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Development of polyploid genotypes in *Mentha spicata* using *in vitro* somatic polyploidisation

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Abstract

Mentha spicata (spearmint) is a widely utilised aromatic herb belonging to the lamiaceae family. It is grown across the globe for its wide range of pharmacological uses. Traditionally, it has been used to treat various respiratory and gastrointestinal conditions. Consequently, numerous research has been done on the breeding of *Mentha* spp. using traditional methods, although very few studies have explored the scope of synthetic polyploidisation in *Mentha* spp. and none in *Mentha spicata*. Hence, the aim of the current study was to obtain polyploids of *Mentha spicata* using oryzalin via *in vitro* somatic polyploidisation. Nodal segments were cultured under *in vitro* conditions on Murashige and Skoog (MS) for 48 hours prior to oryzalin treatment. Thereafter, nodal segments were treated with three oryzalin concentrations (20, 40, and 60 M) for 24 and 48 hours. Flow cytometry and direct chromosome counting were then used to confirm the ploidy of the treated plant. Obtained polyploids were micro-propagated and transferred to be grown under field conditions. Thereafter, morphological, anatomical, and biochemical data were collected for further statistical analysis. Oryzalin treatment yielded a total of 7 polyploids across all treatments. Oryzalin at 40 M concentration for 48 h was found to be the most effective treatment with a polyploid induction rate of 8%. Furthermore, obtained morphological, anatomical, and biochemical data exhibited a significant difference between triploid and hexaploid plants. For instance, the leaf area, and thickness increased by almost 50 percent, and a higher trichome density was achieved in hexaploidy plants compared to the mother triploid plant. Additionally, larger stomata size and higher chlorophyll content indicate a higher photosynthetic capacity in polyploids. The results obtained provide valuable insights into the breeding possibilities in *Mentha spicata* and related species.

Keywords: Autopolyploidy, hexaploidy, *in vitro*, *Mentha spicata*, oryzalin, polyploidisation