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Full Length Research Paper

Effect of stem cuttings and hormonal pre-treatment on propagation of *Embelia tsjeriam* and *Caesalpinia bonduc*, two important medicinal plant species

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In the present study, the growing behavior was evaluated for stem cuttings of two ethno-medicinally important shrub species *Embelia tsjeriam* and *Caesalpinia bonduc*. *In vivo* experiment was conducted on the effect of cutting length (two and four nodes), cutting diameter (thick - 0.65 cm, medium - 0.52 cm and thin - 0.43 cm) and pre-treatment of exogenous hormones in powder formulation (Indole 3 Acetic Acid, α- Naphthalene Acetic Acid and Indole 3 Butyric Acid) on percent cutting sprouted, node sprouted and survival. Results revealed a significant effect of all factors on sprouting and survival of both plant species under green house conditions. Maximum percentage of sprouting, node sprouting and survival were recorded when cuttings were treated with NAA and IBA @ 1000 ppm for *E. tsjeriam* and *C. bonduc* respectively irrespective of diameter and length. Sprouting and survival rate of *C. bonduc* cuttings were considerably higher than *E. tsjeriam* cuttings. Thin and medium stem cuttings at four nodal lengths found to be the best for higher sprouting and survival of both plant species. Our study concluded that vegetative propagation of these two important plant species is feasible through application of exogenous hormone on stem cuttings.

Key words: *Embelia tsjeriam*, *Caesalpinia bonduc*, ethno-medicinal herb, stem cutting, sprouting, hormones.

INTRODUCTION

Embelia tsjeriam (Roem. and Schult.) DC. and *Caesalpinia bonduc* (L.) Roxb. the two ethno-medicinally important shrub species were recorded as ‘Vidanga bheda’ and ‘Gataran’ respectively in Ayurvedic *Materia Medica* (Shah, 1996). *E. tsjeriam* and *C. bonduc* belonging to family Myrsinaceae and Caesalpiniaceae, respectively are widely used among local traditional healers in India.

The fruits of *E. tsjeriam* are used to treat worm infestation, anaemia, oedema, ringworm and other skin diseases, fever, anorexia, urinary calculi, and vomiting (Somashekhar and Sharma, 2002). The powdered seeds are used as an antihelmintic and stomachic whereas, the dried bark of the root is used to relieve toothache. Roots of *C. bonduc* are very effective as an antispasmodic agent (Chopra et al., 1956), while the bark is useful as an antihelmintic and febrifuge. The leaves of this plant are found to be a useful remedy as an emmenagogue (Baquar, 1989) whereas; the seeds have a reputation for various pharmacological actions like antipyretic, febrifuge and asthmatic (Dhar et al., 1968; Nadkarni and Nadkarmi, 1976).

The pharmaceutical industry is largely dependent upon the wild populations for supply of these plant species for the extraction of their intrinsic bioactive components. Moreover, local and forest dwellers often use these plant for medicinal purposes. Due to the indiscriminate collection of the plant materials from forests and insufficient attempts either to allow the replenishment or cultivation, these important flora are rapidly disappearing. As a consequence, *E. tsjeriam* reported as the vulnerable and *C. bonduc* as an endangered plant species in India (Sharma et al., 1991). Therefore, there is an urgent need to develop effective propagation methods for cultivation

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of these important medicinal floras which will ultimately lead to their conservation as well as supply for commercial use.

More often, plant species are hard to propagate sexually and also show complexities and undesirable characters. Thus, vegetative propagation through stem cuttings is the most vital and sole method to reproduce these plants species conserving their innate desirable characters (Nanda and Kochhar, 1987). Establishment and growth rate of the stem cutting depends upon many factors, like seasonal and age variation, portion and diameter of stem, growing media, moisture level, nutrient status and temperature etc. (Kristiansen et al., 2005). Moreover, the use of plant growth regulatory hormones or 'auxins' plays a vital role in influencing the sprouting and survival of stem cuttings.

Auxins, a class of plant growth substances are often called as phytohormones or plant hormones and play an essential role in coordination of many growth and behavioral process in the plant life cycle (Delker et al., 2008; Hobbie, 1998). Indole-3-Acetic Acid (IAA), α-Naphthalene Acetic Acid (NAA) and Indole-3-Butyric Acid (IBA) are typically the principal auxins which are available commercially and can be applied with liquid (liquid formulation) or in talc (powder formulation) for rooting and sprouting of stem cuttings (Ercisli and Guleryuz, 1999; Hopkins, 1999).

Keeping in view of the above facts the present investigation has been conducted with a view to study the vegetative propagation of *E. tsjeriam* and *C. bonduc*.

MATERIALS AND METHODS

All the experiment was conducted under green house conditions at T. C. B. College of Agriculture and Research Station, Bilaspur (Chhattisgarh), India during September, 2008. Disease free evenly matured *E. tsjeriam* and *C. bonduc* were collected from the medicinal plant nursery established in this centre. Stem cuttings were prepared at two length sizes, that is, with two nodes and four nodes while each type maintained at three different diameters *viz.* thick (0.65 cm), medium (0.52 cm), thin (0.43 cm).

Three plant growth regulators that is Indole-3-Acetic Acid (IAA), α- Naphthalene Acetic Acid (NAA) and Indole-3-Butyric acid (IBA) were selected as exogenous hormones. Powder formulations were prepared by mixing the growth hormones in required quantities to inert talc powder and adjusted to a concentration of 500 ppm and 1000 ppm following the procedure of Hartmann and Kester (1983). Cuttings dipped in sterile distilled water (water dipping) and without any treatment (control) were included in the experiment for comparison. Hartmann and Kester (1983) method' was used to apply the exogenous hormone formulations to stem cuttings. Individual stem cuttings were overlaid with respective hormone formulations and kept for overnight at room temperature followed by planting in the nursery bed. Irrigation water was applied for 24 h by overhead sprinklers during the experimental period. After 25 days of planting the number of sprouted cuttings and number of sprouted nodes were counted whereas, their survival was recorded at 60 days after plantation. All the recorded data were expressed in percentage.

The experimental design was arranged in randomized complete block design with ten replications for each treatment and the data was analyzed for the variance.

RESULTS AND DISCUSSION

Data offered in Tables 1 and 2 indicated a wide spectrum of efficacy of growth hormones on per cent sprouting, per cent node sprouting and per cent survival of stem cuttings of both *E. tsjariam* and *C. bonduc*. The data also represent a significant ($P \leq 0.05$) effect of cutting length and diameter on sprouting. Though, the results were not consistent within treatments as well as among the plant species yet, it was observed that irrespective of all treatments, exogenous hormone application superiorly induced sprouting, node sprouting and survival percent-tage in *C. bonduc* (59.3, 33.9 and 34.7%) than from *E. tsjariam* (23.6, 11.0 and 8.5%) (Tables 1 and 2).

Interestingly, it was found that water dipping greatly induced the sprouting in stem cuttings *E. tsjariam* as can be seen by the high per cent sprouting (40.0%) and per cent node sprouting (17.5%) whereas, the per cent survival was recorded zero (Figure 1). Among the growth hormones, NAA at 1000 ppm was found superior ($P \leq 0.05$) followed by IAA at 1000 ppm in terms of mean per cent sprouting, node sprouting and survival of *E. tsjariam* cuttings. Similar trend was recorded for hormonal applications at 500 ppm concentration. Chawdhry and Khan (2000) reported that NAA and IAA are known to promote the expansion of roots in cutting thereby increasing the survival rate. Stem cuttings of *C. bonduc* showed unlike behavior than *E. tsjariam* cuttings. The *C. bonduc* cuttings were significantly ($P \leq 0.05$) and positively affected by exogenous hormones than the water dipping and control treatment. The survival of stem cuttings in the control or water dipping treatments was found to be either zero per cent or extremely low. Application of IBA at 1000 ppm brought the highest per cent sprouting, node sprouting and survival of cutting irrespective of their length and diameter. This was followed by application of NAA @ 1000 ppm and at 500 ppm also (Figure 2). Sun et al. (1998) reported the higher effects of plant growth regulators (NAA, IAA, IBA) on sprouting of rose buds. Growth hormones causes enlargement of plant cells, cell division, laterals branching of shoots and roots, vascular differentiation and early embryonic development (Hobbie et al., 2000). IBA were in conformity with the reports of its effectiveness as com-pared to several naturally occurring auxins in promotion of adventitious roots (Hartmann and Kester, 1983). Moreover, Butola and Badola (2004, 2007) have recommended IAA and IBA as promising treatments to improve rooting, growth and biomass in *Angelica glauca* and *Heracleum candicans*.

Powder formulated hormonal application, irrespective of type, concentrations and length of cutting revealed that thin (diameter 0.43 cm) stem cuttings of *E. tsjariam* were considerably more inducible whereas, it was least in medium cuttings (diameter 0.52 cm) (Table 3 and Figure 3).

Table 1. Effect of cuttings length, diameter (D_1 thick; D_2 medium; D_3 thin) and powder formulated exogenous hormone application on per cent sprouting, node sprouted and survival of stem cuttings of *Embelia tsjeriam*.

			4 Node			2 Node			Mean (2 Node and 4 Node)		
T	ppm	Dm	Cutting sprouted (%)	Node sprouted (%)	Plant survival (%)	Cutting sprouted (%)	Node sprouted (%)	Plant survival (%)	Cutting sprouted (%)	Node sprouted (%)	Plant survival (%)
Indole acetic acid	1000	D_1	30.0	10.0	20.0	40.0	20.0	20.0	35.0	15.0	20.0
		D_2	20.0	7.5	12.5	15.0	15.0	0.0	17.5	11.3	6.3
		D_3	60.0	17.5	42.5	20.0	10.0	10.0	40.0	13.8	26.3
	500	D_1	14.3	4.8	9.5	18.6	9.3	9.3	16.4	7.0	9.4
		D_2	9.5	3.6	6.0	7.0	7.0	0.0	8.3	5.3	3.0
		D_3	28.6	8.3	20.2	9.3	4.7	4.7	18.9	6.5	12.4
a-Naphthalene acetic acid	1000	D_1	30.0	7.5	22.5	20.0	10.0	10.0	25.0	8.8	16.3
		D_2	47.5	20.0	27.5	30.0	15.0	15.0	38.8	17.5	21.3
		D_3	40.0	12.5	27.5	40.0	30.0	10.0	40.0	21.3	18.8
	500	D_1	14.0	3.5	10.5	9.5	4.8	4.8	11.7	4.1	7.6
		D_2	22.1	9.3	12.8	14.3	7.1	7.1	18.2	8.2	10.0
		D_3	18.6	5.8	12.8	19.0	14.3	4.8	18.8	10.0	8.8
Indole buteric acid	1000	D_1	20.0	7.5	12.5	10.0	5.0	5.0	15.0	6.3	8.8
		D_2	10.0	2.5	7.5	0.0	0.0	0.0	5.0	1.3	3.8
		D_3	30.0	15.0	15.0	10.0	5.0	5.0	20.0	10.0	10.0
	500	D_1	9.5	3.6	6.0	4.7	2.3	2.3	7.1	2.9	4.1
		D_2	4.8	1.2	3.6	0.0	0.0	0.0	2.4	0.6	1.8
		D_3	14.3	7.1	7.1	4.7	2.3	2.3	9.5	4.7	4.7
Water dipping		D_1	40.0	17.5	0.0	40.0	20.0	0.0	40.0	18.8	0.0
		D_2	30.0	7.5	0.0	50.0	30.0	0.0	40.0	18.8	0.0
		D_3	40.0	10.0	0.0	40.0	20.0	0.0	40.0	15.0	0.0
Control		D_1	20.0	7.5	12.5	19.0	10.0	9.0	19.5	8.8	10.8
		D_2	30.0	10.0	0.0	60.0	30.0	0.0	45.0	20.0	0.0
		D_3	50.0	22.5	0.0	20.0	32.5	0.0	35.0	27.5	0.0
Mean			26.4	9.3	12.0	20.9	12.7	5.0	23.6	11.0	8.5

T = treatments, ppm = concentration in parts per million.

Table 2. Effect of cuttings length, diameter (D_1 thick; D_2 medium; D_3 thin) and powder formulated exogenous hormone application on per cent sprouting, node sprouted and survival of stem cuttings of *Caesalpinia bonduc.*

			4 Node			2 Node			Mean (2 and 4 Node)		
T	ppm	Dm	Cutting sprouted (%)	Node sprouted (%)	Plant survival (%)	Cutting sprouted (%)	Node sprouted (%)	Plant survival (%)	Cutting sprouted (%)	Node sprouted (%)	Plant survival (%)
Indole acetic acid	1000	D_1	75.0	18.8	80.0	75.0	25.0	80.0	75.0	21.9	80.0
		D_2	100.0	37.5	100.0	75.0	37.5	75.0	87.5	37.5	87.5
		D_3	25.0	6.5	0.0	100.0	62.5	50.0	62.5	34.5	25.0
	500	D_1	50.0	12.5	0.0	75.0	65.0	0.0	62.5	38.8	0.0
		D_2	50.0	25.0	50.0	75.0	100.0	0.0	62.5	62.5	25.0
		D_3	50.0	25.0	0.0	50.0	37.5	0.0	50.0	31.3	0.0
a-Naphthalene acetic acid	1000	D_1	100.0	29.0	72.5	100.0	32.5	76.5	100.0	30.8	74.5
		D_2	100.0	31.3	75.0	100.0	50.0	50.0	100.0	40.6	62.5
		D_3	50.0	12.5	37.5	75.0	32.5	50.0	62.5	22.5	43.8
	500	D_1	75.0	25.0	50.0	50.0	50.0	31.0	62.5	37.5	40.5
		D_2	100.0	50.0	87.5	50.0	75.0	100.0	75.0	62.5	93.8
		D_3	47.5	23.5	25.0	50.0	25.0	27.5	48.8	24.3	26.3
Indole buteric acid	1000	D_1	100.0	80.0	100.0	75.0	35.0	50.0	87.5	57.5	75.0
		D_2	50.0	18.8	100.0	100.0	75.0	66.7	75.0	46.9	83.3
		D_3	100.0	38.8	73.3	100.0	72.5	75.0	100.0	55.6	74.2
	500	D_1	55.0	25.0	55.0	100.0	50.0	25.0	77.5	37.5	40.0
		D_2	100.0	25.0	25.0	82.5	40.0	20.0	91.3	32.5	22.5
		D_3	36.0	44.0	58.3	69.5	34.5	50.0	52.8	39.3	54.2
Water dipping		D_1	52.5	13.8	0.0	50.0	22.5	0.0	51.3	18.1	0.0
		D_2	50.0	12.5	0.0	24.0	50.0	25.0	37.0	31.3	12.5
		D_3	35.0	18.8	0.0	22.5	33.5	0.0	28.8	26.1	0.0
Control		D_1	27.5	18.8	0.0	0.0	0.0	0.0	13.8	9.4	0.0
		D_2	27.5	31.3	0.0	22.5	22.5	0.0	25.0	26.9	0.0
		D_3	22.5	25.0	0.0	25.0	25.0	0.0	23.8	25.0	0.0
Mean			60.1	28.2	38.5	58.6	39.7	30.8	59.3	33.9	34.7

T = treatments, ppm = concentration in parts per million.

Table 3. Analysis of variance at 5% for different treatments for hormone application in powder formulation.

Treatments	*Embelia tsjeriam*			*Caesalpinia bonduc*		
	Sprouting	Node sprouting	Survival	Sprouting	Node sprouting	Survival
Hormones	9.5	5.6	13.7	9.6	11.6	4.9
Diameter	4.1	2.7	7.4	9.4	4.6	5.1
Number of nodes	5.7	1.9	Ns	3.5	3.8	2.6
Hormones x Diameter	16.9	9.5	15.7	12.9	13.8	9.5
Hormones x Nodes	13.8	7.7	12.8	10.5	11.3	7.7
Diameter x Nodes	5.8	5.4	5.4	6.1	6.5	4.5
Hormones x Diameter x Nodes	23.9	13.4	22.2	18.2	19.5	13.4

ns = not significant at 5% level.

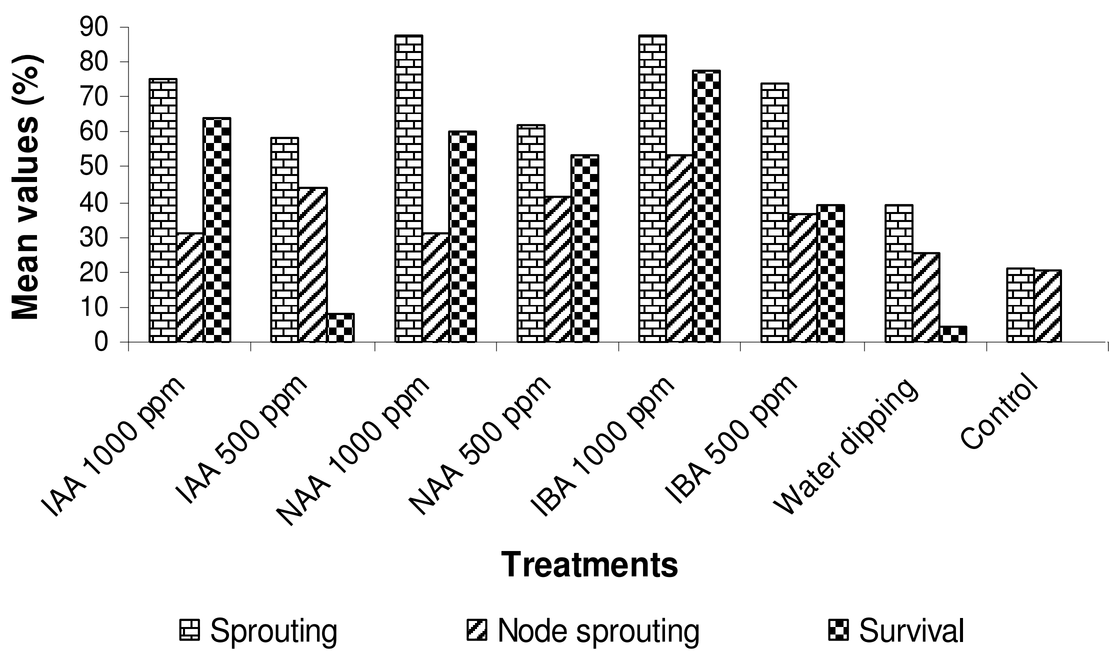

Figure 1. Bars represents mean per cent sprouting, node sprouting and survival of *Embelia tsjeriam* stem cuttings irrespective of length, diameter of cutting after powder formulated hormone application.

For example, mean per cent sprouting (27.8%) and per cent node sprouting (13.6) were highest in thin stem cuttings. On the other hand, hormonal application superiorly stimulated medium stem cuttings of *C. bonduc* followed by thick cuttings (diamt. 0.65 cm) as can be seen by the highest per cent survival (48.4%) (Figure 3). The figure also revealed that exogenous hormone applications were more effective in sprouting and survival of *C. bonduc* cuttings than *E. tsjariam*. For example, the average per cent sprouting, node sprouting and survival of stem cuttings of *C. bonduc* were considerably higher than *E. tsjariam* cuttings irrespective of hormonal applications and cutting length.

Although vegetative propagation of some medicinal plant species through stem tip cuttings were already reported (Hartmann et al., 2002) even if, it was not yet studied in detail for *E. tsjariam* and *C. bonduc*. Market demand of these herbal species for pharmaceuticals and ethno-medicinal utility, are met through harvesting from wild populations. The existing reports on seed germination are not reliable in view of their low germinability and slow growth (Butola and Badola, 2004; Vashistha et al., 2009).

Thus, proliferation by means of stem cuttings is the most economical, easiest and fruitful method for vegetative propagation. Moreover, to improve sprouting, rooting and survival of stem cuttings, plant growth regulators can be wisely and widely used. From the present investigation it

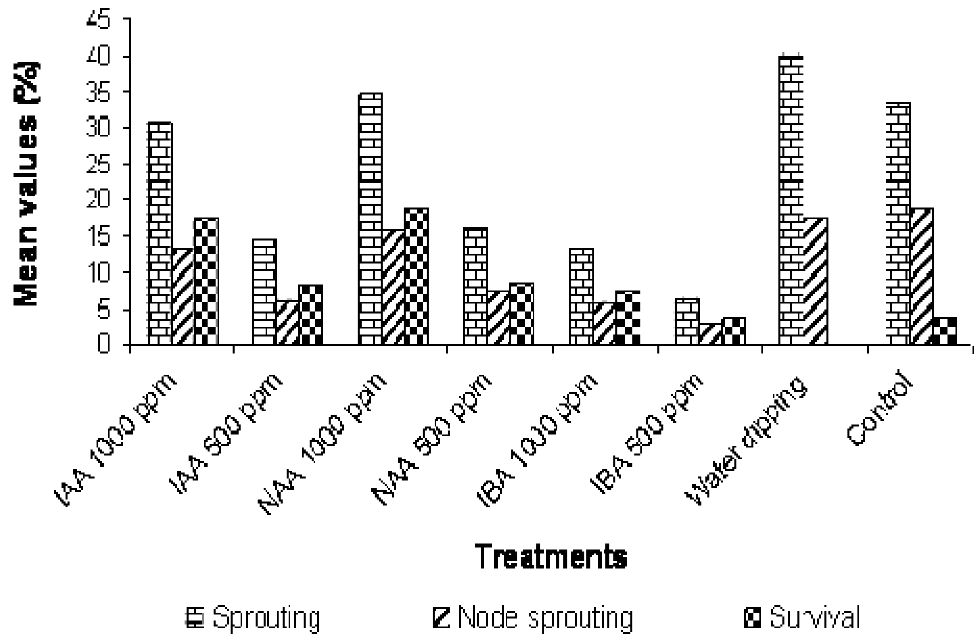

Figure 2. Bars represents mean per cent sprouting, node sprouting and survival of *Caesalpinia bonduc* stem cuttings irrespective of length, diameter of cutting after powder formulated hormone application.

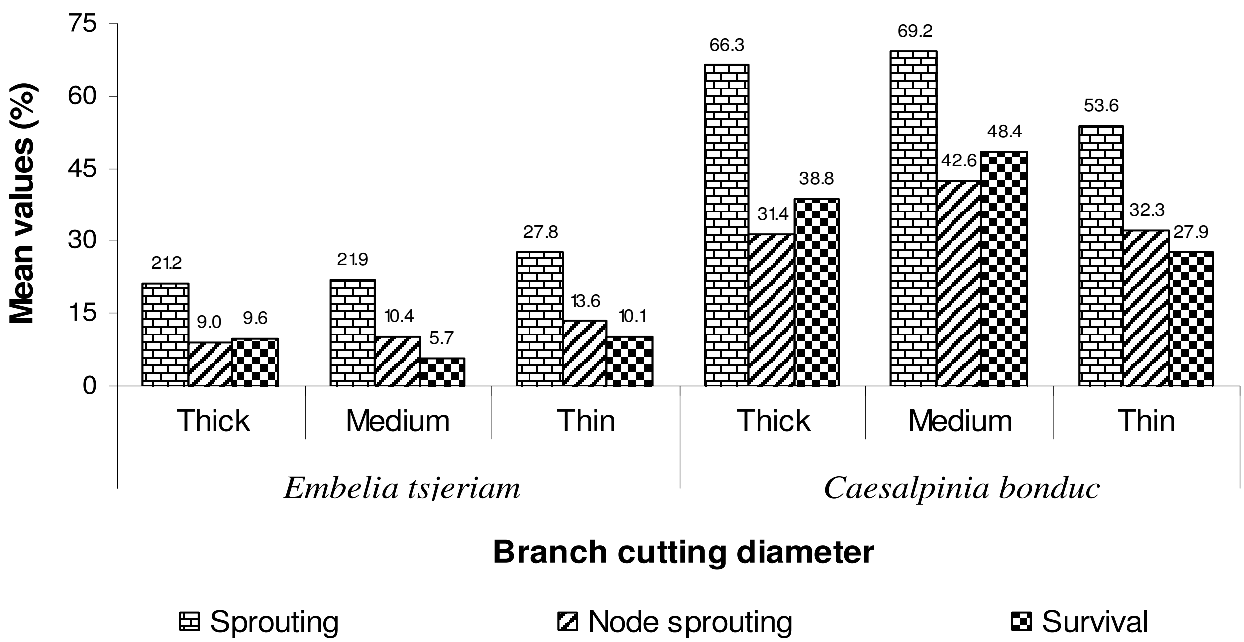

Figure 3. Bars represents mean per cent sprouting, node sprouting and survival of *Embelia tsjeriam*, *Caesalpinia bonduc* stem cuttings at three diameter type (thick= 0.65 cm, medium= 0.52 cm, thin= 0.43 cm) irrespective of cutting length hormone application.

can be concluded that stem cutting with four nodes are appropriate for vegetative propagation irrespective of plant species. Medium cuttings were more suitable for *E. tsjariam* whereas, thin cuttings can be used for propagation in case of *C. bonduc*. More pre-cisely it can be concluded that powder formulation of α- Naphthalene Acetic Acid (NAA) and Indole Buteric Acid should be prescribed for vegetative propagation by stem cuttings of *E. tsjariam* and *C. bonduc* respectively for better sprouting and optimum survival rate. The study laid a strong foundation for the conservation of these two important medicinal plants which are used world wide.

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REFERNCES

Baquar SR (1989). Medicinal and Poisonous Plants of Pakistan. Printas, Karachi, pp. 67.

Butola JS, Badola HK (2004). Effect of pre-sowing treatment on seed germination and seedling vigour in *Angelica glauca*, a threatened medicinal herb. Curr Sci., 87(6): 796-799.

Butola JS, Badola HK (2007). Vegetative propagation of *Angelica glauca* and *Heracleum candicans*. J. Trop. Med. Pl., 8(1): 85-91.

Chopra RN, Nayar SL, Chopra IC (1956). Glossary ofindian Medicinal Plants. CSIR, New Delhi, pp. 43.

Delker C (2008). Auxin dynamics: the dazzling complexity of a small molecule's message. Planta. Apr. 227(5): 929-941.

Dhar ML, Dhar MM, Dhawan HN, Mehrotra BN Ray C (1968). Screening of Indian plants for biological activity.Ind.J.Exptl. Biol. 6: 232.

Ercisli, Guleryuz (1999). A study of the propagation of hardwood cuttings of some rose hips. Tr. J. Agric. For., 23 (Supplement 3): 305-310.

Hartmann HT, Kester DE (1983). Plant propagation principles and practices (4th ed.), Prentice Hall, Inc. England Cliffs, New Jersey, pp 321.

Hartmann HT, Kester DE (1983). Plant propagation principles and practices (4th ed.), Prentice Hall, Inc. England Cliffs, New Jersey, pp 321.

Hartmann HT, Kester DE, Davies FT Jr, Geneve RL (2002). Plant propagation: principles and practices, (7th Ed.). Prentice Hall, Upper Saddle River, NJ. In: Foliage plant production (Ed. Joiner JN. 1981). Prentice-Hall, Inc., Englewood Cliffs, NJ.

Hobbie L, Hurwitz MM, Pierro A, Liu NY, Bandyopadhyay A, Estelle M (2000). The *axr6* mutants of *Arabidopsis thaliana* define a gene involved in auxin response and early development. Development, 127: 22-32.

Hobbie LJ (1998). Auxin: Molecular genetic approaches in *Arabidopsis thaliana* define a gene involved in auxin response and early development. Development, 127: 22- 32.

Hopkins WG (1999). Introduction to Plant Physiology. John Wiley and Sons.

Nadkarni KM, Nadkarni AK (1976). Indian Malaria Medica. Bombay Popular Prakashan, 1: 226.

Nanda KK, Kochhar VK (1987). Vegetative Propagation of Plants. Principles and Practices, India Book Trust.

Shah V (1996). Cultivation and utilization of medicinal plants. Regional research laboratory and CSIR, Jammu-Tawai, pp. 385-411.

Sharma N, Chandel KPS, Srivastava VK (1991). *In vitro* propagation of *Coleus forskohlii* Briq. A threatened medicinal plant. Pl. Cell Rep., 10(2): 67-70.

Somashekhar BS, Sharma M (2002). Identification of selected medicinal plants and their raw drugs. Training manual, Foundation for Revitalisation of Local Health Traditions, Bangalore.

Sun ZF, Chen L. (1998). Effect of cut position and plant growth regulators on growth and flowering in cut roses. Qingdao Institute of Agricultural Science, China. Adv. Hort., 2: 711-715.

Vashistha RK, Chaturvedi AK, Nautiyal BP, Nautiyal MC (2009). Vegetative propagation of *Angelica glauca* Edgew. and *Angelica archangelica* Linn.: two high value medicinal and aromatic herbs of the Himalaya. Nat. and Sci., 7(8): 76-82.