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Evaluation of genetic fidelity of *in vitro* regenerated Sweet flag (*Acorus calamus* L.) using molecular markers

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Sweet flag (*Acorus calamus* L.) is an endangered, perennial wild herb known for its high medicinal properties. An efficient *in vitro* regeneration protocol for sweet flag was developed and the genetic fidelity was assessed across *in vitro* regenerants. Murashige & Skoog media fortified with different plant growth regulators (PGRs) in various concentrations and combinations were used to culture rhizome buds. For surface sterilization of explants, treatment of fungicide (0.2% Bavistin) for duration of 30 min followed by treatment with 70% ethanol for 30 s and mercuric chloride (0.1%) for 15 min was found most effective. MS solid media (0.8% agar) was found more promising than MS semisolid media (0.6% agar) in relation to explants establishment. The maximum number of shoots per explant was observed in solid MS medium supplemented with 15 µM thidiazuron (TDZ) with an average of 7.67 shoots per explants and average explant response of 60%. Shoot length response was maximum in MS containing 10 µM 6-benzylaminopurine (BAP) in combination with 5 µM 1-naphthalene acetic acid (NAA) with an average length of 6.88 cm and percentage explant response of 80%. For root induction, MS medium containing 10 µM indole-3-butyric acid (IBA) gave the maximum number of roots (6.50) and root length (3.50 cm). Plants acclimatized well with 57.14% survivability rate. Forty random amplified polymorphic DNA (RAPD) primers were used to evaluate genetic fidelity among *in vitro* regenerated plants, one from each treatment and compared with mother plant. Out of them, 20 RAPD primers produced a total of 70 fragments ranging from 350-2500 bp. The amplified bands of all *in vitro* plant samples and mother plant were observed to be monomorphic. The results showed that the *in vitro* regenerated plants were genetically stable.

Keywords: Monomorphic amplified bands, Random amplified polymorphic DNA (RAPD)