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In vitro propagation and stoloniferous stem formation of *Thelesperma megapotamicum*

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ABSTRACT

An efficient "in vitro" propagation protocol for *Thelesperma megapotamicum (Spreng.) Kuntnze* was established. Nodal segments were cultured in vitro with different combination and concentration of growth regulators. Microshoots (60-days) cultured in Murashige and Skoog (MS) with the addition of 4 mg/l 6-benzyl adenine (BA) were used as explants and incubated on a half salt concentrations MS/2 medium supplemented with different combination of indol butyric acid (IBA) and sucrose for 120 days.

Explants cultured on a half of Murashige and Skoog salts (MS/2) with the addition of 1,0 mg/L-40 g/L and 1,5mg/L - 30g/L of IBA and sucrose gave shoot number and principal stem length as the control medium with higher stoloniferous stem percentage or development of shoots in stoloniferous stem respectively.

Keywords: Plant tissue culture; *Thelesperma megapotamicum*; stoloniferous stem.

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Introduction

Thelesperma megapotamicum (Spreng.) Kuntnze (Asteraceae), commonly know as "Indian or Pampa" tea, occurs in Argentina. This herb has been gathered and prepared by these American tribes for many centuries. The aerial part of this plant species is used in folk Argentine medicine for renal, digestive affections, and as anaesthesia (Nuñez C at al., 2000; Roig F, 2002 Barboza GE et al., 2006). Luteolin, Luteolin-7-O Glucoside, Marein were isolated of this specie. Luteolin is used as Aldose-Reductase-Inhibitor, antiHIV, anticataract, anticomplementary, antidermatic, antifeedant, antiherpetic, antihistaminic, and antiinflammatory among other properties (Ateya et al., 1982, Dr. Duke's Phytochemical and Ethnobotanical Database).

Studies on the biological activity of this specie showed that organic extracts exhibited a pronounced cytotoxic effect on "in vitro MCF-7 breast tumor line (Bongiovanni G et al., 2006, Goleniowski M et al., 2007).

This work comprise collecting and introduction of germoplasm and in vitro multiplication of this specie to let the "ex situ" conservation. A protocol for *in vitro* plant propagation and stoloniferous stem formation using different combination and concentration of growth regulators was achieved.

Materials and Methods

Plant material and disinfection

Thelesperma megapotamicum (Spreng.) Kuntnze (TM) recollection was carried out at Santa Maria de Punilla, "Sierras de Córdoba". This side is located at approximately 45 km north of "Córdoba", Argentine. Plant samples were collected during spring 2009, immediately transported to the lab and a voucher specimen was deposited in the International Herbarium of the National University of Rio Cuarto, Argentina (RIOC).

The explants were surface sterilized by immersion in tap water for 2 h, 0,1% mercury chloride for 35 min, 70% ethanol for 6 min, and commercial 30% sodium hypochlorite for 15 min (10% active chlorine) with the addition of Tween 20, and rinsed several times with sterile distilled water.

Initiation of plant regeneration

Sterile explants of *T. megapotamicum* were cultured for multiple shoots generation, in basal medium of Murashige and Skoog (MS), supplemented with sucrose 30 g/L, agar 7 g/L, 6-benzyl adenine (BA) 4mg/l and adjusted to pH 5.6. Culture media were sterilized in autoclave at 121 °C and 1.05 atm. for 15 minutes. Incubation was carried out under controlled conditions of temperature (25 ± 2°C) with a 16 h photoperiod (4.35 W.m2).

For the whole plant regeneration, explants of 60 days were transferred to a half salt concentration MS medium (MS/2) supplemented with different combination and concentration of indol butyric acid (IBA) and sucrose as shown in table 1. The plantlets were growing for 120 days in the same conditions before described.

The growth parameters analyzed were shoots numbers, principal shoot length and stoloniferous stem formation percentage.

Table 1: MS/2 medium supplemented with different combination and concentration of sucrose and IBA

Treatment	Sucrose (g/l)	IBA (mg/l)
Control	30	0.0
1	40	1.0
2	50	
3	100	
4	30	1.5
5	40	
6	50	
7	100	

Statistical analysis

Data on principal stem length, number of shoot and formation of stoloniferous stem were recorded after 120 days of culture and reported as means. Differences between treatments were established by ANOVA followed by the LSD Fisher test ($p<0.05$).

Results

After 120 days of cultivation on different induction media, no statistical difference in the principal shoot length was observed (figure 3A). When explants were placed in medium containing IBA (1.5 mg/l) and sucrose (100 gr/l) a higher microshoots percentage was stimulated (Figure 3B).

The stoloniferous stem formation was major at 1.5 mg/l IBA-30 gr/l sucrose and 1.5 mg/l IBA-100 gr/l sucrose, with a percentage of 25 and 21% respectively, (Table 2). The stoloniferous stem length was influenced by the concentration of sucrose in the media. To lower concentration of sucrose induced stoloniferous stem more large (1,0 mg/l IBA-40 y 50 gr/l sucrose and 1.5 mg/l IBA-30 gr/l sucrose; Fig. 4), observing the development of shoots growing of this stoloniferous stem only in the treatment 1 (Fig. 2).

Results from this study showed that both concentrations used of indol butyric acid (IBA) stimulate the stoloniferous stem formation, corresponding the major percentage at a high concentration of IBA (table 2).

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Table 2: Percentage of stoloniferous stem formed in the different culture conditions for 120 days of cultivation

Treatment	Stoloniferous stem formation (%)
Control	0
1	11,8
2	11,1
3	0
4	25,0
5	6,7
6	10,0
7	21,0

Conclusion

The best regeneration plant media corresponded to MS/2 with the combination of IBA and sucrose of 1,0 mg/L-40 gr/l and 1,5mg/l-30 gr/l respectively. Because in these media were obtained shoot number and principal stem length as the control medium and higher stoloniferous stem percentage (1,5mg/l IBA-30 gr/l sucrose) or development of shoots in stoloniferous stem (1,0 mg/L IBA-40 gr/l sucrose).

Note: Part of this study was presented at the 'II Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (Second Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2009.

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Figure 1: Microshoots of *T.Megapotamicum* generated in MS supplemented with 4 mg/l BA for 60 days of cultivation.

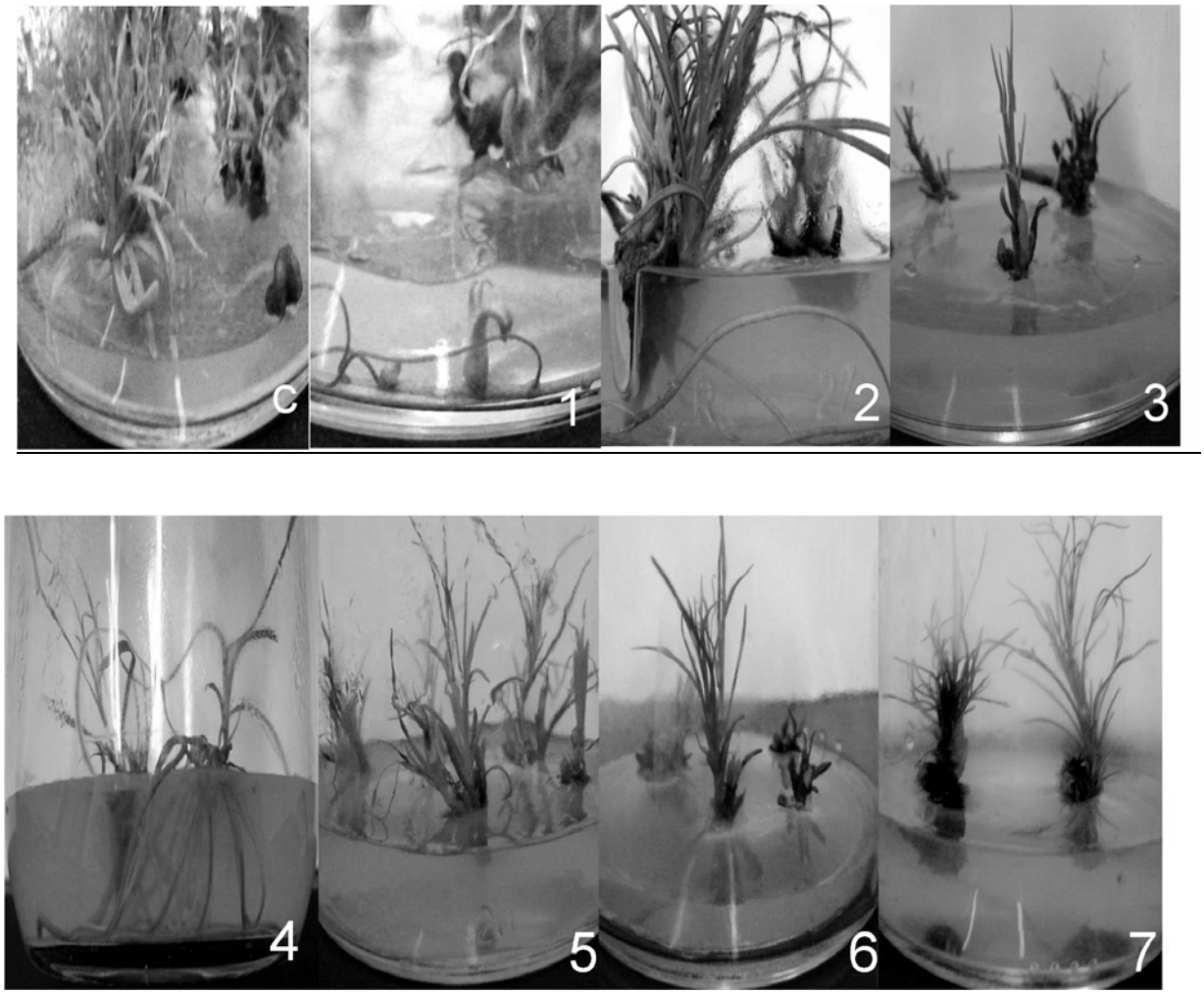

Figure 2: Plantlets of *Thelesperma megapotamicum* grown in medium MS/2 with different combination and concentration of IBA and sucrose after120 days of culture. Treatments C: control, 1) 1,0 mg/l IBA-40 gr/l sucrose, 2) 1,0 mg/l IBA-50 gr/l sucrose, 3) 1,0 mg/l IBA-100 gr/l sucrose, 4) 1,5 mg/l IBA-30 gr/l sucrose, 5) 1,5 mg/l IBA-40 gr/l sucrose, 6) 1,5 mg/l IBA-50 gr/l sucrose and 7) 1,5 mg/l IBA-100 gr/l sucrose.

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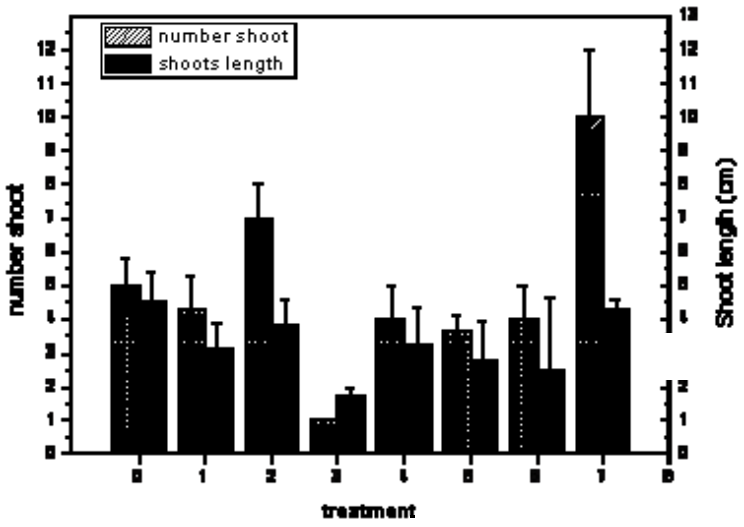

Figure 3: Effect of different combination and concentration of IBA and sucrose on T. megapotamicum growth parameters after 120 days of cultivation

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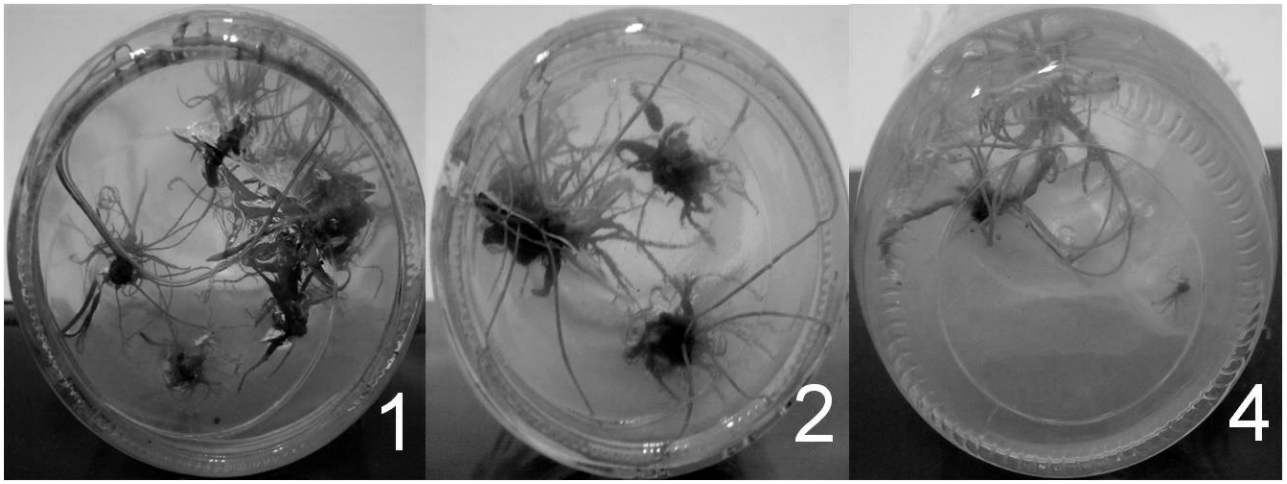

Figure 4: Stoloniferous stem obtained in different culture conditions. Treatments 1) 1,0 mg/l IBA-40 gr/l sucrose, 2) 1,0 mg/l IBA-50 gr/l sucrose and 4) 1,5 mg/l IBA-30 gr/l sucrose