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# Callogenesis and Direct Organogenesis of *Artemisia scoparia*

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**Abstract:** The present study was initiated to develop a protocol for the effective propagation of *Artemisia scoparia*. Shoot tip, leaf and petiole explants were used for callus production and shoot tips were used for direct organogenesis. Murashige and skoog medium supplemented with different concentrations and combinations were used through entire study. MS medium supplemented with 2,4-D and NAA in combination at different concentrations proved best for callus induction from all types of explants. Multiple shoots from shoot tip explants were produced at NAA in combination with BA or Kin. Best root initiation was observed at NAA supplemented in MS medium.

**Key words:** *Artemisia scoparia*, callogenesis, direct organogenesis

## INTRODUCTION

Medicinal plants are source of important therapeutic aid for alleviating human ailments (Dev, 1997). All *Artemisia* species produce aromatic oils and several are culinary herbs or used as flavourings, hallucinogens, vermifuges and pharmaceuticals (Lee and Geissman, 1970; Marco and Barbera, 1990; Heinirich *et al*., 1998; Sy and Brown, 2001) and some are toxic (Burrows and Tyrl, 2001).

*Artemisa scoparia* (worm wood) is known for variety of medicinal uses. Extract of *A. scoparia* is used as antipyretic, antiseptic, diuretic, vasodilator and has strong antibacterial activity against different gram positive and gram negative bacteria (Yeung and Che, 1985; Duke and Ayenus, 1985). Scoparone, from *A. scoparia* extract is known to reduce cholesterol and triglyceride level in blood (Lee *et al*., 2003).

Growth regulators concentrations in culture medium are critical for the control of growth and morphogenesis. Generally, high concentration of auxin and low concentration of cytokinin in the medium promote abundant cell proliferation with the formation of callus (Bennici *et al*., 1988; Chawla and Wenzel, 1987). Regeneration occurs either by somatic embryogenesis or adventitious bud and shoot development with subsequent rooting (Bhaskaran and Smith, 1990), while sometime it may occur either by direct organogenesis (Li *et al*., 1992). Micropropagation of different *Artemisia* sp. has been previously established by using different explants but direct organogenesis is better tool to get high number of plants in short time period. In the present study effect of plant growth regulators on callogenesis from shoot tip, leaf and petiole explants and direct organogenesis from shoot tip explant was studied from *Artemisia scoparia* L. explants.

## MATERIALS AND METHODS

Experimental studies were conducted on *Artemisia scoparia* in the Plant Physiology Lab, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad during 2003-2004. The basal medium used was of Murashige and Skoog (1962) during entire studies. Growth regulators used throughout this experimental work were: 2,4-Dicholorophenoxy acetic acid (2, 4-D), Indol acetic acid (IAA), Indol-3-butyric acid (IBA), α-nephthalene acetic acid (NAA), 6-benzylamino purine (BAP) and Kinetin (Kin). *Artemisia scoparia* explants were collected from Islamabad locality. For sterilization, explant material were rinsed with distilled water and than soaked in 0.1% mercuric chloride solution for 2-3 min. Before cutting and culture, the explants were washed 3-4 times with sterilized distilled water. 0.4-0.5 cm sized explants were used for callogenesis. All the operations and inoculations were carried out under strict aseptic conditions in laminar airflow cabinet. The medium was heated and then dispensed in either test tubes or flasks and autoclaved at a temperature of 121 °C and a pressure of 15 lbs psi for 15 min. The cultures were kept in a culture room incubator with 16 h light cycle in every 24 h. The temperature was regulated at 25±1 °C.

## RESULTS AND DISCUSSION

*Artemisia scoparia* is a plant of great medicinal importance. It is therefore important to develop protocol for efficient propagation through tissue culture to produce quality plants in large number. Results

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regarding to callogenesis of *Artemisia scoparia* explants (shoot tip, leaf and petiole) are presented in Table 1. Callogenic response varies from hormonal concentration/combination and type of explant. The callus texture differed at different hormonal concentration from yellowish to dark green, from soft to compact and hard and from non-embryogenic to embryogenic. Callus formation was excellent at different concentrations of 2,4-D in combination with NAA where non-embryogenic, green in color and compact callus was formed. The callogenic response from shoot tip explant on this combination varies from 80 to 100% while from leaf and petiole explants, that varies from 50 to 100%. Same results were observed by Nin *et al.* (1996) at the same combination. At all concentrations of 2,4-D callus formation was also better but callus texture was poor and callogenic response was also low. The produced callus was green to light green in color. But the callogenic response was not good at any concentration from any explant. Maximum response 90% was observed at 2.0 mg $L^{-1}$ from shoot tip explant while at 2.5 mg $L^{-1}$ from petiole explants. Nin *et al.* (1996) stated that 2,4-D at a dose of 0.90 μM stimulated adventitious root development from 86% of all explant of *Artemisia absinthium* and the callus developed was friable and light green at 1.81 μM 2,4-D.

When 2,4-D in combination with other hormones as BA, Kin and IAA was applied, the callus texture was poor or callogenic response was low. At these combination when cytokinin was used with 2,4-D, embryogenic callus was formed or it turned to be embryogenic after four weeks of culture due to habitutation. At these combinations/concentrations, callogenic response was not so good from any type of explant. Nin *et al.* (1996)

Table 1: Callogenic response of different explants of *Artemisia scopaira* at differential hormonal combinations/concentrations

| | | Shoot tip explant | | | Leaf explant | | |
|---|---|---|---|---|---|---|---|
| PGR | Conc. | Response | (%) | Characteristics | Response | (%) | Characteristics |
| 2,4-D | 0.5 | +++ | 40 | Brown, soft, non-embryogenic | +++ | 40 | Light Brown, soft, non-embryogenic |
| | 1.0 | +++ | 50 | Yellow, soft, non-embryogenic | +++ | 70 | Light grown, soft, non-embryogenic |
| | 1.5 | ++ | 60 | Light green soft embryogenic | ++++ | 50 | Light green soft embryogenic |
| | 2.0 | ++++ | 90 | Yellow soft non-embryogenic | ++ | 60 | White soft non-embryogenic |
| | 2.5 | ++ | 20 | Light greenish yellow, embryogenic | +++ | 60 | White soft non-embryogenic |
| | 3.0 | +++ | 20 | Yellow soft non-embryogenic | +++ | 30 | Light brown soft non-embryogenic |
| 2,4-D + BA | 1.0 + 0.5 | ++++ | 100 | Light green compact hard embryogenic | + | 20 | Light green compact hard embryogenic |
| | 2.0 + 1.0 | ++ | 30 | Dark green compact, hard, embryogenic | ++ | 20 | Green compact, hard, embryogenic |
| | 3.0 + 0.5 | + | 20 | Dark green compact, hard, embryogenic | +++ | 60 | Green soft non-embryogenic |
| 2,4-D + Kin | 1.0 + 0.5 | ++++ | 80 | Light green compact, hard, embryogenic | + | 10 | Light green compact, hard, embryogenic |
| | 2.0 + 1.0 | ++ | 70 | Dark green compact, hard, embryogenic | +++ | 40 | Whitish green compact, hard, embryogenic |
| | 3.0 + 0.5 | + | 10 | Dark green compact, hard, embryogenic | ++ | 20 | Light green compact, hard, embryogenic |
| 2,4-D + IAA | 1.0 + 0.5 | +++ | 60 | Light green compact, hard, non-embryogenic | +++ | 60 | Light green compact, soft, embryogenic |
| | 2.0 + 1.0 | ++ | 20 | Light green compact, soft, embryogenic | ++++ | 80 | Light green compact, soft, non-embryogenic |
| | 3.0 + 0.5 | +++ | 60 | Dark green compact, hard, embryogenic | ++++ | 80 | Light green soft embryogenic |
| 2,4-D + NAA | 1.0 + 0.5 | ++++ | 100 | Dark green compact, hard, non-embryogenic | +++ | 90 | Light green compact, hard, embryogenic |
| | 2.0 + 1.0 | ++++ | 100 | green compact, hard, embryogenic | ++ | 70 | Light green compact, hard, embryogenic |
| | 3.0 + 0.5 | ++ | 80 | Light yellow, soft, non-embryogenic | ++++ | 100 | greenish yellow, soft, non-embryogenic |

| | | Petiole explant | | |
|---|---|---|---|---|
| PGR | Conc. | Response | (%) | Characteristics |
| 2,4-D | 0.5 | ++ | 60 | Dark Brown, soft, non-embryogenic |
| | 1.0 | +++ | 60 | Yellow, soft, non-embryogenic |
| | 1.5 | +++ | 50 | Light green soft non-embryogenic |
| | 2.0 | ++++ | 80 | Yellow, soft embryogenic |
| | 2.5 | ++++ | 90 | Light green, soft, embryogenic |
| | 3.0 | +++ | 60 | Yellowish soft non-embryogenic |
| 2,4-D + BA | 1.0 + 0.5 | ++ | 40 | Light green compact hard embryogenic |
| | 2.0 + 1.0 | + | 10 | Light green compact, hard, embryogenic |
| | 3.0 + 0.5 | +++ | 60 | Light green soft embryogenic |
| 2,4-D + Kin | 1.0 + 0.5 | ++ | 70 | Lush green compact, hard, embryogenic |
| | 2.0 + 1.0 | ++ | 30 | Light green compact, hard, embryogenic |
| | 3.0 + 0.5 | ++ | 50 | Dark green compact, hard, embryogenic |
| 2,4-D + IAA | 1.0 + 0.5 | +++ | 60 | Dark brown, soft, non-embryogenic |
| | 2.0 + 1.0 | +++ | 60 | Whitish, soft, non-embryogenic |
| | 3.0 + 0.5 | + | 50 | Light brown soft non-embryogenic |
| 2,4-D + NAA | 1.0 + 0.5 | ++ | 70 | Light brown soft non-embryogenic |
| | 2.0 + 1.0 | + | 50 | Dark brown soft, non-embryogenic |
| | 3.0 + 0.5 | ++++ | 100 | Whitish green, hard compact non-embryogenic |

Table 2: Direct shooting response from shoot tip explants at different hormonal combinations/concentrations

| Hormone | Conc. | Response | Response (%) | Morphological characters |
|---|---|---|---|---|
| Kin | 2.0 | +++ | 40.9 | 2-3 long shoots with small leaves |
| | 4.0 | ++++ | 72.7 | Multiple shoots with broad green leaves |
| | 6.0 | ++ | 27.2 | Three small shoots with light green leaves |
| BA | 2.0 | + | 36.3 | Single shoot with green leaves |
| | 4.0 | ++ | 18.1 | 1-2 small shoots with green leaves |
| | 6.0 | +++ | 54.4 | Three small shoots with green leaves |
| BA + IBA | 1.0 + 0.5 | ++ | 18.1 | Single shoots with light green leaves |
| | 2.0 + 1.0 | + | 9.0 | Small multiple shoots with pale yellow leaves |
| BA + NAA | 1.0 + 0.5 | +++ | 72.7 | Three shots with small yellow green leaves |
| | 2.0 + 1.0 | ++++ | 90.0 | Small multiple shoots with green leaves |
| BA + IAA | 1.0 + 0.5 | +++ | 63.6 | Two small shoots with green leaves |
| | 2.0 + 1.0 | ++ | 27.2 | Single shoot with green leaves |
| Kin + IBA | 1.0 + 0.5 | + | 18.1 | Single shoot with green leaves |
| | 2.0 + 1.0 | +++ | 27.2 | Two shoots with small leaves |
| Kin + NAA | 1.0 + 0.5 | ++++ | 90.0 | Small multiple shoots with light green leaves |
| | 2.0 + 1.0 | ++ | 18.1 | 1-2 shoots with lush green leaves |

Table 3: Rooting response from *in vitro* grown shoots at different auxin concentrations

| Hormone | Conc. (mg $L^{-1}$) | Response | Response (%) | Characteristics |
|---|---|---|---|---|
| IAA | 1.0 | + | 25.0 | Single root |
| | 2.0 | ++++ | 75.0 | 4-5 roots with root hairs |
| IBA | 1.0 | - | - | No response |
| | 2.0 | + | 12.5 | Single root with no root hairs |
| NAA | 1.0 | +++ | 50.0 | 3-4 roots with +ive and –ive geotropism with roots hairs |
| | 2.0 | ++++ | 87.5 | 5-6 roots with roots hairs, +ive geotropism |

Fig. 1: Direct organogenesis from shoot tip explant at BA and NAA

who stated that the cytokinins (BAP) alone in low concentration did not induce dedifferentiation, but in higher doses were able to induce callus. Benjamin *et al.* (1991) experimented callus induction from shoot buds using BAP plus IAA from *Artemisia pallens*.

Direct shoot proliferation results from shoot tip explant are presented in Table 2. Excellent results were observed on BA, 2.0 mg $L^{-1}$ with NAA (0.5 mg $L^{-1}$) and Kin, 1.0 mg $L^{-1}$ with NAA 0.5 mg $L^{-1}$ where multiple shoot proliferation was observed (Fig. 1). At these combinations the percentage of shoot proliferation was 90.0. Kin in single at 4.0 mg $L^{-1}$ also produced multiple shoots with percentage response 72.2. Geng *et al.* (2001) investigated optimum medium for inducing shoot cluster with flower buds in *Artemisia annua* L. The best result was obtained on MS medium supplemented with 4.0 mg $L^{-1}$ BAP and 0.05 mg $L^{-1}$ NAA. Nam-cheol *et al.* (1992) also reported shooting response at 0.35 mg $L^{-1}$ of NAA with 0.3 mg $L^{-1}$ of BAP in MS medium in *Artemisia annua*. Mackay and Kitto (1988) established a protocol for the optimum proliferation of French Tarragon (*Artemisia dracunculus* L.) at the combination of NAA and BA.

At other concentration of BA or Kin alone or in combination with IAA or IBA, low shoot proliferation was observed. Le (2001) reported new axillary shoots development promoted in *Artemisia annua* by addition of BAP alone in the basal MS medium. It was also observed that during direct shoot proliferation, callus mass was also formed at the base of shoot explant. The callus produced was of different characteristics depending upon hormonal combination and concentration. In some culture the callus formed at the base of explants also produced buds under the synergetic effect of hormones.

NAA and IAA at 2.0 mg $L^{-1}$ proved best for root initiation from proliferated shoots as described in Table 3. At IAA 2.0 mg $L^{-1}$ root initiation was 75% while at same concentration of NAA root initiation was 87.5%. Tsay (1999) observed root initiation at NAA in combination with IBA from *Artemisia formsanus*. Root was generated on MS medium with NAA in *Artemisia annua* reported by Nam-cheol *et al.* (1992). Plantlets were transferred to pots containing soil and peat moss and the percentage of survival was only 33.3.

Results show that it is possible to propagate *Artemisia scoparia* plant through tissue culture. Many researchers had tried different hormonal combination/ concentrations. Results of present investigation and others authors suggest that tissue culture of this plant can be carried out efficiently by direct organogenesis.

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