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Haploid Plant Production from Pollen Grains of Sturt's Desert Pea via Somatic Embryogenesis

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Abstract: Sturt's desert pea (*Swainsona formosa*) is a native Australian legume used for ornamental purposes and in the cut flower industry. High levels of pollen production, petal staining by pollen and self pollination during transport are major obstacles for the cut flower industry. Haploid plantlets have been produced from pollen grains through somatic embryogenesis for the first time in this species. Callii were initiated from pollen grains cultured on Murashige and Skoog (MS) medium supplemented with 1-10 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 60-80 g/l sucrose. Light greenish callii were transferred to growth-regulator free MS medium for embryogenesis. Numerous greenish, globular proembryos were developed from the callus after the second subculture on the same MS basal medium. Somatic embryos were developed from these globular proembryos after 15 days. Somatic embryos multiplied continuously on the same growth regulator-free MS basal medium. Mature somatic embryo germinated into individual plantlets when planted vertically on MS basal medium. This protocol can be used for the mass production of sterile haploid plants.

Key words: Desert pea · Pollen culture · Somatic embryogenesis · Haploid plants · Cut flower

INTRODUCTION

Sturt's desert pea, *Swainsona Formosa* (G. Don) J. Thompson belongs to the botanical family Fabaceae. It is an attractive, wild, flowering plant of Australia. The economic importance of this plant species lies in its potential use as a hanging basket, container or cut-flower plant [1,4]. It is a southern Australian native legume with attractive flowers that are used in the local cut-flower market of Australia and is exported to Japan [1]. Flowers with vibrant colours produce a large quantity of pollen grains, which stain the petals during transport and reduce flower quality significantly [7]. Large amount of pollen production is not desirable in the cut flower industry because they stain hands and cloths of buyers. The pollen production in large amount, petal staining by pollen and self pollination during the transport are the major obstacles that reduces the quality of the flowers in the cut flower industry.

Previous attempt to produce haploid Sturt's desert pea via androgenesis have been unsuccessful. Recently researchers in Australia reattempted to produce sterile haploid plants to overcome the major utilization obstacles in this species [7]. However, there is no published report available on haploid plant production in this species. The researchers from Biotechnology Department of KISR, Kuwait successfully introduced this species and developed a protocol for the mass production of the selected clones via somatic embryogenesis [6] for the first time. Following this research achievement, a research was carried out to produce haploid sterile plants in this species using anther culture technology and a protocol was developed for the production of haploid sterile plants from pollen grains through somatic embryogenesis. The detail of the sterile haploid plant production in this species were reported here.

MATERIALS AND METHODS

Plant material: Recently *Swainsonia formosa* plants were successfully introduced to Kuwait from Southern Australia [6]. Mature unopened flower buds were collected from the healthy plants grew in KISR campus, Kuwait. Anthers were isolated from the flower buds and used as the primary explants.

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Table 1: Effect of different concentrations of 2,4-D with 6% sucrose on *Swainsona* anther culture

2,4-D concentration(mg/l)	Response
0	-
1	-
3	Embryogenic callus
5	Embryogenic callus
10	Embryogenic callus

Sterilization: Mature unopened flower buds were surface sterilized with 20 % commercial Chlorox® containing 1.05% sodium hypochlorite and a drop of Tween-20® for 15 min. After rinsing in sterile distilled water three times, the petals were removed and the anthers were isolated using a sterile forceps and surgical knife.

Culture media and procedure: The Anthers were cut into two segments and placed on the surface of the MS medium [5] containing different concentrations of 2,4-D and sucrose (Table 1). The pH of the media was adjusted to 5.6 and gelled with 0.13% Phytagel®. Media were dispensed into 25x150 mm Pyrex® culture tubes [12 ml/tube) and was autoclaved at 121°C temperature and 15 lb pressure. All cultures were incubated in a growth room at 25±2°C under 16 h light at 1000 lux light intensity and 8 h darkness photoperiods. Cultures were subcultured into fresh media once every 20 days.

Each treatment contained 20 explants and the experiments were repeated twice. Cultures were observed once every five days to record the callus initiation and morphogenetic responses. Embryogenic callii were isolated and subcultured in growth-regulator free MS basal media repeatedly for embryogenesis, embryo maturation and germination.

Acclimatization: Embryolings were carefully removed from the media without damaging the root system and washed in running water to remove the traces of media. Clean embryolings were carefully transplanted into small pots filled with soil mixture after dipping in an aqueous solution of 0.5 % Benlate®. The soil mixture was prepared by mixing sand, peat moss and humus at 1: 1: 2 ratio and aired for two days after autoclaving at 121°C and 15 lb pressure for 45 min. Embryolings, after planting in the soil mix, were kept in the growth room at 25± 2°C and with 3000 lux light intensity under 16 h photoperiod for 20 days prior to the greenhouse transfer. Later, they were gradually acclimatized to the greenhouse environmental conditions by reducing the relative humidity in the incubating containers.

RESULTS

The pollen grains that were exposed through the cut end of the anthers on the surface of the MS media containing 60-80 g/l of sucrose and 3-10 mg/l 2,4- D turned to light green globular proembryos (Fig. 1A) after 30 days (Table1, 2). Anthers failed to form embryogenic callus in media containing 30 g/l sucrose with 1-10 mg/l 2,4-D. These globular proembryos when isolated and subcultured in the MS medium containing 30 g/l sucrose and 1 mg/l 2,4-D produced greenish white, nodulated and friable callus (Fig. 1B). Initially, the callus showed globular proembryos which showed further development and maturation when transferred to the hormone free media with 30 g/l sucrose. Anther explants cultured in all other media combinations turned brown after a month in culture and did not produce callus or embryos even after 3 months.

The callus, that subcultured frequently once in 15-20 days in MS media containing 30 g/l sucrose, grew continuously with out any browning. Browning of the callus was noticed in the cultures that were maintained more than 30 days. Some of the callus turned to pink in color due to the production and accumulation of anthocyanin pigment. Callus transferred from the MS medium containing 10 mg/l of 2,4-D to hormone-free MS medium produced proembryos that produced only primary root (Table 3) and failed to develop into plantlet. The same callus after several sub cultures in the hormone-free

Table 2: Effect of sucrose concentration of MS medium containing 3 mg/l 2,4D on *Swainsona* anther culture

Sucrose (g/l)	Callus initiation %	Type of callus
30	0	-
40	0	-
60	40±0.7	Embryogenic
80	65±7	Embryogenic
100	26±4	Embryogenic

(±) Standard error; data from 20 replicates; experiment was repeated twice

Table 3: Response of embryogenic callus initiated on media containing different 2,4-D concentrations and transferred to Hormone-free supplemented with 30 g/l Sucrose

2,4-D in Initiation Media (mg/l)	Response to Hormone-free Culture Media	No. of haploid embryos
1	Nodular friable embryogenic callus and haploid embryos.	4±0.6
3	Haploid embryos of different stages	24±7.6
5	Nodular callus with roots	-
10	Nodular callus with roots	-

(±) Standard error; data from 20 replicates; experiment was repeated twice

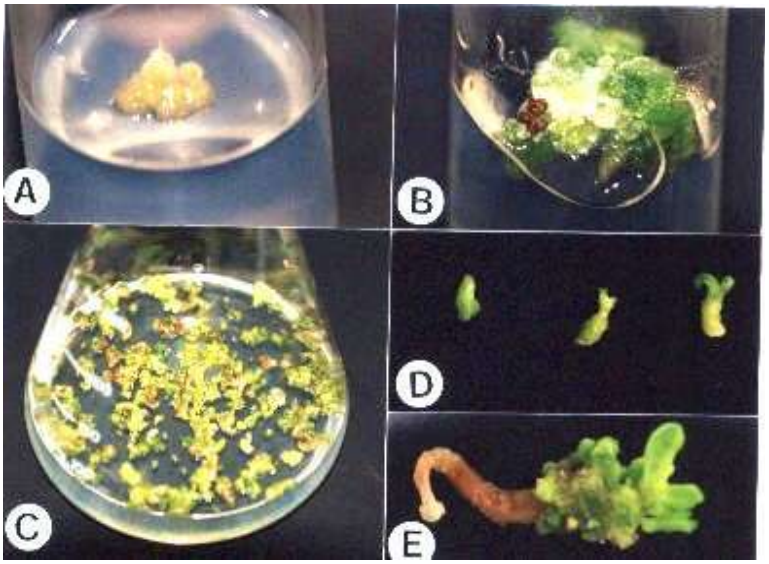

Fig. 1(A-E): *Swainsona formosa* pollen embryogenesis. Fig. A. Embryogenic callus; Fig. B Greenish white nodulated embryogenic callus; Fig. C. Embryogenic callus with different stages of embryo development; Fig. D. Isolated mature haploid embryos; Fig. E. Cotyledon and hypocotyl region of a haploid embryo producing secondary somatic embryos (adventive embryony)

media produced normal globular somatic embryos. The embryogenic callus having globular proembryos (Fig. 1C) when subcultured in the hormone-free media multiplied continuously and produced somatic embryos. Isolated mature somatic embryos (Fig. 1D) germinated into haploid plantlet in the same media. Cotyledons of some of the embryos produced secondary somatic embryos directly (Fig. 1E).

DISCUSSION

Previously attempts were made to produce sterile haploid plants in Sturt's desert pea via anther culture [7], but the production of haploid plants in this species was not reported. A complete protocol has been established in this initial report for the production of sterile haploid plants in this species. Perhaps this is the first report on plantlet production from pollen grains in *Swainsona formosa*.

Sucrose with 2,4-D in the media played a key role in the production of embryogenic callus from the pollen. Three sucrose concentrations in MS media were studied and the results indicated that a high concentration of sucrose followed by a media change to 3% sucrose were effective. Callus developed only in media containing 60-80 g/l sucrose and 1-10 mg/l 2,4-D. For the induction of embryogenic tissue, 2,4-D alone is sufficient [3]. However, embryogenic callusing was not observed in media with 30 g/l sucrose and 1-10 mg/l 2,4-D, which indicated that high concentration sucrose and 2,4-D are the key factors for the initiation of embryogenic callus from microspore of *Swainsona formosa*. Probably, higher concentrations of sucrose ruptured the microspore wall and the 2,4-D induced cell division and the somatic embryogenic callus formation.

Studies have shown that a high level of sucrose is required for the initial microspore survival and division in *Brassica* but a lower level is important for continuation of microapore division [2]. Yang et al. [8] found that 14% sucrose gave the best yield of embryos compared to 6 or 10 %. However in the case of *Swainsona*, 6-8 % of sucrose gave better results.

Recently, Sudhersan and AboEl Nil [6] developed a protocol for the production of somatic embryos in this species from leaf and stem tissue explants. The regenerated plantlets were normal and completely green in color. In the present study, few albino plantlets were noticed among the regenerated haploid plantlets. High proportion of the embryos did not germinate directly but showed abnormal development I. e., callus formation or induction of secondary embryo (adventive embryony). Further studies on refinement of the haploid production technology, acclimatization and field experimentation are in progress. Cytological and morphological studies are also in progress.

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