

## Auxin enrichment for enhanced rooting and biomass production in clonal propagation of thippili (*Piper longum* L.)

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### Abstract

*Piper longum* L., commonly known as long pepper or Thippili, is a valuable medicinal plant widely used in traditional Indian medicine. However, its commercial propagation is limited due to poor seed germination and low rooting efficiency in vegetative cuttings. The study evaluated the effect of different concentrations of Indole-3-butyric acid (IBA) and Naphthalene Acetic Acid (NAA) on rooting and shoot development of *Piper longum* L. cuttings. Among treatments, IBA at 1000 ppm (T<sub>3</sub>) showed the best results with a rooting percentage of 87.79%, primary roots per cutting at 14.85, and secondary roots at 33.05. Fresh and dry root weights were highest at 2.71 g and 1.02 g, respectively, while fresh and dry shoot weights reached 11.02 g and 3.53 g. Leaf area (123.40 cm<sup>2</sup>) and total dry matter production (4.55 g) were also maximized in this treatment. These results suggest that IBA at 1000 ppm significantly enhances rooting and biomass accumulation in long pepper cuttings, making it the optimal choice for propagation.

**Key words :** Thippili, Viswam, IBA, NAA, Rooting, biomass and leaf area.

*Piper longum* L., commonly known as long pepper or *Thippili*, is an important medicinal plant in traditional Indian systems of medicine<sup>11</sup>. Native to the Indo-Malayan region, it grows wild in India, Sri Lanka, Nepal, Indonesia and Malaysia. In India, it is found in scattered pockets across Assam, West Bengal, Maharashtra and the Western Ghats<sup>13</sup>. The commercially traded long pepper includes *P. longum*, *P. peepuloides* and *P. officinarum*. The plant is dioecious, with unisexual flowers

borne on spikes. Only the unripe female spikes and roots are economically and medicinally valuable. Spikes are harvested when blackish-green for maximum pungency. These parts contain volatile oil, piperine (4–5%), piperidin and essential alkaloids. Used in Ayurveda, Siddha and folk medicine, *Thippili* treats bronchitis, cough, indigestion, piles and fever. It is also used post-parturition to aid in placenta expulsion<sup>16</sup>. Despite its medicinal importance, *Piper longum* remains underutilized due to

poor propagation and limited quality planting material. Seed propagation is inefficient, and cuttings often show low rooting and survival. This study evaluates the effect of varying concentrations of IBA and NAA on rooting and shoot development to identify the optimal treatment for large-scale propagation.

The present study entitled “Effect of Plant Growth Regulators on Propagation of Long Pepper (*Piper longum* L.)” was conducted during 2021–2022 at a farmer’s nursery in Karavalli, Namakkal district, Tamil Nadu. The experiment was laid out in a Completely Randomized Design (CRD) with 13 treatments, replicated thrice with 10 cuttings per replication, totaling 390 cuttings. The experiment comprised different combinations of IBA and NAA at varying concentrations, with the following treatment structure. T<sub>1</sub> – IBA @ 250 ppm, T<sub>2</sub> – IBA @ 500ppm, T<sub>3</sub> – IBA @ 1000ppm, T<sub>4</sub> – IBA @ 1500ppm, T<sub>5</sub> – NAA @ 250ppm, T<sub>6</sub> – NAA @ 500ppm, T<sub>7</sub> – NAA @ 1000ppm, T<sub>8</sub> – NAA @ 1500ppm, T<sub>9</sub> – IBA @ 250ppm + NAA @ 250ppm, T<sub>10</sub> – IBA @ 500ppm + NAA @ 500ppm, T<sub>11</sub> – IBA @ 1000 ppm + NAA @ 1000ppm, T<sub>12</sub> – IBA @ 1500ppm + NAA @ 1500ppm and T<sub>13</sub> – Control (Distilled water). The selected variety, ‘Viswam’ is a high-yielding female spike type from the Sri Lankan Cheemathippalli race was used. One-year-old healthy runner vines were cut into 15–20 cm segments with 3–4 nodes, defoliated, and given a slant basal cut. Cuttings were treated with different concentrations of IBA and NAA using the quick dip method (60 seconds) and planted slantingly in polybags containing a 1:1:1 mix of red soil, sand, and vermicompost under shade net. Regular watering was provided, and root

and shoot development were monitored. Observations were recorded from five plants per treatment at 90 DAP and statistically analyzed following Panse and Sukhatme (1985), with significance tested at 5% level.

The present investigation clearly demonstrated that the application of plant growth regulators, particularly Indole-3-butyric acid (IBA), significantly influenced the rooting and biomass parameters of long pepper (*Piper longum* L.) cuttings under nursery conditions.

The maximum number of primary roots per cutting (14.85) and secondary roots (33.05) were observed in T<sub>3</sub>, significantly superior to the control (T<sub>13</sub>) which recorded the lowest values (6.75 and 18.71, respectively). T<sub>3</sub> also exhibited the highest rooting percentage (87.79%), which was 2.42 times greater than the control (36.28%). The enhanced root elongation observed with IBA application may be attributed to its stimulatory effect on cambial activity, which facilitates the mobilization of reserved food materials toward the root initiation site<sup>5</sup>. The availability of these assimilates likely supports the increased elongation of both primary and secondary roots. The increase in root length relative to the control may result from enhanced carbohydrate hydrolysis, auxin-induced accumulation of metabolites, protein synthesis, cell enlargement and cell division triggered by auxin treatment. These findings corroborate those reported by Vigneswari *et al.*,<sup>20</sup> hormone-like substances produced in developing buds are translocated via the phloem to the base of cuttings, stimulating rooting through the enhanced hydrolysis of nutrient reserves, particularly starch. Similar enhancements in root growth parameters have

Table-1. Effect of different auxin concentration on rooting and biomass production of long pepper at 90 DAP

Treatments	Number of primary roots cutting <sup>-1</sup>	Number of secondary roots cutting <sup>-1</sup>	Rooting percentage (%)	Fresh weight of roots (g)	Dry weight of roots (g)	Fresh weight of shoots (g)	Dry weight of shoots (g)	Leaf area (cm <sup>2</sup> )	Total dry matter production (g)
T <sub>1</sub>	9.12	22.44	49.42	1.40	0.41	8.90	2.07	31.81	2.48
T <sub>2</sub>	14.11	31.74	83.54	2.59	0.96	10.79	3.40	110.59	4.36
T <sub>3</sub>	14.85	33.05	87.79	2.71	1.02	11.02	3.53	123.40	4.55
T <sub>4</sub>	10.96	26.31	62.60	1.84	0.59	9.68	2.59	56.36	3.18
T <sub>5</sub>	8.49	21.26	45.16	1.27	0.35	8.65	1.94	25.61	2.29
T <sub>6</sub>	13.09	30.42	79.16	2.42	0.85	10.51	3.21	96.56	4.06
T <sub>7</sub>	12.56	29.19	74.88	2.28	0.80	10.27	3.05	85.11	3.85
T <sub>8</sub>	10.58	25.09	58.31	1.73	0.55	9.43	2.45	48.05	3.00
T <sub>9</sub>	7.82	20.05	40.85	1.17	0.30	8.41	1.76	19.59	2.06
T <sub>10</sub>	11.74	27.81	70.47	2.09	0.70	9.99	2.87	73.07	3.57
T <sub>11</sub>	11.69	27.35	67.05	2.04	0.68	9.97	2.84	67.47	3.52
T <sub>12</sub>	10.16	23.81	53.98	1.58	0.49	9.17	2.32	40.49	2.81
T <sub>13</sub>	6.75	18.71	36.28	1.01	0.24	8.14	1.55	13.21	1.79
S.Ed	0.15	0.27	1.94	0.03	0.01	0.08	0.05	2.94	0.07
CD(p=0.05)	0.33	0.52	3.92	0.08	0.02	0.19	0.12	5.89	0.16

been documented by Akshay *et al.*,<sup>1</sup> in black pepper, Devendra Kumar *et al.*<sup>2</sup> in *Pogostemon cablin* (patchouli) and Madhavan *et al.* 2025a<sup>11</sup> in *Piper longum*.

Fresh and dry weights of roots were also highest in T<sub>3</sub> (2.71 g and 1.02 g, respectively), while the control showed the least values (1.01 g and 0.24 g, respectively). Shoot biomass followed a similar trend, with the maximum fresh weight (11.02 g) and dry weight (3.53 g) observed in T<sub>3</sub>, compared to the control which recorded 8.14 g (fresh) and 1.55 g (dry). This enhanced performance could be attributed to the improved physiological maturity of the cuttings, coupled with effective mobilization of primary metabolites that facilitated better root development under the influence of growth regulators, ultimately leading to greater accumulation of fresh and dry biomass<sup>9</sup>. Similar observations were reported by Sundhariya *et al.*, (2000) in *Gymnema* and by Ingle and Venugopal<sup>7</sup> in *Stevia*. The application of IBA enhanced leaf production, expanded leaf area, and increased chlorophyll and starch content, which collectively contributed to the higher fresh and dry weight of shoots. These findings are in agreement with the reports of Bhattacharya (1998) in scented geranium, and Elhaak *et al.*<sup>4</sup> as well as Hekmat *et al.*<sup>6</sup> in rosemary.

Leaf area was also highest in T<sub>3</sub> (123.40 cm<sup>2</sup>), followed by T<sub>2</sub> (110.59 cm<sup>2</sup>) and T<sub>6</sub> (96.56 cm<sup>2</sup>), with the least being in the control (13.21 cm<sup>2</sup>). This might be due to Auxin application may have necessitated the increased activity of photosynthesis and other activities carried out in the leaves, which in turn may have resulted in the increase of

leaves in the cuttings. The increase in number of leaves per cutting might be due to the reason that the plant might diverted maximum assimilate quantities to the leaf buds, since the leaves are one of the production sites of natural auxins in them besides being very important for vital processes like photosynthesis and respiration<sup>12,20</sup>. Similar findings were reported by Prajapati *et al.*,<sup>15</sup> in black pepper, Rema and Krishnamoorthy<sup>17</sup> in Camphor tree, Diwakar *et al.*,<sup>3</sup> in guggul and Sure *et al.*,<sup>19</sup> in *Guggul*.

Correspondingly, total dry matter production was highest in T<sub>3</sub> (4.55 g), and lowest in T<sub>13</sub> (1.79 g). These results are in agreement with the findings of Ajit Aun Warman *et al.* (2019) in *Piper sarmentosum* and Kumar *et al.*,<sup>8</sup> in *Guggul*.

The application of IBA significantly improved rooting and biomass accumulation in long pepper cuttings by enhancing physiological processes such as cell division, cambial activity, and mobilization of stored metabolites. Auxin-induced stimulation of root primordia, increased chlorophyll content, and enhanced leaf development collectively contributed to better growth and dry matter production. Thus, IBA, particularly at 1000 ppm, is an effective plant growth regulator for the clonal propagation of long pepper, ensuring improved survival and uniform establishment of cuttings.

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**Conflict of interest**

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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