Review of Literature

“If you do not know the history, you don’t know anything; you are a leaf that doesn’t know its part of a tree”

~Michael Crichton
CHAPTER - 2

REVIEW OF LITERATURE

Propagation is one of the most important aspects in forestry. Vegetative propagation methods like cuttings, air layering, budding and grafting are being widely followed to raise plants of desirable genetic constitution and maintain their purity for commercial exploitation.

Among the methods of vegetative propagation, propagation by cuttings is one of the easiest and widely employed method which is usually followed in easy-to-root species. In cuttings, growth substances applied exogenously are found to enhance early and good root formation. Nowadays many of the difficult to root plants are made to root easily by applying plant growth substances. Various classes of growth regulators such as auxins, cytokinins, gibberellins and ethylene influence root initiation in cuttings. Of these auxins have greater effect on root formation in cutting. In addition to these groups, various growth retardants and promoters may have less direct part in adventitious root formation (Krul, 1968).

The research work on the vegetative propagation of Diploknema butyracea is very much limited. While effort has been made to quote works on the D. butyracea and other species of this genus, the paucity of literature on many aspects has let other woody species also to creep in for throwing information on those aspects.
2.1 Study species: *Diploknema butyracea*

*Diploknema butyracea* (Roxburgh) H. J. Lam, (Syn. *Bassia butyracea* Roxburgh, Syn. *Madhuca butyracea* (Roxburgh) Macbride, Syn. *Aesandra butyracea* (Roxburgh) Baehmi, Syn *Illipe butyracea* (Roxburgh) Engler) belongs to family Sapotaceae, many of whose species produce edible fruits and other economic fruits, is a medium sized deciduous tree locally known as Cheura and popularly known as Butter tree. This fast growing multipurpose tree is a native of Nepal and is distributed from India through Nepal to Philippines and from Kumaon eastwards to Sikkim and Bhutan (Manmohan *et al.*, 2009). The population of this species is almost localized in Pithoragarh district particularly the areas bordering Nepal and adjoining areas of Almora, Bageshwar and Champawat. It also occurs sporadically in tropical moist deciduous, semi deciduous and evergreen forests of Andaman Islands. It is a fast growing tree borne oilseed found at an elevation of 400-1400 mtrs mainly along steep slopes, ravines, cliffs and in shady valleys (Negi *et al.*, 1988). In its natural habitat, the mean annual rainfall ranges from 1000-2000 mm. Temperature varies from 24°C to 27°C. Medium soils with deep granules are considered good for Cheura plantations.

It is a medium sized deciduous tree with a straight trunk attaining a height of 15-22 mtrs and a girth of 1.5-1.8 mtr but in Andaman Islands, it reaches a height of 21-36 mtr and a girth of 1.5-2.4 mtr. The bark is dark grey or brown, 1.3 cm thick, fairly smooth. Leaves are 20-35 cm long and 9-18 cm wide and crowded at end of branches. Flowers are 2.0-2.5 cm in diameter, white/ yellow colored with a fragrance. The fruit is a berry, 2.0-4.5 cm in diameter, bright green or blackish, shinning with a thick soft pericarp. Fruits are oval in shape and green in color with three seeds which turns juicy and grey after getting ripened. The
seeds are 1.5-2.0 cm in length which has almond shaped white colored kernels inside (Kureel et al., 2008).

Cheura may be propagated through seeds and cuttings. Fully matured, bold and disease free seeds are collected from the trees during May-June and sown in a week’s time. Cheura starts flowering in the month of October-November at the age of 8-10 years. Generally alternate bearings have been observed. Fruits start ripening in June-July and are harvested in ending July.

Cheura ghee (yosati) is the main source of edible oil for more than one hundred thousand people. The flowers of *D. butyracea* are used as a source of alcohol. The seed contains considerable amount of fat, known as *Phulwara butter* (Annon, 1952).

After removing the pulp, the seeds are cleaned and then sun-dried and steamed. The oil is manually extracted by pounding and macerating between two wooden planks, yielding up to 25-30% of oil. It is white in colour and has a pleasant taste and odour. It remains solid up to 48 °C and does not deteriorate in hot weather. It is used for cooking and is reportedly an ingredient for manufacturing margarine and chocolate in India. Most production is confined to small-scale village or household activities. It is also used as an addictive in animal ghee (Kirtikar and Basu, 1935). Cheura juice is also consumed to quench thirst. The potential use of Cheura products is found in different fields such as confectionary, pharmaceuticals, vegetable ghee production, candle manufacturing and soap making. It has been found effective for rheumatism. The defatted seed contains 25% protein (Mitra and Awasthi, 1962) and saponins (10-25%) which are toxic. The cake produced after processing cheura is used as manure which has pesticide, wormicide, nematicide and insecticide properties (Kureel et al., 2008).
The tree could make substantial contribution for the development of economy of the local people. It would also unfold the many sided advantages besides creation of wealth worth several million rupees, collection and processing of plant material would strength the rural economy. Having such a great economic and medicinal value *D. butyracea* is facing extinction because of relentless anthropogenic pressure. The species is failing to regenerate inspite of reasonable seed propagation. In recent years there have been reports of large scale exploitation of *D. butyracea*. The trend is threatening the genetic resource base of this species. Therefore in order to counteract the continuous degradation of this natural asset and augment the natural regeneration, some artificial regeneration is urgently called for. Thus vegetative propagation is better option, as it ensures purity of clonal material or true-to-type propagation of elite tree.

2.2 **Types of material for vegetative propagation of *Diploknema***

Vegetative or asexual propagation can be defined as the production of a plant so that the offspring will contain the exact characteristics of the mother plant in genotype as well as health status (Macdonald, 1986; Hartmann *et al.*, 2010). Vegetative propagation is an important tool for crop improvement. Its aim is the formation of adventitious roots for successful plant regeneration. According to Hartmann *et al.* (2010), vegetative propagation is possible because the living cells contain genetic information in their nuclei necessary to reproduce the entire plant. In asexual reproduction, growers have the ability to obtain a high degree of crop uniformity and a high quality product is achieved. However, vegetative propagation can sometimes result in intraclonal variation.
which is comparable to variation within a sexually propagated population (Swamy et al., 2001).

Stem cutting propagation is an ancient technique and it has been traced as far back as ancient China. Vegetative propagation by stem cuttings has the ability to produce a large number of young plants from a single parent plant, thus it is a useful technique in conservation of endangered plants (Macdonald, 1986) and rapid propagation of new cultivars.

*D. butyracea* can be multiplied through seeds, stem cutting or layering. Propagation through seeds is the principal method of raising plantations of this species. Seeds are taken during July planted vertically maintaining correct polarity in nursery beds. This method gives rapid, uniform growth and a high survival rate (Tewari and Dhar, 1997).

The stem or branch cutting is the most important and common type of cutting for vegetative propagation of woody species. According to Hartmann et al. (2010), stem cuttings can be grouped into hardwood, semi-hardwood, softwood, and herbaceous. The use of hardwood cuttings is one of the least expensive and easiest methods of vegetative propagation, especially in deciduous plants. Semi-hardwood cuttings are usually employed for woody broad-leaved evergreen species, but leafy summer cuttings from partially matured wood of deciduous plants can also be considered as semi-hardwood cuttings. Softwood cuttings are prepared from the soft, succulent, new spring growth of deciduous or evergreen species. Leafy semi-hardwood and softwood cuttings require to be rooted under high-humidity environment (Hartmann et al., 2010). In propagation by stem cuttings, rooting is influenced by many factors including type of wood, the stage of growth when cuttings are made, the time of year when cuttings are taken, rooting medium, rooting auxin and
physical factors (Macdonald, 1986; Hartmann et al., 2010; Wilson, 1993). Rooting of stem cuttings of Diploknema butyracea is relatively more difficult (Singh and Khan, 2004).

Trees of produced through vegetative means have been reported to be more likely to survive, and possess greater height and breast height diameter than those produced through seed (Bergmann, 2003).

2.3 Effect of cutting diameter on propagation through branch cuttings

Diameter of cutting has a profound influence on regeneration ability. Literature abounds in reports on this aspect of hardwood and semi-hardwood cuttings. The effect may not only be restricted to sprouting, callusing and rooting, but may extend to subsequent growth of the plant as well.

Kumar et al. (2006) carried out studies on effect of root cutting diameter (1, 2 and 3 cm) in conjunction with cutting length (10, 15 and 20 cm) in Paulownia fortunei and found significant effect of cutting diameter and length on sprouting percent. Cuttings of 2 cm thickness gave 48.17 percent sprouting while those of 3 cm diameter recorded 41.18 percent sprouting. Ede et al. (1997) carried out studies to examine the effect of root cutting diameter on rooting ability of Paulownia tomentosa and Paulownia fortunei. New root development was not found to be affected by cutting diameter.

Sprouting, rooting, seasonal growth and survival of Populus ciliata branch cuttings have been found to increase with increasing thickness of cuttings. The cuttings of 20 to 25 mm thickness produced significantly higher number of secondary and tertiary roots. The cuttings of 15 to 20
mm thickness recorded maximum shoot growth (Joshi and Nautiyal, 2002).

Assareh and Hossein (2005) studied the influence of three shoot diameters on rooting of branch cuttings of *Ziziphus spina-christi*. Successful rooting was noticed only with cuttings having greater than 8 mm diameter. Singh *et al.* (2002) investigated the vegetative propagation in *Buchanania lanzan* cuttings. Maximum rooting of 52.7 percent was observed in cuttings with 1.5 to 3.5 mm thickness. The combined effect of cutting thickness and IAA concentration revealed that cuttings with 3.6 to 5.5 mm diameter had maximum rooting percentage with 800 and 1600 ppm IAA.

Zhang *et al.* (2010), studied the effect of cutting diameter on the rooting and sprouting in *Feijoa sellowaina* stem cuttings, the results on the sprouting, rooting rate, rooting traits (sum of adventitious roots and average length of roots) and growth quantity of shoot obtained from using diameter 2.5-3mm are significantly greater than those using diameter 2-2.5 mm and 1.5-2.0 mm.

Sprouting and rooting in Neem (*Azadirachta indica*) cuttings of different diameters (0.5, 1 and 2cm ) with 1 000 ppm IBA treatment was studied by Palanisamy and Kumar (1997) and revealed that 0.5 cm diameter cuttings gave 100% sprouting and rooting which decreased to 97% and 70% in 2cm diameter cuttings respectively. The number of roots however increased with the increase in diameter. Rana and Sood (2012) observed that rooting percentage was significantly higher in large sized (1.25-2.5 cm diameter) cuttings of *Ficus roxburghii*, but the number of shoots wasn`t found significant with small sized (< 1.25 cm diameter) cuttings.
Studies have also been carried out to examine the effect of cutting diameter on the growth of plants. Ede et al. (1997) reported that cutting less than 5 mm diameter produced less shoot growth in *Paulownia fortunei*. The effect of cutting diameter on the vegetative propagation of *Jiga* was studied by Khanam et al. (2007) and reported the significant variation in percent survival between thick (38.9%) and thin (25.9%) cuttings.

Influence of cutting diameter on the rooting of *Coleus* cuttings was studied by Hamilton et al. (2002). They observed that thick branch cuttings with higher carbohydrate reserves tended to root better. Thicker branch cuttings exhibited a non significant trend towards a higher root rating of 4.36 than thin branch cuttings which rated 3.67 on a rating scale where 5 represents good, 4-fair, 3 few roots. The observations revealed by Pandey (2012) in *Gymena sylvestre* showed higher success (52%) in 10-15mm diameter cuttings followed by 5-10 mm and 0-5 mm diameter cuttings (26% and 15%)

Growth parameters like root length, number of roots, root diameter showed considerable increase with the thickness of stem cuttings in *Morus alba* (Irfan et al., 2010). Rin (1975) tested cuttings of 0.5 to 3.5 cm diameter of *Paulownia taiwaniana* in Taiwan. After one year, survival and growth showed increase with increase in cutting diameter. Cuttings less 1.5 cm in diameter were not found to be suitable for propagation.

Kumar et al. (2006), observed plant height for different cutting lengths, when averaged over diameter classes, showed that three centimetre diameter cuttings resulted in the tallest (6.44 m) plants after one year in nursery while one centimetre diameter cuttings produced the shortest (5.61 m) plants
Height and basal diameter of Popular plants increased with increase in diameter of cuttings. Height and basal diameter of cutting raised from 2.8cm diameter were significantly greater than those raised from cuttings of 0.8-2.4 cm diameter (Joshi and Nautiyal, 2002).

It has been shown by Mesen et al. (1997) that an increase in cutting diameter of Cordia alliodora had resulted in significant increase in growth parameters. The bigger diameter cuttings may have more stored reserves for growth than the smaller diameter cuttings. The importance of storage capacity of cuttings for root development and other growth parameters has also been observed by Veierskov and Anderson (1982).

On comparing the performance of semi-hardwood stem cuttings of different diameters, namely 0.5, 1.0 and 1.5 cm, belonging to Commiphora wightii, Kumar et al. (2004) found that the number of branches and primary roots were significantly greater with thick cuttings (1.0 cm or 1.5 cm). Similar results were observed in rooted cuttings of Fagus sylvatica (Beech) by Jurasek (2007).

### 2.4 Effect of cutting length on propagation through branch cuttings

Various studies are reported in the literature on the effect of length of branch cuttings on rooting. The length of cuttings can vary between 5-25 cm depending on the species and method of propagation. Pekka-Rossi (1999) reported that longer the cutting (upto 30-50 cm), better the survival and growth. In practical terms it is advisable to use medium sized (10-20 cm long) cuttings (Girouard, 1974). The readiness of tree species to root is usually more important than the size of the cuttings and the number of the buds on the cuttings. However, Chmelar (1979)
observed that quite small (3 cm long) cuttings of Willow (*Salix* sp.) rooted well and that cuttings of any size could be rooted successfully.

Gautam *et al.* (2010) revealed that size of cuttings in *Psidium guajava* has significant effect on rooting induction. Both callus formation (32%) and root induction (30%) was significantly declined in small cuttings (5 cm), while these differences were insignificant among 10 and 15 cm long cuttings. No additional significant gains could be obtained by increasing the size of cuttings from 15 cm (91%) which indicates that 15 cm is optimum size for root induction in guava.

Palanisamy and Kumar (1997) reported that among the different sizes of Neem cuttings, 25 cm long cuttings gave 100% rooting which significantly decreased to 47% with 12 cm long cuttings and no rooting was observed in 5 cm long cuttings. A significant decrease in sprouting, no. of roots and root length was also observed when length of cuttings was reduced.

Gerakakis and Ozkaya (2005) reported that 3 nodal cuttings gave the highest rooting percentage of 26.67% with respect to 2 noded (18.33%) and 1 noded cuttings of *Oleo europaea* (olive). In 1 noded cuttings there was no rooting. The highest survival rate (60%) was observed in 3 noded cuttings as against 33.33% in 2 noded cuttings.

Irfan *et al.* (2010) studied the effect of cutting size on the rooting of *Morus alba* cuttings and observed that the root length was maximum (22.19 cm) for 2 inch cuttings followed by 19.25 cm, 17.25 cm and 16.60 cm in 4, 6 and 8 inch cuttings respectively. Root diameter also followed the similar trend with 1.50 mm in 2 inch cutting and 1.41, 1.40 and 1.35 mm in 4, 6 and 8 inch cuttings respectively.
The effect of cutting size on the success of the vegetative propagation of Jiga (*Garuga pinnata*) was reported by Khanam *et al.* (2007). The highest percent survival (56.9%) was found in 50 cm long cutting followed by 30.6% and 9.6% in 25 cm and 12.5 cm long cuttings respectively.

Effect of cutting size on rooting and subsequent growth of *Acer rubrum* (Red sunset) cuttings was studied by Smalley *et al.* (1987). Rooting percent (95%), no. of roots (47), length of roots (197 cm) and survival (96%) was found more in 15 cm long cuttings than in 5 cm long cuttings where rooting percent (88%), no. of roots (12), length of roots (77 cm) and survival (92%) was recorded respectively.

The effect of cutting length on the growth and rooting of pomegranate cuttings showed that no. of leaves, no. of shoots and root length was significant. The highest and lowest no. of leaves was observed in multiple bud (19.00) and single bud cuttings (1.67). The highest and lowest shoot number was observed in multiple bud (2.73) and single bud cuttings (1.82) respectively. The longest root length belonged to three bud cuttings (41.56 cm) and the shortest one was observed in single bud cuttings (0.00 cm) (Alikhani *et al.*, 2011).

In a trial on *Paulownia fortunei* at Ludhiana conducted by Kumar *et al.* (2006) during 1999-2000, longer cuttings (15 cm length) in conjunction with 3 cm cutting diameter performed best and produced nursery stock with greater collar diameter (8.36 cm), plant height (6.79 m) and breast height diameter (6.03 cm) in nursery beds. During 2002-2003, longer and thicker cuttings (20 cm length and 3 cm diameter) gave the best performance with respect to the above-mentioned growth parameters in polythene bags as well as in nursery beds with the
exception that 20 cm length with 2 cm diameter recorded the greatest plant height in nursery beds.

In Pakistan, cuttings of 12 cm to 16 cm length belonging to *Paulownia elongata* gave better results in comparison with 8 cm to 12 cm length, averaged over different diameter classes (Khan, 1992). On comparing performance of cuttings of 5 cm to 15 cm length taken from 1-year-old plants of *Paulownia taiwaniana*, cuttings shorter than 5 cm and having less than 5 cm diameter were found unsuitable for propagation.

Kumar (2005) studied the effect of length of hardwood cuttings in conjunction with diameter on the growth in nursery. The cuttings were taken from stem of *Populus deltoides*. Three cutting lengths viz. 15, 20 and 25 cm and six diameters viz., 0.8, 1.2, 1.6, 2.0, 2.4 and 2.8 cm were tested. Cuttings of 25 cm length resulted in plants of significantly more height and diameter as compared to cuttings of 15 and 20 cm length. Longer and thicker cuttings produced plants of significantly more height and basal diameter as compared to smaller and thinner cuttings.

Kumar *et al.* (2004) examined the performance of 15, 20 and 25 cm length of semi-hardwood stem cuttings of *Commiphora wightii* and found the longer cuttings of 20 to 25 cm to be most successful in their field establishment when averaged over different diameter classes.

Saxena and Dhawan (2004) have recommended the use of hardwood cuttings of 30 cm length for propagation of *Jatropha curcas*. Kumar *et al.* (2004) and Puri (2004) have suggested use of 45 cm to 100 cm hardwood cuttings for planting of *J. curcas*. Ratree (2004) employed hardwood cuttings of 5, 10, 20, 30, 40, 50, 60, 70, 80 and 90 cm cutting
length for planting of *J. curcas*. Cuttings of 90 cm length gave the highest seed yield.

Rooting ability and root vigour in apical bud cuttings of *Persea bombycina* have been observed to depend on the number of leaves per apical bud cuttings. A significant positive correlation was observed between the number of leaves per cutting and mean root length (Yadav, 2003).

During propagation of *Tsuga canadensis* and *Tsuga caroliniana*, through softwood cuttings of different lengths, mortality among 6 cm stem cuttings was twice that observed for 3 cm cuttings of both species. However, 6 cm cuttings of *T. canadensis* that did form adventitious roots had more roots and longer total root length compared with 3 cm cuttings (Jetton *et al.*, 2005). Shoot cuttings of 10 to 16 cm length, taken from hedge garden of *Tectona grandis*, have showed better root induction than those of 8 to 9 cm or 20 to 25 cm length (LakshmiKanth, 2003). Ofori *et al.* (1997) also observed lower rooting percentage, number of roots per cutting and greater mortality in longer leafy stem cuttings of *Milicia excelsa*.

In another study softwood cuttings were harvested from 4-year-old *Pinus taeda* hedges in March and September, and placed into a series of factorial combinations of cutting length, diameter class, and the presence / absence of a terminal bud to assess the effects of these characteristics on rooting in the greenhouse and subsequent field performance in a trial in Alabama, USA. Terminal bud status did not appear to influence percentage rooting. Shorter cuttings (5.1 or 7.6 cm) with an average diameter of 2 or 3 mm tended to root better and develop more roots. Field performance of the rooted cuttings through to age 5 years suggests that the original cutting does not require a terminal bud,
but the best set of morphological traits differ depending on bud status. Considering both rooting ability and field growth with an original tip bud present, the best cutting dimensions were 5.1 or 7.6 cm length and 2 or 3 mm diameter. Without a tip bud present, the best cutting dimensions were 7.6 or 10.2 cm length and 3 mm diameter. Number of main roots was a weak predictor of tree height or diameter at breast height at age 5 years (Foster et al., 2000).

2.5 Effect of type of growth regulator on propagation through branch cuttings

Plant growth regulators and others chemicals are used to improve rooting, survival and subsequent growth in plant species when propagated vegetatively (Lundquist and Torrey, 1984; Badola and Badoni, 1990; Bhatt and Tadoria, 1990; Bhatt and Badoni, 1993; Nautiyal and Dhyani, 1994; Nandi et al., 1996, 1997; Tewari and Dhar, 1997; Nautiyal et al., 2001; Pijut and Moore, 2002; Henselova, 2002; Henselova et al., 2002; Khan et al., 2004; Joshi et al., 2004; Das, 2006; Verma et al., 2007). Although some species can be propagated by both the seeds and the vegetative parts, but low germinability (Nautiyal et al., 2002; Joshi and Dhar, 2003; Butola and Samant, 2006), slow growth and long cultivation cycle (Badola and Singh, 2003) are some major constraints while using seeds for their cultivation.

Auxin is one of the most important substances in the adventitious rooting of cuttings. Induction of first root initials is influenced by endogenous or exogenous supply of auxin (Thimann and Poutasse, 1941; Tillberg, 1974). However there are many plant species, cuttings of which do not root even with the application of auxin (Nanda, 1970). Leaves and buds have highest concentration of auxin in cuttings and is believed to be the known sites for auxin production (Moore et al., 1992).
Exogenous application of auxin has been partly or completely successful in stimulating adventitious rooting in absence of bud and/or leaves (Haissig, 1970; Roberts and Fuchigami, 1973; Eriksen and Mohammed, 1974). At first, researchers thought that auxin could only be applied in a semi-natural way, namely by basipetal transport. Practical application of auxin for rooting of cuttings became feasible when it was found that auxin also acts when added to the cut surface of cuttings, i.e., passed through the base (Hitchcock and Zimmerman, 1936). In the same period of time, Indole-3-butyric acid (IBA) and α-naphthalene-acetic acid (NAA) were synthesized chemically, their capability to induce roots was discovered (Zimmerman and Wilcoxon, 1935), and talc powder was introduced as a carrier for auxin (Grace, 1937).

Auxin enters cuttings predominantly via the cut surface even in microcuttings that are known to have a poorly functioning epidermis (Guan and De Klerk, 2000). Auxin is rapidly taken up in cells by pH trapping (Rubery and Sheldrake, 1973) and by influx carriers (Delbarre et al., 1996). Auxin stimulates cell division in the cambium and is important for differentiation of cambial initials into root primordial (Haissig, 1970). Auxins probably are changed chemically (partially oxidized or conjugated with other compounds) before they act physiologically (Gurumurthi et al., 1973). Ethylene may also enhance such binding of auxin (Epstein, 1982). Naturally occurring bound auxins have been shown to promote rooting (Felker and Clark, 1981) and influence embryogenesis (Epstein et al., 1977).

Several investigators (Nanda et al., 1970; Pal, 1980; Nautiyal et al., 1992; Gera et al., 2000) have studied the effect of auxins on rooting of stem cuttings. Pal (1980) found that highest percentage of rooting occurred in cuttings obtained from the basal part of the seedling even without auxin treatment. Nautiyal et al., (1992) reported that 100-200
mg/l IAA, IBA or NAA could root stem cuttings taken from mature tree of *Tectona grandis*. Gera *et al.*, (2000) reported that *Tectona grandis* gave a very poor rooting response (10%) without the assistance of growth hormones.

Seasonal variations in the effectiveness of auxins have been reported by many workers (Nanda, 1970; Roberts and Fuchigami, 1973; Anand and Herberlin, 1975). Thus in the same plant species, an auxin may stimulate rooting of cutting in one season, may be ineffective in the other season and even inhibitory in still other season. Again, the maximum rooting of the same plant species may be caused by two different auxins in two different seasons (Nanda, 1970).

IBA is most commonly used for rooting in commercial productions. The other auxins used commercially are IAA and NAA. Many chemical analogues have been synthesized and examined for auxin-like activity (Jonsson, 1961), but none of them are being used on a large scale for rooting. The observed differences in effectivity between the various auxins may lie in the nature of the compound. For example the affinity to the auxin receptor involved in rooting (Libbenga and Mennes, 1995) or in the concentration of free auxin that is reached in the ‘target’ cells.

Mixtures of root-promoting substances are sometimes more effective than either component alone. For example, equal parts of Indole-3-butyric acid (IBA) and Indole-3-acetic acid (IAA) when used on a widely diverse species were found to induce a higher percentage of cuttings to root and more roots per cutting than either auxin alone (Ellyard, 1981). Adding a small percentage of certain phenoxy compounds to either IBA or IAA increased rooting and produced root systems better than those obtained when phenoxy compounds are used alone (Davis and Haissig, 1990).
2.6 Effect of concentration of Indole-3-butyric acid (IBA) on propagation through branch cuttings

Countless studies have been reported on the stimulatory influence of IBA on the propagation of cuttings of difficult-to-root species. Treating branch cuttings with IBA increases rooting percentage, hastens root formation, and increases uniformity of rooting (Davis et al., 1988). Application of Indole-3-butyric acid (IBA), is one of the most common and effective means to enhance rooting of cuttings (Blazich, 1988; Dirr and Heuser, 2006; Hartmann et al., 2010). IBA has been documented in nearly every aspect of plant growth and development. Some of the processes regulated by IBA, as they pertain to plant propagation include: induction of cell division, stem elongation, apical dominance, induction of rooting, and vascular tissue differentiation (Srivastava, 2002).

A large number of studies have been conducted solely to identify optimal hormone application levels to maximize rooting of hardwood cuttings. Concentrations of 2000-7000 ppm of IBA are typical for the propagation industry (Dirr 1998). Carvalho et al. (1995) and Milind (2008) opined that treating branch cuttings of stevia with 5000 ppm IBA promoted rooting and increased the number of roots. Chalapathi et al. (2001) reported that cuttings treated with IBA 500 ppm was found to be superior with respect to shoot length, number of branches, number of leaves and root length, survival percentage and sprouting percentage. Husen (2003) reported that treating Rauwolfia cuttings with IBA 2000 ppm significantly increased rooting and sprouting percentage against the control. Shah and Bhujwan (1999) obtained the highest rooting percentage (86.66%), maximum number of roots per cutting (10.66) and length of longest root (6.40 cm) in stem cuttings of Berberis when treated with 5000 ppm IBA. Profuse rooting was observed with IBA at 2500 ppm.
within 40 days of planting the stem cuttings (Selvarajan and Madhavaraao, 1982).


There is considerable evidence that IBA act primarily in a regulatory capacity in some phases of the carbohydrate metabolism of plants. A suggestive finding in this connection is that, the introduction of auxins into leaves of cuttings induces a marked hydrolysis of starch (Mitchell and Whitehead, 1940). IBA also appear to participate in some aspects of the respiratory process (Commoner and Thimann, 1941; Berger *et al.*, 1946). Cooper and Went (1938) stated that if one treatment of IBA is not quite sufficient, retreatment of the cuttings can be used successfully.

Panwar *et al.* (2001) observed improved rooting percentage in hard wood cuttings treated with IBA 7500 ppm in pomegranate (*Punica granatum* L.). Farooqi *et al.* (1994) and Bagoury *et al.* (2006) found the increasing trend of rooting percentage, number of roots per cutting and length of the longest root (cm) with increasing concentration of IBA. Bhattacharjee and Balakrishna (1993) obtained cent percent rooting in *Ixora singaporensis* with 4000 ppm IBA.
Application of synthetic IBA to branch cuttings at high concentrations can inhibit bud development and sometimes to the point at which no shoot growth will take place even though root formation has been adequate (Sun and Bassuk, 1993). It is clear that IBA stimulate the formation of roots by an interaction involving organic materials in plants, particularly carbohydrates and nitrogenous materials. This interaction apparently controls the basic step of morphological differentiation at the cellular level (Leopold, 1960). Debnath (2008) studied the effect of IBA on root proliferation of stevia with varying concentration of IBA and found that increasing the concentration of IBA increased sprouting percentage, length of roots and number of roots and callus intensity but to certain limit. Shin and Lee (1979) have reported that higher concentration of IBA inhibited the rooting and sprouting of chrysanthemum cuttings due to the injury caused to the callus tissue.

2.7 Effect of maturity stage of donor plant on propagation through branch cuttings

Maturity stage of donor plants have been reported to have negative influence on the performance of rooted branch cuttings (Mitchella et al., 2004). A general decline in rooting ability, root quality and speed of rooting in the nursery, and a reduction in survival, growth and form in the field, have been associated with donor plants that have reached a state of reproductive or ontogenetic maturity (Patton and Riker, 1958; Hackett, 1985). Clark (1981) identified four areas linking maturation state and rooting. The areas of importance were stem anatomy, which causes physical barriers to root initiation, rooting co-factor levels, endogenous rooting inhibitors and the presence of preformed root initials. The loss severely hinders tree improvement programmes based
on asexual propagation, as proven genotypes typically have lost high adventitious root regeneration potential (Zobel and Talbert, 1984).

Plant growth may be separated into distinct juvenile and mature phases. These phases can be distinguished from one another by a number of morphological and physiological characteristics such as leaf shape, leaf retention, phyllotaxis and pigmentation (Hackett, 1985). The juvenile phase of plant growth is characterised by an inability to initiate flowering (Clark, 1983). Adventitious root formation in woody plants is also intrinsically linked to the developmental age of the plant. The potential to initiate adventitious root formation is one of the characteristics which has been observed to change in many woody plant species with developmental age (Mullins, 1987).

Several types of branch cuttings can be taken from the parent stock, which depends on the maturity of branch the cuttings is taken from. Thus the branch cuttings may be of hardwood, semi-hard wood, softwood and herbaceous types (Hartmann et al., 2010). Propagators usually select healthy, vigorous, well-matured, shoots with viable buds as the source of cutting material. However, research has shown that the maturity of the wood used in making stem cutting play an important role in rooting. Cuttings made from the new growth i-e softwood generally root best and produce roots of higher quality. Thieglas and Hoitink (1972) experimenting with eastern white pine found softwood cuttings as best for rooting whereas, Nienstaedt et al. (1958) reported hardwood cutting as best for maximum rooting in the species. Thimann and Delisle (1939) reported that cuttings removed from juvenile or young plants rooted readily but cutting removed from 3-5 year old sapling of many species rooted poorly. Cuttings taken from 3 year old seedlings of Pine (Pinus sp.), Oak (Quercus sp.) and Larch (Larix sp.) rooted considerably better than cuttings from 10 year or 20 year old plants (Komissarov,
Helgonal and Espagne (1987) recorded 70 percent rooting success for 1-3 month old seedlings and this dropped to a mere 6 percent for 20 year old trees. The most significant difference in rooting ability of *Quercus acutissima* was produced by variation in ortet age; cuttings from 1 year old seedlings showed 84.4 percent rooting, while those from 3 year old seedlings showed 58.3 percent (Moon et al., 1987).

Gurumurti et al. (1988) reported that the stem cuttings made from mature trees of *Eucalyptus tetricornis* completely failed to root whereas, the coppice shoot cuttings made from 30 month old trees responded to rooting to extent of 65 percent. Paton et al. (1970) also reported that *Eucalyptus grandis* cuttings collected from mature branches were difficult to root whereas, cuttings from young coppiced shoots rooted easily. Further, cuttings from seedlings and 3 to 7 year old hedge stock plants of *Pinus taeda* rooted better than cuttings from 3 year old tree from donors and it was concluded that loss of rooting capacity can be arrested by shearing stock plants to low hedges (Hamamm, 1988).

Problems associated with maturation are more severe in some clones and cultivars than others (Kester, 1976). Geneve et al. (1991) stated the control of maturation is mainly a function of the development of the vegetative meristem, which is determined mainly by the number of cell divisions rather than its chronological age. There is evidence that loss of rooting potential often is not marked by the arrival of the capacity for sexual reproduction and therefore can be related to other factors. Clark (1981) contends the loss of rooting ability and the capacity to form flowers are distinctly different physiological processes. Williams et al. (1984) observed that poor-rooting ability in woody plant species was related to suberization of the cortex. Favre (1970) observed that too much or too little lignification gave minimal rooting, while Dalet and
Cornu (1988) found lignification retarded the emergence of adventitious roots in the easy-to-root cherry clone.

Roberts and Moeller (1978) examined rooting potential of Douglas-fir as related to achieving reproductive maturity and found the decrease in rooting not dependent on flowering. The reduced rooting potential was instead correlated to other factors related to physiological maturity.

Another phenomenon associated with ageing is that tissues found at different locations on the same tree differ in juvenility (Phillion and Mitchell, 1984). Paradoxically, tissues at the top of the tree are vegetatively mature but are the youngest tissues in chronological age. Conversely, the oldest tissues found near the base of the tree tend to be more juvenile (Kester, 1976). In Douglas-fir, the rooting potential of the upper two-thirds of the tree was found to be significantly less than the lower one-third of the crown (Roberts and Moeller, 1978). This would appear to support the argument that the lower portion of the crown is more juvenile. Schwabe (1976) implicated proximity to roots as a factor in juvenility of shoots. Specifically, juvenility is related to a gibberellin-like factor associated with the roots.

Branch order also plays a significant role in rooting potential. Secondary lateral branches tend to root better than do primaries (Farrar and Grace, 1942). However, secondary’s exhibit more plagiotropic growth, which is a common problem encountered in propagation programs. However, Miller (1982) found in Fraser fir cuttings that differences in rooting responses attributed to secondary and primary shoots were eliminated when exogenous auxins were applied to cuttings harvested from them.
2.8 Effect of different seasons on propagation through branch cuttings

Season of the year may sometimes have dramatic influence on the rooting of cuttings (Bassuk and Howard, 1981). Kachecheba (1976) demonstrated that seasonal changes in rooting response of the branch cuttings were related to changes in the levels of endogenous regulatory substances and nutritional status of the cuttings. Rooting response in different season also depends upon the nature of cuttings. For soft-wood cuttings of deciduous species, best results are generally obtained if cuttings are collected in the spring, while for broad-leaved evergreens, spring to late autumn collection is most successful. Soft-wood cuttings rooted better in spring but older ones in winter (Hartmann and Brooks, 1958). In many species there is an optimal period of the year for rooting (Anand and Heberlein, 1975). For instance, in *Rubus idaeus*, regeneration from cuttings taken from autumn to spring was almost 100 percent successful. Cuttings taken during the summer months failed to survive (Hartmann et al., 2010).

In an experiment to optimise cutting frequency for rooting, a 5-year-old stand of *Tectona grandis* was coppiced in 1999 and converted into a vegetative multiplication garden. Subsequently, three harvesting regimes for the collection of single node branch cuttings were imposed: (1) once: in March, (2) twice: in March and September and (3) three times: in March, July and November. Rooting ability was affected by the frequency of stock plant pruning, with cuttings from stock plants pruned twice per year having the greatest rooting percentage (27.8 ± 3.8 percent) and the most roots (9.2 ± 4.8) (Singh et al., 2006).

An annual rhythm in rooting response has been reported by some workers (Chauhan and Sehgal, 1982; Nautiyal et al., 1991). The branch
cuttings of *Olea europea* (Hartmann and Loreti, 1965), *Salix atrocinera* (Vieitez and Pena, 1968) and *Populus nigra* (Nanda and Anand, 1970) exhibit annual rhythm in rooting response. Further Nanda and Kochhar (1985) reported that regeneration of cutting was influenced by both external and internal factor and suggested that rooting of cutting occurred in a particular season and time and not before or after that.

In propagation of *Colutea istria* from stem cuttings, autumn cuttings showed better rooting capacity than winter cuttings (Andres et al., 2005). Shamet (2000) propagated *Pinus roxburghii* under open nursery conditions. Cuttings were taken in the summer (May) and rainy season (July). The results showed that the stem cuttings gave the highest rooting success in summer when treated with 5,000 ppm IBA + 2,500 ppm NAA (71.4 %) and in rainy season when treated with 10,000 ppm IBA (61.4 %).

The effect of season of cutting collection and origin of branch cuttings on vegetative propagation of *Osyris lanceolata* was evaluated in Tanzania. Cuttings were collected during December, February, June and September. The results revealed that juvenile branch cuttings collected in September, originating from the basal portion had the best rooting (43.8 ± 3.9 %). This was supposedly related to the high levels of stored food in the plant after undergoing active photosynthesis during the rainy season, November-May. Application of IBA between 50 and 100 ppm further enhanced rooting success (Teklehaimanot et al., 2004).

Negash (2004) described an experiment on *Olea europea* subsp. *cuspidata* in which cuttings were harvested from 14-week-old to 15-week-old sprouts. Branch cuttings derived from intact, 4-year-old trees, resulted in highest percent rooting. Cao and Gao (2003) evaluated four dates for collection of cuttings of *Simmondsia chinensis*. The best cutting
period turned out to be July to August. The main reason put forth for low rooting response of semi-hardwood cuttings was that they contained rooting inhibitors. Nevertheless, rooting was increased by soaking in water for 12 hours.

Thakur (1999) subjected shoot cuttings of *Alnus nitida* to four collection seasons: February, July, September and November. Cuttings collected in November gave little or no rooting with or without auxin treatment. February and July cuttings responded well to auxin treatment. Maximum percentage rooting was in February cuttings treated with 200 ppm IBA, with best sprouting and numbers of roots per cutting in the same treatment with July and February cuttings, respectively. In softwood cuttings of *Alnus maritima*, cuttings collected in August rooted more frequently (41%) than did cuttings collected in June (8 percent) (Schrader and Graves, 2000).

In *Juniperus horizontalis*, the best time for collection of cuttings proved to be April and the plants attained greatest number of roots per rooted cutting as well as root length (Sawwan and Shraim, 1999). Frison (1967) and Nesterov (1967) reported that rainy season was the best for propagating *Picea abies, Prunus malus, Prunus persica, Pyrus communis and Populus deltoides*. Many conifer species rooted better when cuttings are taken in autumn or winter (Wyman, 1930) and this pattern has not altered either by use of root promoting substance or by changing the environment of cuttings (Lanphear and Meahl, 1963).

There were marginal differences in callus formation (85-92%) and rooting potential of cutting (82-90%) raised during different seasons in Guava. However during extreme hot (june) and Cold (January) months, inhibition of root formation was recorded (10-20%) due to non availability of actively growing tips of terminal shoot cuttings.
The effect of season on the success of the vegetative propagation of Jiga (*Garuga pinnata*) was reported by Khanam *et al.* (2007). The highest percent survivability (59.3%) was found in March plantation followed by April (48.1%) and then by May (22.2%). June plantations were found to be unsuccessful.

The influence of season (time) on vegetative propagation of *Gymnema sylvestre* was revealed by Pandey (2012). The cuttings sprouted every month and sprouting percentage varied from 51-89% whereas, rooting percentage varied from 18.5-44.5%. March was found to be the best month for planting of *Gymnema sylvestre* cuttings as it showed maximum sprouting (89.6%) and rooting (44.5%). July was also found to be equally good for the purpose with sprouting 86.4% and rooting 41.5%. The lowest rooting was observed in the cutting planted in the month of December (18.5%).

The response of cuttings to rooting in *Doiscorea sps* was studied by Behera *et al.* (2009) month-wise for the development of propagules by planting in nursery bed. *D. alata* rooted quickly i.e. in 7.87 days during August and longest time was taken during May (27.16) days. Survival percentage was highest during rainy months of July to October (88.18%). The number of primary roots was highest during October (4.37) and lateral roots was highest during August (47.15) with maximum root length (21.26cm). After transferring the material from nursery bed to field, survival percentage was highest in rainy season (86.94%).

Effect of season on the rooting of *Juniperus* cuttings was studied by Jesinger, (1967) and Sawwan and Shraim (1999). All the cutting of *Juniperus chinensis* (Hetzii) rooted when taken in June. Good rooting was also obtained on cuttings taken in April, August and October. Similarly *Juniperus horizontalis* (glomerata) rooted best in June August and...
October. With both of these *Juniperus*, rooting of cutting taken in the remaining months was improved at the higher temperature of rooting medium.

Influence of season on the rooting behaviour of green bamboo by cutting was studied by Razvi *et al.* (2011) and found that rooting was obtained in three seasons except winter season and the variation in all the physiological characters with season were significant at $p< 0.001$. Maximum (62%) rooting and (24.41 cm) root length was found in rainy season followed by summer season with (50.33%) rooting and (15.31 cm) root length. However (47.33%) rooting and (16.80 cm) root length was achieved in spring season.

Effect of different time of collection of *Plantus orientalis* cuttings was studied by Zencirkiran *et al.* (2012) and found that different times of collection of cuttings were effective on the rate of rooting. The highest rooting was seen in November (36.40%) and December (23.47%), it was followed by cuttings prepared in October (22.53%). It was noticed that cuttings prepared in April failed to root. The mean root number was found to be highest in the cuttings prepared in October, followed by November. The primary root length was found to be longest in the cuttings prepared in December and November.

The effect of cutting dates on the rooting of semi hardwood cuttings from the Kiwi fruit (*Actinidia deliciosa*) were investigated by Ucler *et al.* (2004). The results of the study revealed that cutting taken in July had better rooting ability in terms of mean root numbers, mean root length of the longest 5 roots and rooting area. The rooting levels were between 76.6 % and 100% for the 1st group of cuttings taken on July and between 26-63.3 % for the cuttings taken in March.
2.9 **Effect of propagation environment on propagation through branch cuttings**

In plant propagation, the different propagation environments viz., nursery bed (open conditions), glasshouse, greenhouse, polyhouse, non-mist propagation chamber and mist chamber have been widely used for rooting of branch cuttings. Development of non-mist chamber is a major breakthrough in propagation of plants (Prolings and Therios, 1976). A fine water spray by rose-can reduces the transpiration and also temperature to a lower level thereby, help creating a favourable condition for rooting of cuttings (Good and Turkey, 1966). Creating humid atmosphere by means of artificial mist around the planted cuttings either in concealed pot culture house or in open conditions has proved to enhance the process of rooting (Adriance and Brison, 1955; Singh et al., 1957 and Vijayakumar, 1973). Experimental evidences have shown that the success of branch cuttings was markedly influenced by the humidity of propagation bed (Lynn and Hartmann, 1957). Raines (1940) was the first to report the use of propagation chamber for rooting of cuttings. Beneficial effects of humidity on root formation have been recorded in various difficult to root plants (Hartmann and Whisler, 1956).

Chalapathi *et al.* (1999) stated that direct planting of stevia cuttings in the field have limited success. Farooqi *et al.* (1998) found that the medium cost green house was good for propagation through cuttings in view of the relatively high temperature and high humidity. Extent of rooting of medicinal and aromatic plants on an average under medium cost greenhouse was 76.30 percent when compared to shade net conditions (25.00%).

Loach (1997) studied the rooting of cuttings of rhododendron under mist and polythene. He stated that propagation under polythene
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gave better results than mist in the lower radiation conditions. Vijayakumar (1973) obtained successful rooting of guava cuttings under high humidity conditions with IBA at 5000 ppm concentration. It has been further established that when the non-mist propagation was coupled with certain hormonal treatments, the cuttings gave better rooting than with humidity alone. Rooting was highest (17.46%) in stem cutting of plum (Prunus domestica) under high humidity as compared to 9.8 percent in open (Chauhan and Reddy, 1974). Korematsu and Shinno (1975) stated that non-mist propagation increased the percent of rooting and reduced rooting time in many garden trees and shrubs. Beneficial effects of high humidity conditions over open field conditions have been recorded in the propagation of many tropical fruit trees (Garner and Chaudhary, 1976).

Singh (1979) reported that the rooting response of cuttings of Jasminum sambac was found to be good under high humidity. Selvarajan and Madhavarao (1982) reported that non-mist chamber provides most favorable environment for better rooting of patchouli cuttings. Mukhopadhyay and Bose (1979) standardized the propagation of cuttings in some ornamental plants under high humidity and found favourable influence of humidity on root formation. Studies on the effect of light environment during propagation of stem cutting according to Mesen et al. (1997), confirm that photosynthesis occurs on cuttings grown under propagation systems. The same study further relates rooting ability to lower irradiance depending on the leaf area of the cutting.

The importance of propagation medium for the rooting of branch cuttings is also widely recognized (Hartmann et al., 2010). According to Ofori-Gyamfi (1998), rooting performance depends on the type of medium used in the propagating structure. This is so because the various
materials and mixes of materials that can be used in rooting of cuttings provide physical support, oxygen and water (Hartmann et al., 2010; Larsen and Guse, 1997; Andersen, 1986). Tree species differ in their response to different media (Leakey et al., 1990). For example, in *Zruingia gabonensis* (Shiembo et al., 1996a). *Ricinodendron heudelotii* (Shiembo et al., 1996b) and *Militia excelsa* (Ofori et al., 1996) higher rooting percentages were recorded in sand than in the other media tested. In general, media with relatively high water contents, such as sand, are associated with higher rates of water uptake in the cutting and consequently higher rooting percentages. However, water can present a major diffusion barrier to oxygen, and excess water may thereby result in anoxia within the cutting base (Loach, 1986).

Ozawa and Kikugawa (1953) observed better rooting of carnation in sand and vermiculite mixture than either of them or various sand soil mixtures. They concluded that the best physical conditions of the media for rooting were 19-35 percent soil moisture, 60 percent porosity and 30-40 percent air. Ermakov et al. (1962) revealed that cuttings of grape cultivars “Frankenthal” and “Foster” planted in sand (Particle size 0.5-1.00 mm) showed 81.5 and 76.3 percent rooting respectively and those in clay substrates showed 50 and 43 percent rooting respectively. Mukherjee et al. (1979) working under Calcutta conditions, recorded highest percentage of rooting and large number of roots per cutting by using rooting medium of coarse sand followed by a mixture of sand and moss in the proportion of 1:1 or 1:3 in a number of popular ornamental plants. Coarse sand was the most suitable medium for rooting of chrysanthemum cuttings (Kher, 1976).

Lal and Danu (1985) reported that maximum rooting (50.0-76.6%) was obtained with sand alone which was attributed to its high pH (8.02) and lower water holding capacity (21.0%). Saleh and Hossni (1995)
reported that sand (1:1 or 2:1 v/v) resulted in the highest rooting percentage, root number per cutting with best quality as compared to other planting media. Shirol et al. (2001) studied the influence of different locally available rooting media viz., red soil, sand, potting mixture, vermicompost and compost on rootability of Dwarf Poinsettia cuttings. They observed that highest percentage of rooting (67.0%) was obtained in sand alone followed by sand + vermi-compost (1:1). The compost showed poor results (27.0%).

2.10 Effect of incubation on propagation through branch cuttings

Temperature wields considerable influence on metabolic activities responsible for induction and development of shoots and roots in cuttings. Manipulation of temperature of the cutting environment, if it can be done within the available resources and time, may be one of the means for achieving greater success in clonal propagation.

Rooting ability of cuttings can be modified to a certain extent by varying the temperature regime to one, which is optimum for the plant species. Zhu et al. (1986) advocated raising of temperature in storage environment (0°C to 10°C) of cuttings to 12°C to 18°C one week before planting of cuttings so as to accelerate growth and formation of root. They can be taken out when the cuttings ‘heal’ showing bark cracks and few roots emerge. This can be done in hot beds having a media of moist sand and horse manure covered with plastic sheet (Zhu, 1996).

Hartmann et al. (2010) have suggested incubating hardwood cuttings of woody species in moist condition at 18°C to 21°C to stimulate callus development which may lead to quick root initiation upon planting in nursery. Ughini and Roversi (2005) compared two bottom-heating
temperatures of 21°C and 27°C for rooting of hazelnut cuttings and found better percentage of rooting with 27°C.

Singh and Dhiman (1999) used mulch as a means for increasing temperature of the cutting environment. The mulches included black or white plastic sheets or paddy straw. The plastic sheet mulches increased soil temperature and conserved soil moisture, which helped in increasing the sprouting percentage, height and diameter growth and survival. The black plastic sheet mulch gave the best results although the plants thus treated were not significantly different from those obtained with white plastic sheet. In another study on use of plastic film as mulch, reported by Chen et al. (1984), the seedlings emerged earlier and more evenly under plastic and the rate of emergence were higher. Seedling growth was faster and root system was better developed.

Sprouting success of the branch cuttings has been recorded to be 95 percent in the greenhouse and ranged from 19 to 58 percent in the field. The better success in greenhouse was associated with soil moisture conditions at planting time (Stringer, 1994). Lakshmi Kanth (2003) on the other hand compared greenhouse (minimum temperature 24°C, maximum 30°C) and polyglobule (minimum temperature 22°C, maximum 35°C) for rooting leafy stem cuttings of Tectona grandis. The polyglobule environment proved more effective resulting in faster sprouting and root induction.

It has been suggested that poor rooting under unfavourable temperature conditions may be due to diversion of more plant nutrients towards shoots and less towards roots (Hoffmann and Kummerov, 1966) or poor mobilization of starch reserves under low temperature (Nanda, 1970). Bala et al. (1969) considered that poor rooting at supra-optimal temperatures is due to decrease in the content of endogenous auxin at
these temperatures. However, Jain and Nanda (1972) demonstrated that unfavourable temperatures decreased rooting by disturbing the balance between auxin and nutrition that is required for optimal production of roots. Dykeman (1976) tested rooting ability at 25°C and 30°C. He observed that more rapid rooting and more roots per cuttings at 30°C, but root elongation, root diameter, and root hair development were superior at 25°C. Thus, higher temperatures favoured primordium initiation whereas lower temperatures favoured root development.

The beneficial influence of higher temperature on initiation may be due to the related increase in respiration (Ooishi et al., 1978) and catabolism of simple sugars that would have been stored in starch at lower temperature (Veierskov and Andersen, 1982). In most cases warm air and a slightly warmer rooting medium promote most vigorous rooting; for many species an air temperatures of 15-20°C and temperature of 20-30°C of the rooting medium is optimal for glasshouse propagation (Brix, 1973).

Pronounced varietal differences exist in the response of primordium initiation and development to temperature (Dykeman, 1976; Ooishi et al., 1978). Percentage of cutting rooted increased when temperatures of propagating beds were raised above 20°C and 28°C was optimal for most of clones of *Triplochiton scleroxylon*, especially if treated with auxins. While cutting survival declined above 28°C, although up to 38°C surviving cutting rooted equally well (Leakey et al., 1982). A rooting temperature of 16°C delayed the rate of root production compared with the rate at higher temperature, but the final rooting percentage was the same over the range from 16 to 28°C. In addition to this, root branching increased with temperature (Welander, 1995).
2.11 Effect of wounding on propagation through branch cuttings

All cuttings are by definition wounded, however increasing the area of mechanical wounding has been proven to enhance wound responses, including the division of the affected cells, which may lead to adventitious root formation (MacKenzie et al., 1986). Wounding of branch cuttings has been thought to increase rooting efficacy in plant taxa for least the last 50 years. When used in combination with rooting hormone formulations, rooting percentages may again be amplified. The general purpose of wounding is to stimulate the initiation of roots from cortex and phloem cells by exposing a greater surface area of these cells than is provided by the cut base in an unwounded cutting. Furthermore, cells exposed by wounding may take up the hormone solution more readily, as well as some water and other growth factors from the rooting medium (Macdonald, 1986).

The effect of wounding in stem cuttings of Arbutus andrachne was studied by Al-Salem and Nabila (2001). Rooting was significantly affected by wounding. Rooting percentage, number of roots and length were almost three, six, and eight times respectively, as great in wounded as in non-wounded cuttings. Basal wounding of cuttings was also beneficial for rooting of apple (Malus domestica Borkh.) rootstock (Mackenzie et al., 1986) and Parika biglobosa (Teklehaimanot et al., 1996).

To investigate the effect of wounding and Indole-3-butyric acid (IBA) concentrations (0, 500, 1000, 1500 and 2000 mg l⁻¹) on rooting ability of hard wood cuttings of Plumbago capensis, Yousif and Faizy (2013) indicated that the wounding of cutting bases significantly increased the rooting percentage (60.79%) as compared with non wounded cuttings (45.84%), also the wounded cuttings significantly gave
a high values of number of roots, roots length, number of shoots, shoots length and number of leaves as compared with non wounded cuttings which gave the lowest values in all studied features. The interaction between wounding and IBA concentrations indicated that treatments of wounded cuttings with 1500 and 2000 mg l$^{-1}$ of IBA gave the best results of rooting percentage 77.73 and 73.70%, number of shoots 1.90 and 1.80, shoots length 11.90 and 11.55 cm and number of leaves 54.33 and 51.67 respectively as compared with other interactions.

To study the effect of wounding the cuttings base, two planting dates, December and March after treating the cuttings with IBA+NAA mixture in Gardenia thunbergia cuttings. Al-Atrakchii and Saleh (2008) observed that planting date had a great effect on rooting ability, planting cuttings in March gave higher rooting percentage 58.89%, and best results of all rooting characters studied. Wounded cuttings gave higher values of rooting percentage 58.11% and all other characters. On the other hand, cuttings treated with IBA+NAA mixture at 2000+1000 mg.l$^{-1}$ gave 58.33% rooting percentage, 3.91 roots per cutting, longest root length 7.98cm, 1.58 shoots number and 1.25cm shoot length. In general wounding apical cuttings taken in March and treated with 2000+1000mgl$^{-1}$ IBA+NAA mixture proved most effective treatment in rooted Gardenia thunbergia cuttings.

Zhou (2005) studied wounding treatment in a number of experiments conducted on a Bald cypress. Cuttings were incision wounded by cutting 1cm along the vertical axis. Wounding treatment significantly affected ($P \geq 0.05$) rooting percentage and root density ranking (RDR) (a qualitative method of ranking root system quality). Overall wounded treatments produced cuttings with a 1.6 and 1.8 times greater rooting percentage and RDR, respectively, than did non wounded treatments.
In the reports on rooting of jojoba cuttings by Assaf 1990; Birnbaum et al. (1984) and Howard et al. (1984), wounding increased both rooting percent and number of roots. The studies found increase of 60% and 62% in rooting percent for wounded and IBA treated cuttings compared to unwounded IBA treated cuttings. Wounding had a larger effect on the length of jojoba stem cuttings from which roots emerged. With wounding, roots emerged along 17.3 mm of the stem, while without wounding, emergence occurred only along 7.0 mm of the stem. The better root system resulting from wounding would likely result in better performance of the cutting when transplanted to field (Palzkill and Feldman, 1993).

Wounding, Indole-3-butyric acid (IBA) and trimming of cuttings to a basal node were evaluated for their effects on rooting of Buxus sempervirens L. (American boxwood), Buxus sempervirens L. 'Suffruticosa' (English boxwood), Buxus microphylla var. koreana Nakai. (Korean boxwood), and Buxus microphylla var. japonica (Mull. Agr.) Rehd. and Wils. (Japanese boxwood) cuttings by Banko and Stefani (1986). Rooting of all cultivars was improved by a 5 second dip in a 0.4% aqueous solution of the potassium salt of IBA. Wounding the basal end of the cuttings only improved rooting for Korean and Japanese boxwoods. However, the combination of wounding and IBA dip gave the best results for all cultivars.

The effect of different wounding treatments on the rooting of ‘Domat’ Olive cuttings was examined by Isfendiyaroglu and Ozeker (2012). Shallow incision wounding significantly augmented the rooting of cuttings. The highest rooting percentage and root number were 68% and 4.5 respectively with incisions while unwounded cuttings gave 21% rooting and 1.0 root. Shallow slice wounding gave the longest roots (36.8
mm). The highest number of secondary roots (2.6) was also obtained with incision wounding.

The effects of wounding treatments and hormone application on rooting of callused Leyland Cypress cutting was studied by Heike De Silva (2002). Combination of different wounding treatments (no wound, single wound and double wound) and IBA application (0, 5000 and 10 000 ppm IBA) were applied. A double wound in combination with 10 000 ppm IBA increased rooting percentage by 47%, mean number of primary roots from 0.7 to 3.7 roots per cutting. The percentage of cuttings that developed roots with good symmetry was 39% higher than the control treatment.

To investigate the effect of wounding on the rooting of semi hardwood cuttings from the kiwi fruit (Actinidia deliciosa), Ucler et al. (2004) revealed that split wounding at the base of the cuttings has no significant difference in main root numbers, the mean root length of the longest five roots and rooting area. Although it had a significant effect on callus formation.

The rooting of stem cuttings of Protea ‘Susara’ was examined with regard to auxin application, basal wounding and position on the shoot. Three levels of Indole-3-butyric acid (IBA) (0, 2000 and 4000 ppm) and four types of cuttings were used. The type of cutting did not have an overall effect on rooting (P > 0.05). The presence of auxin, or basal wounding, or a combination of both did not improve rooting significantly. Wounding negatively affected rooting (Rodriguez-Perez et al., 2009).

On the basis of experiments conducted on the rooting of dawn redwood cuttings it was found that the application of Seradix B No 1 increased the number of rooted cuttings by 14.5% in comparison to the
control. Incision wounding of cuttings at their base had an adverse effect on the rooting percentage and the length of secondary roots. It results from the conducted observations that at the position of the incision wounding roots are not formed. It can be seen from the performed cross-section that dawn redwood regenerates roots from the cambium (cambial ring) and not from the callus. Hence, incision wounding of cuttings is not recommended in the propagation of dawn redwood (Kolasinski, 2006).

2.12 Effect of different provenances on propagation through branch cuttings

Genetic variation is a necessary pre-requisite for any future adaptive change or evolution. Genetic variation is essential if populations are being successfully reintroduced to a new habitat. The populations with highest genetic diversity are most important to conserve from genetic standpoint (Godt and Hamrick, 1995).

Plants produced from stem cuttings of candidate plus trees showed significant increase in height, collar diameter, root length, number of leaves and dry matter production in *Jatropha curcas* (Kaushik, 2003). High variability in growth performance was also reported in *J. curcas* usually in all the field sites of South India (Montobbio, 2009). The phenotypic and genotypic variance, their coefficients of variability and broad sense heritability showed a sizeable variability. High heritability coupled with moderate genetic gain, was observed which signifies strong genetic control and good amount of heritable additive genetic component can be exploited for improvement of the species (Ginwal *et al.*, 2005).

The study was conducted to assess the genetic variability in phenotypical characters and nursery growth parameters among the different altitudinal provenances of *J. curcas*. The study revealed high
genotypic coefficient of variation, heritability and genetic advance in characters like number of fruits per tree, seed yield per tree and oil content (Pant et al., 2009). Rao et al. (2008) evaluated variability in propagation and growth characters in candidate plus trees (CPTs) of *J. curcas* from 11 locations. Significant trait differences were reported in morphology, oil content, plant height and seed yield in the progeny trial. Sunil et al. (2009) reported variability in *J. curcas* from four ecogeographic zones of India. The variability in the plant height, number of primary branches, crown diameter, number of fruits per clusters and oil content was considerably large. Prasanthi et al. (2009) reported high phenophytic and coefficients of variation for number of leaves per plant, number of branches per plant and seed yield per plant. In different geographic regions, *J. curcas* showed wide phenotypic variability and significant differences in oil content in their accessions (Singh et al., 2010).

Das et al. (2010) conducted a study on heritability in sixteen *Jatropha curcas* genotypes collected from four states and found significant differences in most component traits. Kumar et al. (2011) investigated natural genetic variation at intraspecific level in the wild genotypes of *J. curcas* collected from different parts of northeast India. Analysis of molecular variance (AMOVA) showed 68.88 percent of variation at intra-population level, whereas 31.12 percent variation was recorded at inter-population level.

Yan et al. (2008) studied propagation and growth of plants on *Broussonetia papyrifera*. The results of an analysis from 14 locations and 4 regions in Taiwan shows highly significant differences between trees (for all the characters) and between locations (for morphological characters). Hegde et al. (2004) tested the genetic variation in teak collected from selected trees representing different regions of peninsular
India. Significant variations were obtained in all the characteristics viz. plant height, collar diameter, number of leaves. Lahiri and Choudhary (1990) carried out a provenance trial of *P. caribaea* with 8 provenances. FRI 660 provenance recorded better survival (76.55 percent), height and diameter growth.

Gulcu and Ucler (2008) in *Pinus nigra* recorded large genetic variation within population for the traits of juvenile, one and two-year old seedlings and suggested that selection within populations will yield rapid genetic improvement. It is likely that selection within populations will be much more effective than selection between populations. Richardson (2006) observed that a pronounced phenotypic plasticity is in itself a genotypic trait that allows the plant to respond to different environment through morphological and physiological changes for its survival.

The predominance of environmental factors over genetic factors has been reported by Kaushik *et al.* (2006, 2007) within the small genetic resource base. Growth and yield of the species considerably depend upon genetic characters, climatological conditions, soil environment etc., for establishment of better stand. Thus, testing of provenance variation under a set of similar conditions acquires paramount importance.

Variability studies in different sources of *Pinus wallichiana* were undertaken by Rawat and Bakshi (2011). These traits revealed significant variability. Genotypic variance (*V_g*) and genotypic coefficient of variance (GCV) were found to be higher than corresponding environmental (*V_e*) and environmental coefficient of variability (ECV) for most of the traits except cone length and collar diameter which indicate the dominance of environment on the expression of these traits. Assogbadjo *et al.* (2011) evaluated the extent of variation in *Adansonia digitata*. Trees were sampled in each of the three climatic zones of Benin.
There were significant differences in tree characteristics not only between climatic zones but also between individuals from the same zone and within-trees.

White ash (*Fraxinus americana*) from many geographic origins was planted in 19 locations throughout the natural range of the species in eastern USA and Canada. Each plantation contained 36 to 52 families and 16 to 27 provenances from portions of the range near its geographic location. Survival after 5 years ranged from 97 to 46 percent and differed among provenances in 12 plantations. Provenances accounted for up to 55 percent of the variation in survival, and the variance component for provenances was greater than the family component in 14 plantations. Average tree height after 5 years ranged from 53 cm to 373 cm and differed among provenances in 12 plantations. Provenance variation was generally larger than family variation and provenances accounted for up to 75 percent of the variation in height. Neither survival nor height was generally correlated with latitude of seed origin. In some plantations the family variance component was much greater than the provenance component, and in others the family component increased from the 3rd to the 5th year. This suggests that gains are also possible through family selection once suitable provenances have been identified (Clausen, 1984).

### 2.13 Propagation through air layering

Air layering is a rooting method where the rooting medium is wrapped around the aerial stem, causing it to produce adventitious roots while the stem is attached to the mother plant (Macdonald, 1986; Thompson, 1989; Hartmann et al., 2010). This technique is one of the oldest methods of vegetative propagation which was used to propagate plants in China (Macdonald, 1986) and it is still being used to multiply
plants, especially those which are difficult to root by cuttings and grafting (Thompson, 1989; Das et al., 1996; Eganathan et al., 1999). Philip (1984) indicated that air layering is easily accomplished without special facilities such as mist or polyethylene enclosures and it seems to be more applicable to small farms. The success of air layering as a practice in propagation is probably due, in part, to the effect of endogenous auxins accumulating at the base of the girdled shoots (Cameron and Thompson, 1969).

Mahabir et al. (1995) investigated on the effect of IAA, IBA, NAA, IBA + NAA + IBA or NAA + IBA + NAA (2500, 5000 or 10000 ppm), white or black polythene wrappers on rooting and growth of air layers. The best plant growth regulator treatment for air layering found was IBA in most respects and this treatment was on par with IAA + IBA + NAA. Barholia (1995) investigated the effect of IAA, IBA and NAA at 5000 ppm, 10,000 ppm and 15,000 ppm respectively on jackfruit. The highest percentage rooting (92.5%), length of primary root (29.7 cm) and numbers of primary roots (29.00) were obtained with IBA at 15,000 ppm. Application of IBA at 5000 ppm produced the highest number of rooted layers (90%), primary and secondary roots per layer (19.00 and 46.00, respectively), length of primary (11.39 cm) and secondary roots (8.27 cm) (Sengupta and Thakur, 2001). Similar results were obtained by Singh and Singh (2004); Hore and Sen (1991); Pawan et al. (2004); Sharma et al. (1991) and Lal et al. (2007)

Mishra and Singh (1995) opined that multiple air-layering is feasible for increasing the efficiency of guava propagation. Kamleshkar and Jain (1996) concluded that the highest (78.75%) percent of rooting, number of primary and secondary roots, length of the longest root were obtained when the etiolated shoots were treated with IBA 6000 ppm during the month of July. Gowda et al. (2006a) reported that layers
treated with 5000 mg/l IBA recorded maximum rooting of 66.5 percent during August and it was lowest in untreated control (0.0) during October. Application of IBA at 5000 ppm in July resulted in the highest percentage rooting (98.3%) in *(Syzygium javanic)* air layers. Percentage rooting was also higher in layers treated with NAA at 10,000 ppm in June (Hore and Sen, 1993).

Rahman *et al.* (2000) reported that maximum numbers of roots per plant (9.94), root length (10.94 cm), number of leaves per plant (10.55) were recorded in litchi air-layers treated with 2500 ppm of IBA. The highest rooting percentage (94.93%) and number of roots (52.00 per layer) were obtained with 5000 ppm NAA. In general, root length was improved by the application of 5000 ppm IBA (Sinha and Ray, 2002). Namita *et al.* (2006) observed that, IBA at 1000 ppm significantly increased plant height, primary roots per layer, length of longest roots, mean root length. Maximum values on various shoot and root parameters were noted in 1000 ppm IBA and root trainer treatment combination. Similar results were observed by Smarsi *et al.* (2008) in litchi. Alila *et al.* (2000) in jack fruit; Mukherjee *et al.* (1986) in Bael; Hegde and Sulikeri (1989) in pomegranate; Gowda *et al.* (2006b) in khirni (*Manilkara hexandra*).

Success in air layering depends on the temperature and relative humidity of the atmosphere, leaf area and activity of root producing tissue of the air layered shoots. Some of the plant factors influencing root formation in air layering are, food supply, the hormone or auxin supply and activity of root producing tissue at the upper end of the girdled surface. Adequate supply of carbohydrates and certain growth regulators in minute quantities are also necessary for root production (Bhullar, 1962).
Application of 10000 ppm IBA during different months of layering was found to be significantly effective in rooting of air layers in guava. Air layers prepared during June month treated with 10,000 ppm of IBA gave maximum rooting percentage (90%) with better root characters (Chandrappa and Nache Gouda, 1998). Animesh and Ghosh (2006) investigated on the effect of IBA at 1000 ppm and 2000 ppm wrappers and date of air-layering. Air layers prepared during June and July showed maximum rooting success, number of primary and secondary roots. The results of Ghosh and Ranjan (2005) revealed that air layering conducted in September, October and November months the resulted in higher rooting success of (85%) guava. Duarte and Sachini (2003) reported air layering on Brewster Lychee (*Litchi sinensis* Sonn) in different seasons with and without IBA at 3000 ppm. Air layers with mature terminal leaves and 5000 ppm IBA were superior to those with immature leaves.

The growth regulators such as IAA, IBA and NAA significantly influenced the root formation. Roots appeared in shortest time in air layers treated with 10,000 ppm of IBA. The maximum number of roots with higher root length and diameter was obtained with 10,000 ppm of IBA during August (Suryanarayana and VenkateshwarRao, 1984). Similar findings were observed by Sharma *et al.* (1978).

Bhandary and Kololgi (1960) stated that Indole-3-butyric acid (IBA) is good for early rooting of air-layers during February to August period and reported that, 10,000 ppm and 15,000 ppm concentrations were optimum to induce rooting in shortest period of five to six weeks. It was also reported that 20,000 ppm had toxic effect while 5,000 ppm was found to be suboptimal. The rainy season (August-September) proved more favourable compared to spring. Sixty eight percent of the layers
done during rainy season showed callus development and root initiation within a month compared to 30 to 40 percent in spring (Ahamed, 1964).

Rajan and Ram (1985) studied the changes in root promoting co-factors and inhibitors during different stages of root development in mango air-layers. They observed that endogenous rooting co-factor activity of aqueous fraction increased conspicuously at callusing and root emergence stages, while the rooting inhibitor level decreased.

2.14 Variation in oil content in natural populations


In general *Diploknema* contains about 42 to 47 percent oil, which is low for commercial extraction. The maximum oil in *Jatropha curcus* is stored in the cotyledons (Dhyani *et al.*, 2011), which has maternal inheritance, and hence, improvement in this trait through hybridization is lesser than the selection of high-yielding lines having high oil content. Therefore, extensive survey and selection of high yielding lines can increase oil content. The dry matter distribution ratio between fruit coat and seeds might be good criteria for increasing seed yield and oil content can also be used for selection of best accessions (Jongschaap, 2007).

Sunil *et al.* (2009) reported oil content between ranges between 22 to 42 percent in 162 accessions of *Jatropha*. Other researchers reported oil content ranges such as: 43 to 59 percent by Gubitz *et al.* (1999); 28
percent to 38 percent by Wani et al. (2006); 28 to 39 percent recorded by Kaushik et al. (2007); 29 to 39 percent recorded by Sharma (2007); 28 to 36 percent Shekhawat et al. (2007). The variation found in oil content by Rao et al. (2008) presents us with a viable selection alternative at a very early stage (collection of germplasm) from base seed material. This could be of use in improvement programmes especially considering the fact that the crop breeding is still in its infancy.

A nation level assessment of Tree borne oilseeds for oil content was done by Kaushik et al. (2006). They divided accessions of whole country in seven groups on the basis of percent of plants having more than 35 percent (high) oil content. Patil and Singh (1991) carried out studies on seed oil characteristics in detail viz. seed moisture (10.2 percent), kernels content in seed (61.5 percent), oil content in kernels (62.3 percent, dry basis), oil content in whole seed (39 percent, dry basis) etc. Tewari (1994) observed that the kernels (60 to 68 percent of seeds) contain 50.60 percent oil (38 to 40 percent on seed weight basis). The seeds contain some poisonous principles with purgative properties rendering oil unfit for human consumption.

Jain and Mahajan (1996) carried out chemical study of Jatropha curcus oil seed to identify the toxic constituents and to evolve methods of refining the oil to edible grade. Fatty acid composition reveals that the oil is rich in oleic linoleic acids (34.93 and 41.27 percent), palmitic acid (18.79 percent), palmitolic acid (0.59 percent), and stearic acid (4.42 percent). The authors suggested that seed oil can be helpful in solving the problem of shortage of edible oil in the country to some extent if a methodology is worked out to remove constituents responsible for toxicity.
Singh et al., (1996) conducted a study on bio-deterioration of *Jatropha curcus* oil seeds and recorded quality deterioration due to fungal infestation. Fungus infested seed exhibited increased fatty acids, saponification value of oil and protein content in seed cake while oil content, protein and carbohydrates were decreased. Seeds contain 46 to 58 percent of oil on kernel weight basis and 30 to 40 percent on seed weight basis (Subramanian et al., 2005).

Systematic efforts had also been made by NOVOD by incorporating different institutions to increase production by using current knowledge of plant breeding and improved agronomic practices, but still there is lot of work to be done.