ABSTRACT

Tuberose (Family: Amaryllidaceae) and gladiolus (Family: Iridaceae) has been acclaimed as important floricultural crops and are commercially valued as cut flowers. They perpetuate by bulbs and corms respectively. They take about 2-3 years to come to maturity and are often infested by bacteria, fungi, viruses, nematodes etc. This in turn decreases the production rate both qualitatively and quantitatively. To meet the increasing demand of quality product it becomes important to speed up their propagation. Micropropagation in vitro can therefore, be distinctly advantageous for the large-scale propagation of uniform plants with desirable attributes of economic value. Furthermore, in vitro technology coupled with genetic engineering techniques could serve as an important alternative for the rapid propagation of disease-free elite varieties. The present study successfully describes the establishment of an efficient plant regeneration system in tuberose and gladiolus.

The in vitro organogenic competence of leaf cultures of tuberose was studied. Leaf calluses induced on MS medium containing low concentrations of NAA (0.2-0.5 mg/l) in combination with 2.0 mg/l BA were found to be competent for shoot regeneration. A relatively narrow range of NAA/BA combination was capable of inducing shoot buds within 3 weeks of culture. An increased frequency of shoot regeneration was obtained upon transfer of regenerating calluses onto 1.0 and 2.0 mg/l BA supplemented medium. Multiple shoot regeneration was achieved from axillary buds, preconditioned with NAA (0.2-0.5 mg/l) and BA (2.0 mg/l), on MS medium supplemented with BA (0.5-4.0 mg/l) after 4 weeks of culture. Various factors controlling shoot organogenesis were studied. However, they had little or no influence on increasing the shoot regeneration capacity. A histological study indicated the *de novo* nature of shoot organogenesis. The regenerated shoots proliferated and rooted efficiently on MS medium supplemented with 0.2-0.5 mg/l auxin (NAA or IBA) and sucrose (3%, 6% or 9%). Rooted plantlets were successfully transferred to soil-rite mixture and appeared morphologically normal. This work successfully demonstrates the complete regeneration of plants via shoot organogenesis from leaf callus cultures of tuberose for the first time.

An efficient and reproducible regeneration protocol was developed via the formation of protocorm clusters, from leaf derived callus, and somatic embryos, from root segments, in gladiolus. Creamish-yellow, nodular compact leaf callus was obtained on NAA (1.0-4.0 mg/l) supplemented callusing media which proliferated to produce meristematic clusters upon transfer to combinations of NAA (0.2-1.0 mg/l) and BA (2.0-4.0 mg/l). The meristematic clusters consisted of protocorms, which had swollen base with embryo axis-like shoot that appeared to be different from somatic embryos. The protocorms germinated into either single or multiple shoot upon keeping on the same

media indicating differential morphological behaviour. Based on the distinctness of the apex and on the symmetry along the axis the protocorms were categorized into two groups (I and II). Group I consisted of protocorms that were symmetric along the major axis with a distinct apex and thus referred to as normal. Those with flattened and/or curved apex were referred to as malformed and placed under Group II. Manipulation of sucrose and NO_3^- / NH_4^+ ratio in the induction medium affected the protocorm differentiation. Regenerated plantlets with single shoot upon transfer to NAA, IBA and IAA (0.2-1.0 mg/l) supplemented media resulted in well-developed roots within 2 weeks of culture. Corm development was noted after another 4-6 weeks. Plantlets under combination of NAA (0.2 and 0.5mg/l) and ancymidol (1.0-4.0 mg/l) developed shorter and thick roots. Somatic embryogenesis could be achieved from the thick peg-like roots on NAA (0.2 or 5.0 mg/l) and ancymidol (1.0-4.0 mg/l) supplemented MS medium. The differentiation of the somatic embryos was continuous and asynchronous. Histological analysis revealed that somatic embryos have the morphology of typical grass embryos with distinct coleoptile, scuttellum, and root shoot meristems. Germination of somatic embryos was evident with the emergence of shoots under the same cultural conditions used for embryo induction and development. Somatic embryo germination into root and shoot axes occurred on MS-0 medium with 0.8% agar and 3% sucrose within 2 weeks of incubation. This study for the first time demonstrated a unique developmental pattern in gladiolus and somatic embryogenesis from in vitro raised root segments.

The dimorphic nature of gladiolus protocorms prompted us to develop an image analysis system that could separate the group I and group II protocorms. Such a system when coupled to a large-scale production unit would be of immense value for non-invasive, low cost separation of the viable protocorms. A vision-guided system has successfully been developed to separate the viable propagules. It has immense potential in automation of gladiolus micropropagation.