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Standardization of type of cuttings and concentration of growth regulators for rooting of cuttings in java tea (*Orthosiphon stamineus* Benth.)

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Abstract
Java Tea *(Orthosiphon stamineus* Benth.) is an important medicinal plant most commonly used for its diuretic, hepatoprotective and antibacterial properties. Since, it is an emerging crop for large scale commercial cultivation, standardization of propagation using various types of cuttings and different concentrations of plant growth regulators were attempted in this study. Three types of stem cuttings *viz.*, Terminal, middle and basal cuttings (15 cm length with 3-4 nodes) were prepared and treated with different concentrations of plant growth regulators *viz.*, IAA (1000, 1500 and 2000 ppm) and IBA (1000, 1500 and 2000 ppm) for 3-5 seconds and subjected to rooting under mist chamber conditions. Observations on days taken for rooting, rooting percentage, number of roots, root length, fresh root weight, dry root weight, shoot length, number of shoots, fresh shoot weight, dry shoot weight and survival percentage were recorded. The results of the experiment portrayed that the basal cuttings treated with IBA 2000 ppm under controlled conditions resulted in better rooting percentage (95%), root length (11.68cm), fresh weight of root (0.94g), dry weight of root (0.32g), fresh weight of shoot (3.00g), dry weight of shoot (1.12g), number of shoots (2.53) and survival percentage (88%). Thus, this present investigation revealed that, the basal cuttings of java tea treated with IBA 2000 ppm under mist chamber conditions had significant effect on most of the root and shoot parameters and it was found to be most suitable for successful propagation of java tea.

Keywords: Java tea, cuttings, plant growth regulators**,** rooting, propagation

Introduction
Java Tea *(Orthosiphon stamineus* Benth.) is a medicinal herb belonging to the family Lamiaceae, largely grown in Southeast Asia. Leaves of java tea are commonly used in Southeast Asia and European countries for herbal tea preparation (Indubala, 2000) [8]. The other names of this medicinal herb include Arjak, Barbiflore, Poonai meesai mooligai, Indian kidney tea, Seeraghatulasi, Vantulsi. The plant can be identified by its white or purple flowers bearing long, protruding stamens that resemble cat's whiskers. Hence, it is also called as cat's whiskers plant.

The genera *Orthosiphon* is derived from two words *i.e.* Ortho (straight), while siphon (tube or cylindrical). Both words when combined mean a straight tube-like flower produced by the species in the genus *Orthosiphon*. The characteristic of this straight tube is considered to be one of the main characteristics of the Lamiaceae family (Keng and Siong, 2006) [13].

O. stamineus is identified to be a perennial herb. It grows upto a height of 0.3–1m. The stem is quadrangled, while the leaves are simple, lanceolate-like, elliptical or rhomboid, 2–4cm wide and 4– 7cm long. The flowers have stamens of more than 2cm length that extend from the corolla-tube (Wiart, 2000) [3].

Based on the structure of flowers, *O. stamineus* is grouped into two varieties namely purple type and white type. It is reported that purple type has higher bioactive compounds content than white type (Lee, 2004) [14]. Studies show that the plants of this family possess pharmacological properties such as antioxidant, antibacterial, hepatoprotective, anti-inflammatory, cytotoxic, diuretic, antihypertensive and vasodialative properties (Masuda, *et al.*, 1992; Tezuka *et al.*, 2000; Beaux *et al.*, 1999) [7].

Orthosiphon stamineus, aids in reducing the excessive fluid retention and flushing out of sodium in kidney. There by, it supports kidney function and acts as a natural diuretic supplement (Maheswari *et al*., 2015) [1].

The proportion of extract was highly yielded in *O. stamineus* leaf (16.70%) followed by stem (16.64%) and roots (9.52%).

These extracts have the potential to be used as food and feed additives to protect the intestine from oxidative stress (Cai *et al*., 2018). The leaves of *Orthosiphon stamineus* contains important components like polyphenols: the polymethoxylated flavonoids: sinensetine, eupatorine, etc. and the caffeic acid derivatives: rosmarinic acid, cichoric acid, caffeic acid, etc. (Olah *et al*., 2003) [2].

Although no known side effects were reported as of modern drugs, it cannot be relied on as a complete treatment for the ailments. Unlike European companies which use java tea extracts in modern medical formulations, it is largely promoted as functional herbal tea in South East Asian regions (Himani *et al*., 2013).

Materials and Methods

Studies on standardization of type of cuttings and concentration of growth harmones for rooting of cuttings in java tea (*Orthosiphon stamineus* Benth.) was carried out in the Department of Medicinal and Aromatic crops, Tamil Nadu Agricultural University, Coimbatore during 2019-2020. The cuttings of java tea (*Orthosiphon stamineus* Benth.) plant was collected from Mangalapuram village of Salem district, Tamil Nadu located at 11º59´ N and 78º38´ E. The planting materials were collected during September, 2019. Three portions of java tea stem cuttings *viz.*, Terminal, middle and basal cuttings of 15cm length with 3-4 nodes were prepared for the study.

The growth regulator solutions were prepared by dissolving the required quantity of IAA and IBA in ethyl alcohol individually and made up with distilled water as per treatment concentrations along with control. Twenty cuttings were used per replication in each treatment. The cuttings were dipped in growth regulator solution by quick dip method (3-5 seconds). The treated cuttings were planted in the protrays containing rooting medium (mixture of red soil, sand, well decomposed farmyard manure (2:1:1), vermicompost, coir pith and *Pseudomonas fluorescens*) and kept under fully automated mist chamber. Frequent misting was done to maintain 90% R.H. The mean maximum temperature maintained inside the chamber was 35 ˚C whereas mean minimum temperature was 25 ˚C.

Five cuttings from each replication were maintained separately to observe the root parameters. Rooting percentage was calculated by number of rooted cuttings from total number of cuttings multiplied with hundred and expressed in percentage. Number of roots was recorded in randomly selected five plants in each replication of all treatments and the mean was expressed in numbers. Length of the roots was measured from the base of the cutting to the tip of the root from five plants in each replication and the mean values were expressed in centimetres. Fresh root weight per plant was recorded in randomly selected five plants from each replication and washed thoroughly before weighing using a weighing scale and the average was expressed in grams. Dry root weight was recorded from root samples of five plants in each replication by drying in hot air oven for a week at 80±2˚C and was expressed in grams. Days taken for sprouting were counted in ten plants from each replication. Shoot length was measured in ten plants of each replication using a measuring scale and expressed in centimetres. Number of shoots per plant were counted from ten plants in each replication and were expressed in numbers. Number of leaves per plant from ten plants in each replication were counted and expressed in numbers. Fresh shoots were collected randomly from ten plants in each replication and shoot weight per plant weighed using a weighing scale and the mean was expressed in grams. Shoots of ten plants from each replication were dried in hot air oven at 80±2˚C and expressed in grams. Survival percentage was calculated by number of plants survived from total number of cuttings planted multiplied with hundred and expressed in percentage. The experiment was laid out in Factorial completely randomized design (FCRD) with twenty-one treatment and three replications. Two factors considered for the experiment were different portion of cuttings and plant growth regulators.

Treatment	Treatment details
A_1B_0	Terminal cuttings (control)
A_1B_1	Terminal cuttings + IAA 1000 ppm
A_1B_2	Terminal cuttings + IAA 1500 ppm
A_1B_3	Terminal cuttings + IAA 2000 ppm
A_1B_4	Terminal cuttings + IBA 1000 ppm
A_1B_5	Terminal cuttings + IBA 1500 ppm
A_1B_6	Terminal cuttings + IBA 2000 ppm
A_2B_0	Middle cuttings (control)
A_2B_1	Middle cuttings + IAA 1000 ppm
A_2B_2	Middle cuttings + IAA 1500 ppm
A_2B_3	Middle cuttings + IAA 2000 ppm
A_2B_4	Middle cuttings + IBA 1000 ppm
A_2B_5	Middle cuttings + IBA 1500 ppm
A_1B_6	Middle cuttings + IBA 2000 ppm
A_3B_0	Basal cuttings (control)
A_3B_1	Basal cuttings + IAA 1000 ppm
A_3B_2	Basal cuttings + IAA 1500 ppm
A_3B_3	Basal cuttings + IAA 2000 ppm
A_3B_4	Basal cuttings + IBA 1000 ppm
A_3B_5	Basal cuttings + IBA 1500 ppm
A_3B_6	Basal cuttings + IBA 2000 ppm

Results

From table 1, the portion of cuttings and the concentration of growth regulators significantly influenced the days taken for rooting, and the number of roots produced. A_1B_3 (Terminal cuttings + IAA 2000 ppm) recorded earlier rooting (12 days) followed by A_1B_5 (Terminal cuttings + IBA 1500 ppm) and A_2B_0 (Middle cuttings- Control) (13 days). A_1B_1 (Terminal cuttings + IAA 1000 ppm) and A_3B_4 (Basal cuttings + IBA 1000 ppm) noted delayed rooting (15 days). Highest rooting percentage (90%) was registered in treatment A_3B_6 (Basal cuttings + IBA 2000 ppm) (95%) followed by A_2B_5 (Middle cuttings + IBA 1500 ppm), A_3B_2 (Basal cuttings + IAA 1500 ppm) and A_3B_3 (Basal cuttings + IAA 2000 ppm). A_1B_0 (Terminal cuttings - Control) registered the lowest rooting percentage (72%). Highest number of roots (15.06) was recorded in A_3B_6 (Basal cuttings + IBA 2000 ppm) followed by A_3B_5 (Basal cuttings + IBA 1500 ppm) (11.95) which was on par with the treatments A_3B_0 (Basal cuttings - Control) (11.67), A_3B_2 (Basal cuttings + IAA 1500 ppm) (11.61) and A_2B_0 (Middle cuttings - Control) (11.53). The treatment A_2B_4 (Middle cuttings + IBA 1000 ppm) recorded the lowest number of roots (6.72). Highest root length (11.68cm) was registered in the treatment A_3B_6 (Basal cuttings + IBA 2000 ppm) followed by the treatment A_3B_5 (Basal cuttings + IBA 1500 ppm) (9.95cm). The treatment A_3B_1 (Basal cuttings + IAA 1000 ppm) recorded the lowest root length(6.30cm). Highest fresh root weight (0.94g) was recorded by A_3B_6 (Basal cuttings + IBA 2000 ppm) followed A_3B_4 (Basal cuttings + IBA 1000 ppm) (0.63g). A_2B_5 (Middle cuttings + IBA 1500 ppm) spotted the lowest fresh root weight (0.27g). Highest dry root weight (0.32g) was recorded in treatment A_3B_6 (Basal cuttings + IBA 2000 ppm) followed A_3B_4 (Basal

cuttings + IBA 1000 ppm) (0.20g) and A_3B_5 (Basal cuttings + IBA 1500 ppm) (0.19g). The lowestdry root weight (0.09g) was observed in the treatments A_1B_4 (Terminal cuttings + IBA 1000 ppm), A_1B_6 (Terminal cuttings + IBA 2000 ppm) and A_2B_5 (Middle cuttings + IBA 1500 ppm).

From table 2, earlier sprouting (7.82 days) was recorded in A_1B_0 (Terminal cuttings - Control) followed by A_1B_1 (Terminal cuttings + IAA 1000 ppm) (7.85 days). The treatment A_3B_3 (Basal cuttings + IAA 2000 ppm) took more number of days for sprouting (9.51 days). A_3B_4 (Basal cuttings + IBA 1000 ppm) was found to have the longest shoot length (23.59cm) followed by the treatment A_3B_5 (Basal cuttings + IBA 1500 ppm) (20.27cm) which was on par with A_3B_6 (Basal cuttings + IBA 2000 ppm) (20.26cm). The treatment A_1B_0 (Terminal cuttings - Control) (13.92cm) recorded the shortest shoot length. The highest number of shoots (2.61) was exhibited in treatment A_3B_1 (Basal cuttings + IAA 1000 ppm) followed by A_3B_6 (Basal cuttings + IBA 2000 ppm) (2.53) which was on par with the treatment A_3B_5 (Basal cuttings + IBA 1500 ppm) (2.45). Out of all, the treatment A_2B_0 (Middle cuttings - Control) recorded the lowest number of shoots (1.88). A_1B_6 (Terminal cuttings + IBA 2000 ppm) (3.45g) recorded the highest fresh root weight followed A_3B_6 (Basal cuttings + IBA 2000 ppm) (3.00g). The lowest fresh shoot weight was registered in A_1B_5 (Terminal cuttings + IBA 1500 ppm) (1.60g). Highest dry root weight (1.29g) in treatment A_1B_6 (Terminal cuttings + IBA 2000 ppm) followed by the treatment A_3B_6 (Basal cuttings + IBA 2000 ppm) (1.12g). The treatment A_1B_5 (Terminal cuttings + IBA 1500 ppm) (0.60g) recorded the lowest dry shoot weight. A_3B_6 (Basal cuttings + IBA 2000 ppm) (88%) recorded the highest survival percentage followed by A_2B_6 (Middle cuttings + IBA 2000 ppm) (85%). A_2B_2 (Middle cuttings + IAA 1500 ppm) noticed the lowest (69%) survival percentage.

Discussion

The basal cuttings recorded better rooting but poor rooting was observed in terminal cuttings of java tea. Ramtin *et al.* (2011) [5] confirmed that the basal cuttings resulted in better growth due to stored foods in plant tissue and also presence of hydro carbon substances, nucleic acids, proteins and natural phytoharmone (IAA). Auxin controls the postembryonic activity of cells within the root meristem resulting in profuse growth of roots (Kamil *et al.*, 2007). The usage of auxins improves the activity of hydrolytic enzyme and transport sugars from starch and nutrients to base of the cutting, thereby initiating and development of roots (Deepak *et al*., 2015). Dettweiler (1942) [9] noticed greater metabolic activity as a result of application of growth regulators. The initiation of rooting in cuttings triggered by mobilization of sugars and nitrogenous substances from shoots. Plant growth regulators induce root formation by initiating the synthesis of structural or enzyme proteins in chrysanthemum (Singh and Chettri, 2013). Similar finding was reported in *Lippia javanica* by Soundy *et al.* (2008). The rooting increases with the increase in concentration of plant growth regulators which may be due to the exogenous auxin in the cuttings treated with higher concentration. The formation of root primordia is hastened by auxins thereby facilitating rooting of cuttings (Stoutmeyer, 1954 and Haissing, 1972) [11, 12]. The cuttings with two to three leaves might provide necessary auxin, carbohydrates and other rooting co factors which in turn results in rooting success. (Haissig, 1974). `The combination of two factors, basal cuttings and IBA 2000 ppm performed way better in most of the parameters. Similar results were observed by Ramtin *et al.* (2011) [5] and revealed that the better rooting performance was a result of combined effect of the basal cuttings and treatment in higher concentration of IBA (1000 ppm) in poinsettia.

In case of root parameters except days taken for rooting, the basal cuttings gave better results than terminal and middle positions of cuttings. This may be due to the presence of more starch in basal cuttings as it is thicker in diameter than middle cuttings followed by terminal cuttings. This was in line with the findings of Leaky and Mohammed (1985), who stated that thin cuttings contained less starch in the stem than thicker cuttings thereby thicker cuttings rooted well than thinner ones. Even at congenial environment (mist chamber), the poor rooting of cuttings in other plant growth regulator concentrations may be due to nutrient leaching and poor quality of cuttings (Good and Turkey, 1966) [6]. Poor performance of terminal cuttings may be due to the lesser amount of stored food reserves (Singh and Chettri, 2013).The maximum number of rootswas found in basal cuttings **(**15.06) treated with IBA 2000 ppm. The regeneration of roots on cuttings was influenced by auxins, however, it varies with the concentration and nature of auxin (Hartman *et al*., 2011).The maximum root length was recorded in IBA 2000 ppm (11.68). This may be due to loosening of cell wall and cell elongation caused by hydrogen bonds breakage between cellulose micro fibrils by proteins from IBA. This was confirmed by Kumar *et al.* (2015). According to Kamil *et al.* (2007), the production of cells in the meristem and the elongation of the cells post exit of meristem determine the growth rate of roots. Application of IBA improves histological features like callus formation and vascular tissue differentiation. The translocation of metabolites and carbohydrates metabolism is also influenced by this. This was in close proximity with the findings of Patel and Patel (2018). The acidification process due to auxin treatment might be increasing the root length. Susila *et al.* (2013) revealed that the auxin induces cell enlargement and cell division at the spot of auxin applied due to hydrolysis of carbohydrates and the metabolite accumulation which induces more roots. Vascular tissues undergo the diagnostic differentiation which increases the movement of materials in the vessels and lead to the increase in the fresh and dry weight of the shoots and roots. (Rahdari *et al.*, 2014) [10].

In the present study, the maximum survival percentage (88%) was found due to combined effect of basal cuttings and IBA 2000 ppm. The uptake of water and nutrients drawn by increase in number and length of roots of basal cuttings might be the reason for high survival rate. The results corroborated the findings of Patel and Patel (2018).The basal cuttings treated with IAA 1000 ppm recorded maximum number of shoots (2.61). This may be due to thicker and larger diameter of basal stem cuttings when compared to terminal and middle cuttings. This was in line with the findings of Kouakou *et al.* (2016).

Table 1: Effect of type of cuttings and growth regulators on root parameters at 60 days after planting (DAP)

Treatments	Days taken for rooting	Rooting percentage (%)	Number of roots	Root length (cm)	Fresh root wt. (g)	Dry root wt. (g)
A_1B_0	14.78	72	7.06	6.64	0.37	0.12
A_1B_1	15.11	78	7.50	8.40	0.38	0.12
A_1B_2	14.78	83	8.56	7.69	0.35	0.14
A_1B_3	11.56	85	8.17	7.27	0.50	0.14
A_1B_4	13.39	77	7.72	6.87	0.29	0.09
A_1B_5	13.28	80	8.35	7.46	0.49	0.15
A_1B_6	14.56	85	7.22	6.34	0.29	0.09
A_2B_0	13.11	78	11.53	8.05	0.41	0.14
A_2B_1	13.89	87	7.89	9.05	0.48	0.15
A_2B_2	13.94	83	7.04	7.17	0.42	0.14
A_2B_3	13.39	83	8.33	8.10	0.32	0.10
A_2B_4	14.00	80	6.72	6.57	0.33	0.11
A_2B_5	14.61	90	7.56	7.93	0.27	0.09
A_1B_6	14.06	87	9.39	9.30	0.36	0.11
A_3B_0	14.00	78	11.67	7.51	0.48	0.15
A_3B_1	14.33	88	7.22	6.30	0.38	0.13
A_3B_2	14.50	90	11.61	7.86	0.50	0.16
A_3B_3	14.22	90	7.83	7.35	0.31	0.10
A_3B_4	15.22	85	10.50	9.06	0.63	0.20
A_3B_5	13.72	88	11.95	9.95	0.61	0.19
A_3B_6	13.67	95	15.06	11.68	0.94	0.32
SE (d)	0.40	1.57	0.19	0.33	0.01	8.68
CD (P=0.05)	NS	3.12	NS	0.66	0.03	NS

Table 2: Effect of type of cuttings and growth regulators on shoot parameters at 60 days after planting (DAP)

Treatments	Days taken for sprouting	Shoot length (cm)	Number of shoots	Fresh shoot weight (g)	Dry shoot weight (g)	Survival percentage (%)
A_1B_0	7.82	13.92	1.96	1.72	0.64	71
A_1B_1	7.85	16.27	2.24	2.28	0.85	72
A_1B_2	8.01	16.60	2.12	1.95	0.73	81
A_1B_3	8.21	17.68	2.40	1.77	0.66	82
A_1B_4	8.70	16.11	2.21	2.07	0.77	73
A_1B_5	8.55	19.53	2.12	1.60	0.60	79
A_1B_6	8.69	16.31	2.07	3.45	1.29	82
A_2B_0	8.19	18.50	1.88	2.41	0.90	77
A_2B_1	9.10	16.73	2.04	2.01	0.75	77
A_2B_2	8.96	18.68	2.33	2.31	0.86	69
A_2B_3	8.72	15.38	2.03	2.16	0.81	81
A_2B_4	8.72	19.02	2.09	2.14	0.80	71
A_2B_5	8.55	17.26	1.96	1.82	0.68	84
A_1B_6	8.89	17.18	2.16	2.42	0.90	85
A_3B_0	9.03	18.39	2.19	2.28	0.85	74
A_3B_1	8.62	20.09	2.61	2.56	0.96	71
A_3B_2	8.83	19.37	2.08	1.99	0.74	83
A_3B_3	9.51	18.45	2.12	1.75	0.65	84
A_3B_4	8.98	23.59	2.20	2.34	0.87	74
A_3B_5	8.30	20.27	2.45	2.24	0.84	83
A_3B_6	8.69	20.26	2.53	3.00	1.12	88
SE (d)	0.40	1.57	0.19	0.33	0.01	8.68
CD (P=0.05)	NS	3.12	NS	0.66	0.03	NS

Conclusion

The results of the experiment portrayed that the basal cuttings treated with IBA 2000 ppm under controlled conditions resulted in better rooting percentage, root length, fresh weight of root, dry weight of root, fresh weight of shoot, dry weight of shoot, number of shoots, survival percentage.

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