were more effective with respect to elevation in contents of total sugars, starch and other carbohydrates than Vipul, but all these bioregulators showed increase in all contents including carbohydrates as compared to control. Chowdhary *et al.,* (2009) studied the triacontanol induced changes in water chestnut (*Trapa bispinosa* L.) fruit. They observed decrease in soluble carbohydrates level in fruit due to TRIA application. Except roots of local variety, the starch content is found reduced due to Vipul treatment in the two plant parts of both the cultivars. Similar trend is also shown by soluble sugar content. Thus the carbohydrate biosynthesis and accumulation in roots and leaves of improved Vetiver cultivar as well as leaves of local Vetiver cultivar is not promoted by foliar treatment of triacontanol.

Inorganic constituents

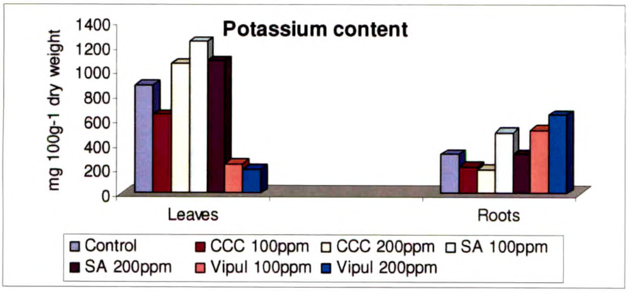
a. Potassium

Effect of foliar spray of plant growth regulators (CCC, SA and Vipul) on potassium content in leaves and roots of both the Vetiver varieties- KS1 and local is recorded in the Fig. no. 11. Application of all the PGRs has lowered the potassium content in leaf tissue of local cultivar. Except SA treatment and treatment with 200ppm CCC similar trend is seen in cultivar KS1 also. Vipul treatment has caused increase in K content in the roots of both the cultivars. Both concentrations of CCC and higher concentration of SA have caused decrease in K content in the roots cultivar KS1 and local respectively. The root potassium content is increased by 100ppm SA and 200ppm SA in cultivar KS1 and local respectively while at the other concentrations an apposite trend is noticeable.

Among different elements found in plants major element potassium occupies a unique place. It is the most essential mineral element for plant life. It is an univalent cation with 0.331nm hydrated ionic radius and 314 J mol^{-1} hydrated energy. Lindhauer (1989) explained the detailed role of K^+ in cell extension, growth and storage. Kochian (2000) studied that K^+ plays a role in cellular and whole plant functions like serving as an osmoticum for stomatal growth and stomatal function, balancing the charges of diffusible and nondiffusible anions, activating more than 50 plant enzymes, oxidative metabolism and protein synthesis with numerous metabolic processes. Another very important function of K^+ is translocation of photosynthates to the tuberous roots. The preferential uptake of potassium is also one of the features contributing to mechanism of salt tolerance in halophytes. The deficiency of potassium causes distinct reduction in root growth which leads to doubling of shoot root ratio (Sircar and Datta, 1959). The



KS1



Local

Fig. No.11. Effect of PGRs (CCC, Salicylic acid and Vipul) on potassium content in leaves and roots of Vetiver cultivars(KS1 and Local) deficiency of potassium is reported to cause rotting of roots in rice (Tanaka and Yoshida, 1970). Epstein (1972) and Munson (1998) consider the concentration of Grasses is especially rich in potassium. But it is evident from observation that potassium content in both cultivars of Vetiver grass is rather low and below the optimal level recorded by Epstein. However no K^+ deficiency symptoms were evident in both the cultivars.

The application of 2,4 Dichlorophenoxyacetic acid (2,4D) caused increase in potassium content in top part while decrease in K^+ in root part in tobacco plant, along with variable changes in another mineral elements (Wildon *et al.,* 1957). The treatment with ABA caused decrease in K^+ content while same effect was seen in response to BA and ABA along with BA, in duckweed '*Lemna gibba* L. (Dekock *et al.,* 1978). Monge *et al.,* (1994), observed an increase in content of K^+ in response to GA3 treatment while decreased K^+ content due to paclobutrazol treatment in adult peach tree. Kinetin and auxin were found to cause increase in the content of potassium in wheat grain (Weirzbowska and Bowszys, 2008).

The effect of CCC on mineral constituents was studied by Hamza and Helaly (1982) in some ornamental plants. It is noticed that CCC caused increase in K^+ content. On the other hand effect of CCC on K^+ content observed in lower yellowing leaves (Tuma *et al.,* 2007). In case of Vetiver grass, CCC treatment has caused reduction in K^+ contents of leaves and roots of local cultivar and cultivar KS1 respectively. But there is marked increase in K^+ level in the leaves of cultivar KS1 due to 200ppm CCC treatment.

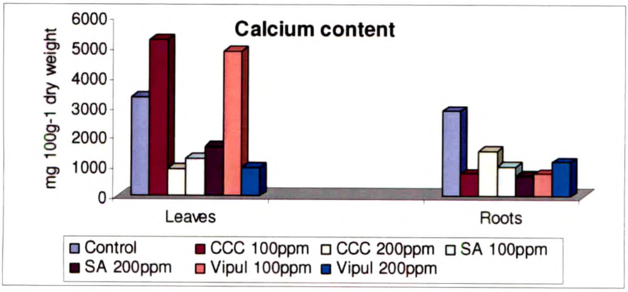
The treatment of brassinolide and SA as seed soaking and as foliar spray on NaCl stressed maize plants resulted in increase in K^+ quantity while Na/K and Na/Ca ratio decreased (Samia *et al.,* 2009). In Vetiver grass SA treatment is quite effective in promoting K^+ uptake in cultivar KS1.

Effect of triacontanol along with potassium on tomato (*Solanum tuberosum* L.) was investigated by Khan *et al.,* (2009). They found that the K^+ content in leaves were increased significantly at different concentrations of TRIA + K^+. In case of Vetiver grass, Vipul treatment alone has caused increase in K^+ contents in the roots. On the other hand in the leaf tissue there is a marked decrease in K^+ status from root to shoot is probably affected by Vipul treatment. The accumulation of potassium in the root tissue can definitely prove beneficial for promotion of root growth and metabolism in view of key role of this mineral element.

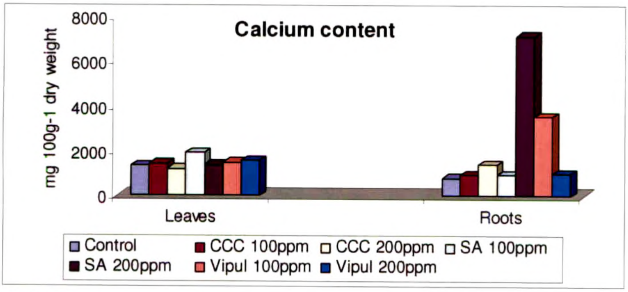
b. Calcium

The calcium content in the leaf and root tissue of the leaf and root tissue of the two Vetiver cultivars subjected to foliar treatments of the three PGRs are depicted in Fig.no. 12. It is clear from figure that all treatments of all the PGRs have caused accumulation of calcium in root tissue of local cultivar while an apposite trend is cleanly evident in case of improved cultivar. Application of SA, Vipul and 100ppm CCC has also caused elevation of calcium level in the leaf tissue in local cultivar. Except 100ppm Vipul treatment and 100ppm CCC treatment, all other treatments of PGRs have caused a decline in cultivar KS1.

Calcium is one of the most important, major inorganic elements in the plants which plays both structural and metabolic role in the plant cells. It is a divalent cation with hydrated ionic radius of 0.412nm and a hydration energy of 1577 J mol^{-1} (Marschner, 1986). Calcium occurs in plant cells in Ca^{++} free form and absorbed to indiffusible ions such as carboxylic, phosphorylic and phenolic hydroxyl groups. It is also present in the form of salt such as Ca-oxalate, carbonates and phosphates (Mengal and Kirkby, 1982). According to Burstrom (1968) Ca^{++} element is essential in cell elongation and cell division. Ca^{++} plays important role in membrane stability with respect to uptake of ion and other metabolic processes (Mengal and Kirkby, 1982). The activity of enzymes like α-amylase, phospholipase and ATPase increases due to Ca^{++} (Wyn jones and Lunt, 1967). Rensing and Cornelius (1980) suggested that Ca^{++} can stimulate membrane bound enzymes, mostly the ATPase at the plasma membrane of roots of certain



KS1



LOCAL

Fig. No.12. Effect of PGRs (CCC, Salicylic acid and Vipul) on calcium content in leaves and roots of Vetiver cultivars(KS1 and Local) plant species (Kuiper *et al.,* 1974) .A special type of calcium binding protein calmodulin is present in plants as well as animals and this protein plays a key role in signal transduction process. In view of Clarkson and Hanson (1980), Ca^{++} influences organization, enzyme confirmation and regulation of many metabolic processes through calmodulin. Cytosolic Ca^{++} play very important role as secondary messenger in plant signal transduction through calmodulin and Ca dependant protein kinase. From such messengers, signals like touch, wind, temperature shock, fungal eliciters, wounding, oxidative stress, anaerobiosis, ABA, osmotic stress and mineral nutrition get transduced. The action of major photo morphogenetic pigment, phytochrome is also found to be mediated through calcium.

Marschner,(1986)suggested the normal range of Ca^{++} found in plants which varies from 1.0-5.0 % of dry weight which depends on the growth condition, plant species and plant organ. Calcium mostly accumulates in old senescent leaves due to its relatively immobile nature (Tsujita *et al.,* 1978 and Hocking *et al.,* 1980). The deficiency of calcium causes weakening of plants and many structural disorders especially in the fruit tissue. It also causes leaf burn. Our observations indicates that calcium uptake potential in improved cultivar KS1 is considerably more than that in local cultivar since in both leaf and roots the calcium level is quite in higher the improved cultivar.

The effect of 2,4D on the different mineral elements in tobacco plant was studied by Wildon *et al.,* (1957). They found that the calcium content from top part and root part get decreased due to treatment of 2,4D. Calcium content was found to be increased in response to ABA, BA and ABA+BA in the duckweed (*Lemna gibba* L.) (Decock *et al.,* 1978). Smith (1978) observed different PGRs like 2,4D; 2,6D; GA etc did not affect the Ca^{++} concentration significantly in tobacco cells cultured in liquid medium.GA_3 caused significant decrease in Ca^{++} content in adult peach trees (Monge *et al.,*1994). On the other hand GA3 and auxin caused increase in calcium content in spring wheat (Weirzbowska and

Bowszys, 2008). Thus it is clear that plant species differ in their response to PGR with respect to calcium nutrition. Our findings indicate that there are intervarietal differences with the plant species also in this respect.

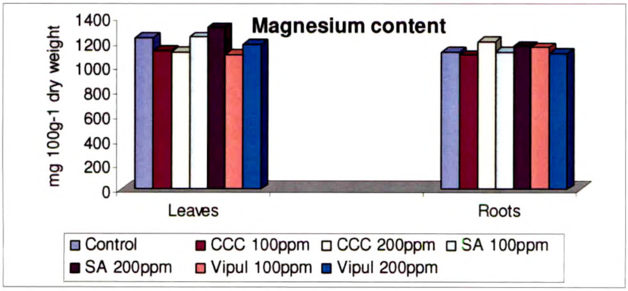
An increase in content of calcium was found in tomato in response to CCC treatment (Castro *et al.*, 1983). CCC also caused increase in calcium content in all part *Avena sativa* (Tuma *et al.*, 2007). CCC caused increase in calcium content in potato leaves (Wang *et al.*, 2010). In case of Vetiver grass such trend is noticed in the plant treated with 100ppm CCC.

Calcium content recorded an increase in salt stressed maize plant in response to BR and SA (Samia *et al.*, 2009). Salicylic acid treatment is also effective in increasing calcium uptake in local cultivar of Vetiver grass. Similar is the one of Vipul (tracontanol) treatment such increase in calcium content may prove advantageous for the plant especially under stress conditions.

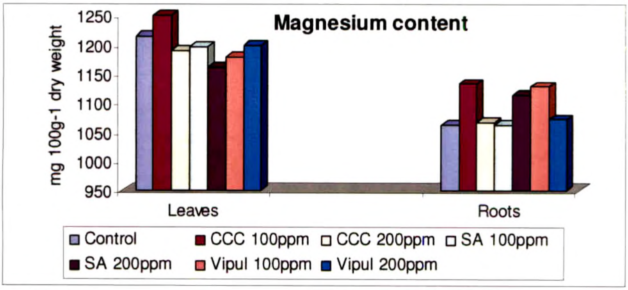
c. Magnesium

The effect of three plant growth regulators like CCC, SA and Vipul of lower and higher concentrations (100ppm and 200ppm) on Vetiver grass of variety KS1 and local is shown in following Fig. no. 13.

Magnesium is the abundant divalent element in soil which is strongly electropositive. It has hydrated ionic radius of 0.428nm with high hydration energy of 1908 J mol^{-1} (Marschner, 1986). Since it is a constituent of chlorophyll, it is considered as very essential element. The internal concentration of Mg in stroma regulates the activities of photosynthetic enzymes such as RUBISCO, fructose 1-6 bisphosphatase and phosphoribulokinase. The function of magnesium depends on its mobility within the cell (Marschner, 1986). This cation can interact with strongly nucleiophilic ligands (ex. Phosphoric groups) through ionic bindings, due to which it play role as bridging element and /or forms different complexes of having stabilities. The enzymes from subgroups kinase require to form ADP-Mg complex before starting reactions, therefore such enzyme's Mg



KS1



LOCAL

Fig. No.13. Effect of PGRs (CCC, Salicylic acid and Vipul) on magnesium content in leaves and roots of Vetiver cultivars(KS1 and Local) requirement is absolute, which is in range ~0.5% of dry weight of the vegetative parts (Mengel and Kirkby, 1982 and Marschner, 1986). Deficiency of magnesium causes decrease in root- shoot dry weight ratio, which is often observed (Marschner, 1995). Chlorosis is general symptom of magnesium deficiency because it is constituent of chlorophyll element. The normal level of magnesium in plant tissue is estimated to be 0.25-1% on dry weight basis (Munson, 1998). The concentration of magnesium in plant tissue is low as compared to calcium and potassium. Our observations indicate that the magnesium level in roots and leaves of both Vetiver cultivars is quite optimum and there is not much difference in the magnesium status among the plants of two cultivars.

Both active and passive mechanisms are involved in magnesium uptake of plants (Russel and Clarkson, 1976 and Pitman, 1976). In rice, magnesium absorption starts with plant seedling stage but rate is low until the formation of flower primordia, but afterwards rate increased upto the heading time with almost 90% total absorption (Ishizuka, 1964).

The application of 2,4 D on tobacco plant caused increase in magnesium content (Wildon *et al.,* 1957). In duckweed Decock *et al.,* (1978) observed increased magnesium content due to ABA treatment while magnesium level decreased due to BA application. Combined application of both PGRs- BA and ABA caused increase in magnesium content. PBZ treated peach were found to have higher magnesium content than control plants (Monge *et al,* 1994). In spring wheat the magnesium content was increased in response to GA while kin and auxin caused decrease of the magnesium (Weirzbouska and Bowszys, 2008).

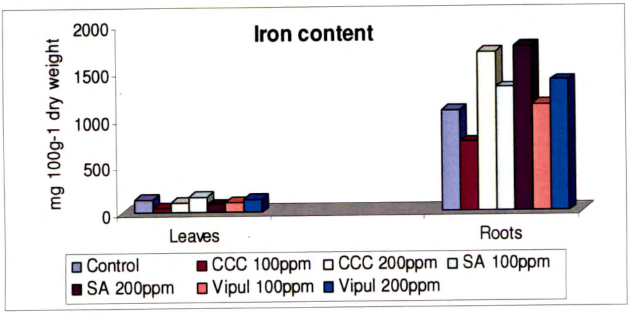
Castro *et al.,* (1983) noticed an increase in magnesium content in response to CCC. Similar result obtained in *Avena sativa* (Tuma *et al.,* 2007). The increased quantity of magnesium in response to SA and BR application was demonstrated in maize plants which were subjected to salt stress (Samia, 2009).

It is evident from the present investigation that magnesium nutrition is not significantly altered due to foliar treatments of all the three PGRs in general.

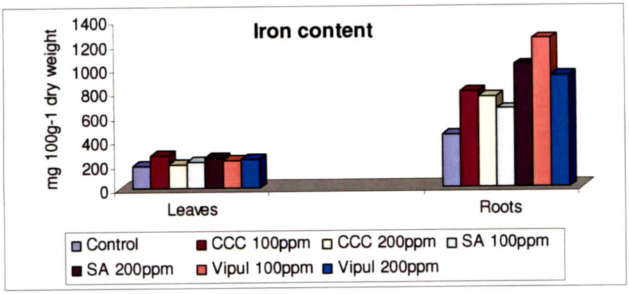
d. Iron

The effect of plant growth regulators (CCC, SA and Vipul) of both concentrations (100 and 200ppm) on iron contents of roots and leaves of two Vetiver varieties-local and KS1 is shown in following Fig. no. 14. It is evident from the figure that the foliar treatments of Vipul and SA have caused increase in root iron level in both the cultivars. Both doses of CCC are effective in bringing about similar effect in local cultivar while higher concentration of CCC is effective in case of cultivar KS1. All the three PGRs have caused increase in leaf iron status in local cultivar. On the other hand except 100ppm SA all other PGR treatments have caused lowering of leaf iron in cultivar KS1.

Iron is very important micronutrient, which play important role in various metabolic activities in the cell. It is an indispensable constituent of heme proteins like cytochrome and iron sulphur proteins which play major role in electron transport during respiration and photosynthesis. Iron is essential constituent of a sulphur protein- ferredoxin which is a member of electron transport in chloroplasts. Another important function of iron is formation of coordination complexes (chelates) and its reaction ex. Reversible oxidation–reduction reaction system (Fe (II) = Fe (III) + e^-). In case of plants, ferrous ion [redacted] (Fe(II)) is the ‘physiologically available’ form of iron which undergoes reversible Fe(III)/Fe(II) oxidoreduction (Marschner, 1986). Around 80% Fe is located in chloroplast in case of leaf (Marschner, 1995). This element can bring about activation of various types of enzymes such as catalase, peroxidase, cytochrome oxidase etc. Iron is required for the action of enzyme aconitase in mitochondria and for the action of enzyme superoxide dismutase (Fe-SOD) in plastids. Iron is an important constituent of nitrogenase enzyme system which is fundamental for biological nitrogen fixation. Campbell (1999) stated that each nitrate reductase enzyme contains two metal- irons and manganese in each subunit. Iron chlorosis is a typical deficiency disorder in case of iron deficiency in many crop plants and due to this disorder a great loss in crop productivity has occurred.



KS1



LOCAL

Fig. No.14. Effect of PGRs (CCC, Salicylic acid and Vipul) on iron content in leaves and roots of Vetiver cultivars(KS1 and Local)

An increase in iron content in 2, 4-D treated tobacco plants was observed by Wildon *et al.,* (1957). Iron content was increased in response to ABA and ABA+BA while it was decreased in response to BA in *Lemna gibba* L. (duckweed) (Decock *et al.,* 1978). Different types of bioregulators including GA caused negligible effect on Fe content in leaves and that of fruit (Belakbir, 1996). At the same time the Fe content registered increase in response to GA_3 in barley under saline condition (Akman, 2009). Nenova and Stoyanov, (2000) noticed increase in Fe content in hydroponically growing maize plant in response to treatment of CCC, IAA and BA and he also found that the iron level was found to be elevated in young iron deficient maize plants due to application of CCC, IBA or BA. CCC also caused increase in Fe content in potato (*Solanum tuberosum* L.) (Wang *et al.,* 2010). In case of local Vetiver cultivar same trend is clearly evident.

The iron accumulation in the root tissue of both Vetiver cultivars is stimulated by application of all the three PGRs and in this respect Vipul is most effective. The increase in iron level in the root tissue can favorably influence on the overall metabolism in view of its key role in various biochemical pathways. Similar situation can also prevail in leaf exerted negative influence on leaf iron status.

Essential oils

Effect of foliar application of the three PGRs on the composition of essential oils in two Vetiver cultivars is recorded in Table no. 2 and 3. The preliminary TLC analysis reveals that there are 9 constituents in essential oil of control roots of lacal Vetiver variety while there are 8 different compounds in essential oil in the roots of cultivar KS1. Some constituents such as benzoic acid, cinnamic acid, bisabolol oxide, propyl cinnamate, azulene and fernesene are present in both the cultivars. Application of CCC and higher dose of Vipul have caused increase in the level of many of these constituents in case of local cultivar.

In case of improved cultivar KS1 such trend is noticed in case of Vipul treatment and treatment with 200ppm SA.

An essential oil is concentrated liquid containing volatile aroma compounds from plants. They are also known as volatile, ethereal oils or aetherolae or simply 'oil of' plant from which they were extracted ex. Oil of clove, khus oil or Vetiver oil. The essential oil producing species are almost equally present in both dicots and monocots, ranging from Gramineae (aroma grasses) to Rosaceae (roses). Litchenthaler (1999) suggests that the biosynthetic pathway of essential oil terpenoid having prokaryotic ancestry with basis of biogenetic pathway of mono and sesquiterpenoid essential oils. Recently from many references, it is discovered that eubacteria type of biosynthetic pathway of essential oil is operated (isopentenyl pyrophosphate) in oil plants like mints, geranium, basil, thyme and chamomile etc. rather the composition of essential oil is very complex. This oil is produced in varied type of plant parts ex. leaves, reproductive structures, stem and roots etc. The content of essential oil is mainly terpenoids but other chemicals like phenylpropanoids are also present in some oil plants. Our observations with Vetiver essential oil also indicate a similar trend. In essential oil chemical components broadly categorized into two groups- terpenoids and phenylpropanoids but the terpenoids are the very common and gives characteristic flavour and odour to the oils.

Somchai *et al.*, (2008) carried out pharmacognostic specification studies of *C. zizanioides* (L.) Roberty roots cultivated in Thialand. They carried out TLC and GC fingerprint of volatile oil of *C. zizanioides* (L.) Roberty roots. They compared the developed spots on TLC plate under short wave in day light, long wave ultra violet light and also sprayed the plate with specified detecting reagent. In TLC fingerprint they showed Rf values of different spots as hRf. They also stated that Cameroonan 7-α-ol and silphiperfol-6-ene were major constituents of *C. zizanioides* (L.) Roberty volatile oils.

Plate No. 3a. Effect of PGRs (CCC, Salicylic acid and Vipul) on the essential oil composition of Vetiver cultivar KS1 as revealed by TLC

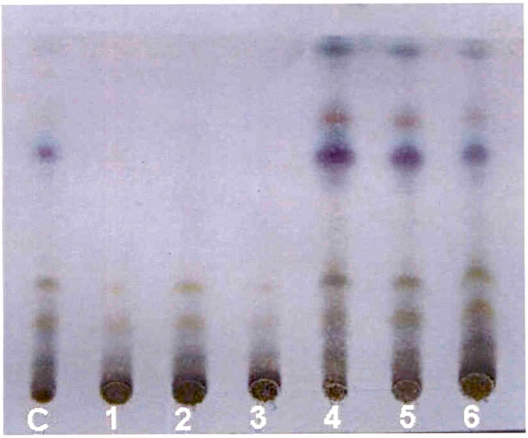
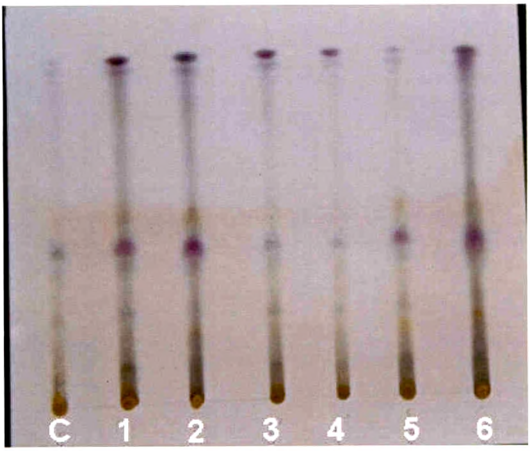


Plate No. 3b. Effect of PGRs (CCC, Salicylic acid and Vipul) on the essential oil composition of Local Vetiver cultivar as revealed by TLC



C- Control, 1- CCC 100 ppm, 2- CCC 200 ppm, 3- SA 100 ppm, 4- SA 200 ppm, 5- Vipul 100 ppm, 6- Vipul 200 ppm.

The biosynthesis of terpenoid is widely investigated by Chappel (1995). Terpenoids are synthesized from five carbon units of isopentenyl pyrophosphate (IPP) and its isomer dimethyl allyl pyrophosphate (DMAPP). Sangwan *et al.*, (2001) have given an outline of biosynthetic pathway of terpenoids which is presented in Fig.no.15 .

Based on radiotracer studies it has been speculated that biosynthesis and utilization of photosynthates controls oil production. Sucrose and CO_2 are preferred as exogenous precursor for essential oil synthesis. Mallavarapu *et al.*, (1999) studied the influence of plant growth stages on the essential oil content and composition in Davana (*Artemisia pallens* Wall). They observed the highest essential oil content at the full emergence of flower heads. Various attempts were made to study chemical components from different essential oils of different plants. Ex. Gurudatt *et al.*, (2010) studied changes in chemical composition of essential oil during annual growth that of *Origanum vulgare* L. from Kumaon, Himalaya. They also observed the highest oil content at full bloom stage (1.3%). Such studies help in fixing the essential oil harvest season of the plant.

The effect of different plant growth regulators on the essential oil contents of different plants was studied by different workers. Koseva – kovacheva and Staev, (1978) observed increased essential oil content of *Mentha piperita* in response to sodium salt of NAA and IAA. Foliar application of cytokinin, kinetin and diphenyl urea (1-10ppm) caused increase in essential oil yield of *M. piperita* and *M. spicata*. Foliar application of triacontanol and mixtalol caused increase in essential oil content in rose scented geranium (Bhattachrya and Rajeswara Rao, 1996). Farooqi and Sharma, (1998) observed increase in essential oil in Japanies mint in response to CCC but ethephon did not show any changes in oil content. The PGRs like AMO1618 and DCPA caused increase in essential oil content in sage (*Salvia officinalis*) (El-Keltawi and Croteaua, 1986). Safari *et al.*, (2004) observed the decreased oil content in *Rosa damascene* Mill in response to Alar, kinetin, NAA and cycocel; while exactly apposite results was observed in *Thymus* *vulgaris* in response to GA, IBA and BA (Reda *et al.,* 2007). Farooqi *et al.,* (2010) revealed that drought stress reduced essential oil content in Vetiver grass and CCC and etheral caused ameliorative effect on oil content of drought stressed Vetiver plant. CCC, etheral and PBZ also caused increased content of essential oil in unstressed Vetiver plant. Further they also recorded increase in khusiol content due to application of CCC.

It is evident from the preliminary qualitative analysis of Vetiver oil constituents with TLC performed in the present investigation that foliar application of PGRs are effective in bringing about qualitative changes in the composition of essential oil in both the cultivars. In case of local cultivar CCC is highly effective in this respect while in case of improved cultivar application of Vipul is highly effective. At the same time it must be admitted have then only a detailed analysis of essential composition with advanced analysis technology will throw more light on this aspect. Further understanding of exact mechanism as significance of such changes needs a very thorough investigation at molecular level.

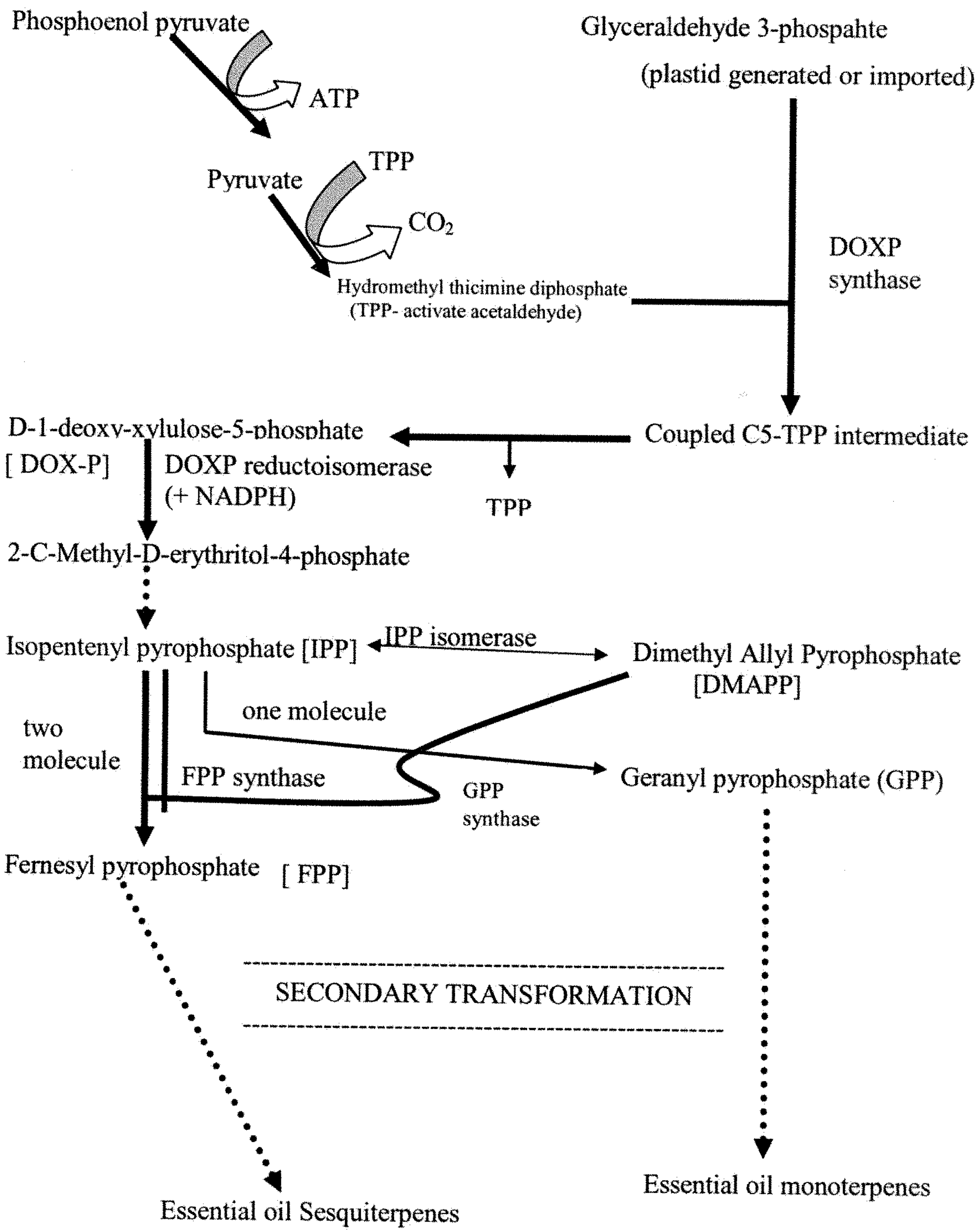


Fig. no. 15. Outline of terpenoid biosynthesis (After Sangwan *et al.* 2001).

Table no. 2. Influence of CCC, SA and Vipul on composition of essential oil in local Vetiver cultivar.

L	Rf Value	Probable identification	C	1	2	3	4	5	6
1	0.08	Benzoic acid Cinnamic acid	+	++++	+++	++	++	+	++++
2	0.13-0.15	U	+	+++	++	+	+	+	++++
3	0.19-0.21	Bisabololoxide	+	++++	++	+	+	+	+++
4	0.24-0.26	Propyl cinnamate	+	+++	++	+	+	+	+++
5	0.38	U	+	+++	+++	+	+	+	+++
6	0.45-0.48	Pulegone	+	++	+++	+	+	+	+++
7	0.51-0.53	Polyines	+	++	++	+	+	++	+++
8	0.85-0.88	Anethol Safrole	-	++	++	+	+	+	++
9	0.90-0.92	U	+	++	++	+	+	++	+++
10	0.94-0.95	Azulene	-	++	++	++	+	+	+++
11	0.98	THC, Fernesene	+	++++	++++	+++	+	++	++++

U = unidentified

- = not detected (visual analysis)

+ = present (visual analysis)

++ = present in greater quantities (visual analysis)

+++ = prominent (visual analysis)

++++ = very prominent (visual analysis)

C - control

1 - CCC 100ppm

2 - CCC 200ppm

3 - Salicylic acid 100ppm

4 - Salicylic acid 200ppm

5 - Vipul 100ppm

6 - Vipul 200ppm

Table no. 3. Influence of CCC, SA and Vipul on composition of essential oil in Vetiver cultivar KS1.

Sr. No.	Rf value	Probable identification	C	1	2	3	4	5	6
1	0.44-0.05	Benzoic acid Cinnamic acid	+	+	+	+	+++	++	++
2	0.09-0.1	Benzoic acid Cinnamic acid	+	+	+	+	+++	++	++
3	0.16	U	+	-	-	-	+++	+	+
4	0.19-0.2	Bisaboloxide Propyl cinnamate	+	+	+	+	++	+	+
5	0.29-0.31	Menthol Linalool	+	+	+	+	+++	++	++
6	0.34	Cineol	-	-	-	-	+++	++	++
7	0.65	Citronellal	++	-	-	-	++++	+++	+++
8	0.69	Menthone	+	-	-	-	++	++	++
9	0.75	Methyl acetate	+	-	-	-	++	++	++
10	0.78	Benzyl acetate	-	-	-	-	++	++	+
11	0.95	Azulene	-	-	-	-	++	+	++
12	0.98	Fernesene	-	-	-	-	++	+	+

U = unidentified

- = not detected (visual analysis)

+ = present (visual analysis)

++ = present in greater quantities (visual analysis)

+++ = prominent (visual analysis)

++++ = very prominent (visual analysis)

C - control

1 - CCC 100ppm

2 - CCC 200ppm

3 - Salicylic acid 100ppm

4 - Salicylic acid 200ppm

5 - Vipul 100ppm

6 - Vipul 200ppm