Chapter: 3

Studies on the vegetative propagation of *Costus pictus*

3.1 Introduction

Plant propagation is the process of multiplying or increasing the number of plants of the same species, and at the same time perpetuating their desirable characteristics. Plants may be propagated mainly by two methods: sexual and asexual or vegetative propagation. Sexual propagation is the common method of reproduction and multiplication of plants. This is usually achieved through the production of seeds or spores. Asexual propagation is the development of a new plant without the fusion of male and female gametes and does not involve the production of seeds. Since it does not involve sex, *i.e.*, sexual reproduction, it is commonly referred to as asexual reproduction or vegetative or clonal propagation. Vegetative propagation takes place when a part of the (mother) plant is used to propagate the same. This could be achieved through stem cuttings, tubers, corms or any other part of the plant.
3.1.1 Vegetative propagation

Vegetative propagation is an irreplaceable tool for domestication and breeding, and its advantages and implications have been widely treated in literature (Wright 1976, Zobel and Talbert 1984, Park et al., 1989). Programmes involving indigenous species and impoverished communities have become important in the last decades (Leakey et al., 2005) and the development of low cost vegetative propagation technologies is one of the most relevant aspects (e.g., Tchoundjeu et al., 2004, Atangana et al., 2006). Despite the advances in tissue culture, for many conservation, domestication and breeding programmes; low cost macro-propagation methods continue to be the basis of most convenient approaches, even when human and financial resources are not scarce (e.g., Wollemia, 2007).

The plantlets produced by vegetative propagation are clonal, i.e., genetically similar to the parents. There are two methods of vegetative propagation in common use, namely, natural and artificial vegetative propagation. In natural methods, a portion of the plant gets detached from the body of the mother plant and grows into an independent plant. The parts may be stem, root, leaf or even flower. Humans have taken advantage of this natural phenomenon and have artificially propagated plants vegetatively by using the specialized parts or by the use of cuttings, grafting and layering. When we use the vegetative parts for propagating crops or ornamental plants artificially, it is termed as artificial vegetative propagation. For vegetative propagation, the use various kinds of plant growth regulators or phytohormones is common to aid and enhance the process of rooting, shooting and budding. Application of plant growth regulators, particularly growth retardants, may maintain internal hormonal balance and efficient sink-source relationships and thus enhance crop productivity (Singh et al., 1987). Phytohormones play essential roles throughout the lifespan of plants. During the past decades, plant researchers have made efforts to elucidate the biological roles and mechanism(s) of function or action of phytohormones in various plant responses (Wolters and Jurgens, 2009). And thus, the phytohormones have been known to mediate a whole range of developmental processes, as well as to interact with environmental factors.

3.1.2 Effect of phytohormones on plant growth

Plant hormones (phytohormones), also known as plant growth substances (PGSs) or regulators (PGRs) are chemicals that regulate plant growth. Phytohormones in very small
amounts are able to promote and influence the growth, development and differentiation of cells and tissues. A large number of related chemical compounds have been synthesized and are used for regulating the growth of cultivated plants, weeds, and in vitro grown plants and plant cells; these man-made compounds are generally called Plant Growth Regulators (or PGRs for short).

Phytohormones are vital to plant growth; affecting processes in plants from flowering to seed development, dormancy, and germination. They regulate which tissues grow upwards and which grow downwards, leaf formation and stem growth, fruit development and ripening, as well as leaf abscission and even plant senescence leading to death. Phytohormones have been used for many years, primarily as a tool to obtain height control or to promote rooting. Today, there are many fascinating and innovative ways to use the tools of yesterday. The efficiency and effectiveness of various phytohormones and plant growth regulators are different, based on the target part of the plant, climatic conditions etc. The activity of these phytohormones does not necessarily have similar effect on different plants or plant parts. The variability in the performance of phytohormones may be due to various environmental factors that may affect their growth and exert their effects on plant. It is reported that triacontans were ineffective in germination of lettuce seeds under high temperature conditions. Wilen et al., (1995) and Dhaubhadel et al., (1999) observed that the brassinosteroids increased the tolerance of cabbage and tomato seedlings against high temperature stress. In addition, it was found that in many plant species exposed to high temperature, there was an increase in the endogenous polyamine contents (Roy and Gosh, 1996, Bouchereau et al., 1999).

Plant growth regulators are now being used as seed soaks, bulb dips, media sprays and rooting agents, etc. Just as exciting are the new products being developed for specific tasks such as reducing leaf yellowing, promoting the growth of offsets, reducing the shattering of flowers, increasing the longevity of flowers and increasing chloroplast efficiency. The plant growth regulator revolution is in full gear (GHPN, 2000).

The appropriate selection of individual phytohormone or the right combination of different phytohormones or PGRs is vital to achieve the desired behavioural characteristics of cells and the productive development of plants as a whole. PGRs are chemicals applied by horticulturists to regulate plant growth. In plant propagation, cuttings are dipped in a “rooting hormone” to stimulate root development. In greenhouse production, many potted flowering plants (like
poinsettias and Easter lilies) may be treated with plant growth regulators to keep them short. Seedless grapes are treated with plant growth regulators to increase the size of the fruit. Phytohormones (auxins and cytokinins in particular) are also important for tissue culture.

The five main classes of endogenous phytohormones include the auxins, e.g. indole-3-acetic acid (IAA), cytokinins (CKs) e.g. zeatin, gibberellins (GAs), ethylene and abscisic acid (ABA). Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MJ), collectively named jasmonates, brassinosteroids, etc. are other natural compounds that regulate plant growth and development (Sasaki et al., 2005).

Auxins regulate many aspects of plant development by controlling cell elongation, division, and differentiation through the regulation of the expression of specific gene subsets (Woodward and Bartel, 2005). Homologous or orthologous loci for Arabidopsis auxin synthesis, and for polar transport have been characterized in maize (McSteen et al., 2007; Barazesh and McSteen, 2008; McSteen, 2010) and rice (Morita and Kyozuka, 2007), and their mutation leads to defects in floral architecture/organ initiation, thereby showing a conservation of auxin biosynthesis, transport, and signaling for lateral organ initiation across widely diverging species. Auxins also promote cell division, for example, in lateral organ formation (Reinhardt et al., 2000), and cell elongation by acid growth (Rayle and Cleland, 1992) or by stimulating expansion, which loosens cell walls (Hutchison et al., 1999), or they can inhibit cell elongation as in root gravitropism (Abas et al., 2006). The effect of ethylene to inhibit cell elongation often occurs via its effect on auxin synthesis and transport, as in Arabidopsis roots (Ruzicka et al., 2007).

In addition to auxins, cytokinins are involved in the regulation of sink-source relations and leaf senescence (Singh et al., 1992 a,b; Werner et al., 2008). Both phytohormones also act as important controllers of meristematic activity (Muller and Sheen, 2008), and current evidence confirms a crosstalk between them (Palni et al., 1988) in the establishment of endogenous levels and regulation of normal root development (Moubayidin et al., 2009; Werner and Schmulling 2009; Perilli et al., 2010). The most common CKs include zeatin (Z), zeatin riboside (ZR), dihydrozeatin (DHZ) and its riboside (DHZR), and isopentenylenzal adenine (IPA) and its riboside (IPAZ) (Letham and Palni, 1983; Wang et al., 2005). Kinetin (KN), the first cytokinin to be discovered does not occur naturally; benzyl adenine (BAP) and its riboside (BAPR), originally
known as “synthetics” are now known to occur endogenously (Nandi et al., 1989a, b). In addition, exogenous CK application to vegetative plants grown in short-day conditions can induce various cellular and molecular changes in the shoot apical meristem that are normally associated with the floral transition (Chang and Chang, 2003). CKs are also known to induce the growth of auxiliary buds leading to development of lateral shoots (Letham and Palni, 1983).

Gibberellins (GAs) are acidic diterpenes with phytohormonal characteristics that control various processes during the plant life (Crozier et al., 2000); GAs are essential for the development of stamens and petals (Koornneef and van der Veen 1980), and many mutants affected in GA synthesis have underdeveloped floral organs (Olszewski et al., 2002).

The gaseous plant hormone, ethylene, regulates a wide range of developmental processes and the response of plants to stress and pathogens (Potuschak et al., 2003). Ethylene is a plant hormone involved in a wide range of plant developmental processes, including seed germination, leaf expansion, root hair formation, fruit ripening, timing of vegetative senescence, and response to stress and pathogens (reviewed by Johnson and Ecker, 1998; Wang et al., 2005). Furthermore, ethylene regulates floral sex determination in cucumbers (Kahana et al., 1999; Duan et al., 2008). Ethylene plays a role in various physiological processes throughout the life cycle of the plant (Mattoo and Suttle, 1991; Abeles et al., 1992). Its involvement in such agronomically important processes as senescence, abscission and fruit ripening has made ethylene a target for manipulation by chemical and biotechnological methodologies (Mattoo and Suttle, 1991; Abeles et al., 1992; Schaller, 2003).

Abscisic acid (ABA) is a plant hormone generally associated with dormancy, and also involved in plant responses to stress (Dood and Davies, 2005). It has been well documented that ABA is involved in plant tolerance and adaptation to a variety of stresses (Zhang et al., 2006), as well as stomatal closure, seed dormancy, salt tolerance, flowering, and photosynthesis (Zeevaart and Creelman, 1988). Jasmonic acid (JA) and abscisic acid (ABA) are among those plant hormones, which mediate in certain types of stress responses and their action results also in a negative regulation of plant growth. ABA is involved in many aspects of water-limiting stresses such as drought, salt stress and cold (Xiong et al., 2002), whereas JA function is mainly attributed to wounding and pathogen response (Creelman and Mullet, 1997). JA and SA (salicylic acid) are major regulators of plant response to pathogen attack (Delaney, 2004). Apart
from these compounds, other chemicals like brassinosteroids, salicylic acid, plant peptide hormones, polyamines, bavistin, and even nitrogenous compounds like nitric oxide, KNO₃, etc. are used to affect plant growth.

### 3.1.3 Propagation of *Costus pictus*

Insulin plant (*Costus pictus*) is a relatively new entrant to Kerala and India. The plant, a late entrant to Kerala Ayurvedic medicinal herb scene, has come mainly from USA. The catchphrase of this plant is ‘a leaf per day keeps diabetes away’. Its propagation is by stem cutting and rhizome. In natural conditions it either multiplies itself by stem or rhizomes. The rhizome is quite an effective means of propagation. Plant produces “propagules” (plantlets, offsets) viviparously also through old inflorescence. With regards to the seeds the plant is self-incompatible and the seed set in nature is rarely found. Commercial exploitation for the production and conventional propagation is hampered due to its poor seed set, viability, low rate of germination and rooting ability of vegetative cuttings. Alternative propagation methods would be beneficial in accelerating large scale multiplication, improvement and conservation of the plant. Limited tissue culture work has been done on *Costus* species. The literature reveals that there are different regeneration systems for mass propagation of many *Costus* species. In vitro propagation of *C. pictus* in MS medium, with growth regulators, has also been successfully achieved (Ahmed and Arun, 2009).

Although *C. pictus* is cultivated, using vegetative (clonal) propagation by stem cuttings and rhizome pieces, problems of delayed rooting and shooting are frequent. Application of plant growth regulators has been widely recommended to overcome problems such as delayed or poor rooting and to enhance shooting in various plants. The present and ever increasing demand of this promising plant necessitated efforts to develop a simple protocol for the mass propagation of this plant. Keeping this in view an effort has been made for developing an effective and simple method of its propagation. A study was, therefore, undertaken to evaluate the effects of selected plant growth regulators on the growth and propagation of *C. pictus* under normal conditions.

### 3.2 Materials and methods

#### 3.2.1 Plant material
C. pictus (one year old) plants were collected from the garden in Sitapur (where it has been cultivated by the candidate from propagules brought over from Kerala as mentioned in chapter 2). The plant material was washed under running tap water to remove the adhered soil particles, particularly from the rhizome. After removing the leaves, the stems were cut into long pieces (10-15cm) leaving about three nodes in each cutting.

3.2.2 Preparation of plant growth regulators

The plant growth regulators/ chemicals selected for the treatment induced IBA, BAP, GA₃, ABA, KNO₃, and Bavistin. The required quantities of various solutions were prepared stock by weighing required quantities of PGRs/ chemicals. The concentrations (10, 100μM) of IBA, BAP, GA₃ & ABA, (5mM, 50mM) of KNO₃ and (0.05%, 0.5%) of Bavistin were prepared by suitably diluting the stock solutions. All these chemicals were procured from authentic suppliers, and stored at 4°C before use.

3.2.3 Treatment of the stem cuttings

The total treatments included 4 plant growth regulators and 2 chemicals: viz., GA₃, IBA, BAP, ABA (10, 100μM) KNO₃ (5mM, 50mM) and Bavistin (0.05%, 0.5%) together with control (treated with water). The cut ends of the stem cuttings were treated with the target plant growth regulators; stem cuttings were allowed to dip in various plant growth regulators for 24 hours. The treatment was conducted in April at Sitapur in the college campus. Every treatment was consisted of 5 replicates. The treated shoots were planted in polythene bags of (30cm x10cm) containing garden soil, and kept under shade with adequate watering.

3.2.4 Observation and Data collection

The polythene bags with planted cuttings were observed frequently to record sprouting/shooting and also to ensure the availability of sufficient moisture in the soil. Various growth parameters were measured two months after the treatment. Data for sprouting, shooting, length and width of stem, leaf, and number of leaves, rhizome size (length & diameter) were recorded on 2 months after the treatment. The plants of each treated group were harvested for the estimation of biomass. The entire plants were washed thoroughly to remove adhering soil particles from the root, and the fresh weight and growth measurements were recorded. The plants
were then oven dried at 65°C for 72h or till constant weight was achieved, and final observations were made using an electronic balance.

3.3 Results

3.3.1 Effect of PGRs on shooting:

The results regarding the effect of PGRs on shoot growth and related parameters have been presented (Table: 3.1); it can be seen that BAP and IBA are marginally and positively effective in the emergence (sprouting of buds), number and length of shoots. The highest value (82.4%) in terms of per cent sprouting of shoot was affected by BAP (10µM) treated cuttings. The IBA treated group (10µM) showed the next best value (81.3%) in this respect. Both BAP and IBA treatment at 100µM gave similar results (80.6% and 80.1%). The untreated control resulted in 79.4% sprouting; treatment with KNO₃ (5 mM, 50 mM) resulted in 78.6%, 75.2% sprouting, respectively. Treatment with GA₃ also resulted in 75.7% and 75.6% sprouting at 10µM and 100µM concentrations respectively; values being lower than control. The Bavistin treated group also attained values (74.3% and 75.6%) at concentrations of 0.05%, 0.5% (active ingredient; w/v) correspondingly. As expected the lowest values (72.9%, 71.4%) were obtained in ABA treated cuttings at 10µM and 100µM concentrations, respectively.

The results of the effect of selected plant growth regulators/chemicals on the emergence of clumps showed very little or no variation. The values in terms of the average number of clumps obtained is 3.1 using the both concentrations (10µM, 100µM) of IBA; even this is similar to that of control. Treatments with BAP, KNO₃, GA₃ and Bavistin resulted in values between 2.8 and 2.4. The treatment with ABA (both concentrations) clearly showed reduced number of clumps, the value being 2.0.

The plant growth regulators/chemicals used have resulted in little or no effect on shoot length, e.g. 22.6cm, 21.8cm IBA (10µM, 100µM) treatment against 21.8 cm in the control plants. The values for shoot length in other treated groups (BAP, ABA, GA₃, KNO₃ and Bavistin) at both the concentrations also showed no effect over control.
3.3.2 Effect of PGRs (chemicals) on leaf characters:

The recorded values of leaf characters e.g. number of leaves per shoot; leaf area, leaf length, and leaf width have been depicted in the Table 3.2. All the treated and untreated water control groups showed limited difference in the average values for number of leaves per shoot which were found in the range of 6.1-7.5 (control = 6.7). The average number of leaves per shoot higher than in control (7.5, 7.4, 7.2, 7.1, and 7) were produced by IBA (10μM), KNO₃ (5mM), BAP (10μM), KNO₃ (50mM), IBA (100μM) and BAP (100μM), respectively. The remaining treatments resulted in values with essentially no difference. The findings regarding leaf area have shown negligible difference among various treatments with different plant growth regulators. The average values of leaf area across all treatments, including control appeared in the range of 53.1-56.6 cm². KNO₃ (5mM, 50mM) treated plants were found to result in maximum leaf area (56.6 cm²; 56.5 cm²). The lowest value (53.1 cm²) for leaf area was obtained with Bavistin (0.05%).

The effect of selected plant growth regulators /chemicals on leaf length however, exhibited some significant difference. KNO₃ (5mM and 50mM) treated groups exhibited the highest values (16.55cm, 16.52cm), and these were followed by IBA (10μM, 100μM - 16.43cm, 16.41cm), BAP (10μM, 100μM - 16.40cm, 16.23cm), and with control (15.23cm). GA₃, ABA, and Bavistin treatments resulted in leaf length from 14.48cm to 14.32cm - lower than the control value.

The values in respect of leaf width measurements showed some variations. The average values of width of leaves were found within the range of 8.25-7.26cm. The maximum values (8.25cm, 8.20cm, 8.17cm, 8.15cm, 8.14cm and 8.12cm) were exhibited by 6 treatments, namely KNO₃ (5mM, 50mM), IBA (100μM, 10μM) and BAP (10μM, 100μM). All other groups (GA₃, ABA and Bavistin) showed leaf width values very similar to the value (7.57cm) obtained in the control group of plants.
Table: 3.1. Effect of plant growth regulators on shoot of *Costus pictus*

<table>
<thead>
<tr>
<th>PGRS/Conc.</th>
<th>% of sprouting axillary buds</th>
<th>Number of clumps (average)</th>
<th>Shoot length (average)</th>
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<tbody>
<tr>
<td>IBA</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>10μM</td>
<td>81.3</td>
<td>3.1</td>
<td>22.6</td>
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<tr>
<td>100μM</td>
<td>80.6</td>
<td>3.1</td>
<td>21.8</td>
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<tr>
<td>BAP</td>
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<tr>
<td>10 μM</td>
<td>82.4</td>
<td>3</td>
<td>20.4</td>
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<tr>
<td>100 μM</td>
<td>80.1</td>
<td>3</td>
<td>21.6</td>
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<tr>
<td>GA3</td>
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<tr>
<td>10 μM</td>
<td>75.7</td>
<td>2.8</td>
<td>21.2</td>
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<tr>
<td>100 μM</td>
<td>75.6</td>
<td>2.4</td>
<td>20.3</td>
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<tr>
<td>ABA</td>
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<td>10 μM</td>
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<tr>
<td>50mM</td>
<td>75.2</td>
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<tr>
<td>Bavistin</td>
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<tr>
<td>0.05%</td>
<td>74.3</td>
<td>2.4</td>
<td>20.3</td>
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<tr>
<td>0.5%</td>
<td>75.6</td>
<td>2.8</td>
<td>20.4</td>
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<tr>
<td>Control</td>
<td>79.4</td>
<td>3</td>
<td>21.8</td>
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</tbody>
</table>
Table: 3.2 Effect of plant growth regulators on leaf characters of *Costus pictus*

<table>
<thead>
<tr>
<th>PGRS/ Conc.</th>
<th>No. of leaves per shoot</th>
<th>Leaf area (cm²)</th>
<th>Leaf length (cm)</th>
<th>Width (cm)</th>
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<tbody>
<tr>
<td><strong>IBA</strong></td>
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<tr>
<td>10μM</td>
<td>7.5</td>
<td>56.4</td>
<td>16.4</td>
<td>8.2</td>
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<tr>
<td>100μM</td>
<td>7</td>
<td>55.5</td>
<td>16.4</td>
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<tr>
<td><strong>BAP</strong></td>
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<tr>
<td>10 μM</td>
<td>7.2</td>
<td>56.5</td>
<td>16.4</td>
<td>8.1</td>
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<tr>
<td>100 μM</td>
<td>7</td>
<td>54.3</td>
<td>16.2</td>
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<tr>
<td><strong>GA3</strong></td>
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<tr>
<td>10 μM</td>
<td>6.1</td>
<td>53.4</td>
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<td>100 μM</td>
<td>6.3</td>
<td>53.7</td>
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<tr>
<td><strong>ABA</strong></td>
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<td>10 μM</td>
<td>6.2</td>
<td>53.2</td>
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<tr>
<td>100 μM</td>
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<td>53.6</td>
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<td><strong>KNO3</strong></td>
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<tr>
<td>5mM</td>
<td>7.4</td>
<td>56.6</td>
<td>16.6</td>
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<tr>
<td>50mM</td>
<td>7.1</td>
<td>56.5</td>
<td>16.5</td>
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<tr>
<td><strong>Bavistin</strong></td>
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<tr>
<td>0.05%</td>
<td>6.3</td>
<td>53.1</td>
<td>14.8</td>
<td>7.3</td>
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<tr>
<td>0.5%</td>
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<td><strong>Control</strong></td>
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<td></td>
<td>6.7</td>
<td>54.5</td>
<td>15.2</td>
<td>7.6</td>
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</tbody>
</table>
Table: 3.3 Effect of plant growth regulators on the rhizomes of *Costus pictus*

<table>
<thead>
<tr>
<th>PGRS/ Conc.</th>
<th>Rhizome fresh weight average(g)</th>
<th>Rhizome diameter average (cm)</th>
<th>No. of roots average</th>
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</thead>
<tbody>
<tr>
<td><strong>IBA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10μM</td>
<td>24.7</td>
<td>2</td>
<td>6.7</td>
</tr>
<tr>
<td>100μM</td>
<td>23.4</td>
<td>1.9</td>
<td>5.3</td>
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<tr>
<td><strong>BAP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 μM</td>
<td>24</td>
<td>1.9</td>
<td>5.4</td>
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<tr>
<td>100 μM</td>
<td>23.5</td>
<td>1.7</td>
<td>5.7</td>
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<tr>
<td><strong>GA3</strong></td>
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<tr>
<td>10 μM</td>
<td>23.1</td>
<td>1.2</td>
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<td>100 μM</td>
<td>23.1</td>
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<td><strong>KNO3</strong></td>
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<td>2.6</td>
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<td>50mM</td>
<td>23.6</td>
<td>2.4</td>
<td>6.4</td>
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<td><strong>Bavistin</strong></td>
<td>0.05%</td>
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<td>1.7</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>21.1</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td>23.1</td>
<td>1.8</td>
</tr>
</tbody>
</table>
Table: 3.4 Effect of plant growth regulators on biomass (dry weight) of *Costus pictus*

<table>
<thead>
<tr>
<th>PGRS</th>
<th>Wt. of total leaves/ plant (g)</th>
<th>Shoot biomass/plant (g)</th>
<th>Above ground biomass/plant (g)</th>
<th>Under ground biomass/plant (g)</th>
<th>Total Biomass/Plant (g)</th>
</tr>
</thead>
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<tr>
<td>IBA</td>
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<td>4.3</td>
<td>6.6</td>
<td>1.0</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>100μM 2.1</td>
<td>3.9</td>
<td>6.0</td>
<td>1.1</td>
<td>7.2</td>
</tr>
<tr>
<td>BAP</td>
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<td>6.2</td>
<td>1.1</td>
<td>7.2</td>
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<tr>
<td></td>
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<td>4.1</td>
<td>6.1</td>
<td>1.0</td>
<td>7.2</td>
</tr>
<tr>
<td>GA3</td>
<td>10 μM 2.1</td>
<td>3.9</td>
<td>6.1</td>
<td>1.1</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>100 μM 2.0</td>
<td>4.0</td>
<td>6.0</td>
<td>1.1</td>
<td>7.2</td>
</tr>
<tr>
<td>ABA</td>
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<td>1.0</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>100 μM 2.0</td>
<td>4.0</td>
<td>6.0</td>
<td>1.1</td>
<td>7.1</td>
</tr>
<tr>
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<td>1.1</td>
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<tr>
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<td>4.0</td>
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<tr>
<td>Control</td>
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<td>4.0</td>
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<td>1.0</td>
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</tr>
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Fig. 3.1 Effect of growth regulators on propagation of *Costus pictus* in
Sitapur

50
Fig. 3.2 Effect of Growth Regulators on propagation of *Costus pictus* in GBPIHED, Green House at Almora
3.3.3 Effect of PGRs/ chemicals on underground parts

After two months of growth the underground parts, i.e. rhizomes and roots harvested, washed and the fresh weight, diameter of rhizomes and number of roots were recorded (Table: 3.3). The IBA (10μM) treated plants were found to achieve maximum rhizome weight (24.7g) compared with plants treated with other PGRs/chemicals. The minimum rhizome weight (21.1g, 21.5g) was found in Bavistin (0.5%), and ABA (10μM) treated group of plants. The remaining treatments, i.e., BAP (10μM), KNO₃ (5mM, 50mM), BAP (100μM), IBA (100μM), Bavistin (0.05%), ABA (100μM), GA₃ (10μM), and GA₃ (100μM) resulted in rhizome weight (24g, 24g, 23.6g, 23.5g, 23.2g, 23.2g, 23.1g, 22.5g) similar (23.1g) to that in untreated control plants.

The average values for diameter of the rhizome were found to be quite similar between the different treatments. The rhizome diameter in control group of plants (1.8 cm) was lower than in KNO₃ (5 Mm, 50Mm) and IBA (10μM) treated group of plants (2.6, 2.4 and 2cm, respectively). The lowest value (1.2, 1.3 and 1.3cm) was obtained in GA₃ (10μM), ABA (10μM, 100μM) and Bavistin (0.5%) treated group of plants.

The record in respect of (Table: 3.3) average number of roots produced in each group of plants indicated the maximum effect (6.7, 6.5, 6.4) for IBA (10μM) and KNO₃ (5Mm and 50mM) treated group of plants. The minimum effect (4.3, 4.6, 4.7) was displayed by GA₃ (10μM) and ABA (100μM and 10μM) groups respectively. All other treatments resulted in similar values or with small differences; control plants gave intermediate value (5.7).

3.3.4 Effect of PGRs/ chemicals on biomass

The treatment with selected plant growth regulators/ chemicals did not appear to result in difference in the overall biomass values. The values (Table: 3.4) recorded for various treatments showed more or less similar findings across all observed parameters like above ground, below ground and total biomass. The maximum values (7.901g, 7.704g, and 7.601g) were found in KNO₃ (5mM, 50mM) and IBA (10μM) treated group of plants; the minimum values (6.997g, 7.006g, and 7.081g) were obtained with Bavistin (0.05%, 0.5%) and ABA (10μM) treatments, respectively; values being similar to that in the control plants (7.087g).
3.4 Discussion

One of the most appropriate actions for safeguarding the conservation status of over exploited species is to improve propagation techniques and for conservation through commercial scale cultivation. The mode of regeneration of *C. pictus* is by rhizome as well as stem cuttings. Since the plant is subjected to severe winter conditions in north India and perennates through underground parts for some duration in the year, the period of growth is confined from spring through summer and rainy months up to autumn, i.e., April to October-November. The improved vegetative multiplication can help in promoting large scale propagation of this species.

Plant growth regulators (phytochemicals) are mediators of crucial importance during the whole process of development plants (Davies, 1987). The synthetic PGRs are commonly used in plant propagation through stem cuttings to influence and promote growth (Helgi, 2005). The commercial exploitation of *C. pictus* for the preparation of antidiabetic formulations (and/or other purposes) is in some ways dependent on the generation of large number of propagules of the plant, and the existing conventional propagation is hampered due to poor seed set, viability, low rate of seed germination, and thus improved methods for rooting of vegetative cuttings could be very desirable as a simple means of clonal propagation. Alternative propagation methods are always beneficial in accelerating large scale multiplication, improvement of cultivable stock (selected elites) and overall conservation of the plant. Very limited tissue culture work has been done on *Costus* species. The current study was attempted to help in the mass propagation of *C. pictus*, a plant of tremendous value in the antidiabetic therapeutic field.

In the present study, however, not much differences were observed in the effect of selected plant growth regulators/chemicals on various parameters; somewhat stimulatory influence was observed on shoot characteristics by IBA (auxin), BAP (cytokinin) treatments at both the concentrations (10μM, 100μM). The IBA and BAP treatments exhibited maximum values (Table: 3.1) in percentage sprouting of axillary buds, number of clumps (shoots) and the overall shoot length. Similar response was observed in experiments with excised stem or coleoptile segments of plant name which showed auxin mediated cell expansion (Kutchera et al., 1987). In a single cell model system Stickens et al., (1996) have explored the positive response of cells to auxins and cytokinins and the possibility to induce cell division or expansion.
Protocorms proliferation of *Vanda helvola* was observed on KC basal medium supplemented with single hormone, NAA (auxin) or BAP (cytokinin) (David *et al.*, 2008).

Both IBA and BAP are also known to induce enhanced bud proliferation in shoot tips of *Cattleya* (Melissa *et al.*, 1994), shoot tips and flower stalk buds of *Phalaenopsis* and *Doritaenopsis* (Tokuhara and Mii, 1993), foliar meristem of *Renanthera imshoottiana* and *Vanda coerulea* (Seeni and Latha, 1992) and axillary buds from stem nodes of *Vanda spathulata* (Deeruse *et al.*, 2003). In the present study also BAP (10 μM) was found to be the most efficient for bud proliferation (sprouting) and shoot development. This result is in agreement with the findings of Sureyya (2010) who reported the maximum effect of BAP (0.5mg) on shoot propagation of *Hypericum retusum*.

The effect of selected plant growth regulators/chemicals, used in this study were found to result in negligible differences among treatments on leaf characteristics. IBA (10μM), BAP (100μM) and KNO₃ (5mM) produced maximum number of leaves, while GA₃ and ABA (10μM) were less effective. All other PGRs treatments, showed similar results to that found in untreated control plants. The promotory effect of KNO₃ (5 mM, 50 mM) on leaf area, leaf length and leaf width (Table: 3.2) was in line with the slight stimulatory effect of IBA and BAP, at both the concentrations. Nitrogen plays a major role in growth and differentiation, such as stem elongation and leaf development and that could be the possible reason of the observed promotory effect of these, KNO₃ in particular on leaf characters. Auxins regulate vegetative growth and organ growth and cytokinins facilitates cell division and cell sprouting (Pan, 2001).

The results of average rhizome weight, diameter and number of roots, etc. (Table: 3.3) did not show much difference among variously treated group of plants. The IBA (10μM) BAP (10μM) and KNO₃ both concentrations could stimulate the growth of rhizome and roots compared to untreated control group of plants as also in relation to some shoot and leaf related parameters. There is a substantial body of evidence that auxins contribute to root initiation (Jarvis 1986; Blakesley 1994; Muller *et al.*, 2005; Osterc *et al.*, 2009). Since their discovery, the exogenous auxin treatment of cuttings has become routine in horticultural practice to ensure and enhance rooting (Hartmann *et al.*, 2002). Ramak *et al.*, 2011 found the best condition for rooting included the presence of IBA in the medium during studies on seed germination and *in vitro* shoot multiplication of *Satureja khouzistanica*, an important medicinal plant. 6-
Benzylaminopurine (BAP) significantly stimulated rhizome induction in *Cyperus serotinus* (Hiroyoshi, 1992) and this group of phytotropes also affected in vitro propagation of *Aconitum balfourii*, on alpine herb of medicinal value (Pandey *et al.*, 2004). Shoot regeneration was induced from leaf discs on the MS medium containing sucrose and agar, supplemented with IBA, kinetin (KN), and BAP; well-developed shoots (3-4 cm in length) were rooted on the same MS medium supplemented with 0.5 mg/L IBA only (Nguyen and Kiet, 2011). Tamta and Palni (2004) have also reported use of PGRs on in vitro propagation and rooting of Cedar. Root formation in stem cuttings of Oak with application of plant growth regulators has also been reported (Purohit *et al.*, 2005).

The influence of plant growth regulators on biomass of *C. pICTUS* has been reported in the results section. It shows KNO₃ has maximum stimulatory effect on biomass (above ground, below ground and total biomass) production, while minimal biomass production was seen in ABA treated group. The induced effect of KNO₃ in plant growth is reported by many researchers. Nitrogen in the form of KNO₃ significantly improved proliferation rate of ginger under *in vitro* conditions using both full and half strength medium (Cecilia, 2010). Generally the essential element K has a great regulatory role within plant cells and organs such as, activating more than 50 enzymes, osmosis regulation and photosynthesis, and loading and unloading of sugars in phloem (Mengel, 2007). The foliar application of KNO₃ significantly increased leaf area, its fresh and dry weight of sunflower and safflower leaves, irrespective to the growth of plant under non saline or saline conditions (Jabeen and Ahmad, 2011). The mechanism of action is still obscure; however, as it has been mentioned earlier, the element K has important regulatory roles, both inside and outside of plant cells. The enhanced effect of KNO₃ seen on the leaf area in treated group of *C. pICTUS* plants, as compared to the control is probably due to the promotory role of potassium in overall growth. A source of readily available potassium might have caused the growth along with increased leaf area. Saied *et al.*, (2012) also observed the stimulatory effect of KNO₃ on plant growth in the study on the influence of foliar application of volk oil, dormex, GA₃ and KNO₃ on vegetative and reproductive growth of Strawberry. This finding supports the results of the present study, the enhanced effect of KNO₃ on vegetative growth of *C. pICTUS*.

In the overall assessment of response of selected plant growth regulators and chemicals on the growth of *C. pICTUS*, stimulatory effect was observed with KNO₃, IBA and BAP. On the
other hand minimal growth observed in GA3, ABA and Bavistin treated group of plants might be due to the inhibitory effect of these plant growth regulators on the vegetative growth of C. pictus. Similar effects of IBA & BAP were reported earlier in in vitro propagation of C. pictus (Ahmed and Arun, 2009) and they observed maximum number of shoots from rhizome explants and multiple shoots were formed on MS medium containing BAP and KN. High frequency rooting was obtained in rhizome derived shoots on half strength MS medium supplemented with IAA and IBA. The effects of auxins and cytokinins on shoot multiplication have been reported earlier (Cellarova et al., 1992; Moura, 1998). Regulation of cell division and morphogenesis are widely regarded as the most significant functions of cytokinins (Letham and Palni, 1983). The mechanisms by which biosynthesis and degradation of auxin and cytokinin molecules occur are important for future agricultural applications. Information regarding auxin metabolism will most likely lead to genetic and chemical manipulation of endogenous phytohormone levels resulting in desirable growth and differentiation of important plant species. Ultimate aim is to explore the possibility to regulate plant growth without the use of hazardous herbicides and chemical fertilizers (Nandi and Palni, 1989; Davies, 1995).

In conclusion, the results from the present investigation reveal the effects, both stimulatory and inhibitory of a range of plant growth regulators and KNO3 on the rooting and growth of C. pictus cuttings. Rhizome, shoot fresh weight, number of leaves and shoot length, width, etc., are all influenced by the treatment of PGRs and KNO3 and the interactions had effect on the growth of C. pictus in normal outdoor conditions. These preliminary findings should pave the way for future work on improvement, conservation and mass propagation of C. pictus, to fulfill the ever increasing demand of plants for commercial level cultivation of this important antidiabetic plant.