



Standardization of time and size of cutting and IBA concentration for the stem cutting technique of dragon fruit (*Hylocereus costaricensis* Britton and Rose)

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ABSTRACT

Dragon fruit saplings prepared from the cuttings of 10, 15 and 20 cm sizes collected in January and April and treated with 100, 250, 500 and 0 ppm IBA were evaluated for root, shoot and biochemical parameters with an objective to choose the best combination of time and size of cuttings and IBA concentration for the stem cutting technique of dragon fruit. The results revealed that 20 cm cuttings taken in April and treated with 250 ppm IBA led to best root and shoot parameters with early (22.33 DAP) shoot initiation, highest root (20.83) and shoot (4.4) numbers, highest chlorophyll (0.51 mg/g) and nitrogen (2.75 %) content, highest vascular cambium thickness (0.15 mm) and highest survival (100 %). The root formation was early (14 DAP) in 20 cm cuttings taken in April and treated with 500 ppm IBA. So, the results suggest that T₂₂(20cm cuttings+April+250 ppm IBA) is the best combination for the root growth and subsequent vegetative growth of dragon fruit stem cuttings.

1. Introduction

Dragon fruit is an emerging fruit crop of the 21st century which originated in the tropical and subtropical forests of Central and South America (Britton and Rose, 1963; Mizrahi *et al.*, 1997). It received massive popularity because of its attractive appearance, luscious tastes and health benefits. The fruits are high in antioxidants (phyto albumins), which help to fight against carcinogenic free-radicals forming in the body. It is rich in Vitamin-C and minerals, especially calcium and phosphorus. They are also low in calories and high in fiber, while the seeds are having high polyunsaturated fatty acids. The seeds of the fruit are said to help in controlling blood glucose levels in people with non-insulin-dependent hyperglycaemic conditions (a kind of diabetes). It is also used to treat stomach and endocrine problems. Dragon fruit also improves eyesight and prevents hypertension (Jeronimo *et al.*, 2015). Dragon fruit can be commercially cultivated upto an altitude of 1700 m above mean sea level with an annual rainfall requirement of 500 - 2000 mm. The suitable temperature ranges from 20° C to 30° C. It grows well in slightly acidic soils with pH 5.5- 6.5 (Gunasena *et al.*, 2007). Due to the presence of xerophytic adaptations of Crassulacean Acid Metabolism (CAM) and presence of a

waxy layer over its succulent stems, these plants can even be grown in desert conditions with low rainfall and temperature as high as 38°C- 40°C (Trivellini *et al.*, 2020). Dragon fruit plants have shallow roots (less than 40 cm) due to which they are less choosy to specific soil requirements and can be grown in varied soil conditions free from water logging. Dragon fruit can be propagated sexually through seeds and asexually through grafting, micropropagation and stem cuttings (Ahmed, 2006; Dhruve, 2017 and Gomez *et al.*, 2000). But, stem cutting is preferred for its propagation due to its ability to provide true to type plants with early yielding capacity (within 2 years after planting) (Hartmann *et al.*, 2011). Moreover, stem cutting technique is cheaper and can be performed by a layman. It was forecasted that world dragon fruit market will register a Compound Annual Growth Rate of 3.6 % during 2021 to 2026 (Mordor Intelligence, 2020) and in India the acreage of dragon fruit increased from 400 ha in 1991 to 4000 ha in 2020 (Wakchaure *et al.*, 2020). Therefore, India with varied agroclimatic conditions have huge prospects for dragon fruit cultivation but, there is scarcity of planting materials to meet the growing demand. So, the current study was undertaken to standardise the time and size of cuttings and IBA concentration for the stem cutting technique of dragon fruit.

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2. Materials and Methods

The experiment was conducted in the Experimental Farm of Department of Horticulture, Assam Agricultural University, Jorhat, which falls in the Upper Brahmaputra Valley Agroclimatic Zone of Assam. The geographical coordinates of the experimental plot was Latitude- 26°44' N and Longitude- 94°12' E with an altitude of 87 m above mean sea level. The experimental site experiences hot humid subtropical summers during July to August (32° C – 34° C) and comparatively cool and dry winters during December to January (9.9° C- 10.9° C). The experiment was laid out in a Factorial Completely Randomised Design (CRD). It consisted of 3 factors (Time of cutting, Size of cutting and IBA concentration), 24 treatment combinations and 3 replications. There were 2 times of cuttings : January (M₁) and April (M₂), 3 sizes of cuttings : 10 cm (L₁), 15 cm (L₂) and 20 cm (L₃) and 4 IBA concentrations : 100 ppm (I₁), 250 ppm (I₂), 500 ppm (I₃) and 0 ppm (I₄). The one year old cuttings having 4 to 5 nodes each were procured from the 5 year old dragon fruit plantation of Krishi Vigyan Kendra (KVK), Jorhat. The cuttings were then sized to the desired lengths by providing a slant cut at the base. The sized cuttings were treated with 0.1 % Carbendazim WP solution and later shade dried for one day prior to planting to dry the ooze coming out of the cuttings. The rooting media used was FYM, Sand and Soil mixture @ 1:2:1 ratio. The rooting mixture was drenched with 0.3 % Captan WP solution as a prophylactic measure to kill the fungal spores present in the mixture. The pH of the rooting mixture was 6.2 and contained 0.86 % organic carbon and 287 kg/ha nitrogen. The cuttings were planted in polybags of 20 cm x 22 cm size and raised in shade net house providing 50 % shading.

The mean data were recorded on root parameters (days required for root formation, number of roots, length of the longest roots, root lengths, diameter of the longest root, root fresh weight, root dry weight, rooting percentage), shoot parameters (days required for shoot initiation, number of shoots, length of primary shoots, length of secondary shoots, shoot fresh weight, shoot dry weight, root to shoot ratio) and survival percentage. Days required for root formation was determined by uprooting the cuttings on alternate days after planting whereas days required for shoot initiation was determined by regular observation of the cuttings after planting. Other root and shoot parameters were recorded at 40 and 60 days after planting (DAP).

The mean data were recorded on chlorophyll, nitrogen and protein content and vascular cambium thickness of the cuttings at 40 and 60 days after planting (DAP). Nitrogen content was estimated by Kjeldahl method

(Thimmaiah, 1999) using the following formula:

$$\text{Nitrogen \%} = \frac{(\text{ml HCL in sample}) - (\text{ml HCL in blank}) \times \text{normality of acid} \times 14.01 \times 100}{\text{Weight of sample (mg)}}$$

Protein content was estimated using the following formula (Thimmaiah, 1999):

$$\text{Protein \%} = \text{Nitrogen \%} \times 6.25$$

Total chlorophyll content was determined using acetone method as suggested by Thimmaiah (1999) using the following formula:

$$\text{Total chlorophyll content (mg/ g)} = 20.2 (A_{645}) + 8.02 (A_{663}) \times V / (1000 \times W)$$

Where, A = Absorbance at specific wavelengths

V = Final volume of chlorophyll extract in 80 % acetone

W = Fresh weight of the tissue extracted

Vascular cambium thickness (mm) of shoot was measured by preparing microscopic slides of stem cross-sections and observing under ocular micrometer (Johanson, 1940).

3. Results and discussion

The time of taking cuttings plays an important role in the success of cuttings as the level of endogenous auxins and carbohydrates varies at different seasons of the year. Among the January and April months it was found that April cuttings led to early saplings as the April cuttings required less time for root (18.09 days) and shoot (33.44 days) initiation (Table 1 and 2). Moreover, the survival percentage (98.13 %) of the April cuttings was higher which may be due to the higher root (12.79) and shoot (4.66) number along with longer (12.79 cm) and thicker (0.77 mm) roots. The higher fresh and dry weight of root (1.42 g; 0.62 g) and shoot (64.27 g; 10.88 g) signified higher food material accumulation in the April cuttings. The biochemical analysis of the shoots revealed that the April cuttings had higher chlorophyll (0.44 mg/g), nitrogen (2.53 %), protein (15.84 %) and vascular cambium thickness (0.08 mm) which may justify the April cuttings to be healthy as compared to the January cuttings (Table 5). Nandi *et al.* (2019) reported highest success rate in the January-March cuttings. Whereas, the slight variance in the result of the present study may be due to the change in the geographical location. Although, the January cuttings comparatively resulted in poor result but the outcomes were enhanced when the cuttings were treated with IBA solution (Table 3, 4 and 6). The larger cuttings normally provides higher success rate whereas small sized cuttings are preferred in bulk production to make the production process economic. Among the three sizes of cuttings (10, 15 and 20 cm), the 20 cm cuttings provided best results (Table 1 and 2). The results were supported by the works of Malswamkimi *et al.* (2019) and

Kakade *et al.* (2019) wherein they also found better rooting and shooting with the larger sized cuttings. The 20 cm cuttings exhibited higher survival rate (99.17 %) which may be attributed to its ability to form early roots (18.46 days) and shoots (29.58 days) along with higher number of root (14.81) and shoot (4.43). Moreover, the roots were longer (14.81 cm) and thicker (0.87 mm) in 20 cm cuttings thus enabling the saplings to accumulate more fresh (74.52 g) and dry (11.52 g) weight. The higher vascular cambium thickness (0.11 mm), chlorophyll (0.45 mg/g), nitrogen (2.63 %) and protein (16.42 %) also signifies the ability of the 20 cm cuttings to form rapid active growth. The 10 cm and 15 cm cuttings also performed well when treated with IBA (Table 3, 4 and 6).

The IBA concentrations (0, 100, 250 and 500 ppm) depicted significant variation in the growth of the saplings (Table 1 and 2). The 500 ppm IBA resulted in early (15.06 days) rooting which was at par with the 250 ppm IBA (18.26 days). The root dry weight (0.63 g), shoot dry weight (11.21 g), root number (15.97) and shoot number (5.01) increased gradually upto 250 ppm IBA whereas a slight decrease was observed in the 500 ppm IBA (0.62 g; 10.83 g; 13.11; 4.2). The 250 ppm IBA treated cuttings were also vigorous as the shoot (72.86 g) and root (1.56 g) fresh weight, the lengths of roots (15.97 cm) and shoots (16.3 cm) and the diameter of the roots (0.91 mm) were significantly higher. Moreover, the 250 ppm IBA treated cuttings would provide better field performance as the chlorophyll (0.45 mg/g), nitrogen (2.6 %), protein (16.23 %) and vascular cambium thickness (0.1 mm) (Fig. 1) were at optimum levels. The results were at par with the works done by Ahmad *et al.* (2016), Fumuro (2011), Minz (2020), Seran and Thiresh (2015) and Siddiqua and Thippesha (2018). The action of IBA got enhanced when the treatment was done on the 20 cm cuttings of April. In T₂₂ (M₂L₃I₂) the root and shoot initiation time were shortened by 1.06 and 7.11 days respectively and the survival percentage was enhanced by 0.83 %. Moreover, the root number, shoot number, root length, shoot length, root dry weight and shoot dry weight were enhanced by 4.86, 0.72, 4.86 cm, 6.4 cm, 0.03 g and 2.22 g respectively. Even the chlorophyll, nitrogen, protein and vascular cambium thickness were increased by 0.06 mg/g, 0.15 %, 0.95 % and 0.05 mm respectively in T₂₂ (M₂L₃I₂) as compared to the 250 ppm IBA treatment.

4. Conclusion

Thus, it may be concluded that 20 cm size cuttings of dragon fruit taken in the month of April treated with 250 ppm IBA appeared to be the best treatment combination which registered minimum days for root formation and shoot initiation, highest root and shoot growth parameters, highest nitrogen and chlorophyll content, highest vascular cambium thickness along with 100% survival of saplings.

5. Authors' contributions

Conceptualization of research (A. Borchetia and M. Neog); Designing of the experiments (A. Borchetia and M. Neog); Contribution of experimental materials (A. Borchetia and M. Neog); Execution of field/lab experiments and data collection (A. Borchetia and M. Neog); Analysis of data and interpretation (A. Borchetia and M. Neog); Preparation of the manuscript (A. Borchetia and M. Neog).

6. Declaration

We wish to draw the attention of the Editor to the following facts which may be considered as potential conflicts of interest and to significant financial contributions to this work.

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author.

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Table 1. Effect of time and size of cutting and IBA concentration on root growth parameters

Treatment	A	B		C		D		E		F		G		H	
		40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP
Time of cutting															
M ₁ - January	20.73	8.35	10.43	9.76	12.93	8.58	10.17	0.42	0.64	1.01	1.23	0.36	0.58	80.09	95.07
M ₂ - April	18.09	10.79	12.79	11.94	14.94	10.79	12.79	0.52	0.77	1.09	1.42	0.40	0.62	82.15	97.15
SEd (+)	1.11	1.17	1.13	0.96	0.91	1.09	1.16	0.02	0.02	0.02	0.02	0.01	0.01	1.02	1.02
CD (5%)	2.21	2.34	2.27	1.92	1.82	2.15	2.27	0.04	0.04	0.05	0.05	0.02	0.02	2.05	2.05
Size of cutting															
L ₁ - 10 cm	22	7.25	9.25	8.15	11.15	7.25	9.25	0.4	0.65	0.91	1.24	0.32	0.53	78.75	93.75
L ₂ - 15 cm	21.33	10.49	12.48	12.67	15.67	10.48	12.49	0.53	0.78	1.09	1.41	0.37	0.59	81.98	96.98
L ₃ - 20 cm	18.46	12.81	14.81	14.37	17.37	12.81	14.81	0.62	0.87	1.26	1.58	0.42	0.63	83.75	98.75
SEd (+)	1.36	1.43	1.38	1.17	1.12	1.33	1.38	0.02	0.02	0.03	0.02	0.02	0.02	1.25	1.25
CD (5%)	2.73	2.87	2.79	2.35	2.24	2.64	2.79	0.04	0.04	0.05	0.05	0.03	0.03	2.52	2.52
IBA concentration															
I ₁ - 100 ppm	21.72	9.82	11.82	10.64	14.02	9.83	11.83	0.52	0.78	1.11	1.43	0.36	0.58	79.87	94.87
I ₂ - 250 ppm	18.26	13.97	15.97	13.64	16.65	13.97	15.97	0.66	0.91	1.23	1.56	0.41	0.63	85.23	99.19
I ₃ - 500 ppm	15.06	11.11	13.11	14	17	11.11	13.11	0.59	0.84	1.21	1.53	0.40	0.62	84.17	99.17
I ₄ - 0 ppm	25.89	5.82	7.82	7.82	10.82	5.82	7.82	0.29	0.54	0.79	1.12	0.29	0.51	77.78	92.78
SEd (+)	1.57	1.65	1.6	1.35	1.28	1.65	1.56	0.02	0.02	0.03	0.03	0.02	0.02	1.44	1.44
CD (5%)	3.15	3.32	3.21	2.71	2.58	3.32	3.21	0.05	0.05	0.06	0.06	0.03	0.04	2.91	2.91

A-Days required for root formation, B-Root number, C-Length of the longest root (cm), D- Root length (cm), E-Diameter of the longest root (mm), F-Root fresh weight (g), G-Root dry weight (g) and H-Rooting percentage

DAP- Days after planting

Table 2. Effect of time and size of cutting and IBA concentration on shoot growth parameters

Treatment	I	J		K		L		M		N		O	
		40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP
Time of cutting													
M ₁ - January	34.78	1.99	3.33	11.96	13.3	7.03	8.36	59.47	62.22	8.04	9.54	0.06	0.07
M ₂ - April	33.44	3.32	4.66	13.29	14.63	8.36	9.7	61.44	64.27	9.38	10.88	0.09	0.10
SEd (+)	0.43	0.43	0.58	0.39	0.56	0.26	0.54	0.89	0.96	0.28	0.32	0.01	0.01
CD (5%)	0.71	0.86	1.15	0.78	1.08	0.64	1.07	1.77	1.92	0.55	0.63	0.02	0.02
Size of cutting													
L ₁ - 10 cm	38.92	1.97	2.71	9.68	11.01	6.16	7.49	51.52	54.35	7.52	9.02	0.06	0.07
L ₂ - 15 cm	33.83	2.9	4.23	11.48	12.81	7.75	9.08	59.1	61.93	8.6	10.1	0.07	0.08
L ₃ - 20 cm	29.58	3.1	4.43	16.73	18.06	9.18	10.52	71.68	74.52	10.02	11.52	0.09	0.10
SEd (+)	0.43	0.43	0.71	0.46	0.63	0.36	0.66	1.08	1.18	0.34	0.39	0.01	0.01
CD (5%)	0.87	1.05	1.42	0.92	1.35	0.72	1.3	2.18	2.33	0.68	0.78	0.02	0.02
IBA concentration													
I ₁ - 100 ppm	34.78	2.58	3.91	12.93	14.26	7.81	9.14	62.44	65.28	8.76	10.26	0.08	0.09
I ₂ - 250 ppm	29.44	3.18	5.01	14.97	16.3	8.69	10.02	70.02	72.86	9.71	11.21	0.11	0.12
I ₃ - 500 ppm	30.45	2.87	4.2	14.04	15.38	9	10.33	67.25	70.01	9.33	10.83	0.1	0.11
I ₄ - 0 ppm	41.78	1.78	2.54	8.57	9.9	5.29	6.62	43.36	46.19	7.04	8.54	0.07	0.08
SEd (+)	0.5	0.51	0.82	0.53	0.74	0.45	0.76	1.25	1.37	0.39	0.45	0.01	0.01
CD (5%)	1	1.21	1.64	1.08	1.47	0.79	0.91	2.51	2.71	0.78	0.9	0.02	0.02

I- Days required for shoot initiation, J- Shoot number, K- Primary shoot length (cm), L-Secondary shoot length (cm), M- Shoot fresh weight (g), N- Shoot dry weight (g) and O- Root to shoot ratio
DAP- Days after planting

Table 3. Interaction effect of time and size of cutting and IBA concentration on root growth parameters

Treatment Combination	A	B		C		D		E		F		G		H	
		40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP
T ₁ (M ₁ L ₁ I ₁)	23	4.73	6.73	6.12	9.12	4.73	6.73	0.34	0.59	0.87	1.19	0.26	0.48	74.17	89.17
T ₂ (M ₁ L ₁ I ₂)	21.33	8.6	10.6	8.72	11.72	8.6	10.6	0.53	0.78	1.02	1.34	0.33	0.54	83.5	96.5
T ₃ (M ₁ L ₁ I ₃)	18	9.9	11.9	11.98	14.98	9.9	11.9	0.46	0.71	1	1.32	0.38	0.59	82.5	97.5
T ₄ (M ₁ L ₁ I ₄)	28	3.63	5.63	4.92	7.92	3.63	5.63	0.23	0.48	0.72	1.05	0.24	0.45	72.5	87.5
T ₅ (M ₁ L ₂ I ₁)	19.67	11.4	13.4	12.72	15.72	11.4	13.4	0.56	0.81	1.11	1.44	0.38	0.6	80.83	95.83
T ₆ (M ₁ L ₂ I ₂)	18	14.23	16.23	15.72	18.72	14.23	16.23	0.65	0.9	1.21	1.54	0.40	0.62	83.33	98.33
T ₇ (M ₁ L ₂ I ₃)	16.33	9.9	11.9	14.52	17.52	9.9	11.9	0.57	0.82	1.19	1.51	0.38	0.61	83.33	98.33
T ₈ (M ₁ L ₂ I ₄)	26.33	4.07	6.07	6.85	9.85	4.07	6.07	0.31	0.56	0.8	1.12	0.29	0.52	77.5	92.5
T ₉ (M ₁ L ₃ I ₁)	21.33	11.53	13.53	14.85	17.85	11.53	13.53	0.65	0.9	1.31	1.63	0.42	0.62	82.5	97.5
T ₁₀ (M ₁ L ₃ I ₂)	16.33	17.4	19.4	15.85	18.85	17.4	19.4	0.77	1.02	1.44	1.76	0.48	0.7	85	100
T ₁₁ (M ₁ L ₃ I ₃)	14.67	11.53	13.53	14.85	17.85	11.53	13.53	0.72	0.97	1.41	1.73	0.42	0.65	85	100
T ₁₂ (M ₁ L ₃ I ₄)	21.33	7.93	9.93	11.05	14.05	7.93	9.93	0.32	0.57	0.83	1.16	0.31	0.54	80.83	95.83
T ₁₃ (M ₂ L ₁ I ₁)	22.67	5.54	7.53	6.55	9.55	5.53	7.53	0.36	0.61	0.89	1.22	0.27	0.5	75.83	90.83
T ₁₄ (M ₂ L ₁ I ₂)	19.33	9.63	11.63	9.15	12.15	9.63	11.63	0.55	0.8	1.03	1.36	0.34	0.56	84.17	99.17
T ₁₅ (M ₂ L ₁ I ₃)	17.67	11.13	13.13	12.42	15.42	11.13	13.13	0.47	0.73	1.02	1.34	0.39	0.61	84.17	99.17
T ₁₆ (M ₂ L ₁ I ₄)	26	4.8	6.8	5.35	8.35	4.8	6.8	0.24	0.49	0.74	1.07	0.25	0.47	74.17	89.17
T ₁₇ (M ₂ L ₂ I ₁)	22.67	12.7	14.7	13.15	16.15	12.7	14.7	0.58	0.83	1.13	1.45	0.40	0.62	81.67	96.67
T ₁₈ (M ₂ L ₂ I ₂)	17.67	15.13	17.13	16.15	19.15	15.13	17.13	0.67	0.92	1.23	1.56	0.42	0.63	85	100
T ₁₉ (M ₂ L ₂ I ₃)	16.33	11.17	13.17	14.95	17.95	11.17	13.17	0.59	0.84	1.21	1.53	0.40	0.62	85	100
T ₂₀ (M ₂ L ₂ I ₄)	27.67	5.27	7.27	7.28	10.28	5.27	7.27	0.33	0.58	0.81	1.14	0.29	0.52	79.17	94.17
T ₂₁ (M ₂ L ₃ I ₁)	21	13.03	15.03	15.28	18.28	13.03	15.03	0.66	0.91	1.32	1.65	0.40	0.64	84.17	99.17
T ₂₂ (M ₂ L ₃ I ₂)	16	18.84	20.83	16.28	19.28	18.83	20.83	0.78	1.04	1.46	1.79	0.50	0.71	85	100
T ₂₃ (M ₂ L ₃ I ₃)	14	13	15	15.28	18.28	13	15	0.73	0.98	1.42	1.75	0.45	0.66	85	100
T ₂₄ (M ₂ L ₃ I ₄)	26	9.2	11.2	11.48	14.48	9.2	11.2	0.33	0.59	0.86	1.18	0.34	0.55	82.5	97.5
S _{Ed} (+)	3.83	4.04	3.91	3.3	3.15	3.76	3.56	0.06	0.06	0.07	0.07	0.04	0.04	3.54	3.54
CD (5%)	7.71	8.1	7.87	6.64	6.33	7.45	7.53	0.12	0.12	0.13	0.14	0.08	0.08	7.12	7.12

A-Days required for root formation, B-Root number, C-Length of the longest root (cm), D- Root length (cm), E-Diameter of the longest root (mm), F-Root fresh weight (g), G-Root dry weight (g) and H-Rooting percentage

DAP- Days after planting

Table 4. Interaction effect of time and size of cutting and IBA concentration on shoot growth parameters

Treatment Combination	I	J		K		L		M		N		O	
		40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP
T ₁ (M ₁ L ₁ I ₁)	41	1.2	1.56	9.17	10.5	5	6.33	48.73	51.57	6.73	8.23	0.06	0.07
T ₂ (M ₁ L ₁ I ₂)	37.67	1.67	3	10.03	11.37	5.83	7.17	58.67	61.5	7.73	9.23	0.07	0.08
T ₃ (M ₁ L ₁ I ₃)	35.67	1.47	2.8	9.63	10.97	7.27	8.6	56.13	58.97	7.4	8.9	0.08	0.09
T ₄ (M ₁ L ₁ I ₄)	44	0.87	1.2	7.2	8.53	3.87	5.2	39.87	42.7	5.53	7.03	0.06	0.07
T ₅ (M ₁ L ₂ I ₁)	34.67	2.13	3.47	10.5	11.83	7.47	8.8	61	63.83	8.33	9.83	0.08	0.09
T ₆ (M ₁ L ₂ I ₂)	29	2.8	4.13	12.83	14.17	8.23	9.57	66.67	69.5	8.8	10.3	0.07	0.08
T ₇ (M ₁ L ₂ I ₃)	31.67	2.53	3.87	12.13	13.47	8	9.33	63.73	66.57	8.47	9.97	0.08	0.09
T ₈ (M ₁ L ₂ I ₄)	42.67	1.07	1.28	7.77	9.1	4.63	5.97	42.33	45.17	6.13	7.63	0.07	0.08
T ₉ (M ₁ L ₃ I ₁)	30.67	2.4	3.73	17.1	18.43	8.97	10.3	75.6	78.43	9.2	10.7	0.07	0.08
T ₁₀ (M ₁ L ₃ I ₂)	23.67	3.07	4.4	20.03	21.37	10	11.33	82.73	85.57	10.6	12.1	0.07	0.08
T ₁₁ (M ₁ L ₃ I ₃)	26	2.6	3.93	18.37	19.7	9.73	11.07	79.87	82.7	10.13	11.63	0.07	0.08
T ₁₂ (M ₁ L ₃ I ₄)	40.67	1.17	1.31	8.73	10.07	5.37	6.7	45.87	48.7	7.47	8.97	0.06	0.07
T ₁₃ (M ₂ L ₁ I ₁)	39.67	2.53	3.87	10.5	11.83	6.33	7.67	50.07	52.9	8.07	9.57	0.07	0.08
T ₁₄ (M ₂ L ₁ I ₂)	36.33	3	4.33	11.37	12.7	7.17	8.5	60	62.83	9.07	10.57	0.08	0.09
T ₁₅ (M ₂ L ₁ I ₃)	34.33	2.8	4.13	10.97	12.3	8.6	9.93	57.47	60.3	8.73	10.23	0.08	0.09
T ₁₆ (M ₂ L ₁ I ₄)	42.67	1.2	1.53	8.53	9.87	5.2	6.53	41.2	44.03	6.87	8.37	0.07	0.08
T ₁₇ (M ₂ L ₂ I ₁)	33.33	3.47	4.8	11.83	13.17	8.8	10.13	62.33	65.17	9.67	11.17	0.07	0.08
T ₁₈ (M ₂ L ₂ I ₂)	27.67	4.13	5.47	14.17	15.5	9.57	10.9	68	70.83	10.13	11.63	0.09	0.1
T ₁₉ (M ₂ L ₂ I ₃)	30.33	3.87	5.2	13.47	14.8	9.33	10.67	65.07	67.9	9.8	11.3	0.09	0.1
T ₂₀ (M ₂ L ₂ I ₄)	41.33	1.12	1.33	9.1	10.43	5.97	7.3	43.67	46.5	7.47	8.97	0.06	0.07
T ₂₁ (M ₂ L ₃ I ₁)	29.33	3.73	5.07	18.43	19.77	10.3	11.63	76.93	79.77	10.53	12.03	0.06	0.07
T ₂₂ (M ₂ L ₃ I ₂)	22.33	4.4	5.73	21.37	22.7	11.33	12.67	84.07	86.9	11.93	13.43	0.09	0.1
T ₂₃ (M ₂ L ₃ I ₃)	24.67	3.93	5.27	19.7	21.03	11.07	12.4	81.2	84.03	11.47	12.97	0.09	0.1
T ₂₄ (M ₂ L ₃ I ₄)	39.33	1.31	1.43	10.07	11.4	6.7	8.03	47.2	50.03	8.8	10.3	0.06	0.07
SEd (+)	1.21	0.83	0.84	1.25	1.8	1.19	1.86	3.06	3.32	0.96	1.09	0.01	0.01
CD (5%)	2.45	1.66	1.68	2.5	3.6	2.16	2.69	6.22	6.64	1.92	2.18	0.02	0.02

I- Days required for shoot initiation, J- Shoot number, K- Primary shoot length (cm), L-Secondary shoot length (cm), M- Shoot fresh weight (g), N- Shoot dry weight (g) and O- Root to shoot ratio

DAP- Days after planting

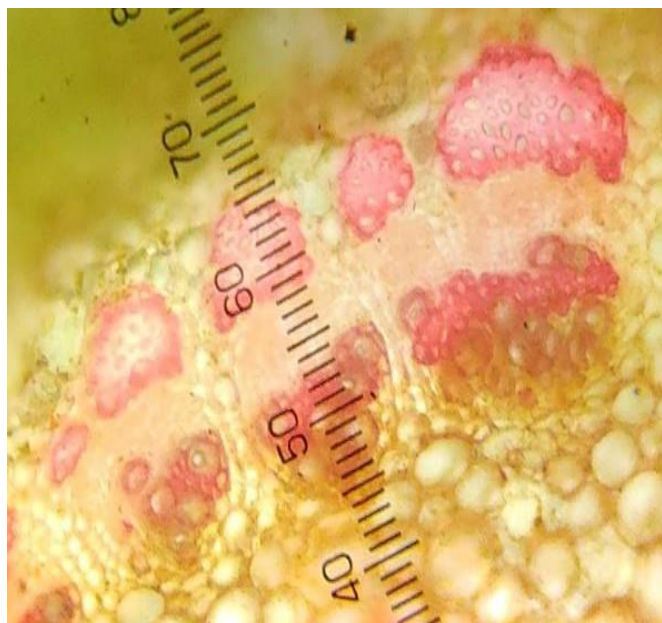
Table 5. Effect of time and size of cutting and IBA concentration on chlorophyll, nitrogen and protein content, vascular cambium thickness and survival of the cuttings

Treatment	Chlorophyll content (mg/g)		Nitrogen content (%)		Protein content (%)		Vascular cambium thickness (mm)		Survival (%)
	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	
Time of cutting									
M ₁ - January	0.34	0.38	2.46	2.47	15.15	15.28	0.04	0.05	95.01
M ₂ - April	0.38	0.44	2.5	2.53	15.6	15.84	0.07	0.08	98.13
SEd (±)	0.01	0.02	0.02	0.02	0.11	0.12	0.01	0.01	1.17
CD (5%)	0.02	0.04	0.04	0.04	0.22	0.23	0.02	0.02	2.33
Size of cutting									
L ₁ - 10 cm	0.33	0.37	2.4	2.44	14.98	15.22	0.04	0.05	95.42
L ₂ - 15 cm	0.36	0.42	2.47	2.51	15.43	15.67	0.06	0.07	98.13
L ₃ - 20 cm	0.39	0.45	2.59	2.63	16.18	16.42	0.1	0.11	99.17
SEd (±)	0.02	0.02	0.02	0.02	0.13	0.14	0.01	0.01	1.43
CD (5%)	0.03	0.04	0.04	0.05	0.26	0.28	0.03	0.02	2.88
IBA concentration									
I ₁ - 100 ppm	0.36	0.34	2.48	2.52	15.52	15.76	0.05	0.07	95.67
I ₂ - 250 ppm	0.39	0.45	2.56	2.6	15.99	16.23	0.08	0.1	99.17
I ₃ - 500 ppm	0.37	0.43	2.54	2.58	15.86	16.1	0.06	0.08	98.61
I ₄ - 0 ppm	0.32	0.33	2.36	2.4	14.73	14.97	0.04	0.06	95.03
SEd (±)	0.02	0.02	0.02	0.03	0.15	0.16	0.01	0.01	1.65
CD (5%)	0.03	0.04	0.05	0.05	0.30	0.32	0.02	0.02	3.32

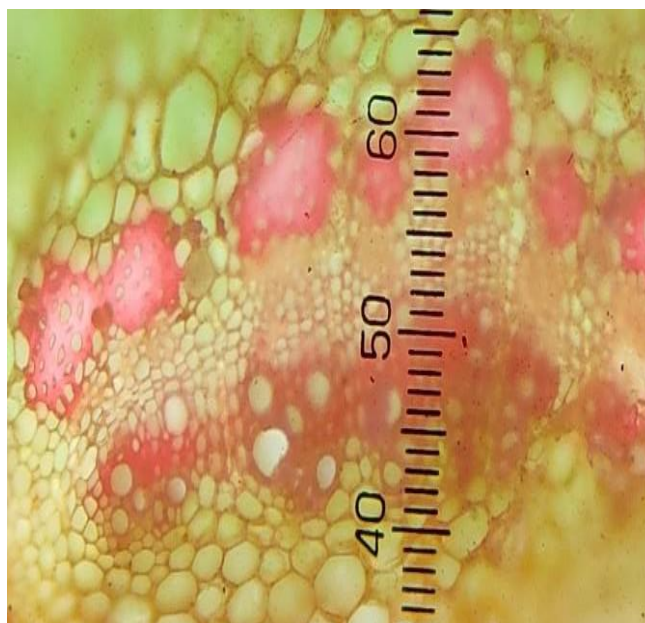
Table 6. Interaction effect of time and size of cutting and IBA concentration on chlorophyll, nitrogen and protein content, vascular cambium thickness and survival of the cuttings

Treatment	Chlorophyll content (mg/g)		Nitrogen content (%)		Protein content (%)		Vascular cambium thickness (mm)		Survival (%)
	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP	
T ₁ (M ₁ L ₁ I ₁)	0.3	0.29	2.37	2.41	14.79	15.03	0.04	0.04	90.09
T ₂ (M ₁ L ₁ I ₂)	0.32	0.38	2.42	2.46	15.15	15.39	0.04	0.05	97.5
T ₃ (M ₁ L ₁ I ₃)	0.31	0.37	2.43	2.47	15.19	15.43	0.04	0.05	95.83
T ₄ (M ₁ L ₁ I ₄)	0.27	0.28	2.32	2.36	14.5	14.74	0.03	0.04	90
T ₅ (M ₁ L ₂ I ₁)	0.33	0.39	2.47	2.51	15.42	15.66	0.05	0.06	90.09
T ₆ (M ₁ L ₂ I ₂)	0.37	0.43	2.53	2.57	15.81	16.05	0.07	0.09	98.33
T ₇ (M ₁ L ₂ I ₃)	0.35	0.41	2.49	2.53	15.56	15.8	0.06	0.08	98.33
T ₈ (M ₁ L ₂ I ₄)	0.29	0.29	2.34	2.38	14.63	14.86	0.05	0.06	90.92
T ₉ (M ₁ L ₃ I ₁)	0.38	0.44	2.58	2.62	16.15	16.39	0.09	0.1	99.17
T ₁₀ (M ₁ L ₃ I ₂)	0.4	0.46	2.69	2.73	16.79	17.03	0.11	0.13	100
T ₁₁ (M ₁ L ₃ I ₃)	0.39	0.45	2.66	2.7	16.63	16.86	0.1	0.12	100
T ₁₂ (M ₁ L ₃ I ₄)	0.31	0.37	2.38	2.42	14.85	15.09	0.07	0.09	91.17
T ₁₃ (M ₂ L ₁ I ₁)	0.35	0.41	2.39	2.43	14.94	15.18	0.03	0.05	94.17
T ₁₄ (M ₂ L ₁ I ₂)	0.37	0.43	2.45	2.49	15.29	15.53	0.04	0.06	99.17
T ₁₅ (M ₂ L ₁ I ₃)	0.36	0.42	2.45	2.49	15.33	15.57	0.04	0.06	97.5
T ₁₆ (M ₂ L ₁ I ₄)	0.33	0.39	2.34	2.38	14.65	14.89	0.03	0.05	94.17
T ₁₇ (M ₂ L ₂ I ₁)	0.38	0.44	2.49	2.53	15.56	15.8	0.05	0.07	98.33
T ₁₈ (M ₂ L ₂ I ₂)	0.41	0.47	2.55	2.59	15.96	16.2	0.08	0.09	100
T ₁₉ (M ₂ L ₂ I ₃)	0.39	0.46	2.51	2.55	15.71	15.95	0.07	0.08	100
T ₂₀ (M ₂ L ₂ I ₄)	0.34	0.4	2.36	2.4	14.77	15.01	0.05	0.07	97.5
T ₂₁ (M ₂ L ₃ I ₁)	0.43	0.49	2.61	2.65	16.29	16.53	0.09	0.11	99.17

$T_{22} (M_2L_3I_2)$	0.45	0.51	2.71	2.75	16.94	17.18	0.12	0.15	100
$T_{23} (M_2L_3I_3)$	0.44	0.5	2.68	2.72	16.77	17.01	0.1	0.12	100
$T_{24} (M_2L_3I_4)$	0.36	0.42	2.4	2.44	15	15.24	0.08	0.1	97.5
SEd (+)	0.04	0.06	0.06	0.06	0.37	0.39	0.02	0.02	4.04
CD (5%)	0.07	0.12	0.12	0.13	0.74	0.79	0.03	0.04	8.08



Vascular cambium thickness = 0.09 mm
Scale [1 ocular division = 0.015 mm] at 10x magnification



Vascular cambium thickness = 0.11 mm
Scale [1 ocular division = 0.015 mm] at 10x magnification

Figure 1. Vascular cambium thickness of $T_{22} (M_2L_3I_2)$