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"Bridging the gap between increasing knowledge and decreasing resources"

Assessment of Genetic Fidelity of Micropropagated Plants and *in vitro* Polyploidisation in *Monarda didyma* L.

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Abstract

Crimson beebalm (*Monarda didyma* L.) is a medicinal plant belonging to the family Lamiaceae, native to North America. Crimson beebalm has a high content of thymohydroquinone, dithymoquinone and thymoquinone. The main objective of this study was the development of an appropriate protocol for *in vitro* propagation of Crimson beebalm by using nodal segments and to obtain tetraploid plants (2n=64 chromosomes) from diploid plants (2n=32) by *in vitro* induced mitotic polyploidisation. For micropropagation the nodal segments were cultured on basal MS medium supplemented with different concentrations of 6-benzylaminopurine (BAP), kinetin (KIN), indolyl-acetic acid (IAA) and naphthalene acetic acid (NAA) and with cytokinins/auxins combination of BAP with IAA and KIN with NAA for shoot and root induction. For the polyploidisation nodal segments of *Monarda* were exposed to 40, 60 and 80 μM oryzalin for 24 and 48 h. Genetic fidelity in regenerated plants was assessed using RAPD (Randomly Amplified Polymorphic DNA) markers.The highest multiplication rate was obtained from MS medium containing 0.5 mg l^{-1} of KIN (1.90±0.31 shoots per plant) and 1.5 mg l^{-1} of KIN (5.60±2.16 new nodes on longer shoots). The best root induction was achieved on medium supplemented with 1.0 mg l^{-1} IAA (6.70 ±4.84 roots per plant). Cultivation time was 60 days. The percentage of survival of plantlets under *ex vitro* conditions was 30 %. Tetraploid plants (2n=64) were obtained in concentration of 40 and 60 μM of oryzalin with treatment duration of 24 h. Triploid plant (2n=48) was obtained in concentration of 60 μM of oryzalin with treatment duration of 48 h. In total, the polyploidisation efficiency was 1.92 %. RAPD analysis confirmed the genetic stability in micropropagated and polyploid plants.

Keywords: Genetic fidelity, *in vitro*, Lamiaceae, micropropagation, *Monarda didyma*, oryzalin, polyploidisation, root induction, shoot induction

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