Journal of Global Biosciences
ISSN 2320-1355
Volume 8, Number 4, 2019, pp. 6146-6159
Website: www.mutagens.co.in

Research Paper

INDIRECT ORGANOGENESIS IN COMMON BEAN: OTHER MODEL OF RECALCITRANT SPECIES?

Juan Chero-Ayay, Consuelo Rojas-Idrogo and Guillermo E. Delgado-Paredes

Facultad de Ciencias Biológicas y Laboratorio General de Biotecnología-UNPRG
Universidad Nacional Pedro Ruiz Gallo,
Ciudad Universitaria, Juan XXIII No 391, Lambayeque,
Peru.

Abstract

Common bean (*Phaseolus vulgaris* L.), one of the most important legume species for human nutritions, is recalcitrant to the induction of several *in vitro* morphogenic process. The study was carried out to investigate the effect of sodium chloride on the callus induction and shoot regeneration of common bean. Two cultivars of common bean, Bayo Florida and Blanco Local, were tested for callus induction and plant regeneration. Hypocotyls and cotyledons from *in vitro* seedlings were incubated on MS (Murashige and Skoog) media containing 2.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 2.0 mg/L kinetin (KIN). It was also studied the effect of NaCl (0 to 250 mM) on relative growth of hypocotyls and cotyledons callus, as alternative strategy to induce indirect organogenesis by salinity stress, after four weeks of culture. The MS culture medium with 2.0 mg/L 2,4-D and 2.0 mg/L KIN was optimum for proliferation of calli. However, in all posible combinations of plant growth regulators AIA-BAP, AIA-KIN, AIA-2iP, ANA-BAP, ANA-KIN and ANA-2iP, in the auxins concentrations (0.1 to 1.0 mg/L) and citokinins (0.2 to 2.0 mg/L), supplemented or not with 0.2 mg/L GA_3, no shoots regeneration was observed. In *Phaseolus vulgaris* cv. Bayo Florida and Blanco Local an efficient callus induction protocol, even in culture medium with high concentrations of NaCl, was developed; however, a protocol for *in vitro* shoot regeneration via indirect organogenesis no was established.

Key words: Callus induction, cellular sensitivity, NaCl stress, *Phaseolus vulgaris*, plant regeneration.

INTRODUCTION

The Fabaceae family with 20,000 species is the third largest familiy in the plant kingdom and second most important after Poaceae as mainstays for human food and protein resources [1]. Common bean (*Phaseolus vul*garis L.) is an important "*pulse cro*p",

including others legumes as *Pisum sativum*, *Cicer arietinum*, *Lupinus* sp., *Lens culinaris*, *Vigna unguiculata*, *Vigna radiata*, *Vigna mungo* and *Cajanus cajan* [2]. "*Pulse crops*" are increasingly being recognized for their role in promoting good health as a primary and affordable source of both primary and secondary metabolites [1].

The use of tissue culture for plant regeneration, by organogenesis and/or somatic embryogenesis, and the introduction of foreign genes that confer pathogens resistance or drought tolerance are a good option for common bean cultures [3]. In fact, organogenesis *in vitro* consists of several aspects such as phytohormone perception, dedifferentiation of differentiated cells (callus induction) to acquire organogenic competence, re-entry of quiescent cells into cell cycle, and organization or *de novo* differentiation of cell division to form specific organ primordia and meristems [4]. Most of the recent protocols on *in vitro* plant regeneration of common bean are based on indirect or direct organogenesis. A protocol was established for regeneration via indirect organogenesis for cv. Negro Jamapa, using as explants apical meristems and cotyledonary nodes diseccted from the embryogenic axes [5]. An improved method for *in vitro* regeneration from embryos of four different varieties in media containing BAP and adenine was also developed [6]. Embryogenic axes of the several Costa Rican cultivars were cultured on MS medium supplemented with BAP and adenine sulphate, noting that the number of regenerated shoots and leaves differed significantly among the cultivars evaluated [7]. Likewise, an efficient plant regeneration using the transverse thin-cell-layer (tTCL) method and TDZ, BAP and silver nitrate was developed [8]. In another work, the effect of BAP and TDZ on shoot development of embryogenic axis from commercial common bean varieties, Bayomex and FM199, in the absence of callus was studied [3]. However all these protocols did not always yield regeneration of the common bean plants, and still lacked some practical tools for genomic research, such as routine genetic transformation [9], and in other cases only callus cultures in white seed mutant of common bean were initiated from the axillary leaves, axillary shoots, nodes, internodes, and root segments, on MS medium with IAA and BAP [10].

On the other hand, the major abiotic stresses that affect the production and productivity of many crops comprise salinity, drought, water logging, heat, frost and mineral toxicities by aluminum, iron, cadmium, and others. Excess salt in soil or in solutions

interferes with several physiological and biochemicals processes causing, mineral deficiency, ion toxicity and oxidative stress, which affects DNA, proteins, lipids and pigments in plants [11], and thus impedens the growth and development of the majority of crops. Few studies have been carried out on callus induction and plant regeneration of common bean in culture medium with NaCl. The effect of salinity (0, 0.2, 0.4, 0.6, 0,8 and 1.0%) on callus formation and partial organogenesis (roots formation) of *P. vulgaris* cv. Red Kidney was studied [12]. Likewise, in a preliminar study callus of common bean was exposed to different levels of salt mixtures, NaCl, $CaCl_2$ and $MgCl_2$ in a ratio of 2:2:1 at concentrations of 0, 50, 100, 200 and 250 mM, and different levels (0, 0.05, 0.1 or 0.5 mM) of the chemical mutagen sodium azide were used for period of time of 30, 60 or 90 min [13].

The aim of this research was to study the response of two common bean genotypes, Bayo Florida and Blanco Local, to callus induction and to evaluate the response of regenerated plants to NaCl stress using tissue culture technique.

MATERIALS AND METHODS

1.1 Plant materials

The two cultivars of common bean (*Phaseolus vulgaris* L.) cv. Bayo Florida and Blanco Local were obtained from National Institute of Agrarian Research (INIA), Lambayeque, Peru. These genotypes are widely cultivated in Peru, especially on the northern coast. The experiment were conducted in the General Biotecnology Laboratory at the Pedro Ruiz Gallo National University, Lambayeque, Peru, between march to november 2017.

1.2 Preparation of culture media and culture conditions

Half salts strength of MS [14] culture medium incluiding vitamins, 100 mg/L myo-inositol and 1.0 mg/L thiamine. HCl and 2.0% sucrose was used for *in vitro* seed germination, while full strength MS, supplemented with 2.0 mg/L 2,4-D and 2.0 mg/L KIN, was used for callus induction and experimental treatments with NaCl (0.0, 62.5, 125.0 and 250.0 mM).

All media pH were adjusted to 5.8±0.1 with 1N KOH or 1N HCl, then solidified with 0.7% agar prior to autoclaving at 121 ^{o}C at 105 kPa for 20 minutes. Each treatment comprised 10 explants and was performed twice. The experiments were evaluated after four weeks and in all treatments 150x25 mm test tubes were used.

The plant material was kept in a growth room with a controlled temperature of 26±2 ^{o}C, photoperiod of 16 hours light, luminous intensity of 60 µmol.m^{-2}.s^{-1} via cool white fluorescent light and a relative air humidity of 80%. The treatments of callus induction and relative growth of callus in NaCl conditions, were established in total darkness.

1.3 Callus induction and roots formation

In common bean, the explants used were both hypocotyls (1 cm in length) and cotyledons (1 cm^2), diseccted from plantlets of germinated seeds. After four weeks of growth calli were transferred to plant regeneration culture medium containing several auxin/cytokinin combinations supplemented with giberellins (GA_3). In the determination of the relative growth of callus (G) the following formula was used: G = 100 (F – I)/I, where I is the initial fresh weight and F is the final fresh weight [15]. Formed roots were observed macroscopically.

1.4 Statistical analysis: Results were processed and analyzed by ANOVA factorial arrangement and Tukey's HSD multiple-range test ($p \leq 0.05$). All the statistical analyses were performed using the software package Statgraphics Plus®, version 5.0 (StatPoint, Virginia, USA).

RESULTS

2.1 Callus induction

In the cv. Bayo Florida the highest increase in fresh weight of callus was reached in cotyledons (1.446 g) with respect to hypocotyls (1.234 g), observing a similar result in cv. Blanco Local, with the highest increase in fresh weight in cotyledons (1.786 g) with respect to hypocotyls (1.680 g) (Table 1); however, the highest relative growth of calluses corresponded to cv. Blanco Local with 2 705.76% for hypocotyls and 1 774.93% for cotyledons, while in cv. Bayo Florida was only reached 2 304.80% for hypocotyls and 1 219.56% for cotyledons (Table 2). These results indicated the

occurrence of a significant varietal effect among cultivars and among types of explants, highlighting widely cv. Blanco Local on the cv. Bayo Florida and hypocotyls on cotyledons.

2.2 Effect of NaCl on callus induction

The increase in the concentration of NaCl (0 to 250 mM) significantly affected the final fresh weight in hypocotyls and cotyledons of cv. Bayo Florida and cv. Blanco Local, resulting in greater tolerance to salinity cv. Bayo Florida and hypocotyl callus (Table 3). The highest relative growth of callus was observed in hypocotyls of cv. Bayo Florida with 40.49% (62.5 mM NaCl), while the lowest relative growth of callus was observed in cotyledons of cv. Blanco Local with 0.33% (250 mM NaCl) (Table 4).

2.3 Plant regeneration (roots formation)

Several combinations of auxins-cytokinins were evaluated in the regeneration of common bean plants from callus with or without NaCl treatments. These combinations were the following: AIA-BAP, AIA-KIN, AIA-2iP, ANA-BAP, ANA-KIN and ANA-2iP, where the auxins were used in the concentrations of 0.1 and 1.0 mg/L and the cytokinins in the concentrations of 0.2 and 2.0 mg/L, in all possible combinations. These treatments were supplemented or not with gibberellin GA_3 0.2 mg/L. No regeneration of shoots was observed and only sporadically the formation of small and filamentous roots (Table 5), which in some way made it possible to suppose that the common bean callus are highly recalcitrant to the organogenic process.

Table 1: Increase in fresh weight of hypocotyls and cotyledons callus of *P. vulgaris* cv. Bayo Florida and Blanco Local, after four weeks of culture, in MS medium supplemented with 2.0 mg L^{-1} 2,4-D and 2.0 mg L^{-1} KIN, without NaCl.

Cultivar	Hypocotyls (g)			Cotyledons (g)		
	Initial weight	Final weight	Increase	Initial weight	Final weight	Increase
Bayo Florida	0.054	1.288	1.234 ± 0.22[b]	0.119	1.566	1.446 ± 0.18[b]
Blanco Local	0.062	1.743	**1.680 ± 0.15[a]**	0.101	1.886	**1.786 ± 0.21[a]**

The value of each treatment consisted of mean SD of 10 replicates.
Means followed by the same letter at the same column are not statistically significant by Tukey's test (P<0.05).
2,4-D, 2,4-dichlorophenoxyacetic acid; KIN, Kinetin

Table 2: Relative growth of hypocotyls and cotyledons callus of *P. vulgaris* cv. Bayo Florida and Blanco Local, after four weeks of culture, in MS culture medium supplemented with 2.0 mg L^{-1} 2,4-D and 2.0 mg L^{-1} KIN, without NaCl.

Cultivar	Explant	Response (g)		Relative growth (%)
		Initial weight	Final weight	
Bayo Florida	Hypocotyls	0.0535	1.2877	2 304.80
	Cotyledons	0.1186	1.5650	1 219.56
Blanco Local	Hypocotyls	0.0621	1.7429	**2 705.76**
	Cotyledons	0.1005	1.8858	**1 774.93**

2,4-D, 2,4-dichlorophenoxyacetic acid; KIN, Kinetin

Table 3: Effect of NaCl (0 to 250 mM) on relative growth of hypocotyls and cotyledons callus of *P. vulgaris* cv. Bayo Florida and Blanco Local, after four weeks of culture, in MS culture medium supplemented with 2.0 mg L^{-1} 2,4-D and 2.0 mg L^{-1} KIN.

Cultivar	Explant	Treat.	Level (NaCl) (mM)	Response (g)		Relative growth (%)
				Initial fresh weight	Final fresh weight	
B. Florida	Hypocotyls	T1	0.00	1.2877	1.8223	**41.52**
		T2	62.5		1.8091	40.49
		T3	125.0		1.7821	38.39
		T4	250.0		1.7595	36.64
	Cotyledons	T5	0.00	1.5650	1.7605	**12.49**
		T6	62.5		1.7116	9.36
		T7	125.0		1.6666	6.49
		T8	250.0		1.6134	3.09
B. Local	Hypocotyls	T9	0.00	1.7429	1.9059	9.35
		T10	62.5		1.8713	7.36
		T11	125.0		1.8137	4.06
		T12	250.0		1.7968	3.09
	Cotyledons	T13	0.00	1.8858	2.0157	6.89
		T14	62.5		1.9310	2.40
		T15	125.0		1.9141	1.50
		T16	250.0		1.8920	0.33

BF, cv. Bayo Florida; BL, cv. Blanco Local
2,4-D, 2,4-dichlorophenoxyacetic acid; KIN, Kinetin

Table 4: Order of merit on the effect of NaCl (0 to 250 mM) on relative growth of hypocotyls and cotyledons callus of *P. vulgaris* cv. Bayo Florida and Blanco Local after four weeks of culture, in MS culture medium supplemented with 2.0 mg/L 2,4-D and 2.0 mg/L KIN.

Order of merit	Treatment	Callus growth (g)	Treatment	Relative growth (%)
1	**T1 BL-Ct**	2.0157^{a}	T1 BF-Hy	41.52^{a}
2	T2 BL-Ct	1.9311^{b}	T2 BF-Hy	40.49^{a}
3	T3 BL-Ct	1.9141^{bc}	T3 BF-Hy	38.39^{ab}
4	**T1 BL-Hy**	1.9059^{bc}	T4 BF-Hy	**36.64^{b}**
5	T4 BL-Ct	1.8920^{bc}	T5 BF-Ct	12.49^{c}
6	T2 BL-Hy	1.8713^{cd}	T6 BF-Ct	9.36^{d}
7	T1 BF-Hy	1.8223^{de}	T9 BL-Hy	9.35^{d}
8	T3 BL-Hy	1.8137^{e}	T10 BL-Hy	7.36^{e}
9	T2 BF-Hy	1.8091^{e}	T13 BL-Ct	6.89^{e}
10	T4 BL-Hy	1.7968^{ef}	T7 BF-Ct	6.49^{e}
11	T3 BF-Hy	1.7821^{ef}	T11 BL-Hy	4.06^{f}
12	T1 BF-Ct	1.7605^{f}	T8 BF-Ct	3.09^{fg}
13	T4 BF-Hy	1.7596^{f}	T12 BL-Hy	3.09^{fg}
14	T2 BF-Ct	1.7116^{g}	T14 BL-Ct	2.40^{g}
15	T3 BF-Ct	1.6666^{h}	T15 BL-Ct	1.50^{h}
16	T4 BF-Ct	1.6134^{i}	T16 BL-Ct	0.33^{i}
		CV = 1.19% **ALS = 0.0443**		**CV = 1.19%** **ALS = 0.0443**

BF, cv. Bayo Florida; BL, cv. Blanco Local; Ct, cotyledons; Hy, hypocotyls
2,4-D, 2,4-dichlorophenoxyacetic acid; KIN, Kinetin

Table 5: Effect of various combinations of auxins - cytokinins and gibberellins in the induction of indirect organogensesi in callus of hypocotyls and cotyledons of *P. vulgaris* cv. Bayo Florida and Blanco Local, after eight weeks of culture.

Auxins (mg/L)		Cytokinins (mg/L)			Gibberellin (mg/L)	Response (indirect organogenesis)
IAA	NAA	BAP	KIN	2iP	GA_3	
0.1		0.2			0.0	0
0.1		0.2			0.2	0
1.0		2.0			0.0	Roots
1.0		2.0			0.2	Roots
0.1			0.2		0.0	0
0.1			0.2		0.2	0
1.0			2.0		0.0	Roots
1.0			2.0		0.2	Roots
0.1				0.2	0.2	0
0.1				0.2	0.2	0
1.0				2.0	2.0	Roots
1.0				2.0	2.0	Roots
	0.1	0.2			0.0	0
	0.1	0.2			0.2	0

	1.0	2.0			0.0	Roots
	1.0	2.0			0.2	Roots
	0.1		0.2		0.0	0
	0.1		0.2		0.2	0
	1.0		2.0		0.0	Roots
	1.0		2.0		0.2	Roots
	0.1			0.2	0.2	0
	0.1			0.2	0.2	0
	1.0			2.0	2.0	Roots
	1.0			2.0	2.0	Roots

Roots, scarce formation of filamentous roots
IAA, indole-3-acetic acid; NAA, 1-naphthaleneacetic acid; BAP, 6-benzylaminopurine; KIN, kinetin; 2iP, 2-Isopentenil-adenina; GA_3, gibberellic acid.

DISCUSSION

Callus induction in common bean was observed in several types of explants (embryos, axillary leaves, axillary shoots, nodes, internodes, and root segments) and genotypes, in MS culture medium supplemented with 2,4-D [13] or with BAP-IAA [10].

These results were very similar to that obtained for hypocotyls callus of cv. Bayo Florida, but much lower for cotyledons callus of cv. Bayo Florida and for both hypocotyls and cotyledons callus of cv. Blanco Local, and demonstrate the high totipotency of various common bean explants to induce callus in several genotypes. In addition, Thiagarajan et al. (2013) [12] observed a gradual decrease in callus growth as the salinity increased, in a study about the effect of salinity on callus formation and organogenesis (roots induction) of cv. Red Kidney beans, using explants from epicotyls, in MS culture medium with 2,4-D.

A. Trewavas in 1981 and 1991, indicated that despite intensive research effort, the role of growth substances in the life of the intact growning plants is far from clear, due to two reasons: growth substance concentration failed to correlate easily with changes in development, and variations in sensitivity could help explain the abscence of correlations [16,17]. This is, that there is no evidence that plant hormones act via changes in the amount or concentration of the hormones, and that all change in response must be attributed to changes in the sensitivity of the tissue [18,19]. The discovery and signaling through cell surface receptors in plants is pivotal for cells to communicate with each other and to interact with the environment [20-22]. In the

present study the observed responses would be related to the state of cellular sensitivity of the tissues at the time of *in vitro* culture.

Many examples of common bean regeneration pathway may be found in the literature. In the case of *in vitro* propagation, in cv. Negro Jamapa, a protocol for *in vitro* regeneration, using apical meristems and cotyledonary nodes, dissected from the embryonic axes was established, and this protocol was reproducible for others nine common bean cultivars tested, suggesting a genotype independent procedure [5]. An efficient protocol for the *in vitro* regeneration using cotyledonary nodes, cotyledonary nodes with one cotyledon and cotyledonary nodes with two cotyledons dissected from the embryonic axis, were established, although different responses were observed depending on genotype [23]. Likewise, the effect of 20 mg/L adenine sulphate in the regeneration and elongation of common bean shoots cv. CIAP 7247F was achieved in cotyledonary nodes dissected from the mature germinated seeds [24], and in another study, cotyledonary nodes of common bean cv. GS012 were used as primary explants, and the optimum treatment for shoot induction was MS salts and B5 vitamins culture medium supplemented with 11.1 µM BAP and 0.5 µM NAA [25]; however, the main disadvantage found in those procedure was that the regeneration from a pre-existing meristematic tissue [26], therefore, this protocol is considered strictly of propagation and not of regeneration, since regeneration is *de novo* formation of shoots (organogenesis) or somatic embryos (somatic embryogenesis), direct (without callus formation) or indirect (with callus formation). Indirect organogenesis opens up the possibility of somaclonal variation with induction of genetic and/or epigenetic variability [27]. This has been clearly pointed out in the recent literature review on *P. vulgaris* by Hnatuszko-Konka et al. (2014) [9].

In the case of organogenesis, several examples of a direct organogenesis pathways may be found in the literature, however there are only a few protocols based on indirect organogenesis of the *P. vulgaris*. In four cultivars of common bean, an efficient regeneration method (83 to 100%) was achieved in embryo axes from mature seeds when explants were incubated on Gamborg culture media supplemented with 10 mg/L BA, without adenine hemisulphate [27]. In two common bean cultivars, Flor de Mayo Anita and Pinto Saltillo, inoculated with different *A. tumefaciens* strains, five-day old

hypocotyls regenerated organogenic buds 5 d after inoculation in culture medium of the same formulation [28]. Likewise, embryogenic axes of the several Costa Rican common bean cultivars were cultured on MS culture medium, and independently of the cultivar, the induction medium supplemented with BAP and adenine sulphate resulted in the higher average of shoots formation [7]. A method was designed to optimize high frequency direct shoot regeneration of the common bean, using the transverse thin cell layer (tTCL) method, with pretreatment of seeds with 10 µM TDZ that significantly increased bud regeneration frequency on tTCL [8]. Also, *in vitro* plants of common bean were obtained by direct organogenesis using embryonic axis from commercial varieties, Bayomex and Flower of May 199 in MS culture medium supplemented with BAP or TDZ [3].

In general, in *in vitro* regenerative processes of common bean (organogenesis and somatic embryogenesis), those obtained from callus induced in explants with differentiated tissues are of greater relevance as hypocotyls, cotyledons, leaves, stems and roots, where a *de novo* regenerative process is induced in competent cells. In the case of in zygotic embryos or embryogenic axes the cells are meristematic and highly competent. However, the influence of factores as plant competence, precultuvation, age factors, morphological integrity, elicitation by abiotic stress, and others [9], can significantly influence in the *in vitro* morphogenetic processes of common bean, so more research is needed.

For these reasons, *P. vulgaris* remains recalcitrant to both routine *in vitro* breeding (somaclonal variation and anther culture) and genetic engineering (transgenic plants).

CONCLUSION

In the present study, it was established an efficient, simple, and reproducible *in vitro* callus induction protocol, from hypocotyls and cotyledons for common bean cv. Bayo Florida and Blanco Local in MS culture medium supplemented with NaCl (0 to 250 mM). However, shoot regeneration was not observed in all posible combinations and in several concentrations of plant growth regulators AIA-BAP, AIA-KIN, AIA-2iP, ANA-BAP, ANA-KIN and ANA-2iP.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. Alain Monsalve-Mera and Dr. Alexander Huamán Mera for English improvements. This research was funded by the General Biotechnology Laboratory-VRINV (UNPRG).

REFERENCES

[1] Cannon, S. B., May, G.D., and Jackson, S.A. 2009, Three sequences legume genomes and many crops species: rich opportunities for translational genomics. *Plant Physiology, 151*:970-977. doi: https://doi.org/10.1104/pp.109.144659

[2] Gatti, I., Guindón F., Bermejo C., Espósito A., and Cointry E., 2016, In vitro tissue culture in breeding programs of leguminous pulses: use and current status. *Plant Cell Tissue and Organ Culture*, *127*:543-559. https:doi.org/10.1007/s11240-016-1082-6

[3] Martínez, B., Rodríguez J. L., Mascorro O., and Iturriaga G., 2015, *In vitro* plants of common bean (*Phaseolus vulgaris* L.) obtained by direct organogenesis. *The Journal of Agricultural Science, 7*:169-179. http://dx.doi.org/10.5539/jas.v7n11p169

[4] Sugiyama, M, 1999, Organogenesis *in vitro*. *Current Opinion in Plant Biology*, *2*:61-64.

[5] Arellano, J., Fuentes, S. I., Castillo-España, P., and Hernández G., 2009, Regeneration of different cultivars of common bean (*Phaseolus vulgaris* L.) via indirect organogenesis. *Plant Cell Tissue and Organ Culture, 96*:11-18. doi:10.1007/s11240-008-9454-1

[6] Quintero-Jiménez, A., Espinosa-Huerta, E., Acosta-Gallegos, J. A., Guzmán-Maldonado, H. S., and Mora-Avilés, M. A., 2010a. An improved method for *in vitro* regeneration of common bean (*Phaseolus vulgaris* L.). *Agrociencia*, *44*:57-64.

[7] Gatica, A., Muñoz, J., Ramírez, P., and Valdez M., 2010, *In vitro* plant regeneration system for common bean (*Phaseolus vulgaris*): effect of N^6-benzylaminopurine and adenine sulphate. *Electronic Journal of Biotechnology, 13*:1-8. http://dx.doi.org/10.4067/S0717-34582010000100006

[8] Cruz de Carvalho, M. H., Van Le B., Zuily-Fodil, Y., Pham, A. T., and Tran Thanh

Van K., 2000. Efficient whole plant regeneration of common bean (*Phaseolus vulgaris* L.) using thin-cell-layer culture and silver nitrate. *Plant Science, 159*:223-232. https://doi.org/10.1016/S0168-9452(00)00346-0

[9] Hnatuszko-Konka, K., Kowalczyk, T., Gerszberg, A., Wiktorek-Smagur, A., and Kononowicz, A. K., 2014, *Phaseolus v*ulgaris – Recalcitrant potential. *Biotechnology Advance, 32*:1205-1215. doi: 10.1016/j.biotechadv.2014.06.001

[10] Mahamune, S. E., Bansode, R. P., Sangle, S. M., Waghmar, V. A., Pandhure, N. B., and Kothekar V. S., 2011, Callus induction from various explants of French bean (*Phaseolus vulgaris* L.). *Journal of Experimental Sciences, 2*:15-16.

[11] Zhu, J. K. 2002, Salt and drought stress signal transduction in plants. *Annual Review of Plant Biology, 53*:247-273. doi:10.1146/annurev.arplant.53.091401.143329

[12] Thiagarajan, T., Recinos, H., and Tillett, A., 2013, Effect of salinity on callus formation and organogenesis of Red Kidney beans (*Phaseolus vulgaris* L.). *Euopean Science Journal 9*:356-362.

[13] Hamad, R. I. and Al-Oubaidi, H. K. M., 2017, Increasing salt tolerance of *Phaseolus vulgaris* L. callus using sodium azide *in vitro*. *European Journal of Biomedical and Pharmaceutical Sciences, 4*:22-26.

[14] Murashige, T., and Skoog, F., 1962, A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiology Plantarum, 15*:473-497. doi: 10.1111/j.1399-3054.1962.tb08052.x

[15] Smith, K.M., and Mc Comb J. A., 1981, Effect of NaCl on the growth of whole plants and their corresponding callus cultures. *Australian Journal of Plant Physiology, 8*:267-275. https://doi.org/10.1071/PP9810267

[16] Trewavas, A., 1981, How do plant growth substances work? *Plant Cell & Environment, 4*:203-228. https://doi.org/10.1111/1365-3040.ep11610983

[17] Trewavas, A., 1991, How do plant growth substances work? II. *Plant Cell & Environment, 14*:1-12. doi: 10.1111/j.1365-3040.1991.tb01366.x

[18] Davies, P. J., 1995, Plant Hormones. Physiology, Biochemistry and Molecular Biology. Dordrecht: Kluwer Academic Publishers. 796 p. doi: 10.1007/978-94-011-0473-9

[19] Gaspar, T. H., Kevers, C., Faivre-Rampant, O., Crèvecoeur, M., Penel, C.L., Greppin, H., and Dommes, J., 2003, Changing concepts in plant hormone action.

In Vitro Cellular & Developmental Biology – Plant, 39:85-106. doi:10.1079/IVP2002393

[20] Geldner, N., and Robatzek, S., 2008, Plant receptors go endosomal: a moving view on signal transduction. *Plant Physiology*, *147*:1565-1574. doi: https://doi.org/10.1104/pp.108.120287

[21] Chow, B., and McCourt, P., 2006, Plant hormone receptors: perception is everything. *Genes & Development, 20*:1998-2008. doi:10.1101/gad.1432806

[22] Engelhard, C., Wang, X., Robles, D., Essen, L. O., Batschauer, A., Bitti, R. and Moldt, J., 2014, Cellular metabolites enhance the light sensitivity of *Arabidopsis* crytochrome through alternate electron transfer pathways. *Plant Cell, 26*:4519-4531. doi: 10.1105/tpc.114.129809

[23] Collado, R., Veitía, N., Bermúdez-Caraballoso, I., García, L., Torres, D., Romero, C., Rodríguez J.L. and Angenos G., 2013. Efficient *in vitro* plant regeneration via indirect organogenesis for different common bean cultivars. *Scientia Horticulturae, 153*:109-116. https://doi.org/10.1016/j.scienta.2013.02.007

[24] García, L., Bermúdez-Caraballoso, I., Veitía, N., Collado, R., Torres, D., and Romero, C., 2012, Efecto del sulfato de adenine en la regeneración y elongación de brotes de frijol común. *Biotecnología Vegetal, 12*:59-62.

[25] Thị Thảo, N., Phượng Thảo, N.T., Hassan, F., and Jacobsen, H.J., 2013, *In vitro* propagation of common bean (*Phaseolus vulgaris* L.). *Journal of Science & Development, 11*:868-876.

[26] Mukeshimana, G., Ma, Y., Walworth, A.E., Song, G., and Kelly, J.D., 2013. Factors influencing regeneration and *Agrobacterium tumefaciens*-mediated transformation of common bean (*Phaseolus vulgaris* L.). *Plant Biotechnology Report, 7*: 59-70. doi: 10.1007/s11816-012-0237-0

[27] Gepts, P., Aragão F. J. L., De-Barros E., Blair M. W., Brondani R., Broughton W., Galasso I., Hernández G., Kami J., Lariguet P., McClean P., Melotto M., Miklas P., Pauls P., Pedrosa.Harand A., Porch T., Sánchez F., Sparvoli F., and Yu K., 2008, Genomics of *Phaseolus* beans, a major source of dietary protein and micronutrients in the tropics. In: Genomics of Tropical Crop Plants. P. Moore and R. Mings (eds.). Berlin: Springer, 2008; pp: 113-143. doi:10.1007/978-0-387-71219-2_5

[28] Espinosa-Huerta, E., Quintero-Jiménez A., Cabrera-Becerra K. V., and Mora-

Avilés, M. A., 2013, Stable and efficient *Agrobacterium tumefaciens* mediated transformation of *Phaseolus vulgaris*. *Agrociencia, 47*:319-333.