Effect of Cytokinin and Kind of Explant upon Friar’s Crown In Vitro Shoot Formation

Embrapa Tropical Agroindustry
Rua Dra. Sara Mesquita 2270, Bairro Pici
CEP 60511-110, Fortaleza, Ce
Brazil

J.P.S. Morais
Embrapa Cotton
Rua Oswaldo Cruz, 1143, Centenário
CEP 58428-095, Campina Grande, PB
Brazil

Keywords: Melocactus zehntneri, Caatinga, cactus, micropropagation

Abstract
Friar crown or coroa-de-frade (Melocactus zehntneri) is a globular cactaceae with high ornamental value. They mainly occur at Brazilian Northeast and Bahia State has the highest diversity. They are slow-growth species, whose reproductive stage begins around 10 years old. Wild populations do not present lateral shoots. Caatinga degradation and illegal commercialisation of them has threatened some of these species. Thus, tissue culture techniques are an alternative way as to conserve as to multiply plants with agronomic interest. In this work, in vitro 19-months-old friar crown seedlings about 3 cm high were used. The plants were transversally cut in three kinds of explants: apical, median and basal. A completely randomized design in factorial scheme 5×3 was used, in which factor A was BAP concentration (0, 1, 2, 4, 8 mg L\(^{-1}\)) and factor B was the different kinds of explants, in a total of 15 treatments, with 5 repetitions of 5 flasks with one explant each. Culture medium JADS was used with 30 g L\(^{-1}\) of sucrose and 2 g L\(^{-1}\) of Gelrite\(^\circledR\). The flasks had an internal volume of 250 ml and were filled with 40 ml of culture medium. The experiment was conducted for 60 days in a growth chamber at 27±2°C, photoperiod of 12 hours and photosynthetically active radiation of 30 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). The statistical analysis showed that both factors were significant. The greater shoot number was at 8 mg L\(^{-1}\) of BAP in accordance to a sigmoidal curve and the explants have relationship to the natural cytokinin distribution, with the greater number of shoot formation at basal explants.

INTRODUCTION
In the Brazilian Northeast semi-arid region, there are several cactaceae with great importance for the native flora and fauna, as they are cattle feed plants mainly during the drought times (Cavalcante and Resende, 2006). Most of the species belong to the genus Cereus, Pilosocereus and Melocactus.

The species of the genus Melocactus are commonly named friar’s crown (coroa de frade) and are attractive plants due to their globular shape and the development of a structure, at the apex of the plant, from which the flowers are grown and where the berries with seeds are produced, named cephalium. The plants are found in Mexico, Caribbean and South America. From all the 36 known species of Melocactus (Anderson, 2001), 19 species occur in Brazilian East, and 17 are endemic ones (Zappi et al., 2010). In the Bahia State there is the highest concentration of the species (Taylor and Zappi, 2004). Melocactus zehntneri is one of the three species that are found in Ceará State (Taylor and Zappi, 2004) and, nowadays, is the most common species in the Ceará Caatinga (Coelho et al., 2010).

Caatinga degradation and illegal friar’s crown sale for ornamental purposes have threatened the species.

The plants are mainly reproduced by seed. There is a lack of knowledge about the seed needs and about the factors affecting seed germination (Lone et al., 2007). Additionally, wild populations do not present lateral shoot formation. Thus, tissue culture could be a valuable alternative way for the conservation and in vitro multiplication of plants with agronomic importance. Several endangered species, including cactaceae, are
already micropropagated under laboratory conditions, as for instance, *Coryphantha minima* (Malda et al., 1999), *Notocactus magnificus* (Medeiros et al., 2006), *Ésobaria minima*, *Mamilaria pectinifera* and *Pelecyphora aselliformis* (Giusti et al., 2002).

It is reported that endogenous phytohormones could affect the response of the explants to exogenous growth regulator applications. Usually, plants produce auxin at their apexes, both stem and root ones, and afterwards the phytohormone is distributed to the whole plant, creating a gradient. Endogenous cytokinins are formed in the roots, with a decreasing concentration from the basal part of the explant to the apical one (Taiz and Zeiger, 2002).

The aim of this work was to evaluate the effect of the level of the growth regulator and the kind of explant over the shoot and root formation from young friar’s crown in in vitro cultured plants.

**MATERIALS AND METHODS**

Seedlings of friar crown (*Melocactus zehntneri*), which were in vitro cultured for 19 months and with about 3 cm of height, were transversally cut, creating three kinds of explants: apical, median and basal (Fig. 1).

A completely randomized design was used, in a factorial scheme 5×3 (A×B), in which the A factor was five different concentrations of BAP (6-Benzylaminopurine) (0, 1, 2, 4, 8 mg L⁻¹) and the B factor was three different kinds of explants, in a total of 15 treatments. They were five-times replicated with five 250-ml flasks of one explant per replicate.

The culture medium JADS was used (Correia et al., 1995) supplemented with 30 g L⁻¹ of sucrose, 2 g L⁻¹ of Gelrite® and BAP as a function of the treatment. The growth regulator and Gelrite® were purchased from Sigma-Aldrich and all the other drugs were purchased from national manufacturers.

The flasks were filled with 40 ml of culture medium. The experiment was carried out for 60 day in a growth chamber at 27±2°C, photoperiod of 12 hours of light and photosynthetically active radiation of 30 μmol m⁻² s⁻¹.

The average shoot number and the rooting percentage were evaluated after 60 days of culture by unaided eye and without flask opening. The data of average shoot number as a function of the kind of explant were transformed to (x+1)⁰.⁵ and analysed through variance analyses and Tukey’s test. The rooting percentage and the shoot multiplication as a function of BAP concentrations were adjusted to a logistic function.

**RESULTS AND DISCUSSION**

The shoot multiplication number is higher in basal and medium explants than in apical ones, as reported in Table 1. This behaviour could be related to the plant endogenous phytohormones gradient (Souza et al., 2003). Since auxin if formed in the stem apex, the phytohormone concentration is decreasing from the apical part of the explant to the basal one, while cytokinins have a decreasing concentration from the basal part of the explant to the apical one (Taiz and Zeiger, 2002).

Considering these two biological events, it is expected that the highest cellular division rates and, consequently, shoot formation must be greater in basal explants than in apical ones. Our data are in accordance with this assumption. The median explants in this cactus species, under the tested culture conditions, showed an auxin-cytokinin balance that is closer to the basal one (Table 1).

Shoot multiplication in friar’s crown (*Melocactus zehntneri*) is related to the kind of explants and BAP concentration in the culture medium (Fig. 2).

In Figure 3 is shown that the three kind of explants have curves of shoot number as a function of BAP concentration that fit to the logistic function, a good regression model for biological growths (Choi et al., 2009). This model is currently used in biological assays. Low doses of the independent variable lead to low answers for the dependent variable. As the doses are increased, one can note the increase, almost linear, of the answer, until it reaches a plateau, when the increases of the doses do not affect the
biological answer, in a sigmoidal-like curve (Berkson, 1944). For the apical and median explants, the beginning of the plateau phase is about 4 mg L\(^{-1}\) of BAP, although there still is a slight increase of shoot number by the BAP at 8 mg L\(^{-1}\), while for the basal one, it might be noted that the shoot number increase is more pronounced. This observation could be a function of the synergism of endogenous cytokinin with the exogenous one.

In accordance with Figure 4, the basal and apical explants fitted to the logistic model. They present for the rooting percentage a decreasing behaviour as a function of the cytokinin increasing. One can note that at 4 mg L\(^{-1}\) the minimal rooting level was reached. For the median one, the logistic function could not be fitted, since the rooting percentage was not influenced by the cytokinin concentrations, staying in a very low level. Because cytokinin presents an antagonist action in comparison to auxin action for rooting, the reducing in rooting percentage follows the awaited behaviour.

**CONCLUSIONS**

It is suggested the use of BAP at 4 mg L\(^{-1}\) for *Melocactus zehntneri* shoot formation from apical and median explants and BAP at 8 mg L\(^{-1}\) for the basal one.

The shoot formation is an increasing sigmoidal function for median and apical explants, while the root formation is a decreasing exponential function for basal and apical ones as a function of cytokinin increasing concentration.

The apical explant is the most susceptible kind to phytohormone modulation.

**ACKNOWLEDGEMENTS**

We thank Banco do Nordeste do Brasil and Embrapa for funding this research.

**Literature Cited**


Tables

Table 1. Shoot multiplication in friar’s crown (*Melocactus zehntneri*) as a function of the
kind of explant after 60 days of in vitro culture.

<table>
<thead>
<tr>
<th>Explant Kind</th>
<th>Shoot number (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>3.23 a</td>
</tr>
<tr>
<td>Median</td>
<td>3.01 a</td>
</tr>
<tr>
<td>Apical</td>
<td>1.21 b</td>
</tr>
<tr>
<td>F</td>
<td>31.36</td>
</tr>
<tr>
<td>CV(%)</td>
<td>35.51</td>
</tr>
</tbody>
</table>

Means which are followed by the same letter do not differ statistically by Tukey’s test (P<0.05); data
transformed to $(x+1)^{0.5}$.

Figures

![Fig. 1. In vitro cultured 19-months-old friar’s crown (*Melocactus zehntneri*) seedling with
about 3 cm of aerial part height. The arrows indicate the kind of explants (apical,
median and basal) which were employed in this experiment.](image)
Fig. 2. Shoot multiplication in friar’s crown (*Melocactus zehntneri*) as a function of the kind of explant (basal, median and apical) and BAP concentrations (0, 1, 2, 4, 8 mg L⁻¹), after 60 days of in vitro culture.
Fig. 3. Average shoot number in friar’s crown (*Melocactus zehntneri*) as a function of kind of explant and cytokinin concentration after 60 days of in vitro culture.

Fig. 4. Rooting percentage in friar’s crown (*Melocactus zehntneri*) as a function of kind of explant and cytokinin concentration after 60 days of in vitro culture.