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MORPHOLOGICAL AND AGRONOMIC RESPONSE IN GREENHOUSE THE *In Vitro* PLANTLETS OF TWO VARIETIES OF CUBAN POTATO

Respuesta morfoagronómica en casa de cultivo de plantas cultivadas *in vitro* de dos variedades de papa cubana

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ABSTRACT. The use of cuban potato varieties for the production of original seed by biotechnological methods help to reduce imports by the concept of buying seed. The present study was to determine the morphological and agronomic response of cultivated *in vitro* plantlets of two varieties of Cuban potato. Grettel and Yara varieties from breeding program of the National Institute of Agricultural Sciences (INCA) were used and planted in the greenhouse. The plant height and number of stems per plant was measured. Subsequently at the time of harvest the number, weight and size of the tubers per plant were quantified. Furthermore, the form of tubers and the skin color in each variety were evaluated. As a result, to produce of original seed of two Cuban varieties included in the national seed production program were gotten. The morphological differences between varieties of Cuban potato Yara and Grettel in greenhouse from *in vitro* plantlets were found. The Grettel variety had the highest number of tubers per plant but smaller calibre than Yara. These results allow to plan the production of original seed potatoes of two varieties by biotechnological methods.

Key words: seed, *Solanum tuberosum*, tubers

RESUMEN. El empleo de variedades de papa cubanas para la producción de semilla original mediante métodos biotecnológicos contribuye a disminuir las importaciones por el concepto de compra de semilla. El presente trabajo se realizó con el objetivo de determinar la respuesta morfológica y agronómica de plantas cultivadas *in vitro* en dos variedades de papa cubanas. Para ello se emplearon plantas de papa cultivadas *in vitro* de las variedades Grettel y Yara procedentes del programa de mejoramiento genético del Instituto Nacional de Ciencias Agrícolas (INCA), las cuales fueron plantadas en casa de cultivo. Se midió la altura de las plantas y el número de tallos por planta. Posteriormente en el momento de la cosecha se cuantificó el número, peso y calibre de los tubérculos por planta. Además se evaluó la forma de los tubérculos y el color de la piel en cada variedad. Como resultado se logró la producción de semilla original de dos variedades cubanas incluidas en el programa Nacional de producción de semilla. Se constataron diferencias en cuanto a la altura de las plantas (cm) y el número de entrenudos entre las dos variedades de papa en casa de cultivo a partir de plantas obtenidas *in vitro*. La variedad Grettel presentó el mayor número de minitubérculos por planta pero de menor calibre que los de la Yara. Estos resultados permiten planificar la producción de semilla original de papa de ambas variedades mediante métodos biotecnológicos.

Palabras clave: semilla, *Solanum tuberosum*, tubérculo

INTRODUCTION

Potato crop ranks the fourth worldwide place with a production of 325 million ton (1). This seed is one of the most important links of tuber production chain in Cuba. Considering its nutritional value and

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people's demand as an essential diet food, the seed is imported from Europe and Canada to our country (2).

In Latin America and several countries, *in vitro* grown plants, microtubers and minitubers are the basis for pre-basic or original seed production (3, 4).

The use of potato seeds derived from tissue culture enables to start producing tubers from healthy material. Besides, it promotes a quick multiplication within a short period of time and seed storage as well as its transportation is relatively easier, due to a small tuber size, so increasing international exchange (5).

Laboratory phases (pre-basic seed), greenhouse (basic) and field cycles are included in the process of certified seed production (6). Potato production in greenhouses is performed with the purpose of obtaining seeds free of pests and diseases, showing its potential in the field. Therefore, bigger tubers than 10 mm diameter are required, so that they can grow on the farm to ensure its emergence and more stems per meter, in order to achieve an adequate crop establishment and higher productivity (7).

Potato varieties grown in Cuba result from introducing and selecting foreign material adapted to our climatic conditions (8). Thus, in 1985, the National Institute of Agricultural Sciences (INCA) of Cuba began working on potato breeding through hybridization and selection, to obtain new varieties with high yields adapted to the climatic conditions of our country. Meanwhile, the Institute of Plant Biotechnology (IBP) was working on crop breeding by means of using biotechnological methods and *in vitro* mutagenesis, achieving mutants with promising field results (9), as well as enhancing research studies related to *in vitro* production and evaluating seed tubers in greenhouse and field (10).

However, regardless of the efforts, the production of potato seeds coming from Cuban varieties has not been reached on a large scale, due to the lack of seed tubers produced in Cuba. So, it is very important to consider the possibilities offered by biotechnological techniques to attain potato seeds of high genetic quality and plant protection. Consequently, this work was aimed at determining the morphological and agronomic response of *in vitro* grown plants from two Cuban potato varieties, Grettel and Yara (registered in the official list of commercial varieties), obtained at the breeding program of INCA, which were planted in a greenhouse.

MATERIALS AND METHODS

In vitro grown potato plants derived from Cuban varieties Grettel and Yara (obtained at the breeding program of INCA) were used; plants were established and *in vitro* multiplied according to a methodology proposed by IBP's researchers. Seeding was carried out within 2012-2013 season.

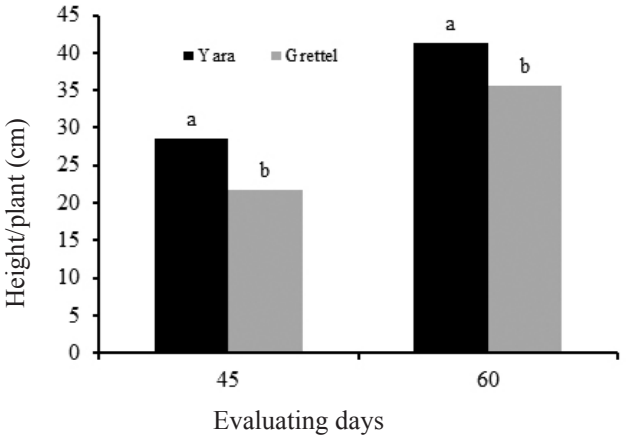
Previous to greenhouse seeding, plants were acclimatized for 14 days under semi-controlled environmental conditions with reduced light intensity to 60,0-70,0 %, using a saran mesh with 1,5x1,5 mm pore size and light intensity values of about 116 umol m^{-2} s^{-1}. Plants of both varieties were put in polyeturane containers of 247 alveoli of 32 mL capacity, with a substrate made up of 85 % filter cake (v/v) and 15 % zeolite, a basal dressing formula 10-13-21 and a dose of 2,5 kg m^{-3}, following the technical instruction for potato production in Cuba (11). After 15 days, plants were transferred to greenhouse beds containing 100 % filter cake using a complete fertilizer formula 9-13-17 at a rate of 0,67 t ha^{-1}. Transplanting was performed by hand to a 0,40x0,10 m plantation frame. A randomized complete design was used with two treatments (variety) and two repetitions at a rate of 525 plants/bed. Plants were irrigated by microsprinkler at a flow rate of 35 L h^{-1} with two pressure bars. Then, 15 microsprinklers were located per each bed with a standard irrigation of 20,46 m^3 per hour for 0,046 ha. Harvesting was manually done at 90 days.

The following variables were measured at 20, 45 and 60 days after bed plantation: stem length (cm) from its base up to the apical bud through a calculated ruler; the number of internodes and stems per plant. After 90 days, the number of minitubers per plant, weight (g) and size per plant were determined and classified as follows: size I (minitubers with smaller diameter than 21 mm), size II (minitubers with diameter between 22 and 36 mm), size III (minitubers with diameter between 37 and 45 mm) and size IV (minitubers with longer diameter than 45 mm). Tuber shape and skin color were evaluated according to a descriptor (12) in 60 plants per treatment for both morphological and agronomic evaluations performed.

Data collected on plant height, internode number, stem number per plant, tuber number and weight were compared by means of a nonparametric *Mann-Whitney* test for p<0,05, previously verifying the supposed variance normality and homogeneity using *SPSS* statistical package for Windows (13).

RESULTS AND DISCUSSION

Differences were recorded at the morphological response of Cuban potato varieties evaluated in a greenhouse after 45 and 60 days; however, no significant differences were observed after 20 days between both varieties, from the morphological viewpoint. The main differences were associated to plant height (cm) and internode number. The greatest values of plant height were registered in Yara with significant differences from Grettel (Figure 1). Such values attained after 45 days were similar to those described at *in vitro* grown plants of Desiree variety developed on zeolite substrate in a greenhouse (14). These authors were able to reach height values of 33,2-40,1 cm, depending on the treatments used.



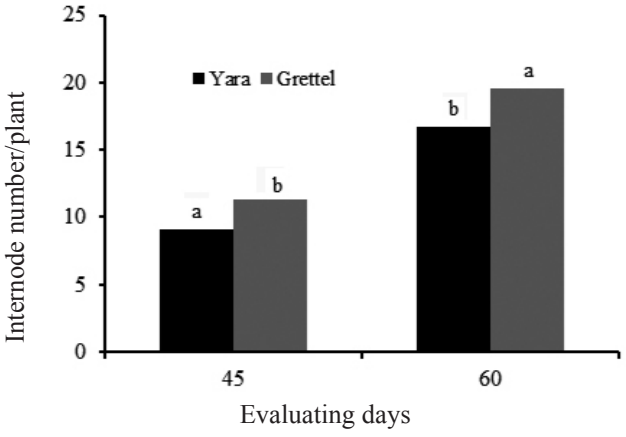
Bars with different letters per each evaluation differ significantly by nonparametric Mann Whitney test for p<0,05

Figure 1. Height of *in vitro* potato plants grown in a greenhouse

According to a report on these new varieties (15), Grettel can exceed stem height values of 45 cm and reach up to 60 cm. However, its values recorded at *in vitro* grown plants of this investigation did not surpass 40 cm. Meanwhile, Yara can attain a mean height of 50-60 cm (16), although its *in vitro* grown plants did not exceed 45 cm. These results could be related to temperatures recorded in the greenhouse during this period, which ranged between 12 and 28 °C in the morning, whereas in the afternoon, minimum temperatures reached values of 22 °C and maximum of 37 °C.

Such temperature rise has an accelerating effect on both chemical and often biological processes up to reaching an optimal value; in the case of potato crop, it is between 20 and 25 °C, after which a growth decrease is observed depending on temperature. This leads to a poor foliage formation that is not enough to completely capture the solar energy required for dry matter formation. Together with the effect on foliage growth and plant development is the effect of temperature on breathing, since its losses are proportionally greater. Therefore, net energy profits per unit of intercepted radiation are lower, so that the energy is less efficiently converted into dry matter (17).

Regarding internode number, Grettel was characterized by showing higher values with significant differences from Yara (Figure 2). Results of this study are consistent with those described in literature (18), as significant differences were found in comparative studies with minitubers from four potato varieties. These authors stated that this variable is an indicator of plant vigor, health and high yields under several environmental conditions.



Bars with different letters per each evaluation differ significantly by nonparametric Mann Whitney test for p<0,05

Figure 2. Internode number of *in vitro* potato plants grown in a greenhouse

Concerning stem number per plant, there were no significant differences between both varieties, either at 45 or 60 days. Values ranged from 1,20 for Yara to 1,45 for Grettel. The same authors reported similar values in terms of stem number per plant of four potato cultivars coming from minitubers with values of 1,38 to 1,72 (18).

In relation to minituber number per plant, Grettel showed the highest values with significant differences from Yara, its values ranging between three and six minitubers per plant respectively (Table). Scientific literature refers that it is possible to obtain from two to ten minitubers at *in vitro* grown plants, its size depending on plant density per m^2 (19). However, other authors argued that from two to five minitubers can be obtained at *in vitro* grown plants (20). For example, field studies with *in vitro* grown plants from Atlantic variety achieved 5,40 minitubers per plant (21). Nevertheless, other studies with Desiree variety reported values of 2,70 until 5,30 minitubers per plant in a greenhouse using elicitors (22).

Results of this study showed that Grettel yielded a greater minituber number than Yara, which could be related to the genetic characteristics of each variety. Breeders of Grettel have indicated 10-15 tubers per plant whereas those of Yara reported 8-10.

Table. Minituber number per plant of two Cuban potato varieties derived from *in vitro* grown plants in a greenhouse

Varieties	Minituber number per plant^{-1} (u)	Mean ranges
Yara	3,30±0,348 b	14,38±0,348 b
Grettel	6,68±0,790 a	26,63±0,790 a

Mean ranges with different letters differ significantly by nonparametric Mann Whitney test for p<0,05

When analyzing minituber weight per plant, it was found that both varieties showed similar values of 57,10 g for Yara and 5655 g for Grettel. However, when considering minituber size structure, Yara presented the highest percentage of sizes III (37-45 mm) and IV (>45 mm). Meanwhile Grettel presented sizes II and III, which fit to those established for seed production (Figure 3).

These results indicate that *in vitro* grown plants from Yara yielded 30 % minitubers with >45 mm size, which should be taken into account in seed production, considering plant spacing or harvesting time.

Seeds obtained from *in vitro* grown plants are determined by several factors, such as variety, irrigation, light and planting density, so it is necessary to perform these studies both in greenhouses and the field (23). In general, Yara presented fewer minitubers per plant with larger size, while Grettel presented more minitubers with smaller size.

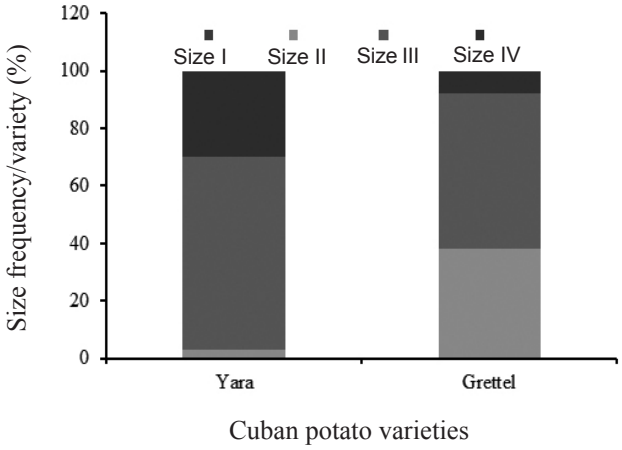


Figure 3. Size frequency of minitubers obtained from *in vitro* grown plants of two Cuban varieties, Yara and Grettel, in a greenhouse

In vitro grown plants coming from both Cuban varieties yielded oval-oblong shaped-minitubers, Grettel with yellowish skin whereas Yara with pink skin (Picture).

The use of biotechnological techniques has affected seed tuber production in Cuba, reducing production cycles with an adequate seed number and a high healthy level of basic plant material. On the other hand, these results enable to plan original seed production, as according to IBP's greenhouse capacity, a biofactory should produce 70,000 *in vitro* grown plants between both varieties to obtain 80,850 and 209,475 Yara and Grettel minitubers with sizes II and III.

Variety Yara

Variety Grettel

Minitubérculos obtenidos en casa de cultivo de dos variedades de papa cubanas

This amount of original seeds would enable to sow 2 ha of Yara and 5 ha of Grettel for obtaining basic seed and continue producing until reaching consumption and certified seed. Therefore, this paper is a contribution to seed production program taking place at IBP, by introducing and evaluating potatoes varieties obtained in the country, particularly at National Institute of Agricultural Sciences.

CONCLUSIONS

- Original seed production obtained from two Cuban varieties included at IBP's seed production program, derived from *in vitro* grown plants.
- Morphological and agronomic differences observed between two Cuban potato varieties, Yara and Grettel, in a greenhouse, derived from *in vitro* grown plants.
- Grettel variety showed the highest minituber number per plant with sizes between 22 and 45 mm adjusted to seed sizes.

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