AXILLARY BUD DEVELOPMENT FROM NODAL CULTURES OF BEAN SEEDLINGS (*Phaseolus vulgaris* L.)[1]

*I. S. MARTINS**
*M. R. SONDAHL**

Resumo

*As condições ótimas para a indução de desenvolvimento e crescimento de gemas axilares de plantulas de feijão (*Phaseolus vulgaris*) consistem na germinação em presença de 6-BA (20 µM) e cultura da região nodal em meio básico B5 com adição de 6-BA (5 µM) e IBA (0.25 µM). O enraizamento das plantulas derivadas das gemas axilares foi obtido na presença de IBA (10 µM) durante 10 dias, seguido de cultura em meio básico B5. A presença de luz foi essencial para o desenvolvimento de gemas axilares a partir de explantes da região nodal de feijão. A frequencia de indução de gemas axilares e o crescimento das plántulas derivadas destas gemas apresentaram diferenças de acordo com a posição do nó e o genótipo. O cultivar Puebla-153 apresentou maior frequencia de indução de gemas axilares, enquanto que no cultivar Carioca observou-se melhor crescimento das plántulas induzidas. O número máximo de gemas desenvolvidas por axila foliar foi seis (6) para o cultivar Moruna (no 1), sugerindo que 12 seria o total de gemas dormentes para cada nó cotiledonar de plántulas de feijão.*

Introduction

The maintenance of bean germplasm requires suitable installations and periodic sowing in order to assure seed viability. Plant tissue culture techniques may help germplasm banks of this species through continuous *in vitro* bud culture or storage in liquid nitrogen. Aseptic cultures of buds also allow for exchange of genetic material between countries or distinct regions without the risk of introducing new phytopathogens. Plants derived from bud cultures are genetically uniform since they are generated by a vegetative process.

The possibility of maintaining genotypes of *Lotus corniculatus* by nodal cultures was demonstrated by Tomes (5). Among 100 genotypes studied, 34% were lost during the 1977-1978 winter due to field conditions. However, all of these genotypes were available from *in vitro* cultures kept at low temperatures during the same period. The time required to raise an adult plant from *in vitro* culture was the same as from germinating seeds. In soybean, Cheng *et al.* (1), (4) and Saka *et al.* (4) showed that the development of axillary buds from nodal cultures was induced by different treatments with 6-benzyl-aminopurine (6-BA) and indole-3-butyric acid (IBA) in the culture medium. This paper presents a methodology for the development of axillary buds from nodal cultures and plant recovery in beans (*Phaseolus vulgaris* L.).

Materials and methods

Seeds from the bean germplasm bank maintained at the Genetic Department of the Institute of Agronomy were utilized for this study. Following washing with detergent (Neo dish 1%) and tap water, the seeds were surface sterilized with ethanol 96° GL for 10 min., and then in NaOCl 4% for 20 min. Glassware and culture media were all sterilized by autoclave (120°C, 20 min.). All the culture bottles were sealed with a PVC film (16.5 µM thickness).

1 Received for publication on December 23, 1983. Supported by FAPESP and CNPq.

* Dept. Genetics, Institute of Agronomy, P. O. Box 28, Campinas, SP, Brazil. Present address of MR Sondahl: DNAP, 2611 Branch Pike, Cinnaminson, NJ 08077, USA.

Different cultivars and culture conditions were tested for the induction of the development of axillary buds from nodal cultures of bean seedlings (*Phaseolus vulgaris* L.)

Seeds of the cultivars Moruna, Piratã-1 and Puebla-153 were germinated in an aqueous solution containing 6-BA (5 or 10 μM). Half of the replicates were kept under a 12 h photoperiod and the other half was cultivated in the dark during an initial period of 9 days. After 12-15 days of germination, evaluations were made pertaining to the number and height of axillary buds developed in the nodal regions of the bean seedlings. The nodal region containing the cotyledonary leaves was coded "node-I", whereas "node-II" comprises the region of the primary leaves. In this first experiment 30 replicates/cultivar/6-BA concentration/culture conditions were performed.

During a second experiment, seeds of the cultivars Moruna and Carioca were germinated in bottles containing the following basic medium: B5 inorganic salts (12); thiamine (30 μM); pyridoxine (5 μM); nicotinic acid (10 μM); meso-inositol (550 μM); sucrose (20 g/l); agar (6 g/l); pH 5.8. To the above basic medium were added 6-BA (50 μM) and IBA (0.025 μM). After 19 days of germination, all axillary buds developed in the nodal regions (nodes I and II) were removed and coded B1. The original nodal explants were transferred to the basic B5 medium supplemented with 6-BA (20 μM) and IBA (0.025 μM). After a period of 17 days of secondary culture, all the axillary buds developed (B2) were removed. The original explants were transfered to the basic B5 medium without growth regulators. The buds developed after 25 days in this tertiary culture were coded B3. These successive cultures were adopted in order to avoid possible growth inhibition of new axillary buds due to the presence of developed buds in the same node. Twenty replicates for each cultivar studied were prepared.

In a third experiment, seeds of the cultivar Puebla-153 were germinated in the basic medium supplemented with 6-BA (20 μM) and IBA (0.025 μM). Following 16 days of culture, nodes I and II were excised and cultivated in the basic medium supplemented with 6-BA (20 μM) and IBA (0.025 μM) or 6-BA (5 μM) and IBA (0.025 μM). Thirty replicates were prepared for each treatment.

Seed germination and nodal cultures for experiments 2 and 3 were performed under a 12 h photoperiod, 400-800 lux and temperature of 26 ± 3°C. During the evaluation of the experiment the number and height of axillary buds developed were recorded.

Results and discussion

Experiment 1

The techniques described for nodal cultures of soybean (*Glycine max*) by Cheng *et al.* (1) and Saka *et al.* (4) were tested with *P. vulgaris*. Seeds of the cultivars Moruna, Piratã-1 and Puebla-153 were germinated in an aqueous solution of 6-BA (5 or 10 μM) in the dark or under a 12 h photoperiod. After 9 days it was observed that all treatments in the presence of light carried axillary buds (1-2 mm) in the nodal regions. On the other hand, the cultures maintained in the dark did not have any sign of bud development. Taking into account the beneficial effect of the light on the axillary bud development, all the remaining cultures were transfered from dark to light. After 12-15 days of culture, the treatments were evaluated (Table 1). In the presence of 6-BA (5 μM) the number of buds developed at node I and node II was one half in the cultures kept for 9 days in the dark in comparision to the ones under 12 h photoperiod. The cultures exposed to 6-BA (10 μM) presented the same frequency of bud development in both conditions (light and dark-light). These data demonstrated the interaction of 6-BA and light in the development of axillary buds from nodal regions of beans. The frequency of bud development varied according to the genotype in relation to node-II in the presence of 6-BA (10 μM). Twice as many axillary buds were observed in the cultivar Puebla-153 than in Moruna in both conditions (light and dark-light). Bean seedlings initiated in the dark (9 days) and transfered to light presented an average of 1.8 and 0.9 axillary buds/node-II for Puebla-153 and Moruna, respectively. Cultures grown in the light gave values of 2.0 and 1.0 axillary buds/node-II for Puebla-153 and Moruna, respectively. It was also observed that Puebla buds were more developed (5-7 mm height) than buds of the two other cultivars (3-4 mm heigh) after the same period of culture.

Experiment 2

This experiment was designed to determine the maximum number of axillary buds inducible by this method in nodal regions of bean seedlings (Table 2). The total number of developed axillary buds in node-I was 5.0 and 4.3 for Moruna and Carioca, respectively. The values for node-II were 4.5 (Moruna) and 4.8 (Carioca). The maximum number of axillary buds observed was six in one axil of the node-I of Moruna, suggesting that the total expected number for this node-I for *P. vulgaris* is twelve, i.e. six for each cotyledonary axil.

Table 1. Development of axillary buds from cotyledonary node (node I) and primary leaf node (node II) excised from seedlings of *Phaseolus vulgaris*.

Cultivar	Culture Condition (a)	6-BA (μM)	Period of Culture (days)	No. buds/node I		No. buds/node II	
				Total	Average	Total	Average
Moruna	dark/light	5	15	15/24	0.6	10/24	0.4
Moruna	dark/light	10	15	34/25	1.4	23/25	0.9
Moruna	light	5	12	31/26	1.2	23/26	0.9
Moruna	light	10	12	29/22	1.3	23/22	1.0
Piratã-1	dark/light	5	15	10/16	0.6	7/16	0.4
Piratã-1	light	5	15	12/12	1.0	16/12	1.3
Puebla-153	dark/light	10	15	38/26	1.5	46/26	1.8
Puebla-153	light	10	12	30/24	1.25	48/24	2.0

a Germination in 6-BA solution during 9 days in the dark followed by 12 h photoperiod, or germination directly under 12 h photoperiod.

Table 2. Sequential axillary bud development from cotyledonary node (node I) and primary leaf node (node II) from seedlings of *Phaseolus vulgaris*

Cultivar	Nodal region	Axillary buds (a)	Replicates (no.)	Bud induction per culture (%)	Frequency bud development (no.)	Average shoot height (mm)
Moruna	I	B1	20	100	1.7	3.2
Moruna	I	B2	20	10	1.0	6.5
Moruna	I	B3	16	69	2.3	7.6
					5.0	
Moruna	II	B1	20	100	1.4	2.5
Moruna	II	B2	20	50	1.4	9.5
Moruna	II	B3	15	93	1.7	9.9
					4.5	
Carioca	I	B1	16	75	1.5	2.8
Carioca	I	B2	15	60	1.3	13.4
Carioca	I	B3	11	73	1.5	14.7
					4.3	
Carioca	II	B1	16	94	1.9	3.6
Carioca	II	B2	15	67	1.6	10.6
Carioca	II	B3	11	54	1.3	8.9
					4.8	

a B1: buds excised after 19 days of culture in the presence of 6-BA (50 μM) and IBA (0.025 μM). B2: buds excised after 19 days of primary culture followed by 17 days in the presence of 6-BA (20 μM) and IBA (0.025 μM). B3: buds excised after 19 days of primary culture, 17 days of secondary culture and 25 days in the basic medium.

A genotypic difference was observed once again pertaining to the height of shoots derived from node-I. The average height (mm) of Moruna shoots was 3.2 (B1), 6.5 (B2) and 7.6 (B3) whereas for Carioca shoots, values of 2.8 (B1), 13.4 (B2) and 17.4 (B3) were observed. Note that differences appeared during second and third culture.

The results regarding the cultures of hypocotyl and epicotyl segments of the common bean suggest that *P. vulgaris* does not have meristematic regions along the hypocotyl and epicotyl axis. Therefore, dormant buds are only found at the nodal regions. Kameya and Widholm (3) demonstrated development of buds from hypocotyl explants of *Glycine*

canescens and discussed the possibility of such buds being derived from pre-existing meristematic centers along the hypocotyl axis. This hypothesis was based on the fact that higher frequencies of buds were found on hypocotyl segments close to the cotyledonary node. In the same paper they indicated that buds could not be observed from the culture of hypocotyl segments without nodal regions in *G. max*. This suggests that, as in commom beans, dormant buds in soybean are only present in the nodal regions.

Experiment 3

The effect of 6-BA on the development of axillary buds from bean seedlings described in experiments 1 and 2 was further studied by using different 6-BA concentrations during primary culture of nodal regions of the cultivar Puebla-153. Nodal explants cultivated with 6-BA (20 μM) developed innumerous small buds (1-2 mm) with a frequency of 30-50 buds/node. This number represents the total number of buds including primary, secondary, and tertiary buds. Primary buds were designated as the ones developed from the nodal explant, and secondary buds as those derived from the nodes of the primary shoots, and so on. On the other hand, nodal segments cultivated in the presence of 6-BA (5 μM) presented excellent bud development (Figure 1). Among 28 cotyledonary nodes (node I), 27 nodes (96%) developed axillary buds with an average frequency of 4.2 buds/node and 9.1 mm of average height after 33 days of culture. During the culture of 25 primary leaf nodes (node II), 100% bud development was observed, with an average of 4.8 buds/node and 8.2 mm of average height following 33 days of culture. These data suggest that the best sequence for the development of axillary buds from nodal regions I and II of bean would be: (a) germination in the presence of high levels of 6-BA (20 μM); and (b) subsequent development of induced buds in the presence of low levels of 6-BA (5 μM). Similar observations were made in soybean cultures by Cheng *et al.* (1). The development of axillary buds was stimulated by the subculture of soybean nodal explants from high levels of 6-BA (10-50 μM) to low levels of 6-BA (1 μM). During the experiment 2, axillary buds were developed from nodal cultures in the presence of 6-BA (20 μM), without secondary or tertiary buds. Perhaps the excision of the developed buds at each subculture would facilitate the induction of new primary buds. A genotypic difference among nodal cultures from bean seedlings may also explain this behavior. This would represent another example of medium specificity for different cultivars. A sequential reduction of 6-BA concentrations from germination to nodal cultures seems very important in order to assure optimum growth of axillary buds.

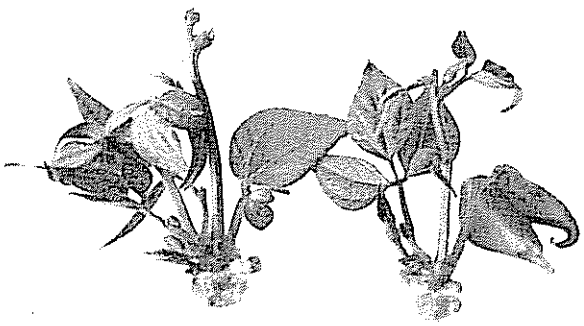

Fig. 1 Axillary bud development from cotyledonary nodes of bean seedlings (*Phaseolus vulgaris* cv. Puebla – 153), following 18 days of culture.

Rooting of the shoots derived from axillary buds was promoted by culturing in B5 medium, supplemented with IBA (10 μM) for 10 days. Following transfer to B5 medium without growth regulators, a fast growing root system was observed after 20 days.

Summary

The best conditions for the induction of development and growth of axillary buds of bean seedlings (*Phaseolus vulgaris*) consist of germination in the presence of 6-BA (20 μM) and culture of derived nodal regions in the basic B5 medium supplemented with 6-BA (5 μM) and IBA (0.25 μM). Rooting of the shoots derived from axillary buds was induced by IBA (10 μM) for 10 days, followed by culturing the plantlets in the basic B5 medium without growth regulators. The presence of light was essential to the development of axillary buds from nodal explants of bean seedlings. The frequency of axillary bud induction and the shoot growth were different according to the node position and genotype. The cultivar Puebla-153 presented higher induction frequencies, whereas better shoot growth was observed for the cultivar Carioca. The maximum number of buds developed per leaf axil was six (cv. Moruna, node I) suggesting a total of twelve dorment buds at each cotyledonary node of bean seedlings.

Literature cited

1. CHENG, T. Y., SAKA, H., and VOQUI-DINH, T. H. Plant regeneration from soybean cotyledonary node segments in culture. Plant Science Letters 19:19-99. 1980.

2 GAMBORG, O. L., MILLER, R. A., and OJIMA, K. Nutrient requirements of suspension cultures of soybean root cells. Expermental Cell Research 50:151-158. 1968.

3 KAMEYA, T., WIDHOLM, J. Plant regeneration from hypocotyl sections of *Glycine* species. Plant Science Letters 21:289-294. 1981

4 SAKA, H., VOQUI-DINH, T. H., and CHENG, T. Y. Stimulation of multiple shoot formation on soybean stem nodes in culture Plant Science Letters 19:193-201. 1980.

5 TOMES, D. T. A tissue culture procedure for propagation and maintenance of *Lotus corniculatus*. Canadian Journal of Botany 57:137-140. 1979

Reseña de libros

JANZEN, D. H. Costa Rican Natural History. The University of Chicago Press, Chicago, 1983. 816 p.

Este libro representa el aporte de 174 autores bajo la coordinación del Dr. Daniel H. Janzen, ecólogico tropical de amplia experiencia. Se trata del mejor intento formal, que se haya publicado hasta la fecha, para recopilar el conocimiento acumulado sobre la historia natural de este país.

Costa Rica, a pesar de ser un pequeño país, por su posición geográfica y su relieve, ofrece al científico en ciencias naturales una amplia variedad biológica producto de un fuerte proceso de endemismo. Esto ha hecho de este país mesoamericano un lugar de convergencia de numerosos investigadores internacionales que han contribuido a mejorar el conocimiento de los diversos aspectos biológicos y ecológicos.

Como en todo libro de carácter enciclopédico, no debe el investigador esperar una obra maestra en el capítulo donde se es especialista. Debe, en su lugar, valorarla como una obra global muy valiosa, precisamente en aquellos capítulos donde no se es especialista. Considero que esta obra es muy apropiada, principalmente para aquel naturalista extranjero que se quiera informar de manera muy actualizada, y relativamente profunda, acerca de ese pequeño y ahora famoso país de numerosos parques nacionales, estaciones de campo y reservas biológicas.

Hacer una revisión completa de una obra de esta envergadura es una tarea difícil. Sin embargo, resumiendo groseramente el contenido, tan variado como valioso, podríamos iniciar diciendo que los primeros cinco capítulos ofrecen un bosquejo introductorio acerca del desarrollo histórico de las ciencias naturales en este país. También se incluye dentro de los capítulos introductorios, valiosa información sobre biogeografía de América Central, clima, historia geológica y una lista someramente anotada de los tipos de suelo, que quizá mereció un desarrollo más profundo.

El capítulo seis nos ofrece un interesante análisis de la agricultura de Costa Rica: su historia, tipo de distribución y explotación de la tierra, así como de los principales cultivos. El análisis de la producción agrícola de Costa Rica, visto con ojos extranjeros, lleva al lector costarricense a la meditación. Al final de este capítulo, así como de muchos de los subsiguientes, se incluye una valiosa lista comentada e ilustrada con magníficas fotografías de las principales especies biológicas tratadas.

A partir del sétimo capítulo, el enfoque de la obra se vuelve más biológico y cada apartado del libro posee al inicio una magnífica introducción, donde quizá estén muchos de los principales aportes que ofrece el tratado y encontramos también el análisis individual, o ensayo corto, sobre cada especie considerada importante. El sétimo capítulo sobre las plantas, se inicia con un interesante análisis sobre el sistema de clasificación de zonas de vida de Holdridge y colaboradores y las plantas existentes en Costa Rica. Seguidamente se ofrece una descripción de los principales sitios existentes en este país para el estudio biológico, así como

su relieve, geología y vegetación; vale decir: Parque Nacional Santa Rosa, Refugio Palo Verde, Parque Nacional de Corcovado, Reserva Biológica La Selva, Reserva de Monteverde, Las Cruces y el Cerro de la Muerte. Posteriormente se incluyen varias páginas sobre diversos e interesantes aspectos y patrones fisionómicos propios de las plantas tropicales. Antes de introducir al lector en una voluminosa lista comentada de especies de plantas tropicales, se incluye una lista de las especies de árboles presentes en los siete principales sitios de estudio citados.

El capítulo ocho trata sobre reptiles y anfibios. Aquí los autores de esta sección ofrecen un análisis ordenado de los patrones reproductivos, evolución, adaptaciones defensivas y hábitos alimentarios de los anfibios de Costa Rica. También se ofrecen algunas páginas dedicadas a las tortugas y los cocodrilos, lo mismo que una lista reciente de la herpetofauna costarricense, basada en las especies registradas principalmente en seis localidades. En la lista comentada de las principales especies herpetológicas se incluyen, además de anfibios, tortugas e iguanas, a las serpientes venenosas.

El capítulo nueve se refiere a los mamíferos. Se ofrecen aquí, en la introducción, aspectos interesantes acerca de la diversidad de este grupo de vertebrados en Costa Rica, su estacionalidad, frugivorismo en carnívoros, la abundancia tropical de los murciélagos y adaptaciones ecológicas de algunos grupos, entre otros. Al final de este capítulo, se incluye una extensa lista de la mastofauna costarricense, acompañada de algunas localidades. Posteriormente el análisis individual de las especies.

El capítulo diez, nos ofrece una interesante parte introductoria. Se hace énfasis aquí en la riqueza de especies de aves presentes en Costa Rica. Posteriormente presenta un análisis acerca de la composición de los principales grupos representados, las especies residentes y las migratorias, nos señala aspectos diversos sobre la zoogeografía, patrones de distribución, comunidades, estructuras tróficas, efectos climáticos, estacionalidad, etc. El mismo autor de la parte introductoria presenta, posteriormente, una extensa lista anotada de las especies de la avifauna costarricense discutidas en el capítulo.

El capítulo correspondiente a los insectos, de por sí es uno de los más difíciles de escribir, presenta un enfoque muy interesante. Como el autor mismo lo advierte, el conocimiento que hoy todavía se tiene acerca de la ecología de los insectos de Costa Rica es proporcionalmente insignificante. En la parte correspondiente a la introducción, el autor inicia su capítulo con aspectos meramente ecológicos y la influencia de tales factores sobre las poblaciones de insectos. Por lo tanto, se analizan aspectos tales como estacionalidad, comportamiento reproductivo de algunos grupos y estrategias de protección a condiciones estacionales adversas del ambiente. También se dedican algunas páginas a los tipos de polinización por insectos observados en Costa Rica, insectos de islas y finalmente algunas apreciaciones dedicadas a las especies de insectos carroñeros.

Posteriormente se agregan listas de algunos grupos de insectos y, como última parte del capítulo, los ensayos cortos sobre diversas especies consideradas, por alguna razón, importantes.

En resumen, puede afirmarse que este es uno de los mejores legados que el Dr. Janzen le deja a los estudiosos de la ecología tropical y a Costa Rica. La coordinación y edición de esta obra general que desde ahora se convertirá en "clásica" y será de gran utilidad para los que se inician y los que quieren información proveniente de alguno de los numerosos especialistas que participaron en confección de esta obra sería importante que sus futuras ediciones de este libro se incluyan capítulos nuevos sobre temas ya estudiados en Costa Rica y que fueron omitidos en esta primera versión.

LUIS FERNANDO JIRON
MUSEO DE INSECTOS
UNIVERSIDAD DE COSTA RICA