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Effect of Indole-3-butyric acid and time of stem cuttings on rhizogenesis of *Chamaecyparis pisifira*

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Abstract
The studies on "Effect of Indole-3-butyric acid and time of stem cuttings on rhizogenesis of *Chamaecyparis pisifira* were carried out during the year 2020-2021 at the experimental farm of the Division of Floriculture and Landscape Architecture, SKUAST-K, Shalimar campus to investigate the effects of dipping cuttings in rooting hormone (IBA) and different time of planting on rooting ability of *Chamaecyparis pisifira*. The experiments comprised of 12 treatment combinations replicated thrice and were laid under Completely Randomized Design. Each treatment comprised of twenty cuttings. Treatment combinations consist of four levels of IBA (0ppm, 1500 ppm, 3000 ppm and 4500 ppm) and three different months of planting. Cuttings were given quick dip treatment of (50-60 seconds) with various concentrations of IBA. The cuttings of *Chamaecyparis pisifira* were planted in three different months *viz*., Ist week of July, Ist week of August and Ist week of September. The cuttings planted in the Ist week of July was most effective in inducing earlier root formation and maximum number of primary adventitious roots, number of secondary adventitious roots, length of primary root per cutting, root volume, length of cutting, collar diameter, percent cuttings rooted and survival of rooted cuttings. Among various levels of IBA, 4500 ppm IBA was most effective than other concentrations of IBA (1500 ppm & 3000 ppm).

Keywords: *Chamaecyparis pisifira*, IBA, Time of planting

Introduction
The propagation of plants is a basic occupation of human kind. Early civilization developed when humans were able to propagate and grow few domesticated plant species that can be used for food and provide shelter, clothing, recreation, and aesthetic fulfilment. Plant propagation and plant breeding both involve genetic selection. The role of plant breeder is to revive patterns of genetic variations in its various forms which are useful to humans. While as the role of plant propagator is to propagate the selected cultivars so that the genetic characteristics of the original population is maintained (Hartman *et al.*, 2015) [1]. Naturally plants propagate by seeds. Root sprouting, layering and suckering are other means of natural reproduction. Humans propagate plants of economic and aesthetic importance through various techniques including vegetative propagation by cuttings. Cutting are prepared from the vegetative portions of the plant including stems, modified stems (rhizomes, tubers, corms and bulbs), leaves or roots. Stem cuttings can be divided into four groups as hardwood, semi-hardwood, softwood and herbaceous. After leaves abscise, hardwood cuttings are made of matured, dormant firm wood. The hardwood cutting is one of the cheapest and easiest methods of vegetative propagation. Hardwood cuttings, prepared during the dormant season (Hambrick *et al.*, 2015), are easy to prepare and require little or no special equipment during rooting (Fourrier, 1984) [3].
Cupressus, the classical name for Mediterranean cypress, is derived from the Greek word Kyparissos (Eckenwalder, 2009) [4] or Cyparissus (Berens, 2009) [5]. The genus Cupressus is the second largest genus in the cypress family with more than 25 species of tall trees or small shrubs. Collectively, the species of Cupressus are very important from mythical, historical, religious, ornamental, horticultural, ecological, economical, and medicinal viewpoints (Giovanelli and De Carlo, 2007) [6]. *Chamaecyparis pisifira* belongs to the family Cupressaceae and is commonly known as sawara Cypress. It is native to Central and Southern Japan, on the islands of Honshu and Kyushu. There are six species in the genus Chamaecyparis worldwide, and sawara cypress (*Chamaecyparis pisifira*) is an evergreen coniferous tree that grows mainly in central Japan. It reaches upto 30m in height and is 1 m in

diameter at breast height (DBH). Sawara false cypress should be grown in full sun to partial shade on moist, well-drained, non-alkaline soil in regions with moderate to high humidity. Although moderately drought tolerant, it is not especially happy in very hot summers unless provided with some irrigation. The plants transplant reasonably well when root pruned. It must be given full sun so lower branches remain on the tree to provide the best appearance. It is best used as a specimen planting for a large, open area of a commercial or large residential landscape. Although it looks great in the nursery, it often grows too wide for a small residential lot It is also suitable for ornamental usage such as garden hedges.

Vegetative propagation of ornamental plants through stem cuttings is usually desirable method for multiplication. It is because of all the living cells of a plant have a capacity to regenerate into a full plant under the favorable environmental conditions. The plants produced through vegetative means are genetically identical and similar to the mother plant. Many kinds of plants can be propagated through cuttings. Great care should be taken while selecting the cuttings from the parent plant. A poor growing, spindly plants are unlikely to produce a prized specimen from a cutting. Similarly, deceased or unhealthy plants also do not produce good cutting. Therefore, healthy and strong growing plants should be selected for the propagation of cuttings. In plant propagation, the induction of roots is a process regulated by environmental and endogenous factors such as temperature, light, plant growth regulators (especially auxin), carbohydrates, mineral salts and other molecules (Gehlot *et al*., 2014) [7]. The cuttings of *Chamaecyparis pisifira* have a reputation for being difficult to root. The root promoting hormones contribute significant role to the process of regeneration of roots in the cutting and their survival.Indole-3-butyric acid (lBA), a synthetic auxin, has been used commercially since its discovery in the mid-1930s. It is known to promote root formation in cuttings of many species (Edwards and Thomas, 1979) [8]. Auxins have been associated with division and elongation of meristematic cells, differentiation of root primordial as well as mobilization of carbohydrates to the base of the cutting (Bhattacharya, 1988) [9]. Specific concentration of IBA is required to start production in rooting of the plants. Use of IBA at 3000 ppm for semi-hard wood cutting and 6000 ppm for hardwood cutting induces better rooting in many ornamental plants. Generally, quick dip method of basal portion of cutting for five seconds to 120 seconds in 1000 to 10,000 ppm solution of IBA gives good rooting in cutting of ornamental plants (Hartman *et. al*., 2015) [1]. Cupressus of various kinds are used in a variety of landscape situations globally.

Since no work has been reported on the propagation of *Chamaecyparis pisifira* in Kashmir although the demand of these plants is steadily increasing. Therefore, the present study entitled as "Effect of IBA and Time of Stem Cuttings on Rhizogenesis of *Chamaecyparis pisifira* was conducted with an aim to generate basic information with an objective to evaluate the effect of IBA and time of cuttings on rhizogenesis of terminal cuttings of *Chamaecyparis pisifira* cv. Boulevard.

Materials and Methods

The present investigation entitled "Effect of IBA and Time of Stem Cuttings on Rhizogenesis of *Chamaecyparis pisifira* was conducted during the year 2020-21, in the propagation chamber at the Experimental Field of Division of Floriculture and Landscape Architecture, Sher-e-Kashmir University of Agriculture Sciences and Technology of Kashmir Srinagar. Srinagar district of Jammu and Kashmir is situated between 35.5 º to 34.7 º North latitude and 74º.8 º to 74º.9 º East longitude at an altitude of 1588 meters above mean sea level. The district is guarded on south-east and north-east sides by the lofty Himalayan ranges. The SKUAST-K, campus is situated at the base of these ranges towards the north-east side, about 15km from the main city. The hardwood cuttings were collected from *Chamaecyparis pisifira* plants obtained from local nursery of Shalimar, Srinagar. 5 – 10 cm long cuttings were taken from the healthy branches of the plant. The experiment was conducted in the propagation chamber under the canopy of chinar trees situated at the experimental farm to maintain optimum temperature for rooting. The propagation chamber was covered with the transparent polythene sheet. Coarse sand (as rooting media) was filled in the propagation chamber. The rooting medium (sand) was drenched with 0.02% Bavistin before inserting the cuttings in order to avoid spread of fungal and other diseases.

The stock solution of IBA was prepared by dissolving weighed quantity of this substance in ethanol and then diluted with distilled water to prepare the required concentrations. At the basal end of each cutting a clean horizontal cut was given below a node. The basal ends of cuttings were given a quick dip treatment for 5 seconds with various concentrations of IBA *viz*., 1500 ppm, 3000ppm and 4500 ppm. Twenty cuttings from each treatment were treated with the IBA and repeated thrice in completely Randomized Design. Cuttings under control were not dipped in any solution and were directly dibbled in the propagation chamber after given the horizontal cut to the cuttings. The cuttings of *Chamaecyparis pisifira* that were treated with IBA were immediately planted in the sand filled propagation chamber with the help of a dibbler at a distance of 3 cm from cutting to cutting and 5 cm from row to row. These cuttings were planted at three different times i.e., Ist week of July, Ist week of August and Ist week of September. Care was taken to keep the rooting medium moist by spraying water on leaves by the hand sprayer. Besides weeds and dead cuttings were occasionally removed from the propagation chamber. Observations were recorded on rooting behaviour after 25 days of planting of cutting.

Results and Discussions

The data presented in table 1 concluded that days taken to root emergence in *Chamaecyparis pisifira* were significantly affected by IBA concentrations. As the level of IBA concentrations increased from 0 ppm to 4500 ppm, a significant decrease in time taken to root initiation was observed. Among different IBA concentrations, the maximum number of days (35.33 days) taken to root emergence in cuttings were found in case of control, followed by IBA 1500 ppm (32.89 days).The minimum number of days taken to root emergence (26.44 days) was obtained when cuttings were treated with IBA 4500 ppm. There was a significant effect of time of planting of cuttings on number of days taken to emergence. Maximum days (33.50 days) taken to root emergence was observed when cuttings were planted in Ist Week of September (Table 1.1), while as, minimum number of days (28.50 days) taken to root emergence was found when planted in Ist Week July planting. The interaction between IBA concentrations and time of planting on number of days taken to root emergence was found significant. Minimum time (24.67 days) taken to rooting at IBA 4500 ppm when planted in Ist week of July in contrast to remaining treatment concentrations (Table 1). Maximum days (37.33 days) to rooting was found in control when planted in Ist week of September. The shortest time taken to root emergence by higher IBA concentration can be explained by the fact that the IBA increased the meristematic activity. With the increase in meristematic activity of cells the callus was formed quickly as compared to control. Root primordia originates from the callus which later on induced root initials and thus forms adventitious roots. The two processes of increasing the size of the cell, which require auxins and oxygen, are the water uptake and expansion of the cell wall. Thimann (1969) reported that auxin activates messenger RNA, which helps in the formation of certain enzymes to aid in cell wall expansion by allowing additional materials to be inserted in to the cell wall. The results obtained in this result are well documented by the researchers like Bharathy (2001) [10] in carnation (*Dianthus caryophyllus*), Tripathi *et al.* (2003) [11] in poinsettia cuttings and Tantary (2004) [12] in *Olea europea*.

Number of primary adventitious roots in *Chamaecyparis pisifira* was significantly affected by different levels of IBA concentrations. Maximum number of primary adventitious roots (10.33) was reported with cuttings treated with IBA 4500 PPM, while as, minimum number of primary adventitious roots (0.67) were obtained under control (Table 2). The data shown in Table 2 on effect of planting time were significant on number of primary adventitious. Highest (7.17) and lowest number of primary adventitious roots (3.00) were found with Ist Week of July and Ist Week of September respectively. The perusal of data (Table 2) on number of secondary adventitious roots revealed that there was a significant effect due to IBA concentrations. The maximum number of secondary roots (31.89) was depicted at IBA of 4500 ppm, followed by IBA 3000 ppm (23.78), while as, minimum number of secondary adventitious (0.78) was found under control. Time of planting showed significant effect on number of secondary adventitious roots (Table 2) with maximum number of secondary adventitious roots (19.5) recorded when planted in Ist Week of July. Whereas, minimum number of secondary adventitious roots (15.75) was observed when planted in Ist Week of September. The highest number of primary and secondary adventitious roots can be linked to the fact that the external application of IBA at optimum levels might brought about certain anatomical and physiological changes in number of roots due to enhanced cell divisions by which numerous root primordia are originated from root initials (Rolston *et al.*,1996) [13]. Thus the IBA might have caused the vascular differentiation of cells and produced more number of roots. The results obtained were similar to the findings of Phuyal *et al*., 2018 [16] in *Zanthoxylum armatum* stem cuttings, Jing Li *et al*., 2018 [14] in *Cupressus sempervirens* and Silva *et al*., 2005 [15] in Leyland cypress (*Cupressocyparis leyland*).

Different concentrations of IBA had significant effect on length of primary root per cutting (cm). The highest length of primary root per cutting (11.17 cm) was observed in cuttings which were treated with IBA of 4500 ppm. The shortest length of primary root per cutting (1.94 cm) was found under control. Lengths of primary root per cutting (cm) were significantly affected with different time of planting (Table 2). Minimum length of primary root per cutting (6.14 cm) was obtained when planted in Ist Week of September, while as, maximum length of primary root per cutting (8.59 cm) was found when planted in Ist Week of July. The application of IBA might have increased the cell elongation, cell division as well as vascular tissue differentiation which ultimately helped in increasing the root length. Moreover in case of IBA treated cuttings there was early production of roots which had helped these cuttings to absorb water and nutrients from soil earlier, thus increasing the length of primary root. These results obtained during the present investigation were also found in conformity with the findings of Jing Li *et al*., 2018 [14] in *Cupressus sempervirens* and Phuyal *et al*., 2018 [16] in *Zanthoxylum armatum.*

The data presented revealed that different IBA levels had significant effect on root volume (ml). The maximum root volume (5.37 ml) was found in cuttings treated with IBA of 4500 ppm. The minimum root volume (1.03 ml) was found under control. The results presented in Table 2 further revealed that the root volume was more (3.52 ml) in cuttings when planted in Ist week of July as compared with cuttings planted in other months. The minimum root volume (2.90 ml) was obtained in Ist week of September. The maximum root volume in cuttings at higher concentrations can be explained by the fact that at higher concentration of IBA there was higher number of primary and secondary roots in cuttings. Thus the increase in length of roots and maximum number of roots at higher concentrations might have increased the root volume in cuttings at higher concentrations. The data obtained is also supported by the findings of Muttaleb *et al*., 2017 in *Piper betle*.

The data shown in Table 3 revealed that different IBA levels had significant effect on collar diameter (mm). IBA 4500 ppm recorded maximum collar diameter (1.90 mm). The minimum collar diameter (1.53 mm) was found under control. Collar diameter (mm) was significantly affected with different time of planting. Minimum collar diameter (1.62 mm) was obtained when planted in Ist Week of September, while as, maximum collar diameter (1.80 mm) was found when planted in Ist Week of July.

From the data presented in Table 3 it is evident that length of cutting (cm) increased significantly as the concentration of IBA increased from 0 ppm to 4500 ppm. IBA 4500 ppm recorded longest length of cutting (14.21 cm), while as control produced shortest length of cutting (11.32 cm). Length of cutting (cm) was significantly affected with different time of planting. Minimum length of cutting (12.39 cm) was obtained when planted in Ist Week of September, while as, maximum length of primary root per cutting (12.92 cm) was found when planted in Ist Week of July. The increase in the length of cuttings and collar diameter at higher concentration could be because of early formation of roots, higher number of roots and utilization of nutrients and water.

The data revealed that all the concentrations of IBA significantly influenced rooting percentage as compared to control. Among all concentrations highest rooting percentage (84.47%) followed by 3000 ppm (78.31%). Control produced only 32.24% of rooting. The results presented in Table 3 depicted that rooting percentage was significantly affected by the time of planting. The minimum rooting percentage (63.33%) was obtained when planted in Ist Week of September, while as, maximum percentage of rooting (70.33%) was found when planted in Ist Week of July. The increase in rooting percentage at higher concentrations of IBA can be due to the fact that the IBA play a variety of roles in meristem division and elongation, cambial initial differentiation into root primordia, and reserve food mobilisation by boosting the activity of hydrolysing enzymes.

Weaver, 1972 reported that the IBA translocates poorly and is retained near the site of application and thus it is one of the most effective rooting stimulators. Another reason could be related to the fact that the hardwood cutting contains more starch which in turn bring about favourable conditions for root initiation and more rooting percentage coupled with positive response of IBA. The results obtained in the present investigation was supported by the findings of Jing Li *et al*., 2018 [14] in *Cupressus sempervirens,* Dumitrascu *et al*., 2003 [18] in *Chamaecyparis pisifira* and Bayraktar *et al*., 2018 [19] in *Taxus baccata.*

The results presented in Table 3 revealed that different IBA concentrations had significant effect on survival of rooted cuttings (%). The maximum survival of rooted cuttings (88.46%) was observed with IBA 4500 ppm, while as, minimum survival of rooted cuttings (22.73%) was found under control. Furthermore, it is evident from the data that the survival of rooted cuttings were significantly affected with different time of planting (Table 3). Minimum survival of rooted cuttings (64.00%) was obtained when planted in Ist Week of September, while as, maximum survival of rooted cuttings (69.64%) was recorded when planted in Ist Week of July. Highest survival of rooted cuttings at higher concentration of IBA might have resulted due to development of effective root system, increase in number of primary and secondary roots and also due to increase in length of primary root per cutting that may have increased the uptake capacity of water and nutrients. While as in case of untreated cuttings, the survival of rooted cuttings decreased which may be due to formation of less number of primary and secondary roots. The current findings are similar to the findings of Patel *et al.*, 2018 in Fig (*Ficus carica*).

Thus considering the effect of planting date, the data shown in Tables 1, 2 & 3 demonstrated that the cuttings planted in July month significantly produced the higher values of rooting percentage and other rooting characteristics, followed by cutting planted in August, where as, lowest values of rooting percentage and other rooting characteristics was recorded in cuttings planted in September. Better rooting characteristics in July may be attributable to increased availability of carbohydrates and nitrogenous substances in cuttings at the time of their collection. Furthermore, it could be related to favourable meteorological circumstances for roots in July. The temperature is higher in the month of July than August and September. According to Hartmann *et al.* 2015 [1], cell division is favoured by raising the temperature, thus it aids in the formation of roots. Where as, reduction of rooting in August and September might be due to influence of stress caused by decrease in temperature and lower levels of humidity during this period. Thus the low temperature in the month of August and September decreases the metabolism, leading to lower production of roots, takes more time for rooting, decrease in rooting percentage and other rooting characteristics. The results obtained are well supported by the findings of Safaa *et al*., 2014 [21] in Button wood tree (*Conocarpus erectus* L.) and Singh, 1993 [22] in Bougainvillea plants.

Table 1: Effect of IBA concentration and time of planting on days taken to root emergence in C*hamaecyparis pisifira*.

IBA concentration (ppm)	Days taken to root emergence		
	Ist week of July	Ist week of August	Ist week of September
Distilled water	33.33	35.33	37.33
IBA (1500ppm)	28.67	34.33	35.67
IBA (3000ppm)	27.33	30.33	32.67
IBA (4500ppm)	24.67	26.33	28.33
Mean	28.50	31.58	33.50
CD($p \leq 0.05$) IBA (I)	0.632		
Time of planting (T)	0.574		
Interaction (IxT)	1.094		

Table 2: Effect of IBA concentration and time of planting on number of primary adventitious roots, number of secondary adventitious roots, length of primary root per cutting and root volume in C*hamaecyparis pisifira*.

IBA concentration (ppm)	Number of primary adventitious roots			Number of secondary adventitious roots			Length of primary root per cutting (cm)			Root Volume (ml)		
	Ist week of July	Ist week of August	Ist week of September	Ist week of July	Ist week of August	Ist week of September	Ist week of July	Ist week of August	Ist week of September	Ist week of July	Ist week of August	Ist week of September
Distilled water	1.00	0.67	0.33	1.33	0.67	0.33	2.43	1.79	1.62	1.17	1.03	0.89
IBA(1500ppm)	4.67	3.33	1.67	17.33	13.33	11.67	8.67	6.94	5.58	3.10	2.85	2.51
IBA(3000ppm)	8.67	5.67	3.67	25.33	24.33	21.67	10.31	8.30	7.59	3.82	3.46	3.31
IBA(4500ppm)	14.33	10.33	6.33	34.00	32.33	29.33	12.95	10.75	9.78	5.98	5.20	4.91
Mean	7.17	5.00	3.00	19.5	17.67	15.75	8.59	6.95	6.14	3.52	3.14	2.90
CD($p \leq 0.05$) IBA (I)	0.375			0.828			0.095			0.15		
Time of planting (T)	0.281			0.745			0.074			0.13		
Interaction(IxT)	0.656			1.573			0.169			0.26		

Table 3: Effect of IBA concentration and time of planting on collar diameter, length of cutting and percent cuttings rooted, in C*hamaecyparis pisifira*.

IBA concentration (ppm)	Collar diameter (mm)			Length of cutting (cm)			Percentage cuttings rooted			Survival of rooted cuttings (%)		
	Ist week of July	Ist week of August	Ist week of September	Ist week of July	Ist week of August	Ist week of September	Ist week of July	Ist week of August	Ist week of September	Ist week of July	Ist week of August	Ist week of September
Distilled water	1.67	1.52	1.39	11.50	11.43	11.03	38.45	33.11	31.15	25.13	23.28	19.79
IBA(1500ppm)	1.72	1.65	1.49	12.41	11.92	11.79	72.24	71.49	67.18	75.77	73.14	70.68
IBA(3000ppm)	1.85	1.78	1.76	13.28	12.87	12.74	81.85	78.03	75.05	86.09	81.71	79.66
IBA(4500ppm)	1.96	1.88	1.84	14.48	14.15	13.99	88.76	84.72	79.93	91.55	87.95	85.88
Mean	1.80	1.71	1.62	12.92	12.59	12.39	70.33	66.84	63.33	69.64	66.52	64.00
CD($p\leq$ 0.05) IBA (I)	0.078			0.186			0.040			0.454		
Time of planting (T)	0.068			0.161			0.035			0.393		
Interaction(IxT)	0.146			0.347			0.069			0.786		

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