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The Effect of UV-B And UV-C Radiation on Contamination Rate and Shoot Proliferation of Tamban Pineapple Crown Explants (*Ananas comosus* L. Merr.)

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ABSTRACT

This study examines the effect of ultraviolet-B (UV-B) and ultraviolet-C (UV-C) radiation on contamination rate and shoot proliferation of Tamban pineapple crown explant. The experimental design was nested and completely randomized with a separate control. The first factor was the type of UV light, namely UV-B and UV-C. The second factor was the duration of UV light exposure, namely 10, 20, 30, and 40 min. This study was carried out from March to June 2023 at the Plant Tissue Culture Laboratory, Faculty of Agriculture, Lambung Mangkurat University, South Kalimantan, Indonesia. Observations were made on the contamination percentage, survival percentage, time of first shoot formation, percentage of explants able to regenerate shoots, and number of shoots. The results showed that UV light treatment decreased the contamination rate. Increasing the duration of UV light exposure decreased the contamination rate, delayed the formation of the first shoot, and affected the number of shoots. UV-B light exposure produced a higher number of shoots than UV-C light. These results suggest that UV-B and UV-C radiation have the potential to optimize surface sterilization protocol and promote somaclonal variation.

Keywords: Fruit, plant tissue culture, radiation, somaclonal variation

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INTRODUCTION

Tamban pineapple is a local superior variety of pineapple from Barito Kuala Regency, South Kalimantan, Indonesia. It has the advantage of being quite tolerant of low soil acidity under 4.0. Besides that, it is known to tolerate high levels of iron (Fe) and aluminum (Al) (Balai Penelitian Pertanian Lahan Rawa [Balittra], n.d.). The pineapple

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is shade tolerant and is usually planted as an intercrop in oil palm, coconut, and rubber plantations (Cahyana & Destina, 2013).

According to the Badan Pusat Statistik (BPS) (2024), the three largest pineapple-producing regions in Indonesia in 2021 were Lampung (705,883 tons), South Sumatera (476,074 tons), and Riau (354,878 tons). Meanwhile, pineapple production in South Kalimantan in the same year was only 13,788 tons. It showed that the production of Tamban variety pineapples in South Kalimantan is still quite far from pineapple production in other regions. Additionally, the quality of Tamban pineapples (shelf life and vitamin C content) is still lower than other pineapple varieties, such as the MD2 variety, which is one of the superior and popular pineapple varieties on the international market. Thus, both the quantity and quality of Tamban pineapples need to be improved.

Ultraviolet (UV) radiation is a component of non-ionizing radiation in the electromagnetic spectrum, where 8-9% of total solar radiation is UV radiation (Hollósy, 2002). Rai and Agrawal (2017) mentioned that UV radiation is divided into three based on its wavelength range, namely UV-A (315-400 nm), UV-B (280-315 nm), and UV-C (200-280 nm). UV-A radiation has been observed to have a smaller effect than UV-B radiation on the morphology of *Ocimum basilicum* (Qian et al., 2023). Similarly, Sarghein et al. (2011) reported that red pepper (*Capsicum longum* A.DC.) was more sensitive to UV-C radiation than UV-A radiation. Furthermore, UV-A radiation has been proven to have an indirect impact on DNA because it is not easily absorbed by DNA (Mohamed et al., 2016). However, both UV-C and UV-B have sufficient energy to destroy chemical bonds, causing photochemical reactions, which are the main cause of the biological effects on plants (Kovács & Keresztes, 2002).

Several authors have reported the effects of UV-B and UV-C radiation on various plants. Metwally et al. (2019) reported that *Spathiphyllum cannifolium* plantlets treated with 45 min of UV-B radiation had the highest values on growth parameters, such as shootlet length, number of shoots, root length, and survival rate (%) when compared to other treatments (0, 15, and 30 min). In addition, Sadeghianfar et al. (2019) revealed that exposure to 12 hr UV-C light caused a significant increase in the germination rate and radicle length of maize (*Zea maize* L.) seeds. Meanwhile, UV-C light exposure on Persian violet shoots in vitro with low intensity (30 $\mu W/cm^2$) for 4 hours showed the maximum increase in the number of roots, root length, plantlet height, and number of shoots compared to other treatments (including control group) (Phanomchai et al., 2021).

According to several previous research, UV-B and UV-C radiation affected plants' morphological changes. Therefore, this research was conducted to investigate (a) the difference between the effect of control and UV light exposure on the morphological development of Tamban pineapple crown explants, (b) the effect of UV-B and UV-C light on the morphological development of Tamban pineapple crown explants, and (c) the effect of the duration of UV light

exposure nested in a type of UV light on the morphological development of Tamban pineapple crown explants.

MATERIALS AND METHODS

Plant Materials and Sterilization of the Explant Surface

Crowns of Tamban pineapples were obtained from a single source in Tamban Village, Mekarsari District, Barito Kuala Regency, South Kalimantan, Indonesia. The crown leaves were removed, and the crowns were washed under running tap water. The crowns were sterilized with 0.3% bactericide solution (Agrept®, Indonesia) for 15 min and 0.5% fungicide solution (Bendas, Indonesia) for 30 min. The crowns were then rinsed with sterile distilled water. Next, the crowns were transferred to a laminar airflow cabinet. The explants were later soaked in 70% alcohol (OneMed, Indonesia) for 1 min and 0.1% mercuric chloride ($HgCl_2$) solution (Merck, Germany) with several drops of Tween 20 (Merck, Germany) for 5 min. The crowns were later rinsed three times in sterile distilled water. The sterilized crown explants were split into two parts and then treated with UV light. The explants were then cultured on Murashige and Skoog (MS) media with 2 mg/L 6-benzylaminopurine (BAP, Glentham Life Sciences, United Kingdom).

Experimental Design and Statistical Analysis

This study used a nested, completely randomized design with a separate control. The treatment factor consisted of two factors. The first factor was the type of UV light, which consisted of 2 levels: UV-B light and UV-C light. Artificial UV-B light comes from 2 UV-B lamps (each with a power of 18 W), while UV-C light comes from a 30 W UV-C lamp. The distance between the lamp and the explants is ± 20 cm. The second factor was the duration of UV light exposure, which consisted of 4 levels, namely 10, 20, 30, and 40 min. The nested arrangement is in the form of the time of UV light exposure nested in the type of UV light. Besides that, the control group was non-treated crown explants.

Data were collected and expressed as the mean of the cultures of three replicates, each containing five explants. The effect of the control and UV light treatment was analyzed using orthogonal comparison. Meanwhile, the effect of the type of UV light and the duration of UV exposure were analyzed using variance analysis.

RESULTS AND DISCUSSION

Effect of UV-B and UV-C Exposure on the Percentage of Contamination and Survival Explants

Based on Table 1, the recorded contamination rate of the control group was 33.33% at 4 WAP (weeks after planting) and 60.00% at 8 WAP. Furthermore, the UV light treatment resulted in a significantly reduced contamination rate of 7.50% at 4 WAP and 38.33% at 8 WAP. The same pattern was observed in the sterilization protocol of bear's garlic (*Allium ursinum*) explants,

where the combination of UV-C exposure for 40 min and sodium hypochlorite solution (ACE, United Kingdom) for 10 min showed a high sterilization efficiency with a contamination rate of 10% (Tomaszewska-Sowa et al., 2015). Apart from that, Sriana et al. (2022) found that no significant difference was observed between UV light exposure and various sterilization agents on the contamination rate of Talas banana (*Musa paradisiaca* L. var. *sapientum*) corm explants. They concluded that sterilization of Talas banana corm explants could be carried out using only UV light exposure treatment. Overall, the reduction of contamination rate due to UV light exposure indicates UV light potential to enhance the surface sterilization protocol.

Takada et al. (2017) found a bactericidal effect from UV-B light exposure, with a decrease in the percentage of living bacteria as the duration of UV-B light exposure increased. Abdelrahman et al. (2018) reported that UV-C light exposure for 118 J/cm^2 was sufficient to decrease the fungal contamination of *Penicilliun commune* and *Chaetomium globosum*. Other studies reported that 15 to 60 min of UV-C light exposure is effective for fungal decontamination of *Aspergillus niger* and *Aspergillus flavus* (Jhahan et al., 2022).

Table 1
Mean contamination rate (%) and mean survival rate (%) of Tamban pineapple crown explants after control and ultraviolet (UV) light treatment

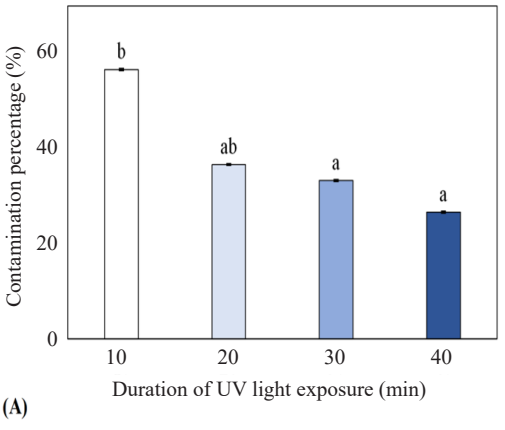
Treatment	Mean percentage of contamination (%)		Mean percentage of survival (%)*
	4 WAP	8 WAP	
Control	33.33b	60.00b	100.00
UV light	7.50a	38.33a	100.00

Note. Means followed by the same letter(s) within each column are not significantly different ($p \leq 0.05$) using orthogonal comparison; WAP = Weeks after planting; * = Data were collected after 8 weeks

The mechanism of the germicidal effect of UV light is related to the absorption of UV light by the nucleic acid components of a microorganism (Gurzadyan et al., 1995). As a result, damage to a microorganism's DNA occurs due to the dimerization of thymine molecules, which subsequently produces cyclobutane pyrimidine dimers (CPDs). The production of these CPDs makes it difficult for nucleic acids to replicate and causes cell death. Even when replication occurs, defects frequently prevent the microorganism from surviving (Dai et al., 2012).

According to the result of the variance analysis, no significant difference was detected between UV-B and UV-C in response to the contamination rate. However, the different durations of UV light exposure showed significant differences. As for the contamination rate in different durations of UV light exposure (Figure 1A), the results show values ranging between 26.67% (40 min UV light exposure) and 56.67% (10 min UV light exposure). Thus, increasing the duration of UV light exposure from 10 to 40 min decreased the contamination rate. A

similar result has been reported by Ferreira et al. (2021) that a longer UV-C exposure of 3 hr increased the efficiency of reducing the fungal colonies and mycotoxin levels compared to 1 hr exposure. Abdelrahman et al. (2018) also found that the fungicidal effect of UV radiation depends on the concentration of fungal spores and the dose of UV radiation. Similarly, Mengmeng et al. (2022) reported that the dose of UV radiation positively correlates with the bactericidal rate.



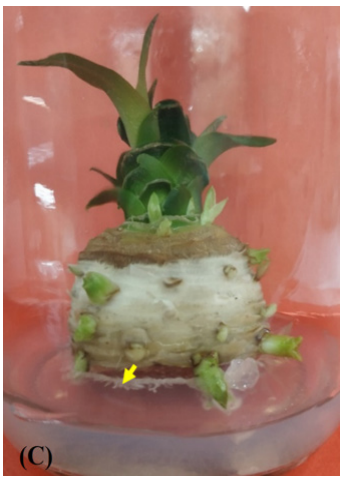


Figure 1. (A) Contamination rate of Tamban pineapple crown explants at 8 weeks after planting (WAP) after ultraviolet light treatment. Different letters indicate statistically significant differences between factors (Duncan's multiple range test at $p \leq 0.05$); (B) (C) Contamination on Tamban pineapple crown explant at 8 WAP. The blue arrow shows fungal contamination, and the yellow arrow shows bacterial contamination

Note. UV = Ultraviolet

Effect of UV-B and UV-C Exposure on Shoot Proliferation

The first shoot formation in UV light treatment was significantly slower than the control group, with the time of first shoot formation respectively of 14.82 days after planting (DAP) and 10.67 DAP (Table 2). In terms of the percentage of explants able to regenerate shoots, UV light treatment was significantly lower than the control at 2 WAP. All explants of the control group were able to regenerate shoots at 2 WAP, while UV light treatment resulted in 100% of explants being able to regenerate shoots at 4 WAP. These results indicated that UV light treatment on Tamban pineapple explants significantly slowed down the formation of the first shoot.

Table 2
Shoot proliferation of Tamban pineapple crown explants after control and ultraviolet light treatment

Treatment	Time of first shoot formation (DAP)	Mean percentage of explants able to regenerate shoots (%)		Mean number of shoots per explant			
		2 WAP	4 WAP	2 WAP	4 WAP	6 WAP	8 WAP
Control	10.67a	100.00b	100.00	2.80b	5.73b	7.27	8.47
UV light	14.82b	60.83a	100.00	1.93a	4.94a	7.05	8.60

Note. Means followed by the same letter(s) within each column are not significantly different ($p \leq 0.05$) using orthogonal comparison; DAP = Days after planting; WAP = Weeks after planting

The effect of UV-B and UV-C was not significantly different in terms of the time of first shoot formation and the percentage of explants able to regenerate shoots (Table 3). Based on Figure 2, the time of first shoot formation in the different durations of UV light exposure ranged from 13.30 DAP (10 min UV light exposure) to 16.40 DAP (40 minutes UV light exposure). This result indicated that increasing the duration of UV-B and UV-C exposure delayed the time of first shoot formation. In addition, increasing the duration of UV light exposure also reduced the percentage of explants able to regenerate shoots at 2 WAP (Figure 2).

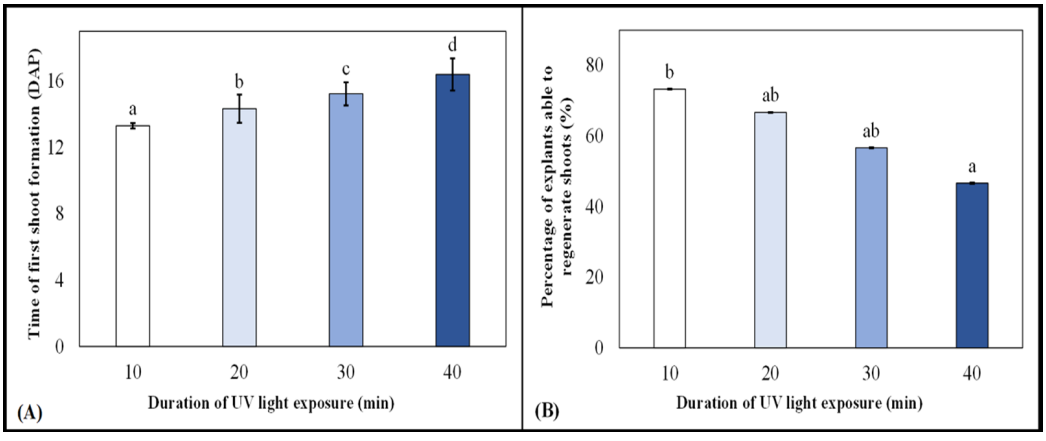


Figure 2. (A) Time of first shoot formation on Tamban pineapple crown explants after different durations of ultraviolet (UV) light exposure; (B) Percentage of explants able to regenerate shoots after different durations of UV light exposure

Note. Different letters indicate statistically significant differences between factors (Duncan's multiple range test at $p \leq 0.05$); DAP = Days after planting

Table 3
Effect of the type of ultraviolet (UV) light on shoot proliferation

Type of UV light	Time of first shoot formation (DAP)	Mean percentage of explants able to regenerate shoots (%)		Mean number of shoots/explant			
		2 WAP	4 WAP	2 WAP	4 WAP	6 WAP	8 WAP
UV-B	14.28	65.00	100.00	2.35b	5.70b	8.20b	10.03b
UV-C	15.35	56.67	100.00	1.50a	4.18a	5.90a	7.17a

Note. Means followed by the same letter(s) within each column are not significantly different ($p \leq 0.05$) using analysis of variance; DAP = Days after planting; WAP = Weeks after planting

Pineapple crown explants have dormant axillary buds located beneath every leaf axil of the crown leaves (Agogbua Josephine & Osuji Julian, 2011). These dormant axillary buds have the potential to grow into shoots (Py et al., 1987). The slowing down of first shoot formation from UV light treatment may be related to the increased dormancy period of axillary buds on Tamban pineapple crown explants.

The dormancy and development of axillary buds can be influenced by reactive oxygen species (ROS) content. Chen et al. (2016) stated that the hydrogen peroxide (H_2O_2) compounds contribute to the inhibition of the axillary bud outgrowth in tomato plants (*Solanum lycopersicum* L. cv Aisla Craig and M2). Similarly, Porcher et al. (2020) reported that increased H_2O_2 caused the axillary buds on rose plants (*Rosa* sp.) to remain dormant. Contrariwise, when the content of H_2O_2 decreases, axillary buds on rose plants begin to form. In addition, Li et al. (2022) also mentioned that the low level of ROS leads to dormancy release.

UV radiation can have an indirect biological impact by increasing ROS production (Tan et al., 2023). Metabolic disturbances from UV-B radiation, such as impaired electron transfer and other quinone components in photosystem II, can induce ROS production in photosystem I (Renger et al., 1989; Vass et al., 1996). UV-B radiation can turn H_2O_2 into highly reactive hydroxyl radicals through photo-conversion, which can lead to oxidative damage (Czégény et al., 2014). Xue et al. (2022) reported that the H_2O_2 content was increased in *Neoporphyra haitanensis* after UV-B light exposure. They also added that the intensity and duration of UV-B light exposure were positively correlated with the H_2O_2 content. A similar result was also recorded in tomato plants in which 20- and 40-min UV-C exposure significantly increased the ROS contents (O_2^-, OH^-, and H_2O_2) than without UV-C exposure (Dawood et al., 2022).

The hormones that play an important role in releasing bud dormancy are gibberellin (GA) and abscisic acid (ABA) (Yue et al., 2018). Meyer et al. (2021) mentioned that UV-B radiation can affect increasing ABA, reducing GA, and increasing ROS. The same pattern was also observed by Pascual et al. (2017), where *Pinus radiata*

plants irradiated with UV-C light showed a decrease in GA and an increase in ABA. Changes in GA and ABA content and increased ROS may be associated with the delayed release of axillary buds' dormancy in Tamban pineapple crown explants after UV-B and UV-C light treatment. It resulted in the highest duration of UV light exposure with the latest time of first shoot formation and the lowest percentage of explants able to regenerate shoots. The shoots formed on the Tamban pineapple crown explants can be seen in Figure 3.

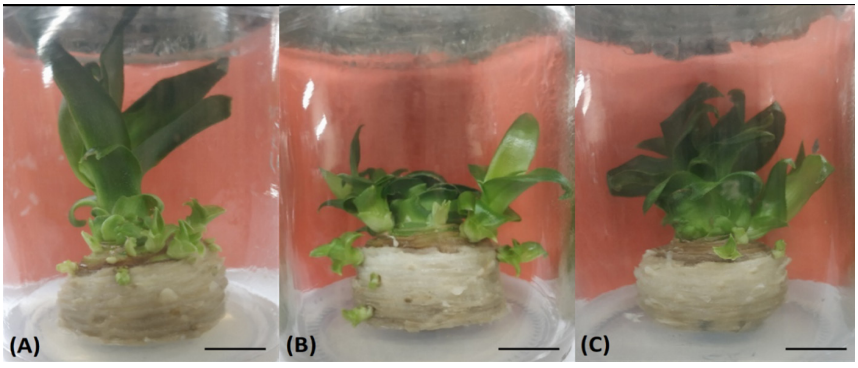


Figure 3. Shoot formation on the Tamban pineapple crown explants as the effect of UV light exposure after 8 weeks of culture. (A) control; (B) Ultraviolet-B (UV-B) light; and (C) UV-C light

Note. Bar = 1.0 cm

The UV light treatment was significantly different from the non-treated explants in terms of the mean number of shoots at 2 and 4 WAP (Table 2). UV-B light treatment produced a higher mean number of shoots than UV-C light treatment at 2-8 WAP (Table 3). Stapleton (1992) mentioned that DNA has maximum absorption in the electromagnetic spectrum range of UV-C light (260 nm). Therefore, the most energetic radiation is UV-C, but as a result, it often causes damage to plants, although at lower doses (Vanhaelewyn et al., 2020).

Increasing the duration of UV-B light exposure from 10 to 30 min increased the number of shoots per explant to 11.20 shoots at 8 WAP, and yet when the duration of UV-B light exposure was further increased to 40 min, the number of shoots per explant decreased to 10.13 shoots (Figure 4A). Meanwhile, increasing the duration of UV-C light exposure from 10 to 40 min increased the number of shoots from 6.40 to 8.27 (Figure 4B). These results were in accordance with the previous study that found an increment in the number of shoots in *S. cannifolium* after UV-B radiation (Metwally et al., 2019). Similar results were also observed in *Amsonia orientalis*, where UV-C light treatment produced a higher number of shoots than without treatment (Acemi et al., 2018).

Mallet et al. (2022) stated that the growth of axillary buds is controlled by a

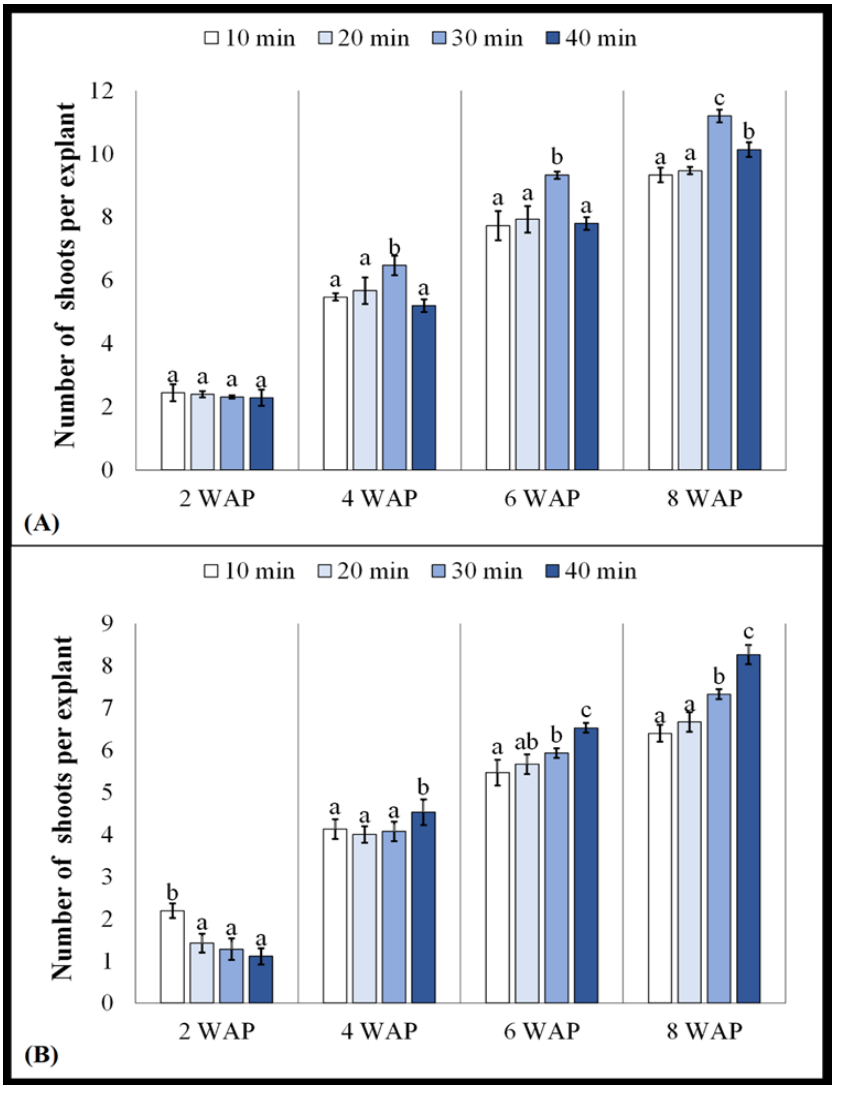


Figure 4. Number of shoots on Tamban pineapple crown explant at 2, 4, 6, and 8 weeks after planting (WAP) after different durations of ultraviolet (UV) light exposure: (A) UV-B exposure; (B) UV-C exposure

Note. Different letters indicate statistically significant differences between factors (Duncan's multiple range test at $p \leq 0.05$)

complex interaction between several main factors, such as hormones, nutrients (sugar and nitrogen), and ROS. The hormones that regulate the induction of axillary shoot growth are auxin (IAA), cytokinin (CK), and strigolactone (SL) (Barbier et al., 2021; Domagalska & Leyser, 2011). In the interaction of these three hormones, CK acts as a promoter of axillary shoot growth, while auxin and SL act as inhibitors of axillary

shoot growth (Gomez-Roldan et al., 2008; Leyser, 2009; Umehara et al., 2008). Auxin inhibits axillary shoot growth indirectly by inhibiting CK synthesis and promoting SL synthesis and signaling (Mallet et al., 2022).

UV light catalyzes the photodestruction of auxin (Ros & Tevini, 1995). UV-B radiation can cause the oxidative degradation of IAA, which begins with a decarboxylation process involving peroxidase on the side chain or oxidation of the indole ring (Berli et al., 2013; Normanly, 2010). Additionally, Hayes et al. (2014) revealed that UV-B radiation leads to the degradation of the PIF4 and PIF5 proteins, which can further inhibit the regulation of auxin biosynthesis.

As a result of UV-B radiation, the auxin content in rice leaf lamina decreased by 17.3% compared to natural light treatment. Increasing the dose of UV-B radiation from 2.5 to 5 kJ/m^2 also decreased the auxin content (Ling et al., 2022). IBA decreased significantly in the second leaves of *Cucumis sativus* L. irradiated with UV-B light (Qian et al., 2021). According to Katerova and Todorova (2011), exposure to low doses of UV-C light (0.1 kJ/m^2.d) on pea plants (*Pisum sativum* L.) caused the IAA content in the second leaf to be higher than the control. Meanwhile, the lowest IAA content was found in plants exposed to high doses of UV-C light (0.3 kJ/m^2.d). Therefore, the response of the different durations of UV-B and UV-C light exposure to a number of shoots may be due to the reducing auxin synthesis.

CONCLUSION

UV light treatment showed better results than the non-treated explants in terms of the contamination rate. However, UV light treatment delayed the formation of the first shoot and reduced the percentage of explants able to regenerate shoots at 2 WAP. UV-B light treatment produced a greater number of shoots than UV-C light treatment. Increasing the duration of UV light exposure reduced the contamination rate, delayed the formation of the first shoot, and reduced the percentage of explants able to regenerate shoots at 2 WAP. Increasing the duration of UV-B exposure to 30 min significantly increased the number of shoots at 8 WAP, but the number decreased at 40 min. Meanwhile, increasing the duration of UV-C exposure to 40 min significantly increased the number of shoots. As ROS and hormone relating were not tested during this study period, future studies should consider that.

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