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Influence of Growth Stimulants on Sprouting and Rooting of Orthotropic Shoots of Black Pepper *(Piper nigrum* L.) in Protrays

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ABSTRACT

An experiment was conducted to assess the influence of growth stimulants on sprouting and rooting of the orthotropes using protrays. Result of the study revealed that among, growth stimulants, a significant differences were found in the orthotropic shoot cuttings treated with Tender coconut water (T_{10}) recorded highest values for sprouting, shoot and root parameters *i.e.*, least number of days to first sprouting (6.00)and complete sprouting (16.58), maximum sprout length (14.67 cm), number of leaves (4.85), total leaf area (427.07 cm^2), leaf area index (7.59), total chlorophyll (2.04(mg/g fr. wt.), fresh (6.69 g) and dry (1.88 g) of shoot, survival (87.04 %), rooting (85.12 %), number of primary (7.56) and secondary (54.34) roots, length of longest primary root (20.67cm), root volume (5.00 cc) and thickness (1.78 mm), fresh (2.70 g) and dry (0.46 g) of root and total dry matter (2.34 g). However highest sprouting (96.29 %) was recorded in T_1 (NAA 100 ppm).

Key words: *Orthotropes, Polyhouse, Protrays, Tender coconut, Growth stimulants.*

Introduction

Black pepper (*Piper nigrum* L.) is known as the "King of spices" and having somatic chromosome number of 2n = 52. It is indigenous to the tropical evergreen forests of the Western Ghats of South India. Black pepper vines develop five types of aerial shoots, namely primary stem, top shoots or orthotropes, runner shoots, lateral branches or plagiotrops and hanging shoots.

Plant propagation using cuttings is an important technique for growing new plants with desirable characteristics. It involves taking a portion of a parent plant, such as a stem or leaf and allowing it to produce roots and develop a new individual.

The vines from the terminal shoots (orthotropes) are found to give better growth and large number of fruit bearing lateral branches, starting right from the base of the vine which ensures complete coverage of the standard with fruiting branches without leaving any gaps and enable the plant to be more productive. This growth habit is missing in the vines raised from runner shoots (Sarma *et al.,* 2013). The potential of orthotropic shoots as planting material has been

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identified and this practice is already widely adopted in most of the pepper growing countries. However, this practice is not popular in India due to the limited availability of orthotropes because most of the plantations are on living standards and the pepper vines growth is unrestricted.

In the rooting of black pepper cuttings, a number of factors such as type of cuttings, pre- treatment of cuttings, environmental conditions during rooting, rooting medium *etc.,* influence the rooting and regeneration of plants from cuttings. Plant growth regulators play an important role for root induction in many crops including vine crops like black pepper. However, there exists a lot of contradiction with regard to optimum concentration and period of auxin treatments. The use of auxins like IBA, NAA *etc*., has been found effective to induce rooting of cuttings in black pepper.

Plant growth stimulators play a vital role in improving the rooting of black pepper cuttings. The maximum rooting percentage was found to increase the establishment of cuttings in nurseries. Growth regulators increase the percentage of success and number of roots in black pepper cuttings. This would improve the vigour of freshly transplanted rooted cuttings in the field, thus reduces the rate of mortality of plants and helps to maintain adequate crop stand in the plantations.

Plastic protray usage in black pepper nursery have several benefits over traditional polybags. Protrays are designed for easy handling and transportation, making them convenient for both nurserymen and farmers during the propagation process. Black pepper plants can easily remove from protrays without disturbing their root systems, which in turn reduces the transplanting shock. While there is an initial investment in protrays, their reusability over multiple propagation cycles can lead to long-term cost savings and it reduces the risk of environmental pollution by decreasing the plastic usage and helps to sustainable black pepper propagation.

Materials and Methods

Experimental site

The present investigations was carried out at the college of Horticulture, Mudigere by using completely randomized design in naturally ventilated polyhouse with eleven treatments replicated thrice during March 2023 to August 2023.

Planting material

Orthotropic shoots were collected from 5 to 6 years old healthy mother vine of Panniyur-1 and single node cuttings were prepared. Orthotropic shoots at upper part of the vine with shorter internodes are carefully separated and top the 1 to 2 nodesare discarded and 5 to 6 nodes below the top two nodes are selected for planting. Semi-hard wood cuttings of pencil thickness (0.8-1.0 cm diameter) were selected and cuttings of 5 to 6 cm length with single node was prepared by giving a slant cut at the bottom to expose the maximum absorption surface area for inducing effective rooting and all the leaves were removed.

Preparation of growth stimulants formulations

Different concentrations of growth regulators were prepared according to the treatments by weighing accurate quantity using high precision digital weighing balance. NAA of three different concentrations *viz.,* 100 ppm, 150 ppm and 250 ppm, IBA of three different concentrations *viz.,* 250 ppm, 500 ppm and 750 ppm and combination of both *viz.,* NAA (250 ppm) + IBA (250 ppm), NAA (250 ppm) + IBA (500 ppm) and NAA (250 ppm) + IBA (750 ppm) were prepared.

The tender coconut water obtained from seven months old nuts were used for dipping of cuttings for two minutes. Coconut water is a rich supplement that naturally contains plant growth regulators such as indole acetic acid (IAA), cytokinin, kinetin, zeatin and gibberellic acid. Tender coconut water also comprise of major minerals such as potassium, sodium, calcium, phosphorus, iron, copper, sulphur and chlorides (Acha *et al.,* 2004 and Agele *et al.,* 2010).

Filling of media into the protrays

Protraysmade with high density polythene material of dimension 275 mm × 535 mm were filled with media (Forest soil: Sand: FYM @ 3:1:1) and watering was done before the planting of cuttings.

Treatment of cuttings

After that cuttings was washed thoroughly and shade dried and then cuttings were quick dipped in prepared growth stimulant solution for better rooting.

Planting of cuttings and preparation of polytunnel

Meanwhile, the polytunnel was made using locally

available bamboo sticks and iron rods. Planting of cuttings was done into the protrays and kept inside the polytunnel for one month. A white polythene sheet (100 micron) was used to cover the tunnel completely.

Aftercare

Immediately after planting, the pro-trays were lightly watered and later, irrigation was done depending on the moisture content. Weeds were removed in the pro-trays as and when noticed. Plant protection measures were taken. copper oxychloride @ 0.1 % was sprayed as and when disease was noticed.

Foliar nutrition was given at 120 days after planting of cuttings. Two sprays of water soluble fertilizers like N:P:K (19:19:19) @ 0.2 %, magnesium, zinc and boron (@ 0.1 % each) was sprayed at upto 150 DAP.

Observations recorded

The observations were recorded onsprout parameters *viz.,* days taken for first sprouting, days taken for complete sprouting and sprouting percentage, growth parameters *viz.,* sprout length (cm), number of leaves, leaf area(cm^2), leaf area index (LAI), total chlorophyll content (mg/g), fresh weight of shoot (g), dry weight of shoot (g) and survival per cent and root parameters *viz.,* rooting per cent, number of primary roots, number of secondary roots, length of longest primary root(cm), root volume (cc), root thickness (mm), fresh weight of roots (g), dry weight of roots (g) and total dry matter production (g).

Results and Discussion

Sprout parameters

Sprouting parameters like number of days taken for first sprouting and complete sprouting and sprouting percentage of orthotropes was significantly influenced by growth stimulants and represented in Table 1.

Among the treatments, the cuttings treated with tender coconut water (T_{10}) showed least number of days for first sprouting (6 days) and which is followed by the treatment T_9: NAA (250 ppm) + IBA (750 ppm) (8.33 days). The minimum number of days for complete sprouting (16.58) was noticed in the cuttings treated with tender coconut water (T_{10}) and it was found *on par* with the T_9(18.38 days). The early sprouting in the cuttings treated with tender coconut water might be due presence of growth promoting substances such as cytokinins, sugars and amino acids. The cuttings treated with NAA and IBA resulted in delayed sprouting which might be dueto interactions between the exogenous rooting hormones and endogenous plant hormones. Usman and Akinyele (2015) also reported that tender coconut water is more effective than IBA and NAA in for sprouting and callus formation of *Massularia acuminata*.

At 60 DAP, the orthotropic shoot cuttings treated with the NAA @ 100 ppm (T_1) showed highest percentage of sprouting (96.29) and it was *on par* with the both the treatments T_{10} and T_2 (90.74 % each). The lower concentrations of NAA are more likely to promote sprouting and shoot growth. High concentrations of NAA could be toxic to plant tissues, potentially causing damage or inhibiting growth. Ahmed *et al.* (2017) noticed that lower doses of NAA found to give the better number of sprouts than higher doses in grapes stem cuttings and Agele *et al.* (2013) reported highest sprouting in cuttings treated with coconut water and NAA in black pepper compared to IBA and IAA.

Shoot parameters

The data pertaining to the growth parameters like sprout length, number of leaves, total leaf area, leaf area index, total chlorophyll content, survival percentage and weight of the shoot was significantly influenced by the growth stimulants at 150 DAP. (Table 1).

The maximum sprout length (14.67 cm) was recorded in tender coconut water (T_{10}) treated cuttings and it was found *on par* with T_9: NAA (250 ppm) + IBA (750 ppm) treated cuttings (13.73 cm). The more number of leaves (4.85) were produced in the cuttings treated with the tender coconut water (T_{10}) and it was *on par* with T_9 (4.57). The maximum total leaf area (427.07 cm^2) and leaf area index (7.59) per cutting was recorded in tender coconut water (T_{10}) treated cutting and it was followed by the treatment T_9 (385.76 cm^2 and 6.86, respectively). The increased sprout length, number of leaves and total leaf areain the tender coconut water treated cuttings could be due to presence of auxins, gibberellins, cytokinins as well as other growth stimulants in tender coconut water stimulated rapid cell division and cell enlargement (Yong *et al.,* 2013 and Ma *et al.,* 2008). This increase in stem length is be due to the activity of the

Table 1. Effect of growth stimulants on sprouting and growth of orthotropic shoot cuttings in black pepper at 150 DAP.

Treatments	Days taken for first sprouting	Days taken for complete sprouting	Sprouting (%) at 60 DAP	Sprout length (cm)	Number of leaves	Total leaf area (cm^2)/ cutting	Leaf area index	Total chlorophyll (mg/g fr. wt.)	Survival (%)	Weight of the shoot (g)	
										Fresh	Dry
T_1: NAA (100 ppm)	9.33	18.46	96.29	13.23	4.24	305.70	5.43	1.79	79.63	5.17	1.63
T_2: NAA (150 ppm)	9.67	19.86	90.74	11.65	3.65	209.67	3.73	1.04	46.29	4.68	1.46
T_3: NAA (250 ppm)	11.67	22.46	85.18	10.90	2.50	124.81	2.22	0.95	42.50	3.24	0.71
T_4: IBA (250 ppm)	10.67	21.43	83.33	10.54	2.92	157.52	2.80	1.20	44.44	3.54	0.84
T_5: IBA (500 ppm)	9.67	19.05	87.03	12.25	3.90	255.19	4.54	1.19	61.11	3.83	1.12
T_6: IBA (750 ppm)	9.67	18.60	88.88	12.98	4.10	284.03	5.05	1.57	66.67	5.07	1.51
T_7: NAA (250 ppm) + IBA (250 ppm)	11.00	18.59	77.77	11.98	3.75	249.35	4.43	1.30	59.26	4.06	1.32
T_8: NAA (250 ppm) + IBA (500 ppm)	10.00	18.52	87.03	12.21	3.95	295.34	5.25	1.42	60.45	4.57	1.38
T_9: NAA (250 ppm) + IBA (750 ppm)	8.33	18.38	88.88	13.73	4.57	385.76	6.86	2.02	83.33	6.37	1.64
T_{10}: Tender coconut water dipping	6.00	16.58	90.74	14.67	4.85	427.07	7.59	2.04	87.04	6.69	1.88
T_{11}: Dipping in tap water - Check	14.00	24.19	77.77	8.78	2.09	85.44	1.52	0.71	40.18	2.89	0.68
S.Em ±	0.7	0.63	2.06	0.35	0.11	8.13	0.13	0.06	2.04	0.16	0.04
CD @ 5%	2.06	1.86	6.05	1.03	0.34	23.83	0.37	0.17	5.98	0.47	0.13

*DAP: Days after planting

benzyl adenine in cell elongation and division. The auxins and cytokinins present in the tender coconut water might have accelerated the differentiation of leaf primordia in the apical growing region and leading to increased production of leaves. The cytokinins found in coconut water supported the cell division, elongation and differentiation. Thus, promoted rapid expansion of leaves, resulted in increased leaf area. The result of the present study is in agreement with reports of Yufdy and Ernawati (1987) reported that black pepper cuttings dipped in coconut water (25 %) for 12 hours increased the shoot length, Karunarathna and Harris (2016) in cuttings of ixora.

The maximum total chlorophyll content (2.04 mg/g fr. wt.) was noticed in the cuttings treated with tender coconut water (T_{10}) and it was *on par* with the T_9 (2.02 mg/g fr. wt.). Auxins present in the tender coconut water enhances the photosynthesis by activating the enzymes involved in the light reaction. The increased leaf area could be due to presence of growth enhancing substances in tender coconut water which might have fixed more nitrogen associatively and also activated more photosynthates, resulting in more chlorophyll content of leaves. Similar results were reported by Mukhtar (2008) in *Hibiscus sabdariffa* with spray of coconut milk (15%) and GA_3(100 ppm).

Orthotropic shoot cuttings treated with tender coconut water (T_{10}) recorded maximum survival percentage of 87.04 and it was found *on par* with the cuttings treated with the T_9: NAA (250 ppm) + IBA (750 ppm) which recorded 83.33 %. The cuttings treated with tender coconut water resulted in development of effective root system in terms of number of primary and secondary roots which might have influenced the uptake of nutrients and water. The overall performance in relation to growth and root parameters were comparatively better in this treatment which ultimately increased the survival percentage. The above results are in conformity with Thankamani *et al.* (2019) in bush pepper, Agele *et al.* (2013) in guava and black pepper and Vidanapathirana *et al.* (2023) in tea cuttings.

The maximum fresh weight of shoot (6.69 g) was recorded in tender coconut water (T_{10}) treated cuttings and which was found *on par* with T_9 (6.37 g). The maximum dry weight of a shoot (1.88 g) was recorded in tender coconut water (T_{10}) treated cutting, followed by T_9 (1.64 g) and T_1 (1.63 g). The increase in fresh and dry weight of shoot might be due

to auxin and cytokinin present in the tender coconut water which activated the stem and leaf growth by uptaking higher nutrients and greater accumulation of photosynthates leading to increase in number of leaves, plant size and fresh bio mass which in turn yields higher fresh and dry matter content. These results are in line with the findings of Holwerda and Ekanayake (1991) in sweet potato.

Root parameters

Root parameters such as rooting percentage, number of primary and secondary roots, length of longest primary root, root volume and thickness, fresh and dry weight of the roots and total dry matter production of orthotropic cuttings was significantly influenced by growth stimulants at 150 DAP and represented in Table 2.

The highest rooting (85.12 %) was recorded in orthotropic shoot cuttings treated with tender coconut water (T_{10}) and which was *on par* with treatments T_9 (82.90 %). Tender coconut water contains growth enhancing substances would have increased the meristematic activity and root differentiation. The slightly acidic to neutral pH and low salinity levels of coconut water create favourable conditions for root initiation and growth. Yong *et al*. (2013) reported that tender coconut water (7-8 months) contains growth regulators *viz.,* auxins, cytokinins, gibberellic acids, sugars, amino acids, potassium, chlorine, sulphur, enzymes *etc.,* which are necessary for rooting and better growth of the plants. The findings of Agele*et al.* (2013) in guava and black pepper; Vidanapathirana *et al.* (2023) in tea and Karunarathna and Harris (2016) in ixora cuttings supports the present results.

The maximum number of primary roots (7.56) and secondary roots (54.34) was recorded in orthotropic cuttings treated with tender coconut water (T_{10}) and which was *on par*with T_9 (7.30 and 52.16, respectively). Tender coconut water contains natural auxins, such as indole-3-acetic acid (IAA) and cytokinins which are known to promote root initiation and development in cuttings. Auxins stimulate the initiation and formation of adventitious roots through downward movement, leading to a higher number of roots and it is being rich in essential nutrients, including vitamins, minerals and sugars, provided the necessary nourishment for the developing roots resulted in higher number of primary and secondary roots. Similar trend was recorded by Thankamani *et al.* (2019) in bush pepper;

Table 2. Effect of growth stimulants on root parameters of orthotropic shoot cuttings of black pepper in 150 DAP.

Treatments	Rooting (%)	Number of primary roots	Number of secondary roots	Length of longest primary root (cm)	Root volume (cc)	Root thickness (mm)	Weight of the root (g)	Total dry matter (g)	
								Fresh	Dry
T_1: NAA (100 ppm)	76.43	6.33	42.33	11.85	2.20	1.35	0.91	0.10	1.73
T_2: NAA (150 ppm)	44.87	3.67	19.33	6.40	0.80	0.82	0.33	0.04	1.50
T_3: NAA (250 ppm)	39.64	3.33	17.67	4.90	0.70	0.64	0.21	0.04	0.75
T_4: IBA (250 ppm)	60.34	3.67	23.33	6.77	0.90	0.92	0.36	0.06	0.90
T_5: IBA (500 ppm)	41.98	4.00	30.67	7.70	1.01	1.08	0.42	0.06	1.18
T_6: IBA (750 ppm)	63.89	5.33	40.67	9.80	2.00	1.12	0.72	0.08	1.59
T_7: NAA (250 ppm) + IBA (250 ppm)	57.87	4.33	32.67	8.40	1.50	1.10	0.51	0.06	1.38
T_8: NAA (250 ppm) + IBA (500 ppm)	58.72	4.67	38.67	8.60	1.80	1.17	0.60	0.08	1.46
T_9: NAA (250 ppm) + IBA (750 ppm)	82.90	7.30	52.16	12.80	4.90	1.72	2.63	0.22	1.86
T_{10}: Tender coconutwater dipping	85.12	7.56	54.34	20.67	5.00	1.78	2.70	0.46	2.34
T_{11}: Dipping in tap water - Check	38.26	2.66	14.67	3.23	0.40	0.55	0.13	0.02	0.70
S.Em ±	1.43	0.11	0.79	0.28	0.06	0.03	0.03	0.01	0.04
CD @ 5%	4.10	0.33	2.31	0.82	0.17	0.08	0.09	0.03	0.09

*DAP: Days after planting.

Agampodi and Jayawardena (2009) in *Dracaena*; Karunarathna and Harris (2016) inixora and Vidanapathirana *et al.* (2023) in tea.

The maximum root length of 20.67 cm was recorded in the cuttings treated with tender coconut water (T_{10}) followed by the treatment T_9 (12.80 cm). The highest root volume (5.00 cc) and root thickness (1.78 mm) was recorded in the cuttings treated with tender coconut water (T_{10}) and which was *on par* with T_9 (4.90 cc and 1.72 mm, respectively). The tender coconut water contains natural plant growth hormones like auxins and cytokinins. These hormones stimulated cell elongation and division in the root tissues, leading to longer and thicker roots. Auxins, in particular, encouraged the cell division in the root meristem through hydrolysis and translocation of carbohydrates and nitrogenous substances at the base of cuttings, resulted in increased number of root cells. Thus, maximum root volume and thickness. The nutrient rich tender coconut water provides essential elements and energy for root growth. These results are in conformity with reports of Agampodi and Jayawardena (2009) in *Dracaena purplecompacta* L.; Usman and Akinyele, (2015) in *Massularia acuminata*; Ibironke (2016) in bougainvillea; Karunarathna and Harris (2016) in ixora and Vidanapathirana *et al.* (2023) in tea.

The treatment with tender coconut water (T_{10}) had maximum fresh weight of root (2.70 g) and which was *on par* with T_9 (2.63 g). The significantly maximum dry weight of root (0.46 g) was recorded in the cuttings treated with tender coconut water (T_{10}) followed by T_9 (0.22 g). Tender coconut water is rich in essential nutrients, vitamins, minerals, and sugars, which provided better nourishment for the developing roots. This nutrient supply enables the roots to grow and accumulate more biomass and better mobilization of primary metabolites lead to better root formation. Auxins present in the tender coconut water increased the number of roots resulting in higher accumulation of fresh and dry matter of roots. The present results are in agreement with the findings of Vidanapathirana *et al.* (2023) in tea where highest fresh weightof roots at 4th and 6th week after application and maximum root dry weight at 6th week after application and Ibironke (2016) noticed increased wet and dry weight of roots in bougainvillea

The significantly maximum total dry matter (2.34 g) was recorded in tender coconut water (T_{10}) treated cuttings followed by T_9 (1.86 g) and T_1 (1.73 g). Tender coconut water is rich in essential nutrients, including vitamins, minerals and sugars. These nutrients provided better source of nourishment for the cuttings, supporting overall growth and biomass accumulation. The tender coconut water also contains natural plant growth hormones such as auxins and cytokinins. These hormones play a crucial role in stimulating cell division, elongation and differentiation, promoting both root and shoot growth. The increased growth of plant tissues contributed in terms of number of leaves, shoot length and fresh weight of biomass. Both hormonal and nutritional properties of tender coconut water encourage the development of a robust root system and vigorous shoot growth. These results get support from the findings of Holwerda and Ekanayake (1991) in sweet potato stem cuttings.

Conclusion

Among different growth stimulants tried, tender coconut water (T_{10}) treated cuttings showed better sprouting parameters like days taken for first sprouting and complete sprouting, shoot parameters like sprout length, number of leaves, leaf area, leaf area index, total chlorophyll content, fresh and dry weight of shoot and survival per cent and root parameters like rooting per cent, number of primary roots, number of secondary roots, length of longest primary root, root volume, root thickness, fresh weight of roots, dry weight of roots and total dry matter production and which is followed by T_9: NAA (250 ppm) + IBA (750 ppm).

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Conflict of interest

The authors declare no conflicts of interest

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