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EFFECT OF LOW GAMMA RADIATION ON THE GROWTH OF *Vanilla planifolia* CULTURES

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ABSTRACT

A study was conducted to determine the effect of low doses of gamma radiation on the regeneration and morphological of *Vanilla planifolia*, a tropical climbing orchid from the family Orchidaceae. The explants were treated with seven different combinations of sterilization agents before inoculating on Murashige and Skoog medium and incubated for three weeks at 25±2ºC. After incubation, the cultures were exposed to three doses of gamma radiation (10 Gy, 20 Gy and 30 Gy). After twelve weeks, the result showed that regeneration of *V. planifolia* was the highest at 10 Gy irradiation, where shoot height was 13.40±0.33 cm compared to the non-irradiated culture with only 6.89±0.21 cm. The leaf and root numbers as well as root length also showed the highest for 10 Gy cultures. The lowest regeneration was obtained from 30 Gy irradiated cultures. However, no morphological changes were detected in all treated cultures. Thus, it can be suggested that low doses of gamma irradiation stimulate the *in vitro* regeneration of *V. planifolia*.

Key words: Tissue culture; *Vanilla planifolia*; Orchidaceae; gamma ray

INTRODUCTION

Vanilla has the greatest contribution to the world of flavors. Vanilla beans rival saffron in intensity and cardamom in aromatic complexity. *Vanilla planifolia* is one of the infamous species among vanilla family that is used widely in commercial products such as foods, fragrances and cosmetics. This plant is a climber that can reach more than 20 m in height, and often flowers at considerable heights above the ground. It is an ancient genus in the Orchidaceae family (Cameron, 2004; 2011). Due to its high demand, various strategies and methods in vanilla cultivation need to be improved and plant tissue culture is an alternative for mass production besides the traditional hand-pollination.

The effect of gamma radiation on plant growth, development and functions highly depends on the dose of irradiation. Moreover, the plant perception and response to a low dose of radiation may be completely different from the one caused by high dose radiation. A low dose of ionizing radiation is more likely to produce a positive physiologically effect in comparison to the high dose radiation, as done in *Capsicum* sp (Wi *et al.,* 2007). Several researchers manage to improved regeneration yield of explants in tissue culture using gamma irradiation such as in carrots (Al-Safadi and Simon, 1996), grapevine (Charbaji and Nabulsi, 1999), potato (Al-Safadi, Ayyoubi and Jawdat, 2000), red pepper (Kim *et al.,* 2004), caper (Al-Safadi and Elias, 2010) and wheat (Tufail, Sabiha and Akhtar, 2010).

The objective of this study was to identify the optimum dose of gamma irradiation for the highest growth of vanilla cultures purposely to improve vanilla cultivation using tissue culture. Thus, this research analyzed four growth parameters, such as shoot height, leaf number, root length and root number after being treated with different doses of gamma radiation.

MATERIALS AND METHODS

Source of explants

Young plant of *Vanilla planifolia* obtained from Hexagon Green Biotech Sdn Bhd, Taman Teknologi Agensi Nuklear Malaysia, Bangi, Selangor, Malaysia. These mother plants were grown in the pots under well-ventilated shade house. The plants were watered once daily.

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Surface sterilization

In this study, excised nodes were used as explants. The vanilla mother plant was washed with tap water to remove dirt and soil. Then, the mother plant was cut into five cm in length and placed in a plastic beaker before washing under running tap water for thirty minutes. The explants were soaked in a few drops of Tween 20™ for twenty minutes before rinsing using sterilized distilled water for three times. Seven combinations of surface sterilization, (**Group 1** – 15% commercial bleach and 70% alcohol; **Group 2** – 20% commercial bleach and 70% alcohol; **Group 3** – 1% fungicide and commercial bleach; **Group 4** – 1% fungicide and 20% commercial bleach; **Group 5** – 1% fungicide, 10% commercial bleach and 70% alcohol; **Group 6** – 1% fungicide, 15% commercial bleach and 70% alcohol; **Group 7** – 1% fungicide, 20% commercial bleach and 70% alcohol) were carried out in laminar airflow to maintain sterility. Lastly, the explants were rinsed three times with sterile distilled water. The explants were dried by using sterile tissue paper and excised to one cm each. The sterility results of vanilla cultures were gathered after five days.

Medium preparation and culture induction

The surface sterilized explants were cultured on MS (Murashige and Skoog, 1962) medium supplemented with 3% sucrose, 0.01% myo-inositol, 0.01% amino acid and 0.37% gelrite. The pH of the medium were adjusted between 5.7 – 5.8 prior to autoclaving at 121ºC for 20 minutes. The explants were incubated at 25 ± 2ºC under a 16 hours/8 hours (light/dark) photoperiods provided by 40 watt cool white fluorescent lights.

Gamma irradiation of explants

Two weeks old *V. planifolia* cultures were exposed to three doses of gamma irradiation, 10, 20 and 30 Gy. The irradiation was performed in a gamma chamber in Agensi Nuklear Malaysia, Bangi, Selangor. The explants were immediately transferred into new MS media and sub-cultured every five weeks.

Statistical analysis

All the data obtained were analyzed using One-way ANOVA followed by Duncan's multiple-range test (DMRT) at the 95% (≤ 0.05) significance level. All data were expressed as mean±SEM (standard error mean) with statistical significance at $p \leq 0.05$.

RESULTS AND DISCUSSION

The vanilla plant nodes were selected for shoot induction due to its high percentage of regeneration in tissue culture. In Table 1, the result of surface sterilization showed Group 5 (1% fungicide, 10% commercial bleach and 70% alcohol) was the best sterilant agent to sterilize *V. planifolia* explants due to 100% explants sterility and 0% mortality. Group 3 also produced 0% explants mortality however the sterility of explants was lower. More, Group 6 and 7 showed a 100% explants sterility however both combinations showed mortality in explants. Fungicide was needed as an addition to the double sterilization of commercial bleach and alcohol to ensure a sterilize culture however, higher concentration caused high percentage of explants mortality.

Table 2 shows that the shoot height of vanilla cultures irradiated with 10 Gy (13.40±0.33 cm) was significantly greater ($p \leq 0.05$) than the non-irradiated cultures (6.89±0.21 cm) and other doses of gamma irradiations. This was followed by the shoot height of 20 Gy irradiated vanilla cultures (7.76±0.37 cm). Meanwhile, the shoot height for 30 Gy irradiated vanilla cultures was the lowest (6.33±0.30 cm) with no significant different ($P \geq 0.05$) between non-irradiated cultures. In similar, the leaf number for 10 Gy irradiated vanilla cultures was also higher than other treatments including the non-irradiated cultures, with 4.05±0.17 leaves followed by leaf number of 20 Gy irradiated vanilla cultures (2.65±0.11). No significant different ($P \geq 0.05$) between the non-irradiated and 30 Gy irradiated vanilla cultures, which were 1.67±0.12 and 1.65±0.13 leaves respectively.

Table 1. Results of different combinations of surface sterilization of *Vanilla planifolia* after five days

Group	Fungicide (%)	Commercial bleach (%)	Alcohol (%)	Sterilization (%)	Mortality (%)
1	0	15	70	50	40
2	0	20	70	70	70
3	1	15	0	70	0
4	1	20	0	80	10
5	1	10	70	100	0
6	1	15	70	100	40
7	1	20	70	100	80

Surface sterilization was performed to 10 explants in MS media.

Table 2. Effect of low doses of gamma irradiation on shoot length and leaf number of *in vitro Vanilla planifolia* in MS media

Doses (Gy)	Shoot height (cm)	Number of leaf
0	6.89±0.21c	1.67±0.12c
10	13.40±0.33a	4.05±0.17a
20	7.76±0.37b	2.65±0.11b
30	6.33±0.30c	1.65±0.13c

The data was expressed as Mean±SEM. Means with the same letters in a column are not significantly different at p>0.05.

Table 3. Effect of low doses of gamma irradiation on root length and number of *in vitro Vanilla planifolia* in MS media

Doses (Gy)	Root length (cm)	Number of root
0	3.37±0.14c	1.59±0.12c
10	7.40±0.45a	3.85±0.13a
20	4.93±0.19b	2.75±0.12b
30	1.80±0.33d	0.65±0.11d

The data was expressed as Mean±SEM. Means with the same letters in a column are not significantly different at p>0.05.

Table 3 shows the result for root length and root number of *in vitro* vanilla in MS media. The longest root growth was found in 10 Gy irradiated vanilla cultures with 7.40±0.45 cm followed by 20 Gy irradiated vanilla cultures with 4.93±0.19 cm. Non-irradiated cultures displayed approximately half of the length when it was compared to the longest root growth (10 Gy) with 3.37±0.14 cm while 30 Gy irradiated vanilla cultures showed the lowest root length with only 1.80±0.33 cm. There was a significant different ($P \leq 0.05$) for root length of all the gamma irradiated vanilla cultures when compared to the non-irradiated cultures. Likewise, the results for the highest root number was also gathered from 10 Gy irradiated vanilla cultures with 3.85±0.13 roots followed by 20 Gy irradiated vanilla cultures with 2.75±0.12 roots when compared to the non-irradiated cultures, where the roots collected was only 1.59±0.12. The number of roots in 30 Gy irradiated vanilla cultures was lower than the non-irradiated cultures with only 0.65±0.11 root. There was a significant different ($P \leq 0.05$) between all gamma irradiated vanilla cultures when compared to the non-irradiated cultures. Figure 1 showed the non-irradiated and irradiated vanilla cultures after twelve weeks of incubation, where figure B (10 Gy irradiated cultures) clearly showed a substantial increase in the regeneration of vanilla cultures compared to figure A (non-irradiated cultures).

The cultures treated with 10 Gy radiation gave the highest growth in term of shoot height, leaf number, root length and root number when compared to the other doses including the non-irradiated cultures. The second dose of gamma irradiation that showed a higher growth rate was 20 Gy irradiated vanilla cultures and the lowest was 30 Gy irradiated vanilla cultures. The use of gamma irradiation especially in low doses to improve growth rate in plant tissue culture has previously been done by other researchers however, the effects on vanilla explant have yet been investigated. In this research, it was revealed that the induced stimulation of plant growth is a result of gamma ray. Gamma irradiation causes changes in biochemical characteristics of target tissue in plant, which directly or indirectly regulate cell division and cell elongation. Thus, changes the morphological aspects and therefore the related physiological characteristics (Singh and Datta, 2010). This can be seen in the shoot height and root length of vanilla cultures where in low dose the shoot and root elongation are much higher compared to the non-irradiated cultures. These results were in line with a research done by Charbaji and Nabulsi (1999) in grapevine, where 5 Gy irradiated Hellwani cultivar grapevine showed a significant increase in root length when compared to the non-irradiated cultures.

Another factor that may contribute to a higher growth in *V. planifolia* cultures is probably the increasing rate of photosynthesis. In a research done by Kim *et al.* (2004), they found that photosynthesis rate in two commercial red peppers, Yeomyung and Joheung, increased after they were treated with low doses of gamma irradiation. Photosynthesis is an important indicator of internal plant metabolism where it is considered as the fundamental process of energy production for growth and development. If the rate of photosynthesis increases so does the rate of metabolism, thus increasing the plant development. However, photosynthesis is sensitive to heavy metals, which in this study was generated from the gamma irradiation, because it directly disturbs the chloroplast function by inhibiting the activities of enzymes of chlorophyll biosynthesis and CO_2 fixation or the aggregation of pigment protein complexes of the photosystem (Chaves, Flexes and Pinheiro, 2009). Thus, only a low dose gamma irradiation can provide a beneficial outcome in plant growth while a higher dose will result in a retard development.

Bhatt *et al.* (2008) suggested that gamma irradiation increases the endogenous hormones in plants. Hormones such as auxins and cytokinins cause a change in the primary metabolism in plant, which can cause shoot and root elongation, budding and flower formation. Irradiation may cause *de novo* synthesis of free forms of hormones or conversion of conjugated forms into free forms to overcome the physical stress (Cho and Song, 2000). Similar results were also obtained in this study where no exogenous

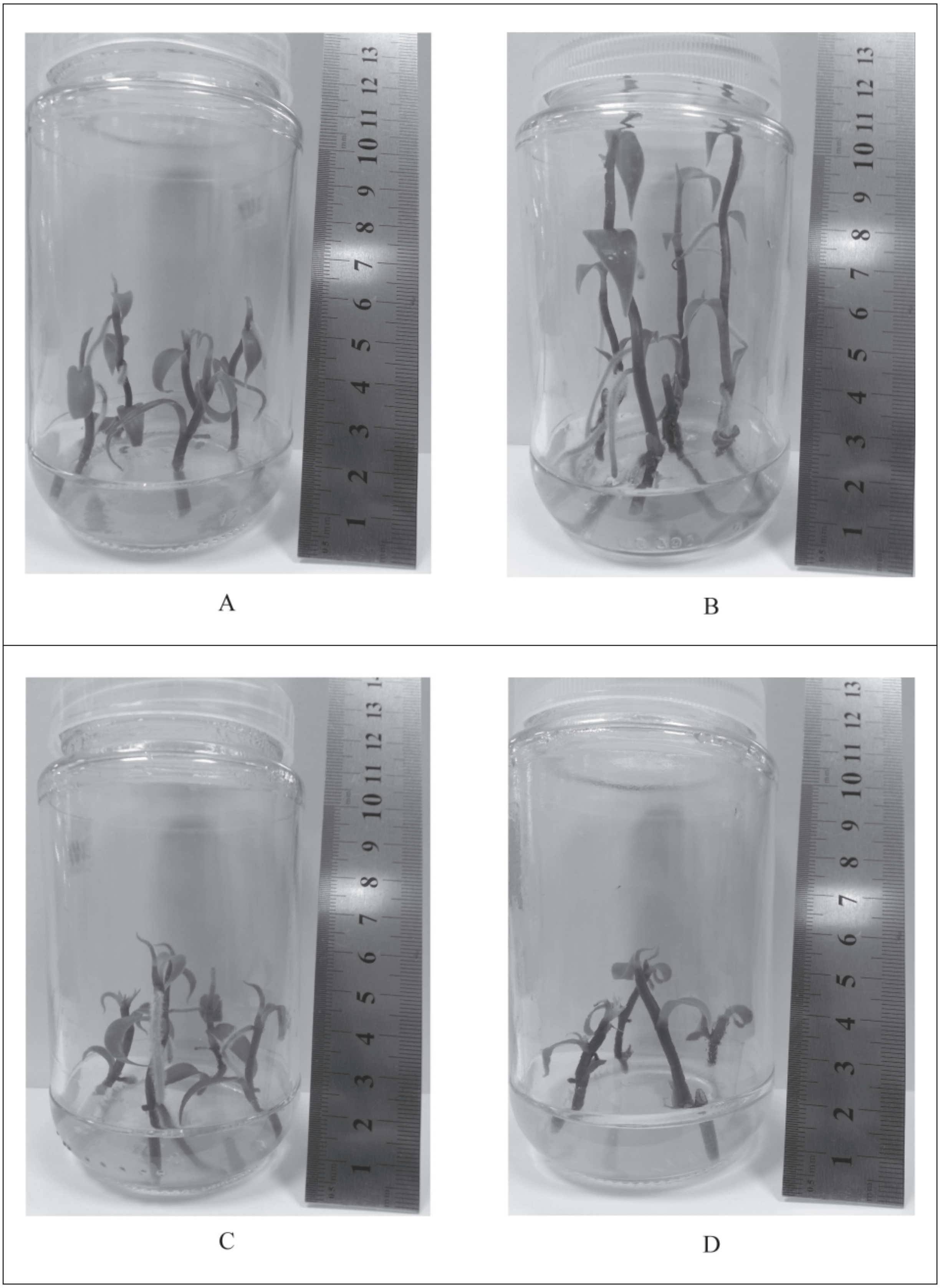

Fig. 1. *In vitro* culture of *Vanilla planifolia* Andrews after twelve weeks of incubation in MS media. A – non-irradiated culture, B – 10 Gy gamma irradiated culture, C – 20 Gy gamma irradiated culture and D – 30 Gy gamma irradiated culture.

hormones were applied to the media but the growth of irradiated cultures increase tremendously compared to the non-irradiated cultures. However, the physiological and biochemical effect of gamma radiation are dose-dependent, which determine the stimulation and/or inhibition of plant growth.

CONCLUSIONS

This study showed stimulation growth of *V. planifolia* cultures after treated with gamma irradiation where at lower doses, gamma irradiation has a potential to stimulate vanilla regeneration *in vitro*. Ten Gy gamma irradiation dose doubled the plant growth (height, leaf number, root number and root length) of *V. planifolia*. This ascertained that gamma irradiation is an excellent alternative source to improve cultivation of vanilla in tissue culture.

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