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# Efficient plant regeneration via somatic embryogenesis from anthers of *Datura stramonium* L.

**Sundar, A.N.[1] and Jawahar, M.[2*]**

[1]Department of Botany, J.J. College of Arts and science, Pudukkottai, Tamil Nadu, India.
[2]Department of Botany, Rajah Serfoji Govt. College (Autonomous), Thanjavur-05, Tamil Nadu, India.

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An efficient protocol was achieved for direct somatic embryogenesis from anther culture of *Datura stramonium* L. The MS medium was supplemented with different concentrations of 2,4-D, (2.26- 18.08µm/l) and combinations of 2,4-D and 13.32µm/l BAP in addition with 3ml/l CW. Maximum number of somatic embryos were obtained on MS medium with 9.04µm/l 2,4-D. Higher frequency of maturation and plant regeneration of embryos were observed on medium containing 9.04µm/l 2,4-D and 13.32µm/l BAP in addition with 3ml/l CW. The well-developed plants were successfully established in the field after hardening.

**Key words:** *Datura stramonium* L., embryo, somatic embryogenesis, maturation, plant regeneration

## Introduction

*Datura stramonium* L., a member of *Solanaceae*, is a small herbaceous plant, and is one of the important medicinal plant cultivated throughout India. The whole plant (leaf, root, flower and seed) is used medicinally, because of the presence of tropane, flavanoids with anolids, coumarins, tannins, allantoin and Vitamin C. It is used as pre-anaesthetic in surgery and child birth, in ophthalmology and prevention of motion sickness. It has long been known in India for their narcotic and antispasmodic properties. The green leaves are reported to use in east Africa for dye cloth. The seeds contain a fixed oil with a disagreeable odour and taste. The leaves contain vitamin "C" (Anonymous, 1952).

*In vitro* on *Datura* species have been reported by many workers (Guha and Maheswari, 1964; Chandy and Narayana, 1971; Cappadocia and Sreeramulu, 1980; Ziv *et al*., 1984; Reddy and Dhamayanthi, 2001). However regeneration of plants through anther culture was poor in many cases. The present investigation was attempted to standardize *in vitro*

*Corresponding author: M. Jawahar; e-mail: somajainn @yahoo.co.in

protocol for plant regeneration via somatic embryogenesis by anthers as the explants.

## Materials and methods

The anthers of *Datura stramonium* L. from young flower buds were collected from college herbal garden. The collection of young buds was made in early morning. The flower buds about 3-5 mm length containing microspores in the mononucleate stage were selected. The collected flower buds were soaked in 2,4-D (2,4- dichlorophenoxy acetic acid) at the rate of 10 mg/l for 6 hrs. at 4°C dark, the surface sterilized using Teepol solution. Thereafter, they were sterilized in 70% ethanol for 10 sec followed by washing with distilled water for 3 times. Surface sterilization was done with freshly prepared 0.1% (w/v) $HgCl_2$ for 5 minutes and finally washed sterile distilled water for 3-4 times. Anthers were then excised from the sterilized flower buds and inoculated onto the MS medium (Murashige and Skoog, 1962), containing 3.0% (w/v) sucrose, 0.7% (w/v) agar and various concentrations of 2,4-D (2.26-18.08 μm/l), and different concentrations and combination of 2,4-D and 13.32 μm/l BAP (6 - benzyl aminopurine) in addition with 3 ml/l coconut water. Then, the cultures were kept under 16 hrs in light 8 hrs and in dark (2400 lux) photoperiod at 25±2°C.

Rooted plantlets were first transferred to plastic cups having vermiculate and garden soil (3:1). The cups were kept in covered glass trays for a week in an incubator at 25±2°C under 16 hrs photoperiod. After a week these plantlets were transferred to the greenhouse and then to the field.

### *Statistical analysis*

Experiments were setup in a Randomized Complete Block Design (RCBD) repeated twice. Data were recorded on the percentage of response and number of embryos per anthers. Mean and standard errors were carried out for each treatment.

## Results and discussion

### *Induction of somatic embryos*

Excised anthers were cultured on MS basal medium containing different concentrations of 2,4-D at the rate of 2.26-28.08 μm/l for induction of somatic embryos. After 10-15 days of inoculation white spots were formed along the surface of the anthers. These organized structures closely resembled globular shaped somatic embryos and directly contact with the anther (Fig. 1A). Among the different concentrations of 2,4-D the highest frequency of somatic embryos (85.00%) and higher number of embryos (42.92 ± 0.89) per

anther were observed on MS medium containing 9.04μm/l 2,4-D. (Table 1; Fig. 1B). Mastsuoka and Hinata (1979) reported that NAA induced a higher frequency of somatic embryos in eggplant using hypocotyls explants. Kamada and Harada (1979) suggested that the somatic embryos formed in response to a wide range of auxins, including IAA, NAA and 2,4-D in carrot. However the highest frequency of somatic embryo proliferatin was obtained on MS medium supplemented with 9.0 μml/l of 2,4-D.

**Table 1.** Effect of 2,4-D on somatic embryo induction in *Datura swamonium* L.

| 2,4-D (μM/l) | Embryos induction (%) | Number of embryos/anthers |
|---|---|---|
| 2.26 | 22 | $10.42 \pm 1.77^{de}$ |
| 4.52 | 45 | $21.27 \pm 1.76^{c}$ |
| 9.04 | 85 | $42.92 \pm 0.89^{a}$ |
| 13.56 | 68 | $30.43 \pm 1.83^{b}$ |
| 18.08 | 52 | $10.83 \pm 1.78^{d}$ |

Each value represents the mean ± SD of 30 replicates and each experiment was repeated of least thrice. Values with the same superscript are not significantly different at the 0.05% probability level according to DMRT.

### *Regeneration of somatic embryos*

The 2,4-D at the rate of 2.26 – 18.08 μml and combined with 13.32 μm/l BAP in addition with 3 ml/l coconut water was tested in either full – strength of half – strength MS for plant regeneration from somatic embryos (Table 2). MS medium supplemented with 2,4-D alone did not lead to embryo maturation and plant regeneration. Somatic embryo maturation and plant regeneration occurred the best in the full-strength MS medium supplemented with 9.04 μm/l 2,4-D combined with 13.32μm/l BAP in addition with 3ml/l coconut water (92.00 ± 3.34 and 86.00 ± 4.56) (Figs. 1 C and D). Somatic embryos regenerated into whole plantlets in the regeneration medium for 4 week after culture (Fig 1E & F). The observation on the synergistic effect of 2,4-D in combination with BAP added coconut water could promote embryo development and plant (Rao *et al*., 1973).. Reynolds (1996) reported that the medium lacking 2,4-D but in addition with low concentrations of cytokinin induced somatic embryogenesis through callus cultures of solanum carolinense. Kamada and Harada (1979) reported that the medium containing auxins, including IAA, NAA and 2,4-D induced higher frequency of somatic embryogenesis in carrot. This study inferred that 2,4-D combined with BAP in addition with 3ml/l coconut water is the more suitable for the induction of somatic embryogenesis.

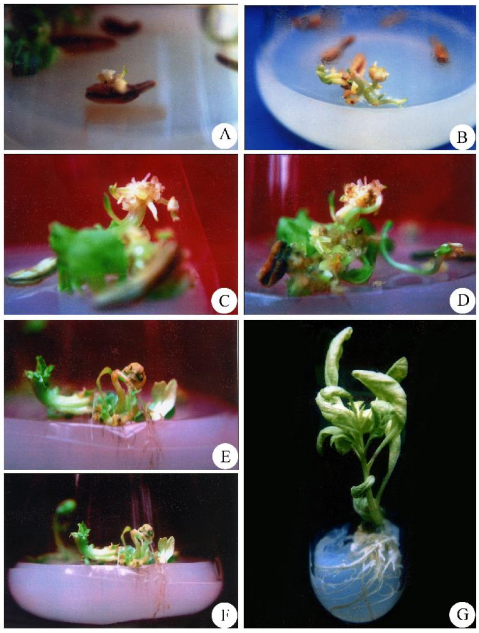

Fig A. Somatic embryo initiation Fig B. Somatic embryo proliferation
Fig C. Embryo maturation Fig D. Regeneration
Fig E & F. Plantlet regeneration Fig G. Well developed plant

**Fig. 1.** Somatic embryos.

**Table 2.** Effect of different concentrations of 2, 4-D and combine with 13.34mm/l BAP in addition with 3ml/l CW on embryo matuvation and Plant re generation from anthers embryos of *Datura stramonium* L.

| 2,4-D (µM/l) | Embryos maturation (%) | Plant regeneration (%) |
|---|---|---|
| 2.26 | $34.00 \pm 4.56^{e}$ | $20.00 \pm 4.00^{e}$ |
| 4.52 | $42.00 \pm 5.21^{d}$ | $38.00 \pm 3.4^{d}$ |
| 9.04 | $92.00 \pm 3.34^{a}$ | $86.00 \pm 4.56^{a}$ |
| 13.56 | $64.00 \pm 2.19^{b}$ | $56.00 \pm 2.19^{b}$ |
| 18.08 | $48.00 \pm 3.34^{c}$ | $42.00 \pm 5.21^{c}$ |

Each value represents the mean ± SD of 30 replicates and each experiment was repeated at least thrice. Values with the same superscript are not significantly different at the 0.05% probability level according to DMRT.

### *Acclimatization and hardening*

The well-developed plantlets (Fig. 1G) were transferred to plastic cups containing vermiculate and garden soil (3:1). The cups were kept in covered glass trays for a week in an incubator at 25±2°C under 16 hrs. photoperiod. After a week plantlets were transferred to the greenhouse and then to the field.

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