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Short Communication

Plant regeneration from inflorescence cultures of *Gynandropsis pentaphylla* DC.

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ABSTRACT

An efficient protocol for shoot regeneration from immature inflorescence of *Gynandropsis pentaphylla* DC (Capparidaceae) is described. Healthy inflorescences were cultured on MS medium supplemented with BAP, Kn, zeatin, TDZ (0.5 - 5.0 mg/l) alone and in combination with IAA, NAA, IBA (0.5 - 2.0 mg/l). BAP (3.0 mg/l) with IBA (1.0 mg/l) as well as Kn (1.0 mg/l) with TDZ (0.1 mg/l) were found to be effective in inducing callus and in production of shoots and roots. The present investigation also describes the histological studies depicting various stages that occurred during the development of embryoids and shoot buds. The species is so potential that the flower buds on the medium induces callus from each part of the flower bud. All the *in vitro* raised shoots were transferred to MS rooting liquid medium supplemented with 0.5 - 1.0 mg/l IAA, NAA and IBA. Well rooted plantlets were transferred to polycups containing soil : vermiculite (1:1) for hardening. Finally the hardened plantlets were transferred to field conditions for maximum survivability. The flower is mixoploid and hence the regenerated roots were squashed in acetoorcein to know the ploidy level of the regenerated shoots. All the plants regenerated were shown to be diploid.

INTRODUCTION

In the last four decades, a number of studies dealing with the *in vitro* culture of flower buds were conducted many of which involved the growth of buds from an early stage of development.*In vitro* culture of immature inflorescence is a useful technique for investigating nutritional and hormonal factors for callogenesis and regeneration of shoot buds.

Gynandropsis pentaphylla, an erect rather showy glandular, pubescent annual herb, is well known for its immense medicinal properties viz., indigenous medicine in the same way as mustard, rubifacient, vesicant and used as counter irritant in headache, neurolgia, rheumatism, anthelmintic, etc. Due to its immense medicinal value the present investigation was carried out to find out its regenerative potentials from inflorescence. A successful method of propagation through tissue culture would be very useful for propagating selected genotypes on a large scale in a short period of time. The success in obtaining normal growth of buds to maturity in both dicotyledons (Nataraja, 1964) and monocots (Guha and Johri, 1966) was limited. Structural analysis is an important step in the study of the organization and changes in plant body and it is an extremely useful approach in the study of plant morphogenesis.

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Tepfer *et al* (1963, 1966) used a complex medium for the growth of young floral buds of *Aquilegia* and although the initiation and early development of the different organs was obtained, the petals did not grow to maturity and stamen primordia aborted at later stages.

MATERIALS AND METHODS

Vigorous floral shoots of *Gynandropsis pentaphylla* were excised, washed with tap water, surface-sterilized with 0.1% mercuric chloride for 5-6 min, rinsed with absolute alcohol for 10 sec and again washed in sterile distilled water 7-8 times. The floral segments were aseptically placed on the initial medium (MS, 1962) supplemented with various growth regulators. All the media were adjusted to pH 5.8 with IN KOH or 1N HCl before autoclaving at 1210C, for 15 min. All the cultures were maintained at 21 ± 20C and under cool, white, fluorescent (16h photoperiod) light providing a quantum flux density of 45 □ mol m2 s-1. To stimulate proliferation the basal medium was supplemented with the cytokinin BAP and auxin IAA or IBA in combination. Embryos showing proliferation were retained on the induction medium until shoots were at least 2 mm in length and were then transferred to basal medium supplemented with GA3 for shoot elongation.

The shoots were excised individually and rooted in MS liquid medium supplemented with IAA or NAA. After rhizogenesis the plantlets were transferred to moist sterilized potting mixture in seedling trays. After acclimatization the plants were then moved to a glasshouse.

`For histological analysis flower buds were fixed at regular intervals like 3-day-old, 5-day-old, 7-day- old and 10-day-old inoculated flower buds in Carnoy's fixative for 24-48 hours and stored in 70% ethanol (Johansen, 1940). Microtome sections at 11 µm thickness were prepared by conventional method and the sections were stained with propionohaematoxylin.

RESULTS AND DISCUSSION

The explants remained quiescent for 1 week and thereafter started callusing (Fig. 1) from each flower bud of the inflorescence on MS + BAP (3 mg/l) + NAA (1 mg/l) and on MS + BAP (3 mg/l) + I BA (1mg/l). About 50% of the cultures responded in producing callus. After 5 weeks of culture in some of the replicates roots were produced. However, further growth of the roots was arrested. The callus became nodular and light brown and differentiated shoots in 20% cultures. These shoots were excised and implanted on a fresh medium. Within 5 weeks the shoots attained a size of 2.0 - 2.5 cm with 3-4 pairs of normal leaves. The shoots started showing senescence effect on hormone-fetched medium. They were, therefore, excised and transferred to MS half strength and full strength liquid medium supplemented with IAA or NAA (0.5 - 1 mg/l) for root induction.

In order to increase the percentage of differentiating shoots, different concentrations of kinetin and TDZ were tried. Maximum number of cultures showing shoots was achieved with Kn (1.0 mg/l) + TDZ (1.0 mg/l) + IAA (1.0 mg/l). These shoots were excised and transferred to liquid medium for rhizogenesis. Roots were regenerated from the base of the excised shoots. These tiny plantlets were transferred to moist sterilized potting mixture and acclimatized when transferred to glasshouse and then to garden soil. Histological studies of the embryogenic callus revealed the presence of cell clusters stimulating early stages in embryogeny which further leads to the development of various shapes of embryoids such as cordate and cotyledonary stages (Fig.2). The vasculature was very well differentiated at the base of each embryoid.

Embryogenesis has been thoroughly defined in the culture of flower buds starting from the callusing of each and every part of the flower. The calyx lobes at first showed curling from day-3 after inoculation. In the 5-day-old flower bud callusing was seen both in petals and sepals. In the 7-day-old flower buds each and every part of the flower bud callused. In the 10-day-old flower bud the histological sections showed the various types of embryoids along with the differentiation of shoot bud (Fig. 3) with a pair of primordial leaves (Fig. 4). In some replicates the gynandrophore in the section showed the development of histones on which a globular embryoid initiated with two-celled-suspensor (Fig.6). Likewise 4-celled-proembryo and a globular embryoid with a single-celled stalk differentiated from the proliferated callus of petal.

Another peculiar feature observed is the development of an embryoid on a globular embryoid with four celled proembryo (Fig.5).Apparently this is the first reported attempt to culture flower bud of *G. pentaphylla* to investigate the embryogeny as there are only few reports from other plants. The requirement for floral bud growth is not surprising in view of the established role of cytokinins as promoters of cell division in cultured tissues. Hicks and Sussex (1970) in tobacco demonstrated the essentiality of kinetin for the growth and development of floral organs to maturity. In floral buds of *Aquilegia*, grown on a medium containing coconut milk, kinetin and gibberellic acid, the initiation of all the organs occurred except the carpels which required the presence of IAA (Tepfer et al., 1966). Quite contrary to this in our investigation the growth of floral organs did not show further growth in a medium containing BAP and NAA instead callusing was observed from each and every part of the flower bud including gynandrophore from its inner and outer layers. Callus cells of gynandrophore showed high potentiality in inducing various types of embryoids.

Developing shoots were first observed on late torpedo stage embryos as a pair of cotyledon-like outgrowths from the surface of the hypocotyl, and subsequently elongated as an apical meristem with leaf primordia. On younger embryos, similar proliferation produced globular somatic embryos with 2-celled-suspensor. From this we are able to attain the *in vitro* vegetative phase from the reproductive phase of *G. pentaphylla* and hence morphogenic development via callusing and somatic embryogenesis can be studied in ease. This has been observed earlier in birches resembling proembryos.

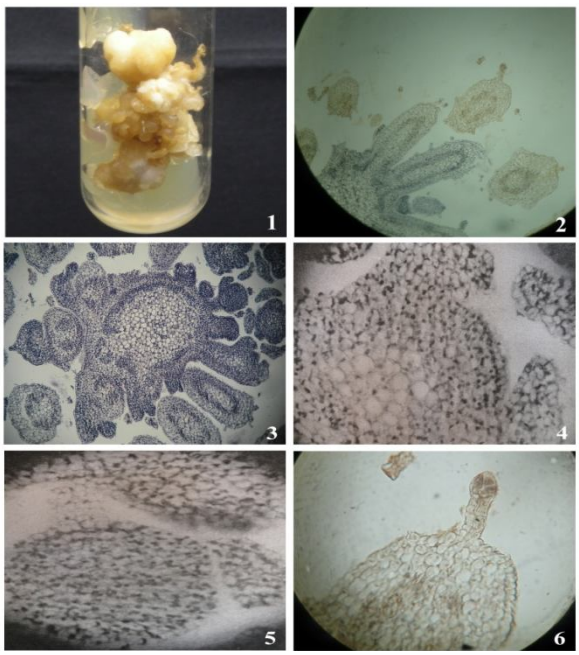

Fig. showing different stages of plant regeneration: 1. Callusing; 2. Various shapes of embryoids; 3. Differentiation of shoot bud; 4 pair of primordial leaves; 5. Four celled proembryo 6. Multicellular suspensor.

CONCLUSION

The fact that normal morphogenesis of floral buds occurred *in vitro* in floral buds of tomato (Rastogi and Sawhney, 1986) is suggestive that flower development is relatively independent of the control by the rest of the plant. Contrarily, in our investigations the growth of the flower buds i.e. normal morphogenesis *in vitro* in *G. pentaphylla* was arrested but induced callus and embryoids suggesting that flower development was controlled by the nutrient medium and the growth regulators and not by the plant itself.

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