In vitro clonal mass propagation of Ximenia americana L.

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Abstract — Introduction. Ximenia americana is a species developed in Africa and South America. This fruit tree is threatened by a dangerous process of genetic erosion. In vitro techniques could be used for its rapid clonal propagation. Since there is still no report on vitroculture of the species, we tested its micropropagation using axillary buds from mature plants of X. americana. Materials and methods. Single node explants of X. americana shoots were cultured on a proliferation medium made up with a MS medium containing different concentrations (2.5–15 μM) of two cytokinins [benzyladenine (BA) or kinetin] used individually or in combination with 0.5 μM of an auxin [2,4-dichlorophenoxyacetic acid (2,4-D) or naphtaleneacetic acid (NAA)]. Data were recorded after 5 weeks of culture. From proliferated shoot clumps, shoot explants (approximately 3 cm in length) were excised and transferred to rooting media made up with MS medium with or without 0.5 μM of indolebutyric acid (IBA) (pH = 5.8). Results. The most rapid and earliest proliferation was observed in media with the lowest concentrations of cytokinins. Absence of growth regulators in media and media with 2,4-D or NAA considerably delayed bud proliferation. The number of shoots per explant increased with the increase of cytokinin. The maximum number of shoots was achieved in 10 μM BA. When shoots were transferred to rooting media, media supplemented with 0.5 μM IBA improved the rooting frequency, root quality and number of roots per cutting. After rooting, the vitroplants were transplanted into small polybags with 1:1 non-sterile soil and sand, then in the field after 4 weeks. Eighty percent of the plants taken from regulator-supplemented media were acclimated versus 15% of those taken from auxin-free media. Conclusion. The rapid clonal propagation of X. americana is possible through in vitro culture of nodal explants. The best cytokinin for shoot multiplication was BA.

Brazil / Ximenia americana / micropropagation / nodes / explants / culture media / plant growth substances

Propagación clonal in vitro de Ximenia americana L.

Resumen — Introducción. Ximenia americana es una especie que se desarrolla en África y América del Sur. Esta fruta está amenazada por un proceso de destrucción genética. Dado el escaso conocimiento sobre su vitrocultivo, se realizó un estudio para propiciar su propagación clonal. Para ello se emplearon brotes axilares de plantas adultas de X. americana. Materiales y métodos. Se cultivo explantes de solo un nudo de ramas de X. americana que se cultivaron en medios de proliferación formado con un medio MS con diferentes concentraciones de cytokininas (2.5–15 μM) [benzaldehído (BA) o kinetina] utilizadas individualmente o en combinación con 0.5 μM de un auxina [ácido 2,4-dichlorofenoxiacético (2,4-D) o naphtalenoacético (ANA)]. Se registraron datos después de 5 semanas de cultivo. De los brotes clústeres, se excisaron y transferirieron a medios de rastrillado, medios suplementados con 0.5 μM IBA mejoraron la frecuencia de rastrillado, la calidad y el número de raíces por explante. Después de rastrillado, las vitroplantas fueron transplantadas en pequeñas bolsas de 1:1 de suelo no estéril y arena, luego en el campo después de 4 semanas. El 80% de las plantas tomadas de medios suplementados con reguladores fueron aclimatados frente al 15% de aquellos tomados de medios con auxina. Conclusión. La propagación clonal rápida de X. americana es posible a través de la vitrocultura de explantes de nudos. La mejor cytokinina para la multiplicación de brotes fue BA.

Brasil / Ximenia americana / micropropagao / nécio / explante / meio de cultura / substância de crescimento vegetal.
1. Introduction

_Ximenia americana_ L., or Hog plum, Tal-ловood plum, Seaside plum, etc. in English and popularly called 'Ameixa' in Brazil, is a species developed in Africa and South America. It belongs to the class Magnoliopsida, subclass Rosidae and order Santalales, family Olacaceae. _X. americana_ occurs abundantly in Rio Grande do Norte state, Brazil. It is a rapidly growing perennial woody plant that can attain a maximum height of 3–5 m after 1–2 years. The fruit has an excellent taste. The major part of the production of late ripening of Ameixa is processed into solid, liquid and frozen products and partly used for direct consumption in the fresh state. _X. americana_ is commonly used in Côte-d’Ivoire by native healers for the medical attention of malaria. Currently, its exploration is still done in extra vista form and very little is known about this native fruitful tree, in agronomic terms. This species is threatened by a dangerous process of genetic erosion. Conventional methods of vegetative multiplication have failed and producing plants through seeds has its limitations.

Clonal propagation of plants using tissue culture technique has many applications in agronomy [1], especially for woody plants that are extremely difficult to propagate by conventional means. Considerable work has been done in the last few years on _in vitro_ propagation of woody species [2–4]. However, there is no report on _vitroculture_ of _X. americana_. Here we describe micropropagation using axillary buds from mature plants of _X. americana_ to provide an alternative for its rapid clonal propagation.

2. Materials and methods

Young shoots, each with 3–4 nodes, were collected from a 2-year-old tree of _X. americana_ (figure 1) in November–December and thoroughly washed in running tap water. Surface disinfection was carried out with 0.1% (w/v) mercuric chloride (HgCl₂) for 10 min after a brief rinse in 70% (v/v) water/ethanol. The material was then washed with sterilized double-distilled water for 10 min, giving four changes.

To induce proliferation, single node explants were then excised and cultured on a MS medium [5] containing different concentrations (2.5–15 µM) of cytokinin, benzyladenine (BA) or kinetin, individually or in combination with 0.5 µM of an auxin, 2,4-dichlorophenoxyacetic acid (2,4-D) or naphtaleneacetic acid (NAA).

After 5 weeks of culture, data on the days taken to bud proliferation, percentage of proliferation cultures, number of shoots per culture and shoot length (cm) were recorded. For each treatment, 20 replicates were used and the experiment was repeated at least twice.

From proliferated shoot clumps, individual shoots (approximately 3 cm in length) were excised and transferred to rooting media made up with a MS medium with half-strength salts and sucrose with or without 0.5 µM of indolebutyric acid (IBA). The pH of the medium was adjusted to 5.8 before autoclaving and solidified with 7 g L⁻¹ Difco Bacto agar.

The explants were cultured singly in 200 mL glass flasks, each containing 30 mL of the culture medium. The cultures were grown at (26 ± 2) °C, with a 16 h photoperiod at a light intensity of 55–60 µmol·m⁻²·s⁻² provided by warm white fluorescent tubes.

After harvesting of microcuttings for rooting, the stock cultures were transferred to fresh medium after removing dead and discolored tissues. In this way, stock cultures of shoots were maintained for continuous production of shoots for several months. No change was observed in this cyclical method of shoot production while the proliferation cultures were maintained.

3. Results

Two to three changes of media during the first week of culture were found to be most beneficial for obtaining a satisfactory response of explants in all instances. Nodal explants cultured on different treatment
combinations showed their first response by enlargement and break of axillary buds. Rapid and early proliferation was observed in media with lower concentrations of cytokinins (Table I). Absence of growth regulators in media considerably delayed bud proliferation. Media with 2,4-D or NAA also delayed proliferation. More than 45% of the explants responded in growth regulator-supplemented media but the survival percentage was only 25% in growth regulator-free media. The number of shoots per explant increased with the increase of cytokinins up to 10 µM and declined with 15 µM. The maximum number of shoots was achieved in 10 µM BA (Figure 2) followed by 10 µM kinetin. Addition of 2,4-D or NAA in the media did not improve shoot proliferation and resulted in callus production at the base of explants, which suppressed the growth of the shoot. Shoot elongation was more or less inverse to the shoot number and maximum shoot length was recorded in 2.5 µM BA.

When shoots were transferred to rooting media, roots produced in auxin-free media were thin and the number of roots per cutting was low. Media supplemented with 0.5 µM IBA improved the rooting frequency, root quality and number of roots per cutting.

After 4 weeks of root induction, the plantlets appeared good enough to be transferred to ex vitro conditions. We observed that, for successful establishment in soil, in vitro rooted shoots needed to be kept at 30 °C with a constant photoperiod for 7–10 d until roots became brown. If the plantlets were transplanted without this
treatment, survival was less than 5%. The plantlets with brown roots were carefully transplanted to small polybags with 1:1 non-sterile garden soil and sand covered with a glass beaker to maintain humidity. After 4 weeks, the potted plants were finally transplanted into the field. Eighty percent of the plants taken from regulator-supplemented media were acclimated versus 15% of those taken from auxin-free media.

4. Conclusion

The results described in this investigation show that rapid clonal propagation of *Ximenia americana* is possible through *in vitro* culture of nodal explants. The best cytokinin for shoot multiplication was BA.

References


