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MICROPROPAGATION OF Maclura tinctoria L.: AN ENDANGERED WOODY SPECIES[1]

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Abstract – Some native species produce seeds with low germination percentage and in most cases with dormancy, which makes the appearance of new individuals by sexual propagation difficult. The Maclura tinctoria *has been considered an endangered species due to the indiscriminate use of its wood and low rate of seed germination. In this context, the objective of the present study was to establish an* in vitro *propagation methodology for this species. Combinations of NAA + BAP, different concentrations of GA_3 and combinations IBA + activated charcoal were evaluated for shoot induction, shoot growth and root formation, respectively. The results indicated that the maximum shoot formation was obtained when 5.37 µM NAA + 4.45 µM BAP was used. The use of 5.48 µM GA_3 promoted shoot growth. Root formation was observed on explants inoculated in WPM with a pH adjusted to 7.0 and supplemented with 23.62 µM IBA + 4.7 g L^{-1} activated charcoal. The use of a 70% light screen for 7 days followed by the use of 50 and 30% light screens also for 7 days each provided 97% plantlet survival.*

Keywords: Maclura tinctoria, Propagation and acclimatization.

MICROPROPAGAÇÃO DE *Maclura Tinctoria* L.: UMA ESPÉCIE LENHOSA EM EXTINÇÃO

Algumas espécies nativas produzem sementes com baixa porcentagem de germinação e, na maioria dos casos, dormência que pode dificultar o aparecimento de novos indivíduos, por meio da propagação sexuada. A *Maclura tinctoria* tem sido considerada como ameaçada de extinção devido ao uso indiscriminado de sua madeira e à baixa taxa de germinação de suas sementes. Nesse contexto, o objetivo deste estudo foi estabelecer uma metodologia de propagação *in vitro* para a espécie. Combinações de ANA + BAP, diferentes concentrações de GA_3 e combinações de AIB + carvão ativado foram avaliadas na indução de brotações, alongamento caulinar e indução de enraizamento, respectivamente. Os resultados indicaram que a máxima formação de brotações foi obtida quando 5,37 mM NAA + 4,45 mM BAP foram utilizados. O crescimento das brotações foi observado com 5,48 mM GA_3. Para a formação de raízes, foi indicado o uso do meio WPM, com pH ajustado para 7,0, suplementado com 23,62 mM AIB e 4,7 g L^{-1} de carvão ativado. O uso de sombrite 70% por sete dias, seguido da utilização de sombrite 50 e 30%, também por sete dias cada, promoveu 97% de sobrevivência de plantas.

Palavras-chave: Maclura tinctoria, Propagação e aclimatização.

1. INTRODUCTION

Maclura tinctoria L. is a woody plant of the Moraceae family, classified as a secondary species adapted to degraded areas from Mexico to southern Brazil (TORRES et al., 1992). Its fruit contains a large number of seeds that become nonviable quickly. Germination rates are low (approximately 30%), but the seeds do not exhibit dormancy. The plant produces a milky liquid in its peel, leaves and stem segments which has been used in folk medicine for healing wounds (VAN DER BERG, 1986) and in the relief of toothaches and hernias (BRAGA, 1976).

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Its wood has been used in furniture manufacturing, decorative coverings, carpentry, fence posts, poles, and in general construction (NOGUEIRA, 1977; PAULA and ALVES, 1997). Extensive harvesting of wood from *M. tinctoria* combined with its low frequency of seed germination have resulted in the reduction of the populations of this species from regions such as the south of the Brazilian state of Minas Gerais (VIEIRA, 1990). In this context, the use of tissue culture techniques may play an important role for species propagation.

Since native woody species have been successfully propagated using *in vitro* procedures (NOGUEIRA et. al., 2007; SOARES, et. al., 2007; LIMA et. al., 2008), the objective of this work was to test an *in vitro* micropropagation system for *M. tinctoria* to provide a continuous supply of this commercially valuable native plant.

2. MATERIAL AND METHODS

2.1. Plant material and surface disinfestation

Young nodal stem segments (1.5 cm in length and approximately 3 mm in diameter) were collected from six-month-old stock plants grown in pots fertilized with 5g NPK (4-14-18), maintained under greenhouse conditions and used as explants. These were disinfested in 70% ethanol for 1 min, and in a water:sodium hypochloride solution (v/v) for 10 min in a laminar flow chamber. Then, explants were rinsed three times (1 min per rinse) in sterile distilled water.

2.2. Shoot induction

Young nodal stem segments were inoculated in culture tubes containing 30 mL of fresh Woody Plant Medium – WPM (LLOYD and MCCOWN, 1980) supplemented with 30 g L^{-1} sucrose, 7 g L^{-1} agar and a pH adjusted to 6.0. The basal medium was supplemented with different combinations of naphthaleneacetic acid (NAA) (0; 2.68; 5.37 and 10.74 µM) and 6-benzylaminopurine (BAP) (0; 2.22 and 4.44 µM). After inoculation, the explants were maintained in a growth room with a light intensity of 43 µmol m^{-2} s^{-1} and temperature of 24 ± 2 °C.

2.3. Shoot growth

The shoots obtained *in vitro* from 20 day old nodal segments were inoculated in culture tubes containing 30 mL of WPM medium supplemented with 30 g L^{-1} sucrose, 6.5 g L^{-1} agar and a pH adjusted to 6.0. The medium was supplemented with gibberellic acid (GA_3) (0; 2.74; 5.48; 10.97 and 16.46 µM). After inoculation, the explants were maintained in a growth room with a light intensity of 43 µmol m^{-2} s^{-1} and temperature of 24 ± 2 °C. After 15 days, shoot growth was evaluated visually by observing the increase in diameter and absence of callus formation in the segment basal side.

2.4. Rooting and acclimatization

Shoots (1.5 cm in length) obtained with the best stem elongation treatment were inoculated in culture tubes containing 40 mL of WPM supplemented with 30 g L^{-1} sucrose, and 6.5 g L^{-1} agar and a pH adjusted prior to autoclaving to 5.4, 6.0 or 7.0. The medium was supplemented with indole butyric acid (IBA) (0; 4.92; 9.84; 19.68 and 29.52 µM) + activated charcoal (0; 0.5; 1; 2; 4 or 6 g L^{-1}). Plantlets were moved to 10 x 10 cm portable trays filled with the commercial soil mix Plant Max, placed in a humidity chamber with a vaporizer and acclimatized by covering them with 70, 50, and 30% light screens for 7 day consecutive periods.

2.5. Statistical analyses

A completely randomized design with 20 replications per treatment was used. Each replication consisted of one tube with 3 explants. All experiments were repeated twice. The effects of different treatments were analyzed using the generalized linear models approach (DEMÉTRIO, 1993; BOX and DRAPER, 1987).

3. RESULTS AND DISCUSSION

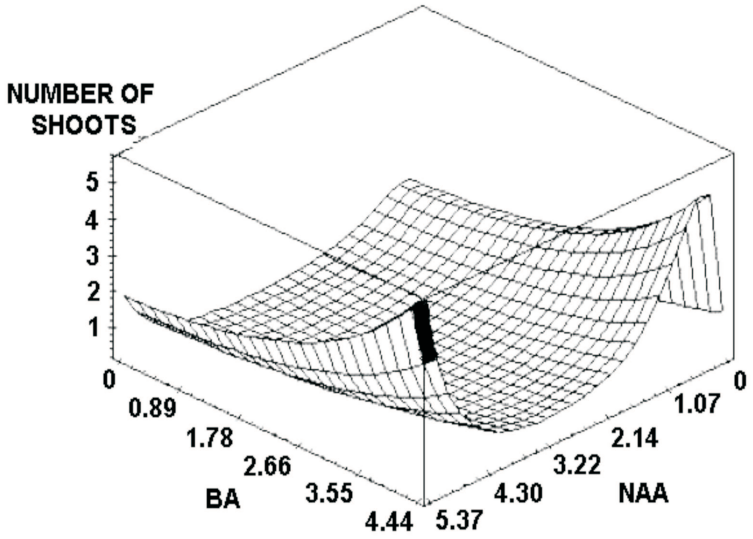
3.1 Shoot induction and growth

The use of concentrations higher than 2.68 µM NAA, inhibited the formation of shoots and induced callogenesis. This result agrees with George (1996), who states that the use of high concentrations of auxins is not adequate for sprouting induction.

Formation of shoots was not observed in the presence of isolated BAP. Conversely, in species such as *Morus australis* (PATTNAIK, 1996), *Garrya elliptica* (WOODWARD, 1996) and *Gmelina arborea* (KANNAN, 1996), the use of BAP favored sprouting induction from nodal segments. In *Acacia mearnsii*, the use of 0.4 µM BAP promoted the highest rate of bud multiplication (3.5 shoots/explant) (BORGES JÚNIOR et al., 2004).

Figure 1 – Surface response model of number of shoots obtained from nodal segments of *Maclura tinctoria* as a function of different concentrations of NAA (µM) and BAP (µM).

Figura 1 – *Modelo de superfície de resposta de número de brotações obtidas a partir de segmentos nodais de Maclura tinctoria em função de diferentes concentrações de ANA (µM) e BAP (µM).*



$$Y = e^{(1,3863 - 22,8989\ NAA + 0,0,3127\ BAP + 13,8225\ NAA^2 + 0,9454\ BAP^2 - 0,1384\ NAA.BAP + 10,6169\ NAA^5)}$$

Maximum shoot production (5) from nodal segments was obtained usin 5.37 µM NAA + 4.43 µM BAP (Figure 1). The combination of auxin + cytokinin for shoot induction has also been reported in *Ficus religiosa* L. (DESHPANDE, 1998), *Alternifolium* sp. (VALLI KHAN, 1996), *Lavandula latifolia* (SÁNCHEZ-GRAS, 1996) and *Cercis canadensis* L. (DISTABANLONG, 1997).

Santos et al. (2006) demonstrated that the use of 0.2 µM NAA + 3.33 µM BAP was the best treatment for shoot induction in *Caryocar brasiliensis* Camb. with an average of 6 shoots/explant.

The combination of BAP and kinetin also favored shoot induction in *Cinnamomum camphora* L. (BABU et al., 2003) and *Annona glabra* L. (DECCETTI et. al., 2005).

When treated with GA_3, shoots obtained from nodal segments inoculated in the presence of NAA + BA presented higher growth. The use of 5.48 µM GA_3, promoted a length growth of 1.5 cm favoring its transference to the rooting induction medium. The use of 2.74 and 4.12 µM GA_3 also promoted shoot growth in *Morus australis* Poir. (PATTNAIK, 1996) and *Paulownia* (BERGMANN, 1997). In *Annona glabra* L., however, GA_3 had no effect on shoot growth (DECCETTI et al., 2005;).

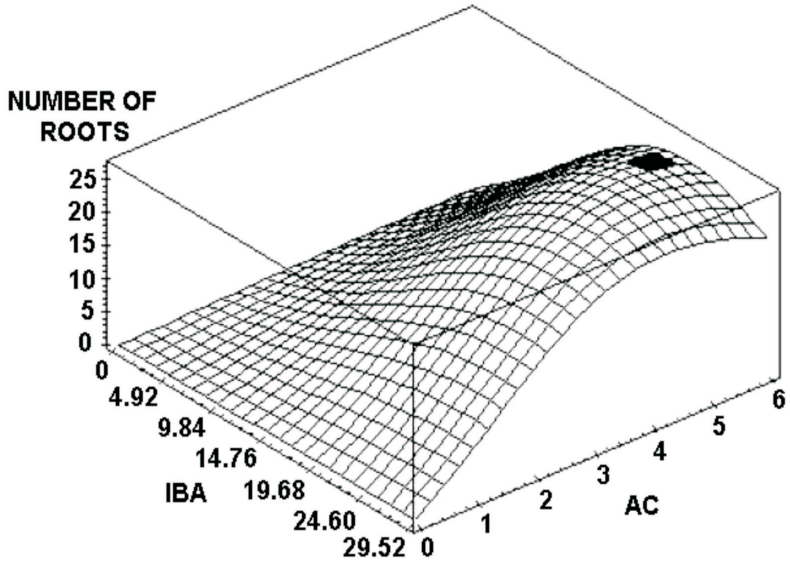
The use of 10.97 µM and 16.46 µM GA_3 also induced satisfactory growth, however with the use of these concentrations it had the formation of callus in the base of the explante. According to George (1996), the formation of callus in the base of the shoots is undesirable; therefore it hinders the vascular connection between the root system and the aerial part.

3.2. Rooting and acclimatization

Rhizogenesis was highly influenced by IBA and pH. The use of WPM supplemented with 23.62 µM IBA, 4.7 g L^{-1} activated charcoal and a pH adjusted to 7.0 provided maximum adventitious root formation (20 roots/explant) (Figure 2). According to McCown (1988), reduced activity of peroxidases in this pH promotes rooting of explants.

Figure 2 – Surface response model of number of roots obtained from nodal segments of *Maclura tinctoria* as a function of different concentrations of IBA (µM) and activated charcoal (AC) (g L^{-1}).

Figura 2 – *Modelo de superfície de resposta de número de raízes obtidas a partir de segmento nodal de Maclura tinctoria em função de diferentes concentrações de AIB (µM) e carvão ativado (CA) (g L^{-1}).*



$$Y = e^{(-3,2602+0,5375\,IBA-0,2841\,IBA^2-0,178\,CA^2-0,0755\,CA.IBA+0,5408\,IBA+2,2968\,CA+0,5584\,(CA.IBA)^{0,5})}$$

The effect of IBA on rooting has also been reported in *Aspidosperma polyneuron* (RIBAS et al., 2005), *Cinnamomum camphora* L. (BABU et al., 2003) and *Annona glabra* L. (DECCETTI et. al., 2005; SANTANA et. al., 2008; OLIVEIRA et. al., 2008). In *Aspidosperma polyneuron*, for instance, IBA has induced 80% rooting.

Root induction in *Caryocar brasiliense* Camb. was also promoted with the use of IBA showing an average of 12.87 roots per explant. Roots developed in the presence of activated charcoal showed greater length (33.16 mm) and a higher number of secondary roots (19.53) (SANTOS et. al., 2006).

The process for acclimatization (70% light screen for 7 days followed by 50 and 30% light screens for 7 days each) resulted in 97% plantlet survival. High plantlet survival has also been reported when explants are treated with IBA. Over 90% survival has been reported in *Cinnamomum camphora* L. (Babu et al., 2003) and 100% in *Ficus religiosa* L. (Dasphande et al., 1998).

4. CONCLUSIONS

Maximum shoot formation was obtained with the use of 5.37 µM NAA + 4.45 µM BAP.

Shoot growth was observed using 5.48 µM GA_3.

The use of WPM supplemented with 23.62 µM IBA + 4.7 g L^{-1} activated charcoal and a pH adjusted to 7.0 promoted root formation.

The use of 70% light screen for 7 days followed by the use of 50 and 30% light screen also for seven days each promoted 97% plantlet survival.

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