



Effect of Auxin and Cow Urine as Nutrient Source on Pineapple Propagation through Crown in Soilless Culture under Protected Condition

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was aimed to investigate the effect of auxin and cow urine on rooting, survival and growth of pineapple through crown propagation with soilless culture under protected condition. The research was conducted in 2020 at the High-Tech Horticultural Park, College of Horticulture, Junagadh Agricultural University, Junagadh, employing a Completely Randomized Design (CRD) as the experimental design. Eight treatments were implemented, including Control (T₁), IBA 400 ppm (T₂), IBA 600 ppm (T₃), IBA 800 ppm (T₄), NAA 600 ppm (T₅), NAA 800 ppm (T₆), NAA 1000 ppm (T₇) and Cow urine 15 % (T₈). The results of this experiment revealed that IBA 600 ppm had a notable impact on various growth parameters. It exhibited the highest percentage of rooted crowns

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(94.44 %), length of root (5.58, 8.26 and 11.08 cm) at 15, 25 and 35 days, respectively, length of leaf (13.35, 16.27 and 19.67 cm) at 15, 25 and 35 days, respectively, no. of leaves (70.58, 80.28 and 91.06) at 15, 25 and 35 days, respectively. Furthermore, IBA 600 ppm significantly influenced the fresh root weight (16.46 g), dry root weight (7.77 g), fresh shoot weight (136.33 g), and dry shoot weight (57.67 g). Additionally, IBA 600 ppm demonstrated the highest survival rate (65.91%) and the lowest mortality rate (24.09%). While T₆ gave maximum no. of roots (70.58, 80.28 and 91.06) at 15, 25 and 35 days, respectively. T₈ gave minimum days required for root initiation (2.67 days). The use of IBA at 600 ppm for a brief dipping period, along with the utilization of NAA at 800 ppm for enhancing root formation, and Cow urine at 15% for accelerated rooting, can be recommended for promoting successful pineapple crown propagation.

Keywords: *Pineapple; propagation; crown; PGR; cow urine and soilless.*

1. INTRODUCTION

Pineapple (*Ananas comosus* L. Merrill) is a significant fruit crop cultivated extensively in tropical as well as subtropical regions in worldwide. In India, pineapple is mainly produced in south Indian and east Indian states such as Assam, West Bengal, Karnataka, Meghalaya, Manipur, Arunachal Pradesh, Kerala, and Bihar. Pineapple is commonly propagated through using slips, ground suckers, side shoots and crowns. However, obtaining large quantities of uniform planting materials can be challenging due to low-quality propagules and limited conventional methods. Therefore, exploring alternative technologies, such as in vitro culture, becomes crucial for producing uniform planting materials.

Commercial pineapple cultivation predominantly utilizes vegetative propagation as the primary method for establishment [1]. In traditional practice, pineapple plantations commonly utilize seedlings derived from ground sucker, aerial sucker, and crown types, all of which emerge from the parent plant. As a result, commercial plantations typically rely on obtaining propagation material from external sources.

Achieving large-scale commercial production of pineapples and ensuring consistent availability of high-quality planting materials is challenging due to the limited availability of uniform propagules and the lack of conventional methods. Consequently, exploring alternative technologies becomes imperative for producing uniform planting materials. Fruit crowns present a viable solution as they can be harvested and employed as cost-effective planting materials. Abundant crown resources can be sourced from fruit processing fields, facilitating their utilization in pineapple cultivation. Furthermore, the transportation and sale of crowns as propagation

material mitigate the costs associated with propagation, thereby reducing pineapple crown wastage in the market and industry. This study addresses the crucial need for sustainable approaches in obtaining uniform pineapple planting materials, optimizing resource allocation and enhancing overall production efficiency.

Plants in soilless culture receive water and nutrients through the dissolution of inorganic chemicals, ensuring their nutritional requirements are met. A fundamental and time-tested approach to soilless culture involves employing a vessel filled with water and inorganic chemicals. By adopting soil-less systems, farmers can effectively minimize resource usage. Notably, the hydroponic system enables plants to achieve 20-25% higher yields compared to soil-based systems, exhibiting 2-5 times greater productivity [2]. This scientific insight underscores the benefits of soil-less culture methods, showcasing their potential for enhancing agricultural efficiency and sustainability.

According to ancient Ayurveda, cow urine holds significant pharmacological importance. It primarily comprises 95% water, 2.5% urea and the remaining 2.5% is a composition of hormones, enzymes and minerals. Cow urine is rich in nutrients such as N at 1%, K₂O at 1.9% and traces of P₂O₅ [3]. Additionally, cow urine contains various elements and compounds, including N, P, K, Na, S, Ca, Mg, Cu, I, NH₃, silver, urea, uric and oxalic acid, lead, hippuric acid, creatinine, eltonin, enzymes, steroids phosphates, lead, propylene oxide, ethylene oxide, glycosides, glucose, citric acid, alkalide, acetate, endosonine, carbolic acid and growth substances [4].

The macro propagation technique offers significant advantages due to its non-technical nature and lack of specialized skill requirements.

Moreover, it proves to be highly cost-effective, enabling the production of uniform planting materials on a large scale within a relatively short timeframe [5]. Consequently, this research study was undertaken to investigate the effects of plant growth regulators (PGR) and cow urine on the growth and survival of pineapple crowns. By examining these factors, valuable insights can be gained to enhance the cultivation practices and overall productivity of pineapple crops.

2. MATERIALS AND METHODS

The research study was conducted in 2020 at the High-Tech Horticultural Park, College of Horticulture, Junagadh Agricultural University, Junagadh. The experimental design employed was a Completely Randomized Design (CRD) with three replications and a total of eight treatments implemented within a polyhouse environment. The primary objective of the study was to evaluate the specific influence of auxin and cow urine on the successful propagation of pineapple through crown in a soilless culture system. By analysing the results obtained from this comprehensive investigation, valuable insights were obtained regarding the growth, survival and overall performance of pineapple crown under the various treatment conditions.

Planting material collection and preparation:

In July, pineapple crowns were obtained from a local fruit store during a period of abundant pineapple availability. The crowns were selected based on their healthy condition, absence of diseases-pests and uniform size. These crowns were sourced from a local pineapple vendor who

would otherwise discard them. To prepare the crowns for the experiment, they were carefully cut at the attachment point of the fruit and had their lower leaves removed to expose the stem. Notably, the presence of brown dots on the base of the crown indicated the specific region from which root formation would initiate. Subsequently, the crowns were sun-dried for a period of 24 hours to eliminate moisture and dry any remaining fruit pulp (Fig. 1).

Application of treatments: Treatments used in this experiment were IBA (400 ppm, 600 ppm and 800 ppm), NAA (600 ppm, 800 ppm and 1000 ppm), cow urine (15%) and control. Following the sun drying process, the pineapple crowns were subjected to a treatment involving the application of various concentrations of auxin solution for a duration of 5 seconds. Additionally, cow urine was applied to the crowns for a duration of 4 minutes. Subsequently, the treated crowns were carefully positioned in jars filled with water, ensuring that only the basal part of the crown was submerged in the water. This method aimed to facilitate the initiation of root development and subsequent growth in the pineapple crowns.

Various parameters were assessed in this experiment, including number of days required for rooting, percentage of rooted crown, length of root, number of roots, fresh and dry weight of root, number of leaves, length of leaves, fresh and dry weight of shoot. All the data collected on these growth parameters were subjected to statistical analysis using the methods recommended by Panse and Sukhatme [6].



Fig. 1. A. Collection of crowns, B. Removing leaves & excess parts, C. Sun Drying of pineapple crown for 24 hrs. D. General layout of experiment

3. RESULTS AND DISCUSSION

Number of days required for root initiation:

The time required for root initiation varied across the different treatments. Crowns treated with T₈ (cow urine 15%) exhibited early rooting within 2.67 days, while crowns treated with T₃ (IBA 600 ppm) showed root initiation after 3 days (Table 1). This observation can be attributed to the presence of auxins in cow urine, as auxins play a crucial role in iron chelation, promoting overall plant growth, health and nutrient absorption, particularly in root system development [7]. Similar findings were reported by Pawar et al. [8] in their study on bush pepper cuttings treated with cow urine 15%. These results emphasize the potential benefits of cow urine as a rooting agent in plant propagation.

Percentage of rooted crown (%): The highest percentage of rooted crown (94.44%) was observed in T₃ (IBA 600 ppm), which was at par with T₄ (IBA 800 ppm), T₅ (NAA 600 ppm), T₆ (NAA 800 ppm), T₂ (IBA 400 ppm) and T₈ (Cow urine 15%) (Fig. 2a). Conversely, the lowest percentage of rooted crown was recorded in T₁ (Control) and T₇ (NAA 1000 ppm) (72.22% and 72.22% respectively). This could be attributed to the application of IBA, as auxins stimulate root regeneration by facilitating hydrolysis, utilization and mobilization of nutrient reserves in the root formation region [9]. On the other hand, the lower number of rooted crowns with NAA 1000 ppm suggests that a higher concentration of auxin inhibits the root initiation process at the base of the crown. Similar results were reported by Darwati et al. [10] in Cashew nut grafting and Ali and Saif (2013) in grape cuttings treated with IBA 600 ppm.

Length of root (cm): The highest length of root (5.58, 8.26 and 11.08 cm) was observed in T₃ (IBA 600 ppm) at 15, 25 and 35 days, respectively (Fig. 2b) and it was at par with T₆ (NAA 800 ppm), T₄ IBA 800 ppm and T₂ (IBA 400 ppm). The minimum length of root (3.7 cm, 6.33 cm and 8.22 cm) was recorded in T₁ (Control) at 15, 25 and 35 days, respectively (Table 1). This phenomenon can be attributed to the auxin's activity, which induces hydrolysis and translocation of carbohydrates and nitrogenous substances at the cellular level, resulting in accelerated cell elongation and division under favorable environmental conditions [11]. Similar

findings were reported by Satpal et al. [12] in cuttings of lemon (*Citrus limon* burm.) cv. Pant lemon-1 cuttings and by Ali and Saif (2013) in grape cuttings treated with IBA 600 ppm and by Tien et al. [13] in *Solanum procumbens* stem cuttings with IBA 500 ppm.

No. of roots: The highest number of roots (31.10, 36.61 and 43.56) were recorded in T₆ (NAA 800 ppm) at 15, 25 and 35 days, respectively (Table 1) and it was at par with T₃ (IBA 600 ppm) and T₄ (IBA 800 ppm). The lowest number of roots (24.83, 29.56 and 34.25) were recorded in T₈ (Cow urine 15%) 15, 25 and 35 days, respectively (Table 1). The observed phenomenon of accelerated rooting with increased IBA concentration can be attributed to the effect of IBA on cell wall elasticity, which promotes cell division and subsequently leads to an increased number of roots up to a certain threshold [14]. His finding aligns with the results reported by Kumar et al. [15] in stem cuttings of Lemon (*Citrus limon* Burm) cv. Pant Lemon-1. The increased cell wall elasticity induced by IBA likely enhances the overall root development process by facilitating cell division and elongation, ultimately resulting in a higher number of roots. Similar results were found by Lancita et al. (2023) in magic fruit (*Synsepalum Dulcificum*) with rooting response of softwood stem cuttings.

Length of leaf (cm): The maximum length of leaves (13.35 cm, 16.27 cm and 19.67 cm) was recorded in treatment T₃ (IBA 600 ppm) (Fig. 2c) and it was at par with T₃ (IBA 600 ppm) and T₄ IBA 800 ppm at 15, 25 and 35 days, respectively. The minimum length of leaf (9.42 cm, 11.57 cm and 14.40 cm) was recorded in T₁ (Control) (Table 2). In the polyhouse environment, characterized by high relative humidity and optimal light intensity, both of which are critical factors for leaf development, the presence of auxin content triggered the synthesis of increased amounts of carbohydrates within the leaves. This likely facilitated leaf elongation through a combination of cell division and cell elongation processes, resulting in greater leaf length compared to the control treatment (Ghosh et al., 2017). These findings suggest that the interplay between environmental conditions and auxin plays a significant role in regulating leaf growth and development, ultimately leading to observable differences in leaf morphology.

Table 1. Effect of different concentration of PGR and cow urine on number of days required for root initiation, rooted crown (%), length of root, no. of roots, fresh root weight and dry root weight in pineapple crown

Treatments	Days required for rooting	Rooted crown (%)	Length of root			No. of roots			Fresh root weight (g)	Dry root weight (g)
			15 th Day	25 th Day	35 th Day	15 th Day	25 th Day	35 th Day		
T ₁ - Control	7.67	72.22	3.70	6.33	8.22	25.27	29.60	34.25	12.08	4.16
T ₂ - IBA 400 ppm	4.00	83.33	4.82	7.18	10.13	28.33	33.72	40.89	13.84	5.31
T ₃ - IBA 600 ppm	3.00	94.44	5.58	8.26	11.08	30.83	35.11	42.11	16.46	7.77
T ₄ - IBA 800 ppm	4.67	88.89	5.10	7.58	10.48	30.29	34.87	41.78	14.17	5.77
T ₅ - NAA 600 ppm	4.00	88.89	4.50	6.84	9.90	28.52	32.41	36.00	15.34	7.02
T ₆ - NAA 800 ppm	4.00	88.89	5.34	7.88	10.75	31.10	36.61	43.56	15.42	6.99
T ₇ - NAA 1000 ppm	5.00	72.22	4.14	6.81	9.08	24.92	30.00	34.83	13.44	5.49
T ₈ - Cow urine 15%	2.67	83.33	3.90	6.79	8.97	24.83	29.56	34.00	11.23	3.95
S.Em. ±	0.167	4.811	0.259	0.342	0.401	0.430	0.632	0.612	0.408	0.334
CD at 5 %	0.50	14.42	0.78	1.03	1.20	1.29	1.89	1.84	1.22	1.00

No. of leaves: The maximum number of leaves (70.58, 80.28 and 91.06) was recorded in T₃ (IBA 600 ppm) it was at par with T₆ (NAA 800 ppm) at 15, 25 and 35 days after crown planting respectively (Table 2). Auxins, such as IBA, play a significant role in promoting the development of a greater number of leaves. IBA not only mitigates stress levels but also enhances the concentration of photosynthetic pigments in vegetative parts, particularly in leaves [16]. Additionally, favorable climatic conditions can contribute to the number of leaves per crown. Similar findings were reported by Satpal et al. [12] in cuttings of lemon (*Citrus limon* burm.) cv. Pant lemon-1, Darwati et al. [10] in Cashew nut grafting and Bahadar et al. [17] in hydrangea and fuchsia cuttings. Furthermore, Rout et al. [18] observed similar results in Ashoka with IBA 500 ppm and Carvalho Pires et al. [19] found similar results in Passion fruit with treatment of IBA 500 ppm. Similar results were found by Tien et al. [13] in *Solanum procumbens* stem cuttings with IBA 500 ppm and by Lancita et al. (2023) in magic fruit (*Synsepalum dulcificum*) with rooting response of softwood stem cuttings with IBA 600 ppm. These studies support the notion that IBA and favourable environmental conditions positively influence leaf production in various plant species.

Fresh root weight (g): The maximum fresh root weight (16.46 g) was observed in T₃ (IBA 600 ppm) and it was at par with T₆ (NAA 800 ppm). The minimum fresh root weight (11.23 g) was recorded in T₈ (Cow urine 15%) (Table 1). Exogenous application of both natural and synthetic auxins has been observed to enhance the development of pre-existing root primordia. This increase in the number, length, and diameter of roots likely contributes to the overall fresh weight of the roots [20]. The application of auxins promotes root growth by stimulating the initiation and elongation of root structures. The mechanism behind this effect involves the activation of cell division and elongation processes in the root tissues. Consequently, the increased root development results in higher fresh weight measurements. The findings of Haissing [20] support the understanding that the application of natural and synthetic auxins influences root characteristics and can contribute to the overall growth and vigour of plants. And also supported by Kaur et al. [21] in grapevine, Murthy et al. [22] in vanilla cuttings, and Bhagya and Sreeramu [23] in *Vitex nigundo* are in agreement.

Dry root weight (g): The maximum dry root weight (7.77 g) was observed in T₃ (IBA 600 ppm) and it was at par with T₅ (NAA 600 ppm) and T₆ (NAA 800 ppm). The minimum dry root weight (3.95 g) was recorded in T₈ (Cow urine 15%) (Table 1). The observed increase in dry weight of the roots may be attributed to the effect of IBA, which promoted greater root length and a higher number of roots. The elongation of roots and their increased abundance likely resulted in a higher accumulation of stored carbohydrates, leading to an overall increase in dry weight. The greater number of roots also contributed to an increased volume per crown in pineapple plants. This suggests that the application of IBA influenced root development, ultimately leading to enhanced carbohydrate storage and greater root biomass. Results are supported by Kaur et al. [21] in grapevine, Murthy et al. [22] in vanilla cuttings, and Bhagya and Sreeramu [23] in *Vitex nigundo* are in agreement.

Fresh shoot weight (g): The maximum fresh shoot weight (136.33 g) was observed in T₃ (IBA 600 ppm) and it was at par with T₆ (NAA 800 ppm) (135.33 g). The minimum fresh shoot weight (101.33 g) was recorded in T₁ (Control) (Table 2). The observed increase in root and shoot length, as well as the number of leaves, likely facilitated the assimilation and redistribution of photosynthates within the crown, leading to higher fresh and dry weights of the shoot. This increase in dry matter assimilation can be attributed to the enhanced growth and development of the plant [24]. Similar findings were reported by Satpal et al. [12] in cuttings of lemon (*Citrus limon* burm.) cv. Pant Lemon-1, by Kaur et al. [21] in grapevine and by Murthy et al. [22] in vanilla cuttings.

Dry shoot weight (g): The maximum dry shoot weight (57.67 g) was observed in T₃ (IBA 600 ppm) and it was at par with T₅ (NAA 600 ppm) and T₆ (NAA 800 ppm). The minimum dry shoot weight (28 g) was recorded in T₁ (Control) (Table 2). The dry shoot weight serves as a comprehensive measure of leaf photosynthesis and nutrient uptake by the roots. The application of IBA resulted in a significant increase in the number of leaves, leaf length and chlorophyll content within the crown. This physiological enhancement led to heightened photosynthetic activity, ultimately translating into a higher dry shoot weight. Similar results were reported by Satpal et al. [12] in lemon (*Citrus limon* burm.) cv. Pant lemon-1 cuttings, by Kaur et al. [21] in

Table 2. Effect of different concentration of PGR and cow urine on length of leaf, no. of leaves, fresh shoot weight, dry shoot weight, mortality and survival rate in pineapple crown

Treatments	Length of leaf			No. of leaves			Fresh shoot weight (g)	Dry shoot weight (g)	Mortality rate (%)	Survival rate (%)
	15 th Day	25 th Day	35 th Day	15 th Day	25 th Day	35 th Day				
T₁ - Control	9.42	11.05	14.40	53.48	58.67	65.57	101.33	28.00	33.33 (35.26)	66.67 (54.74)
T₂ - IBA 400 ppm	10.41	13.92	16.72	59.42	66.33	74.42	108.00	29.67	33.33 (35.26)	72.22 (58.86)
T₃ - IBA 600 ppm	13.35	16.27	19.67	70.58	80.28	91.06	136.33	57.67	16.67 (24.09)	83.33 (65.91)
T₄ - IBA 800 ppm	12.34	15.67	18.24	66.33	76.50	87.67	126.00	42.33	33.33 (35.26)	66.67 (54.74)
T₅ - NAA 600 ppm	12.03	14.52	17.21	66.22	76.29	86.67	128.00	56.33	33.33 (35.26)	66.67 (54.74)
T₆ - NAA 800 ppm	12.82	15.71	18.40	68.58	79.33	90.67	136.33	54.33	27.48 (31.54)	72.22 (58.46)
T₇ - NAA 1000 ppm	10.61	14.01	17.17	60.25	66.75	74.50	127.67	44.67	44.44 (41.75)	55.56 (48.25)
T₈ - Cow urine 15%	9.69	11.57	14.84	58.69	64.72	66.00	103.67	34.00	33.33 (35.26)	66.67 (54.74)
S.Em. ±	0.471	0.520	0.645	3.398	1.431	1.165	2.530	1.307	1.746	1.746
CD at 5 %	1.41	1.56	1.94	10.19	4.29	3.49	7.59	3.92	5.23	5.23

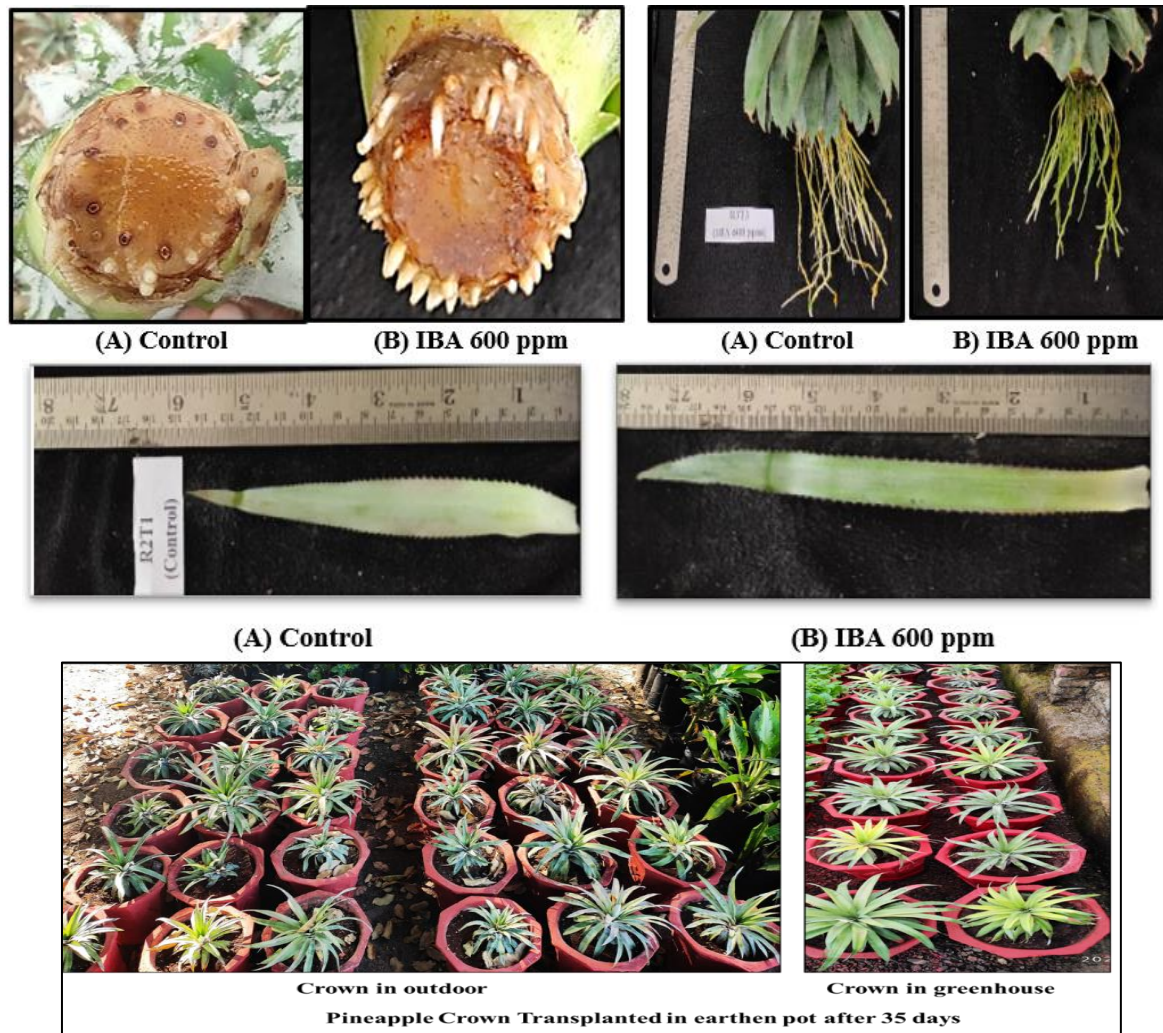


Fig. 2. Effect of IBA 600 ppm on crown growth parameters

A. Percentage of rooted crown (%), **B.** Length of root (cm), **C.** Length of leaf (cm), **D.** Survived crown transplanted in greenhouse and outdoor

grapevine and by Murthy et al. [22] in vanilla cuttings.

Mortality rate (%): Minimum mortality (24.09%) was observed in treatment T₃ (IBA 600 ppm) The maximum mortality (41.75%) was observed in and T₇ (NAA 1000 ppm) (Table 2).

Auxin is a naturally occurring plant hormone found in plants. However, the application of synthetic auxins such as IBA and NAA stimulated and enhanced the growth of pineapple crowns. In the control treatment (T₁), where no auxin was applied, there was no artificial auxin or other hormone source for crown growth, resulting in the highest mortality rate. Furthermore, a higher concentration of IBA hindered crown growth, suggesting an inhibitory effect at higher

concentrations. These findings highlight the importance of auxin supplementation for promoting healthy crown growth in pineapple plants.

Survival rate (%): The maximum survival (65.91%) was observed in treatment T₃ (IBA 600 ppm) (Fig. 2d) The minimum survival (48.25%) was observed in T₇ (NAA 1000 ppm) (Table 2). The higher survival rate observed in treatment T₃ (IBA 600 ppm) (Fig. 2d) compared to other treatments can be attributed to several factors. Firstly, the increased root length, higher number of roots, and early root initiation in T₃ resulted in the development of thicker and stronger roots. These robust root systems likely contributed to enhanced nutrient uptake and overall plant vigour, leading to improved survival rates.

Furthermore, the higher number of leaves and longer leaf length in T₃ played a crucial role in reducing transpiration loss through decreased surface area and increased photosynthetic activity. Reduced transpiration minimizes water stress and helps the plant allocate resources more efficiently, while increased photosynthesis supports the production of essential carbohydrates for growth and maintenance. These findings are consistent with the results reported by Satpal et al. [12] in cuttings of lemon (*Citrus limon* burm.) cv. Pant lemon-1 and by Bahadar et al. [17] in hydrangea and fuchsia cuttings. The similar outcomes highlight the positive influence of these factors on plant survival and reinforce the importance of promoting root development and optimizing leaf characteristics during propagation [25-27].

4. CONCLUSION

The results indicate that a quick dipping of pineapple crown in IBA solution at a concentration of 600 ppm for 5 seconds yielded several positive outcomes. This treatment led to an increase in the percentage of rooted crowns, root length, fresh weight of roots, dry weight of roots, number of leaves, length of leaves, fresh weight of shoots and dry weight of shoots. Furthermore, the application of IBA at 600 ppm resulted in the lowest mortality rate and the highest survival rate among the treatments. Interestingly, the crown treated with NAA at 800 ppm exhibited the maximum number of roots, indicating the effectiveness of this particular hormone in root development. Additionally, the application of Cow urine at a concentration of 15% reduced the time required for rooting on the crowns. In conclusion, the use of IBA at 600 ppm for a brief dipping period, along with the utilization of NAA at 800 ppm for enhancing root formation, and Cow urine at 15% for accelerated rooting, can be recommended for promoting successful pineapple crown propagation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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