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In vitro shoot regeneration studies in *Java citronella*

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Java citronella (*Cymbopogon winterianus* Jowitt.) is a aromatic plant of great economic value and also well known throughout the world due to its repellent properties attributed to its essential oil, rich in citronellal. An efficient protocol for rapid regeneration of *Java citronella* through callus was developed by manipulating growth regulators in the culture medium. Shoot tips and leaves were used as explants source for regeneration and callus induction. The best callus induction was achieved on MS medium supplemented with 2, 4-D 2.5 mg/L. The best rate of shoot regeneration was obtained on MS medium supplemented with BAP 2 mg/L. The multiplication of shoots was observed on MS medium supplemented with BAP 4 mg/L.

Key words : *Java citronella*, *In vitro* regeneration.

INTRODUCTION

Java citronella (*Cymbopogon winterianus*) is volatile oil producing plant. A member of the *Poaceae* family, includes more than 10,000 species and covers a great percentage of ecosystems. The *Java citronella* is originally from Sri Lanka and is called Mahapengeri in the local language.

It is also widely grown in India, Guatemala, Honduras, Malaysia and several other countries. It is, basically, a tropical plant. A suitable humid climate is ideal for the growth of this plant. *Java citronella* is the kind that has higher concentrations of citronellal (Laszlo, 2000). Moreover, citronellal, citronella consists of geraniol, *citronella* oil is overlooked in aromatherapy because of its association with insect repellency. Its use in perfumery is also very popular, and used as a starting point for the synthesis of various active ingredients (Castro and Chemale, 1995). The species can also be used to combat soil erosion.

The sheets of *citronella* were used as a poultice for fever and pain and accelerate healing, and can also be used in the treatment of RSI (injury repetitive). Moreover, in China Citronella is also used for pain rheumatic (Laszlo, 2000). In aromatherapy it is used to increase mental acuity, fight themigraines and headaches, besides being a skin conditioner. Properties antidepressant, antiseptic, tonic, stimulant were also analysed (Laszlo, 2000). Despite the price swing, often low, the demand in production *Citronella* still have been high and the prices achieved yet allow the cultivation of this species in economic scale (Castro and Ramose, 2003).However, it faces problems due to contamination by a fungus of the genus *Fusarium*, which causes great damage to producers. In the search for alternatives to facilitate the spread of certain species several studies on *in vitro* micropropagation have been made. This is technique that aims to cultivate cells, organs and / or tissues isolated from the mother plant, under controlled conditions. Thus, it becomes an important tool, since advantages such as mass production of plants free of viruses and other pathogens (Grattapaglia and Machado, 1998). The present paper describes large scale propagation of *Java citronella* through shoot tips by tissue culture technology.

MATERIALS AND METHODS

Leaf / Shoot tips were given a sterlization treatment, explants were washed thoroughly in a running tap water for 10-15 min to remove dust and phenols and then were treated 1-2 min each with tween 20 and 0.1% mercuric chloride. The explants were then thoroughally washed (4-5) times with sterilized distilled water.The explants shoot tips (0.5-1 cm) were dissected. The explant were inoculated under aseptic condition on sterile culture medium in test tube on murashige and skoog medium supplemented with 3% sucrose, 0.8% agar and plant growth requlators, like BAP, 2,4-D and adenine sulphate

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with different concentrations in each tubes. For callus induction midrib of Leaf of very small size were inoculated in variously augmented MS media with different concentrations of 2,4,-D. Media was adjusted to 5.7 pH before addling agar. Medium was dispensed in glass test tube and autoclave at 15 psi and 121^{0}C for 15 min.After inoculation test tubes were kept in a culture room at temp. of 25-28^{0}C , relative humidity of 60-70 % and light intensity of apooroximate 15 lux provided by cool white fluroscent tubes.

RESULTS AND DISCUSSION

The present investigation entitled "*In vitro* establishment, multiplication and micropropagation was carried out for large scale propagation of *Java Citronella* through shoot tips by tissue culture.

Shoot tips exlplants were inoculated on MS media with 6 different concentration of BAP and adenine sulphate either alone BAP or combination of BAP and adenine sulphate. Among the various treatments the effective results were obtained from the combination given in Table 1 and Fig. 2. Within 5-7 days of inoculation explants turned green and showed establishment, of which best establishment was found in M_4 MS+BAP(2mg/l)+15mg adenine sulphate as in Table 1 followed by M_5 *i.e.* combination of MS + BAP (4mg/l)+15mg adenine sulphate which showed well establishment in 10-12 days.

Subculturing of established explants for multiplication

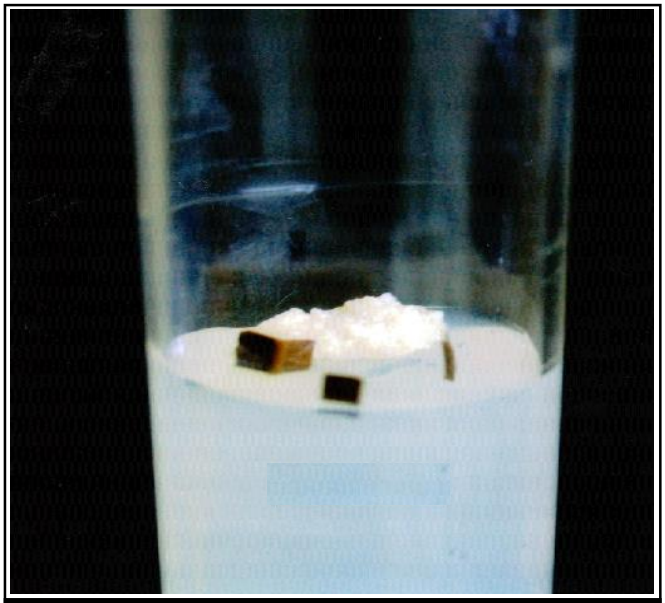

Fig. 1 : Callus induction

on same respective medium induced multiple shoots in 7-8 days and Fig. 3 and 4. M_5 *i.e.* combination of MS + BAP (4mg/l)+ 15mg adenine sulphate showed highest multiple shoot induction (Table 2). Upto 2-3 multiple shoot were observed from single inoculated explants. The proliferating buds were well defined pale green to greenish and 1-2 cm long with bulbous base and pointed tips. Whereas M_4 MS + BAP (2mg/l), M_3 with MS + adenine sulphate (15mg/l) + BAP (1mg/l) have shown multiple

Table1: Effect of different concentrations of plants growth regulators on *in vitro* shoot establishment

Sr. No.	Media treatments	MS media	BAP (mg/l)	Adenine sulphate (mg/l)	No. of explants inoculated	Percentage of plants established
1.	M_0	MS	-	-	10	0%
2.	M_1	MS	1	-	10	30%
3.	M_2	MS	-	15	10	30%
4.	M_3	MS	1	15	10	20%
5.	M_4	MS	2	15	10	60%
6.	M_5	MS	4	15	10	40%

Table 2: Effect of different concentrations of plant growth regulators on i*n vitro* shoot proliferation from shoot tip explants of Java citronella

Sr. No.	Media treatments	MS media	BAP (mg/l)	Adenine sulphate (mg/l)	No. of explants inoculated	No. of shoots per explants
1.	M_0	MS	-	-	10	-
2.	M_1	MS	1	-	10	1
3.	M_2	MS	-	15	10	1
4.	M_3	MS	1	15	10	1-2
5.	M_4	MS	2	15	10	1-2
6.	M_5	MS	4	15	10	2-3

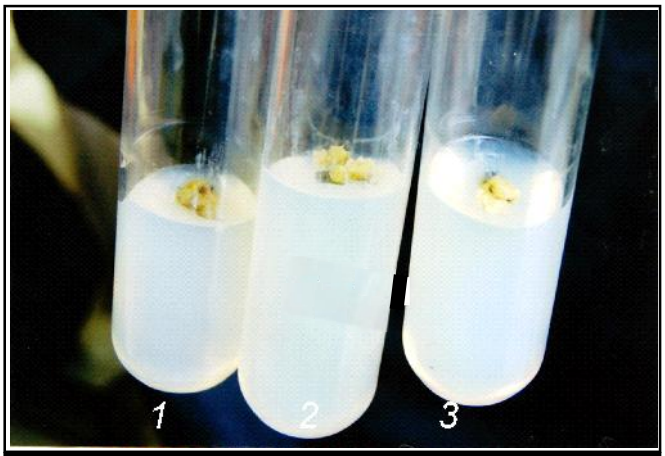

Fig. 2 : Shoot initiation

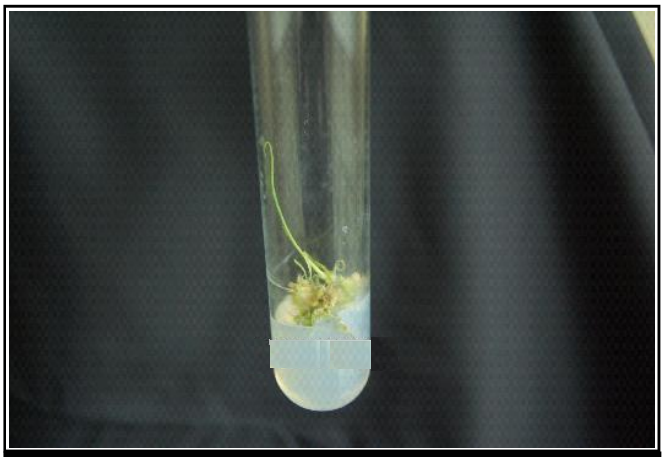

Fig. 3 : Shoot multiplication

Fig. 4 : Shoot multiplication and root induction

shoot induction within 11 – 12 days and showed 1-2 multiples per inoculated explants. Zigiotto (2004) also obtained good results with respect to the sprouting of citronella explants at a concentration of 2.0mg/l BAP.

Table 3: Effect of different concentrations of plant growth regulators for callus induction in *Java Citronella*

Sr. No.	Media treatments	MS	2,4-D (mg/l)	Callus growth
1.	M_0	MS	-	-
2.	M_1	MS	1	-
3.	M_2	MS	1.5	-
4.	M_3	MS	2	-
5.	M_4	MS	2.5	++
6.	M_5	MS	3	-

Note: (+) - Growth
(-) - No growth

Midrib of leaf were inoculated for callus induction on MS media supplemented with different concentration of 2, 4-D. As per Table 3 MS + 2,4-D (4mg/l) showed callus induction in 23-24 days (Fig. 1).

In the study of callus induction, whitish yellow coloured and brownish yellow coloured callus was observed on MS media supplemented with 2,4-D (2.5mg/l) with 15-20 days in midrib and internodal leaf disc explants.

REFERENCES

Castro, L.O. and Chemale, V.M. (1995). *Medicinal plants, herbs and aromatic description and cultivation*. Guaíba p. 177-179.

Castro, L.O. and Ramose, R.L.D. (2003). Major oil-producing grasses Essential: *Cymbopogon citratus* (DC) Staupf., grass cider, *Cymbopogon martinii* (Rox) JF Watson, palm pink, *Cymbopogon nardus* (L) Rendle, *Citronella*, *Elyonurus candidus* (Trin) Hack., lemon grass, *Vetiveria zizanioides* (L) Nash, vetiver. Porto Alegre: FEPAGRO, 2003. 31pp. (Bulletin FEPAGRO, 11).

Grattapaglia, D. and Machado, M.A. (1998). Micropropagation. In: TORRES, AC; CALDAS, LS; BUSO, JA (ed) *Tissue culture and genetic transformation of plants*. Brasília: Embrapa-SPI/ Embrapa-CNPH, pp.183-260.

Laszlo, F. (2000). Grasses lemon grass, lemon and ginger, palmarosa, and Jamrosa citronella. Available at:http://www.aromalandia.org/capins.htm. Access on April 15

Zigiotto, D.C. (2000). Micropropagation of Citronella (*Cymbopogon winterianus*). Marechal Cândido Rondon, Monograph (*completion of course*). Course Agronomy, State University of West of Paraná.