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BIOBRAS-16 IMPROVES THE ACCLIMATIZATION OF SPANISH CARNATION (*DIANTHUS CARYOPHYLLUS* L.) PLANTS OBTAINED IN VITRO

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ABSTRACT

The Spanish carnation (*Dianthus caryophyllus* L.) is one of the most commercialized flowers worldwide. Its accelerated propagation is possible due to the utilization of micropropagation methods, especially if combined with the employment of different bioproducts, between them the brassinosteroid analogous, known as Biobras-16. However, up to the moment, there is a lack of references about the acclimatization of Spanish carnation plants, micropropagated with this brassinosteroid analogue. For this reason, in the present work we proposed the aim of determine the survival of plants of Spanish carnation acclimatized with the employment of Biobras-16. The plants were obtained from meristems, cultured in medium with different concentrations of Biobras-16 (10^{-1}; 10^{-2} and 10^{-3} mg·L^{-1}), and from them, two propagations were made by cuttings, without the addition of Biobras-16 but respecting the treatments of origin of the plants. The plants obtained from the second propagation, were acclimatized under semi controlled conditions, using two assays: the first one, without treatment with Biobras-16, and the second one, with the application to the leaves of a solution with the same concentrations of Biobras-16 used in each treatment of the meristems culture. In general, in the control treatments was obtained an intermediate survival of the plants of Spanish carnation, confirming it is a bounding and transcendental stage for the successful culmination of the micropropagation process. In the first assay, the plants survival overcame significantly to the control in the treatmentwhich employed 10^{-3} mg·L^{-1} of Biobras-16 during the micropropagation. In the second assay, in the treatments where the plants were sprayed with 10^{-1} and 10^{-3} mg·$L^{-1}$of brassinosteroid analogue, were obtained higher survival percentages than the obtained in the first assay. It was appreciated the feasibility of the use of the Biobras-16, because its use in the *in vitro* culture and its spraying during the acclimatization phase of the Spanish carnation vitroplants, it is capable to produce increases in the survival percentage between 12,5% and 17%, which increment the efficiency of the micropropagation process.

Indexingterms/Keywords

Adaptation; brassinosteroids; survival; carnation.

Academic Discipline And Sub-Disciplines

Biotechnology

SUBJECT CLASSIFICATION

Agriculture

TYPE (METHOD/APPROACH)

Experimental

INTRODUCTION

The Spanish carnation (*Dianthus caryophyllus* L.), is one of the cultures most commercialized in the sector of the flower growing worldwide (1), even, in Latin-American countries as Colombia, constitutes one of the principal exportable items (2). From the point of view of the genetic improvement, it presents a great importance for the obtaining of new varieties that overcome the traditional ones in relation to different characters as the coloration, the aroma and the freightage of the flowers. In addition, it constitutes a species widely used in physiological studies, specially recounted to the floral ageing. Therefore, the availability of effective methods of propagation and conservation of the Spanish carnation with the employment of technologies of *in vitro* culture, possesses great interest for different purposes (3, 4).

The methods of micropropagation allow the rapid production of uniform vegetable material, with high quality and free of diseases, independently of the season of the year and the climatic factors. However, one of his major bounding is constituted by the high death rates that present the plants on having been transferred from the laboratory conditions towards the natural conditions, stage known as acclimatization (5). The plants are exposed to conditions different from which there were coming *in vitro*, which can be a reason of abiotic stress (temperature, luminous intensity, humidity) and biotic stress (microorganisms of the soil, interspecific or intraspecific competition, parasites), for what they need to be acclimatized to achieve their establishment and survival (5).

The brassinosteroids, compounds of steroidal nature, are fitohormons that intervene in multiple functions, since: the stimulation of the growth and the cellular division; the differentiation of the xilem; the promotion of the growth of young tissues, particularly the meristems; the curvature of the leaves in the knots and the growth of the root (6). Its application in the agriculture is based on the possibility of increasing the crops, of stimulating the physiological processes in the plants and of allowing the growth of cultures under unfavorable conditions as: high salinity, drought or insufficient nutrients, so they can be called hormones of the stress (7).

In Cuba are produced analogous of brassinosteroids, for example the Biobras-16, which has been used with good results in different cultures as the tomato (*Solanumlycopersicum* L.) (7, 8), the lettuce (*Lactuca sativa* L.) (9) and the bean (*Phaseolus vulgaris* L.) (10), and even in ornamental species as the orchids (*Cattleya* spp. and *Guarianthe* spp.) (11). In different phases of the micropropagation has also been employed successfully this analogous, like in the multiplication and the rooting of the sugar cane (*Saccarumofficinarum* L.) (12), in the somatic embryogenesis of potato (*Solanumtuberosum* L.) (13) and in the acclimatization of banana (*Musa* spp.) (14) and macaw palm (*Acrocomiaaculeata* (Jacq.) Lodd. Ex Martius) (15).

On the other hand, although it have been made studies about the utilization of the Biobras-16 in the micropropagation of Spanish carnation as the only hormonal supplement and as substitute of other fitohormones (16, 17), up to the moment do not exist references on the acclimatization of plants micropropagated with this analogous of brassinosteroid, for what in the present work we proposed as aim, determine the behavior of plants of Spanish carnation acclimatized with the employment of Biobras-16.

MATERIAL AND METHODS

VegetalMaterial

Plants of Spanish carnation (*Dianthus caryophyllus* L.) of two months of age, previously obtained *in vitro* from the germination of seeds, were taken as donors of meristems of 0,1 cm of length (Figure 1), which were sowed in the MS (Murashige-Skoog, 1962) culture medium (18) with sucrose 30 g· L^{-1}, Indol-3 Acetic Acid (IAA) 1.5 mg·L^{-1}, Gibberelic Acid (GA_3) 1 mg·L^{-1} and Kinetin (KIN) 0.8 mg·L^{-1} and Gelrite 2 g·L^{-1} (Control medium).

Figure 1.Culture of meristems of *Dianthus caryophyllus* L. previous to the propagation by cuttings

Meristems were also sowed in three variants of this medium, substituting the KIN for different concentrations of Biobras-16: 10^{-1} mg·L^{-1} (treatment 1); 10^{-2} mg·L^{-1} (treatment 2) and 10^{-3} mg·L^{-1} (treatment 3). At the three months of the establishment of the meristems culture, it was taken all the cuttings from the plants obtained in each variant of culture medium, and it was sowed in the Control medium culture, but keeping the treatments from which they were coming. Three months later, this plants were propagated by cuttings, and it was considered the second propagation (Figure 2) (17).

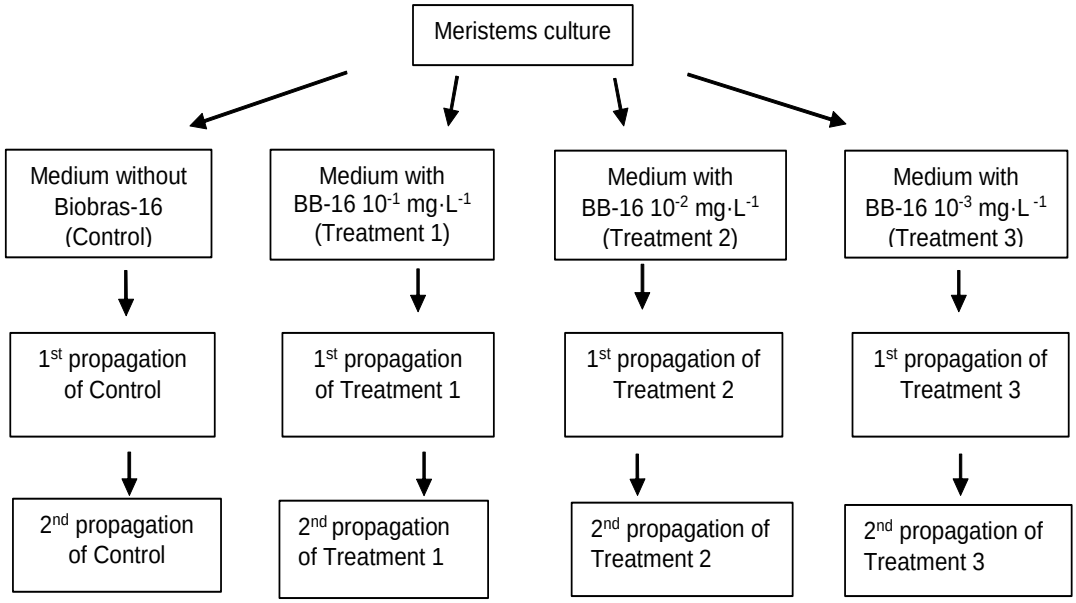

Figure 2. Diagram of the meristems culture and the propagation by cuttings of Spanish carnation (*Dianthus caryophyllus* L.) for the studied conditions. BB-16 means Biobras-16.

Tests of acclimatization

For this study were took plants from the second propagation, when they had 45 days of age and presented five knots, and they were submitted to a wash of the roots with distilled water to eliminate the remains of the culture medium. The plants were sowed in semi-controlled conditions, under a shelter, to an environmental temperature of 30 ± 2 °C, during the months of July and August, with daily irrigation. The containers used were bags of polyethylene with a mixture of organic matter (sloth) and Compacted Ferralitic Red Soil (19), in a relation 1: 2 v/v, and the plants were placed in conditions of humid chamber during the first seven days to diminish the perspiration.

During the stage of acclimatization were carried out two tests:

Test No. 1: Of each treatment were taken 15 plants and were sowed in the conditions previously mentioned, respecting the treatments from which they were coming in the *in vitro* conditions and without make foliar aspersion with the product.

Test No. 2: Of each treatment were taken 15 plants and were sowed respecting the treatments from which they were coming in the *in vitro* conditions, so the plants coming from treatment 1 were sprayed with a solution of 10^{-1} mg·L^{-1} of Biobras-16; the plants coming from treatment 2 were sprayed with a solution of 10^{-2} mg·L^{-1} of Biobras-16 and the plants coming from treatment 3 were sprayed with a solution of 10^{-3} mg·L^{-1} of Biobras-16, ten days after the beginning of the acclimatization.

Statistical analysis

There were made three repetitions of each test, following a Completely Randomized Design. The morphological traits of vigor (good, regular or bad) and color (light green or dark green) were determined 10 days and 40 days after the beginning of acclimatization. At the 40 days it was determined the percentage of survival of the plants in every treatment of each test. For the survival percentage, it was made a Test of Comparison of Proportions using the program Statgraphics version 5.0 for Windows.

RESULTS AND DISCUSSION

In relation to the morphological traits, ten days after the beginning of the acclimatization the vitroplants of carnation of all the treatments from both tests, remained with a light green color and a regular vigor (Figure 3), which changed to dark green color and good vigor, as the time progressed. During the *in vitro* culture the plants grow under an environment with high relative humidity, low luminous intensity, constant temperature, scanty gaseous exchange and a medium rich in organic compounds; these conditions cause changes in the morphology and the physiology of the plants, which provoke that a part of they do not survive the transplant to the environmental conditions. Between this changes are the low functionality of the stomata, the fragility of the rooting system and the poor development of the foliar cuticle (20).

On the other hand, it is well known that vitroplants when are transferred from *in vitro* to *ex vitro* conditions, suffer stress because they go from a heterotrophic or mixotrophic condition, to an autotrophic one (21). High sucrose and salt

containing media, low light level and the carbon dioxide concentration in culture vessel are some of the important limiting factors among various physical micro environmental factors which influence photosynthesis of *in vitro* cultured plants (5). Besides, the plants which are supplemented with an excess of fitohormones, generally show abnormalities in its morphology and physiology and they are called vitrified or hiperhydric plants, a phenomenon very common in carnation (22, 23) that was not observed in the experiment.

Figure 3. Spanish carnation (*Dianthus caryophyllus* L.) plants of 10 days of acclimatization in Test 1, Control treatment.

During the phase of acclimatization of the plants of Spanish carnation, in the Control treatments (where it was not used the Biobras-16 during the micropropagation process), there were obtained percentages of survival that varied between 35 % (Figure 3) and 35,5 % (Table 1), being confirmed that this one is a bounding and transcendental stage for the successful culmination of the process of micropropagation (20). Other studies about the behavior of the Spanish carnation during acclimatization in a different stage of the year (from September to April) in Cuba, inform a survival of the 76 % of the plants with the application of a rooting stimulator product and under controlled conditions of illumination, temperature and relative humidity (24). Similar results were obtained in other study when the carnation plants were transferred to pots with a sterile substrate and covered with polystyrene plastic bags. The plants were kept under the same conditions of the culture room, and after 4 weeks were placed in the greenhouse, where the survival rate reached the 78 % (25).

Table 1. Survival of the vitroplants of Spanish carnation (*Dianthus caryophyllus* L.) previously micropropagated with Biobras-16, acclimatized without foliar aspersion of the analogous of brassinosteroid (Test No. 1) and with foliar aspersion of the analogous of brassinosteroid (Test No. 2).

Treatment	Survival (%)	
	Test No. 1	Test No. 2
Control	35 b	35,5 b
1	42,5 ab	50 a
2	40 b	42,5 b
3	47,5 a	52,5 a
$S_{\bar{X}}$	0,493*	0,514*

In the Test No. 1 (Table 1), the mayor value of survival was reached in treatment 3 (47,5 %), where was employed 10^{-3} $mg \cdot L^{-1}$ of Biobras-16 during the *in vitro* culture, and were detected statistical differences in relation to the rest of the treatments, so it can be inferred that at this concentration, this brassinosteroid analogue had a significant influence in the survival of the plants, achieving an increase up to the 12,5 % over the Control. Although in this Test was not directly used the Biobras-16 during the stage of acclimatization of the plants, it is considered that the result obtained is related to the activation of metabolic processes linked to anti-stress effects, due to the application of the analogue of brassinosteroid in the *in vitro* phase, and also reaffirms that this compounds act at very low concentrations, generally between 0,001- 0,1 ppm (6).

In the treatments 1 and 2 were obtained survival percentages (Table 1) which resulted to be statistically similar to the survival reached in the Control treatment and lower than the percentage reached in treatment 3. This treatments represent the plants obtained from the second propagation of the meristem culture, in the mediums where were employed the concentrations of 10^{-1} mg·L^{-1} and 10^{-2} mg·L^{-1} of Biobras-16, so it is possible that this concentrations employed during the *in vitro* culture haven´t been effective to give the plants an anti-stress effect during the acclimatization stage.

Some authors, when analyzed the effect of Biobras-16 on the growth of coffee plant (*Coffeaarabica* L.), determined that the imbibition of the seeds in a solution with a concentration of 10^{-1} mg·L^{-1}, caused a stimulation on the germination of the seeds, as well as a certain stimulation of the formation of the sixth pair of leaves, in later stages (26).

Results obtained in the acclimatization of macaw palm (*Acrocomiaaculeata* (Jacq.) Lodd. Ex Martius), with the employ of different concentrations of Biobras-16 during the *in vitro* germination, demonstrate that the concentrations of 10^{-2} and 10^{-3} mg·L^{-1} presented a behavior lower or similar to that of the control on the major number of variables associated with the acclimatization, unlike the concentration of 10^{-1} mg·L^{-1}, which had a positive effect on the acclimatized plants (15).

In the other hand, the favorable influence of the concentrations of 10^{-2} and 10^{-3} mg·L^{-1} of Biobras-16 in the tolerance to salt stress on rice (*Oriza sativa* L.) vitroplants was informed by other authors (27). In a first experiment, the plants were sowed in MS medium with NaCl 75 mM and Biobras-16 for 16 days, and in a second experiment, the plants were sowed in MS medium with Biobras-16 for 4 days and then they were sowed in MS medium with NaCl 75 mM for 14 days; in both cases, the Biobras-16 increased significantly the activity of some antioxidant enzymes.

In the test No. 2, is considered that the survival of the plants was superior to the survival obtained in Test No. 1 (Table I). In the treatment 1 (spray with 10^{-1} mg·L^{-1} of Biobras-16) it was reach a medium survival of 50 %, larger than the 42,5 % of survival obtained in the first treatment of test 1. This result could be attributed to anti-stress effects eject by Biobras-16 when it is sprayed over the carnation plants. In fact, some authors found anti-stress effects due to the diminish on the contents of free proline, in spray with the same concentration of Biobras-16 on banana plants previously propagated in vitro and submitted to thermic stress (28). This results coincide with other experiment in banana, in which during the acclimatization the major increases in the survival percentage of the plants, were produced after the spray with 10^{-1} mg·L^{-1} of Biobras-16, in detriment of the concentration of 10^{-2} mg·L^{-1}, which only produced discreet increases of this variable (29). This aspect also coincide with the results obtained in the present study with regard to the plants sprayed with 10^{-2} mg·L^{-1}, which were not favored with the effect of Biobras-16, because the survival values did not differ significantly from the control survival.

In a experiment with coffee, the sprayed of the leaves with 10^{-1} mg·L^{-1} of Biobras-16, increased significantly the growth, favored the hydric state and increased the pigments concentration, and it was probed that spray the leaves for one time during the experiment, was more effective than spray for several times and the immersion of the plants in this solution for different periods of time (29).

The major value of survival (52,5 %) was reach in the treatment 3, which consisted in the plants obtained from the second propagation and sprayed with a concentration of Biobras-16 identic to the used in this treatment for the meristems culture (10^{-3} mg·L^{-1}). This fact can be related to the activation of the brassinosteroids synthesis when they are exogenously applied in the plants and their influence in the survival and consequently the growth and development of the plants (14): in banana plants obtained *in vitro*, the root immersion for 15 minutes before planting and the foliar spraying with a brassinosteroid analogue in a concentration between 0.02 and 0.2 µmol·L^{-1}, 15 days after planting, increased plant survival by 11 % approximately compared to the control plants.

In both tests, it results interesting the fact that the increase in the survival of the plants was not a lineal increase, so in the plants of treatment 1 (coming from the second propagation by cuttings of the meristem culture in medium with 10^{-1} mg·L^{-1} of Biobras-16) and in the plants of treatment 3 (coming from the second propagation by cuttings of the meristems culture in medium with con 10^{-3} mg·L^{-1} of Biobras-16) -the higher and the lower concentrations used in this study- the increase in the survival was superior than the survival in the plants of treatment 2 (coming from the second propagation by cuttings of the meristem culture in medium with 10^{-2} mg·L^{-1} of Biobras-16), where it was employed an intermediate concentration of the brassinosteroid analogue.

This behavior should be related with the fact that each concentration of brassinosteroid analogue can eject a differentiated effect on the vegetable explant (29). For example, in an experiment where it was made the transplant of the forest specie *Robiniapseudoacacia* L., it was demonstrated that the immersion of the plants roots in brassinolid solutions and the foliar spray of the plants, increased significantly the survival at the concentrations of 0,2 and 0,3 mg·L^{-1}, while the survival at the concentrations of 0,1 mg·L^{-1} (10^{-1} mg·L^{-1}) and 0,4 mg·L^{-1} reached values statistically similar to the control (Li et al., 2008, quoted by Núñez, 2012(21)). The results obtain to the date, demonstrate that the effectiveness of the brassinosteroids and brassinosteroids analogues depends not only on its chemical structure, but also on the concentration, the methods and the moments of application (28).

At the light of our current knowledge, it is the first time that is reported the utilization of Biobras-16 for the acclimatization of Spanish carnation (*D. caryophyllus* L.). In a general way, it was appreciated the feasibility of the employment of this brassinosteroids analogue during the phase of acclimatization of the plants of Spanish carnation, since its employ during the *in vitro* culture and spray in this stage, is capable of produce an increase in the percentage of survival of the plants, which guarantees major efficiency in the process of micropropagation.

CONCLUSIONS

It was obtained an increase up to 12,5% in the survival of the Spanish carnation plants acclimatized with the employment of Biobras-16 during the micropropagation at the concentration of 10^{-3} mg•L^{-1}. It was also obtained an increase in the survival of the plants when the leaves were sprayed with the solution of Biobras-16 at the concentrations of 10^{-1}mg·L^{-1}(14,5%) and 10^{-3} mg·L^{-1}(17%) during the acclimatization.

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REFERENCES

[1] Abu-Qaoud, H. 2013.Adventitious shoot formation and plant regeneration from leaf explants of carnation (*Dianthus caryophyllus* L.). African Journal of Biotechnology. 12: 3244-3249.
[2] Soto, J. C. andFilgueira, J. J. 2012. Evaluation of the reproduction proficiency of carnation (*Dianthus caryophyllus* L.) hybrids and varieties as search of useful parentals for a breeding program. RevistaFacultad de CienciasBásicas. 8: 184-195.
[3] Casas, J. L., Olmos, E. andPiqueras, A. S. M. 2010. *In vitro* propagation of carnation (*Dianthus caryophyllus* L.). In: S. M. Jain y S.J. Ochatt (eds.). Protocols for In Vitro Propagation of Ornamental Plants, Methods in Molecular Biology. Humana Press, vol. 589.
[4] Mohammed, L.S.,Abdulhammed S. and Mohammed, A. 2014. Effect of different explant and different concentrations of plant growth regulators on micropropagation of *Dianthus caryophyllus* L.Journal of Agriculture and Veterinary Science.7: 1-9.
[5] Kumar, K. and Rao, I. U. 2012. Morphophysiological problems in acclimatization of micropropagated plants in - *ex vitro* conditions- a Review. Journal of Ornamental and Horticultural Plants. 2: 271-283.
[6] Núñez, M. 2012. Brasinoesteroides y sus análogos: aplicaciones prácticas en la agricultura. Ediciones INCA, San José de las Lajas, Mayabeque., 74 p. ISBN: 978-959-7023-59-3.
[7] Reyes, Y., Mazorra, L. M., Martínez, L. and Núñez, M. 2010. Efecto del análogo de brasinoesteroides (Biobras-16) en la germinación y el crecimiento inicial de las plantas de dos variedades de tomate en condiciones de estrés salino. Cultivos Tropicales. 31: 82-88.
[8] Ruiz, J., Tejeda, T., Terry, E. and Díaz, M. M. 2009. Aplicación de bioproductos a la producción ecológica de tomate. Cultivos Tropicales. 30: 60-64.
[9] Terry, E., Ruiz, J., Tejeda, T., Reynaldo, I. and Díaz, M. M. 2011. Respuesta del cultivo de la lechuga (*Lactuca sativa* L.) a la aplicación de diferentes productos bioactivos. Cultivos Tropicales. 32: 77-82.
[10] Rosabal, L., Martínez, M., Reyes, Y. and Núñez, M. 2013. Resultados preliminares del efecto de la aplicación de Biobras-16 en el cultivo del frijol (*Phaseolusvulgaris* L.). Cultivos Tropicales. 34: 71-75.
[11] Suárez, L. 2007. Efecto que ejercen las aspersiones foliares de una mezcla de oligogalacturónidos (Pectimorf) y la formulación a base de un análogo de brasinoesteroides (Biobras-16) en dos especies de orquídeas (*Cattleyaleuddemanniana*y*Guariantheskinneri*). CultivosTropicales.28: 87-91.
[12] De la Fe, C., Ortiz, R. and Jiménez, M. 1998. Aportes a la tecnología de micropropagación de la caña de azúcar en Cuba. II. Efecto de análogos de brasinoesteroides en la multiplicación, el enraizamiento y la adaptación de las vitroplantas. Cultivos Tropicales. 19: 45-48.
[13] Hernández, M. M., Moré, O. and Núñez, M. 1999. Empleo de análogos de brasinoesteroides en el cultivo *in vitro* de papa. Cultivos Tropicales. 20: 41-44.
[14] Izquierdo, H., González, M. C., Núñez, M.,Proenza, R. and Álvarez, I. 2012. Efectos de la aplicación de un análogo espirostánico de brasinoesteroides en vitroplantas de banano (*Musa*spp.) durante la fase de aclimatización. Cultivos Tropicales. 33: 71-76.
[15] Borcioni, E. and Bonato, R. R. 2012. Aplicação de análogo de brassinosteroide (Biobras 16®) sobre a germinação e crescimento*in vitro* de embriõeszigóticos e aclimatização de plântulas de bocaiuva. Ciencia Rural. 42: 270-275.
[16] Castilla, Y., Pinares, A., Xiqués, X., Pérez, D. and Torres, M. 2004. Efecto del Biobras-16 en la propagación *in vitro* del clavel español (*Dianthuscaryophyllus* L.). In: Taller de Biotecnología Vegetal (2004, 9 al 12 de noviembre: La Habana). Memories of the XIV Scientific Congress of INCA. La Habana, 2004. ISBN 959-7023-27-X.
[17] Castilla, Y. and González, M. E. 2011. Efecto de un análogo de brasinoesteroides en la propagación por esquejes de clavel español (*Dianthuscaryophyllus* L.) a partir de plantas obtenidas por cultivo de meristemos. Temas de Ciencia y Tecnología. 15: 31-36.
[18] Murashige, T. andSkoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology Plantarum. 15: 473-497.
[19] Hernández, A. 1999. Nueva versión de clasificación de los suelos de Cuba. Instituto de Suelos. AGRINFOR, Ministerio de la Agricultura, Ciudad de La Habana, Cuba. 64 p.URL: http://mst.ama.cu/id/eprint/615
[20] Agramonte, D., Jiménez, F. and Dita, M. A. 1998. Aclimatización. In: Pérez Ponce, J. N. Propagación y mejora genética de plantas por biotecnología. Santa Clara: Instituto de Biotecnología de las Plantas. 400 p.
[21] Nuñez, M. 2012. Brasinoesteroides y las respuestas de las plantas al estrés ambiental. INCA. ISBN: 978-959-7023-57-9.
[22] Kharrazi, M., Nemati, H., Tehranifar, A., Bagheri, A. andSharifi, A. 2011. *In vitro* culture of carnation (*Dianthus caryophyllus*L.) focusing on the problem of vitrification. Journal of Biological and Environmental Sciences. 5: 1-6.

[23] Lăpădătescu S., Petolescu C., Lazăr A. and Bălă M.2012. Influence of growth regulators on morphogenetic processes under *in vitro* condition.Journal of Horticulture, Forestry and Biotechnology. 16: 197-202.
[24] Montes, S., Ramírez, L., Hernández, M. M., Santana, N., Martínez, M. and Lara, D. 1997. Micropropagación de variedades de clavel (*Dianthuscaryophyllus L.* y*Dianthusplumarius* L.) mediante el cultivo de meristemos. Cultivos Tropicales. 18: 75-81.
[25] Bakrudeen, A.,Mohajer, S.,Elnaiem, E. M. and Taha, R. M. 2012. *In vitro* regeneration, acclimatization and antimicrobial studies of selected ornamental plants. In: Kumar, N. Plant Science. URL: <http://www.intechopen.com/books/plant-science/in-vitro-regeneration-acclimatization-and-antimicrobial-studies-of-selected-ornamental-plants>.
[26] Soto, F., Tejeda, T. and Núñez, M. 1997. Estudio preliminar sobre el uso de brasinoesteroides en cafetos. CultivosTropicales. 18: 52-54.
[27] Nuñez, M., Reyes, Y. and Martínez, M. 2013. Análogos de brasinoesteroides, nuevos productos para la agricultura. Editorial Académica Española. ISBN: 978-3-659-08256-6.
[28] González, J. L. 2005. Efecto de un análogo de brasinoesteroide sobre plántulas de FHIA-18 expuestas a un estrés térmico. InfoMusa. 14: 18-20.
[29] Núñez, M. 2003. Actividad biológica *in vitro* y potencialidades antiestrés de análogos de brasinoesteroides cubanos. Informe Final Proyecto MES. INCA.URL: <http://bivia.inca.edu.cu/document/pd/IFIE0310.pdf>.

Author' biography with Photo

YanelisCastilla Valdés was born in March, 1982, in La Habana city, Cuba. In 1999 she graduated from Bachiller studies and in the same year she started to study Biology in the Faculty of Biology in the Havana University. After her graduation, in 2004, she started working as a researcher in the National Institute of Agricultural Sciences (INCA), where she has worked in Projects of Biotechnology related to Micropropagation of Ornamental Plants and Conservation of Coffee. She has participated in national and international courses, events and trainings about different issues of the Plant Biotechnology. In 2009 she got the degree of Master in Science in Plant Biology, mention Plant Biotechnology in Havana University, with a thesis about the *in vitro* propagation of Spanish carnation (*Dianthus caryophyllus* L.) with the use of Biobras-16, an analogue of brassinosteroids. Nowadays she develops her PhD in INCA, in the thematic of the *in vitro* conservation and cryopreservation of coffee (*Coffea* spp.) genotypes by different methods.