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MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF GAMMA IRRADIATED *Vanilla planifolia* ANDREWS ORCHIDS

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Vanilla planifolia is the ultimate source of natural vanilla flavour, which makes it an economically important plant. However, due to its limited genetic diversity, hybrid production is difficult in *V. planifolia*. A feasible means to improve the *V. planifolia* genome is using gamma (γ) radiation. The objectives were to investigate the changes imposed by γ radiation on the morphology of *ex vitro* M_1V_1 (mutation 1 in the first vegetative generation) and *in vitro* M_1V_3 (third generation) and the physiology of *ex vitro* M_1V_1 and M_1V_3 *V. planifolia*. Cuttings were exposed to five doses of γ radiation (10, 20, 30, 40, and 50 Gy), each with five replicates, and propagated *ex vitro* to generate M_1V_1 and M_1V_2. *In vitro* studies were performed by culturing *ex vitro* M_1V_2 explants. Photosynthetic pigments of *ex vitro* propagated M_1V_1 and M_1V_3 plants were measured spectrophotometrically. The extraction of vanillin and vanillic acid from M_1V_1 leaves and roots was done using two methods, and the identification and quantification of the extract contents were performed using liquid chromatography-mass spectrometry-ion trap-time of flight (LC-MS-IT-TOF) and high performance liquid chromatography (HPLC). The result showed that the radiation affected the induction of M_1V_1 compared to M_1V_0 (irradiated cuttings or generation zero). Morphologically, *ex vitro* M_1V_1 shoot height and M_1V_3 *in vitro* root length were significantly reduced ($p < 0.05$), while the root length of M_1V_1 and shoot height of

M_1V_3 were insignificantly affected ($p > 0.05$) when compared with the control (0 Gy). However, the photosynthetic pigments were significantly increased ($p < 0.05$) by the treatment in M_1V_1 and reduced significantly ($p < 0.05$) only in M_1V_3 (30 Gy), except for pigment ratios, which were insignificant amongst the treatments. The presence of vanillin and its derivatives was confirmed in the extract from both methods using LC-MS-IT-TOF. Furthermore, there was a negligible disparity in the quantity of vanillin between the two methods. Using the catalysis method, vanillin quantity was significantly enhanced ($p < 0.05$) in the leaves of M_1V_1. Nevertheless, an insignificant effect was seen on root vanillin content. The amount of vanillic acid was insignificant, regardless of the treatment or plant organ. In summary, γ radiation treatment enhanced photosynthetic pigment levels and vanillin content in the leaves of M_1V_1. Furthermore, the *in vitro* morphology of M_1V_3 plantlets was not impaired morphologically.

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PENCIRIAN MORFOLOGI DAN FISIOLOGI ORKID *Vanilla planifolia* ANDREWS TERSINAR GAMMA

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Vanilla planifolia adalah sumber utama penghasilan perasa vanila semulajadi. Walau bagaimanapun, penghasilan hibrid adalah sukar bagi *V. planifolia* kerana kepelbagaian genetik yang terhad. Cara yang boleh dilaksanakan bagi menambah baik genom *V. planifolia* adalah dengan penggunaan sinaran gamma (γ). Objektif kajian adalah untuk menyiasat perubahan yang berlaku akibat sinaran gamma pada morfologi *ex vitro* M_1V_1 (mutasi 1 dalam generasi vegetatif pertama) dan *in vitro* M_1V_3 (generasi ketiga) dan fisiologi *ex vitro* M1V1 dan M1V3 *V. planifolia*. Keratan didedahkan kepada lima dos sinaran gamma (10, 20, 30, 40, dan 50 Gy) dengan lima replikasi dan dibiakkan secara *ex vitro* untuk menjana M_1V_1 dan M_1V_2. Kajian *in vitro* dilakukan dengan mengkultur eksplan *ex vitro* M_1V_2. Pigmen fotosintetik keratan yang dibiakkan secara *ex vitro* pada M_1V_1 dan M_1V_3 diukur secara spektrofotometri. Dua kaedah digunakan bagi pengekstrakan vanillin dan asid vanilik daripada daun dan akar pada M_1V_1 dan pengenalpastian dan kuantifikasi kandungan ekstrak menggunakan Kromatografi Cecair Spektrometri Jisim-ion-perangkap-masa penerbangan (LC-MS-IT-TOF) dan Kromatografi Cecair Prestasi Tinggi (HPLC) telah dilakukan. Hasil kajian menunjukkan bahawa sinaran gamma memberi kesan kepada generasi teraruh berbanding M_1V_0 (Keratan diradiasi atau generasi kosong). Secara morfologi, ketinggian pucuk *ex vitro* M_1V_1 dan panjang akar *in vitro* M_1V_3 telah berkurangan

dengan ketara ($p < 0.05$), manakala panjang akar M_1V_1 dan ketinggian pucuk M_1V_3 tidak terjejas dengan ketara ($p > 0.05$) berbanding kawalan (0Gy). Walau bagaimanapun, pigmen fotosintesis meningkat dengan ketara ($p < 0.05$) pada rawatan M_1V_1 dan berkurangan dengan ketara ($p < 0.05$) hanya pada M_1V_3 (30Gy), kecuali nisbah pigmen antara rawatan, di mana ianya tidak signifikan antara rawatan. Kedua-dua kaedah dapat mengesan vanillin dan derivatifnya dengan menggunakan LC-MS-IT-TOF. Tambahan pula, terdapat perbezaan yang tidak ketara ($p > 0.05$) dalam tahap vanillin antara kedua-dua kaedah. Kaedah katalisis mengesan kuantiti vanillin yang tinggi ($p < 0.05$) pada daun M_1V_1.Namun begitu, kesan yang tidak ketara ($p > 0.05$) didapati pada akar. Kuantiti asid vanillik adalah tidak ketara ($p > 0.05$) tanpa mengira rawatan atau organ tumbuhan. Secara ringkasnya, rawatan sinaran gamma meningkatkan tahap pigmen fotosintesis dan kandungan vanillin dalam daun M_1V_1. Tambahan pula, morfologi *in vitro* plantlet M_1V_3 juga tidak terjejas secara morfologi.