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CAN CRYOTREATMENT BE A TOOL USEFUL IN MUTATION BREEDING?

The impact of cryo-treatment (storage in liquid nitrogen) on the stability of plant material is still ambiguous. It would be interesting to learn, whether liquid nitrogen could be a potentially valuable tool in mutation breeding. Therefore, the aim of the study was to evaluate the stability of three chrysanthemum cultivars cryopreserved via encapsulation-dehydration technique. Shoot tips were precultured on MS medium supplemented with 10 µM ABA for 7 days, encapsulated in sodium alginate, and then, osmotically dehydrated in sucrose gradient for four days, followed by 3-hour air desiccation. The pre-treated shoot tips were stored in liquid nitrogen (-196oC). After thawing, the recovered micro-shoots were acclimatised and cultivated in glasshouse conditions. The plants were subjected to molecular, cytogenetic, biochemical and phenotypical analysis. By applying RAPD and ISSR markers, 18 polymorphic bands within 5-6% of cryopreservation-obtained chrysanthemums were detected. Usually no changes in the flower pigment content were detected. Only one plant produced a completely new phenotype, i.e. with mosaic leaves (forma variegata) and a double-coloured inflorescence (Figure). Interestingly, the colours of the flower got reversed in the second vegetative generation, and no alternation of this parameter was observed in the third generation, as compared with the control. The variegated leaves, however, remained stable the whole time. The recovered after cryopreservation and control plants had the same diameter and fresh weight of inflorescences, as well as, ray florets length. On the other hand, the analysis revealed that the bottom and central leaves of all cryopreservation-derived plants were shorter and thinner, and had a reduced amount of chlorophyll, while their internodes were shorter as compared to the untreated control. Furthermore, inflorescences of one cultivar opened slower, but faded faster.

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