EVALUATION OF TWO BRASSINOSTEROID ANALOGUES ON CALLUS FORMATION AND SHOOT INDUCTION IN SWEET POTATO (*Ipomoea batatas* (L.) Lam.)

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ABSTRACT. Leaf explants were put in Murashige and Skoog medium with growth regulators 2,4-D (0.01 mg.L^{-1}), 6-BAP (0.2 mg.L^{-1}) and brassinosteroid analogues Biobras-6 (BB-6) or MH-5 at different concentrations (0.001, 0.01, 0.05 and 0.1 mg.L^{-1}) and the brassinosteroid analogues used only or combined with 2,4-D in the medium for callus induction: Callus percentage was evaluated after four weeks. For studying the influence of selected analogues on shoot induction, four-week-old calli from control medium (2,4-D + 6-BAP) were used and transferred to media containing BB-6 or MH-5, either alone or combined with IAA (0.05 mg.L^{-1}) or with IAA (0.05 mg.L^{-1}) and Kinetine (0.2 mg.L^{-1}). Calli were kept in incubation chamber under sunlight conditions, at the temperature of 25 ± 2^0C, 80-90 % relative humidity and 72-90 µmol.m^{-2}.S^{-1} light intensity. After four weeks, different quantitative and qualitative aspects were evaluated in calli and shoots obtained. The best results were recorded when using growth regulators 2,4-D (0.1 mg.L^{-1}) and 6-BAP (0.2 mg.L^{-1}) alone in the medium, as well as combined with BB-6 or MH-5 brassinosteroid analogues at 0.01 mg.L^{-1} concentration. Shoot induction was favored, in general, when brassinosteroid analogues were combined with IAA (0.05 mg.L^{-1}) and Kinetine (0.2 mg.L^{-1}), compared to separate analogues in the culture medium.

Key words: callus, *1pomoea batatas*, regeneration, brassinosteroid

RESUMEN. Se seleccionaron explantes del limbo foliar y se sembraron en el medio de cultivo de Murashige y Skoog con 2,4-D (0.1 mg.L^{-1}) y 6-BAP (0.2 mg.L^{-1}), combinados con los análogos de brasinoesteroides Biobras-6 (BB-6) o MH-5 a diferentes concentraciones (0.001, 0.01, 0.05 y 0.1 mg.L^{-1}) y los análogos de brasinosteroides independientes o combinados con 2,4-D para inducir la formación de callos. Después de cuatro semanas se evaluó el porcentaje de callos formados. Para estudiar la influencia de los análogos seleccionados en la inducción de regenerantes, se utilizaron callos de cuatro semanas obtenidos en el medio control (2,4-D + 6-BAP) y se transfirieron a medios que contenían BB-6 o MH-5 solamente o en combinación con AIA (0.05 mg.L^{-1}) o con AIA (0.05 mg.L^{-1}) y Kinetina (0.2 mg.L^{-1}). Los callos se mantuvieron en cámara de incubación en condiciones de luz solar a la temperatura de 25 ± 2°C, humedad relativa de 80-90 % e intensidad luminosa de 72-90 µmol.m^{-2}.S^{-1}. Transcurridas cuatro semanas, se realizaron mediciones cuantitativas y cualitativas a los callos y los regenerantes procedentes de los callos obtenidos. Se lograron buenos resultados en la formación de callos morfogénicos, al emplear 2,4-D (0.1 mg.L^{-1}) y 6-BAP (0.2 mg.L^{-1}) como únicos reguladores del crecimiento en el medio de cultivo y al ser combinados con los análogos de brasinoesteroides BB-6 o MH-5 a la concentración de 0.01 mg.L^{-1}. La inducción de regenerantes se vio favorecida, de manera general, con el empleo del análogo de brasinoesteroides combinado con AIA (0.05 mg.L^{-1}) y Kinetina (0.2 mg.L^{-1}), en comparación con los análogos independientes en el medio de cultivo.

Palabras clave: callo, *Ipomoea batatas*, regeneración vegetal, brasinoesteroides

INTRODUCTION

Sweet potato is an economically profitable crop to many countries, especially Cuba, for human and animal nutrition, as well as in industry. The consumption of its roots and leaves by humans and animals, respectively, is the most important use of such crop.

Current difficulties, in this regard, are related to low yields in some regions, due to phytosanitary problems, pest and disease attack, as well as poor availability of seeding material. Therefore, it is necessary to find some efficient ways for obtaining new superior clones to those

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currently employed. For this purpose, *in vitro* techniques are recommended, since they have been a powerful tool in trade exploitation and have favored micropropagation of different species. In this sense, available results indicate that micropropagation will substitute conventional multiplication systems, bringing about the necessity of studying callus formation and shoot induction of sweet potato, using an appropriate culture medium for achieving an efficient process of indirect organogenesis. Growth regulators constitute one of the key and more expensive elements used for *in vitro* propagation. That is why, they must be optimized or substituted for more efficient and cheaper bioregulators (1, 2).

There are reports on the application of brassinosteroids and their analogues to different crops for obtaining morphogenic calluses, as well as plant regeneration and embryogenic callus formation, where a well-marked activity of *in vitro* processes was observed, opening new prospects for Cuban agriculture (3). Such bioregulators are effective in morphogenetic processes of different species and, differently from traditional growth regulators, the best results are recorded when the lowest concentrations of the product are used, allowing a reduction in costs of the culture medium (2, 4, 5).

These compounds perform physiological functions in plant growth and development (3), which suggests that they could play an important role in regulating regenerative growth. Previous studies (6) have shown that responses to brassinosteroids include its effects on cell elongation, division, vascular and reproductive development, membrane polarization and proton pumping, source-sink relationships, as well as on stress modulation.

Brassinosteroid promoting effects on cell elongation of vegetable tissues have been observed in many species (7), from which only a reduced group has been studied in detail, in order to determine the role of such effects in cell culture. On the other hand, regulation of gene expression by brassinosteroids has been studied (8) whereas, recently, good results have been reported by applying different brassinosteroids to various crops (9, 10).

Therefore, the objective of this work was to evaluate the effect of two brassinosteroid analogues, as growth regulators, on callus formation and shoot induction in different sweet potato clones.

MATERIALS AND METHODS

The present work was carried out in the "Center for Vegetable Biotechnological Studies", at the University of Granma, in collaboration with the National Institute of Agricultural Sciences. For this purpose, tuberous roots from Censa 78-354, Invit B 90-1, Invit B 93-1, Yabú-8 and Jewel clones were collected from seed banks, taking into account their health and size uniformity. Afterwards, they were placed in glass flasks containing water under semicontrolled lab conditions and their shoots were cut for selecting leaf limb explants, keeping them at a temperature of 27 ± 2^0C, 75-80 % relative humidity and 80-95 ìmol.m^{-2}.S^{-1} light intensity.

Experiment 1: Callus formation by using 2,4-D, 6-BAP and brassinosteroids. For evaluating effectiveness in morphogenic callus formation, two brassinosteroid analogues were employed: BB-6 or MH-5 (0.001, 0.01, 0.05 and 0.1 mg.L^{-1}), synthesized in the "Faculty of Chemistry", at the University of Havana. Such analogues were used as only growth regulators, combined with 2,4-D (0.1 mg.L^{-1}) or with 2,4-D (0.1 mg.L^{-1}) + 6-BAP (0.2 mg.L^{-1}) in a MS culture medium containing mioinositol (100 mg.L^{-1}), sucrose (3 %) and phytagel (0.25 %). In all cases, pH was adjusted to 5.8 ± 0.01 before adding the solidifying agent. A control treatment with traditional regulators was used. 1cm^2 explant per flask containing 20 mL of medium were placed and 25 flasks per treatment were used. Culture was placed in sunlight aseptic chambers, at a temperature of 25± 2^0C, 80-90 % relative humidity and 72-90 ìmol.m^{-2}.S^{-1} light intensity.

After four weeks, the following evaluations were performed: percentage of formed calli, callus development (according to Santana's scale (11)), color and strength. Proportion-comparing analysis was applied to calli belonging to the third degree on the scale.

Experiment 2: Effect of brassinosteroids, kinetine and IAA on shoot induction. A second experiment was carried out, with the objective of evaluating the effect of brassinosteroid analogues on shoot induction. In this case, calli obtained from Murashige and Skoog basal medium, supplemented with 2,4-D (0.1 mg.L^{-1}) and 6-BAP (0.2 mg.L^{-1}), were transferred to media containing the formerly mentioned brassinosteroids alone, using the same concentrations as in the previous experiment, or combined either with IAA (0.05 mg.L^{-1}) or with IAA (0.05 mg.L^{-1}) + kinetine (0.2 mg.L^{-1}). A control treatment containing growth regulators alone was applied using such concentrations. After four weeks, 15 calluses per treatment were evaluated, taking into account the following indicators: percentage of calli with shoots, number of shoots per callus and percentage of calli with roots.

A proportion-comparing analysis was applied to the first and third indicators; whereas a one-way classification variance analysis was applied to the second one, using the fixed effect model. In cases where significant differences were found, Duncan's Multiple Range Test was applied at 5 %.

RESULTS AND DISCUSSION

Experiment 1: Callus formation by using 2,4-D, 6-BAP and brassinosteroids. When using brassinosteroid analogues as growth regulators independently, no results on morphogenic callus formation belonging to the second and third degree of the scale used were obtained. Only with 0.01 mg.L^{-1} and 0.05 mg.L^{-1} BB-6, cicatrization callus at the edges of explant (first degree) was obtained in some clones. Morphological changes were neither observed and the explant kept its green color.

As it is seen in Table I, when BB-6 or MH-5 brassinosteroids were combined with 2,4-D (0.1 mg.L^{-1}) in culture medium, a better behavior was observed than when they were added as only growth regulators. The highest values (88.0 %) were reached when using 0.01 mg.L^{-1} of either brassinosteroid, indicating the highest stimulation of callus formation when analogues were combined with 2,4-D in the culture medium. When BB-6 or MH-5 were used at a concentration of 0.01 mg.L^{-1}, combined with 2,4-D and 6-BAP, 100 % callus formation was achieved in all clones and no significant differences were found with the control (2,4-D and 6-BAP), in which high values of morphogenic callus formation were obtained.

When performing the qualitative analysis of calluses, all treatments presented similar morphological features and third-degree morphogenic calluses were cream yellow, nodular and friable. Calli placed in media with BB-6 or MH-5 were higher than those in the medium containing 2,4-D and 6-BAP alone, which could be associated with brassinosteroid effect on cell elongation. It should be highlighted the fact that, when using brassinosteroid analogues as only growth regulators in the culture medium, roots appeared in some cases, even when callus reached the first degree only. This coincided with other authors (2), who affirmed that brassinosteroids or their analogues in the culture medium usually include the control of *in vitro* root induction.

In previous works on potato callus formation (12), different concentrations (0.25, 0.1, 0.01, 0.001 mg.L^{-1}) of BB-6 or MH-5 analogues combined with 2,4-D were studied and a favorable effect was observed on callus growth, development and quality; BB-6 standing out at a concentration of 0.01 mg.L^{-1}, as well as MH-5 at 0.001 mg.L^{-1}. There was no response when analogues were used as auxin substitutes.

When MH-5 behavior was studied in culture medium for coffee callus formation (13, 14, 15), it was stated that when brassinosteroid analogues were added to the medium, a strong biological activity in callus formation was recorded, increasing fresh callus weight under extreme conditions of hormonal imbalance. Changes in auxin levels, caused by brassinosteroids and their analogues, could amplify the final effect of auxin-cytokinin relationship on vegetable tissues. However, according to other authors (16, 17, 18), brassinosteroids combined with auxins could achieve more effective callus growth than auxins and cytokinins.

On the other hand, previous works (9) on sugar cane callus recovery, submitted to water and salt stresses, have shown that callus growth was favored when they were placed in a culture medium supplemented with BB-6, as a substitute for cytokinin, revealing antistress potentialities of such analogue.

Experiment II: Effect of brassinosteroids, kinetine and IAA on shoot induction (Tables II and III). In general, the highest capacity was manifested when BB-6 or MH-5 brassinosteroid analogues were used independently in the culture medium at 0.01 mg.L^{-1} in all clones evaluated, the control being superior in most cases. It should be highlighted that calli with roots were abundant and big in most cases, probably because of the brassinosteroid analogue used, which favored root emission in all clones, as well as in most concentrations evaluated, they being lower at superior concentrations to 0.05 mg.L^{-1}.

After evaluating BB-6 analogue combined with IAA (Table IV), it was found that the lowest concentrations favored shoot induction per callus, but they never surpassed the control, in which far superior values were obtained. Calluses with roots appeared at all concentrations when BB-6 was combined with IAA. It seems that, when combined with BB-6 brassinosteroid analogue, auxin concentration favored root emission, they being more abundant at the lowest concentrations of brassinosteroid analogues.

Table I. Results of morphogenic callus formation (%) by using 2,4-D, 6-BAP and brassinosteroids

Clones	BB-6 (mg.L^{-1}) combined with 2,4-D (0.1 mg.L^{-1}) and 6-BAP (0.2 mg.L^{-1})								
	0.001 2,4-D	0.00l 2,4-D 6-BAP	0.01 2,4-D	0.01 2,4-D 6-BAP	0.05 2,4-D	0.05 2,4-D 6-BAP	0.1 2,4-D	0.1 2,4-D 6-BAP	2,4-D 6-BAP
Cemsa 78-354	20.0 d	32.0 c	76.0 b	100 a	36.0 c	40.0 c	16.0 d	20.0 d	92.0 a
Inivit B 90-1	12.0 d	36.0 c	84.0 b	100 a	20.0 d	24.0 cd	16.0 d	24.0 cd	100 a
Inivit B 93-1	16.0 d	36.0 c	88.0 b	100 a	12.0 d	20.0 d	12.0 d	12.0 d	100 a
Yabú-8	0 e	8.0 d	72.0 b	100 a	16.0 cd	20.0 c	0 e	12.0 cd	92.0 a
Jewel	0 e	12.0 cd	80.0 b	100 a	0 e	12.0 cd	0 e	4.0 d	100 a
Clones	MH-5 (mg.L^{-1}) combined with 2,4-D (0.1 mg.L^{-1}) and 6-BAP (0.2 mg.L^{-1})								
	0.001 2,4-D	0.00l 2,4-D 6-BAP	0.01 2,4-D	0.01 2,4-D 6-BAP	0.05 2,4-D	0.05 2,4-D 6-BAP	0.1 2,4-D	0.1 2,4-D 6-BAP	2,4-D 6-BAP
Cemsa 78-354	20.0 e	28.0 d	80.0 b	100 a	40.0 c	48.0 c	24.0 de	28.0 d	92.0 a
Inivit B 90-1	16.0 de	20.0 d	80.0 b	100 a	12.0 e	16.0 de	20.0 d	32.0 c	100 a
Inivit B 93-1	16.0 e	24.0 d	84.0 b	100 a	12.0 e	20.0 de	20.0 de	36.0 c	100 a
Yabú-8	0 e	8.0 d	68.0 b	100 a	0 e	0 e	0 e	16.0 c	92.0 a
Jewel	0 e	16.0 c	76.0 b	100 a	0 e	0 e	0 e	8.0 d	100 a

Key for all tables: Callus inducing medium: 2,4-D (0.1 mg.L^{-1}) and 6-BAP (0.2 mg.L^{-1}). (*means with common letters do not differ significantly at 0.05%) Santana's scale (1982). Means are followed by the same letter in lines (Table I) and in columns (Tables II, III, IV, V, VI and VII)

Table II. Shoot induction (%) from calli using BB-6 brassinosteroid analogue as the only growth regulator in culture medium

Clones	Concentration ($mg.L^{-1}$)			Calluses with shoots	Shoots per callus	Calluses with roots
	BB-6	AIA	Kin.	(%)	(X)	(%)
Cemsa 78-354	0.001	0	0	26.7 ef	1.8 e	26.7 e
	0.01	0	0	66.7 bc	1.6 e	66.7 c
	0.05	0	0	0 g	0 g	93.3 ab
	0.1	0	0	0 g	0 g	33.3 de
	0	0.05	0.2	93.3 a	3.0 ab	100 a
Inivit B 90-1	0.001	0	0	73.3 abc	1.8 e	53.3 cd
	0.01	0	0	93.3 a	2.0 de	73.3 bc
	0.05	0	0	0 g	0 g	73.3 bc
	0.1	0	0	0 g	0 g	66.7 c
	0	0.05	0.2	100 a	4.0 a	13.3 ef
Inivit B 93-1	0.001	0	0	53. 3 cd	1.9 de	53.3 cd
	0.01	0	0	66.7 bc	2.3 cd	66.7 c
	0.05	0	0	13.3 fg	1.0 f	0 f
	0.1	0	0	0 g	0 g	0 f
	0	0.05	0.2	93.3 a	3.0 ab	6.7 ef
Yabú-8	0.001	0	0	26.7 ef	1.8 e	40.0 de
	0.01	0	0	66.7 bc	2.6 bc	100 a
	0.05	0	0	0 g	0 g	13.3 ef
	0.1	0	0	0 g	0 g	0 f
	0	0.05	0.2	86.7 ab	2.0 de	13.3 ef
Jewel	0.001	0	0	40.0 de	3.0 ab	53.3 cd
	0.01	0	0	86.7 ab	3.1 a	100 a
	0.05	0	0	0 g	0 g	100 a
	0.1	0	0	0 g	0 g	40.0 de
	0	0.05	0.2	100 a	4.0 a	0 f
	VC= 4.77					
	SE= 0.140					

Table III. Shoot induction (%) from calli using MH-5 brassinosteroid analogue as the only growth regulator in culture medium

Clones	Concentration ($mg.L^{-1}$)			Calluses with shoots	Shoots per callus	Calluses with roots
	MH-5	AIA	Kin.	(%)	(X)	(%)
Cemsa 78-354	0.001	0	0	0 d	0 g	26.7 fg
	0.01	0	0	60.0 ab	1.8 e	100 a
	0.05	0	0	60.0 ab	2.0 de	80.0 ab
	0.1	0	0	0 d	0 g	53.3 cde
	0	0.05	0.2	93.3 a	3.0 ab	100 a
Inivit B 90-1	0.001	0	0	33.3 c	1.0 f	66.7 bc
	0.01	0	0	66.7 a	2.3 cd	66.7 bc
	0.05	0	0	13.3 cd	1.0 f	20.0 fgh
	0.1	0	0	0 d	0 g	0 h
	0	0.05	0.2	100 a	4.0 a	13.3 ef
Inivit B 93-1	0.001	0	0	13. 3 cd	1.2 f	40.0 def
	0.01	0	0	60.0 ab	2.6 bc	60.0 bcd
	0.05	0	0	33.3 c	1.6 e	53.3 cde
	0.1	0	0	0 d	0 g	0 h
	0	0.05	0.2	93.3 a	3.0 ab	6.7 f
Yabú-8	0.001	0	0	0 d	0 g	33.3 efg
	0.01	0	0	80.0 a	2.8 b	26.7 fg
	0.05	0	0	20.0 cd	1.9 de	40.0 def
	0.1	0	0	0 d	0 g	13.3 gh
	0	0.05	0.2	86.7 ab	2.0 de	13.3 ef
Jewel	0.001	0	0	40.0 bc	2.3 cd	66.7 bc
	0.01	0	0	73.3 a	3.0 a	100 a
	0.05	0	0	0 d	0 g	13.3 gh
	0.1	0	0	0 d	0 g	40.0 def
	0	0.05	0.2	100 a	4.0 a	0 f
	VC (%)= 4.19					
	SE= 0.130					

Table IV. Shoot induction (%) from calli using BB-6 brassinosteroid analogue combined with IAA in culture medium

Clones	Concentration (mg.L^{-1})			Calluses with shoots	Shoots per callus	Calluses with roots
	BBA-6	AIA	Kin.	(%)	(X)	(%)
Cemsa 78-354	0.001	0.05	0	13.3 ef	1.4 cd	100 a
	0.01	0.05	0	33.3 cde	3.0 a	100 a
	0.05	0.05	0	0 f	0 e	100 a
	0.1	0.05	0	0 f	0 e	66.7 bc
	0	0.05	0.2	93.3 a	3.0 ab	100 a
Inivit B 90-1	0.001	0.05	0	53.3 bc	1.8 bc	100 a
	0.01	0.05	0	66.7 ab	2.8 a	100 a
	0.05	0.05	0	0 f	0 e	80.0 ab
	0.1	0.05	0	0 f	0 e	53.3 cd
	0	0.05	0.2	100 a	4.0 a	13.3 ef
Inivit B 93-1	0.001	0.05	0	20.0 ef	1.3 cd	66.7 bc
	0.01	0.05	0	46.7 bcd	3.0 a	93.3 a
	0.05	0.05	0	0 f	0 e	53.3 cd
	0.1	0.05	0	0 f	0 e	33.3 de
	0	0.05	0.2	93.3 a	3.0 ab	6.7 f
Yabú-8	0.001	0.05	0	0 f	0 e	53.3 cd
	0.01	0.05	0	13.3 ef	1.9 b	66.7 bc
	0.05	0.05	0	0 f	0 e	40.0 de
	0.1	0.05	0	0 f	0 e	40.0 de
	0	0.05	0.2	86.7 ab	2.0 de	13.3 ef
Jewel	0.001	0.05	0	53.3 bc	3.0 a	80.0 ab
	0.01	0.05	0	80.0 a	3.0 a	100 a
	0.05	0.05	0	66.7 ab	1.1 d	33.3 de
	0.1	0.05	0	26.7 de	1.0 d	26.7 e
	0	0.05	0.2	100 a	4.0 a	0 f
	VC (%)= 4.37					
	SEx= 0.130					

On the other hand, response to shoot induction and number per callus was found at certain concentrations, when BB-6 was combined with IAA and kinetine (Table V), although efficiency was not superior to the one found by other researchers in previous experiments, where different regulators were used (2). The highest values in shoot induction were found in the medium without brassinosteroid analogues (control) and with 0.01 mg.L^{-1} BB-6. Genotype influenced *in vitro* response to brassinosteroid analogues, used independently or combined with traditional growth regulators.

Also, shoot and root induction occurred simultaneously, so that there were more favorable results when using low concentrations, and surpassed the control in some cases. This could be owing to the fact that, increasing brassinosteroid analogue concentrations in culture medium, simultaneous shoot and root emission is inhibited. When brassinosteroid analogue at 0.01 mg.L^{-1} was combined with IAA and kinetine, a superior shoot index per callus was obtained in relation to the remaining treatments, except for the control, root emission being abundant in all treatments evaluated.

In previous works, different concentrations of BB-6 brassinosteroid analogue were applied to culture medium, in order to prove its auxin effect on shoot growth, achieving its good development. Some authors (1, 3) suggested a possible brassinosteroid action as promoters of cell differentiation, although mechanisms by means of which this occurs are still unknown.

When MH-5 was combined with auxin, the best shoot emission was obtained with 0.01 mg.L^{-1} in most clones studied (Table VI). It should be highlighted that, although results are neither shown nor of great concern in the present study, roots were favored in almost all concentrations and clones studied, reaching values up to 100 % in some cases. The former issue indicates that root emission was favored when MH-5 was combined with auxin. Root responses to brassinosteroids are diverse and physiologically different from shoot responses (21).

When using MH-5 brassinosteroid analogue combined with IAA and kinetine, shoots and roots were obtained in all clones studied, as well as in most treatments evaluated (Table VII). Shoot induction was favored with most concentrations applied, starting from 0.01 mg.L^{-1} when combined with growth regulators, achieving similar results when the control medium was used. This indicates that there is a relationship in organogenic response starting from calluses, between brassinosteroid analogue, combined with auxin and cytokinin, and the genotype used. It is necessary to point out that brassinosteroid analogues are effective at *in vitro* morphogenic processes, having the advantage of obtaining the best results in their way of action, as well as in their effectiveness, when using low concentrations of the product (2, 4, 12, 14, 22).

Table V. Shoot induction (%) from calli, using BB-6 brassinosteroid analogue combined with IAA and kinetine

Clones	Concentration ($mg.L^{-1}$)			Calluses with shoots	Shoots per callus	Calluses with roots
	BBA-6	AIA	Kin.	(%)	(X)	(%)
Cemsa 78-354	0.001	0.05	0.2	26.7 ef	2.0 de	100 a
	0.01	0.05	0.2	66.7 bc	3.3 b	100 a
	0.05	0.05	0.2	0 g	0 h	33.3 efg
	0.1	0.05	0.2	0 g	0 h	13.3 gh
	0	0.05	0.2	93.3 a	4.0 a	100 a
Inivit B 90-1	0.001	0.05	0.2	20.0 ef	1.8 ef	60.0 bcd
	0.01	0.05	0.2	80.0 ab	3.4 b	100 a
	0.05	0.05	0.2	13.3 f	1.6 ef	53.3 cde
	0.1	0.05	0.2	0 g	0 h	26.7 fg
	0	0.05	0.2	100 a	3.0 bc	13.3 gh
Inivit B 93-1	0.001	0.05	0.2	53.3 cd	1.9 ef	80.0 b
	0.01	0.05	0.2	100 a	3.3 b	100 a
	0.05	0.05	0.2	26.7 ef	1.4 fg	13.3 gh
	0.1	0.05	0.2	26.7 ef	1.0 g	26.7 fg
	0	0.05	0.2	93.3 a	3.9 a	6.7 h
Yabú-8	0.001	0.05	0.2	26.7 ef	2.3 d	26.7 fg
	0.01	0.05	0.2	80.0 ab	4.1 a	66.7 bc
	0.05	0.05	0.2	13.3 f	1.0 g	0 i
	0.1	0.05	0.2	0 g	0 h	40.0 def
	0	0.05	0.2	86.7 ab	2.8 c	13.3 gh
Jewel	0.001	0.05	0.2	20.0 ef	3.0 bc	53.3 cde
	0.01	0.05	0.2	100 a	4.3 a	100 a
	0.05	0.05	0.2	80.0 ab	1.8 ef	40.0 def
	0.1	0.05	0.2	40.0 de	1.0 g	20.0 fgh
	0	0.05	0.2	100 a	4.0 a	0 I
	VC(%)= 2.85					
	SEx= 0.160					

Table VI. Shoot induction (%) from calli using MH-5 brassinosteroid analogue combined with IAA in culture medium

Clones	Concentration ($mg.L^{-1}$)			Calluses with shoots	Shoots per callus	Calluses with roots
	MH-5	AIA	Kin.	(%)	(X)	(%)
Cemsa 78-354	0.001	0.05	0	40.0 e	1.2 f	66.7 bc
	0.01	0.05	0	53.3 de	2.4 d	93.3 a
	0.05	0.05	0	0 f	0 g	100 a
	0.1	0.05	0	0 f	0 g	0 e
	0	0.05	0.2	93.3 a	3.0 ab	100 a
Inivit B 90-1	0.001	0.05	0	40.0 e	1.8 ef	100 a
	0.01	0.05	0	80.0 abc	3.0 c	100 a
	0.05	0.05	0	0 f	0 g	46.7 cd
	0.1	0.05	0	0 f	0 g	40.0 d
	0	0.05	0.2	100 a	4.0 a	13.3 ef
Inivit B 93-1	0.001	0.05	0	40.0 e	2.0 e	100 a
	0.01	0.05	0	86.7 ab	2.6 cd	100 a
	0.05	0.05	0	0 f	0 g	53.3 cd
	0.1	0.05	0	0 f	0 g	66.7 bc
	0	0.05	0.2	93.3 a	3.0 ab	6.7 f
Yabú-8	0.001	0.05	0	0 f	0 g	66.7 bc
	0.01	0.05	0	40.0 e	1.8 ef	86.7 ab
	0.05	0.05	0	0 f	0 g	100 a
	0.1	0.05	0	0 f	0 g	33.3 d
	0	0.05	0.2	86.7 ab	2.0 de	13.3 ef
Jewel	0.001	0.05	0	66.7 bcd	3.8 b	100 a
	0.01	0.05	0	93.3 a	4.0 a	100 a
	0.05	0.05	0	60.0 cde	1.1 f	93.3 a
	0.1	0.05	0	0 f	0 g	33.3 d
	0	0.05	0.2	100 a	4.0 a	0 f
	VC (%)0 4.44					
	SEx= 0.130					

Table VII. Short induction (%) from calli using MH-5 brassinosteroid analogue combined with IAA and kinetine

Clones	Concentration ($mg.L^{-1}$)			Calluses with shoots	Shoots per callus	Calluses with roots
	MH-5	AIA	Kin.	(%)	(X)	(%)
Cemsa 78-354	0.001	0.05	0.2	40.0 b	1.6 cd	80.0 abc
	0.01	0.05	0.2	80.0 ab	3.1 b	100 a
	0.05	0.05	0.2	20.0 cd	1.8 c	60.0 cde
	0.1	0.05	0.2	0 e	0 e	0 i
	0	0.05	0.2	93.3 a	3.0 b	100 a
Inivit B 90-1	0.001	0.05	0.2	13.3 d	1.8 c	66.7 bcd
	0.01	0.05	0.2	100 a	3.4 b	100 a
	0.05	0.05	0.2	13.3 f	1.3 cd	46.7 def
	0.1	0.05	0.2	20.0 cd	0 e	40.0 efg
	0	0.05	0.2	100 a	4.0 a	13.3 hi
Inivit B 93-1	0.001	0.05	0.2	46.7 b	2.0 c	46.7 def
	0.01	0.05	0.2	100 a	3.3 b	100 a
	0.05	0.05	0.2	40.0 b	1.0 de	60.0 cde
	0.1	0.05	0.2	0 e	0 e	53.3 de
	0	0.05	0.2	93.3 a	3.0 b	6.7 hi
Yabú-8	0.001	0.05	0.2	0 e	0 e	53.3 de
	0.01	0.05	0.2	86.7 ab	3.2 b	86.7 ab
	0.05	0.05	0.2	13.3 d	1.8 c	20.0 ghi
	0.1	0.05	0.2	0 e	0 e	0 i
	0	0.05	0.2	86.7 ab	2.0 c	13.3 hi
Jewel	0.001	0.05	0.2	86.7 ab	3.0 b	100 a
	0.01	0.05	0.2	100 a	3.4 b	100 a
	0.05	0.05	0.2	33.3 b	1.6 cd	66.7 bcd
	0.1	0.05	0.2	26.7 bc	1.0 de	26.7 fgh
	0	0.05	0.2	100 a	4.0 a	0 I
	VC(%)= 3.30					
	SEx= 0.170					

There are recent reports in Cuba on the application of BB-6 or MH-5 brassinosteroid analogues under field conditions (18) and, up to now, there is no evidence of its use on sweet potato tissue culture. Therefore, this is the first time that a detailed and integral study on morphogenic callus formation and sweet potato seedling regeneration is carried out, using brassinosteroid analogues in marketable sweet potato clones. In this sense, novel results have opened new possibilities in this crop, as well as in the use of brassinosteroid analogues as growth regulators. Good results have been also recorded in potato (*Solanum tuberosum* L.) callus differentiation, yam (*Dioscorea* sp.) *in vitro* tuber formation, conversion and adaptation of papaya (*Carica papaya* L.) plants from somatic embryos, as well as in potato vitroseedling adaptation (3). Analogues have been also used for evaluating weight increase of embryogenic calluses, from fertilized ovules of Cleopatra mandarin (*Citrus rehni Hort* e. Tan) to low concentrations (0.01 $mg.L^{-1}$) in culture medium, obtaining good results (23).

In a recent work (24), two brassinosteroid analogues (BB-6 and MH-5) were used in callus formation and lettuce plant regeneration, showing that combining 0.001 $mg.L^{-1}$ BB-6 or 0.01 $mg.L^{-1}$ MH-5 with 0.1 $mg.L^{-1}$ 6-BAP not only stimulated callus formation, but also plant regeneration. However, as it is seen in the present work, using such analogues as only growth regulators in the culture medium did not favor callus induction. These authors also reduced 6-BAP concentrations to 50 % and added BB-6 or MH-5; recording stimulation of callus formation but reduction in plant regeneration, which reveals the importance of cytokinin in the medium. The former issue confirms what other authors (7) have expressed, that cell auxin and cytokinin endogenous content is critical to determine brassinosteroid effect.

Results from these bioassays, related to the capacity of brassinosteroid analogues for activating metabolic and growth processes in vegetable tissues at very low concentrations, confirmed other results from several research works on different species (2, 19, 23, 24). This opens new prospects on their use as substitutes for growth regulators in the different biological processes (3).

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