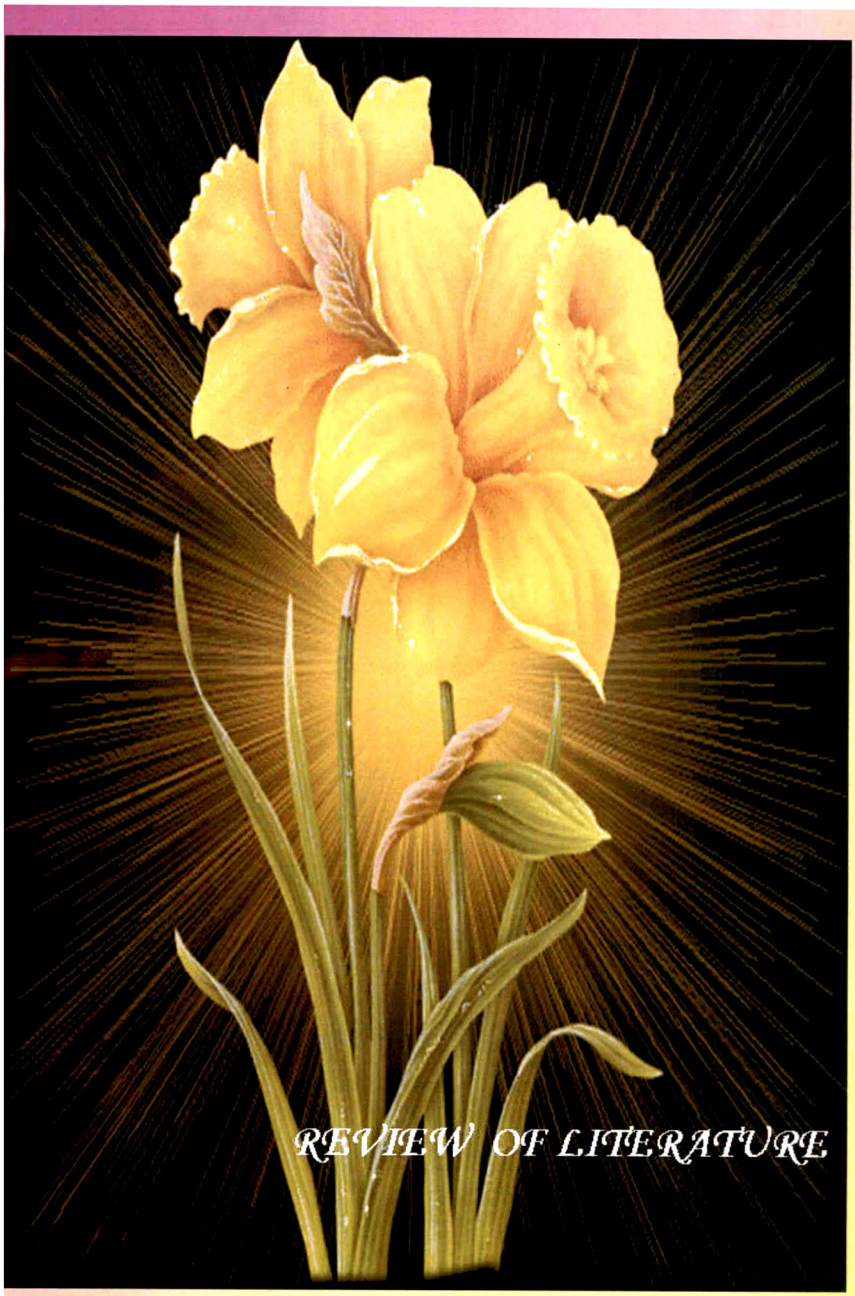
REVIEW OF LITERATURE

Chrysopogon zizanioides(L.) Roberty is a member of family Poaceae and all the information regarding this genus is described in following words.

Taxonomy

The systematic position of vetiver is as follows (Cronquist, 1981)-

Classifiacation

Division: Magnoliophyta Cronquist, Takhtajan and Zimmermann.

Class: Liliopsida Cronquist, Takhtajan and Zimmermann.

Order: Cyperales G. T. Burnett.

Family: Poaceae Barnhart

Sub-family: Panicoideae

Tribe: Andropogoneae

Genus: *Chrysopogon* Trin.

Species: *Chrysopogon zizanioides* (L.) Roberty.

Upto 1999, the Vetiver grass was known in the taxanomic World by the name *Vetiveria zizanioides* Nash. In 1999 Veldkamp merged genus *Vetiveria* under the genus *Chrysopogon* and the species was named as *Chrysopogon zizanioides*(L.) Roberty.There were different synonyms for this grass species. These are as follows-

Andropogon zizanioides Linn

Andropogon squarrosus Hack

Andropogon muricatus Retz

Andropogon nardus Blanco

Andropogon nigricatus Stapf

Andropogon festucoides Presl

Andropogon echinulatum Koenig

Anatherum zizanioides Linn

Anatherun muricatum Beauv

Agrostis verticillata Lam

Phalaris zizanioides Linn

Vetiver grass has various local names in different languages (Grimshaw, 1990)-

English	Vetiver grass, cus-cus, khus-khus, botha grass.
Marathi	Vala.
Hindi	Bala, Bena khus, Panni.
Punjabi	Panni.
Benali	Khas-Khas.
Telugu	Avurugaddiveru, Kuruveeru, Lamajjakamuveru, Vettiveeru, Vidavaliveru.
Tamil	Ilamichamver, Vettiver, Vilhalver, Viranam.
Malayalam	Ramaccham, Ramachehamver,Vettiveru.
Gujarati	Valo, Sugandhivaalo.
Kannada	Lavancha, Mudivala, Kuruvelu,Kaadu.
Sanskrit	Shit-sugandhi-mulak, Ushira, Vernum.
Orissa	Usheera, Bena khus.

Distribution

Vetiver is Asian tropical plant. According to Greenfield (1988), the distribution of *C. zizanioides* (L.) Roberty is as follows- India, South-East Asia (Thailand, Malaysia, Philippines, Indonesia), Pakistan, Polynesia (Samoa, New Caledonia, Fiji, Tonga), Nepal, Tropical Africa as well as South Africa, Burma, Sri- Lanka, Guyana, New Guinea, French Guyana, Argentina, West Indies (Haiti, Cuba, Jamaica, Puetto Rico, Antigua, St. Vincent, Martinique, Barbados (Trinidad), Colombia (Santa Maria), Brazil (Rio Janeiro, Para, Bahia), Paraguay (Central Paraguay), China (Fujian and Jiangru Provinces, Hainan, Island). Zimbabwe, Kenya, Somalia, Nigeria, United Kingdom are the countries where Vetiver is introduced from different sources.

In India, it is distributed in Assam, Bihar, Madhya Pradesh, Meghalaya, Orissa, Tamil Nadu, Uttar Pradesh, West Bengal (Maharashtra state Gazetteers, 1961). But practically Vetiver grass is cultivated all over India.

In Maharashtra, it is found in wild state at following regions- Bhandara, Mumbai, Chandrapur, Gondiya, Kolhapur, Nagpur, Nasik, Pune, Raigad, Ratnagiri, Satara, Sindhudurg and Yavatmal (Potdar, 2006).

Cultivation practices

From ancient time, Vetiver grass has been cultivated for its pleasant oil yielding roots even before the rose scent from petals was obtained. The Annual World Trade of Vetiver oil is around 250 tons (Lavania, 2003). But now a days Vetiver grass is also cultivated on large scale in different parts of the World for the phytoremediation purpose and to control the soil erosion. Especially in this second context, Vetiver System (VS) is developed for systematic cultivation of Vetiver in about 100 countries. Application Technical Reference Manual is published by Truong *et al.*, (http://www.vetiver.org/TVN-Manual_Vf.pdf). At the same time Central Institute of Aromatic and Medicinal Plants (CIMAP) has standardized cultivation method for raising Vetiver as an oil yielding crop and these are described in Handbook of Medicinal and Aromatic plants. These cultivation practices can be briefly described in following words.

Selection of variety

For oil extraction purpose, there are different improved varieties like KS-1, Gulabi, Dharini, Kesar etc have been developed by CIMAP. These improved varieties have good quality and quantity of oil as compared to local cultivars. But if the targets are phytoremediation, rehabilitation or soil erosion control then the sterile, noninvasive Vetiver cultivar is used.

Soil

Vetiver grass can be easily grown in any type of soil but the well drained loamy or sandy soil is most suitable. Lands should be prepared slightly sloping to avoid waterlogging condition.

Methods for propagation

a) Bare root slips

Firstly plants are cut above 25-30 cm and then slips are separated out. Each slip includes 2-3 tillers. These are separated from mother clump. Slips with roots are then dipped into various treatments like growth rooting hormones, manure slurry (cow or horse tea), clay mud or in shallow water pools, until new roots arise. For good results, slips should be kept in wet and in light until they are used for plantation.

b) Plant parts

Vetiver culms, tillers and crown or corms are also used for propagation. The old corms with more mature buds and nodes are used by removing old leaf covers. These are cut into 30-50mm (1-2'') lengths and 10-20mm (4-8'') below the nodes. These culms are used for propagation and sowed into the soil. Effect of tiller number on the growth of Vetiver grass (as indicated by maximum tiller number) has been studied by some workers. Xia (2003) observed as many as 51 tillers with the triple tillers in two month period. Moula *et al.*, (2008) noticed that the maximum number of tillers per clumps is produced from corms with double tillers. They found 81 tillers with the double tillers.

c) Bud multiplication or Micropropagation

Le Van and Truong (2006) suggested a protocol consisting of four micro-propagation stages, all in liquid medium.-

1) Inducing lateral bud development
2) Multiplying new shoots
3) Promoting root development on new shoots

4) Promoting growth in shade house or glass house

Initial nursery

In nursery, the slips are sown into the soil. Nursery is raised on light soil so that plants can be removed easily. It is manured by FYM or compost 20-25 t / ha. To encourage fast tillering, DAP 75 kg/ ha is also applied. Slips are generally planted 30X 30cm apart. Urea (60 kg / ha) is applied after second weeding.

The slips are also planted in small pots or small plastic bags containing soil and compost. In this way plantlets are maintained for about six weeks. When new tillers appear the plantlets are ready to be planting. The new planting strips with specially lined long furrows are also used for preparation of planting which is convenient for transportation.

Land preparation

Land for Vetiver cultivation should be free from weeds and shrubs and deep tilled. With the onset of pre-monsoon shower, final land preparations are performed.

Planting

The plantlets are planted in lines at 45 X30 cm spacing in flat bed followed by ridging. Planting is carried out in holes upto 5-6 cm deep, the soil around is pressed and leveled. One hectare area requires 75,000 slips. In off rainy season, watering of the field is essential.

Manuring and fertilization

Ten to twelve t/acre FYM or compost supplemented with urea applied after one month of planting stimulates growth of Vetiver. Further topdressing with 20 kg N at about four months after planting followed by 40 kg N at next year after rain in June.

Further care

Two or three weedings at an interval of about a month are needed during initial phase of Vetiver growth. Application of herbicide, Atrazin 0.5 al/ha (50% al) is used before transplanting which results in weed free condition for 75-90 days and during this time Vetiver grows and covers the ground surface.

The cutting of shoots at winters (November) increase tillering and simultaneously gives more roots per plant.

Harvesting

Harvesting is started in between 15-18 months of growth to get fully developed root system and high quality of oil. Oil content of roots starts decreasing after 20 months considerably. While harvesting before 15 months the roots may remain immature with low quality and quantity of oil. Harvesting is commenced from moist area in the field.

Land Development Department, Bangkok, Thailand carried out research on selection, propagation and cultivation techniques of Vetiver grass and their application (Morakul *et al.*, 2000).The department has given a chart for production of the propagating material of Vetiver by tissue culture.

Selection of suitable ecotype

↓

Preparation of seedling by root division

(Kept for one month)

↓

Operation on tissue culture in the lab, Germplasm

(Kept in agar and fed with nutrient formulae 1, 2 and 3 consequently for 3 months)

↓

Transplant plantlets in plastic chamber

(kept carefully for one month)

↓

Transfer all mini seedlings to the nursery near or within the Vetiver growing area

↓

Transplant all plantlets into small vinyl bags

(kept in nursery for one month)

↓

Cultivation of Vetiver in the field

Following this technique, ambitious large scale programme of Vetiver production was undertaken by the department in Thailand. In 1993; 6,500,000 plants were available and in 1999; 99,500 000 plats were available.

To avoid grass waterways by gully or rill erosion theVetiver grass was planted in inverted V shape across the channel or grass waterways with apex pointed against upstream. They also planted Vetiver grass against edges or excavated ponds, irrigation canals and dikes on dam to avoid clean up to form rills and gullies. For this, two lines of Vetiver were planted on edges of reservoir and one line along irrigation canals and natural waterways. The vertical interval is of 20 cm and that of between two lines is 50 cm. Chemical fertilizer with compost was usually applied in planting furrow.

The Vetiver grass has been also cultivated on side slopes of highways or roads with vertical interval of 20- 50 cm in between two rows to prevent erosion. However, Truong *et al.,* (*http://www.vetiver.org/TVN-Manual_Vf.pdf*) has commented that this technique is rather expensive.

Morphology

Vetiver grass is perennial, strong and densely tufted grass, propagated asexually by root divisions. Rhizome or stolon is absent. Rootstock is spongy and branched from which erect culms arise. The height of plant is upto 80-200cm (Lakshmanperumalsamy *et al.*, 2008)

Potdar (2006) described the morphology of Vetiver grass in following words. The grass is perennial, culms tufted, terete, 80-200cm tall, rhizomatous, simple, glabrous, nodes glabrous. Leaf sheath terete, 20-25cm long, keeled, ligule narrow hairy rim. Leaf blade linear, 10-80X0.5-1cm, densely hairy on upper side, apex acuminate. Panicle 10-40cm long, effuse, rachis stout, smooth, branches filiform, glabrous. Joints slender, linear, 4-5mm long. Sessile spikelet elliptic, 4-5X1-1.2mm, awn less. Lower glume coriaceous, narrowly ovate-oblong, 3.5-4X0.5-0.7mm, margins inflexed, 5-nerved muriculate on back, keels spinulose, margins ciliate in upper half, apex sub acute. Upper glume hyaline, broadly elliptic oblong, 3.5-4X0.8-1mm, 3 nerved, keel covered with scabrid hair, margin ciliate, and apex acute. Lower lemma membranous, narrowly obovate, 3-3.5X0.6-0.7mm, glabrous, margin ciliate, apex acute. Palae hyaline, oblong 2-2.2X0.4-0.5mm, apex rounded. Lodicules two, stamens-3, anthers 2-2.2mm long. Pedicels linear, 45mm long. Pedicillate spikelet narrowly ovate, 3-4X0.6-0.8mm, unawned. Lower glume coriaceous, narrowly ovate, 3-4X0.6-0.7mm, 5-7 nerved, keels spinulose, apex acute. Upper glume coriaceous, narrowly elliptic, 3-4X0.7-0.8mm, glabrous, 3 nerved, 1 keeled, spinulose on keel, margins ciliate, apex acute. Lower lemma hyaline, broadly elliptic, 3-3.5X0.5-1mm, glabrous, 2 nerved, apex acute. Palae hyaline, oblong, 1.5-2X0.2-0.3mm, apex rounded. Lodicules 2, stamens 3, anthers 5, 1.5-1.8mm long.

Anatomy

Leaf anatomy

The first report on anatomical study of *C. zizanioides* (L.)Roberty is by Pratt (1937). Afterwards the anatomy of leaf was studied by Mettcalfe in 1960. Balloon like micro hair are visible on abaxial epidermis while large prickly hair are present at leaf margins. The abaxial epidermis has several stomata, ranging between 27-33 μm in length, arranged in rows with dome shaped or triangular subsidiary cells with narrow guard cells. Variety of cells is found under the abaxial

epidermis in which ground mass has narrow elongated cells. Epidermis on both sides of blade is single layered. On abaxial surface of leaf bulliform cells are present. The cross section of blade reveals palisade and parenchyma with less differentiation. Mesophyll cells beneath both upper and lower epidermis, surrounded vascular bundles. There are intercellular cavities are found within assimilatory tissue upto below surface of abaxial epidermis.

Most of the members of family Poaceae are C4 plants and Vetiver grass is not exception to this trend. It possesses Kranz leaf anatomy which was studied in detail by Rajendrudu and Das, (1981). They observed that in bundle sheath cells, the position of starch containing chloroplast is in uniform centrifugal position. The shape of bundle sheath cells of Vetiver grass is shorter than that of those of *Cymbopogon*. Leaf interveinal distance is very short. Cramer *et al.*, (1991) carried out further investigation on the C4 nature of Vetiver; they studied leaf ultra structure which showed NADP-ME bundle sheath Kranz cell anatomy. Evolution of O_2 was absent as indicated by absence of granal thylakoid and peroxisomes in bundle sheath Kranz cells. In C.S. of minor veins, one layer of sheath cells was found to surround vascular bundle sheath. Esau (1977) reported that in case of Vetiver the mestome sheath (MS) does not appear between Kranz cells and metaxylem vessel elements as in case of other grasses of Pooidae. The bundle sheath chloroplast is with reduced grana development or it was noticeably absent. In the BS agranal chloroplast are present along with the suberised lamella of cell wall. Chloroplasts were found to contain numerous starch grains.

Bertea *et al.*, (2001) performed RUBISCO immunolocalization studies using rabbit polyclonal antibodies raised against RUBISCO and labeled gold particles. They observed that labeling occurred in bundle sheath chloroplast, gold particles distributed in entire organelle except the starch grains while it was absent in mesophyll chloroplasts, which is typical character of C4 plant.

Root anatomy

Pratt in 1937 for the first time attempted study of root anatomy of Vetiver. Afterwards many workers studied this aspect in detail of Vetiver. Vipunngeun *et al.*, (2005) studied differentiated microscopic characters of underground parts of *Imperata cylindrica* and *Chrysopogon zizanioides* (L.) Roberty. They observed following characters- trichome present on epidermis, starch grains is large in size in epidermis and vascular bundles are arranged as polyarch from the centre. Sclerenchyamatous tissue is in between parenchyma cells spread randomly and there is pith.

Mc Donald *et al.*, (2002) studied the similarity and diversity in root anatomy in a range of wetland and dry land grass species including Vetiver grass. They found that *C. zizanioides* (L.) Roberty and *V. fillips*, both had a variant of the root anatomy which is found in rice, which may be beneficial to increase these traits in waterlogging intolerant crops.

Cytogenetical studies

Sreenath (1983) studied nuclear cytology of callus culture in which he found rhizome calli of a triploid (2n=3X=30) variety of Vetiver. They found that few meristematic cells in calli were small in structure with large nuclei and nucleoli and dense cytoplasm. They observed normal mitotic divisions in those cells. The balloon like cells were also seen with thin cytoplasm, small nuclei and insignificant nucleoli and these cells did not show mitosis. Some cells (1-2%) from this showed nuclear budding which lacked nucleoli.

Nuclear karyomorphological studies and DNA analysis in *C. zizanioides* (L.) Roberty was performed by Lavania (1985) in 20 different collections. He found the somatic chromosome number is 20 in all collections and karyotype were found to be nearly symmetrical with chromosome having median to submedian centromere. Variation was found in nuclear DNA content and chromosome size.

They also noticed that 2C value ranges from 2.02 pg to 2.56 pg and total haploid chromatin length varies from 25.6 μm to 38.7 μm.

Chromosome morphology of root cells of 15 ecotypes of Vetiver collected from different parts of Thailand was investigated by Kongprakhon *et al.*, (2003). They classified these ecotypes in group of eight *C. zizanioides* (L.) Roberty and group of seven *V. normalis.* For raising healthy root tips, they used humic acid solution. Cutting the root tips was carried out between 11.00a.m to 1.00p.m.for further fixation. They observed same chromosome number as 2n=2X=20 in all ecotypes. They found 3 B chromosomes only in one ecotype while, they also observed variation of satellite chromosome with respect to its number and morphology in six ecotypes. The total length of chromosome ranged from 1.8-8.4 μm while the karyotype mostly consisted of metacentric and sub metacentric chromosomes. They also localized the heterochromatin regions on the chromosome of Vetiver by using modified C band and N band. This study helped to characterize each pair of the Vetiver chromosome and thus they identified all 10 pairs of chromosome by observing banding patterns with their length and arm ratios. Each ecotype showed whole dark band containing at least one chromosome which indicates presence of constitutive heterochromatin but the remaining chromosome showed variation in all ecotypes.

Shanthamma and Narayan, (1976) studied meiosis in 13 panicoidae and 9 pooidae members with detiled microsporogenesis.They found that *C. zezanioides* (L.) Roberty (Sexual) had 50% pollen viability, and noticed the presence of univalents, laggards, bridge fragments and micronuclei in meiotic preparations.

Vetiver grass is originated from India and South Africa continent but due to it's wide applications regarding phytoremedation, water purification and soil erosion, this grass is now cultivated in many countries throughout the world. But due to various environmental conditions, adaptation of plant, domestication their forms a new ecotypes of Vetiver grass. Many workers studied the genetical differences and similarities amongst these Vetiver ecotypes.

Dong *et al.*, (2003) collected 13 ecotypes of Vetiver grass from eight countries and studied genetic relationship by using RAPD molecular markers. Neighbor-Joing (NJ) cluster analysis showed that there were two groups, one included seven ecotypes named as Sunshine, Zomba, Domesticated type, Wild type, Capitol, Lilongwe and Malaysia with bootstrap value (100 %), these all having same character i.e. earlier earing trait except in Capitol. Another group included Huffman, Parit buntar; Kandy and Karnataka having bootstrap value (58 %) and all having lower earing rate in earing stage.

Adams *et al.*, (1998)[a] studied accessions (n = 121) of *C. zizanioides* (L.) Roberty from its native and around the world by using RAPDs. They found that the genotypes Sunshine accounts for nearly all germplasm used outside South Asia. Their study revealed that *Vetiveria* and *Chrysopogon* were not separated by their RAPDs, so in this way this observation support the merging of *Vetiveria* into *Chrysopogon.*

Adams *et al.*, (1998)[b] also studied DNA genetic diversity of *C. zizanioides* (L.) Roberty cultivars from Panama by using RAPDs. They observed several accessions which were different from commonly utilized Sunshine cultivars.

Comparative analysis of four elite genotypes- BDP, BMH, MBR 5 and KS 1 of *C. zizanioides* (L.) Roberty through RAPD profiling was made by Shasany *et al.*, (1998). They observed genetic stability and homogeneity at population level without polymorphism. They grouped KS 1 with BDP 1 due to similar khus essence while other two BMH 1(Kesar) and MBR 5(Rose) grouped together.

Vetiver genome analysis was carried out by Srifah *et al.*, (2000) using different methods like RAPD and SSCP in different cultivars from Thailand. This study of intron region in Δ 9 stearoyl-acyl carrier protein desaturase gene for oleic fatty acid synthesis lead them to distinguish each ecotype of *C. zizanioides* Roberty Nash. and *Vetiveria.normalis* A.Camns.

Physiological studies

Seed production and germination were studied by Parihar *et al.*, (1998).They found that after 45 days after starting monsoon commencement in the last week of June there was initiation of inflorescence with peaked density at 139.6 /m^2 with yield of pure germinating seeds (PGS) as 648 / ha in year one and 150.2 /m^2 density with (PGS) 418kg / ha in year two. Acropetal anthesis was lasted for 5days and shedding was basipetal which started after 21 days of anthesis. Seed dormancy was noticed in freshly collected spikelets which lasted for three months. Dormancy caused by hull was broken by removal of caryopsis from the enclosing husk. Application of GA and potassium nitrate also proved effective in breaking seed dormancy.

Several studies involving growth analysis of Vetiver grass have been conducted. Lavania (1988) found that the biomass of both diploid and autotetraploid plants was not changed distinctly, only marginal increase was found in autotetraploids. Hongto *et al.*, (1996) made a comparative study of growth, root system and yield of different ecotypes of Vetiver grass (6 from *V. normalis* and 4 from *C. zizanioides* (L.) Roberty).They observed that rooting system and root's yield of *C. zizanioides* (L.) Roberty was higher than *V. normalis*. Study on extent of root depth and density of Vetiver grass was performed by Luekaewma *et al.*, (1996).They selected 8 promising ecotypes for this study.They observed that ecotype Buri Ram had highest root depth upto 315cm and root density with average dry weight 1000g/ clump found in ecotype Loei. They observed shallow root depth and lowest density in ecotype Kwang- Chiang Mai. Dukpa *et al.*, (1996) studied effect of environment on adaptability, growth and development of two Vetiver types- South Indian type (SIT) and North Indian type (NIT) at different altitudes that is 640, 1640 and 2200m in Bhutan. They observed that NIT grows vigorously and show good adaptability than SIT. Both produced seeds but SIT produced less viable seeds. Jin- xiang *et al.*, (2005) observed that sprouting rate was 10-30 % maximum upto 50 % and average tillers of seeding reproductive

Vetiver grass was 21 to 30 per plant after nine months of growth, plant height (130-200cm), leaf length (55-100cm), leaf width (0.5-1cm), diameter of the root (0.06-0.18cm), root corona ratio(46.15-55.5%), panicle length (15-39cm), seed length (1.5-2.5mm), seed width (0.6-0.8mm), the thousand grain weight of topmost, first and second side branch was 0.363g, 0.280g and 0.381g respectively. Singh *et al.*, (2008) followed out hydroponic technique to grow Vetiver plants. They used micropropagated plants to grow in hydroponics and studied effect of phenol on plant growth. They found that plant growth was reduced in response to phenol.

Root characteristics and root distribution studies of Vetiver grass were studied by Payamanonta *et al.*, (1996) using P32 tracer technique. They used two Vetiver grasses *C. zizanioides* (L.) Roberty Surat Thani and *V. normalis*- Pimai and planted the slips. After 1 and 2 .5 months the root growth was upto 150 and 200cm depths respectively and more root density was found at the depth 30-100cm. After 9.6 months, the root length found upto 400cm depth and highest root density was found at the depth of 150, 250 and 350cm respectively. They also observed that the root length of both Vetiver species was upto 500cm at 25 months old plant. Nix *et al.*, (2006) evaluated growth rate of Vetiver and root and oil distribution. They found that at each sampling date, the amount of Vetiver oil in roots was increased. At the end of study, they found root length was upto 2m and 25cm wide with 0.48g of dry weight. Uprooting resistance of Vetiver grass was studied by Mickovski *et al.*, (2005). This study was carried out in Spain. Shoot and root morphological characteristics were used to correlate the uprooting resistance. Morphological differences in roots were observed in Vetiver plants which were planted in its native and in sub-humid environment. Result showed that the roots of both plants were having strength against torrential runoff but the plants growing in native place showed good results.

The discovery of C4 pathway by Hatch and Slack, (1966) has opened a new chapter in photosynthetic research. Initially presence of this pathway was detected

No need to give all details using 21 lines

in sugarcane and some grasses. Rajendrudu and Das, (1981); studied in details, photosynthetic carbon assimilation in leaves of *C. zizanioides* (L.) Roberty and *Cymbopogon.* While studying anatomy of these plants under light and scanning electron microscope, they observed that the leaf sections show typical Kranz leaf anatomy with centrifugally placed starch in Bundle Sheath cell's chloroplasts. They also carried out studies of carbon isotope discrimination with Mass Spectrophotometry technique and measured CO_2 compensation point by using infra red gas analyzer (IRGA). Analysis of photosynthetic $^{14}CO_2$ assimilation products was also performed with two dimensional ascending paper chromatography. The results of above experiments showed that oxygen had no effect on photosynthetic CO_2 assimilation.CO_2 compensation point is below 5µlit/lit at 21% O_2 and rapid incorporation of $^{14}CO_2$ into the C4 acids, mainly malate took place. They also observed massive movement of 14 C from malate to 3 phosphoglycerate and other C3 cycle intermidates. About 50% of malate was found to be converted into 3 PGA within 30 sec. chase period, which is followed by further increasing radioactivity in Calvin cycle intermediates with steady decrease in that of PGA. All these findings revealed typical characteristics of C4 photosynthesis in *Chrysopogon.* They indicated that there is resemblance between *Chrysopogon* and NADP malic enzyme type C4 species like sugarcane, *Sorghum* and maize. They also observed narrow interveinal distances which can accelerate the translocation of photosynthetes from the mesophyll and bundle sheaths cells to phloem tissue. Maffei *et al.,* (1985) studied activites of photosynthetic enzymes in *C. zizanioides* (L.) Roberty, cultivated in temperate climate. They determined the δ13 values and phosphoenol pyruvate carboxylase, ribulose 1, 5 biphosphate carboxylase and glycolate oxidase activities. This experiment also demonstrated that Vetiver posses C4 phtosynthetic mechanism and same mechanism prevails under humid temperate climate after cultivation. The fact that Vetiver grass posses C4 NADP ME type photosynthetic pathway was further confirmed by Bertea *et al.*, (2001). These workers carried out leaf anatomical study by using light

microscopy and TEM, imminogold localization study of RUBISCO determination, study of photosynthetic enzyeme activites and CO_2 assimilation of stomatal conductance of Vertiver from native tropical and sub tropical areas and plants cultivated in temperate climates. They made detailed study of Kranz leaf anatomy in Vetiver. They found that NADP ME enzyme activity was 124 fold higher than NAD ME type. According to these workers, all these evidences indicate that *C. zizanioids* (L.) Roberty is NADP ME type C4 plant.

The essential oil is the economically most important component of Vetiver grass. Effect of ploidy level on essential oil content in Vetiver has been investigated by some workers. The percent oil content of control (diploid) and artificial tetraploid (obtained through colchicines treatment) was compared by Lavania (1988). It was noticed that in tetraploid, % oil content was 1.4% while in the control and check it was 0.98% and 1.15% respectively.Gurnani *et al.*, (2003) suggested that the essential oil yield is mainly influenced by the number of tillers per plant, root length per plant, root yield per plant. Massardo and Senatore, (2006) correlated the Vetiver oil production with early root growth. It was further noticed that the oil production was constant during first six months but in next two months, it increased two fold and decreased in cold months.

Effect of different concentrations of Hoagland nutrient medium on level of different mineral element in Vetiver plants was investigated by Ta-un *et al.*, (1996).They studied the essential element accumulation potential of Vetiver grass. They raised Roi- et variety of Vetiver grass in six different nutrient solutions that is 0, 0.5, 1, 2, 4 and 8X of standard Hoagland solution. The plants were taller which grow in low concentration of nutrient solution than that of higher concentration, but they possessed less tillers after cuttings. In 2X concentration, there was best root growth while in 4X concentration; it gave highest root/ shoot weight. Lower root/shoot ratio was seen in higher nutrient concentrations. Nutrient concentration in order as in roots were N, K, S, Mg, P and Ca whereas in shoot it

was in following order K, N, Ca, S, Mg and P. There was greater accumulation of N, Mg and S in roots than shoot while P accumulation was similar in both.

Mucciarelli *et al.*, (2009) studied sugar metabolism of cells of *C. zizanioides* (L.)Roberty, cultured with Amberlite XAD-4. The result showed that after addition of the polymeric adsorbent XAD-4 to the culture medium there was significant increase of cell wall invertase activity (7.5 fold more than control) and was attributed to sugar hydrolysis into the media. Result of labeling experiment showed that the cells were able to take [U^{14}C] sucrose, [U^{14}C] D-glucose and [U^{14}C] fructose from the cell suspension medium. This uptake was found to be active uptake. The XAD-4 caused increased in sucrose affinity with increase V max. XAD also caused doubling of glucose consumption while radiolabelled glucose was found 25 fold inside the Vetiver cells with respect to control. They also observed increased activity of both glucose transporters and cytosolic or vacuolar invertase activity. XAD-4 did not alter the uptake rate of fructose.

Sompong *et al.*, (2000) studied the association of the N_2 fixing bacteria with Vetiver. This bacterium (*Azospirillum*) was isolated from soil in Vetiver root zone. They further noticed that the bacterium fixes N_2 and also produces a plant growth hormone Indole 3 Acetic Acid (IAA).Glufosinate is a potent herbicide whose primary target is a key enzyme of ammonia assimilation -glutamine synthase. Prasertsongskun *et al.*, (2002) isolated herbicide glufosinate resistant Vetiver cell lines through tissue culture which had high glutamine synthetase activity and the enzyme was resistant to glufosinate. These cells could grow in culture medium containing $5X10^{-5}$ glufosinate with I_{50} values were $4.2X\ 10^{-5}$ M.

Liu *et al.*, (2004) investigated changes in photosynthesis and physiological index of Vetiver grass in response to heat and drought stress. Under stress conditions, there was decrease in soil water content, relative leaf water content and the chlorophyll content while increased in dissociated proline content. Enzymatic study of SOD, POD was increased first and then decreased. A stress induced decrease in net photosynthic rate of leaf and intracellular CO_2 concentration was

evident in early stages. As the stress level increased, there was decrease in PS II effective phytochemistry quanta output (FV'/FM'), photochemical quenching (qP), electron transport rate (ETR) and non-photochemical quenching (qN). All these findings suggest that the heat and drought stresses cause decrease in photosynthetic rate in this hardy grass.

Li-ya *et al.*, (2008) studied responses of *C. zizanioides* (L.) Roberty to waterlogging condition. They gave two waterlogging treatments- shallow (10cm) and deep (40cm) waterlogging depths. The control plants were not subjected to waterlogging condition. They also found balanced free radical condition, with small increase in MDA and without changing membrane permeability under stress. Liu and Wang, (2005) also studied effect of waterlogging on Vetiver grass. They did not notice any distinct change in photosynthetic rate, transpiration rate and water use efficiency during stress condition and after relieving the stress. The above two reports throw light on the mechanism of waterlogging tolerance in Vetiver grass. These observations clearly reveal that Vetiver has strong capacity to resist waterlogging.

Vetiver is highly reputed for its hardiness and it can grow in varied types of environments. Many workers studied the effect of different types of environmental stresses on growth and/or other aspects of Vetiver grass. Yuttapong *et al.*, (1995) studied the effect of soil salinity with shading on the growth of Vetiver. They raised the Vetiver plants in plastic bags in normal and saline soil which had electrical conductivity of 7.6ds/m. Then they kept these bags in 100% sunlight vs 50% sunlight with shading and then observed different types of growth parameters like number of tillers per crown, shoot dry weight, root dry weight and whole plant dry weight. They found that the plants grown under saline condition showed poorer growth than normal. Relative performance of different aromatic grasses under saline condition was studied by Tomar and Minhas, (2004). In this study they compared the performance of Citronella, lemon grass, palmrosa and Vetiver, which were planted on calcareous sandy loam soil and irrigated with canal and

saline water with ECw- 8.5 ds/m alternatively. Results showed that maximum biomass was produced by Vetiver (90.9 tones/ha dry weight basis) followed by palmarosa (29.1 tones/ha) and lemon grass (16.1 tones/ ha) but death of citronella occurred. Among these species Vetiver was least affected by salinity. Zhou and Yu, (2009) studied the changes in free, conjugated and bound polyamine content in relation to salt adaptation of Vetiver grass. They found that treatments with 100, 200 and 300 m mol/L of salts for 9 days caused decrease in growth as compared to control. They also observed that the amount of bound polyamines and total bound polyamines was decreased in both roots and leaves under salt stress. Tran (http://www.Vetiver.org/VNN_invitrio_r.pdf) selected salt tolerant callus clones by sub culturing *in vitro* plants 5 times on the MS medium with gradually increasing concentration of NaCl (0.5-2.5%). They found 8.89% of callus clones survived and thus formed high salt tolerant Vetiver plantlets. Dagar *et al.*, (2004) raised three aromatic grasses palmarosa, lemon grass and Vetiver grass, in different alkali soils. They noticed that the grasses could produce essential oil for industrial purpose in alkali soil with pH 9.2. Among these 3 grasses, Vetiver showed good yield without much loss in highly alkaline soil.

It is now very well established that heavy metal tolerance in plants is mediated through the production of phytochelatins. Induction of lead binding phytochelatins in *C. zizanioides* (L.) Roberty was studied by Andra *et al.*, (2009). They put forth a hypothesis that Vetiver produces Pb and phytochelatin complexes after exposure to Pb. They found that exposure to Pb for 7 days caused accumulation of Pb in shoot tissue upto 3000mg kg^{-1} while in root it was upto 20, 000mg kg-1, without symptoms of phytotoxicity. Pb depositions were observed in vascular tissue of root and shoot under SEM that Pb was translocated to shoot. HPLC ES-MS analysis showed presence of four unique phytochelatins (n=14) based on their respective sequence of amino acids. This was also confirmed by using ES-MS and Pb mass isotopic pattern.

Allelopathy is regarded as a special type of plant-plant interaction. Certain allelochemicals leached out from dry plant litter from any plant can influence the growth of same or another neighboring plant. Vimala *et al.*, (2008) treated Vetiver grass with pre analyzed laechates of *Jatropha curcas*, *Ricinus communis* and *C. zizanioides* (L.) Roberty in 1:10, 1:20 and 1:50 v/v concentrations. They observed increase in total chlorophyll and root nitrogen content and a decrease in shoot peroxidase activety in the plantlets of Vetiver after new leaf emergence which is in response to Vetiver leachates. *Jatropha* leaves caused increase in root nitrogen and shoot peroxidase activity. They also observed that growth of *Jatropha* seedling was promoted by Vetiver laechates while inhibited the seedling growth of the *Ricinus* was inhibited.

Evaluation of antioxidant activities of Vetiver oil and its components was done by Kim *et al.*, (2005). They used *in vitro* assay methods like DPPH free radical scavenging assay and Fe++ metal chelating assay with BHT and tocopherol as standards. The result showed that Vetiver oil (10ml/ml methanol solution of Vetiver oil) having 93% free radical scavenging activity in DPPH assay and 34% in metal chelating assay. These results indicate that Vetiver oil may become alternative for natural antioxidants with wide applications. In root bag grown Vetiver plants, Xu *et al.*, (2008) studied activities of antioxidant enzymes in response to Zn, Cd and EDTA applications. Antioxident potential of the Vetiver roots was studied by Luqman *et al.*, (2009). They have used spent root extract of two Vetiver varieties KS-I and Gulabi and then subjected to *in vitro* assays of ferric reducing antioxidant power(FRAP); 1-1 diphenyl , 1-2 picryl hydrazil (DPPH), total phenolic analysis, total antioxidant capacity (TAC) and reducing power (RP). They found that KS I genptype had higher value of FRAP, DPPH inhibition, TPC and RP potential as compared to gulabi and also observed increased antioxidant activity with increasing concentration of the extracts (10-1000µg/ml). KS I oil at 100 µg/lshowed protective effect. On the basis of these observations these workers concluded that cv KS I showed better antioxidant

capacity than cv gulabi. Singh *et al.*, (2008) studied effect of phenol on antioxidant enzymes like superoxide dismutase, peroxidase and catalase in tissue cultured Vetiver plants. They found increased activity of enzymes superoxide dismutase and peroxidase in response to phenol treatments.

Effect of plant growth retardants (CCC, PBZ and etheral) and water stress on physiology of Vetiver grass was studies by Farooqi *et al.*, (2010) at CIMAP, Lucknow. They observed decrease in plant height, root yield, relative content of leaves and water potential due to water stress. It also caused decrease in NR activity while increase in activity of peroxidase. Protein content was reduced under water stress while proline content was increased but the oil yield was reduced under drought. Plant growth retardants showed ameliorating effect on growth characters and oil content of Vetiver plant which was subjected to drought stress. In same plants, NR activity increased by CCC while all other growth retardants caused increase in peroxidase activity. Proline content was increased in response to PBZ and etheral in stressed Vetiver plants. In unstressed plants, CCC and etheral caused increase in root yield, peroxidase and NR activity while the same growth retardants caused reduction in protein content. Oil content was increased by action of CCC, etheral and PBZ in unstressed plants..

Phytochemical studies

Since essential oil is economically the most important constituent of Vetiver roots, numerous investigation have been carried out on this aspect in Vetiver grass. The first such study was performed about 70 years ago by Pfau and Plattner (1940). Analysis of Vetiver oil in relation to taxonomy was performed by Anderson (1970). He studied oil of Vetiver samples of Haiti, Reunien and North Indian origin by using GLC technique and concluded that North Indian variety (khus oil) is found distinct race with different characters. Bor (1960) suggested that the smell of Vetiver oil is mainly due to ketonic sesquiterpne from which α-vetivone and β- vetivone were detected earlier. Bhatwadekar *et al.*, (1982)

detected 29 compounds of Vetiver oil with gas chromatography. They studied the chemical structure of major and minor sesquiterpenes and also noticed that the chemical composition varies on the basis of Vetiver source. Chowdhary *et al.*, (2009) recently made chemical analysis of essential oil of Vetiver roots and found that the oil contains sesquiterpene and sesquiterpene derivatives mainly γ-cadinene, clovene, α-amorphene, aromadendrene, junipene, β-hmachelene, franesene, β-bisabolene, cis-caryophylene, khusimol, epiglobulol, spathulenol, khusinol, khusinone, khusimone and khusinol acetate which give characteristic odour to Vetiver oil. It also contains eugenol, isoeugenol and khusinol acetate.Ganguly *et al.*, (1978) studied the nature of khusiol components from Vetiver oil. It is a tricyclic saturated, crystalline antipodal terpenic alcohol which is considered to be a biogenetic precursor of zizaene and prizizaene. Kalsi *et al.*, (1985) investigated the structure and absolute configuration of khusitoneol, a 14C ketolalcohol from Vetiver oil. They have used North Indian Vetiver oil and isolated a new 14C terpenoid norkhusinol. It is a first report in which biological evaluation was used as a tool to confirm stereol structure of a naturally occurring terpenoid. Kalsi and Talwar, (1987) studied the stereostructure of vetidiol isolated from North Indian Vetiver oil by using spectral data and chemical correlation. Weyerstahl *et al.*, (1996) isolated four new sesquiterpene ethers from Vetiver oil named as 7-beta,10-beta-epoxy-4-beta-H-eremophila-1,11(12)-diene, 10-beta,11-epoxy-4-beta-H-eremophil-1-ene,4-alpha,7-epoxy-10-beta-H-spirovetiva-2,11(12)-diene and 6-alpha,12-epoxy-7-beta-H,10-alpha-H,11-beta-H-spiroax-4-ene. They isolated these components from the unpolar part of Haitian Vetiver oil using with the help of spectral data. Weyerstahl *et al.*, (1997) also isolated a new cis-eudesma-6, 11-diene from Vetiver oil as a main constituent of the sesquiterpene hydrocarbon fraction. They further studied six derivatives of it. Weyerstahl *et al.*, (2000) studied a medium polar part of commercial Haitian Vetiver oil, particularly of carbonyl compounds. They reviewed many new natural compounds and identified by NMR data which are as follows 1,7-cyclogermacra-

1, 4-dien-15-al; 10-epi-acor-3-en-5-one ; 10-epi-acora-3,11-dien-15-al ; (E)-opposita-4, 7(11)-dien-12-al; 13-nor-opposit-4(15)-en-11-one ; 7-epi-cis-dracunculifoliol (ax-4(15)-en-7-ol, 11); elema-1,11-dien-15-al (2 epimers); 6,12-epoxyelema-1,3-diene ; eremophila-1, 6-dien-12-al (2 epimers); 15-nor-funebran-3-one; 7,15-epoxyprezizaane; 15-nor-prezizaan-7-one; 12-nor-preziza-7(15)-en-2-one; prezizaan-15-al; cyclocopacamphan-12-al (2 epimers); 5,6-seco-6,7-furoeudesman-5-one; 11,12,13-tri-nor-cis-eudesm-5-en-7-one; 11,12,13-tri-nor-cis-eudesma-5,8-dien-7-one; 13-nor-eudesm-5-en-11-one (2 epimers); and 13-nor-trans-eudesma-4,7-dien-11-one. Two oxiranes, 13-nor-4, 5-epoxyeudesm-6-en-11-one and 13-nor-7, 8-epoxy-trans-eudesm-4-en-11-one, which were also isolated, might be artefacts. The polar part of the oil was converted to the methyl ethers. Distillative and chromatographic separation furnished, among others, beta-funebrenyl methyl ether 23', prezizaenyl methyl ether 50', khusimyl methyl ether 51', and cyclocopacamphanyl methyl ethers 58a',b' (epimers). Weyerstahl *et al.*, (2000) further analyzed the polar fractions of Haitian Vetiver oil. They studied polar part of oil which was converted into methyl ethers which was separated by distillation and repeated flash chromatography. Further they studied and identified the compounds by their 1-H and 13-C- NMR spectra. The names of compounds are 12-nor-ziza-6(13)-en-2beta- and -2alpha-ol, eudesma-4,7-dien-3beta- and -2beta-ol, eudesma-3,5-dien-1alpha-ol, eremophila-1, 4(15)-dien-2alpha-ol, eremophila-1,11-dien-2alpha-ol (nootkatol), guaia-1, 11-dien-3-ol, spirovetiva-3,7(11)-dien-12-ol , preziza-7(15)-en-3alpha-ol and helifol-1-en-14-ol (syn. khusien-14-ol). Furthermore, hemi-acetal, 10-epoxy-salvialan-10-ol was identified. Karkhanis *et al.*, (1978) studied minor sesquiterpene alcohol of North Indian Vetiver oil. They determined the structure of nootkatane based primary alcohol, iso valencenol and isovetiselineol, on the basis of chemical transformation and spectral evidences. Martinez *et al.*, (2004) carried out volarization of Brazillian Vetiver roots. They developed extraction method for oil and studied the chemical composition of the extracts, their sensorial characters and possibility of

chemical transformation of the product. They found that Brazilian oil sample have greater acid amount than other commercial Vetiver oils.They extracted this oil into basic medium to remove main acid (zizanoic acid) which was further chemically transeferred into khusimol with desirable sensorial properties. Huang *et al.*, (2004) studied chemical components of *C. zizanioides* Roberty volatiles by using SPME and GC-MS technique. They found that main component was valencene (30-36%) while in shoots and leaves they found presence of 9-octadecenamide (33-50%), 2,6,10,15,19,23 hexamethyl-2, 6, 10,14,18,22 tetracosahexane (27-46%) and 1, 2-benzenedicarboxylic acid, diisooctyl ester (18-29%). The results indicated that many terpenoids are volatiles. In shoot volatiles there are 3 monoterpenes, 2 sesquiterpenes and 1 triterpene while in roots most of the volatiles were sesquiterpenes. Pripdeevech *et al.*, (2006) studied highly volatile constituents of *C. zizanioides* (L.) Roberty, roots grown under different cultivation conditions. They used steam distillation and solvent extraction (SDE) method for oil extraction for oil separation. Two dimensional gas chromatography (GC X GC) and solid phase microextraction (SPME) techniques used. GC X GC analysis of crude essential oil showed a total of 156 and 48 well resolved components. They found similarity in component profile of oils from roots obtained from normal soil and semihydroponic cultivation while differences were only seen in some major volatiles when microbes were used in cultivation system.

Some workers also made attempts to study the biosynthetic pathways of Vetiver oil constituents. Dauben and Hart, (1977) standardized method of preparing fractionalized spirocycles synthesis of spiro vetivane sesquiterpene. They found that spirocycle is result of reaction between sodium salt of alpha-formylcycloalkanones and 1-carbethoxycyclopropyl triphenyl phosphonium tetrafluroborate. This reaction is very important because it provides common intermediate for synthesis of sesquiterpene like Beta vetivone (present in *C. zizanioides* (L.) Roberty) (+-) hanesol, (+-) beta-vetispirine and (+-) alpha vetispirine. Buchi *et al.*, (1977) studied the synthesis of khusimone which is non-

sesquiterpene with zizaene skeleton. The synthesis proceeds through 2 epimeric tricyclic ketones 14 and 15, formed by intramolecular Diels-Alder cyclization of the trienone ketal. Akhila *et al.*, (1987) investigated the biosynthesis of khusimol and allokhusiol in *C. zizanioides* (L.) Roberty. They conclude that mavalonic acid was incorporated into tricyclic sesquiterpene alcohols and degradation of exomethylene group of khusimol and prizizaene (obtained from allo khusiol) showed that a stereospecific 1,2 methyl shift takes place during the formation of khusimol skeleton *in vivo*. This study also indicated that khusimol and allokhusiol biosynthesized occur by different routes but with a common intermediate. Sathya Shankar and Subba Rao, (1994)described total synthesis of (+-) allo cedrol (khusiol) from ketone 11 which involves the Lewis acid catalysed rearrangement of the prezizaene analogue 8 as the key step. Adams *et al.,* (2004) made preliminary comparision of Vetiver root essential oil from cleansed (bacteria and fungus free) versus non cleansed (normal) Vetiver plants by using tissue culture method they obtained cleansed fungus free plants .For oil extraction steam distillation was carried out and for oil profile analysis GCMS technique was used. Oil yield of cleansed plant (0.02%) with clear oil while that of non cleansed Vetiver was 0.35% with light yellow coloured oil. GCMS oil profile of non cleansed Vetiver oil was typical while that of cleansed Vetiver had large amount of (19-20)alkanes plus several alkanols along with typical Vetiver oil compounds but lacked presumed fungal metabolites such as beta funebene, prezizaene, alpha – amorphene and beta-trispirene. The above observations indicate that phytochemistry of Vetiver oil is a highly interesting topic and many advances are expected in future.

Besides essential oils, other phytochemical constituents in Vetiver grass have been also investigated by some workers. Pawadee *et al.,* (2003) studied hemicellulosic polymer from Vetiver grass and its physicochemical properties. For this purpose, gravimetric method was used and it was found that the main polysaccharides in samples were hemicelluloses (ca 38%), followed by cellulose

(ca 27%). The molecular weight of obtained hemicellulose was 30,000 and showed the decomposition at 310^0C temperature. According to them 8h treatment with 4M NaOH at ambient temperature was ideal for maximum extraction of hemicellulose.They also found approximately 5% protein which studied by using bicinchoninic acid assay with bovine serum albumin (BSA) as a standard. Acid chlorine method was used to determine lignin content which was approximately 10%. The ash content was 3% which mainly constitutes silica (ca 50%).Oraphin *et al.*, (2004) elucidated structure of hemicelluloses from Vetiver grass. They confirmed the structure of hemicelluloses by using TFA hydrolysis and methylation analysis with 13C NMR and FTIR spectroscopic methods, which gave details of the anomeric linkage configuration. The confirmed structures are having arabinoxylan which contains (1 -4)-B-D-xylan backbone substituted in 0-2&1 or 0-3 by single 2-L- arabinose residue, single 2-D glucuronic acid residue and short chains of sugar residues containing arabinose.

Pathology

Vetiver is found to be resistant to many diseases and insects. Fungal diseases like brown spot disease caused by *Carvularia trifolia*, rust, smut and *Fusarium* have been noticed on Vetiver (Sreenath *et al.*, 1994). Vanky (2005) reported a smut fungus from Ustilagomycetes – *Macalpinomyces effuses* on Vetiver grass. Damage to Vetiver grass is found to be cause by termites, white grubs (*Eupledia* species), stem borers, *Holotrichia serrata* and rats. It is worth mentioning that this grass species is resistant to root knot nematode, which is one of the serious problems in sub tropical areas (Vietmeyer, 1993).

Tissue culture

Tissue culture studies in Vetiver grass have been performed by number of workers. Mathur *et al.*, (1989), cultured leaves, stem, leaf sheath and mature leaf blade segments as an explants on MS medium supplemented with or without 0.1-5 mg/ l 2,4 D combined with 0.1-2 mg/ l kinetin or used as alone, but the explants did not form callus. Leaf sheath as explants forms callus in presence of 1-2 mg/l 2, 4 D within 3-4 weeks of inoculation. Addition of kinetin (0.25-1 mg /l) in 2,4D containing media enhanced proliferation of the induced callus. Sreenath and Jagadishchandra, (1989) found that embryogenic and non embryogenic callus was formed from the leaf tissues on MS medium which contained 2, 4 D with BAP or kinetin. They also found that the callus only formed in presence of 2,4D with or without cytokinin. The embryogenic callus was hard, finely nodular and pale yellow while non embryogenic callus was soft, non-nodular and white. The calli were maintained upto two years by sub culturing at two monthly intervals on medium which contained 2,4 D. Leupin *et al.*, (2000) used crown and leaf slices of *in vitro* generated plantlets from Java and produced compact calli to regenerate plantlets. They cultured these explants on modified MS medium combined with 2.26 µM 2,4D; 2.24 mM 6 BA and 75 g/l sucrose which lead to callus formation. For further regeneration callus was transferred to regenerate medium. They raised upto 100 plantlets by following this procedure. Mucciarelli *et al.*, (1993) cultured leaves on MS medium supplemented with 9 µM 2,4D; 5.7 µM IAA and 4.6 µM kinetin. They found shoot initiation from 14 day old fast growing callus and also observed embryo like structure. Roots developed after transferring into the basal medium and regenerated plant successfully in soil.

By using inflorescence as an explant, Sreenath and Jagadishchandra, (1990) established calli with addition of 2,4 D with or without cytokinin. They observed 100% callus formation when spikelet primordia of young inflorescence were used. They used 2, 4 D and BAP or kinetin additionally with sub culturing every 6-8 weeks. The embryonic calli further developed into somatic embryos and retained

their embryonic capacity even after eight total subcultures. Prasertsongskun *et al.*, (2002) used Vetiver cells derived from an inflorescence and cultured on modified N6 liquid medium supplemented with 10 µM 2,4 D and 10 mM proline. They observed exponentially growing cell suspension which was subcultured for further study. Prasertsongskun (2003) developed cell suspension cultures from calli developed from inflorescence of Vetiver from Surat Thani germplasm source.They used liquid N6 medium supplemented with 10 µM 2,4D and 10 µM proline. They observed highest small colonies or cell suspension when plated on solid MS medium which contained 0.45 µM 2,4D. They obtained 65% of plantlets when transferred to regeneration medium. Sangduen *et al.,* (2009) also cultured Surat Thani ecotype of Vetiver and obtained cell suspension culture by using inflorescence as explant. They used MS medium supplemented with 10-15 µM 2, 4 D and observed two types of proliferation–embryogenic and nonembryogenic. By using SEM, they made detailed study of structure of both types of calli. They observed that embryogenic calli were compat, nodular knobs, white or creamy while nonembryogenic calli were soft, friable, unorganized light stained cells, translucent, watery and light yellow. Nonembryogenic cells were tubular long and loosely arranged while embryogenic cells were nodular and knobby, quite deeply embedded and tightly packed.

Sreenath *et al.*, (1994) observed intact roots of rhizome form callus. Calli produced from different parts of rhizome were soft and non-nodular but failed to undergo further morphogenesis. They used media containing 2,4D (1-5mg/l).

George and Subramanian, (1999) cultured mesocotyl part of young seedling of Vetiver on MS medium supplemented with 2,4D and kinetin and noticed callus induction and high frequency regeneration. They observed shoot formation from nodular pale yellow callus after 40-50 days when cultured on MS basal medium with or without BA. Rooting was initiated by addition of NAA. These plantlets were then successfully planted into the soil.

Zhen-rong *et al.*, (2003) studied factors which affect somatic embryogenesis and plant regeneration. They used axillary buds and aseptic adventitious buds as explant and cultured them on MS medium supplemented with 2,4D and 6 BA .On this medium explant got regenerated. They further observed that callus induction frequency was more in Vetiver cv Zomba and lowest in Vetiver cv Malysia. The embryonic calli lost embryogenic differentiation ability at 3-7^0 C temperature in which 40-50% of them could regenerate. Yang *et al.*, (2006) used axillary and aseptic adventitious buds as explants and cultured them on MS medium supplemented with 2,4 D and 6BA. They observed somatic embryogenesis and concluded that for such regeneration 2,4D was more important with or without 6 BA.

Prasertsongskun and Songklanakarin, (2004) isolated protoplasts from cell suspension from inflorescence of Vetiver of Surat Thani germplasm. They found that optimum medium for this experiment was 2% cellulose Onozuka R 10, 2% macerozymes R 10, O.5 % pectinase in 0.4 M mannitol and 7mM $CaCl_2$, $2H_2O$ at pH 5.8. The tissue was incubated for 10 h in the dark at 50 rpm on rotary shaker. Maximum protoplast yield was at rate 8.4 X 10 ^{4} protoplasts/ml PCV. Protoplast division was also observed but only in liquid medium. They found average division 5.0 % in the N6 medium supplemented with 1.0 mg/l 2,4D and 0.5 mg/l BA and optimum density for culture division was 1x 10^5 protoplasts/ml. They also noticed that first cell division started three days after culture initiation.

Be *et al.*, (2008) developed a low cost micropropagation method for Vetiver grass. They found that the liquid MS medium is best for multiplication when supplemented with 2-4mg/l BA. They carried out proliferation and rooting in net house rather than in growth chamber and found 100% survivals after 10 weeks. These plants were cheaper than tissue culture raised plants. They also noted that the quality of both kinds of plantlets growing under different conditions was similar.

Leupin (2001) attempted to obtain essential oil variants via tissue culture. He successfully regenerated a callus after 18 weeks in 55% of the crown slices and 60% of the leaf slices resulting in about 100 plantlets per slice. He used different concentrations of growth regulators like 2,4D and 6BA and by adding different concentrations of sucrose with different environmental conditions like light and dark. He found that starting from *in vitro* plantlets upto harvesting, 15-22 months are required. He further extracted oils from roots using different methods and investigated composition of Vetiver oil of different variants raised through tissue culture with TLC and GC. Prasertsongskun *et al.*, (2002) cultured inflorescence of Vetiver and obtained cell suspension culture and further subcultured on a selection medium which contained a herbicide, Glufosinate (ammonium-DL homoalanine-4-yl (methyl) phosphinate). They selected Glufosinate resistant cells which had I_{50} value as $4.2X\ 10^{-5}$ and which could grown in medium containing $5X10^{-5}$ glufosinate.

Antibacterial, antifungal, antipest, antitermite activities

Antibacterial activities

Antibacterial activity of some essential oils was studied by Gangrade (1990). They used three essential oils-*Cymbopogon martini* var *motva* (palmarosa), *Pimpenella anisum* (anise) and *C. zizanioides* (L.) Roberty (Vetiver) with different dilutions in dimethyl sulphoxide (DIMSO) and studied them against *Staphylococcus aureus, Streptococcus pyogens, Escherichia coli* and *Corynebacterium ovis*. They used penicillin and streptomycin as standard inhibitors. They found that Vetiver oil and its dilutions with DIMSO (1:1000) were highly effective in retarding the growth of *Staphylococcus aureus* as compared to other oils, while at higher dilutions (1:1000), Vetiver oil and palmarosa oil had similar effect on the growth of *Streptococcus pyogens*. All diluted and pure essential oils showed 45% inhibition of growth of *E. coli*. Luqman *et al.*, (2005) detected antibacterial activity in spent roots of two

genotypes (gulabi and KS-I) of Vetiver.They studied antibacterial acticivity against drug- resistant strains of *Mycobacterium smegnatis* and *Escherichia coli* using disk diffusion and micro broth dilution method. They used inflorescence, intact roots and spent roots in hexane extracts and hydro distillation extracting the essential oils from *C. zizanioides* (L.) Roberty in two genotypes. They found that hexane extracts of intact roots and spent roots after distillation of both varities were causes potent activity against the *M. smegmatis* and *E. coli*, at concentration of 0.5mg/ ml to 60mg/ ml. The extracts of inflorescence did not show any antibacterial activity. They also observed that the Vetiver cv gulabi had better inhibitory activity than cv KS-I. Putiyanan *et al.*, (2005) demonstrated antimicrobial activity of *C. zizanioides* (L.) Roberty against *Pseudomonas aeruginosa* ATCC 278533, *E. coli* ATCC 25922 and *Staphylococcus aereus* ATCC 25923. They used six varieties of Vetiver grass and found that Pong-Bong variety was most effective as compared to other ecotypes.

Antifungal activity

Antifungal action of some essential oils against animal pathogen was tested by Dikshit and Husain, (1984). They used 28 different types of essential oils of different plants including *C. zizanioides* (L.) Roberty and tested against fungi like *Microsporum gypseum, Trichophyton equinum* and *T. rubrum.* They observed toxicity effect of Vetiver oil on all the tested organisms. Gangrade *et al.*, (1991) tested the effect of three essential oils extracted from aniseed (*Pimpinella anisum*), seeds of Palmarosa (*Cymbopogon martini* var motia) and roots of *C. zizanioides* (L.) Roberty (Vetiver) in pure and diluted form (four dilutions with dimethyl sulphoxide 1:10, 1:100, 1:1000 and 1:10000) against *Aspergillus niger, A. flavus, Fusarium oxysprum* and *Penicillum spp.* They used cyclohexamide and hamycin as standard inhibitors. They observed that upto 70-80% inhibition of all pathogens occureed in response to 3 essential oils in pure form, as compared to standards. These activities were reduced below 50% after 1:10 dilution of oils with dimethyl

sulphoxide.Cho *et al.*, (2002) studied fungicidal activities of 67 herb derived oils with respect to six phytopathogenic fungi (*Pyricularia grisea, Phytopthora infestans, Puccinia recondite*) etc. They noticed that Vetiver oil displayed a strong fungicidal activity against *Puccinia recondite.* Putiyanan *et al.*, (2005) demonstrated antifungal activity of six varieties of *C. zizanioides* (L.) Roberty against *Trichophyton mentagrophytes.* They found Pong-Bong variety is most effective as compared to other ecotypes.

Antipest activety

Effect of South Indian variety oil against the immatures of *Culex quinquefasciatus Diptera Culcidae* SAY was studied by Murty and Jamil, (1987). To study the larvicidal activity against the potential filariasis carrier *Culex quinquefasciatus* SAY 2nd, 3rd and 4th instar larvae was studied under lab conditions. They used these results for comparision with detested mosquito species as a control. Udomporn (1996) studied the effect of root extracted substances from Vetiver grass on black moth- *Plutella xylostella* L. They followed two methods- Topical application (contact poison) and feeding method (stomach poison) and tested with 3rd instar larvae of diamond black moth. They studied the mortality regularly for four days. The higher concentrations (100%) of extract killed (37.14% and 51.52%) larvae by contact poisoning and stomach poisoning method respectively. Treatment with lower concentration (below 30%) of root extract did not show any difference in mortality.

Antitermite activity

Chen *et al.*, (2003) studied termiticidal activities of Vetiver oil against the formosan subterranean termite- *Coptotermes formosanus.* They found that at low concentration of oil (5g/g sand) termite tunneling was decreased and at higher concentration of oil (25g/g) sand entirely inhibited the termite tunneling. (Zhu *et*

al., 2001) studied the comparative effect of eight essential oils of Vetiver grass, cassia leaf, clove bud, cedar wood, *Eucalyptus globulues*, *E. citrodora*, lemon grass and geranium on this termite species. They found that along with clove ($50g/cm^2$), Vetiver oil at (5g/g concentration) decreased the activity of termite tunneling, but at 25g/g Vetiver oil concentrations there was total absence of tunneling and paper consumption by termites. Nix *et al.*, (2006) studied the effect of Vetiver oil against Formosan subterranean termites. They used 25% Vetiver root mulch treatment and observed decreased tunneling, wood consumption and increased mortality of termites. Zhu *et al.*, (2001) studied the effect of Vetiver oil constituent- nootkatone on the *Coptotermes formosanus* subterranean termite. They found that the concentration of nootkatone even below 100µg/gm substrate could increase the mortality of termites upto 90%.The effect of nootkatone and disodium octaborate tetra hydrate on *Coptotermes formosanus* termite and its symbiotic fauna like protozoa was studied by Maistrello *et al.*, (2002).Extract of population of termite tunneling, feeding activity, survival and symbiont protozoa were the parameters selected. They found the nootkatone gave better result than Vetiver oil and disodium carbonate with respect to termite control. Further it was noticed that nootkatone remained viable for more than 12 months as compared to Vetiver oil. According to these workers nootkatone will become a promising natural alternative to control invasive pest.Vetiver oil, termite repellant and toxicant have been patented in USA on the basis of the key role of nootkatone as termite repellant and toxicant.

Medicinal use

Vetiver is well known as an aromatic grass because this plant contains some chemical constituents having fragrances. But alongwith this character, it has many medicinal uses. Chomchalow (2001) reviewed the medicinal uses of Vetiver in different nations. In India, various tribes used different parts of this grass for

case of mouth ulcers, boils, epilepsy, burns, snake bite, scorpion strings, rheumatism, fever, headache etc. Paste of fresh roots is used in burns, snake bites, scorpion strings while decoction of the roots used as tonic for weakness used by Santhal tribe of West Bengal and Bihar. The Lodha tribe of West Bengal region uses the root paste against headache, rheumatism and sprain while stem decoction is used in Urinary tract infection. The tribal people of Mandla ThBastar from Madhya Pradesh uses juice of leaf which is used as anthelmentic and it is also used for boils, burns, epilepsy, fever, scorpion string, snake bite and mouth sour while the root extract is taken for relief from cure of headache and toothache. Tribals from Varanasi are found to apply root vapour for malarial fever. Oranon tribe uses root ash of Vetiver against acidity. In Indonesia, Vetiver roots are used for rheumatism, for this purpose they use mixture of ¼ handful Vetiver roots, 10 leaves of betel pepper, 15 leaves of Gandarasa, 10 leaves of Glentang Warak, 10 cm of Canar Babi root, 20 cm of Kelingtang root, which is ground and mixed with calcium oxide. In Pakistan, Vetiver is used as medicine against cardiac debility, palpitation, fainting, fever, inflammation, irritability of the stomach, cholera etc.Vetiver roots mixed with lotus seeds are used in polydipsia in children. In Senegal, the Vetiver is boiled with rice is found to relaxe and relieve stress and it is widely used there. It is also used in skin care, wounds, open sores etc. In SriLanka Vetiver is employed for skin care with sandal wood cure of urinary tract infections, urinary calculi and for cooling the body etc. In Thailand, roots of Vetiver are utilized to improve functioning of heart, nourishing blood, treating nervous disorders, it is also used in fever and fainting and disease related to bile, the gall bladder and stomach. Sen *et al.*, (2001) observed that Vetiver grass was among the twenty medicinal plants which were commenely used against dysurea in Bargarh district of Orissa. Deshpande *et al.*, (2008) reviewed the medicinal importance of Vetiver grass. He stated that the roots of *C. zizanioides* (L.) Roberty are aromatic, refrigerant, depurative, digestive, carminative, haemostatic, haemantic, stomachic, antiemetic, febrifuge, diuretic, expectorant, emmenagogue,

anthelmintic, stimulant, alexeteric, tonic, antispasmodic and soporific These are used in pitta and vata, in burns, hyprdipsia, flatulence, colic , anemia, haemoptysis, hemorrhages, cough, stragury,dyspepsia, hiccoughs, asthma, biloious, insomnia, diarrhea, amentia, hyperhidrosis, cardiac debility, dysmenorrhoea, amenorrhoea, erysipelas, spasmodic affections, general debility, and emaciation.

Shealy (1998) mentioned that in Ayurveda, Vetiver oil was used to increase 'Pitta'and 'kapha' and to reduce 'Vatha. He also mentioned that this oil is utilized for skin care because it is antiseptic, tonic and detoxifier, helps in acne, promote skin regeneration, strengthen the connective tissue and heals the wounds and benefits in aging skin.

Sellar (1992) stated that the Vetiver oil is effective in fertifying the red blood corpuscles which are important in transporting oxygen to all parts of the system and thus revitalizes the body. In this way increased blood flow can overcome the problem of muscular aches and pains and thus useful in cases of rheumatism and arthritis. Chomchalow (2001) while describing therapeutic uses of Vetiver oil in Thailand reported that, the main action of this oil is on the nervous system and has both sedative and strengthening effect. This oil is mainly used in treatment of depression, nervous tension, debility, insomnia, and many stress related diseases. He further added that it stimulate circulatory system and hence is used as massage oil. It is used in anemia because the oil stimulates the production of red blood cells. The oil used for deep massage to relieve mascular aches and pains, sprains, rheumatism, stiffness and arthritis. It is used to balance the secretion of sebum in skin care purpose. It is an antiseptic and also used in lotions.

Chomchalow and Hicks, (2001)described the method for making Vetiver root drink or herbal drink as follows, handful of Vetiver root mixed with leaves in equal amount and then boiled in four glasses of water which is followed by boiling concentrate the liquid to a quarter of a glass.

In many parts of India, roots of Vetiver grass are washed and dried and kept in drinking water, to reduce the heat of body. It imparts cooling effect with sweet fragrance.

Pharmacognosy

Putiyanan *et al.*, (2005) attempted pharmacognostic identification of Vetiver root. They used six different varities like Surat Thani, Phimai, Wiang-chai, Pang Bong, Rachaburi and Indonesia. Their studies revealed that starch grains accumulated in both inside and outside of parenchyma cells, while oil granules and calcium oxalate crystals were also found to be accumulated inside and outside the cells. They also observed presence of large vessels, fiber cells, stone cells and sclerides. The difference was found in single hair cells (trichomes) in all six strains. Somchai *et al.*, (2008) studied pharmacognostic specification of *C. zizanioides* (L.) Roberty roots in Thailand. They used PHEAK-HOM variety and studied cross section of the root and powdered drug under microscope. They also found starch grains, variable in size in root parenchyma. Their analysis indicated that powdered drug contains 8.48% w/w water content, 10.63% w/w total ash, 9.1%w/w acid insoluble ash, 48.03% w/w water soluble extractive, 4.98% w/w ethanol extractive, 0.3%w/w foreign matter, 9.34% w/w loss on drying, 0.23% w/w volatile content and.

Pharmacology

Chatterjee *et al.*, (2005) studied effect of nourishing baby oil which contains oil of *Olea europea*, oil extracts of *Sida codrifolia, Withania somnifera, C. zizanioides* (L.) Roberty and *Aloe vera* on 1-12 months 30 babies. They observed that 15(50%) babies had a significant reduction in skin dryness after one application. After a week they found overall improvement in the skin dryness in all babies. They did not notice any type of hypersensitivity reaction like erythema

edema, pruritus or urticaria. Acute and sub acute toxicity studies of Vetiver oil on rats were performed by Tripathi *et al.*, (2006). They exposed rats for 90 days to Vetiver oil and found a hematotoxic effect on rats due to prolonged exposure. According to them it might be due to alterations in lipid metabolism of rats by Vetiver oil.

Phytoremediation

Phytoremediation is highly promising emerging technology where green plants are utilized for reduction or amelioration of the soil pollution. According to Cull *et al.*, (*http://prvn.rdpb.go.th/files/icv/CP-5-9.DOC*) the most important characters of Vetiver in this respect is its high tolerance to any soil constraint such as acidity, alkalinity, sodicity and of high levels of Mg, Al, Mn, As, Cd, Cr, Ni, Pb, Hg, Se and Zn in soil. It can also withstand higher N and P supply with high efficiency of absorbing N and P from polluted water. Due to these reasons Shu *et al.*, (2002) employed Vetiver and other three grasses for revegetation of Pb/ Zn mine tailings, in South China. These tailings contained high levels of heavy metals like Pb, Zn, Cu and Cd and low levels of major nutrient elements like N, P, K and organic matter. They used four treatments; tailings were covered with 10 cm of domestic refuse + complex fertilizer (N PK) as treatment A, 10 cm domestic refuse (treatment B), complex fertilizer (treatment C). Tailings without any addition served as control (treatment D). Four grass species (*Paspalum notatum, Cynadon dactylon, Imperata cylindrical* and *C. zizanioides* (L.) Roberty) were raised and their growth performance was evaluated of above grasses. They found that all grasses showed good response to treatment B and C and better response for A. The growth of Vetiver grass was quite normal with height 220cm and yield was 2.1kg dry wt m^{-2}. They concluded from results that *C. zizanioides* (L.) Roberty gave best results than other three species. Phytoremediation with four grasses (including *C. zizanioides* (L.) Roberty) in oil shale mined land was carried out by Xia (2004). They found highest survival rate of Vetiver upto 99% followed by

other three grasses like Bahia, St. Augustine grass and bana grass as 96%, 91% and 92% respectively. They also observed slight accumulation of Pb and Cd. Growth performance of *C. zizanioides* (L.) Roberty and *Phragmities australis* on Pb/ Zn and Cu mine tailings with manure compost and sewage sludge in greenhouse was studied by Chiu *et al.*, (2006). They found that Vetiver produced higher yield than common reed. Rotkittikhun *et al.*, (2007) studied growth and lead accumulation by the Vetiver grass with *Thysanolaena maxima* in lead accumulated soil with addition of pig manure and fertilizer under glasshouse conditions. The results showed that both these plants could tolerate high Pb concentrations in soils (10-750 mg kg^{-1}) with good growth performance but Vetiver had good biomass. Angin *et al.*, (2008) studued effect of humic acid addition on B and Pb phytoextraction by Vetiver grass. They used boron in pot culture and Pb in field and then applied humic acid solution. From results they concluded that addition of humic acid (400 kg/ ha) was very effective in this respect. Zhuang *et al.*, (2005) studied phytoextraction of heavy metals of contaminated soil using these plant species like *C. zizanioides* (L.) Roberty, *Viola baoshanensisand* and *Rumex patientia*. They found that application of EDTA in such contaminated soils improved phytoavailability of Pb and Zn. Phytoaccumulation of lead by using sunflower, tobacco and Vetiver was investigated by Benjaporn *et al.*, (2005) in hydroponics. The medium contained Pb $(NO_3)_2$ at concentration of 0.25 and 2.5 mM Pb in presence or absence of chelating agents like EDTA and DTPA. To study localization and transport of Pb, they used SEM with X- ray photometers (SEM-EDS). They noted that 17 fold greater accumulation of Pb in shoot region of *C. zizanoides* (L.) Roberty raised in 2.5 mM Pb- EDTA treatment. They found accumulation of Pb was more in *Hibiscus* than in Vetiver which was followed by tobacco. Response of *C. zizanioides* (L.) Roberty to Pb^{++} stress was studied by Lu *et al.*, (2005) using water culture technique. They found that increasing concentration of Pb badly affected the growth of Vetiver. Phytoremediation of induced lead toxicity was improved in

Vigna mungo (L.) Hepper when intercropped with Vetiver grass. It was further evident that roots of Vetiver plant grown in lead treated soil (9.0, 10.0 and 11.0 mg/L) accumulated lead (Gupta *et al.*, 2008).

Efficiency of arsenic removal from soil by *C. zizanioides* (L.) Roberty and *V. normalis* (Balansa)A. canus was tested by Srisatit *et al.*, (2003). They applied five concentrations of sodium arsenate as 50, 75, 100, 125 and 150mg As/ kg soil in pots. They studied that the plant growth and arsenic accumulation in leaves, stem and root of both plant species. They found that in *C. zizanioides* (L.) Roberty more amount of arsenic was accumulated than *V. normalis*. The study of Truong and Baker, (1998) revealed that growth of *C. zizanioides* (L.) Roberty was decreased by 250mg As/kg soil, due to high accumulation of arsenic in different plant parts, mainly the leaves. Singh *et al.*, (2007) found that *C. zizanioides* (L.) Roberty could tolerate and grow in soil with 500 mg/ kg of As. But at higher concentration of As (1000-2000mg/kg), the plants were died. They also observed decrease in soil As concentration from 500mg/ kg to 214 mg/ kg after six months of phytoextraction by Vetiver. Wong *et al.*, (2007) studied the extent of mycorrhizae association with Vetiver grown in Pb/ Zn contaminated soils. They observed that the Pb concentration in soil is negatively correlated with mycorrhizal association of Vetiver. They noticed that mycorrhizae could reduce the toxicity effect of Pb and Zn on *C. zizanioides* (L.) Roberty. Phytoextraction studies of ^{137}Cesium and ^{90} Strontium from solutions and low level nuclear wastes by *C. zizanioides* (L.) Roberty was carried out by Singh *et al.*, (2008). They used ^{90}Sr and ^{137}Cs (5 X 10 3 k Bq/l) and found 94% removal of ^{90} Sr and 61% removal of ^{137}Cs from solutions after 168h by Vetiver. They also noticed more accumulation of ^{137}Cs and ^{90}Sr in root and shoot respectively. They found that potassium application reduced the uptake of ^{137}Cs and Calcium caused ^{90}Sr accumulation by *C. zizanioides* (L.) Roberty. Copper tolerance of Vetiver grass Elephant grass (*Pennisetum purpureum*) and the upland reed (*Phragmites australis*) in soil culture was studied by Liu *et al.*, (2009). They studied changes in

dry weight, Cu content, chlorophyll content and photosynthesis rate. They found that Elephant grass showed better tolerance followed by Vetiver grass and upland reed respectively.

Influence of heavy metals and soil amendments on Vetiver grass grown in Zinc mine soils was studied by Roongtanakiat *et al.*, (2009). They used amendments like compost and chelating agents such as EDTA and DTPA, and then raised Vetiver in mine area. They found that compost did not influence any growth character of Vetiver while EDTA increased the efficiency of uptake of Zn, Mn and Cu except Fe by Vetiver while DTPA increased the concentration of these heavy metals, but not their uptake. Both amendments did not affect Mn and Zn translocation. In short, they concluded that EDTA was highly useful for soil amendment in Zn mine soils with respect to phytoremediation.

Potentials of Vetiver for phytoremidiation of petroleum hydrocarbon contaminated soils in Venezuela were evaluated by Brandt *et al.*, (2006). They found that Vetiver could grow in crude oil contaminated soil at 5% (w/w) concentration but this plant was unable to biodegrade the crude oil in soil. But they noticed that this plant has potential to grow and adopt in such toxic environments with high rates of tillering. They observed reduction in fine root number with higher root diameter in plants raised on contaminated soils. Paquin *et al.*, (2002) studied removal of polycyclic aromatic hydrocarbons (PAH) by Vetiver grass. Vetiver grass shows high level of tolerance to herbicide and pesticide such as Diuron or Atrazine herbicide at concentrations up to 2000mg/l level (http://prvn.rdpb.go.th/files/icv/CP-5-9.DOC). Markis *et al.*, (2007) observed high uptake of mutagenic and carcinogenic substances 2, 4, 6 trinitrotoulene by Vetiver grass. They observed that in hydroponics with 40 mg TNT l^{-1}, the normal growth of Vetiver was not affected for 8 days and no toxic effects were observed, also noticed affinity of Vetiver for TNT.

Wastewater treatment

Vetiver grass is also employed for the purpose of wastewater treatment. Li *et al.*, (2006) noticed the degradation of benzo (a) pyrene [B(a)P] with increase in experimentally contaminated paddy soils by Vetiver grass. They observed disappearance of B (a) P with increase in soil microbial biomass in contaminated soil as well as in flooded soil by application of Vetiver grass.Kantawanichkul (1999) noticed that *C. zizanioides* (L.) Roberty and *Cyperus flabelliformis* Rotts were good candidates for treating domestic wastewater as well as wastewater. They can construct wasteland in tropical areas with Vetiver grass under hydraulic and organic loading rates within 121 mm/d and 191 kg COD/ ha d^{-1}, respectively. Liao *et al.*, (2003) tested abilities of *C. zizanioides* (L.) Roberty and *Cyperus alternifolius* for pig farm wastewater treatment. They found that both these grasses influenced the COD, BOD and NH_3-N forms from pig farm wastewater - COD 825 mg/l, BOD 500mg/l and NH_3-N 130 mg/l. They also noticed higher content of phosphorus in Vetiver grass than *Cyperus*. Lavania *et al.*, (2004) emphasized the importance of all Vetiver system technologies for water quality improvement and environment enhancement in different situations. Roongtanakiat *et al.*, (2007) studied removal of heavy metals from industrial wastewater by 3 ecotypes- KamPhaeng phet 2, Sri Lanka and Surat Thani Vetiver. Wastewater sources were milk factory (w1), battery manufacturing plant (w2), electric lamp plant (w3) and an ink manufacturing unit (w4).They observed that Vetiver could grow well in all these types. Growth was best in milk factory wastewater while poor growth was evident in ink manufacturing factory due to contamination of Mn, Fe and Cu. They also concluded from the results that uptake of Zn and Fe by Vetiver was more in treatment w1 while uptake of Mn and Cu was more in w4. Toxic effect of Cu on root growth was noticed. According to them among these 3 ecotypes Sri Lanka was better with higher heavy metal removal efficiencies. Laxmanperumalsamy (2008) studied the application of Vetiver for water restoration. In Noyyal River, continuous toxic effluent discharge from dying and

bleaching units increased toxicity of that river which was situated in Tamil Nadu. They suggested that Vetiver technology could be applied in such domestic as well as dye contaminated wastewater.

Smeal *et al.,* (2003) studied application of Vetiver system for industrial wastewater treatment and disposal at Gelita Apa, Qeensland, Australia. They noticed that Vetiver requires less land sustainable irrigation in both N and effluent volume than the *Pennisetum clandestinum* and *Chloris Guyana*. Truong (2008) has highlighted the utility of Vetiver system (VS) for prevention and treatment of polluted water. He mentioned that VS can reduce the volume or dispose polluted wastewater by following ways- seepage control, land irrigation and wetland. The VS can minimize the pollution from domestic and municipal sewage effluent, landfill leachate, wastewater from animal farm, industrial wastewater and mining seepage. VS can improve wastewater by trapping debris, sediments and particles, by absorbing particles ex. nutrients, heavy metals, agrochemical detoxification etc. According to him VS reduces pollution and leads to rehabilitation and phytoremidiation. He pointed out that nowadays the Vetiver grass is used in computer modeling to treat industrial wastewater.

Soil Erosion

One of the important role of Vetiver grass recognized in recent years is related to prevention of soil erosion. Truong (2000) mentioned various characters of Vetiver grass which are responsible for capacity of Vetiver to aid water and soil conservation. According to him Vetiver can grow in extreme climatic variations such as prolonged drought, flood, submergence and extreme temperature from -22 to 60^{o}C He also mentioned that Vetiver can be considered as a nurse plant on disturbed lands. This plant can also be easily irradicated by simple spraying glyphosate herbicide or by uprooting by hand or by machine.

Truong (2000) summarized adaptability range of Vetiver in Australia and other countries (Table no. 1) in following words-

Table No. 1. Adaptability Range of Vetiver in Australia and other Countries

Adverse soil conditions	**Australia**	**Other countries**
Acidity	pH 3	pH 4.2(with high level solubleA1)
Aluminium level(Al sat %)	68-87%	80-87%
Manganese level	>578 mg kg^{-1}	
Alkalinity (highly sodic)	pH 9.5	pH 10.5
Salinity (50% yield reduction)	17.5 $mScm^{-1}$	
Salinity (survived)	47.5 $mScm^{-1}$	
Sodicity	48%(exchange Na)	
Magnesicity	2400 mg kg-1(mg)	
Heavy metals		
Arsenic	100-200 mg kg^{-1}	
Cadmium	20 mg kg^{-1}	22 mg kg^{-1}
Nickel	50-100 mg kg^{-1}	
Chromium	200-600 mg kg^{-1}	
Copper	35-50 mg kg^{-1}	174 mg kg^{-1}
Mercury	>6 mg kg^{-1}	
Lead	>1500 mg kg^{-1}	3123 mg kg^{-1}
Selenium	>74 mg kg^{-1}	
Zinc	>750 mg kg^{-1}	3418 mg kg^{-1}
Lattitude	$15^{O}S$- $37^{O}S$	$41^{O}N$- 380S
Altitude		2800m
Annual rainfall (mm)	450-4000	250-5000
Frost(ground temp.)	-11 ($12^{O}F$)	$-22^{O}c$(7.6^{O} F)
(soil temp.)		$-10^{O}c$ ($14^{O}F$)
Heat wave	$45^{O}c$ ($113^{O}F$)	$60^{O}c$ ($140^{O}F$)
Drought (Without effective rain)	15 months	
Fertilizer		
Vetiver can be established on very infertile soil	N & P(300kg/ha DAP)	N and P farm manure
Palatability	Dairy cows, cattle, hors	Cows, cattle, goats,
	Horse, rabbits, sheep	Sheep, pigs, carp
	kangaroo	

Nutritional value	N= 1.17 %	Crude proteins3.3%
	P= 0.17%	Crude fat 0.4%
	K= 2.2 %	Crude fibre 7.1%

Dalton *et al.,* (1996) developed Vetiver grass hedge to control soil erosion on a cropped flood plains and made assessment of hydraulic characteristics of Vetiver grass and carried out number of trials at various discharges and depths. From the results, they calculated the maximum Vetiver grass hedge spacing required to control soil erosion. Vetiver and other grasses hedge grow and mulch was tested for soil conservation capacity under field stimulated rainfall by Rodriguez *et al.,* (1993). They found that Vetiver grass and ferns were more efficient hedge grows than lily and lemongrass, due to their highly dense vegetation structure. On the other hand, study of Misra *et al.,* (1997) *Cymbopogon martini* was superior to *C. zizanioides* (L.) Roberty as vegetative hedge because of its higher soil conservation value.

Le Viet Dung (2003) reported that establishment of Vetiver hedge rows has provided effective soil erosion control measure and bank stability in Mekong river Delta against current and wave erosion caused by motorized boats in fresh water, brackish water, rivers and canals as well as sulphate acidity.

For the stabilization and protection of infrastructure (roads, rail roads and building sites) Vetiver system is proven effective, efficient and low cost when compared to other 'hard' engineering alternatives (cement + rock and steel) e.g. Xie (1997) mentioned the use of Vetiver for highway stabilization in Jian Yang County. Further Vetiver system protects ponds, reserviours and river banks from erosion caused by wave action. It strengthens earthen dams against collaps and it reduces maintainance costs and ensures the integrity of dam walls, canal and river banks and drains. Jaspers-Focks *et al.,* 2006 mentioned the usage of Vetiver for the protection river bank.

It is evident from the foregoing account that besides providing a fragrant pleasant essential oil through its root system, Vetiver grass has got several other important advantages for mankind. However, review of literature indicates that in spite of its great value, relatively little work has been done on physiological aspects of this grass species.

Other uses of Vetiver grass

Essential oil in perfume industry

Vetiver is highly repeated for the essential oil present in its root system. Vetiver oil is widely utilized in India, in preparation of scents (attar) quite before the rose scents. About 250 tons of Vetiver oil is produced annually in different regions of the World. The oil is produced on large scale in Indonesia (Java), China, India, Brazil, Japan, Haiti and the main consumers are USA, Europe, India and Japan. Oil produced from Haiti and Indonesia has the best quality. The aroma of Vetiver oil is basically heavy, woody having earthy character, pleasant and persistent. This oil complex mixtures of sesquiterpene alcohols and hydrocarbons and it is viscous with less volatility. This essential oil is having varied odor characters depending on source of origin. Reunion (Bourbon) and Haitian oil have a roseate note therefore regarded as best quality, while the oil from wild 'khas' of India is considered as best due to its balsamic woody note. Slow evaporation and solubility into the alcohol, which improves the miscibility with other perfume material have made Vetiver oil unique perfume source.

In India, about two year old plants are harvested for extraction of essential oil. The roots of Vetiver are obtained by manual digging. According to Chamchalow (2000); Vetiver should be grown into the poly bags and other containers, to avoid soil loss or erosion due to digging.

Different methods are followed for essential oil extraction like hydro distillation, hydro steam distillation, steam distillation, CO_2 supercritical fluid extraction etc. Lavania (2003) explained the traditional method of oil extraction-

Bhapka system, which is used in North India. It requires 4-5 h longer time as compared to other methods but it led to recover a superior quality of oil. In this method, round bottom copper still 'Deg' and receiver 'Bhapka' which is made up of copper are used. These two equipments are connected with each other by bamboo called 'Chonga' which serve passage for steam. The 'Bhapka' is put in a small water tank for cooling. The oil obtained from this method is light to dark green colored where slow fire is used. Luu (http://www.Vetiver.org/AUS_Research%20Proposal%20vet%20oil.pdf) demonstrated different methods for essential oil extraction e.g. in solvent extraction method, Soxhlet extractor is used with solvent hexane for 30g of dried roots, while in steam distillation method, modified Clevenger is employed with water as a solvent. Martinez *et al.*, (2004) also reported hydro distillation method in which they used Clevenger apparatus with water as solvent. Leupin (2001) studied various methods to obtain essential oil from variants of *C. zizanioides* (L.) Roberty obtained via tissue culture technique. He used water distillation and solvent extraction methods and observed that distillation time could be reduced by using 0.5 M phosphate buffer at pH 8 instead of water.

Synthetic substitutes for Vetiver oil are not yet available. This oil is used in both fine perfumery and in a many ranges of soaps, skin lotions, deodorants and other cosmetic applications.

Forage / livestock grazing

The leaves of Vetiver grass are also consumed as forage in some parts of World (Poathong *et al.*, 1996) and other workers verified the forage value of freshly cut Vetiver leaves. Although the grass has significant amount of toxic substances which are not harmful to the livestock.

Decorative hedge

Vetiver grass is used for decorative purpose in hedges.It is also used as beautiful ornamental plant for gardens, patioc, decks etc

Agriculture related activities

Vetiver is used in mulching. The grass is also utilized as compost and with humic acid, it enhances soil fertility. Due to chemical constituents like cellulose, hemicellulose, lignin and crude proteins with various minerals it is used as a supporting medium in mushroom cultivation. Chamchalow (2000) highlighted the traditional use of Vetiver grass for pesticide activity.

Handicrafts

From leaves and culms of Vetiver grass , different types of handy accessories like bags, hats, belts, brooches, containers like baskets, pots, boxes, utility bowls; decorative materials like clocks, picture frames, lamp shades, dolls, animal figures, flowers; home appliances such as chairs, stools, room partitions, tables are prepared.

In India, dried Vetiver roots are woven into 'pardaa' or 'tatti' and sprinkled with water in summer which provides a cool and fragrant air. Vetiver roots are also used to make fans, cloth hangers etc.