In vitro propagation of *Ocimum basilicum* L. (Lamiaceae)

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ABSTRACT. O*cimum basilicum* L. (sweet basil), of the family Lamiaceae, is rich in aromatic essential oils and valuable for its pharmaceutical, aromatic and culinary properties. The present study describes the procedure for micropropagation of *O. basilicum* using cotiledonary leafs from *in vitro* geminated plants. Cotyledons from *in vitro* germinated seeds were used as initial explants, put in MS (Murashige and Skoog, 1962) medium with 0.2mg.L^{-1} NAA (1-Naphthalene acetic acid) in combination with 0–5mg.L^{-1} BAP(6-Benzyl aminopurine) and kept at 28 ± 1°C, 16-h light photoperiod and 48µmol.m^{-2}.s^{-1} luminous density flow, for 45 days. The highest efficiency of shoot formation after 45 days occurred in the medium containing 5mg.L^{-1} BAP and 0,2mg.L^{-1} NAA. The presence of NAA inhibited root formation, when combined with different concentrations of cytokinin (BAP, 1 to 5mg.L^{-1}). Higher BAP concentrations induced an increase in the number of explants with shoots and a higher number of shoots/cotyledon.

Key words: *Ocimum basilicum,* tissue culture, micropropagation, basil, growth regulators.

RESUMO. Propagação *in vitro* de *Ocimum basilicum* L. (Lamiaceae). *Ocimum basilicum* L. (manjericão) pertencente à família das Lamiaceae é rico em óleos essenciais aromáticos e valorizado por suas propriedades farmacêuticas, aromáticas e culinárias. Neste trabalho descrevemos o procedimento para micropropagação de *O. basilicum* utilizando cotilédones de sementes germinadas *in vitro*. Estas foram utilizadas como explante inicial, colocadas em meio MS (Murashige e /skoog, 1962), com 0,2mg.L^{-1} de ANA em combinação com BAP nas variações de 0–5mg.L^{-1} e mantidas à 28 ± 1°C, fotoperíodo de 16h de luz e densidade de fluxo luminoso de 48µmol.m^{-2}.s^{-1} por 45 dias. Maior eficiência na formação de brotos foi obtida utilizando 5mg.L^{-1} BAP e 0,2mg.L^{-1} ANA. A presença de ANA inibiu a formação de raízes quando combinada com diferentes concentrações de citocinina (BAP, 1 a 5mg.L^{-1}). Altas concentrações de BAP induziram aumento no número de explantes com brotos e no número de brotos/cotilédone.

Palavras-chave: *Ocimum basilicum,* cultura de tecidos, micropropagação, manjericão, reguladores de crescimento.

Introduction

Ocimum basilicum L. (sweet basil) of the family Lamiaceae is a herbaceous species rich in aromatic essential oils and is valuable for its pharmaceutical, aromatic and culinary properties. The plant is stomatic, antihelminthic, antipyretic, diaphoretic, expectorant, carminative, stimulant and pectoral (Sahoo *et al.,* 1997; Phippen and Simon, 1998). Roots, bark, and leaves are cyanogenetic. The seeds have demulcent, stimulant, diuretic and diaphoretic properties (Sahoo *et al.,* 1997).

The current commercial basil varieties show a wide range of leaf shapes, leaf textures and colors, flower colors, plant heights and structures, and fragrances. Purple varieties of basil have recently been shown to be rich sources of anthocyanins and many of them may be a potential source of antioxidants (Phippen and Simon, 1998). However, a major difficulty in the use of Lamiaceae species for pharmaceutical purposes is the individual variability, due to genetic and biochemical heterogeneity (Shetty, 1997; Vieira *et al.*, 2001).

In vitro micropropagation is an effective mean for rapid multiplication of species in which it is

necessary to obtain a high progeny uniformity. Therefore, the interest in using these techniques for rapid and large-scale propagation of medicinal and aromatic plants has been significantly increased (Sahoo *et al.*, 1997). Many *in vitro* studies have been conducted on Lamiaceae species, including the *Ocimun* genus, using different explants, like nodal segments (Ahuja *et al.*, 1982; Shahzad and Siddiqui, 2000; Begun *et al.*, 2000), leaf explants (Phippen and Simon, 2000), young inflorescence (Singh and Sehgal, 1999) and axillary buds (Begun *et al.*, 2000, 2002). The present study describes the procedure for the micropropagation of *O. basilicum* using cotiledonary leafs from plants geminated *in vitro*.

Material and methods

Seeds from *O. basilicum* were surface sterilized with a solution of 70% alcohol for 2min and rinsed 3 times with sterile distilled water, followed by 15min in a solution of sodium hypochlorite and, finally, rinsed 3 times with sterile distilled water. These surface sterilized seeds were explanted onto Murashige and Skoog (1962) germination culture medium (MS) (supplemented with 3% sucrose, 100mg.L^{-1} myo-inositol, 0.7% agar and pH 5.8) using 6 seeds/flasks and 35 flasks.

Cotiledonary leaves were excised and used as initial explants 12 days after planting. MS culture medium (1962) was supplemented with sucrose (3% w/v), myo-inositol (100mg.L-^{1} w/v), and different hormone combinations. The pH of the medium was adjusted to 5.8 before gelling with agar (0,7% w/v), and 30mL of medium were dispensed in 150mL flasks. These were capped with aluminum foil and autoclaved at 1.5 atm of pressure, 121°C for 20min.

Cultures were maintained in the culture room at 28 ± 1°C, under 16h photoperiod provided by cool white fluorescent light (48µmol.m^{-2}.s^{-1}), for 45 days. The experimental design used was random, and each treatment constituted of different dosages of BAP (0, 1, 2, 3, 4 and 5mg.L^{-1}) in combination with 0.2mg.L^{-1} of ANA. Six repetitions per treatment were used, and each repetition was represented by 5 cotyledons. The data were submitted to variance analysis and the media of the treatments were statistically compared by Duncan test, or analyzed by polynomial regression, through the Statistic Program SANEST-*Sistema de Analise estatística* (Zonta and Machado, 1984). The data expressed in percentage were transformed in sine arc of the square root and, the data from the average number of shoots from each explant were transformed in square root of (x+0.5). Regenerated shoots (1,5cm long) were removed and transferred to 1/2 MS without growth regulator supplement. After 45 days, rooted shoots were transferred to soil.

Results and discussion

Cotyledons were a suitable explant source for *Ocimum* micropropagation. Depending on the culture media hormonal balance, adventitious shoots, callus and roots were formed. Low auxin concentration (0.2mg.L^{-1}, NAA) combined with different levels of cytokinin (BAP) was effective for callus induction (Figure 1). Although it was possible to regenerate shoots in all the different media combinations tested, there was a large difference in the response of the tissue on the various media. These differences were related to cytokinin dosis .

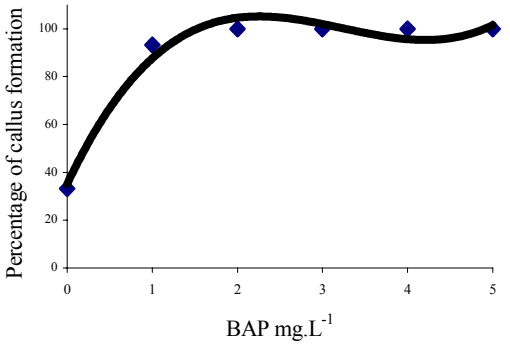

Figure 1. Percentage of callus formation from cotiledonary explants in different concentrations of BAP.

Compact and organogenic green tissue was observed mainly in the higher concentration media, whereas creamy friable tissue proliferated in the lower BAP doses. Cotyledons cultured on medium containing 0.2mg.L^{-1} NAA and 5mg.L^{-1} BAP for 45 days gave the highest efficiency of shoot formation per explant (66, 7%) and a higher number of shoots per explant (3,46), as can be seen in the Figures 2 and 3.

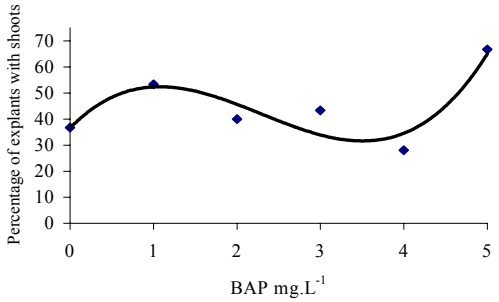

Figure 2. Percentage of basil explants with shoots induced from cotyledons on different concentrations of BAP.

The second most responsive medium was the one containing 1mg.L^{-1} BAP and 0,2mg.L^{-1} NAA. Singh and Seghal (1999) observed direct regeneration and multiple shoot induction incubating *O. sanctun* inflorescences on MS containing 0,5 to 3,0mg.L^{-1} of BAP. In their studies, the medium containing 1mg.L^{-1} of BAP and 0,05 proved to be most effective.

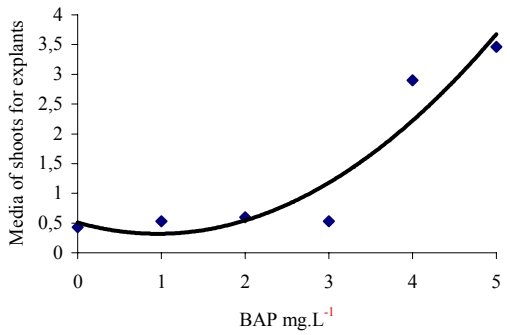

Figure 3. Average number of shoots per explant on different concentrations of BAP.

Several authors have observed a strong co-relation between the auxin/citokinin ratio in the media and shoot formation using different explant sources and genotypes (Pattnaik and Chand, 1996; Khanna and Raina, 1998; Singh and Sehgal, 1999; Shahzad and Siddiqui, 2000).

Phippen and Simon (2000) used high cytokinin levels to successfully stimulate leaf organogenesis in basil. However, the tested media were not proper to stimulate shoot regeneration from cotyledons, petioles or stems.

In the present study, the presence of NAA (auxin) inhibited root formation even when combined with different concentrations of cytokinin. Growth regulators balance in the shoot induction media had a controlling effect on the morphogenic process. Previous reports agreed that the adjustment of exogenous auxins and cytokinins levels play an important role on shoot induction and development, drastically affecting the morphogenesis (Rout *et al.*, 2000).

More than 90% of the shoots quickly developed roots when transferred to MS hormone free media and in 45 days several plants were successfully potted.

Conclusion

The protocol established in this study will enable future works on developing *Ocimum in vitro* propagation systems. These would enable the development of genetic transformation protocols to address concerns about diseases and pest resistance, and also to start a biochemical selection, focusing secondary metabolites production.

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