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SUCROSE, SALICYLIC ACID, AND PEG IMPACT ON SHOOT MULTIPLICATION OF GRAND NAIN BANANAS AND HPLC DETECTION OF MELATONIN

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SUMMARY

This study happened in 2021–2023 at the Plant Tissue Culture Laboratory, College of Agriculture, University of Basrah, Basrah, Iraq. The presented study determined the effect of adding sucrose, salicylic acid, and polyethylene glycol (PEG) to shoot multiplication of the Grand Nain banana plant. The results revealed the concentration of sucrose (90 g L^{-1}) was significantly superior by recording the highest number of shoots, plant height, the number of leaves, and the leaf width with values of 18.33 shoots, 7.10 cm, 6.33 leaves, and 3.83 cm, respectively. In contrast, the utmost concentration of melatonin emerged with the sucrose treatment 120 g. L^{-1}. The treatment of salicylic acid (2.0 mg L^{-1}) provided the best results for studied qualities. The salicylic acid concentration of 3.0 mg L^{-1} resulted in the maximum concentration of melatonin (41.56 mg g^{-1} dry weight). The PEG concentration of 1.0 g L^{-1} was significantly superior by recording the highest values, while the PEG concentration at 3.0 g L^{-1} gave the topmost value of melatonin (43.32 mg g^{-1} dry weight).

Keywords: Maize (*Zea mays* L.), water stress, silicon, yield, carbohydrate, protein, oil, stress-tolerance index

Key findings: Banana Grand Nain (*Musa acuminata* L.), isolation melatonin, micropropagation

INTRODUCTION

The banana plant *Musa acuminata* L. is a hybrid Grand Nain cultivar of the commercial banana cultivar currently distinguished by the Chiquita, a widely cultivated banana in Central America and India. Its length ranges between 6–8 meters and produces many clusters of fruit. The weight of the bunch may reach 2368 kg in the United States. The Grand Nain hybrid banana plant is considerably one of the attractive plants in the US due to its possible

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landscaping use and good resistance to winds. It is one of the primary fruit crops for consumers in rural areas in tropical and subtropical countries and an important source of income (Justine *et al*., 2022).

Appraised as a plant, bananas are giant monocotyledonous herbaceous plants bearing flowers on the panicles as clusters arranged in a spiral. Each cluster of flowers appears in two rows shaping like a palm, covered with red scaly leaves. Bananas have vital roles in the economy of many countries. In addition, banana fruits have high nutritional value and are popular among many consumers. It is also available in markets year round, easily storing and transporting its fruits (Liao and Hung, 2015). Plant tissue culture has great dimensions as it has been functional in vital fields. This includes the production of secondary compounds because of the inability of traditional agriculture to meet the increasing demand for these compounds in some plants and the difficult process of inducing many natural compounds when using traditional methods (Mahood, 2021).

The technique of shoot multiplication is usable through tissue culture and using some laboratory stimulants, which are highly effective in stimulating the production of secondary compounds aside from being safe (Al-Qatrani *et al*., 2021). Melatonin is a considered antioxidant found in many plants and foods. It is beneficial for the health. The melatonin content in the blood increases significantly when eating foods rich with it. Its symbol is N-acetyl-5-methoxytryptamine. It is a hormone produced in the pineal gland and is the primary initiator in forming the amino acid tryptophan (Boden *et al*., 2013). Considered a substance, it facilitates the growth process in humans, with its secretion and production in the dark; hence, called the hormone of darkness (Keijzer *et al*., 2014). In the mid-nineties, melatonin appeared in pharmacies and markets as a nutritional supplement. A great interest in its activities arose as a good medicine for treating cancer and preventing aging (Tan *et al*., 2012).

The possibility of stimulating cultivated explants to increase the induction of secondary compounds transpires with their exposure to biotic and abiotic stress (Al-Ameri and Nasser, 2021). This study aimed to investigate the role of water stress compounds in the shoot multiplication of banana plant tissues through the plant tissue culture technology and the induction of effective compounds represented by melatonin.

MATERIALS AND METHODS

The study commenced in 2021 until 2023 at the Plant Tissue Culture Laboratory, College of Agriculture, University of Basrah, Basrah, Iraq. The apical buds of the banana plant Grand-Nain hybrid cultivar (Figure 1) were the specimens used in the study. The experiment

Figure 1. Plant parts of the banana plant, the Grand-Nain hybrid cultivar.

grew explants on the 4.33 g L^{-1} MS medium (Murashige and Skoog, 1962) supplemented with 500 mg L^{-1} polyvinylpyrrolidone (PVP), 1 mg L^{-1} each of vitamin glycine, 0.5 mg L^{-1} proline, the cytokinin growth regulator TDZ, and 0.2 mg L^{-1} auxin NAA. The pH of the MS medium had the range of 5.7–5.8. Then, adding the agar to the MS medium had a 5 g L^{-1} concentration.

Studying the effect of sucrose on the shoot multiplication of banana plants proceeded with the sucrose addition at concentrations of 30, 60, 90, and 120 g L^{-1}. Ten replications comprised each treatment, and after eight weeks, the data recording ensued on the number of shoots, shoot length (cm), number of leaves, and leaf width (cm).

The assessment of salicylic acid (SA) effects on the multiplication of banana plants included adding it at concentrations of 0.0, 1.0, 2.0, and 3.0 mg L^{-1}. Mixed with MS salts, 10 replications of consisted each treatment, with the same measurements recorded as stated previously after eight weeks.

The effect of PEG8000 on the multiplication of stunted banana plants sustained evaluation when added at concentrations of 0.0, 1.0, 2.0, and 3.0 g L^{-1}, having 10 replications for each treatment. After eight weeks, recording the same measurements followed, as with the sucrose and SA treatments.

Diagnosis of the melatonin compound

The mobile phase consisting of Acetonitrile and Water HPLC continued into the injection site in an amount of 30 µl to ensure of non-contamination in the injected solution (Figure 2). Later, withdrawing 10 µl from the amount of 30 µl of the melatonin standard solution proceeded injecting into the liquid chromatography device. Using the high-performance HPLC type 100 LC-UV 100 Plus by the Angstrom Company helped determine the retention time and the height of the sample band area for the standard solution. The detention time for the melatonin compound was 3.20 min to pass through the separation column. Meanwhile, the peak distance for the compound was above the 1985.00 for a volume of 10 microliters at the injection site located in the detection unit of the device. The concentrations of nanocomposites determined quantitatively used a comparison between the standard compound and the model under the same laboratory conditions employing the following equation (Albu *et al.*, 2020).

Model space Sample concentration (mg g^{-1}) =

$$\frac{Model\ area}{Area\ of\ standard\ solution} \times standard\ solution\ concentration \times number\ of\ dilutions$$

Statistical analysis

The study experiments had a completely randomized design (CRD), with the results of the study analyzed using analysis of variance. Comparing the averages of the coefficients was according to the Revised Least Significant Difference test (RLSD), with a probability level of 0.01%. The ready-made statistical analysis program Genstat V.12 served to analyze the results.

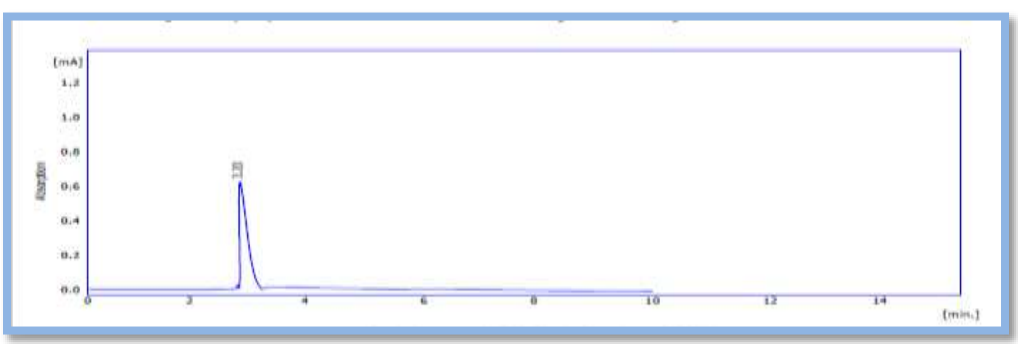

Figure 2. The standard solution of melatonin.

Table 1. Effect of different concentrations of sucrose with the cytokinin TDZ at a concentration of 0.5 mg L^{-1} on shoots multiplication of the banana plant cultivar Grand – Nain.

Sucrose treatment gm l^{-1}	Number of shoots	shoots length (cm)	Number of leaves	Leaf width (cm)
30.0	c8.00	b6.00	b2.33	b2.50
60.0	b12.67	b6.16	b3.33	b2.67
90.0	a18.33	a7.10	a6.33	a3.83
120.0	bc11.00	ab6.66	b3.67	ab3.00
R-LSD P ≥ 0.01	3.11	0.91	2.85	1.11

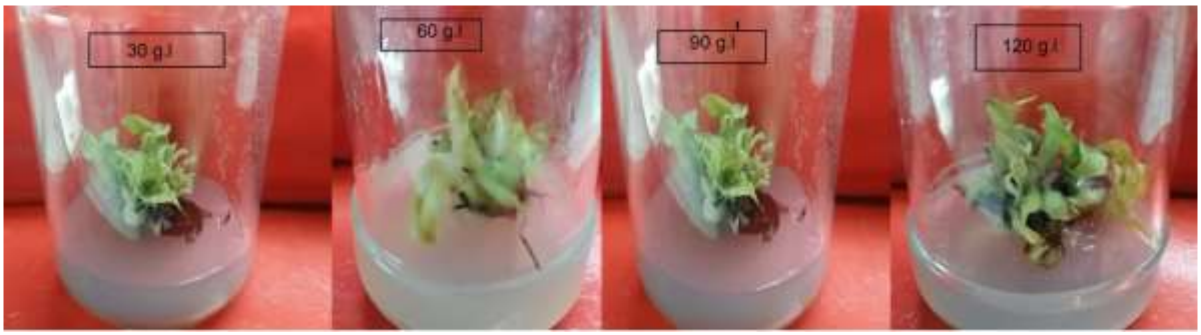

Figure 3. Effect of different concentrations of sucrose with cytokinin TDZ at a concentration of 0.5 mg L^{-1} on the vegetative multiplication of banana plants of the Grand-Nain cultivar.

RESULTS AND DISCUSSION

Sucrose effect on vegetative multiplication

The results showed the effect of different concentrations of sucrose on the vegetative shoot multiplication of banana plants' apical buds grown under 16-h lighting for eight weeks (Table 1, Figure 3). The results exhibited when increasing sucrose concentration in the MS nutrient medium, an increase in the number of vegetative shoots appear. The 90.0 g L^{-1} sucrose treatment excelled on all treatments and produced 18.33 shoots. The findings in the same table also indicate the highest rate of shoot length, number of leaves, and leaf width occurred at the concentration of 90.0 g L^{-1} sucrose compared with the control treatments, reaching 7.10 cm. 6.33 leaves, and 3.83 cm, respectively. It was also evident a decrease in the average number of vegetative shoots happens with a decrease in sucrose concentration in the MS nutrient medium, with a concentration of 30.0 g L^{-1} reaching 8.00 shoots.

Sucrose is one of the factors creating a negative osmotic potential in the nutrient medium at high concentrations. The accumulation of these substances stimulates tissues to amass organic solutions and sucrose to regulate osmosis, contributing to cell specialization, and thus, increasing the number of vegetative shoots (Gago *et al*., 2021). Adding sucrose to the nutrient medium activates the process of unfolding and obtaining vegetative shoots from the cultivated plant part. It also affects the process of nutritional metabolism in the cells. Therefore, the increase in sugars leads to an upsurge in the reserve supporting plant growth (Al-Drisi *et al*., 2022). These results agreed with the findings of Mujib *et al*. (2009) on the digitalis plant, Rosc confirming when the concentration of sucrose in the food medium increases, it also increases the average number and length of vegetative shoots.

Table 2. Effect of different concentrations of salicylic acid on the vegetative multiplication of banana plants of the Grand-Nain cultivar after eight weeks of culture in the light.

Treatments (mg L-¹)	Number of shoots	Plantlets' length	Number of leaves	Width leaf
0.0	c3.67	c3.27	b2.67	2.43
1.0	bc4.33	b5.27	b2.67	2.67
2.0	a13.67	a7.00	a5.00	3.50
3.0	b6.67	ab5.67	b3.33	2.67
R-LSD P ≥ 0.01	2.85	1.82	1.18	N.S

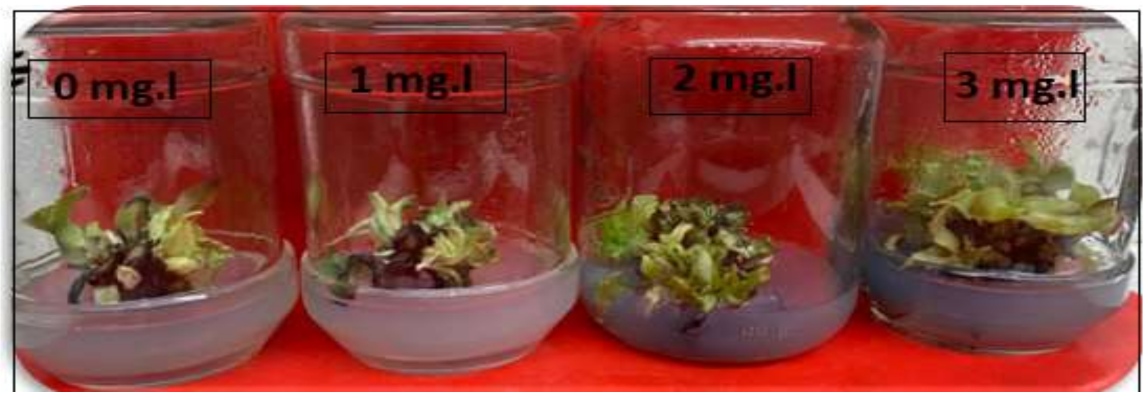

Figure 4. Effect of different concentrations of salicylic acid with cytokinin TDZ 0.5 mg Lµ¹ at a fixed concentration and auxin NAA 0.2 mg Lµ¹ on the reproduction of the Grand-Nain banana plant.

Salicylic acid effect on vegetative multiplication

The results revealed the effect of different concentrations of salicylic on the multiplication of the banana plant after eight weeks of incubation in the light (Figure 4, Table 2). The outcomes also showed the 2.0 mg L -¹ treatment excelled on all treatments, with the average number of vegetative shoots reaching 13.67 shoots plant-¹. It differed significantly from the rest of the treatments. Meanwhile, the MS nutrient medium without salicylic acid recorded the lowest average number of shoots, reaching 3.67 shoots (Table 2). The highest average of shoot length emerged at the concentration of 2.0 mg L. - ¹ of salicylic acid compared with the rest of the concentrations, as it reached 7.00 cm.

The findings also showed significant differences between the treatments in the average leaf width. This is due to the role of salicylic acid in encouraging the process of photosynthesis and its contribution to regulating the activities of enzymes, such as, DNA replication, cell division, and membrane stability. Cellular cells have stimulating functions related to plant growth and development (Hayat *et al.*, 2007). The results are consistent with the findings of Suhim *et al*. (2023) on date palms. They stated adding salicylic acid to the MS nutrient media led to an increase in the average number of shoots and leaves and increased the length of vegetative shoots.

PEG effect on vegetative shoots

The results enunciated the effect of different levels of the compound - PEG8000 with the presence of both the growth regulator cytokinin at a concentration of 0.5 mg L^{-1}-TDZ and auxin at a concentration of 0.2 mg L^{-1}-NAA (Table 3). It was apparent a negative effect resulted on the average number of multiplied vegetative shoots and the average length. The shoot with moderate concentrations, particularly 1 g L^{-1} excelled and recorded the highest number of shoots, which reached 3.67 shoots. However, it had a nonsignificant

Table 3. Effect of different concentrations of PEG on the vegetative multiplication of banana plants of the Grand-Nain cultivar after eight weeks of culture in the light.

PEG_{8000} treatment (g L^{-1})	Number of shoots	Plantlets' length (cm)	Number of leaves	Width leaf
0.0	a3.00	a4.67	2.00	1.13
1.0	a3.67	a4.50	2.67	1.17
2.0	b2.33	b3.20	2.00	1.93
3.0	c1.33	b2.83	2.67	1.50
RLSD P ≥ 0.01	0.93	1.26	N.S	N.S

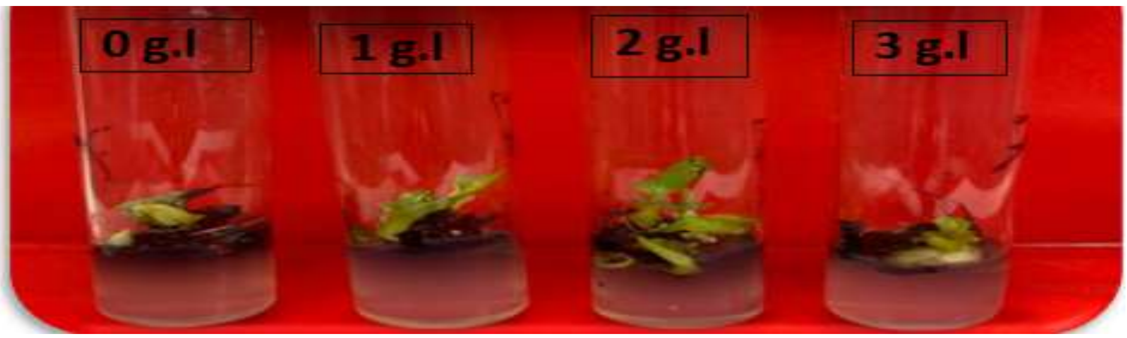

Figure 5. Effect of different levels of PEG on the multiplication of vegetative branches of the banana plant variety Grand - Nain eight weeks after the beginning of cultivation in the light.

difference from the control treatment, which amounted to 3 shoots. The lowest rate reached came from the concentration of 3 g L^{-1}, recording 1.33 shoots (Figure 5). The comparison treatment also recorded the topmost rate of shoot length, reaching 4.67 cm, with a nonsignificant difference from the concentration of 1 g. The accumulation of many compounds inside the plant when exposed to stress treatments leads to high stress on cells, causing difficulty in absorbing the nutrients necessary for the photosynthesis process, which leads to plant weakness or even death.

Among these compounds are free radicals (ROS), which are unstable. It can interact with cell components, such as proteins and DNA. In removing the negative effect of these compounds, the plant resorts to many methods, including producing and assembling antioxidant enzymes and some chemical that can interact with free radicals to remove them (Rashid, 2023). In addition, the cells lose their water content, which affects their growth. The importance of water lies in cell division and elongation, and when the water content decreases, the cell's vital processes stop, leading to a decrease in its growth (Hussain *et al*., 2019). Researchers Tian and Lei (2006) also confirmed these results. When adding PEG at a concentration of 15% to wheat seedlings, it negatively influenced the antioxidant system, while accumulating large amounts of organic solutions and mineral elements due to water stress compounds, causing a toxic effect inhibiting growth processes (Errabii *et al*., 2007).

Diagnosis of the melatonin compound

The results indicated the concentration of sucrose notably altered in increasing the concentration of the melatonin compound in banana plants' leaves grown on the MS nutrient medium after eight weeks of cultivation (Figure 6). The results further showed that the concentration of 120 g L^{-1} was superior in giving the highest percentage of the melatonin compound. It reached 36.88 mg g^{-1} dry weight, followed by a concentration of 90 g L^{-1}, which reached 33.65 mg g^{-1}. Meanwhile, the control treatment recorded the lowest concentration of the melatonin compound, reaching21.82mg g-

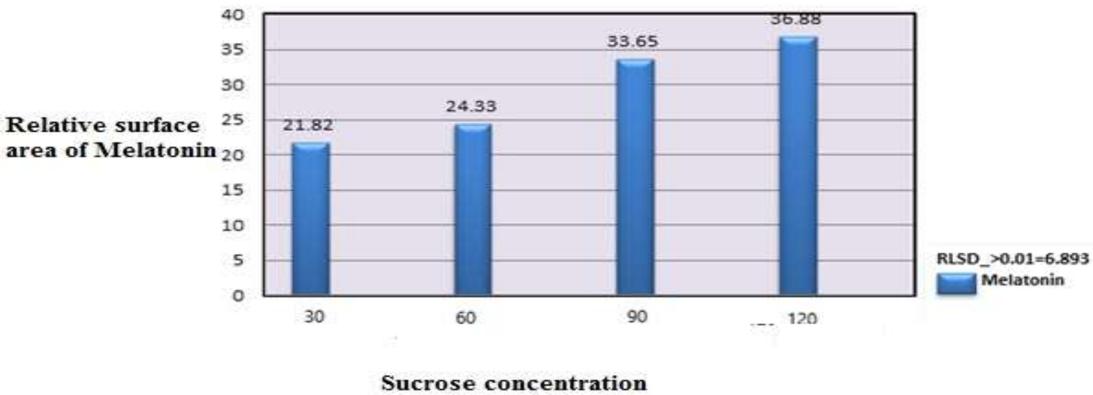

Figure 6. Effect of different levels of sucrose on the melatonin content of banana leaves.

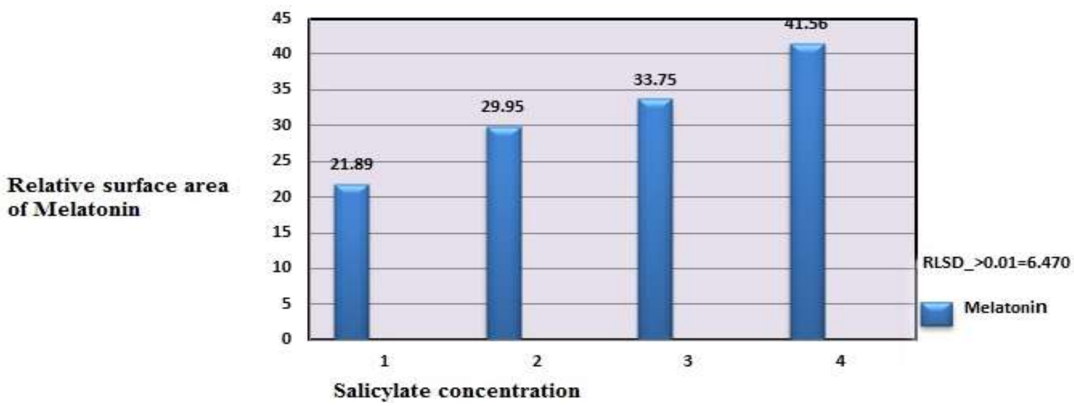

Figure 7. Effect of different levels of salicylic acid on the melatonin content of banana leaves.

^{1}. Sucrose is one of the crucial components of the nutritional medium for growth. Its effect is limited to the plant part selected for agriculture, which is a source of carbon and energy needed to complete various vital processes, in addition to its relevant role in osmotic regulation and maintaining osmotic pressure (Zhou *et al*., 2019). The gradual stress resulting from sucrose affects the work of enzymes due to the process of dehydration from the protoplasm, stimulating cells to produce secondary compounds (Patil *et al*., 2013). The results align with Ahmad *et al*. (2022), as their findings showed osmotic stress agents, such as sucrose in the MS nutrient medium, increase the stimulation and production of secondary compounds.

Salicylic acid effect on melatonin content

The outcomes showed the concentrations of salicylic acid have a significant effect in increasing the concentration of the melatonin compound in banana leaves (Figure 7). It was also evident that the 3.0 mg L^{-1} treatment was superior in giving the highest concentration of the melatonin compound, reaching 41.56 mg g^{-1} dry weight, followed by the concentration of 2.0 mg L^{-1} at 33.75 mg g^{-1} dry weight. Meanwhile, the control treatment recorded the lowest concentration of the melatonin compound, reaching 21.89 mg g^{-1} dry weight. It highlights the role of salicylic acid in maintaining the stability of cellular membranes and increasing the representation of CO_2.

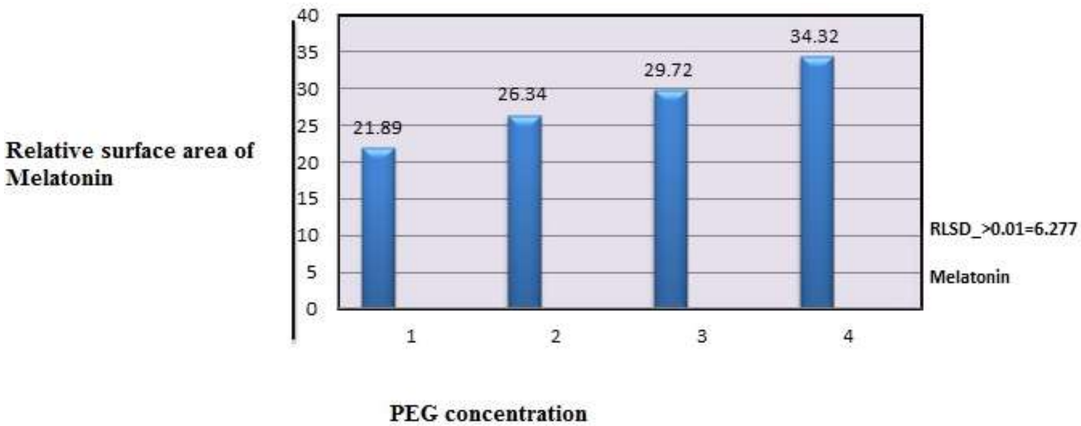

Figure 8. Effect of different levels of PEG on the melatonin content of banana leaves.

Moreover, it accumulates dry matter and increase absorption of water, nutrients, and minerals under the influence of stress through its possession of the plant's systemic acquired resistance mechanism, which formulate the organic chemistry of the plant cell from toxic secretions (Zhang *et al*., 2011).

PEG effect on melatonini content

The results detailed the treatment with PEG remarkably affected the accumulation of melatonin in the leaves of banana plants after eight weeks of the cultivar (Figure 8). It was also noteworthy that the 3.0 g L^{-1} treatment excelled in giving the maximum concentration of melatonin, reaching 34.32 mg g^{-1} dry weight, followed by the 2.0 g L^{-1} treatment, where the melatonin concentration reached 29.72 mg g^{-1} dry weight. Inversely, the control treatment provided the minimum concentration of the melatonin compound, reaching 21.89 mg g^{-1} dry weight. The plant thrives in a state of stress resulting from lack of water. The critical levels lead to manipulating the change in plant biology and plant biochemistry. Their cellular receptors' activation initiates a signal transmission chain that alters physiological processes and changes in the metabolic process, which in turn, leads to secondary signaling (Laxa *et al*., 2019; Sianipar *et al.*, 2024). The findings of this study agree with the same outcomes from Ahmad *et al*. (2020) on their study in the stevia plant. They showed the greater the concentration of PEG in the MS nutrient media, the greater the accumulation of secondary metabolite compounds.

CONCLUSIONS

The hybrid banana Grand Nain had the possibility of propagating its plants, as determined by plant tissue culture. The sucrose gave a good response to the growth of the plant using 90 mg L^{-1} concentration. The salicylic treatment also encouraged growth, in addition to the role of environmental stress compounds, such as, salicylate and PEG, in stimulating the formation of secondary compounds, especially melatonin. The processing of the nutrient medium with a concentration of 2 mg L^{-1} of salicylic acid and 1 g L^{-1} of the PEG compound gave the maximum content of the melatonin compound, which helps the plant's tolerance to environmental stresses.

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