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RESEARCH NOTE

## Benzylaminopurine and indol-3-acetic acid concentrations in *in vitro* proliferation of *Agave angustifolia* adventitious shoots

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### Abstract

**S. Ríos-Ramírez, J. Enríquez-del Valle, G. Rodríguez-Ortiz, and J. Ruíz-Luna. 2017. Benzylaminopurine and indol-3-acetic acid concentrations in *in vitro* proliferation of *Agave angustifolia* adventitious shoots. Cien. Inv. Agr. 44(3): 285-294.** Today, a micropropagation method for *Agave angustifolia* exists, but for the multiplication of propagules, more information is needed on the diverse components of the culture medium, cytokinins and auxins and their effect on organogenetic response. The objective of this study was to evaluate dosages of benzylaminopurine and indol-3-acetic acid (IAA) in a culture medium and their effect on the formation of new adventitious shoots (organogenesis). The explants used were clusters of shoots on a common base of *A. angustifolia* stem tissue. Clusters of three to four shoots were established in different variants of a Murashige and Skoog culture medium that had different concentrations of benzylaminopurine (BAP; 0.5, 1, 2, and 4 mg L<sup>-1</sup>) and IAA (0.1, 0.3, and 1 mg L<sup>-1</sup>). After 16 wk, it was found that a higher proliferation of shoots was positively related to the concentration of growth regulators. Explants formed a greater quantity of shoots in a culture medium with an increase in BAP of up to 4 mg L<sup>-1</sup> and in IAA of up to 1 mg L<sup>-1</sup>, and there was a synergistic effect with BAP to induce the formation of the greatest number of shoots.

**Keywords:** Benzylaminopurine, explant, growth regulator, indol-3-acetic acid, organogenesis.

### Introduction

Agaves are species with morphological and physiological characteristics (Crassulacean acid metabolism, CAM) that make them capable of growing on sloping terrain; in shallow, rocky and infertile soils; and in environments with limited availability of water during long periods of the year where other crops cannot prosper (Cruz *et al.*, 2013).

In the state of Oaxaca, Mexico, several species of Agave, such as *A. angustifolia*, *A. potatorum*, and *A. karwinskii*, are used to produce a distilled alcoholic beverage called mescal. Between 2006 and 2013, Oaxaca had an average of 13,572 ha planted with Agave, and *A. angustifolia* occupied up to 70% of this area, with an average of two thousand plants per ha. Because the crop cycle of this species of Agave is seven to nine yr, 2.6 million plants are necessary to replenish the area that is harvested every year. Since 2014, there has been a deficit in the supply of plants. Said species is propagated

conventionally using rhizome shoots that are locally called “hijuelos” (“plant sprouts”). It is also propagated using bulbils from the inflorescence (Arizaga and Ezcurra, 2002). However, since 1986, plant tissue culture techniques have been proposed for propagation of this species.

*In vitro* culture is a biotechnological tool consisting of an aseptic culture of different explants in a culture medium and incubation to make use of the totipotentiality of the cells to induce cell division, morphogenesis and plant development (Fortes and Pais, 2000; Siddique *et al.*, 2015).

Propagating *Agave* using tissue culture can complement conventional methods of propagation to satisfy the demand of commercial plantations for quality plant material. A tissue culture propagation method for *in vitro* tissue culture (CTV) would also be applicable to several wild mescal agave species, some of which are in danger of extinction. Increasing plant production *in vitro* of these species would complement seed collection for nursery propagation (Martínez-Palacios, 2003).

Micropropagation was made possible four decades ago when technical innovations were adopted for intensive commercial propagation of numerous species of commercial importance. In the case of agaves, the main challenges have been to increase productivity at each stage, reduce production costs, and produce the quantity of quality plants necessary to establish plantations (Dominguez-Rosales *et al.*, 2008a). The quality of one plant can be evaluated through normal morphologic characteristics of a healthy and strong plant of the species.

*In vitro* culture has been used for several economically important *Agave* species: *Agave inaequidens* Koch (Aureoles-Rodríguez *et al.*, 2008), *A. grijalvensis* (Sánchez-Urbina *et al.*, 2008); *A. fourcroydes* Lem. (Garriga *et al.*, 2010); and *A. tequilana* (Valenzuela-Sánchez *et al.*, 2006).

In Oaxaca, México, since 1986, a plant tissue culture method has been developed for propaga-

tion of *A. angustifolia* Haw., and this method of asexual propagation includes the use of leaf and stem tissue of selected plants as explants. Data are available on the different stages of *in vitro* propagation, acclimatization in a greenhouse of micropropagated plants, and plant development in a nursery. In 1987, the first twelve thousand plants were established in the field.

In *in vitro* clonal propagation of any species, somatic tissues are established in a culture medium containing mineral salts, carbohydrates, vitamins and growth regulators. Auxins and cytokinins are the most-used growth regulators in *in vitro* culture, and the concentrations and types used depend on the species and the stage of propagation. The proportion of auxins/cytokinins during *in vitro* tissue culture can play a critical role in inducing a morphogenic response (García *et al.*, 2008). Morphogenic potential under *in vitro* culture may also depend on certain specific interactions between the auxins and cytokinins (Garriga *et al.*, 2010).

Still, more information on the diverse components of the culture medium, cytokinins and auxins and their effects on organogenetic response and shoot proliferation is needed for propagation of *A. angustifolia*. For *in vitro* proliferation of agave shoots, it is common to add cytokinins to the culture medium. Among those that have been used are benzylaminopurine (Reyes-Zambrano *et al.*, 2016), kinetin or 6-FAP, 6-furfurylaminopurine (Nikam *et al.*, 2003), 2-isopentenyladenine (2iP), and thidiazuron (TDZ) (Portillo *et al.*, 2007). In addition to cytokinins, use of auxin in a culture medium has been reported (Tejavathi *et al.*, 2007).

The objective of this study was to evaluate, during the propagule multiplication stage, the effect of dosages of benzylaminopurine and IAA in a culture medium on formation of new multiple adventitious shoots (organogenesis) from explants that had shoot clusters on a common base of *Agave angustifolia* Haw. stem tissue.

## Materials and methods

### *Plant material*

Clusters of 6 to 12 adventitious shoots of different sizes formed on *A. angustifolia* stem tissue were the plant material for the experiment. To transfer the shoots to culture media for the propagule multiplication stage, under aseptic conditions in a horizontal laminar flow filtered air chamber and using sterilized tweezers and scalpel, the clusters were extracted from the initial culture medium and placed in a sterilized glass Petri dish, 10×100 mm, where they were divided into smaller clusters of 3 to 4 shoots with 9 to 15 leaves on a base of stem tissue from which they initially emerged. One cluster of shoots (experimental unit) was established in each 145 cm<sup>3</sup> of recipient containing 20 mL of one of several variants of culture medium to induce proliferation of the adventitious shoots. These culture medium variants contained 100 mg L<sup>-1</sup> myo-inositol, 30 g L<sup>-1</sup> sucrose, 0.4 mg L<sup>-1</sup> thiamine-HCl, mineral salts (MS), diverse quantities of IAA (0.1, 0.3, 1 mg L<sup>-1</sup>) and N<sup>6</sup>-benzylaminopurine (BAP; 0.5, 1, 2, 4 mg L<sup>-1</sup>). The pH of the culture medium variants was adjusted to 5.8 before adding 5.6 g L<sup>-1</sup> of agar. The agar was dissolved by heating and shaking and distributed in the 145 cm<sup>3</sup> glass recipients which were capped with a polypropylene stopper and sterilized in an autoclave for 17 min at 120 °C and 1.2 kg cm<sup>-2</sup> pressure.

After planting the clusters, the polypropylene stopper was again placed on the recipients and sealed with polyethylene adherent. The cultures were kept for 16 wk in the incubation area, where they were exposed to white fluorescent illumination (35 μmol m<sup>-2</sup> s<sup>-1</sup>) in photoperiods of 16 h light and 8 h dark; the temperature varied from 15 to 29 °C.

At the end of the experiment, seven experimental units from each treatment were harvested and the following variables were evaluated as follows: 1) number of leaves, the total number of leaves of the shoots that formed the cluster on the common

base of stem tissue; 2) number of new shoots; 3) size of the largest shoot, measured using a precision ruler in 0.1 cm increments; 4) fresh weight, determined on an analytical balance with 0.1 mg accuracy (DENVER INSTRUMENT®, USA); and 5) total dry weight, where the samples of plant material were placed in paper bags for drying in a convection oven (Felisa® Fabricantes Feligneo, S. A. de C.V. México) at 70 °C for 72 h and later weighed on an analytical balance.

### *Experimental design and evaluation*

The experiment was set up in a completely randomized design with a 3 (IAA 0.1, 0.3, 1) × 4 (benzylaminopurine 0.5, 1, 2, 4) factorial array. The experimental unit was one cluster of shoots, and there were 12 replications per treatment. The data were subjected to a variance homogeneity test using the Bartlett test and the Shapiro-Wilk normality test. The variable number of shoots was transformed to  $\log_{10}(x)$  to comply with assumptions of variance normality and homogeneity. The data of each variable were subjected to analysis of variance and a test of comparison of means (Tukey, 0.05). For the variables of increase in number of leaves and shoots, the statistical models of exponential regression  $y = b_0 e^{b_1(1/x)}$  were carried out to obtain models that described the increase in shoots or leaves as a function of the variation in growth regulator concentrations. Later, graphs of response areas were constructed using Minitab 15® software (Minitab. Inc., State College PA, USA).

### *Results and discussion*

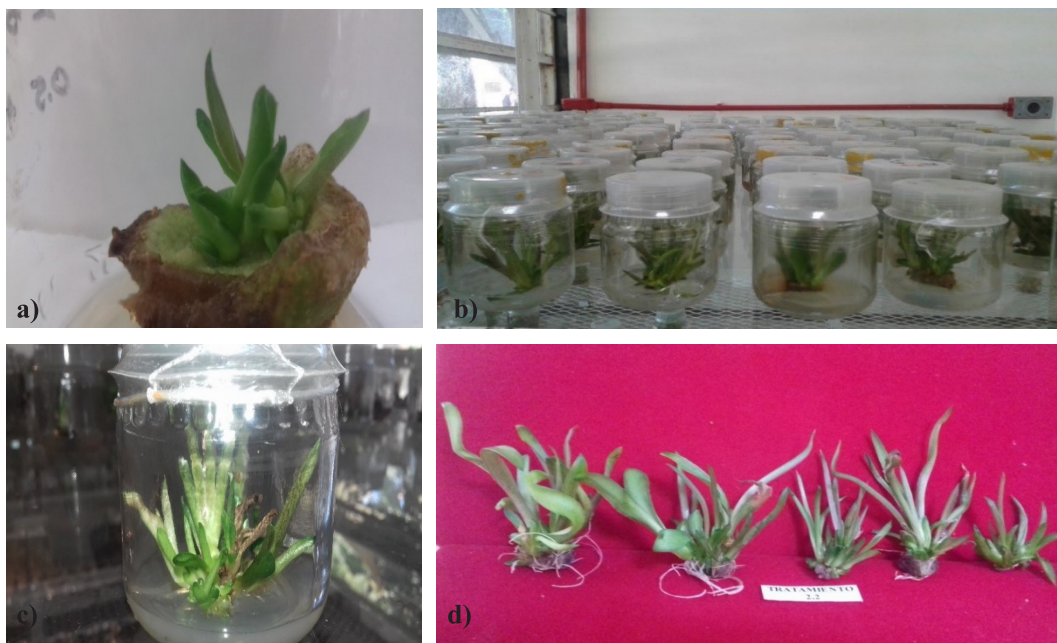
The results of this experiment indicate that *Agave angustifolia* Haw. explants subjected to the tested treatments had more shoots and more leaves, but the magnitude of the increases was conditioned by growth regulator concentrations. At the beginning of the experiment, each experimental unit consisted of clusters of 3 to 4 shoots with a total of 9 to 15

leaves and heights of 3 to 4 cm (Figure 1a). The analyses of variance showed that the concentrations of benzylaminopurine (BAP) had highly significant ( $P \leq 0.01$ ) effects on leaf number, leaf length, number of shoots, fresh weight and total dry weight, while the concentrations of IAA had highly significant ( $P \leq 0.01$ ) effects on total dry weight and a significant ( $P \leq 0.05$ ) effect on number of shoots and total fresh weight. The interaction of BAP and IAA had a highly significant effect on number of shoots and total dry weight and a significant effect on total fresh weight (Table 1). There was an increase in the number of shoots that was in a positive relationship with increasing concentration of BAP and IAA (Figure 1b, 1c and 1d). Several studies that have achieved organogenesis *in vitro* report that the growth regulators are the critical factor for growth, quantity or size of the propagules (Ramírez-Malagón *et al.*, 2008; Meratan *et al.*, 2009; Shimizu *et al.*, 2009).

At the beginning of the experiment, the explants had 3 to 4 shoots with a total of 9 to 15 leaves. After 16 wk of incubation, the explants established

in the different variants of culture medium had formed more shoots and leaves, but this increase had a significant ( $P \leq 0.001$ ) positive exponential relationship with the BAP and IAA concentration, with an  $R^2$ -adj for leaves and shoots of 0.76 and 0.61, respectively. The number of new shoots and leaves is conditioned by the BAP concentration in the culture medium, and the synergy that occurred with the IAA auxin increased the effectiveness of the cytokinins (Figures 2a and 2b). In this way, the explants established in the culture medium with  $0.5 \text{ mg L}^{-1}$  BAP +  $0.1 \text{ mg L}^{-1}$  IAA had 7.8 shoots with 24 leaves, 4.5 g total fresh weight and 0.21 g total dry weight, while the explants established in the culture medium with  $4 \text{ mg L}^{-1}$  BAP +  $1 \text{ mg L}^{-1}$  IAA had 32.8 shoots with 45 leaves, 9.1 g total fresh weight and 0.58 g total dry weight (Table 2). These figures, in each case, were significantly different (Tukey, 0.05).

In a scheme of *in vitro* propagation of agaves, during the propagule multiplication stage, it is necessary to know what types and concentrations of growth regulators to use in the culture



**Figure 1.** a) Clusters of 6 to 12 adventitious shoots formed on explants from *Agave angustifolia* stem tissue and transferred to propagule multiplication; b) *in vitro* cultures of *A. angustifolia* shoots incubated for 16 weeks; c) *A. angustifolia* shoot; d) harvest of *A. angustifolia* shoots 16 weeks after *in vitro* establishment.

**Table 1.** Summary of the analysis of variance of the characteristics of *Agave angustifolia* Haw. shoots established in culture media with different concentrations of growth regulators.

Sources of variation	Degrees of freedom	Mean Squares and significance				
		NL <sup>2</sup>	LL (cm)	NS	TFW (g)	TDW (g)
BAP <sup>1</sup>	3	786.5 **	12.8*	0.4**	14.5**	0.05**
IAA	2	20.0 ns	7.1 <sup>ns</sup>	0.04 <sup>ns</sup>	13.3*	0.07**
BAP× IAA	6	77.7 ns	5.6 ns	0.06 <sup>ns</sup>	6.9*	0.03**
Error	48	76.8	4.5	0.03	2.9	0.01
Total	59					

\*Values with significant effects ( $P \leq 0.05$ ), \*\*values with highly significant effects ( $P \leq 0.01$ ), ns: not significant ( $P > 0.05$ ). <sup>1</sup>BAP: N<sup>6</sup>- Benzylaminopurine, IAA: indol-3-acetic acid. <sup>2</sup>NL: number of leaves, LL: length of longest leaf, NS: number of shoots, TFW: total fresh weight, TDW: total dry weight.

**Table 2.** Characteristics of *Agave angustifolia* Haw. adventitious shoots that were established in a culture medium with different concentrations of BAP and IAA for four months.

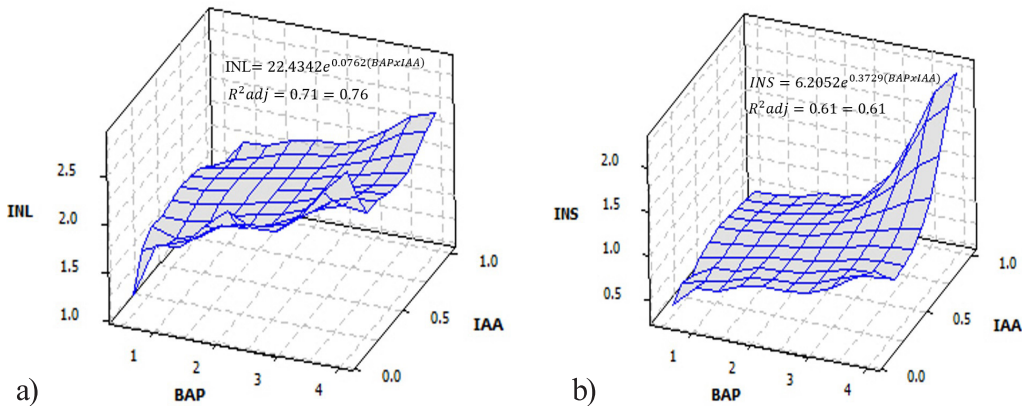
Factor and levels		Variables				
BAP <sup>1</sup> (mg L <sup>-1</sup> )	IAA (mg L <sup>-1</sup> )	NL <sup>2</sup>	LL (cm)	NB	TFW (g)	TDW (g)
0.5	0.1	24.0±5.7 c	8.3±2.6 a	7.8±2.8 c	4.5±2.5 b	0.21±0.03 b
0.5	0.3	30.0±5.5 abc	8.0±1.6 a	8.2±3.3 c	5.4±1.3 b	0.33±0.09 b
0.5	1	27.6±5.9 bc	8.9±0.8 a	8.8±3.3 bc	4.1±1.1 b	0.24±0.08 b
1	0.1	32.2±10.5 abc	7.1±2.3 a	10.6±4.7 bc	4.1±1.4 b	0.21±0.06 b
1	0.3	35.4±7.0 abc	8.7±2.0 a	11.4±3.8 bc	5.5± 2.8 ab	0.30±0.1 b
1	1	33.0±3.3 abc	10.0±1.5 a	9.4±0.8 bc	4.9±0.6 b	0.27±0.05 b
2	0.1	41.8±12.3 abc	10.4±1.1 a	13.0±4.7 bc	5.0±1.2 b	0.28±0.06 b
2	0.3	34.6±8.7 abc	10.1±2.0 a	12.0±8.7 bc	5.4±2.2 b	0.28±0.1 b
2	1	37.2±10.3 abc	10.6±1.2 a	11.2±5.8 bc	7.4±1.9 ab	0.38±0.1 ab
4	0.1	49.0±10.1 a	7.4±2.1 a	18.8±3.4 b	5.3±1.1 b	0.27±0.06 b
4	0.3	39.0±9.5 abc	10.4±1.1 a	14.0±5.9 bc	5.9±1.5 ab	0.31±0.1 b
4	1	45.2±10.8 ab	8.1±4.3 a	32.8±4.2 a	9.1±0.6 a	0.58±0.1 a

In each column, values with the same letter are not significantly different (Tukey, 0.05). The mean is ± standard deviation. <sup>1</sup>BAP: N<sup>6</sup>- Benzylaminopurine; IAA: indol-3-acetic acid. <sup>2</sup>NL: number of leaves, LL: length of the longest leaf, NB: number of shoots, TFW: total fresh weight, TDW: total dry weight.

medium to achieve optimal response, considering the maximum number of quality shoots. Quality shoots are those that are not deformed and do not have hyper-hydrated organs.

For the micropropagation of different *Agave* species, the dose of any cytokinin has been evaluated to induce the formation of shoots in protocols for *in vitro* propagation of *Agave cupreata*, *A. difformis*, *A. karwinskii*, *A. obscura* and *A. potatorum*. Formation of multiple shoots on basal explants has been achieved in an MS medium supplemented with 30 g L<sup>-1</sup> sucrose, 8 g L<sup>-1</sup> agar and several types of cytokinins: BA,

2iP, Cin, TDZ and MT, which also varied in concentration (0.5, 1, 1.5, 2, 2.5, and 3 mg L<sup>-1</sup>). It was found that explants of *A. cupreata* and *A. karwinskii* established in a CM with 1.5 and 1 mg L<sup>-1</sup> BA formed 10.5 and 6.1 shoots each, respectively, while explants of *A. difformis* and *A. obscura* responded best in a CM with 0.2 mg L<sup>-1</sup> TDZ, forming 8.5 and 11 shoots per explant, respectively. *A. potatorum* responded best in a CM with 3 mg L<sup>-1</sup> kinetin, forming 6.9 shoots per explant (Dominguez-Rosales *et al.*, 2008b). However, in other works, the best response was obtained when a combination of cytokinin and auxin was used as follows: 0.002 mg L<sup>-1</sup> 2,4-D



**Figure 2.** Response areas due to the effect of N6-benzylaminopurine (BAP) in combination with indol-3-acetic acid (IAA) in the culture medium on a) an increase in the number of leaves (INL) and b) an increase in the number of shoots (INS).

and 9.9 mg L<sup>-1</sup> BA for *A. americana* (Reyes-Zambrano *et al.*, 2016); 0.011 mg L<sup>-1</sup> IBA and 1 mg L<sup>-1</sup> BA, for *A. duranguensis*, 0.49 mg L<sup>-1</sup> IBA / 1 mg L<sup>-1</sup> BA for *A. oscura*, 0.09 mg L<sup>-1</sup> IBA / 2.99 mg L<sup>-1</sup> BA for *A. pygmaea*, 0.11 mg L<sup>-1</sup> IBA / 1 mg L<sup>-1</sup> BA for *A. salmiana* subspecies *crassispina*, 0.49 mg L<sup>-1</sup> IBA / 0.50 mg L<sup>-1</sup> BA for *A. victoria-reginae* (Ramírez-Malagón *et al.*, 2008), and 0.99 mg L<sup>-1</sup> naphthaleneacetic acid plus 0.19 mg L<sup>-1</sup> zeatin for *A. veracruz* Mill (Tejavathi *et al.*, 2007). The aforementioned results show that cytokinin has the most effect in inducing shoot proliferation, but this effect is increased when an auxin was used in the media.

Additionally, in *in vitro* culture of other plant species, variations in type and concentration of cytokinins and auxins have been used in the culture medium to examine the synergetic effect of the two growth regulators. Siddique *et al.* (2015) achieved *in vitro* propagation of a medicinal species, *Cassia angustifolia* Vahl, and evaluated two cytokinins (BA, Kin) in the concentration of 1.12 mg L<sup>-1</sup> and the auxin IAA (0.01, 0.08 or 0.17 mg L<sup>-1</sup>). They observed that BA was more effective than kinetin in promoting new shoot formation: the explants established in a culture medium with 1.12 mg L<sup>-1</sup> BA and 0.08 mg L<sup>-1</sup> IAA developed 8.7 shoots 5.6 cm in length. Although cytokinins have the effect of promoting new shoot formation, it has also been

observed that this effect increases when an auxin is included to complement the cytokinins. When Uribe-Moraga and Cifuentes (2004) propagated *Legrandia concinna* Phil, they found that the explants established in a culture medium with MS mineral salts and 0.1/0.5 mg l<sup>-1</sup> IBA/BAP, and 12.7 shoots per explant were formed with total fresh and dry weights of 9.1 and 0.55 g, respectively, while the explants established in a culture medium with a similar concentration of BAP but when combined with 1.0 mg L<sup>-1</sup> IAA formed only 7.8 shoots. Koné *et al.* (2013) used explants from *Vigna subterranea* (L.) Verdc. cotyledons established in culture media with different concentrations of BAP (1, 3, and 5 mg L<sup>-1</sup>) and NAA (0.01, 0.05, 0.1, and 0.5 mg L<sup>-1</sup>); on average, 6 to 10 shoots were formed on explants established in a culture medium with 3 mg L<sup>-1</sup> BAP alone or combined with 0.5 mg L<sup>-1</sup> NAA.

All of the shoots established in the diverse culture media variants grew to lengths of from 7.1 to 10.6 cm, but there were no significant differences (Tukey, 0.05) (Table 2). We thus conclude that the dosage of BAP is a determining factor in inducing formation and proliferation of adventitious shoots, and the type and concentration of auxin can increase the effect (Daffalla *et al.*, 2011). When data are grouped as a function of levels of BAP concentration (Table 3), a positive relationship between the number of new shoots and the

concentration of BAP in the culture medium can be observed. Explants established in the culture medium with 0.5 mg L<sup>-1</sup> BAP and explants in the culture medium with 4 mg L<sup>-1</sup> BAP formed 8.3 and 21.0 shoots, on average, with 27 and 44 leaves, which had a total of 4.7 and 6.8 g total fresh weight and 0.26 and 0.39 g total dry weight, respectively. These magnitudes significantly differed from each other (Tukey, 0.05). This effect could be attributed to BAP, which induces cell division of somatic cells and synthesis of specific proteins (Arab *et al.*, 2014) that lead to later formation of meristematic structures that develop in the shoots. However, the quantity of BAP is related to how much meristematic structure can be induced (Siddique and Anis, 2009).

It should be noted that the shoots obtained in our study in the diverse culture medium variants appeared normal and there were no hyper-hydrated shoots as it is not only the quantity of shoots but also their quality that is desirable.

Sánchez-Urbina *et al.* (2008) propagated *A. grijalvensis in vitro* establishing explants in

diverse CM in which BA concentrations varied from 0 to 13.51 mg L<sup>-1</sup>. The highest number of shoots formed on explants established in culture medium with 8.60 mg L<sup>-1</sup> BA. Martínez-Palacios *et al.* (2003) evaluated indirect organogenesis in *A. victoriae reginae* by inducing regeneration of multiple shoots from stem segments cultured in MS medium with from 0.49 to 0.99 mg L<sup>-1</sup> de BA. Aureoles-Rodríguez *et al.* (2008) cultured explants from axillary buds and stem sections of *A. inaequidens* Kochen in a CM with BA; they achieved 1 to 2 shoots on the explants. Miguel *et al.* (2014) established clusters of 2 to 3 shoots with *A. americana*, while in a CM with different concentrations of BAP in the range of 2 to 8 mg L<sup>-1</sup>, they induced formation of 8.4 to 11.7 shoots per explant. They found that the number of new shoots was positively related to the concentration of BAP in the culture medium.

Nikam *et al.* (2003) for 28 d cultured *A. sisalana* Perr. ex. Engelm explants from embryogenic calluses obtained from a portion of a bulbil shoot. The explants established in a CM with 0.1 mg L<sup>-1</sup> kinetin formed somatic embryos, of which 76% germinated.

**Table 3.** Characteristics of *Agave angustifolia* Haw., adventitious shoot clusters established in a culture medium with different concentrations of benzylaminopurine (BAP) and indol-3-acetic acid (IAA).

Variable	Growth regulator (mg L <sup>-1</sup> )			
	N <sup>o</sup> - Benzylaminopurine factor			
	0.5	1	2	4
NL <sup>†</sup>	27.2±5.8 c	33.5±7.1 bc	37.8±10.2 ab	44.4±10.3 a
LL (cm)	8.4±1.7 a	8.6±2.2 a	8.6±1.4 a	10.4±2.9 a
NS	8.2±2.9 b	10.4±3.4 b	12.0±6.2 b	21.8±9.3 a
TFW (g)	4.7±1.7 b	4.8±1.8 b	5.9±2.0 ab	6.8±2.0 a
TDW (g)	0.26±0.09 b	0.26±0.09 b	0.31±0.1 b	0.39±0.1 b
	Indol-3-acético acid factor			
	0.1	0.3	1	
	NL	34.7±13.3 a	35.7±7.9 a	36.7±10.0 a
LL (cm)	8.3±2.3 a	9.3±1.9 a	9.4±2.4 a	
NS	12.5±5.5 ab	11.4±5.7 b	15.5±10.8 a	
TFW (g)	4.7±1.6 b	5.5±1.9 ab	6.4±2.3 a	
TDW (g)	0.2±0.07 b	0.3±0.1ab	0.37±0.1 a	

In each column, values with the same letter are not significantly different (Tukey, 0.05). The mean is ± standard deviation. <sup>†</sup>NL<sup>†</sup>: number of leaves, LL: length of the longest leaf; NS: number of shoots, TFW: total fresh weight, TDW: total dry weight.

By ordering the growth data of the cultures as a function of the IAA concentrations in the culture medium (Table 3), a slight but significant trend can be observed in that a larger number of shoots were formed when the concentration of IAA increased in the culture medium. An average of 12.5 shoots formed on explants that were established in a culture medium with 0.5 to 4 mg L<sup>-1</sup> BAP supplemented with 0.3 mg L<sup>-1</sup> IAA, while 15.5 shoots formed on those established in culture medium with 0.5 to 4 mg L<sup>-1</sup> BAP supplemented with 1 mg L<sup>-1</sup> of IAA. These numbers were significantly different (Tukey, 0.05). Explants established in culture medium with 0.5 to 4 mg L<sup>-1</sup> BAP and 1 mg L<sup>-1</sup> IAA had a total fresh weight of 6.5 g and a total dry weight of 0.37 g, which was 1.36 times the fresh weight and 1.85 times the dry weight of the propagules established in a culture medium with 0.5 to 4 mg L<sup>-1</sup> BAP and 0.1 mg L<sup>-1</sup> IAA, respectively.

Therefore, it can be concluded that in a CM that contains BAP, the auxins in low concentrations contribute to increasing the promoting effect of this cytokinin on adventitious shoot proliferation

in *A. angustifolia*. This agrees with Daffalla *et al.* (2011), who propagated *Boscia senegalensis* (Pers.) by establishing mature zygotic embryos in a CM with MS inorganic salts and varying concentrations of BA (1 to 5 mg L<sup>-1</sup>), TDZ (1 to 5 mg L<sup>-1</sup>), NAA (1 to 5 mg L<sup>-1</sup>) and 2,4-D (1 to 5 mg L<sup>-1</sup>). They found that BA induced shoot formation in two ways: adventitious organogenesis and the development of axillary shoots. Explants established in a CM with the auxin 2,4-D and with the cytokinin TDZ tend to produce calluses, while in a CM with the auxin NAA they developed somatic embryos. The auxin NAA and the cytokinin BA were complementary to one another in promoting morphogenesis.

In the present work, BAP promoted shoot proliferation in *A. angustifolia*, while IAA exhibited synergism with this cytokinin in promoting the shoot formation. The greatest proliferation of *A. angustifolia* adventitious shoots was obtained on explants established in the culture medium with 4 mg L<sup>-1</sup> BAP + 1 mg L<sup>-1</sup> IAA, with an average of 32.8 shoots per explant.

## Resumen

**S. Ríos-Ramírez, J. Enríquez-del Valle, G. Rodríguez Ortiz, y J. Ruíz-Luna. 2017. Concentración de bencilaminopurina y ácido indolacético en la proliferación *in vitro* de brotes adventicios de *Agave angustifolia*. Cien. Inv. Agr. 2017. 44(3): 285-294.** Actualmente existe una metodología para micropropagar a *Agave angustifolia*, y en la multiplicación de propágulos, se requiere mayor información sobre los diversos componentes del medio de cultivo, citocininas y auxinas en su efecto sobre la respuesta de organogénesis. Por lo que el objetivo fue evaluar las dosis de bencilaminopurina y ácido indol-3-acético (AIA) en el medio de cultivo, en su efecto para la formación de nuevos brotes adventicios (organogénesis). Se tomaron explantes que fueron racimos de brotes en una base común de tejidos de tallos de *A. angustifolia*. Se establecieron racimos de 3 a 4 brotes de *A. angustifolia* en diversas variantes de medio de cultivo MS que tenían diferentes concentraciones de bencilaminopurina BAP (0.5, 1, 2, 4 mg L<sup>-1</sup>) y AIA (0.1, 0.3, 1 mg L<sup>-1</sup>). Transcurridas 16 semanas, se obtuvo que la mayor proliferación de brotes fue en relación positiva con la concentración de reguladores de crecimiento, en explantes establecidos en medio de cultivo con 4 mg L<sup>-1</sup> BA + 1 mg L<sup>-1</sup> AIA y explantos en MC con 0.5 mg L<sup>-1</sup> BA + 0.1 mg L<sup>-1</sup> AIA se formaron en promedio 32.8 y 7.8 brotes, con 45 y 24 hojas, respectivamente.

**Palabras clave:** Ácido indol-3-acético, bencilaminopurina, explante, organogénesis, regulador de crecimiento.



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