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EFFECT OF SODIUM NITROPRUSSIDE WITH PLANT GROWTH REGULATORS ON *IN VITRO* PROPAGATION AND GENETIC STABILITY OF 'BARHEE' DATE PALM (*PHOENIX DACTYLIFERA* L.)

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ABSTRACT

Date palm micropropagation still faces many limitations in practical applications due to tissue browning, reduced callus growth and development, low organogenesis and multiplication efficiency, and low rooting rate. This study investigated the effects of sodium nitroprusside (SNP), a source of nitric oxide and plant growth regulators, on the growth attributes and some biochemical constituents of *in vitro* cultured 'Barhee' date palm. The addition of SNP to the culture media was more effective than the individual application of growth regulators regarding callus growth, shoot regeneration, and the number of shoots per jar. The best results (338 mg callus, 80% shoot regeneration, and 17.5 shoots per jar) were obtained in the medium with the addition of 20 µM SNP, plus 90.5 µM 2,4-dichlorophenoxyacetic acid (2,4-D) for callus growth, and 17.7 µM 6-benzylaminopurine (BAP) for shoot regeneration. The above treatment reduced the amount of soluble phenolic compounds in the callus, reducing browning. Effective micropropagation was accompanied by an increase of chlorophyll content and a decrease in the levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2). The use of 20 µM SNP in combination with 5.37 µM naphthalene acetic acid (NAA) was most effective on root induction and the number of roots per shoot (93.34% and 7.14 roots per shoot). Monomorphic banding patterns obtained in RAPD-PCR and ISSR-PCR in tissue culture-derived and parental plants confirmed genetic stability. Based on these results, nitric oxide can be considered as an intermediary of callus stimulation, adventitious shoot regeneration, and root induction.

Key words: adventitious regeneration, callus, nitric oxide, genetic fidelity, molecular markers, rooting

INTRODUCTION

The date palm, or "tree of life", is one of the most beneficial plants in nature, and demand for its fruit is growing. However, date palm production is threatened by aging plantations, pests, and diseases. Traditionally, date palms are propagated by offshoots, which is inefficient because each tree produces several offshoots, especially in some cultivars, and fruiting can take up to seven years (Jasim et al. 2009; Gantait et al. 2018). Al-Mayahi (2021) suggested that to overcome the limitations of traditional propagation methods, propagation through *in vitro* culture is essential. Therefore, the development and improvement of *in vitro* culture techniques in this date palm is of paramount importance. *In vitro* shoot regeneration and multiplication largely depend on the selection of a suitable culture medium (Awad et al. 2020; Al-Mayahi 2022a), including growth regulators (Al-Asadi et al. 2019; Al-Mayahi 2022b). *In vitro* tissue growth requirements may vary depending on species and source of initial explants. In addition to the essential hormonal substances, auxins, cytokinins, and gibberellins, adding other substances to the growth medium can contribute to more effective propagation (Al-Mayahi 2023, 2024a). Nitric oxide (NO), now considered a messenger molecule involved in the regulation of plant growth and development (Neill et al. 2003), has been classified as a phytohormone-like (Leterrier et al. 2012).

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