Research Article

Effect of Low Gamma Radiation and Methyl Jasmonate on *Vanilla planifolia* Tissue Culture

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Accepted on: 30-04-2014; **Finalized on:** 30-06-2014.

ABSTRACT

A study was conducted to determine the effect of low dosage of gamma radiation and methyl jasmonate on the regeneration of *Vanilla planifolia*, a tropical climbing orchid from the family Orchidaceae. *V. planifolia* plant was surface sterilized before inoculated 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 (0 Gy, 10 Gy, 20 Gy and 30 Gy). Then, the cultures were directly transferred into five different concentration of methyl jasmonate (0 µM, 10 µM, 20 µM, 40 µM and 80 µM). After ten weeks, the result showed that regeneration of *V.planifolia* was highest at 20 µM methyl jasmonate media after exposed to 10 Gy irradiation, where shoot height was 14.5±0.4 cm. The number of leaf, root length and root number also showed the highest for 10 Gy cultures in 20 µM methyl jasmonate while the lowest regeneration was collectively obtained from 80 µM methyl jasmonate. However, no morphological changes were detected in all treatments. In conclusion, low doses of gamma irradiation and methyl jasmonate stimulate the *in vitro* regeneration of *V.planifolia* while the highest dose and concentration of methyl jasmonate retarded the growth and development.

Keywords: Tissue culture, *Vanilla planifolia*, Orchidaceae, gamma ray, elicitor.

INTRODUCTION

The genus *Vanilla,* originates from the family Orchidaceae consists of 110 species, ranging from the rare and endangered *Vanilla aphylla* to the commercially important *Vanilla planifolia.* Vanilla is a herbaceous perennial climbing plant that uses its adventitious roots for support and vanilla fruit, commercially known as vanilla bean is one of the priciest spice in the market because growing and harvesting the vanilla plant is labor-intensive and time-consuming.[1]

Vanilla extract is widely used in the food industry, perfumeries, toiletries and pharmaceutical industry.[2] Vanilla has been reported to have anti-metastatic and anti-angiogenic effects when tested on cancer cells of mice as well as cytolytic and cytostatic tendencies that could be useful as a colon-rectal cancer preventive agent.[3,4] Moreover, vanilla extract is a strong antimicrobial and antifungal agent that can prevent food spoilage against *Listeria spp., Zugosaccharomyces bailii*, *Zygosaccharomyces rouxii, Saccharomyces cerevisiae* and *Candida parapsilosis.*[5,6]

Gamma irradiation has been widely used in the biological study of plants, including tissue culture. Gamma ray had been reported to induce cytological, biochemical, genetical, physiological and morphogenetic changes in cells and tissue, thus, influencing the growth and development of the plants.[7] Several studies on the effect of low dose gamma irradiation on the growth and development of tissue culture plant also had been done on banana, oil palm, rose and violet.[8-11]

Methyl jasmonate is a volatile compound originally recognized as a chemical inside *Jasminum grandiflorum* flower but later discovered to be universally distributed in the plant kingdom.[12] Methyl jasmonate has been known to be an important cellular regulator that regulates plant development such as seed germination, root growth, fertility, fruit ripening, and senescence as well as defense mechanism against biotic and abiotic stresses.[13] Several studies on the effect of methyl jasmonate on tissue culture had also been done on tobacco, flax, cabbage and potato.[14-17]

Several researches has been done on tissue culture of other plant species to increase plant growth and development by using low dose of gamma irradiation and methyl jasmonate but none had been done to *V. planifolia* tissue culture. Due to an inadequate supply and the highly usefulness of vanilla, therefore this study was conducted to determine the effect of *V. planifolia* regeneration in methyl jasmonate induced media after a low dose of gamma radiation.

MATERIALS AND METHODS

Source of explants and surface sterilization

Young plants of *Vanilla planifolia* obtained from Hexagon Green Biotech Sdn Bhd, Taman Teknologi Agensi Nuklear Malaysia, Bangi, Malaysia, were grown in pots in a well-ventilated shade house and received water application daily. Surface sterilization was used to achieve sterilized explants. First, the vanilla mother plant was washed with tap water to remove dirt and soil. Then, the mother plant was cut into 5cm in length and then placed in a plastic beaker under a 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. Fourth, the explants were soaked

in 1% fungicide for twenty minutes and rinsed three times with distilled water. The explants were soaked in 10% commercial bleach (5.25% w/v sodium hypochlorite) for ten minutes and washed three times with sterile distilled water. Last, the explants were immersed in 70% alcohol for three minutes before rinsing three times with sterile distilled water. The explants were dried and excised to 1 cm each. Sterilization process was carried out in laminar airflow to maintain sterility.

Medium preparation and culture induction

The 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 twenty minutes. The explants were inoculated onto MS media inside laminar airflow to maintain sterility. The explants were incubated at 25 ± 2ºC under a 16-h light and 8-h dark cycle provided by cool white fluorescent lights. The incubation was performed for two weeks before transferred into new media after radiated.

Gamma radiation of explants

Two weeks old stock of *V.planifolia* was exposed to four different doses of gamma radiation, (0 Gy, 10 Gy, 20 Gy and 30 Gy). The radiation was made in a gamma chamber in Agensi Nuklear Malaysia, Bangi, Selangor. The explants were immediately transferred into treatment MS media and sub-cultured every three weeks.

Methyl jasmonate of explants

The different media treatment used was prepared by using methyl jasmonate stock solution (1mg/ml) adjusted to various concentrations. The stock was prepared by dissolving powdered methyl jasmonate in 70% ethanol and further diluted with distilled water. The solution then was filtered using 0.2 µm microfilter into sterilized MS media in laminar airflow to maintain sterility. The different types of methyl jasmonate concentrations (0 µM, 10 µM, 20 µM, 40 µM, 80 µM) were used in this study. The two weeks old stock of *V.planifolia* were immediately transferred into the treatment media and sub-cultured every five weeks.

Statistical analysis

Multivariate analysis of variance at 5% level was done using SPSS18. All data were expressed as mean±SEM (standard error mean) with statistical significance at $P \leq 0.05$.

RESULTS

Multivariate analysis test (MANOVA) was done at 5% significant level and the result showed that there was a significant difference ($P \leq 0.05$) between all parameters measured which were shoot height, leaf number, root length and root number after ten weeks of incubation. The result indicated that there was indeed a correlation between all parameters in the regeneration of *V.planifolia* after the treatment with methyl jasmonate and gamma radiation.

Shoot Height

Figure 1 showed the shoot height of vanilla cultures in methyl jasmonate media (0 µM, 10 µM, 20 µM, 40 µM, 80 µM) after a low gamma radiation (0 Gy, 10 Gy, 20 Gy and 30 Gy) treatment. The highest shoot height among all the treatments was obtained from vanilla cultures grown in 20 µM methyl jasmonate media after 10 Gy gamma irradiation while the lowest shoot height among all treatments was obtained from 80 µM methyl jasmonate media after 30 Gy gamma irradiation. The vanilla cultures that went through 10 Gy gamma irradiation produced a substantially higher shoot height among all treatments, however the result only occurred within the three lowest concentration of methyl jasmonate (0 µM, 10 µM and 20 µM). Moreover, there was no significant different ($P \geq 0.05$) between 0 µM and 10 µM methyl jasmonate of the 10 Gy gamma irradiated cultures. For the overall results, 40 µM and 80 µM methyl jasmonate media displayed the lowest shoot height among all of the cultures. The multivariate analysis results showed that there was a significant different ($P \leq 0.05$) between the methyl jasmonate treatment and gamma irradiation on the height of the vanilla cultures.

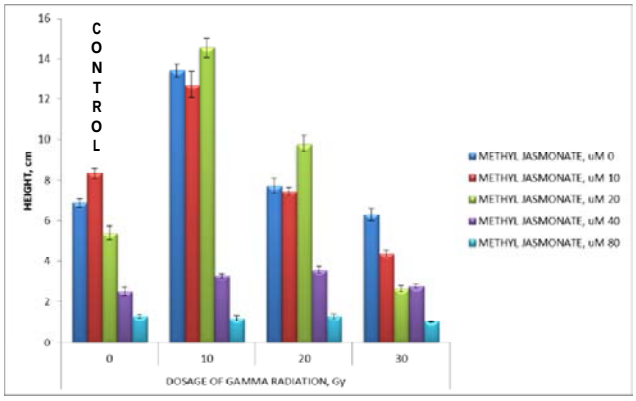

Figure 1: Effect of low gamma radiation and methyl jasmonate on shoot height of vanilla tissue culture after ten weeks of incubation

Leaf Number

Figure 2 showed the leaf number of vanilla cultures in methyl jasmonate media (0 µM, 10 µM, 20 µM, 40 µM, 80 µM) after a low gamma radiation (0 Gy, 10 Gy, 20 Gy and 30 Gy) treatment. Similar to the result in shoot height of vanilla cultures, the highest leaf number among all of the treatments was obtained from vanilla cultures grown in 20 µM methyl jasmonate media after 10 Gy gamma irradiation. Meanwhile the lowest leaf number among treatments was obtained from 80 µM methyl jasmonate media without gamma irradiation treatment. Moreover, vanilla cultures in MS media after being treated with 10 Gy gamma ray also showed a significantly ($P \geq 0.05$) higher leaf number compared to other treatments whereas the rest of cultures were comparable among each other, except for 40 µM and 80 µM methyl jasmonate media,

where they showed the lowest leaf number among all treatments. There was a significant different (P ≤ 0.05) between the methyl jasmonate treatment and gamma irradiation on the leaf number of the vanilla cultures.

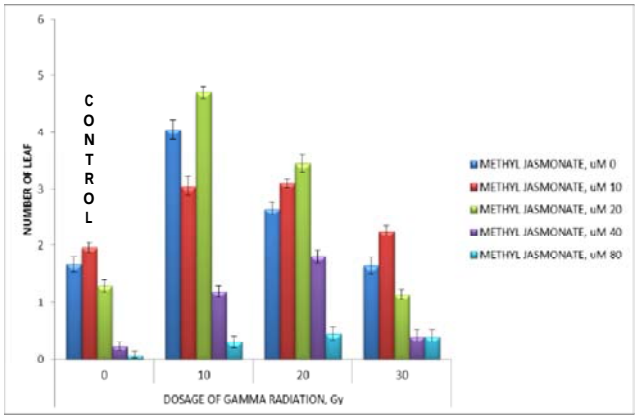

Figure 2: Effect of low gamma radiation and methyl jasmonate on leaf number of vanilla tissue culture after ten weeks of incubation

Root Length

Figure 3 showed the root length of vanilla cultures in methyl jasmonate media (0 µM, 10 µM, 20 µM, 40 µM, 80 µM) after a low gamma radiation (0 Gy, 10 Gy, 20 Gy and 30 Gy) treatment. The vanilla cultures obtained from 20 µM methyl jasmonate media after a 10 Gy gamma irradiation was considerably higher when compared to the other treatments.

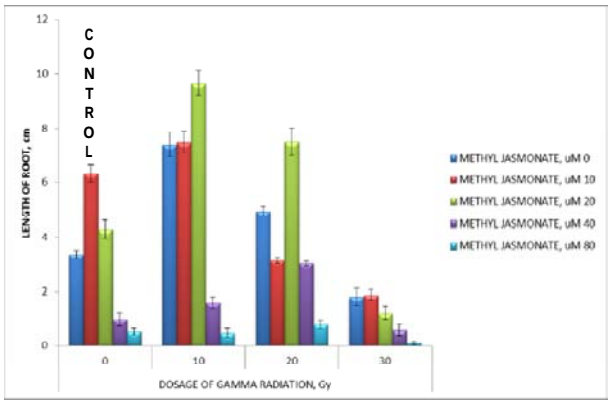

Figure 3: Effect of low gamma radiation and methyl jasmonate on root length of vanilla tissue culture after ten weeks of incubation

A high root length was also obtained from 0 µM and 10 µM methyl jasmonate after a 10 Gy gamma irradiation however there was no significant different (P≥ 0.05) between the two concentrations. A higher root length was also recorded from 20 µM methyl jasmonate media after a 20 Gy gamma irradiation and 10 µM methyl jasmonate media of non-irradiated cultures. The 30 Gy gamma irradiated cultures showed an overall result for the lowest root length, and the cultures in 80 µM methyl jasmonate media were the lowest root length recorded among the treatments. There was a significant different (P ≤ 0.05) between the methyl jasmonate treatment and gamma irradiation on the root length of the vanilla cultures.

Root Number

Figure 4 showed the root number of vanilla cultures in methyl jasmonate media (0 µM, 10 µM, 20 µM, 40 µM, 80 µM) after a low gamma radiation (0 Gy, 10 Gy, 20 Gy and 30 Gy) treatment. Similar to other results, the highest root number was also obtained from 10 Gy gamma irradiated cultures in 20 µM methyl jasmonate media, followed by 0 µM methyl jasmonate and there was a significant different (P ≤ 0.05) between both of the treatments. The 20 Gy gamma irradiated cultures showed a high root number in 0 µM and 20 µM methyl jasmonate media. Meanwhile, the lowest root number was obtained from 80 µM methyl jasmonate media after a 30 Gy gamma irradiation. The overall lowest root number was obtained from 30 Gy gamma irradiated cultures. There was a significant different (P ≤ 0.05) between the methyl jasmonate treatment and gamma irradiation on the root number of the vanilla cultures.

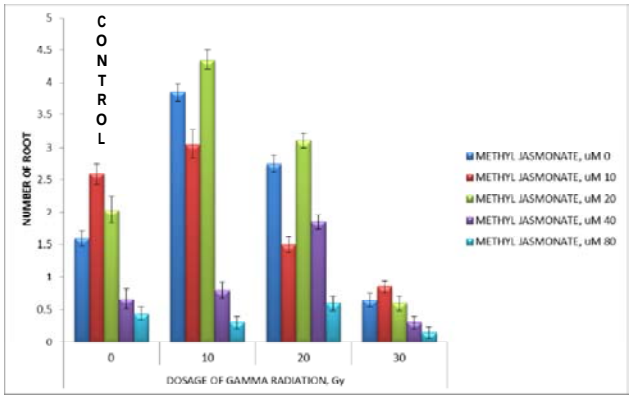

Figure 4: Effect of low gamma radiation and methyl jasmonate on root number of vanilla tissue culture after ten weeks of incubation

DISCUSSION

In comparison to the control, vanilla cultures that undergone gamma irradiation treatment showed more prominent plant regeneration. This is revealed from the overall results, where the highest growth in term of shoot height, leaf number, root length and root number was originated from 10 Gy gamma irradiated vanilla cultures. A research done on two commercial red peppers found that low dose gamma irradiations increased the photosynthetic rate on the plant tissue culture.[18] Internal metabolism in plant depend highly on photosynthesis because photosynthesis is the fundamental source of energy for growth and development. If the photosynthesis rate increases, so does the plant metabolism. However, this only applies for a low dose of gamma irradiation because photosynthesis process is sensitive toward heavy metal, where it disturbs the chloroplast function rendering it useless in CO_2 fixation.[19]

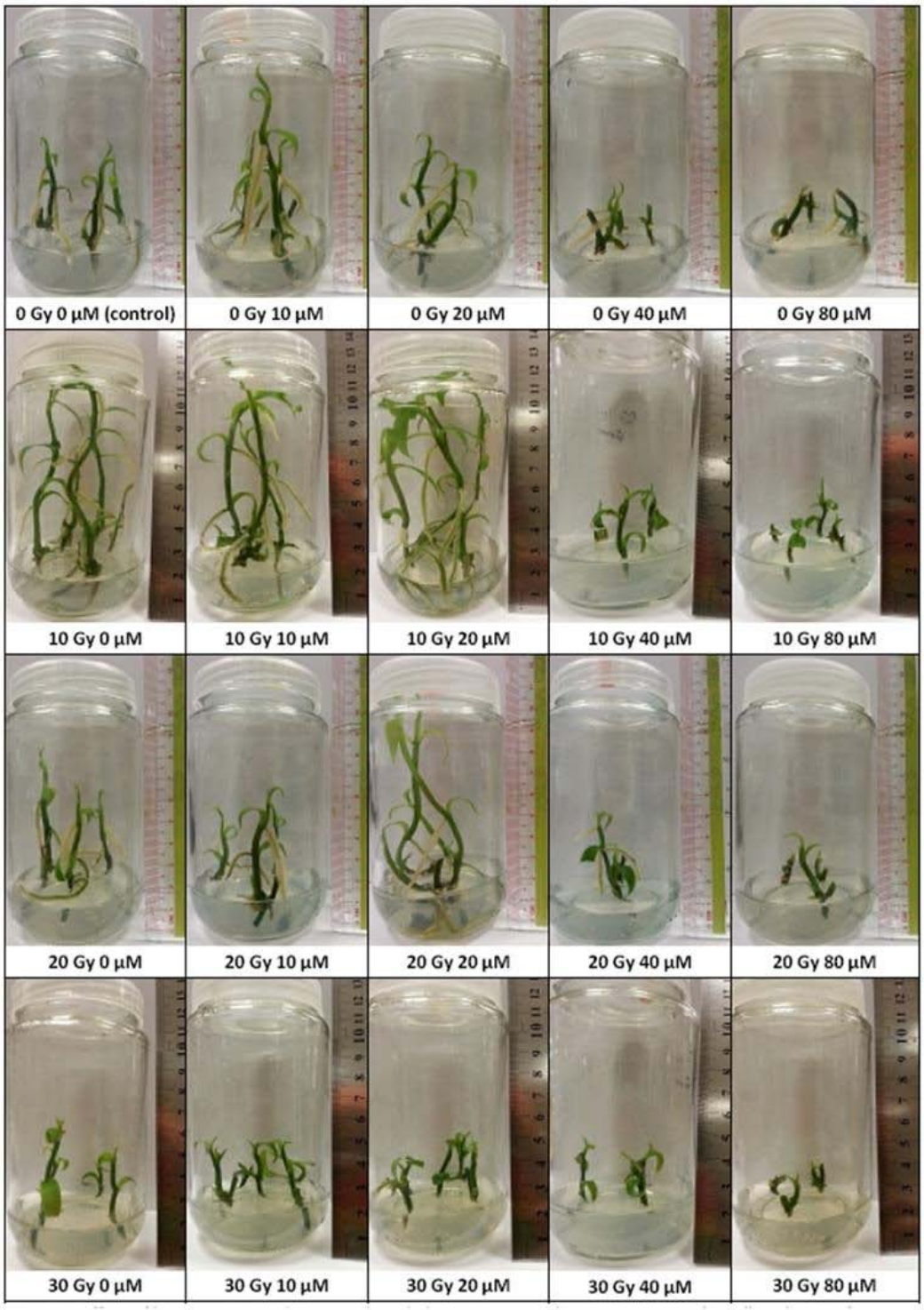

Figure 5: Effect of low gamma irradiation and methyl jasmonate on the regeneration of vanilla cultures after ten weeks.

This is shown in the highest dose, 30 Gy gamma irradiation, where it gave a negative effect that retard the development of the vanilla culture. The other reason gamma irradiation increase the regeneration of vanilla cultures is that radiation significantly influences the protein synthesis and cell metabolism in plant meristem cells. Plants respond to gamma irradiation by developing a defense mechanism that increases the total soluble protein content by altering the protein metabolism, thus influencing the plant cell metabolism and development.[20]

The best methyl jasmonate concentration to improve vanilla cultures regeneration was 20 µM methyl jasmonate media after the cultures irradiated with 10 Gy gamma irradiation. Methyl jasmonate is an elicitor that can stimulate plant defense by acting as a signal that triggered the plant to activate their defense mechanism.[21] This response is crucial for the plant survival as it protects the plant against abiotic stress, wounding and pathogens. However, this only occur at low concentration of methyl jasmonate because at high concentration, the signal caused the activation of cell death program instead of

defense program as seen in the *Arabidopsis* plant.[22] This was shown in all vanilla cultures that were grown in high concentration of methyl jasmonate media (40 µM and 80 µM), where the cultures resulted in a retard regeneration of vanilla. The other reason for increased regeneration of vanilla cultures is that methyl jasmonate up-regulated genes expression of cell wall formation, jasmonate biosynthesis and secondary metabolism.[12]

CONCLUSION

Gamma irradiation and methyl jasmonate has a great potential in stimulating the regeneration of vanilla cultures. However, only low dose gamma irradiation and methyl jasmonate concentration cause a positive result while a at high dose and concentration gave a negative result.

Acknowledgements: The authors would like to thank Hexagon Green Biotech Sdn Bhd and Agensi Nuklear Malaysia for the assistance and cooperation in this research and RMI, UiTM for financial aid, grant no. 600-RMI/DANA 5/3/CG (8/2012).

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Source of Support: Nil, **Conflict of Interest:** None.