J Plant Biochem Physiol 2018, Volume 6
DOI: 10.4172/2329-9029-C1-003

6th Global Summit on Plant Science

October 29-30, 2018 | Valencia, Spain

In vitro conservation of heterozygous plants: A case study of date palm

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With heterozygous plants, vegetative (asexual) propagation is usually used for commercial production/propagation of true to type genotypes. Moreover, special approaches include maintenance in field gene banks and the storage in cold stores of dormant vegetative forms is required for conservation of such germplasm. However, these methods have limitations regarding efficiency, costs and long-term maintenance. Since date palm is a dioecious and heterozygous fruit tree, and for commercial purposes most often vegetatively propagated through offshoots, it is difficult to store or handle its germplasm by conventional means. Biotechnology techniques offer an alternative method for conservation of such plant material. In this respect, we set up protocols for preservation of date palm tissue cultures for short term (3–6 months) by addition of osmotic agents to culture medium and for mid-term (9-12 months) by reducing incubation temperature to 5°C. Moreover, cryopreservation has been recognized for *in vitro* long-term storage of date palm cultures. Undifferentiated tissue and somatic embryos were cryopreserved by freezing methods. A number of steps have been followed for cryopreservation, which includes: pre-culture in media with verification compounds [22% (w/v) glycerol, 15% (w/v) ethylene glycol, 15% (w/v) propylene glycol and 7% (w/v) dimethyl sulfoxide], treatment with cooling, storage at -196°C thawing and recovery. Furthermore, a successful system for preservation of date palm germplasm via artificial seeds was realized. Somatic embryos (at late cotyledon stage) proliferated *in vitro* from shoot tip cultures were encased in sodium alginate (3%) capsules and stored for 12 months and then regenerated to plantlets.

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