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INDUCTION, MULTIPLICATION, AND ELONGATION OF *IN VITRO* SHOOTS OF ADVANCED LINES OF *Phaseolus vulgaris* L., *Phaseolus coccineus* L., AND *Vigna radiata* L.

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ABSTRACT

Beans are the second most important crop in Mexico and the most consumed legume in the human diet. Advances in the knowledge of its genetic diversity have allowed the identification of lines with resistance to biotic and abiotic factors. To exploit this variability, the use of *in vitro* culture techniques aimed at the multiplication of germplasm with economic, cultural, and nutritional value is proposed. In this research, conditions for the induction, multiplication, and shoot elongation of three advanced lines of *Phaseolus vulgaris* L., *Phaseolus coccineus* L., and *Vigna radiata* L. by direct organogenesis were determined. Seeds were disinfected by a sequential treatment that reached 93 % asepsis. Germination was achieved in Murashige and Skoog (MS) medium at 50 % inorganic salts after scarifying the seeds in 1 % hydrogen peroxide for 30 min. To induce organogenesis, explants were grown on MS medium with a full concentration of inorganic salts. The best results were obtained in cotyledonary nodes, with 72 µM of 6-benzyladenine (BA) for *P. vulgaris* (4.1 shoots) and *V. radiata* (6.8 shoots), and with 90 µM of BA for *P. coccineus* (27.9 shoots). Multiplication was most efficient in groups of 3 to 4 shoots per explant, using 72 µM of BA for *P. vulgaris* (6.1 shoots), 54 µM of BA for *P. coccineus* (30.7 shoots), and 90 µM of thidiazuron (TDZ) for *V. radiata* (24.6 shoots). Shoot elongation was achieved on MS medium without regulators for *P. vulgaris* (0.22 cm), with 5.6 µM gibberellic acid (AG_3) for *P. coccineus* (5.53 cm), and with 2.8 µM AG_3 for *V. radiata* (0.8 cm).

Keywords: bean, regeneration, cotyledonary nodes, growth regulators.

INTRODUCTION

Mexico is the center of origin of several crop species with global economic importance. Beans are a legume that, since its domestication, has been an important part of the Mexican people's daily diet and, when combined with corn, provides practically all of

the necessary proteins consumed by the lowest-interest social strata (INEGI, 2024). In 2023, bean production in Mexico was recorded at 724 thousand Mg, which represents a 25 % decrease compared to 2022, mainly due to the drought caused by the delay in rainfall. This figure represents the lowest production in 30 years (SIAP, 2024). More than 85 % of the total area cultivated with beans is worked under rainfed conditions, so the crop is affected by the incidence of biotic and abiotic factors that decrease its production (Pandey *et al*., 2017).

Genetic variability is the foundation for survival and adaptation (Schmitz *et al*., 2023) and is essential in a breeding program, which allows the selection of cultivars for higher yield, higher protein or oil content, and tolerance to biotic or abiotic factors (Morales-Soto and Lamz-Piedra, 2020). *In vitro* culture consists of the isolation of explants (cells, tissues, or organs) from the mother plant and their development in defined culture media under aseptic conditions. These techniques allow the rapid propagation of disease-free and genetically uniform plants in a short time, which benefits production (Segura *et al*., 2024). Direct organogenesis plays an important role in the process of plant tissue culture for common bean regeneration (Yu *et al*., 2021).

The creation of an efficient *in vitro* propagation protocol for *Phaseolus* and *Vigna* species represents a strategic tool in breeding programs, as it overcomes many limitations imposed by traditional breeding methods. Furthermore, it facilitates the rapid and massive multiplication of elite genotypes, especially those with desirable agronomic traits, and the conservation of valuable germplasm, including lines resistant or tolerant to adverse conditions. As a result of research on genetic diversity in the genera *Phaseolus* and *Vigna* and the interest in the creation and use of a germplasm base in breeding programs for the creation of varieties, lines with resistance to abiotic and biotic factors have been developed, while including other favorable attributes such as nutritional quality, earliness, growth habits, and tolerance to high temperatures.

The objective of this research was to characterize the induction, multiplication, and shoot elongation capacity of advanced lines of *Phaseolus vulgaris*, *P. coccineus,* and *Vigna radiata* by direct organogenesis. For this purpose, different culture media, as well as different combinations and concentrations of plant growth regulators, were evaluated in order to exploit and utilize available genetic variation, as well as the capacity to multiply and preserve potential germplasm.

MATERIALS AND METHODS

Advanced lines of *Phaseolus vulgaris* L. (white bean variety), *Phaseolus coccineus* L., and *Vigna radiata* L. provided by the Bean Breeding Program of the Postgraduate Program in Genetic Resources and Productivity (PREGEP-Genetics) of the Postgraduate College were used.

Culture medium and incubation conditions

Murashige and Skoog (1962) basic culture medium (MS medium), supplemented with sucrose (30 g L^{-1}) and solidified with agar (Merck, Mexico, 7 g L^{-1}), was used.

The pH of the medium was adjusted to 5.7 with NaOH or 1N HCl using an Orion 3 Star potentiometer (Thermo Fisher Scientific, Mexico City, Mexico) before adding the agar. The sterilization was carried out in a vertical autoclave (AESA 300, Mexico City, Mexico) at 121 °C and 1.5 kg cm^{-2} pressure for 20 min. The culture flasks with the seeded plant material were kept in an incubation room with a photoperiod of 16/8 h of light/darkness provided by 75 W LED cold white light lamps and luminous intensity of 45 μmol m^{-2} s^{-1}, or in darkness, at a temperature of 25 ± 1 °C sustained by a mini-split (Carrier Mexico, Mexico City, Mexico) and relative humidity of 40 %.

Establishment of the aseptic culture

Seeds were washed with commercial detergent (Roma, Mexico) and tap water for 10 min under continuous agitation. Two disinfection treatments were tested: 1) seeds were immersed in ethanol (70 %) for 1 min, then in commercial sodium hypochlorite (NaOCl; 0.5 % v/v) (Cloralex, Alen del Norte, Mexico City, Mexico) + Tween 20 (five drops per 100 mL of solution) (Thermo Fisher Scientific, Mexico City, Mexico) + colloidal silver-based bactericide (1 % v/v, 0.2 % active ingredient) (Microdyn, Alen del Norte, Mexico City, Mexico) dissolved in sterilized water, and finally, in a fungicide solution (4 g L^{-1} Benlate and 4 g L^{-1} Captan) (Sigma Aldrich, USA); 2) using the same treatment with a variation in NaOCl concentration (1 % v/v).

In both treatments, seeds were shaken continuously and rinsed five times with sterilized distilled water before depositing them in each solution. Three seeds were sown in 250 mL capacity glass vials with 30 mL of MS culture medium with 50 and 100 % concentrations of inorganic salts. After 17 d, the germination rate (%), days to germination, bacterial contamination (%), and fungal contamination (%) were determined. The experiment was set up in a completely randomized design with 12 replicates per treatment. The experimental unit was one seed in each jar.

In vitro germination

Seeds of three bean species were subjected to different scarification times (4, 2, and 1.5 h, plus a control without scarification) using hydrogen peroxide (H_2O_2) at concentrations of 0, 1, and 3 % (v/v). Subsequently, they were seeded on MS medium with 50 % of the concentration of inorganic salts. After three weeks, germination rate (%), days to germination, and seedling height (cm) were quantified. The experiment was set up in a simple completely randomized design with 10 replicates per variety, with five seeds in each jar as the experimental unit.

Shoot induction

Evaluation of different explant types and culture media

Cotyledonary nodes (Cn), cotyledonary axis (Ca), cotyledons (C), and embryonic axis (Ea) were dissected from 14-d-old *in vitro* germinated seedlings of the three species. They were planted on MS and B5 culture medium (Gamborg *et al*., 1968). The latter

is composed of inorganic salts, thiamine (10 mg L^{-1}), and organic components of MS medium. Both culture media were supplemented with 6-benzyladenine (BA, 72 μM). At three weeks, sprouting (percentage of explants that generated shoots), number of shoots per explant, and shoot length were quantified. The experiment followed a completely randomized design with 15 replicates per treatment, using a single explant per culture flask as the experimental unit.

Evaluation of different concentrations of 6-benzyladenine (BA) and thidiazuron (TDZ)

The two types of explants that yielded the best results (cotyledon and cotyledonary node) were sown on MS medium supplemented with 54, 72, and 90 μM 6-benzyladenine (BA) and thidiazuron (TDZ), in addition to a medium without plant growth regulators as a control. After three weeks, sprouting (percentage of explants that generated shoots), number of shoots per explant, and shoot length were quantified. The experiment followed a completely randomized design with 10 replicates per treatment, considering a single explant per culture flask as the experimental unit.

Shoot multiplication

To increase the number of shoots, groups of 3 to 4 shoots with an average length of 0.1 to 0.15 cm, obtained from cotyledon and cotyledonary node explants during the induction stage, were sown in MS medium supplemented with the same BA and TDZ concentrations used previously (54, 72, and 90 μM). At three weeks, sprouting (percentage of explants that generated shoots), number of shoots per explant, and shoot length were quantified. The experiment was set up in a simple completely randomized design with 10 replicates per treatment, considering a single explant per culture flask as the experimental unit.

Shoot elongation

Groups of 3 to 4 shoots from the cotyledon and cotyledonary node of the multiplication stage, with a uniform average length (between 0.05 and 0.1 cm depending on the species), were sown in MS medium with two concentrations of inorganic salts (50 and 100 %) and different concentrations of gibberellic acid (AG_3) (0.0, 1.4, 2.8, 4.2, 5.6, and 7 μM). Each treatment included 12 replicates, with one explant per culture flask serving as the experimental unit. The experiment followed a completely randomized design.

Statistical analysis

With the data of the quantified variables, an analysis of variance was performed with the statistical program R (R Core Team, 2022) using Tukey's test ($p \leq 0.05$) for comparison of means.

RESULTS AND DISCUSSION

Establishment of the aseptic culture

Two weeks after *in vitro* sowing, the treatment with 20 % NaOCl prevented the growth of fungi and bacteria ($p \leq 0.05$). In addition, this treatment showed the best response for aseptic culture, achieving germination rates of 50, 87.5, and 95.33 % in *P. vulgaris*, *P. coccineus*, and *V. radiata*, respectively. After 17 d, the seedlings reached 1.08, 4.64, and 5.15 cm in height. These results are similar to those obtained by Yu *et al*. (2021), who disinfected *P. vulgaris* seeds with 20 % sodium hypochlorite for 10 min. On the other hand, Santalla *et al*. (1998) successfully disinfected *P. coccineus* seeds with 95 % ethanol for 1 min and 1 % sodium hypochlorite for 20 min, while Nupur (2025) established an effective seed disinfection protocol for *V. radiata*, using 0.1 % sodium hypochlorite for 15 min.

In vitro germination

Vigna radiata germinated faster (2.65 d after sowing) than *P. vulgaris* (3.25 d after sowing) and *P. coccineus* (4.01 d after sowing). It was found that 30 min of immersion in hydrogen peroxide caused a decrease in germination time of 3.12 d after sowing in all three species, compared to other treatments (1, 2, and 4 h). The scarification of seeds from all species with hydrogen peroxide caused an increase in germination due to the softening of the seed coat, and germination rates of 85.13, 95.9, and 91.79 % were achieved in *P. vulgaris*, *P. coccineus*, and *V. radiata*, respectively, with a considerable increase in *P. vulgaris* (from 50 to 85.13 %). This is consistent with the findings of Rajashekar and Baek (2014), who accelerated the imbibition of *P. vulgaris* seeds and facilitated seed coat removal by immersing them in peroxide.

Shoot induction

Evaluation of different types of explants and culture media

Significant differences in sprouting were detected among species ($p \leq 0.05$). *Phaseolus coccineus* showed superior sprouting (100 %) and a higher number of explants with shoots (9.08 in MS) in both culture media (MS and B5) (Figure 1). Results for *P. vulgaris* were significantly lower (30.77 % sprouting in MS and 46.15 % in B5) in contrast to *P. coccineus* and *V. radiata* (Table 1). The main cause of this is recalcitrance, which limits the *in vitro* regeneration of this species (Song *et al*., 2020).

In *P. vulgaris*, the explant that showed the best results was the cotyledon axis planted in MS medium supplemented with 72 μM BA. However, its response was not significantly higher than that of cotyledonary nodes, which produced up to 1.23 shoots per explant on MS medium, although with shorter shoot length. These results are lower than those obtained by Yu *et al*. (2021), who generated 2.91 shoots with a length of at least 2 cm per explant in cotyledon nodes sown in MS medium. This difference may be attributable to the variety used in the present study. On the other hand, sprouting reached 64.29 %

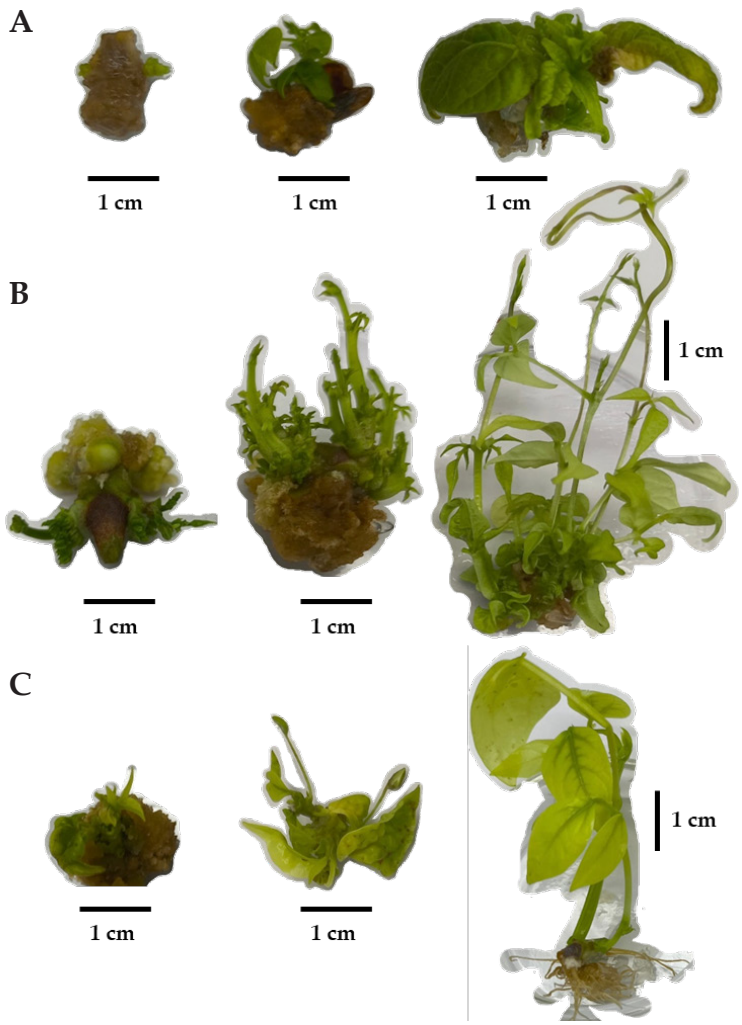

Figure 1. Induction, multiplication, and elongation of shoots *in vitro*. A: *Phaseolus vulgaris* L.; B: *Phaseolus coccineus* L.; C: *Vigna radiata* L.

in MS medium, exceeding the 46.15 % obtained in B5 medium. This contrasts with the findings of Quintero-Jiménez *et al*. (2010), who achieved greater shoot regeneration in B5 medium (98–100 %) compared to MS medium (15–73 %). For *P. coccineus*, the best results were achieved in cotyledon nodes in MS medium supplemented with 72 µM BA, with 12.36 shoots per explant (Table 1). This was similar to that obtained by Malik and Saxena (1992), who obtained up to 11 shoots per explant in *P. coccineus* cotyledons in MS medium supplemented with 30 µM BA.

In the case of *V. radiata*, the highest number of shoots per explant (7.27) was obtained in cotyledons sown in MS medium supplemented with 72 µM BA. In contrast, Avenido and Hattori (2021) found that the cotyledon node had the highest potential for shoot

Table 1. Effect of the interaction between the cotyledon axis, cotyledon node, cotyledon, and embryonic axis of *Phaseolus vulgaris* L., *Phaseolus coccineus* L., and *Vigna radiata* L. in Murashige and Skoog (1962) and B5 (Gamborg *et al*., 1968) culture media on *in vitro* shoot induction after three weeks of culture.

Species	Culture media						
		MS			B5		
	Explant (Type)	Sprouting (%)	Number of shoots per explant	Shoot length (cm)	Sprouting (%)	Number of shoots per explant	Shoot length (cm)
P. vulgaris	Cn	30.77 cde	1.23 bcde	0.04 cd	46.15 abcde	1.15 bcde	0.11 bcd
	Ca	64.29 abcd	1.71 bcde	0.05 cd	46.15 abcde	0.62 de	0.03 d
	C	13.33 de	0.13 e	0.01 d	7.33 e	0.93 cde	0.01 d
	Ea	0.00 e	0.00 e	0.00 d	0.00 e	0.00 e	0.00 d
P. coccineus	Cn	85.71 ab	12.36 a	0.31 a	85.71 ab	8.71 abcd	0.19 abc
	Ca	100.00 a	9.08 abc	0.10 bcd	100.00 a	2.69 bcde	0.22 ab
	C	33.33 bcde	9.13 ab	0.18 abc	6.67 a	0.20 e	0.01 d
	Ea	14.29 cde	2.69 bcde	0.06 cd	14.29 cde	0.79 cde	0.04 cd
V. radiata	Cn	53.33 abcde	3.27 bcde	0.07 cd	40.00 bcde	1.33 bcde	0.09 bcd
	Ca	93.33 a	2.60 bcde	0.09 bcd	93.33 a	1.33 bcde	0.11 bcd
	C	66.67 abc	7.27 abcde	0.06 cd	60.00 abcd	6.20 abcd	0.06 cd
	Ea	20.00 cde	1.00 cde	0.01 cd	20.00 cde	0.67 de	0.02 d

Means with a common letter are not significantly different (Tukey, $\alpha \leq 0.05$). MS: Murashige and Skoog basic culture medium; B5: Gamborg culture medium; Cn: cotyledon node; Ca: cotyledon axis; C: cotyledon; Ea: embryonic axis.

induction by direct organogenesis in *V. radiata*, with 3.3 to 10.4 shoots per explant. In this work, the longest shoots were obtained from the cotyledon axis (0.09 cm in MS medium and 0.11 cm in B5 medium, both supplemented with 72 µM BA). These results are much lower than those reported by Bhajan *et al*. (2024), who obtained shoots of up to 5 cm in cotyledons in MS medium supplemented with 5 µM BA.

Evaluation of different concentrations of 6-benzyladenine (BA) and thidiazuron (TDZ)

To evaluate the effect of BA and TDZ on shoot induction, cotyledon nodes and cotyledons were used as explants due to their quantitative and qualitative results. In this case, *P. coccineus* and *V. radiata* showed a higher percentage ($p \leq 0.05$) of sprouting than *P. vulgaris* (Table 2).

In the case of *P. vulgaris*, the most efficient responses were obtained with cotyledonary nodes planted in MS medium supplemented with 72 µM BA (Table 2), where the highest number of shoots per explant (4.1) was obtained, as well as the longest shoots (0.09 cm). These results are superior in terms of number of shoots per explant (2.91) and inferior in terms of shoot length (2 cm) to those obtained by Yu *et al*. (2021) in cotyledon nodes sown in MS medium supplemented with 7 mg L^{-1} BA and 0.2 mg

Table 2. Effect of the interaction between cotyledon (C), cotyledon node (Cn), 6-benzyladenine (BA), and thidiazuron (TDZ) on the induction of *in vitro* shoots of *Phaseolus vulgaris* L., *Phaseolus coccineus* L., and *Vigna radiata* L. after three weeks of cultivation.

Species	Explant							
			Cotyledon			Cotyledonary node		
	BA (μM)	TDZ (μM)	Sprouting (%)	Number of shoots per explant	Shoot length (cm)	Sprouting (%)	Number of shoots per explant	Shoot length (cm)
P. vulgaris	0	0	30 abc	0.5 f	0.02 d	40 abc	0.8 f	0.03 d
	54	0	10 c	0.6 f	0.01 d	40 abc	1.1 f	0.04 d
	72	0	40 abc	3.1 f	0.05 cd	100 a	4.1 f	0.09 cd
	90	0	10 c	0.6 f	0.01 d	50 abc	1.1 f	0.05 cd
	0	54	60 abc	2.9 f	0.03 d	40 abc	2.2 f	0.04 d
	0	72	10 c	2.3 f	0.01 d	70 abc	2.5 f	0.07 cd
	0	90	20 bc	2.1 f	0.01 d	40 abc	1.0 f	0.04 d
P. coccineus	0	0	60 abc	1.5 f	0.59 abc	100 a	12.2 bcdef	0.83 ab
	54	0	70 abc	10.7 cdef	0.10 cd	100 a	27.3 a	0.37 abcd
	72	0	40 abc	3.6 f	0.09 cd	90 ab	22.3 abcd	0.28 cd
	90	0	60 abc	9.7 def	0.24 cd	100 a	27.9 a	0.44 abcd
	0	54	60 abc	10.4 def	0.17 cd	80 abc	25.9 ab	0.17 cd
	0	72	70 abc	20.6 abcde	0.26 cd	100 a	25.6 abc	0.28 cd
	0	90	70 abc	14.2 abcdef	0.16 cd	90 ab	28.2 a	0.23 cd
V. radiata	0	0	10 c	0.2 f	0.02 d	100 a	5.7 ef	0.86 a
	54	0	40 abc	2.5 f	0.08 cd	80 abc	5.1 f	0.16 cd
	72	0	50 abc	5.5 f	0.04 d	100 a	6.8 ef	0.31 bcd
	90	0	30 abc	3.5 f	0.04 d	80 abc	6.2 ef	0.13 cd
	0	54	70 abc	7.9 def	0.05 cd	90 ab	7.1 ef	0.10 cd
	0	72	40 abc	2.6 f	0.03 d	90 ab	4.3 f	0.17 cd
	0	90	60 abc	8.2 def	0.04 d	100 a	6.2 ef	0.13 cd

Means with a common letter are not significantly different (Tukey, $\alpha \leq 0.05$).

L^{-1} 1-naphthaleneacetic acid (ANA). It is likely that this balance of auxins (ANA) and cytokinins (BA) favored the growth in shoot length.

For *P. coccineus*, the best sprouting response was obtained in cotyledon nodes planted in MS medium supplemented with 90 μM BA (Table 2), where 100 % sprouting and 27.9 shoots per explant with an average length of 0.44 cm were achieved. The number of shoots per explant obtained was higher than that reported by Santalla *et al*. (1998), who achieved up to 13.5 shoots per explant in cotyledon nodes sown in MS medium supplemented with 15 μM BA and 2 μM AG_3. The shoots of *P. coccineus*, both in cotyledon nodes and cotyledons, were significantly superior in size and vigor compared to *P. vulgaris* and *V. radiata* (Figure 1).

Finally, in *V. radiata*, the most efficient response was obtained with cotyledon nodes planted in MS medium supplemented with 72 μM BA, with 100 % sprouting, up to 6.8 shoots per explant, and measuring 0.31 cm in length. These results were superior

to those reported by Bhajan *et al*. (2024), who achieved 5.36 shoots per explant in cotyledon nodes planted in MS medium supplemented with 5 mg L^{-1} BA. This reflects that a higher concentration of BA in the MS medium caused greater shoot regeneration.

Shoot multiplication

Groups of 3 to 4 shoots from cotyledonary nodes and cotyledons of the three species were dissected and sown in MS medium supplemented with the same concentrations of BA and TDZ as in the induction stage. The sprouting behavior showed significant differences ($p \leq 0.05$), with *P. coccineus* (84.29 %) and *V. radiata* (72.86 %) showing a higher sprouting percentage compared to *P. vulgaris* (42.86 %) (Table 3). The unsatisfactory responses in *P. vulgaris* could be mainly due to the recalcitrance of this species, which causes limitations for its *in vitro* cultivation (Chero-Ayay *et al*., 2019).

Table 3. Effect of the interaction between shoots from the cotyledon, cotyledon node, 6-benzyladenine (BA), and thidiazuron (TDZ) on the *in vitro* multiplication of shoots of *Phaseolus vulgaris* L., *Phaseolus coccineus* L., and *Vigna radiata* L. after four weeks of cultivation.

Species	BA (μM)	TDZ (μM)	Original explant					
			Cotyledon			Cotyledon nodes		
			Sprouting (%)	Number of shoots per explant	Shoot length (cm)	Sprouting (%)	Number of shoots per explant	Shoot length (cm)
P. vulgaris	0	0	30 abc	0.6 h	0.03 c	50 abc	1.2 h	0.03 c
	54	0	30 abc	1.5 h	0.03 c	50 abc	1.6 gh	0.06 c
	72	0	40 abc	3.4 gh	0.10 c	100 a	6.1 fgh	0.11 c
	90	0	10 c	0.0 h	0.01 c	50 abc	1.3 h	0.06 c
	0	54	60 abc	3.0 gh	0.05 c	40 abc	3.4 gh	0.05 c
	0	72	10 c	2.3 gh	0.03 c	70 abc	4.0 gh	0.08 c
	0	90	20 bc	2.8 gh	0.03 c	40 abc	1.0 h	0.04 c
P. coccineus	0	0	60 abc	4.0 gh	0.77 abc	100 a	16.7 bcdefgh	1.22 a
	54	0	70 abc	14.7 cdefgh	0.25 bc	100 a	36.9 a	0.64 abc
	72	0	60 abc	8.6 defgh	0.52 abc	90 ab	27.4 abcde	0.57 abc
	90	0	80 abc	14.2 cdefgh	0.76 abc	100 a	30.7 abc	0.64 abc
	0	54	90 ab	21.7 abcdfg	0.54 abc	80 abc	28.2 abcd	0.34 abc
	0	72	80 abc	31.0 abc	0.91 abc	100 a	28.0 abcde	0.54 abc
	0	90	80 abc	27.1 abcde	0.55 abc	90 ab	35.5 ab	0.44 abc
V. radiata	0	0	20 bc	0.8 h	0.04 c	100 a	6.5 fgh	1.17 ab
	54	0	50 abc	5.4 fgh	0.15 c	80 abc	8.3 defgh	0.18 c
	72	0	50 abc	6.7 fgh	0.03 c	100 a	8.0 efgh	0.39 abc
	90	0	40 abc	6.4 fgh	0.12 c	80 abc	9.6 defgh	0.17 c
	0	54	80 abc	15.2 cdefgh	0.08 c	90 ab	11.6 cdefgh	0.12 c
	0	72	50 abc	6.6 fgh	0.07 c	90 ab	9.4 defgh	0.24 bc
	0	90	90 ab	24.6 abcdef	0.13 c	100 a	9.5 defgh	0.18 c

Means with a common letter are not significantly different (Tukey, $\alpha \leq 0.05$).

For *P. vulgaris*, superior sprouting was achieved with shoots from cotyledonary nodes planted in MS medium supplemented with 72 µM BA (Table 3), with the highest increase in shoots per explant (6.1), in addition to shoots with greater length (0.11 cm). This was lower than that reported by Quintero-Jiménez *et al*. (2010), who obtained up to 20 shoots per explant from embryonic axis sown in MS medium supplemented with 10 mg L^{-1} BA.

Explants from cotyledon nodes in MS medium with 54 µM BA showed the most efficient response for *P. coccineus* (Table 3), with 100 % sprouting and an increase of 30.7 shoots per explant with an average length of 0.64 cm. The increase in shoots, considering that four shoots per explant were used as a starting point, was almost 800 %. These results greatly exceeded those obtained by Vaquero *et al*. (1993), who achieved a multiplication of between 200 and 300 % in *P. coccineus* in MS medium supplemented with 1 µM BA, 0.1 µM ANA, and AG_3.

In *V. radiata*, the highest increase in shoots per explant (24.6) was observed in shoots from cotyledons sown in MS medium with 90 µM TDZ, greatly exceeding the results of Patra *et al*. (2018), who achieved up to 7.8 shoots per explant in cotyledon nodes sown in MS medium supplemented with 1.5 mg L^{-1} BA and 1 mg L^{-1} kinetin. It can be deduced that, in *V. radiata*, high concentrations of TDZ are preferable for effective *in vitro* shoot multiplication.

Shoot elongation

In order to accelerate the growth of shoots of the three species, groups of 3 to 4 shoots with an average length of 0.1 to 0.15 cm from cotyledon nodes (Cn) and cotyledons (C) were dissected. Significant differences were detected between the different levels of AG_3 ($p \leq 0.05$) (Table 4).

The shoots of *P. coccineus*, with an average length of 3.19 cm, elongated to a greater extent than those of *P. vulgaris* (0.12 cm) and *V. radiata* (0.18 cm) (Figure 1), with a concentration of 5.6 µM of AG_3 in shoots from cotyledonary nodes, causing maximum elongation (5.533 cm). This is similar to the results obtained by Vaquero *et al*. (1993), who achieved effective shoot elongation of up to 91 % of the explants (cotyledon nodes) planted in MS medium supplemented with 3 µM AG_3. Shoot elongation in MS medium with 100 % inorganic salts was higher (1.35 cm) compared to that obtained in MS medium with 50 % inorganic salts (1.24 cm). This is similar to what was reported by Doğan (2022), who obtained the greatest shoot elongation of *Staurogyne repens* (2.27 cm) with 100 % inorganic salts in MS medium.

On the other hand, in *P. vulgaris*, greater elongation (0.222 cm) was achieved in shoots from cotyledonary nodes sown in MS medium with 100 % inorganic salts without AG_3 (control). This is consistent with the methodology followed by Mohamed *et al*. (1993), who achieved at least 0.5 cm elongation in *P. vulgaris* shoots sown in MS medium without plant growth regulators.

In the case of *V. radiata*, shoots from cotyledon nodes planted in MS medium with 100 % inorganic salts and supplemented with 2.8 µM AG_3 were those that increased their

Table 4. Effect of the interaction between shoots from cotyledon nodes (Cn), cotyledons (C), and gibberellic acid (AG_3) sown *in vitro* in Murashige and Skoog (1962) basic medium (MS) with 50 and 100 % inorganic salts on the elongation of *in vitro* shoots of *Phaseolus vulgaris* L., *Phaseolus coccineus* L., and *Vigna radiata* L. after four weeks of cultivation.

Species	AG_3 (µM)	MS inorganic salts (%)			
		100 Increase in shoot length (cm)		50 Increase in shoot length (cm)	
		C Shoots	Cn Shoots	C Shoots	Cn Shoots
P. vulgaris	0.0	0.117 hi	0.222 ghi	0.106 hi	0.161 hi
	1.4	0.211 ghi	0.089 hi	0.039 i	0.094 hi
	2.8	0.139 hi	0.206 ghi	0.117 hi	0.083 i
	4.2	0.044 i	0.117 hi	0.194 ghi	0.056 i
	5.6	0.039 i	0.056 i	0.067 i	0.033 i
	7.0	0.211 ghi	0.078 i	0.011 i	0.044 i
P. coccineus	0.0	1.854 defghi	2.008 defghe	2.460 cdefgh	5.208 a
	1.4	3.187 abdcef	2.675 bcdef	2.608 bcdefg	4.125 abcd
	2.8	5.192 ab	2.025 cdefghi	1.429 defghi	3.967 abcd
	4.2	4.842 ab	4.025 abcd	2.237 cdefghi	2.808 abcdef
	5.6	3.754 abcde	5.533 a	1.125 efghi	2.512 bcdefgh
	7.0	1.475 defghi	5.450 a	2.825 abcdef	4.592 abc
V. radiata	0.0	0.083 i	0.236 ghi	0.056 i	0.161 hi
	1.4	0.071 i	0.617 fghi	0.076 i	0.083 i
	2.8	0.083 i	0.804 fghi	0.050 i	0.075 i
	4.2	0.092 hi	0.042 i	0.104 hi	0.204 ghi
	5.6	0.046 i	0.129 hi	0.058 i	0.129 hi
	7.0	0.100 hi	0.129 hi	0.067 i	0.129 hi

Means with a common letter are not significantly different (Tukey, $\alpha \leq 0.05$).

length to a greater extent (0.804 cm). This elongation was lower than that reported by Bhajan *et al*. (2019), who managed to increase shoots taken from cotyledon nodes grown in MS medium supplemented with 2 µM BA by up to 4 cm.

CONCLUSIONS

The induction, multiplication, and elongation of shoots from advanced lines of *Phaseolus vulgaris*, *Phaseolus coccineus*, and *Vigna radiata* were established using explants from dissected cotyledonary nodes of *in vitro* germinated seedlings. Shoot induction was achieved in Murashige and Skoog (MS) medium with 100 % inorganic salts and 72 µM (*P. vulgaris* and *V. radiata*) or 90 µM (*P. coccineus*) 6-benzyladenine (BA). Multiplication was effective in MS medium with 100 % salts and 72 µM BA (*P. vulgaris*), 54 µM BA (*P. coccineus*), or 90 µM thidiazuron (TDZ) (*V. radiata*). Elongation was achieved in MS

medium at 100 % salts without growth regulators (*P. vulgaris*), 5.6 µM gibberellic acid (AG_3) (*P. coccineus*), or 2.8 µM AG_3 (*V. radiata*).

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