Abiotic and biotic Elicitors in the propagation and induction of callus and the production of secondary compounds of the ginger plant Zingiber officinale var. Roscoe cv. White in vitro

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Summary

The study was conducted in the laboratory of plant tissue culture of college of Agriculture-University of Basra, Iraq and the laboratories of college of Pharmacy – University of Basra for the period from 21/2/2018 to 2/12/2019. This study aims to know "Abiotic and biotic Elicitors in the propagation and induction of callus and the production of secondary compounds of the ginger plant *Zingiber officinale* var. Roscoe cv. White *in vitro* " and the results are as follows:

1. The MS nutritional medium provided with BA 3 mg L^{-1} with NAA 0.5 mg L^{-1} on all treatments in giving the highest average number of vegetative branches amounted to 10.00 branches per vegetable part and the highest average branch length was 5.16 cm and the highest average number of roots reached 5.00 roots per branch, as well as the emergence of callus around the tips of shoots grown in MS medium supplemented with BA at a concentration of 7 and 10 mg L^{-1}.
2. The study also showed that callus could arise from cultivating half-buds with 100% in MS medium supplemented with 2,4-D at a concentration of 1.0 mg L^{-1} with BA at a concentration of 5.0 mg L^{-1} with a short period of 11.67 days in the dark. The same concentration gave the highest soft and dry weights of callus.
3. The results of planting the terminal half-buds of ginger indicate that the MS medium provided with 30 mg L^{-1} NAA was superior with 2ip 3.0 mg L^{-1} and PVP 500 mg L^{-1} in induction and formation of white callus, as the same concentration gave the highest amount of soft weight. And dry callus compared to concentrations 10 and 20 mg L^{-1} NAA, as the response to callus stimulation was 100% over a period of 11.33 days of incubation in the dark.

4. It was also observed when replacing PVP with activated charcoal 500 mg L^{-1} when planting half-buds concentration exceeded 30 mg L^{-1} NAA, as the percentage of half-bud response to callus stimulation was 100% in a time period of 10.67 days, and it gave the same The concentration was higher fresh and dry weight of callus compared with concentrations 10 and 20 mg L^{-1} NAA, and the emergence of an occasional vegetative branch was observed at the end of the incubation period at the same concentration.
5. The treatment outperformed 90 g L^{-1} sucrose with 2,4-D at a constant concentration of 1.0 mg L^{-1} and BA at a constant concentration of 0.5 mg L^{-1}, as the highest fresh and dry weights of callus were 2.38g and 0.17 g, respectively, compared to with concentrations 30, 60, 120 g L^{-1}, and when the growth of callus was continued at sucrose concentrations 30, 60, 90, 120 g L^{-1} for another eight weeks, it was observed that the developing callus in the MS medium supplied with 2,4-D at a concentration of 1.0 mg L^{-1} with BA at a concentration of 0.5 mg L^{-1} and supplemented with sucrose 60 g L^{-1}, It began to separate and the appearance of green bumps scattered on the surface of the callus, formed in the form of small transverse branches with the appearance of thick white roots containing dense capillaries, as was observed when the callus continued to grow at the age of twelve weeks, not mutating into vegetative branches and adventitious roots in the same nutrient medium, and provided with the same concentrations of sucrose with 2,4-D at a concentration of 1.0 mg L^{-1} + BA at a concentration of 0.5 mg L^{-1} for another four weeks in light, the embryo callus granules transformed into embryonic nodes that developed into somatic embryos of different phases and sizes at concentration 30 and 60 g L^{-1} while embryo callus did not develop into somatic embryos at high concentrations 90 and 120 g L^{-1}.

6. The treatment with proline at 150 mg L^{-1} with 1.0 mg L^{-1} 2,4-D at a fixed concentration and 0.5 mg L^{-1} BA at a fixed concentration outperformed it, as it achieved the highest rate of soft and dry weights of callus of 2.73 g and 0 18 g, compared with the rest of the concentrations, and upon the continuation of the growth of callus in MS medium, which was supplied with different concentrations of proline 0, 50, 100, 150 mg L^{-1}, and after twelve weeks after the initial cultivation, the growth and reproduction of a number of primary roots were observed, corresponding to their density. Directly with an increase in the concentration of proline in the nutrient medium, it was observed when the growth of non-mutated callus (at the age of twelve weeks) to adventitious roots on the MS with the same concentrations of proline, and for a period of four weeks in light, it was observed that the callus mutated into embryos of different stages.
7. The treatment inoculated with *A. tumefaciens* superior the LBA4404 strain with a concentration of 10^1 and 10^3 bacterial cells Ml^{-1} with 2,4-D at a constant concentration of 1.0 mg L^{-1} and BA at a constant concentration of 0.5 mg L^{-1}, as it achieved the highest rate of soft and dry weights of callus. C58 and AGL1, the concentration of 10^1 bacterial cells exceeded Ml^{-1} in giving the highest soft and dry weights of callus.
8. The proportion of acclimatized plants reached 40% when planting in sterile peat moss: sand in proportions of 1: 1.
9. The results indicate that the growing callus tissue in the MS medium supplied with sucrose was better at a concentration of 90 g L^{-1} in its carbohydrate content and proline, while the developing callus in the dietary medium MS supplied with proline at a concentration of 150 mg L^{-1} was outperformed in its content of carbohydrates and proline.

10. Callus grown in MS medium supplied with *A. tumefaciens* superior the LBA4404 and C58 strains with a concentration of 10^1 bacterial cells Ml^{-1} in its total carbohydrate content, while the comparison treatment was superior in all treatments of the bacterial strains in its content of proline.
11. The results of the analysis of the HPLC liquid chromatography showed that adding sucrose to the MS medium had a concentration of 90 $g.L^{-1}$ resulted in increased production of secondary compounds in the tissue of ginger callus from Zingerone, 6-gingerol and 6-Shogaol compared with the comparison treatment.
12. It was observed that when adding proline at a concentration of 150 mg L^{-1} to the MS medium, it resulted in an increase in the production of secondary compounds in callus from Zingerone, 6-gingerol and 6-Shogaol compared with the comparison treatment.
13. When treated with *A. tumefaciens*, the strain LBA4404 with a concentration of 105 bacterial cells ML^{-1} led to the accumulation of secondary compounds in the callus tissue, as it gave the highest concentration of Zingerone, followed by the concentration of 10^1 bacterial cells ML^{-1}, which gave the highest concentration of the three secondary compounds compared to the comparison treatment. Treatment 10^1 bacterial cells ML^{-1} for strain C58 gave the highest concentration of Zingerone, followed by a concentration of 10^3 bacteria cells ML^{-1}, which gave the highest concentration of 6-gingerol and 6-Shogaol compared with the comparison treatment. Treatment 10^1 and 103 bacteria cells also exceeded ML^{-1}. AGL1 strain gave the highest concentration of the secondary compounds 6-gingerol and 6-Shogaol in the treated ginger plant callus tissue compared with the comparison treatment.