Efficient In Vitro Plant Regeneration from Internode Explants of *Ibervillea sonorae*: An Antidiabetic Medicinal Plant

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Abstract. *Ibervillea sonorae* is a medicinal plant mainly used to treat diabetes, ulcers, and other metabolic disorders. A regeneration protocol using internode segments containing axillary buds grown on Gamborg medium (B5) supplemented with 0.5 mg L⁻¹ α-naphthalene-acetic acid (NAA), 0.5 mg L⁻¹ 6-nbenzyadenine (BA), and 1.0 mg L⁻¹ indole-3-acetic acid (IAA) successfully regenerated shoots in *I. sonorae* explants. The induction of organogenic calli attained 100% efficiency. The highest percent shoot production was 87.5% with a mean of 9.17 shoots per explant on day 15, and the maximum length of 5.8 cm was observed on day 21. Regenerated shoots induced roots in B5 medium supplemented with 0.5–3.0 mg L⁻¹ indole-3-butyric acid (IBA). The maximum rooting frequency observed in the medium containing 2.0 mg L⁻¹ IBA was 83.3% which promoted long, thick roots on day 21. The plantlets with emerging roots grown at the culture facility attained 50% survival after acclimatization for 30 d. The account describes a simple and efficient protocol for in vitro plant regeneration, and this micropropagation procedure offers an alternative for preservation of this medicinal plant.

From ancient times, flora are source of compounds for the treatment of diseases. It is estimated that around 75% of the world’s population currently depends on plants as source of traditional medicine (Arias et al., 2009; Rao and Ravishankar, 2002). Medicinal plants contain therapeutic molecules, whose active principles also serve as precursors for drug synthesis (Loraine and Mendoza-Espinoza, 2010).

*Ibervillea sonorae* (S. Watson) Greene is a medicinal wild perennial plant usually known as “wereke” or “guareque,” traditionally used to treat diabetes, ulcers, and other metabolic disorders (Johnson et al., 1996; Xolalpa, 2002). It belongs to the Cucurbitaceae family, and it is native to arid areas from northern Mexico (Lira and Caballero, 2002; Xolalpa and Aguilar, 2006). It produces secondary metabolites such as alkaloids, tannins, saponins (Alarcon-Aguilar et al., 2005), flavonoids, phenols, (Zapata-Bustos et al., 2014) and cucurbitacins (Achenbach et al., 1993; Jardón-Delgado et al., 2014). Pharmacological studies show that plant root extracts display hypoglycemic and anti-inflammatory activities (Alarcon-Aguilar et al., 2005; Jardón-Delgado et al., 2014; Rivera-Ramírez et al., 2011; Zapata-Bustos et al., 2014), antioxidant (Estrada-Zúñiga et al., 2012), antimicrobial (Robles-Zepeda et al., 2011), and antifungal activities (Ruiz-Bustos et al., 2009). A recent study conducted on human preadipocyte cells showed that aqueous root extracts from *I. sonorae* stimulate glucose uptake by a PI3K independent pathway (Zapata-Bustos et al., 2014). This evidence supports the antidiabetic properties attributed to *I. sonorae* roots in traditional medicine. The widespread demand of *I. sonorae* roots for therapeutic purposes threatens their survival; therefore, an action is required to protect this species (Gómez-Aiza, 2011).

Cell and tissue culture are valid alternatives for in vitro production of secondary metabolites with biological activity because they are independent of seasonal factors. A standardized protocol for in vitro micropropagation represents a suitable option for the conservation of endangered species or propagation of variants with a desired phenotype (Elías et al., 2015). Many of these protocols have been developed for regeneration of cucurbitaceous species, like cucumber (*Cucumis sativus*) (Kim et al., 2010; Kumar et al., 2003a), winter squash (*Cucurbita maxima* Duch.) (Lee et al., 2003), ash gourd (*Benincasa hispida*) (Thomas and Sreejesh, 2004), summer squash (*Cucurbita pepo* L.) (Kathiravan et al., 2006), spiny gourd (*Momordica dioica* Roxb.) (Devendra et al., 2009), ahatlakai and kaktol (*Momordica tuberosa*) (Aileni et al., 2009), melon gubat (*Melothria maderaspatana* Linn.) (Baskaran et al., 2009), fig-leaf gourd (*Cucurbita ficifolia* Bouche) (Kim et al., 2010), balsam apple (*Momordica balsamina*) (Thakur et al., 2011), telukuch (*Coccinea cordifolia*) (Roy et al., 2012), ridge gourd (*Luffa acantangula* L. Roxb.) (Zohura et al., 2013), and bitter melon (*Momordica charantia* L.) (Sammaiah et al., 2014). Moreover, micropropagation in vitro has been reported using shoot and nodal explants of cucurbitaceous *Cucumis sativus* (*Trichosanthes dioica*) (Ahmad and Anis, 2005; Kumar et al., 2003b). Despite this profuse account on Cucurbitaceae, no records are available for regeneration of *I. sonorae* except for one report on calli induction using leaf explants (Estrada-Zúñiga et al., 2012). Therefore, the aim of this work is to establish a protocol for in vitro regeneration of *I. sonorae* to serve as a plant repository, and to define the growth conditions for subsequent establishment of cell-suspension cultures aiming the production of secondary metabolites as well.

Materials and Methods

**Plant material.** *I. sonorae* (S. Watson) Greene plants (~750 g) were obtained at the local Sonora market, Mexico City, Mexico. The plant internode containing axillary buds (NXB) was selected as the source of explants.

**Explant decontamination** was done according to the procedure modified by Estrada-Zúñiga et al. (2012). The explants were washed with 1% (v/v) Extrran detergent solution (Merrick) for 10 min and rinsed with distilled water, followed by immersion in 70% (v/v) ethanol for 30 s, then transferred to a 1.2% (v/v) sodium hypochlorite solution diluted from a 5% commercial stock (Cloralex®) for 10 min and finally rinsed four times with sterile-distilled water. The explants were excised into 0.4–0.8 cm length fragments that were separately cultured in flasks containing Murashige and Skoog (MS) (Murashige and Skoog, 1962) or Gamborg medium (BS) (Gamborg et al., 1968) supplemented supplementation.
with various concentrations and combinations of plant growth regulators (BA, IAA, and NAA).

Organogenic callus induction and adventitious shoot regeneration. The explants were grown on basal media comprising MS (4.3 g L\(^{-1}\) or BS 3.1 g L\(^{-1}\)) supplemented with 25 g L\(^{-1}\) sucrose, 150 mg L\(^{-1}\) ascorbic acid, 6 g L\(^{-1}\) agar-agar, and growth regulators. The cultures were then incubated in a growth chamber maintained at 25 ± 2 °C under 16 h photoperiod (50 umol-m\(^{-2}\)-s\(^{-1}\), daylight fluorescent tubes). To determine the best concentrations of growth regulators for shoot induction, we applied a two level full factorial design (2\(^4\)) using the growth regulators BA, IAA, and NAA as the three factors (Gardiner and Gettinby, 1998). In this study, the most efficient auxin/cytokinin ratio in MR medium for the production of organogenic callus and regenerated shoots derived from NXB explant was investigated. Besides, the frequency of shoot induction and the number of regenerated shoots per explant were recorded after 15 d of culture.

Conditions for root induction. To establish the culturing conditions for root induction, proliferating shoots (4–6 cm length and 21-d-old) with one or two leaves were excised and transferred to B5 medium supplemented with IBA. Different IBA concentrations were assayed to determine the optimal condition for root induction. These conditions were chosen by factorial design [one factor multilevel, equation: \(Y = 3.62 + 20.8 t\), where \(Y\) is the response (root induction), and \(t\) is the IBA factor] (Gardiner and Gettinby, 1998). The culturing conditions were similar to those described in section “Organogenic callus induction and adventitious shoot regeneration.”

The number of emerging roots and the root length of explants bearing shoots were recorded by day 21. Shoots containing roots were removed from the culture and after rinsing with a 1% (v/v) aqueous nystatin were separately placed inside plastic pots containing a commercial potting soil mix pH 6.0 (Hydro-Environment) containing 20% perlite, respectively placed inside plastic pots containing a commercial potting soil mix pH 6.0 (Hydro-Environment) containing 20% perlite, and growth regulators BA, IAA, and NAA as the three factors (Gardiner and Gettinby, 1998). Therefore, we adopted the optimized conditions as shown on Table 1 (BA 0.5 mg L\(^{-1}\), IAA 1.0 mg L\(^{-1}\), and NAA 0.5 mg L\(^{-1}\)) and named the MR medium in subsequent experiments to induce organogenic calli and regenerate shoot in NXB explants. The dynamic of these events is summarized in Fig. 1A–D. Figure 1A shows green organogenic calli on day 5 of culture and white calli from NXB explants, displaying the largest number of regenerated shoots from green calli (Fig. 1C) and white calli (Fig. 1D).

Furthermore, under this condition, organogenic calli sprouted on day 5 at the ends of internodes in contact with the medium. White friable calli were 2.7 times more abundant than green calli and their masses increased nearly 4-fold by day 15 (Table 2) regardless the color of calli (green calli, Fig. 1A; white calli, Fig. 1B). After culturing for 15 d, the regenerated shoots emerged more frequently (3.5x) in friable white calli than in green calli (Table 3).

Table 1. Effect of medium and concentration of growth regulators on shoot induction in *I. sonorae* NXB explants.

| BA (mg L\(^{-1}\)) | IAA (mg L\(^{-1}\)) | NAA (mg L\(^{-1}\)) | Percent shoot induction B5 | Percent shoot induction MS |
|-------------------|-------------------|-------------------|-----------------------------|-----------------------------|
| 0.5               | —                 | —                 | 0 a                         | 0 a                         |
| 1                 | —                 | —                 | 0 a                         | 0 a                         |
| —                 | 0.5               | —                 | 0 a                         | 0 a                         |
| —                 | 1                 | —                 | 0 a                         | 0 a                         |
| —                 | —                 | 0.5               | 0 a                         | 0 a                         |
| 0.5               | 0.5               | 0.5               | 23.3 ± 7.5 b                | 0 a                         |
| 1                 | 0.5               | 0.5               | 13.3 ± 4.71 ab              | 6.70 ± 4.71 ab              |
| 0.5               | 1                 | 0.5               | 96.70 ± 4.71 c              | 30.0 ± 0 cd                 |
| 1                 | 1                 | 0.5               | 16.70 ± 4.71 b              | 40.0 ± 8.16 d               |
| —                 | 0.5               | 1                 | 0 a                         | 0 a                         |
| 0.5               | 1                 | 0.5               | 46.66 ± 9.42 d              | 13.33 ± 4.71 b              |
| 1                 | 1                 | 0.5               | 13.33 ± 4.71 ab             | 26.70 ± 4.71 c              |

Each value represents the mean ± se of three independent experiments. Values in the same column with different letters are significantly different by Tukey’s test (P < 0.05). Shoot induction was evaluated on day 21. NXB = intermode with axillary buds; BA = N6-benzyladenine; IAA = indole-3-acetic acid; NAA = α-naphthalene-acetic acid.

Upon emerging white-calli, the first shoots appeared between 5–8 d (Fig. 1B). After culturing for 15 d, internodes with axillary buds emerged, and the frequency of shoot induction was 87.5 ± 14.4% (Fig. 1D; Table 3). On the other hand, green calli showed lower frequency of induced shoots (25 ± 4.0%) and their shoots emerged around day 13 (Fig. 1C; Table 3). At the end of the elongation period (15–20 d), the regenerated shoots attained 4–6 cm length.

Figure 1E displays the elongating shoots. Using the optimized MR medium, the addition of fresh growing medium to stimulate elongation of regenerated shoots was not necessary. After 20 d in MR culture, 70.8 ± 19.1% of the regenerated shoots grew up, averaging 9.17 ± 2.9 shoots per explant, exhibiting normal appearance and no signs of hyperhydrycity (Fig. 1F). These shoots were selected for further rooting experiments.

Root induction. Cultures of organogenic white calli from NXB explants, displaying the largest number of regenerated shoots (10 ± 1.9 shoots per explant) and averaging 4.44 ± 0.3 cm (data not shown) were used to evaluate the rooting ability. In the absence of IBA, shoots survived up to 10 d after transplantation without emergence of roots. Successful rooting was observed in regenerated shoots grown in B5 medium supplemented with IBA (Table 4). Between 0.5 and 1.0 mg L\(^{-1}\) IBA, rooting attained 6.6% to 20%, respectively. Most shoots failed to generate...
roots and did not survive beyond day 15. Survivors at 0.5 mg·L⁻¹ IBA exhibited thin primary roots without lateral roots (0.5 ± 0.47 roots) on day 21 (Table 4). Meanwhile, root induction gradually increased to 20% in the medium supplemented with 1.0 mg·L⁻¹ IBA attaining 1.25 ± 0.94 roots per regenerated shoot. At 2.0 mg·L⁻¹ IBA and 21 d of culture, the plantlets displayed fasciculate thin roots (Fig. 2A) and primary thick taproot with lateral roots (Fig. 2B) and yielded the highest rooting frequency of 83.3% (Table 4). The result of the specimen selected for acclimatization on day 25 (Fig. 2C) shows a robust increase in root induction accompanied by larger number of roots (4.25 ± 0.98). At 3 mg·mL⁻¹ IBA, emergence of thin primary fasciculate roots declined by 40% compared with 2 mg·L⁻¹ IBA (Fig. 2D; Table 4). On day 25, regenerated plantlets with primary and secondary roots were transferred to small plastic pots with sterilized soil (Fig. 2E), and 50 ± 0.5% (Table 4) of these plantlets survived after culturing for 30 d (Fig. 2E and F).

**Discussion**

**Callus induction and shoot regeneration.**

Micropropagation is a tissue culture technique to propagate species with slow growth in their natural habitat, production of plant free of pathogens, year around nursery of plantlets, clonal propagation of parental stocks, production of germplasm and implicit with this, preservation of endangered species. The aim of this study was to establish a protocol for micropropagation using NXB explants from *I. sonorae*. Initially, we investigated the most efficient culture media and auxin/cytokinin ratio for callus and shoot induction in *I. sonorae* NBX explants. We found that B5 medium was 3.2-fold more efficacious to induce callus than MS medium under equivalent conditions (unpublished data). Similarly, B5 medium induced 2.2-fold more shoots than MS medium (Table 1). Possibly, this organogenic efficacy of B5 medium is due to its higher content in vitamins (Smith, 2013).

We now demonstrate that optimized MR medium comprising 1.5 mg·L⁻¹ auxin (IAA + NAA) and 0.5 mg·L⁻¹ cytokinin (BA) with molar ratio 3.8:1 induced high percentage of organogenic friable calli in *I. sonorae* NXB explants on day 15 (Fig. 1B) and promoted shoot induction as well (Fig. 1C and D). Inclusion of IAA was based on the notion that it participates during the developmental and growing stages, coordinating plant metabolism (synthesis, conjugation, hydrolysis, oxidation, and transport) in cucurbitaceous, more efficiently than NAA or 2,4-D (Lee et al., 2010; Normanly et al., 1995). The auxins IAA and NAA have been reported to promote callus formation and new shoots in tissue culture of cucurbitaceous *Cucumis metuliferus* (Compton and Gray, 1993). Prior reports showed that high auxin/cytokinin ratio enhances root formation in explants of *Melothria maderaspatana* and during generation of organogenic callus in *Cucumis*

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**Table 2. Frequency and weight of organogenic calli in NXB explants from *I. sonorae*.**

| Calli | Calli induction (%) | Explant wt (g) | Calli wt (g) |
|-------|---------------------|----------------|--------------|
| White | 72.7 ± 2.0 a        | 1.0 ± 0.05     | 4.59 ± 0.72  |
| Green | 27.2 ± 0.5 b        | 0.9 ± 0.28     | 4.02 ± 0.79  |

Each value represents the mean ± SE of three replicates. Values that do not share a letter are significantly different according to Student’s test (P < 0.05). The percent of induced calli and the mass of calli were assessed on day 15. The mass of the explant was measured on the first day.

**Table 3. Frequency of regenerating shoots from calli in NXB *I. sonorae* explants.**

| Calli | Regenerating shoots (%) | Number of shoots per explant |
|-------|-------------------------|------------------------------|
| White | 87.5 ± 14.4 a           | 9.17 ± 2.95                  |
| Green | 25.0 ± 4.04 b           | 2.33 ± 0.57                  |

Each value represents the mean ± SE of three replicates. Values that do not share a letter in the same column are significantly different according to Student’s test (P ≤ 0.05). Percent of regenerating shoots was assessed on day 21.

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**Fig. 1. Regeneration of shoots in *I. sonorae* NXB explants grown in optimized MR medium.** (A) green friable organogenic callus on day 5, (B) white friable organogenic callus on day 7 with regenerated shoots, (C) regenerated shoots after 15 d in green callus, (D) regenerated and proliferating shoots on day 15, (E) elongation of shoots, and (F) regenerating shoots selected for rooting (20 d).
sativus (Baskaran et al., 2009; Kakani et al., 2009; Selvaraj et al., 2007), while low auxin/cytokinin ratio privileges shoot formation, although in this study we observe an increase of shoot production with high auxin/cytokinin values.

In previous studies, the stimulatory effect of BA combined with IAA in Cucumis melo was shown to be more efficient for calli formation than BA plus NAA or 2,4-D (Tabei et al., 1991). However, Valdez-Melara and Gatica-Arias (2009) demonstrated that the superior effect elicited by BA-IAA in C. melo depended on the genotype used. Also, the authors noted that regardless the genotype, supplementation of medium with BA alone without auxins was able to sustain shoot emergence. These results depart from what is observed in I. sonorae where IAA, NAA, and BA are essential for shoot emergence. Differences in the requirement of growth regulators highlight the relevance of variations between species that must be taken into account during micropropagation studies.

Table 4. Effect of IBA concentration on root induction of regenerated shoots from I. sonorae.

| IBA (mg·L⁻¹) | Root induction (%) | Roots per regenerated shoot | Root length | Survival in soil (%) |
|--------------|--------------------|-----------------------------|-------------|----------------------|
| 0            | 0                  | 0                           | 0           | 0                    |
| 0.5          | 6.60 ± 5.7 c       | 0.50 ± 0.47                 | 0.75 ± 0.70 | 0                    |
| 1            | 20.0 ± 10 c        | 1.25 ± 0.94                 | 2.25 ± 1    | 0                    |
| 2            | 83.30 ± 2.3 a      | 4.25 ± 0.98                 | 5.01 ± 0.81 | 50 ± 0.5%            |
| 3            | 43.30 ± 5.7 b      | 3.03 ± 0.81                 | 6.25 ± 0.95 | 0                    |

Each value represents the mean ± SE of three replicates. Values that do not share a letter are significantly different according to Tukey’s test (P < 0.05). Percent root induction, the number of roots per shoot and root length were evaluated on day 21. Plant survival was recorded on day 30. IBA = indole-3-butyric acid.

was shown to be more efficient for calli formation than BA plus NAA or 2,4-D (Tabei et al., 1991). However, Valdez-Melara and Gatica-Arias (2009) demonstrated that the superior effect elicited by BA-IAA in C. melo depended on the genotype used. Also, the authors noted that regardless the genotype, supplementation of medium with BA alone without auxins was able to sustain shoot emergence. These results depart from what is observed in I. sonorae where IAA, NAA, and BA are essential for shoot emergence. Differences in the requirement of growth regulators highlight the relevance of variations between species that must be taken into account during micropropagation studies.

Results of induction of organogenic calli or shoot induction, similar to those reported here were described on cultures using axillary buds of nodal explants in cucurbitaceous Momordica charantia L. (0.5 mg·L⁻¹ BA and 2.0 mg·L⁻¹ NAA) (Agarwal and Kamal, 2007), in hypocotyl and leaf explants of Cucumis anguria L. (0.5 mg·L⁻¹ BA and 1.5 mg·L⁻¹ 2,4-D, 1.5 mg·L⁻¹ IAA) (Ju et al., 2014) during propagation in Momordica balsamina using 1.0 mg·L⁻¹ BA (Thakur et al., 2011) and callus induction and plantlet regeneration of Citrullus colocynthis (Cucurbitaceae) with 0.5 mg·L⁻¹ IAA, 0.5 mg·L⁻¹ 2,4-D, and 1 mg·L⁻¹ BA (Satyavani et al., 2011).

The role of cytokinins and auxins as agents for organogenesis has been discussed earlier. Agarwal (2015) proposed that cytokinins activate totipotent cells in callus for shoot organogenesis whereas in the case of direct organogenesis, these molecules activate preexisting machinery pertaining to somatic cells. Cytokinins in shoots stimulate growth because of the presence of meristematic cells located at the tip of explants, whereas auxins regulate or influence diverse responses at the whole-plant level, by mechanisms such as tropisms, apical dominance and root initiation, and by triggering cellular responses such as cell enlargement, division, and reactivation of differentiated cells to promote additional vascular tissue development and regulating lateral organ formation (Hagen and Guilfoyle, 2002; Mockaitis and Estelle, 2008).

Although the mean number of shoots per explant obtained in I. sonorae was lower when compared with Citrullus colocynthis (Meena et al., 2013) or Momordica charantia (Thiruvengadam et al., 2012), their inception within two weeks is somewhat earlier than in most regenerating systems. Interestingly, most examples describing regeneration of
organogenic callus include two regulators (I auxin and I cytokinin), while in this work we maximize calli production by using 2 auxins and 1 cytokinin.

The rationale for using axillary buds as a vehicle for micropropagation rests on the notion that they are able to preserve the genetic traits due to the presence of meristematic tissue (Souza et al., 2006). This notion is supported by studies, showing that many of the propagated plants in Echinocereus cineascens and Momordica dioica when derived from axillary buds displayed higher genetic stability and uniformity (Elías et al., 2015; Thiruvengadam et al., 2006). Our results showed that the optimized MR medium enhanced the growth of regenerated shoots, and preserved them in fair condition without damage or loss during the 15 d experimental period, obviating the need for further passage to an elongation medium.

Root induction. The efficacy of root induction seen in I. sonorae regenerating shoots at 2.0 mg L⁻¹ IBA was also demonstrated in Stackhousia tryonii, M. dioica, Citrullus lanatus, Momordica cymbalaria, and Cucumis sativus (Bhatia et al., 2002; Selvaraj et al., 2007; Thiruvengadam et al., 2006). In S. tryonii, Bhatia et al. (2002) also reported that IBA was more effective than IAA and NAA for rooting, thus justifying its application as root inducer in many cucurbitaceous. In M. balsamina, a dose dependent effect on rooting has been demonstrated with maximal activity at 1.5 mg L⁻¹ of IBA (Thakur et al., 2011).

IBA has been used in commercial agriculture as well as for its capacity in plants to promote cuttings, root initiation/growth, inhibition of primary root elongation, and stimulation of lateral root formation (Zázmílova et al., 2014). In I. sonorae, regenerating shoots in B5 medium supplemented with 2.0 mg L⁻¹ IBA there was a strong induction of roots containing 83.3% (Table 4). In contrast, Yan et al. (2010) observed no significant difference in rooting rate in vitro and ex vitro in Stratta grovenorii. However, in vitro developed roots in S. grovenorii were thick, fragile, and easily broken while handling. Because of this difference, ex vitro rooting was preferable as it promoted higher percent survival rate. In I. sonorae, we detected 50% survival for plantlets regenerated following treatment with 2.0 mg L⁻¹ IBA and acclimatized in small pots for 30 d.

Interestingly, tuberous root of I. sonorae has been demonstrated to possess pharmacological activity. To date, no report exists on micropropagation or induction of tuberous root of I. sonorae in vitro or even the procedure to modulate its growth in soil. Rooting similar to that observed in this study is found in field specimens of I. sonorae; hence, investigating the presence of biologically active metabolites in vitro grown roots is essential to confirm the presence of hypoglycemic activity. Fan et al. (2011) reported that in Manihot esculenta combinations, auxins and cytokinins are necessary to induce tuberosous roots in vitro, and the absence of one of them in the medium led to their decrease or full absence. According to our results, IBA alone induced thick taproots and moderated the number of fasciculate roots; thus, obviating the need for cytokinins during root development. Future studies aim to investigate if the regenerating roots arising in culture and roots developed in ex vitro plantlets contain the metabolites responsible for the medicinal properties in I. sonorae.

**Conclusions**

This is the first report describing the in vitro micropropagation of I. sonorae through a simple and efficient protocol to induce shoots and rooting. Internode explants from this plant seem to be appropriate for the induction of organogenic calli and regenerated shoots under these experimental conditions. NXB explants grown on MR medium (B5 with 0.5 mg L⁻¹ NAA, 0.5 mg L⁻¹ BA, and 1.0 mg L⁻¹ IAA) promoted shoot proliferation and their elongation during a 3-week period. Furthermore, only one medium was required to induce and elongate shoots by day 15 obviating the need of a further passage. In B5 medium supplemented with IBA, a strong rooting response was observed displaying long thick roots with potential to become tuberous roots. This protocol is of interest for industrial propagation of the species for eventual production of secondary metabolites and for preservation of I. sonorae.

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