Plant cell culture is being considered as a promising technology not only for secondary metabolites production but also for mass production of plantlets. Production of plantlets through plant cell culture on an industrial scale is expected to be an alternative source for supplying seedlings to agriculture in the coming years (Morita et al., 1999).

In the application of biotechnological procedure for crop improvement, one of the limiting factors has been the lack of consistent, stable and dependable regeneration of whole plants from callus or cell suspensions in tissue cultures. Regeneration of intact plants can be obtained by callus cultures, organ culture or cell suspension cultures leading to organogenesis or embryogenesis. Maximum callus production will be of potential use in setting up an experimental system, which will be suitable for basic biochemical studies. In general, Cytokinins as a class of growth promoters appear to have some role in stimulating bud or shoot formation and growth where as auxins tend to favor root differentiation in cultures capable of undergoing organogenesis. Cytokinins and auxins thus frequently appear to be antagonistic to each other at least at physiological levels where either one may inhibit or reduce the commonly elicited morphogenetic response of the other. Differences in growth under in vitro conditions are due to the physiological status of the explants (Yoeman and Forsche, 1980; Lindsey and Yoeman, 1986). There can be a gradient of metabolites and other substances responsible for the differentiation response even with in an
Plant growth regulators are required to induce and establish callus from an explant (Kordan, 1959; Yeoman and Forsch, 1980).

Skoog and Miller (1957) found that shoot formation could be induced predictably from tobacco callus using relatively low levels of auxins and high level of cytokinin in the growth medium. Since this discovery, many aspects of cellular differentiation and organogenesis in tissue and organ culture have been found to be controlled by an interaction between cytokinin and auxin concentrations. Tissues from monocotyledons can be often induced for callus formation by culture in high levels of auxin alone, and cytokinin may be inessential or unimportant. (George, 1993)

Cereals and grasses are propagated efficiently and cheaply from seeds, so that vegetative multiplication is not required except for certain specialized purposes such as the production of clones of individual plants for evaluation and uses as seed parents in breeding programs. Vegetative propagation of annual cereals by traditional means has been either difficult or impossible and although perennial grasses can be increased by division, cuttings or tillers, using these techniques to obtain large number of plants from a single selection can take a long while. Hence, micropropagation is suitable for raising plants of cereals and grasses (George, 1996)

Callus cultures of cereals and grasses can only be initiated from tissues that are young and meristematic. Explants are derived from root tips,
nodes, shoot primordia, seeds and seed embryos and also from young leaf tissue or from immature inflorescences, (Yamada, 1977). Only newly initiated leaves or the basal parts of actively growing leaves are capable of callus proliferation. And as leaves age, their tissues rapidly lose the capability; (Wernicke and Brettel, 1980; Zamara and Scott, 1983). The leaves of graminaceous plants grow from basal meristems and so perhaps only actively dividing leaf cells may be competent to go on growing and dividing \textit{in vitro} (George, 1996).

In the earlier studies with cereals and grasses, among the various explants used for the induction of regenerable callus cultures, young seedlings are the most available donor material since they can be grown \textit{in vitro} and short term frequent supply of explants can be provided (Jahne-Gartner and Lorz, 1996).

In the present investigation the two main basal combination tried were MS and CHU. Out of these MS was found to be suitable for all the three plants selected for the present study. Earlier reports show that in gramineaceous species, MS medium was sufficient for the induction of callus and regeneration (Vasil and Vasil, 1984; Eapen and Rao, 1982). The source of explants has been found to be important for inducing calli. Mature embryos, seedlings, mesocotyl portions, young inflorescences and leaf explants gave rise to embryogenic calli, while rhizome, root and culms gave rise to non-embryogenic calli (Vasil and Vasil, 1981; Sreenath and
Jagadishchandra, 1991). In the present study also, out of the various explants tried for callus induction in the three plants, the mesocotyl segments and immature inflorescences were found to be good for callus induction in Vetiver, *Cymbopogon flexuosus* and *C. citratus* respectively. The basal portion of the stem, young leaves from mature plants and culms didn’t respond well to any of the media combinations tried. They instead released a leachate into the medium, probably of phenolics in nature. In Vetiver, the basal portion of the stem induced very little callus. However, later the growth was retarded due to the accumulation of the leachate in the medium. Similar observations were made by Mathur et al. (1988) in *Cymbopogon winterianus*. In *C. citratus*, immature inflorescence were found to be best for induction of morphogenic calli, while in *C. flexuosus* and Vetiver the mesocotyl portion of 3-4 days old seedlings was found to be suitable.

The callus cultures of cereals and grasses have been found to have different morphogenetic capacity as those derived from broad leafed plants. Somatic embryo formation was very rare and even indirect shoot regeneration was not commonly or readily obtained in grasses. Cultures producing shoots generally gave rise to only a small number of plantlets and their morphogenetic ability soon disappeared, especially if the density of meristematic primordia was too greatly reduced upon subculturing. King et al., (1978) conjectured that shoots regenerated from most cereal and grass cultures may not have arisen adventitiously, but may have been derived from
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shoot primordia which were pre-formed in the original explants and were stimulated to grow and divide under invitro conditions.

MS medium augmented with 1mg/l 2,4-D and 0.1mg/l KN was found to be suitable for initiation of pale yellow nodular calli having morphogenetic potential in C.flexuosus. Vasil (1987) reported that addition of lower level of KN (less than 0.5 mg/l) in 2,4-D containing medium was beneficial for callus growth and development in grass species. Inclusion of low concentrations (0.1-0.3 mg/l) of BA in the induction, growth and regeneration media strongly promoted the callus growth in Kentucky bluegrass (Vander Valk et al., 1995) In C. flexuosus also addition of lower concentration of KN (0.1 mg/l) in 2,4-D containing medium stimulated and enhanced the growth of the calli. The calli become pale yellow and nodular in nature.

In Vetiver however, the results were different. Here, the concentration of 2,4-D and KN remained same (1mg/l each). The addition of 2,4-D and KN at the same concentrations produced morphogenic calli. This is in contrast with the findings of the present investigation with the regeneration pattern of C.flexuosus, another aromatic grass using mesocotyl explants. In C.flexuosus lower concentration of KN was enough for proliferation of morphogenetic calli. From these studies it can be concluded that, the response of same type explants obtained from different members of the same family differs with respect to their genotype.
Callus induction and regeneration from seedling as well as seedling parts have been reported in various plant species of both dicots and monocots. Regeneration and somatic embryogenesis from hypocotyl explants of groundnut (Venkatachalam et al., 1997), micropropagation of curry leaf tree using seedling explants (Bhuyan et al., 1997), plant regeneration from coleoptile tissues in rice (Oinam and Kothari, 1995), plant regeneration in Sorghum from hypocotyl explants (Gendy et al., 1996), plant regeneration in Kentucky bluegrass via coleoptile tissue cultures (Kee and Lee, 1996), shoot regeneration from invitro grown seedling of Alstroemeria (Lin et al., 1997), differentiation of multiple shoots from intact seedlings of switch grass (Dutta-Gupta and Conger, 1998), plant regeneration and multiplication of Juncus accuminatus from seedling explants (Sarma and Rogers, 1998), shoot regeneration via callus cultures from seedling explants of Typha latifolia (Dethier-Rogers et al., 1998), regeneration from seedling explants of Oat (Gless et al., 1998), plant regeneration from seedling explants of two Eucalyptus sps. (Bandhopadhyay et al., 1999), micropropagation of Spathaglottis sps. from seedling explants (Teng et al., 1997) invitro propagation of Dendrocalamus strictus via seedling cultures (Shrigurkar et al., 1996; Ravikumar et al., 1998), callus induction from the mesocotyl region of seedlings has been reported in Distichlis (Warren and Gould, 1982) and in Spartina (Li and Gallagher, 1996) are some of the earlier works.
reported using seedling as an explant source for callus induction and regeneration.

In *C. citratus* immature inflorescences were found to be highly suitable for callus induction and regeneration. MS medium augmented with 1mg/l each BA and 2,4-D was found to be suitable for induction of morphogenic calli. The floret primordia as well as spikelets were involved in callus induction. Somatic embryogenesis and plant regeneration from cultured young inflorescences of *Oryza sativa* (Tsung et al., 1985), callusing and regeneration of plantlets from inflorescences cultures of *Triticum aestivum* (Rajyalakshmi et al., 1988), plant regeneration from cultured immature inflorescences of *Cenchrum* sps (Kacker and Shekawat, 1991), plant regeneration through direct shoot development from immature inflorescences of finger millet (George and Eapen, 1990), plant regeneration from immature inflorescence of Sorghum (George and Eapen, 1988) Plant regeneration from inflorescence cultures of *Poa pratensis* (Vander Valk et al., 1989) are some of the reports on callus induction and regeneration from immature inflorescences.

Plant regeneration from immature inflorescence of *Cymbopogon winterianus* (Sreenath and Jagadishchandra, 1989) has been obtained when cultured on MS medium augmented with 1mg/l each 2,4-D and BA or KN. The same combinations were found to be efficient for callus induction in *C. citratus* also. Immature inflorescences have been recognized as an
important source of totipotent cultures in many cereals, millets and grasses (Vasil, 1982). The main routes of plant regeneration from cultured inflorescences in gramineae have been reported to be through direct shoot differentiation of floret primordia (Heinz and Mee, 1969; Dale et al., 1981; Dale and Dalton, 1983), organogenesis (Dudits et al., 1975) and somatic embryogenesis. (Brettel, 1980)

Callus maintenance medium for Vetiver was found to be MS medium augmented with 1 mg/l 2,4-D + 1 mg/l KN + 100ppm each PVP and CH. In Lemongrass the additives were found to be unsatisfactory for callus maintenance. The morphogenic potential of calli of grasses and cereals was found to be lost during prolonged cultures. The regeneration capacity was often lost within the first few subcultures, after which the calli either failed to grow or reverted to root forming cultures (Vasil, 1987). Similar observations were made in all the three plants selected for the present study after 3rd to 4th subcultures. Hence different additives were incorporated into the medium for maintaining the morphogenic potential of the callus cultures. 100 ppm each CH and PVP together was found to be essential for callus maintenance. After the addition of these substances, the morphogenic potential was found to be maintained till 7-8 subcultures, one subculture every 35-40 days.

PVP is usually added to tissue culture media to adsorb phenolics, which are leached out into the medium during the first phase of cultures.
mainly in higher plants. (Weatherhead, 1978, 1979; Bhat and Chandel, 1991; Kasthuri, 1998) CH is also used as an additive in culture media as a source of amino acids, which is found to be efficient in plant tissue cultures. This has led to the speculation that CH might contain some unknown growth promoting factor (Inoue and Maeda, 1982) CH (100-500 mg/l) was used for callus culture proliferation in *Nigella sativa* (Bannerjee and Gupta, 1976). Zaghmout and Torello (1989) reported in *Red fescue* that along with 2,4-D; addition of CH helped in developing high-density embryogenic aggregates within 2 weeks of cultures. Among the explants used for the induction of regenerable callus cultures, young seedlings are the most easily available donor material, since they can be grown ‘*in vitro*’ and short term, frequent supply of explants can be provided. (Jahne-Gartner and Lorz, 1996; Gless et al., 1998)

**Shooting medium:**

Tissue culture systems have been developed for several gramineae species using 2,4-D to stimulate callus induction in a nutrient medium, followed by reducing or eliminating the 2,4-D level for plant regeneration (Vasil, 1988; Al-Khayami et al., 1989). The efficiency and quality of regenerable callus depends on media composition. The most important component is the auxin; 2,4-D has been used most frequently in tissue
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culture of grasses. (Zimny and Lorz, 1996; Sharma et al., 1980; Armstrong et al., 1983; Jordan and Later, 1984; Stolarz and Lorz, 1986)

In the present study, for regeneration in all the three plants, 2,4-D was used to stimulate callus induction but for regeneration 2,4-D was completely avoided. In Barley callus induction and shoot regeneration was achieved only when seedlings were grown in the presence of high concentration of 2,4-D (Vitanova et al., 1995) In the present study 2,4-D was not required for regeneration. In Vetiver shooting was observed in the medium containing BA as the cytokinin. Microtillering started when the 40-50 days calli obtained from mesocotyl explants was transferred to MS basal medium, devoid of any growth hormones or MS medium fortified with 0.5 mg/l to 2mg/l BA. Addition of lower concentrations of NAA to the medium containing BA suppressed the number of shoots. Baruah and Bordoloi (1991) made similar observations in another aromatic grass C. winterianus.

In C.flexuosus also shooting was observed with the addition of BA. MS +1mg/l BA +0.2 mg/l IBA gave rise to more number of shoots. Here addition of IBA in lower concentrations in the regeneration medium increased the number of shoots while higher concentrations suppressed the number of shoots. However, in C. citratus MS+1mg/l KN was needed for shooting compared to the other two grasses selected for the present study. In nature, inflorescence of many grasses are known to become transformed into shoots instead of producing seeds (Aber, 1934) This phenomenon called
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vivipary, is a mode of reproduction in these plants. Transformation of floral primordia into vegetative shoots may be triggered by phytohormones in the culture medium (George and Eapen, 1990). Multiple shoot production from cultured inflorescences has been suggested as means of clonal propagation in grasses (Lo et al, 1980)

In the earlier reports Barthakur and Bordoloi (1989) induced shoot primordia in *C.winterianus* in MS+2mg/l BA +0.2mg/l NAA. This result is in accordance with the present study in Vetiver. In *C. martinii*, Patnaik et al., (1997) reported plant regeneration obtained from MS+1.5mg/l BA+0.2mg/l KN. Here both BA and KN were needed for shoot regeneration. In the present study however it (addition of two cytokinins i.e. BA and KN) was not required for both the species of *Cymbopogon*, shoot induction was achieved either with BA or KN. But in the same plant Baruah and Bordoloi (1989) reported plant regeneration in MS-2mg/l KN+1mg/l BA+0.1mg/l NAA. In *C.winterianus*, Sreenath and Jagadishshandra (1989) reported regeneration from young inflorescence cultures on MS +1mg/l BA. While in present study with immature inflorescence culture of *C.citratus* KN was found to be good for induction of shoots. With BA the calli greened and failed to regenerate. Nayak et al., (1996) reported plant regeneration from nodal cultures of *C.flexuosus*, here for regeneration MS+5mg/l 2,4-D +0.5mg/l KN+0.1mg/l NAA was required. In the present study with *C.flexuosus* shoots readily developed from calli derived from mesocotyl
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segments when cultured on MS medium with BA alone, here addition of 2,4-D has enhanced the callus growth. BA alone found to be sufficient for shooting. In another aromatic grass *C. winterianus* regeneration was obtained on a medium containing IAA (1mg/l) and KN (0.5mg/l), here the auxin supplied was higher than the cytokinin. In the present study NAA and IBA was used as the auxin in the regeneration medium and that too in lower concentrations (<0.5mg/l) was sufficient to enhance the shoot formation in Vetiver and *C. flexuosus*. This study revealed that the hormonal requirement for regeneration of plantlet in Vetiver largely confirms to that of other grasses and cereals (Ammirato et al, 1984)

In the earlier studies on Vetiver, Mucciiarelli et al., (1993) reported regeneration on MS medium augmented with 2mg/l KN+0.2mg/l 2,4-D from calli derived from nodal explants. While in the present study regeneration was achieved in MS medium with BA and NAA from calli derived from mesocotyl explants.

Somaclonal variation:

For plant breeding experiments both genetic instability and stability are important. Instability is desired in order to obtain genetic variation, but very high degree of variation resulting from multiple events may be difficult to handle in a breeding experiment and require extensive cross breeding. On the other hand stability of cultures and regeneration is essential for most
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stages in breeding programs for commercial propagation of established cultivars and for maintenance of germplasm. The mechanism of somaclonal variation is still not clear, but generally it is linked to the passage of disorganized growth of callus. The most commonly observed morphological variations are plant height, morphology of leaves and whole plants, fertility, flowering pattern etc. (Zimny and Lorz, 1996; Brettel et al., 1986). The use of ‘in vitro’ culture combined with somaclonal variation for the isolation of potentially disease resistant phenotypes has been demonstrated in a number of plants (Wenzel, 1985; Chawla and Wenzel, 1987). Moon et al., (1997) isolated somaclonal variants tolerant to Aluminium in Maize. Mathur et al., (1988) had isolated somaclonal variants in Cymbopogon winterianus having broad leaves and with improved oil quality and quantity. In the present study with C. citratus a somaclonal variant having broad, longer leaves and better growth rate could be observed compared to normal plants obtained through tissue cultures. The variant produced long and healthy roots within 3 days of culture in a hormonal free medium.

Rooting medium:

Rooting of shoots, irrespective of different explants used for induction of shooting, depends as the composition of the culture medium. In C. flexuosus half strength MS basal medium was sufficient for rooting of the healthy shoots, while in C. citratus 0.2-0.5 mg/l NAA or IBA was essential. But in the somaclonal variant of C. citratus MS basal medium was sufficient
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for rooting. In earlier studies with *C. winterianus* rooting was observed with 1mg/l IAA (Mathur et al., 1988). Baruah and Bordoloi (1989, 1991) obtained roots from shoots cultured on half strength MS basal medium or in full strength Whites medium supplemented with 2mg/l IBA or NAA. In the present study, either MS basal medium or MS medium with lower concentrations of auxins was found to be sufficient for rooting in all the three plants. Whites rooting medium tried for all the three plants was found to be giving poor response than MS basal medium. In the present study, higher concentration of auxins (>0.5 mg/l) produced calli at the base of the shoots. In some cases roots emerged from the calli having no connection with the shoots.

In Vetiver riboflavin was added to suppress the growth of the calli after the addition of NAA (from 0.05-0.5mg/l). Riboflavin (Vit, B₂) has been found to inhibit the formation of callus at the base of the shoots, which occurs either with or without the addition of auxins into the medium. The absence of callus may then have either a promotive or inhibitory effect of adventitious root formation, depending on the plant species. (George, 1996). Miller et al., (1982) found that Stabs mixture of vitamins prevented the rooting of a peachroot stock for which Riboflavin was responsible. Rooting of *Eucalyptus ficifolia* shoots was prevented by adding 3.8 mg/l Riboflavin in to the regeneration medium, because it stopped IBA-induced basal callus formation which was an essential requisite for rooting. (de-Fossard et al., 73)
1978; Gorst et al., 1982). Riboflavin increased the rooting frequency in Carica papaya shoots in both light and darkness. It promotes root and shoot growth and decreases the formation of callus so that roots are more likely to be formed (Drew, 1978; Drew et al., 1993) In the present study in Vetiver, by the addition of 10mg/l RBF into the rooting medium (MS+0.1-0.5mg/l NAA) no callus formation at the base of the shoots was observed. This corroborates the view of Drew et al., (1993) that Riboflavin promotes root growth and suppresses callus formation.

Acclimatization:

Acclimatization is the final, but necessary, step in all micropropagation schemes. During these processes plants have to adapt to the new environmental conditions such as lower relative humidity, higher light intensity, fluctuating temperatures and constant disease stress (Preece and Sutter, 1991; Van-Huylenbroeck and Debergh, 1996). Successful micropropagation depends on the efficient establishment of plantlets ex vitro. Acclimatization of in vitro plantlets is often difficult because they possess succulent stems and leaves due to the high humidity within the culture vessel and free water in the medium. (Thomas, 1998; Grout and Aston., 1978; Pierick et al., 1988; Read and Fellman, 1985; Ziv, 1991) Besides, poorly developed cuticle (Dhawan and Bhojwani, 1987; Sutter and Langhans, 1979), reduced differentiation in palisade cells (Brainerd et al., 1981; Dami
and Hughes, 1995; Grout, 1978), large intercellular spaces (Brainerd et al., 1981; Dami and Hughes, 1995) and defective stomata (Blanke and Belcher, 1989; Sutter et al., 1988; Werker and Leshem, 1987) lead to rapid water loss and desiccation once exposed to ambient conditions (Bhojwani and Dhawan, 1988; Ziv, 1986). The transition from in vitro to ex vitro is a critical phase required to make the largely heterotrophic plantlet into a completely autotrophic one through photosynthesis and preparing it to withstand the usual water loss by development of protective layers and regulation of stomatal functioning. The use of increased light intensity and gradual reduction in relative humidity has been demonstrated to facilitate ex vitro establishment. (Driver and Suttle, 1987; Marin and Gella, 1988; Preece and Sutter, 1991). In the present study a similar approach was made and all the rooted plantlets were transferred to a small poly-house to maintain high humidity for 12-15 days.

Comparative studies of the essential oil:

Essential oils consist of mainly terpenoids, particularly the mono, di and triterpenoids. In Lemongrass, the oil quality increases with the increase in terpenes like citronellal, citronellol and geraniol. Where as the presence of elemol, another terpenoid is considered as a negative aspect (Mathur et al., 1988). Therefore plants producing essential oils with higher citronellol, citronellal and geraniol and lower elemol contents are considered as a
superior varieties of high economic importance. In the present study it has been found that a new component is present in the oil obtained from in vitro grown plants of *C. flexuosus*. This clearly indicates an altered metabolism in these plants due to the environmental condition. This is an interesting observation because; it may be possible in future to alter the composition of essential oils by altering their condition of growth.

**Mutation Breeding:**

*In vitro* culture is becoming a mature technology as a complementary tool to conventional mutation breeding in order to accelerate its process and make it more economic. Effective micropropagation of this biotechnology to plant breeding programmes is dependent on the plant species and breeding objectives. A stable morphogenetic system is useful either for mutation induction and selection or for rapid propagation of the potential mutants for field evaluations.

γ-ray irradiation to *Cymbopogon* sps. was found to increase the callus growth but the regeneration potential found to be reduced. (Nayak et al, 1997) In the present study in Vetiver, the calli obtained from mesocotyl parts were subjected to UV irradiation. The response was same as that of normal when compared to callus growth. 25-60 minutes of UV irradiated calli when subjected to regeneration, showed that except for delay in the onset of shoot morphogenesis, other significant variation were not induced.
In cell culture experiments for physical mutagens UV, X-rays and \( \gamma \)-rays are most commonly used. The most used are \( \gamma \)- and X-rays. UV irradiation was used very less, because the penetration power of UV is very less compared to X-rays and \( \gamma \)-rays.