Phenotypic evaluation of two transgenic potato varieties for control of *Tecia solanivora*

Evaluación fenotípica de dos variedades transgénicas de papa para el control de *Tecia solanivora*

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ABSTRACT

Eight genetically modified potato lines with *cry1Ac* gene from *Bacillus thuringiensis* of the Colombian varieties Diacol Capiro and Parda Pastusa that show high resistance to *Tecia solanivora* in the laboratory were evaluated under greenhouse conditions. The following agronomic traits were evaluated: plant height, growth habit, canopy structure, number of stems per plant, flowering, form and size of the mini-tubers, skin texture, skin color, pulp color, presence of abnormalities, and experimental performance. It was established that at least one of the transgenic lines studied has great potential for further study, bearing no morphological variability with respect to the unmodified control line.

Key words: *Solanum tuberosum*, *cry1Ac* gene, plant breeding, morphological differences, GM crops.

RESUMEN

Se evaluaron bajo condiciones de invernadero de bioseguridad, ocho líneas de papa transgénicas con el gen *cry1Ac* de *Bacillus thuringiensis* de las variedades colombianas Diacol Capiro y Parda Pastusa, una alta resistencia frente a *Tecia solanivora* a nivel de laboratorio, mediante el análisis de las siguientes variables agronómicas: altura de la planta, hábito de crecimiento, estructura del follaje, emergencia de floración, número de tallos por planta, forma y tamaño de los minitubérculos, profundidad de los ojos, textura de la piel, color de la piel, color de la pulpa, presencia de anormalidades y rendimiento experimental. Se estableció que al menos una de las líneas transgénicas estudiadas posee un gran potencial para posteriores estudios, al no presentar variabilidad morfológica con respecto a su línea control no modificada.

Palabras clave: *Solanum tuberosum*, gen *cry1Ac*, mejoramiento genético, diferencias morfológicas, cultivos GM.

Introduction

Guatemalan moth, *Tecia solanivora* (Lepidoptera: Gelechiidae), is one of the most harmful insects affecting potato (*Solanum tuberosum*). The plague originated in Central America from where it spread to South America in the late 80's and is currently present in the main potato regions of the country. The moth attacks the tuber precluding its use for both seed and human or animal consumption (Herrera, 1998).

The most common form of pest control is the application of chemical insecticides, however, its effectiveness is low due to the emergence of resistant insects, which cause an increase in the level of use such products (Villamizar *et al*., 2003). Moreover, the use of these chemicals creates pollution and environmental damage. In the long term it will be necessary to replace chemical controls for other control methods such as biological control, fumigation with active compounds of low residual toxicity or the introduction of GM varieties expressing resistance to pests (Villamizar *et al*., 2003).

One form of biological control of this pest is based on the use of *Bacillus thuringiensis* (Bt), a bacterium that has the capacity to produce CRY proteins, which are selectively toxic to insect cells (Maagd *et al*., 1999; Sansinenea, 2000; Schnepf *et al*., 1998), as well as to some nematodes and protozoa (Ely, 1996; Xu *et al*., 2004). *T. solanivora* is susceptible to commercial preparations of *Bt* but its effectiveness is reduced because the it feeds on internal tissues of the tuber (Martínez *et al*., 2003; Villanueva *et al*., 2009).

A tool provided within the integrated management program (IPM) is the use of insect resistant plants using genetic engineering. The introduction of *Bt* genes in crop species

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allows the expression of proteins in the inner tissues of plants and consequently the control of insects could spread to the entire growing season. Additionally, the insect population would be exposed only one that feeds on plants (Ely, 1996).

In previous studies, the Plant Biotechnology group (UNALMED-CIB) developed 36 transgenic lines of potato varieties: Diacol Capiro (11), Parda Pastusa (8) and Pan de Azucar (17), transformed with the *Bt* gene *cry1Ac*. These lines showed up to 100% mortality of *T. solanivora* larvae compared to control lines showing 0 and 8% under laboratory conditions. This was correlated with gene detection by PCR and the expression of Cry1Ac protein by DAS-ELISA (Valderrama *et al*., 2007), concluding that the transgenic lines obtained showed high resistance to insect in the laboratory.

Here we describe the evaluation of morphological characteristics of genetically modified potato lines, grown at a biosafety greenhouse scale complying with the technical and legal requirements, approved in resolution of the ICA 3723 of 30 October 2008. The results presented represent an advance in the development of lines for more efficient control of the Guatemalan potato moth.

Materials and methods

Plant material

Four lines of the variety Diacol Capiro (DC), coded: DC 40.5; DC 40.7a; DC 40.7b and DC 40.72 and four lines of the variety Parda Pastusa (PP): PP 28; PP 28.1; PP 40.a; and PP 40.3, as well as non-GM controls were used. These lines were obtained previously in the Plant Biotechnology Unit at UNALMED-CIB, and showed levels of 100% mortality in laboratory scale (Valderrama *et al*., 2007). The plants were propagated *in vitro*, under conditions of photoperiod light/dark 16/8 h, an average temperature of 19°C±2, relative humidity of 65%±3 and 3,000 lux of light intensity. Propagation medium Murashige and Skoog (MS) supplemented with an antibiotic selection as reported by Valderrama *et al*. (2007) was used. The plantlets were subcultured by transferring nodal segments to fresh medium every two months.

Hardening and culture under greenhouse conditions

Greenhouse phase was carried out taking into account the biosafety standards established by the Instituto Colombiano Agropecuario (ICA) for the management of genetically modified plants. The greenhouse is located in the municipality of el Carmen de Viboral (Antioquia), at an altitude of 2,100 m s.a.l., with an average temperature of 20°C±5 and an average relative humidity of 92%. Fifteen five weeks old *in vitro* plants were planted for each of the 10 lines tested at a planting distance of 20 cm, on beds of 8 m long, 60 cm wide and 40 cm deep, equipped with an automated drip irrigation. The substrate used to harden the material was composed of coal slag and burnt rice hulls, the substrate was chemically sterilized 15 d before planting.

Evaluation of phenotypic characteristics of plants and tubers

Agronomic evaluation was performed on the genetically modified lines. Minitubers both at the time of harvest (160 d), and live plants at 130 d after planting were evaluated on several characters considered as essential features in the identification of potato cultivars (Senasa, 2008). The characters evaluated were: plant height, growth habit, foliage structure, number of stems per plant, average diameter of the stems, time of emergence of flowering, for plants; and size, depth of eyes, skin texture, skin color, pulp color, presence of abnormalities (classification by transverse diameter in mm based on Resolution ICA 2501 of 2003) and experimental yields (grams of minituber per plant) for minitubers.

Data analysis

For analysis of quantitative morphological traits ANOVA test was performed, comparing each of the transgenic lines with non-GM control for each of the characters through the t-student test. All statistical analyzes were performed using SAS® v. 8.

Results and discussion

In this study, a morphological evaluation of genetically modified potato plants of varieties Parda Pastusa and Diacol Capiro was done to preliminarily evaluate the usefulness of these transgenic lines grown under confined greenhouse conditions following the existing regulations in Colombia for the management of these organisms. Some morphological differences were evident between the lines evaluated with a tendency to greater variability in the variety Diacol Capiro.

Differences in plant height were presented among varieties Diacol Capiro and Parda Pastusa (Fig. 1) with heights between 11.208 and 17.646 cm for variety Diacol Capiro (Fig. 2) and between 36.330 and 69.292 cm for Parda Pastusa variety (Tab. 1). A grater height was also observed in the untransformed control lines compared with the transgenic

lines for the two varieties. The number of stems per plant, for the variety Diacol Capiro ranged between 2.0 and 3.5. The lines DC 40.7b and DC 40.5 showed statistically significant differences compared to control (Fig. 2). For variety Parda Pastusa no significant differences between transgenic and control lines was observed, with values between 2.5 and 3.0 stems per plant (Fig. 2).

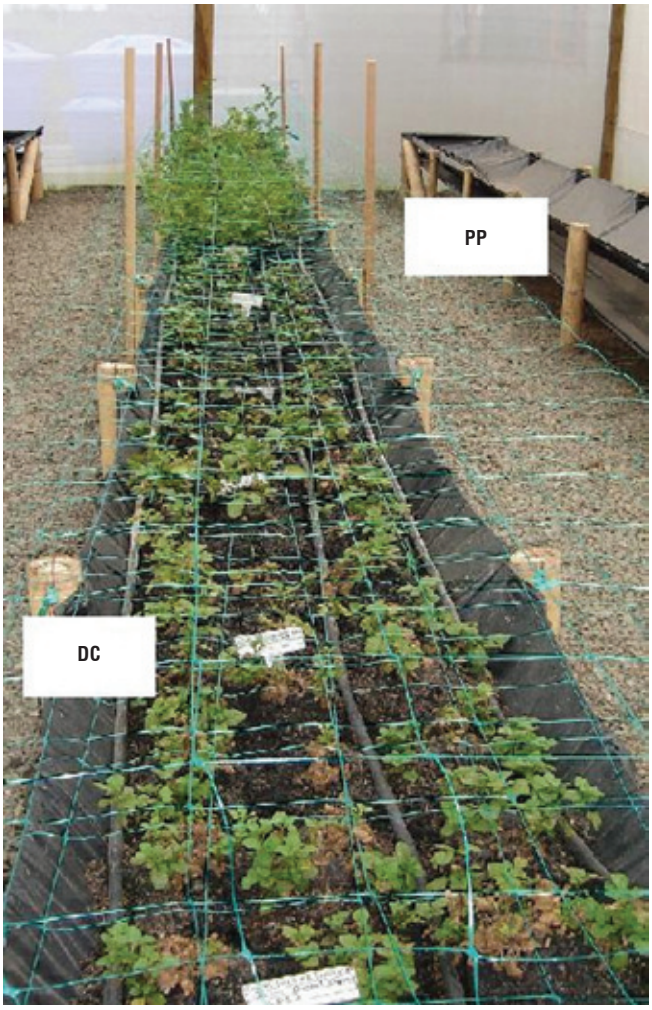

FIGURE 1. Transgenic potato plants varieties Diacol Capiro (DC) and Parda Pastusa (PP) planted under greenhouse conditions, with 130 d of culture.

The stem diameter in the variety Diacol Capiro was between 2.125 and 2.344 mm. None of the transgenic lines showed differences with respect to non-transgenic line (DC) (Fig. 2). In the variety Parda Pastusa the line PP 28 showed an average smaller stem diameter with 2.643 mm diameter and was the only line to present significant differences compared to the control line (PP) (Fig. 2). For parameters: growth habit, foliage structure and emergence of flowering there were no differences for any of the lines in each variety.

The varieties Diacol Capiro and Parda Pastusa showed a growth habit formed into rosettes and foliage structure was of branching type (open foliage, stems clearly visible). With regard to the emergence of flowering, this could not be measured because under greenhouse conditions it was not possible to obtain mature buds and flowers for any genetically modified lines or their respective controls.

Significant differences were observed in size, shape and performance within each of the transformed and untransformed lines. For variety Diacol Capiro tubers showed reduced size and in some cases irregular shapes (enlargement), something not common to the variety (Fig. 3, a to e). In contrast the variety Parda Pastusa did not present such irregularities in the shape of the tubers, them being much more homogeneous and larger (Fig. 3, f to j).

When comparing the productivity obtained for each of the transgenic lines and their respective non-GM controls (Tab. 2), we found that for the variety Diacol Capiro there was a noticeable difference in grams of tubers per plant. Three of the four transgenic lines (DC 40.7a; DC 40.7b and DC 40.72) showed values above 30% compared to their respective control. The exception was the DC 40.5 in which

TABLE 1. Morphological data taken in biosafety greenhouse potato plants of transgenic varieties Diacol Capiro (DC) and Parda Pastusa (PP) and their non GM controls.

Line	Plant height (cm)	Number of stems	Diameter of the stems (mm)
DC	17.646±2.3	2.896±0.2	2.344±0.1
DC 40.7a	13.159±1.6	2.773±0.1	2.344±0.2
DC 40.7b	11.550±1.8	2.000±0.2	2.125±0.2
DC 40.5	11.208±2.1	3.333±0.2	2.250±0.1
DC 40.72	12.023±1.9	2.697±0.3	2.313±0.1
PP	69.292±3.4	3.250±0.3	3.722±0.2
PP 28	28.589±3.1	2.875±0.3	2.643±0.2
PP 28.1	36.333±2.9	2.958±0.1	3.250±0.2
PP 40.3	53.755±3.0	2.536±0.2	3.427±0.3
PP 40.a	61.730±2.3	3.135±0.2	3.365±0.3

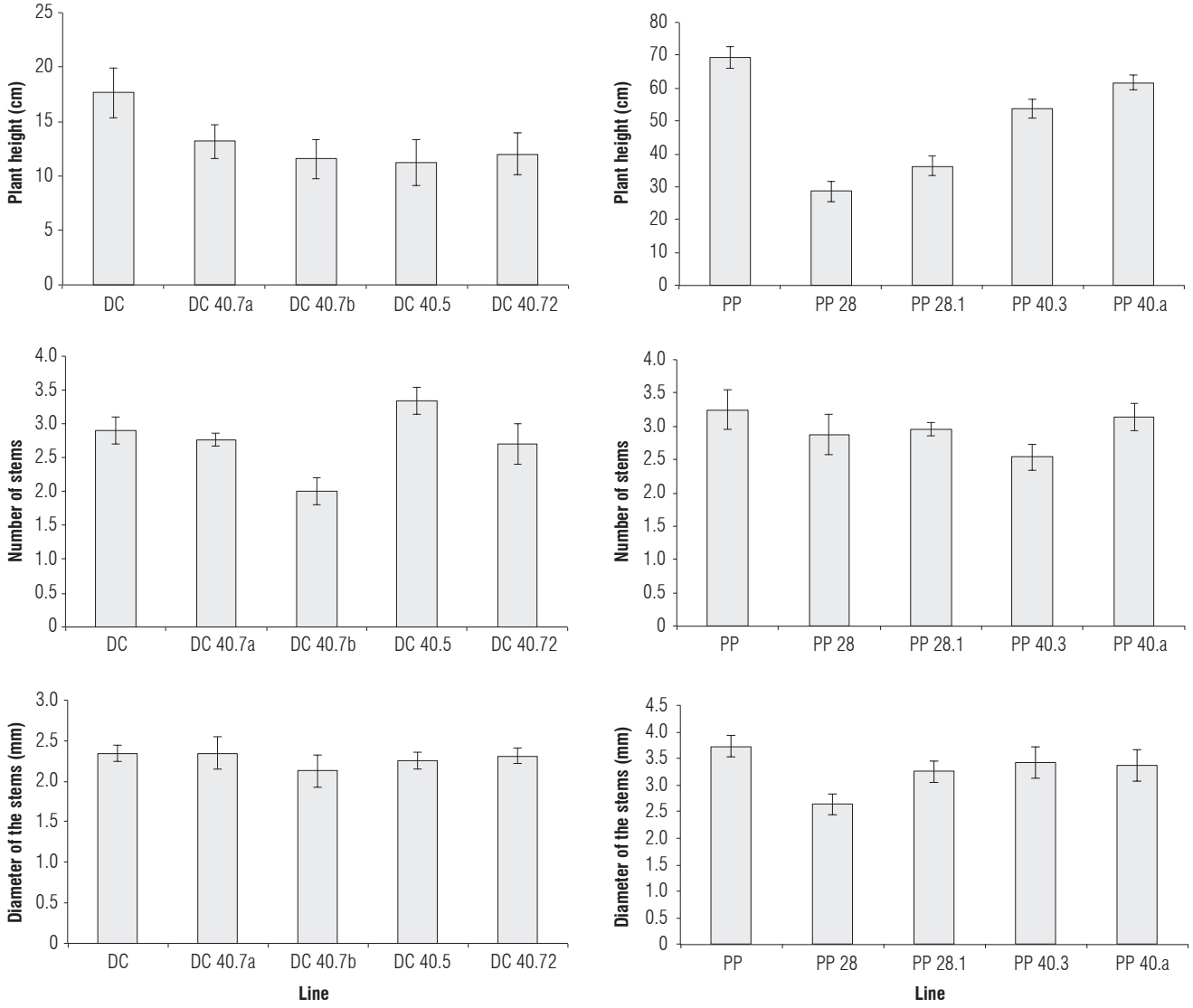

FIGURE 2. Morphological characteristics of plants of the variety Diacol Capiro (DC) and Parda Pastusa (PP). Comparison of genetically modified lines with their respective control untransformed. The bars on the columns indicate standard deviation.

values were far below the control line (less than 28%). On the other hand, in the variety Parda Pastusa, two transgenic lines: PP 28 and PP 28.1 showed results below its control, whereas lines PP 40.a and PP 40.3 shiwed values above the untransformed control line (Tab. 2).

The line that showed the highest amount of minitubers were thenon-transgenic line (DC) of the Diacol Capiro variety with a total of 680 units, followed by lines DC 40.5 with 504, DC 40.7 with 314, DC 40.7b with 291 and DC 40.72 with 289 units of minitubers. In the variety Parda Pastusa the transgenic line PP 28.1 was the one that had the highest number of minitubers with 1,008 units, closely followed by the lines PP 40.3 (967), PP 40.a (950) and the untransformed line PP (894 minitubers) (Tab. 3).

TABLE 2. Number of minitubers in grams per plant (estimated as the total weight of the minitubers of each line divided by the number of plants, n = 15) obtained under biosecurity greenhouse conditions in potato lines of the varieties Diacol Capiro and Parda Pastusa (controls and transgenics).

Line code	Minituber (number)
DC	66.25
DC 40.7a	80.00
DC 40.7b	106.80
DC 40.5	47.72
DC 40.72	94.26
PP	142.10
PP 28	91.27
PP 28.1	110.11
PP 40.a	215.06
PP 40.3	177.65

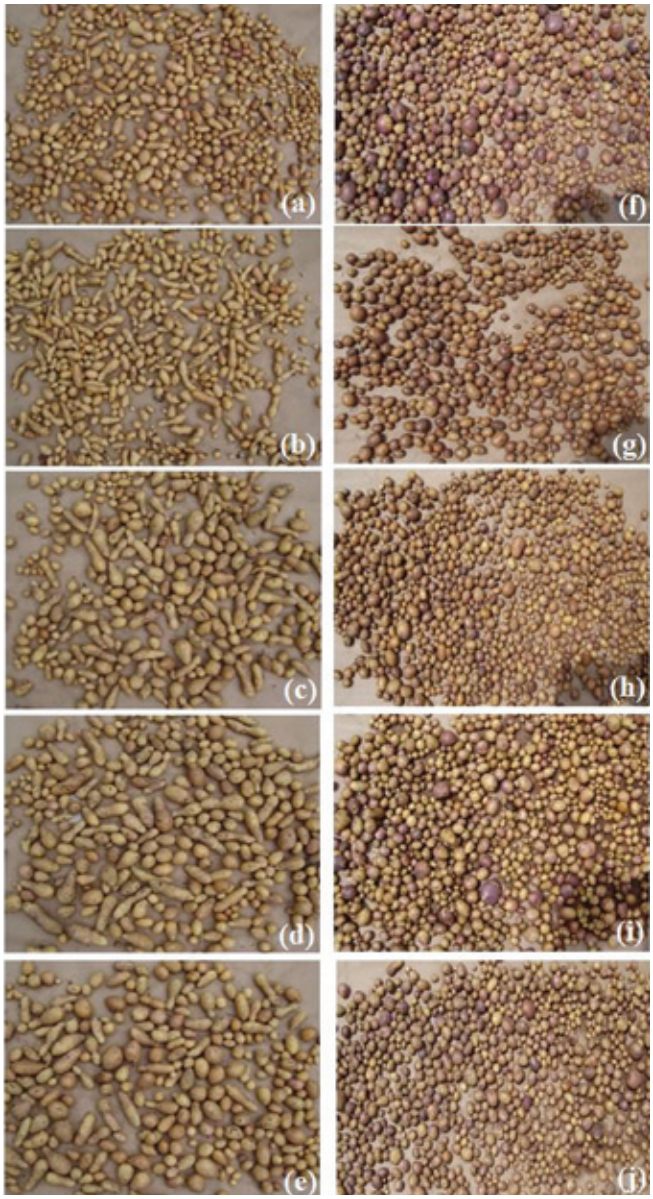

FIGURE 3. Minitubers of the potato lines in Parda Pastusa and Diacol Capiro transgenic: b) DC 40.5, c) DC 40.7a, d) DC b 40.7b, e) DC 40.72, g) PP 28, h) PP 28.1, i) PP 40.3, j) PP 40.a and control a) DC and f) PP, obtained under conditions of biosafety greenhouse.

Regarding the classification of minitubers based on their diameter, line PP had only minitubers of category 0 (> 4 cm). Category 1 (from 3.0 - 3.9 cm) was found on the lines PP and PP 40.a. The highest number of minitubers were of less than 1.9 cm in diameter, representing 58.03% of total, 33.7% were of category 3 and for category 4, respectively (Tab. 3). For variety Diacol Capiro, the untransformed line had the highest number of minitubers of category 3 and the second for category 4, surpassed in this category by DC line 40.5, which did not havea minitubers of category 2 (Tab. 3). In the variety Parda Pastusa, the non-transformated line was the only one which presented minitubers in five categories of classification and only lines PP and PP 40.a had minitubers of category 1 (3.0 to 3.9 cm). As for the lines PP 28, PP 28.1 and 40.3 minitubers were obtained only in categories 2, 3 and 4 (Tab. 3). Most minitubers for all lines in Parda Pastusa were in the range of 1.0 to 1.9 cm in diameter (category 3), for category 4, the line that showed the highest abundance was PP 28.1 with 455 units. In contrast, the line PP 40.a had the lowest amount of minitubers for this category.

Regarding the tuber shape, depth of eyes and the presence of physical abnormalities in minitubers, no significant differences were found for both Diacol Capiro and Parda Pastusa varieties (Tab. 4). For variety Parda Pastusa rounded minitubers were obtained whereas Diacol Capiro tubers were present in two forms: enlarged and oval (Tab. 4). These are not common to this variety and were considered abnormal based (Grupo de Investigación en Papa, 2010) (Fig. 4).

The evaluation of characteristics of the skin and pulp from harvested minitubers did not show significant differences among the lines of each variety. The texture of the skin in all lines of varieties Diacol Capiro and Parda Pastusa was of smooth type (Tab. 5). There were differences in color between the two varieties, Diacol Capiro showed a

TABLE 3. Classification of minitubers produced in greenhouse of the GM lines and their respective controls non-GM by categories based on their diameter.

Line code	Category					Quantity of minitubers
	0	1	2	3	4	
DC	0	0	4	355	321	680
DC 40.7a	0	0	36	228	50	314
DC 40.7b	0	0	34	228	29	291
DC 40.5	0	0	0	149	355	504
DC 40.72	0	0	43	216	30	289
PP	5	12	129	573	175	894
PP 28	0	0	1	281	274	556
PP 28.1	0	0	11	542	455	1008
PP 40.a	0	2	166	620	162	950
PP 40.3	0	0	92	553	322	967

TABLE 4. Evaluation of general physical characteristics of the minitubers produced in greenhouse lines of modified and unmodified varieties Diacol Capiro (DC) and Parda Pastusa (PP).

Potato line	Shape of minitubers	Tuber eye depth	Presence of abnormalities
DC	Oval shaped and enlarged	Superficial	Enlargement of the Minitubers
DC 40.7a	Oval shaped and enlarged	Superficial	Enlargement of the Minitubers
DC 40.7b	Oval shaped and enlarged	Superficial	Enlargement of the Minitubers
DC 40.5	Oval shaped and enlarged	Superficial	Enlargement of the Minitubers
DC 40.72	Oval shaped and enlarged	Superficial	Enlargement of the Minitubers
PP	Rounded	Superficial	None
PP 28	Rounded	Superficial	None
PP 28.1	Rounded	Superficial	None
PP 40.a	Rounded	Superficial	None
PP 40.3	Rounded	Superficial	None

FIGURE 4. Minitubers with elongated shape obtained in the variety Diacol Capiro (DC).

light brown color and Parda Pastusa was predominantly intense brown with some purple spots. Only the line PP 40, presented a light brown tonality, differing from the color obtained for the other lines of this variety (Tab. 5). The color of the pulp that prevailed for all lines of variety Diacol Capiro was light yellow and for variety Pastusa Parda was intermediate yellow, demonstrating that there were no differences between the lines of the same variety, and have been transformed or non-transformed (Tab. 5).

Variability in some morphological traits considered important for these varieties (Grupo de Investigación en

TABLE 5. Evaluation of physical characteristics of the skin and pulp of minitubers produced in greenhouse lines of modified and unmodified varieties Diacol Capiro (DC) and Parda Pastusa (PP).

Potato line	Texture of the tuber skin	Color of the tuber skin	Pulp color
DC	Smooth	Light brown	Light yellow
DC 40.7a	Smooth	Light brown	Light yellow
DC 40.7b	Smooth	Light brown	Light yellow
DC 40.5	Smooth	Light brown	Light yellow
DC 40.72	Smooth	Light brown	Light yellow
PP	Smooth	Intense brown	Yellow intermediate
PP 28	Smooth	Intense brown	Yellow intermediate
PP 28.1	Smooth	Intense brown	Yellow intermediate
PP 40.a	Smooth	Light brown	Yellow intermediate
PP 40.3	Smooth	Intense brown	Yellow intermediate

Papa, 2010) was observed mainly in the lines of Diacol Capiro, both in control and genetically modified lines. This excludes the posibility of changes induced by the transformation event.

The variation found in plants of Diacol Capiro variety are due to a phenomenon occurring before the introduction of the foreign gene. The occurrence of somaclonal variation as result of the maintenance and establishment of plant material under *in vitro* conditions, may explain the phenotypic variation found. This hypothesis is supported by work of Beaujean *et al*. (1998) who developed a system of transformation by *Agrobacterium tumefaciens* in three potato varieties: Bintje, Désirée and Kaptah Vandel. In this work they found between 17 and 19% of abnormal ploidy and 10% of appearance of morphological abnormalities in the regenerated plants after transformation, as a product of somaclonal variation. Similarly Thieme and Griess (1996) produced somaclones of different cultivars of potato and found variability of agronomic traits related to growth, earliness and tuber yield.

To confirm the presence of somaclonal variation in these lines it would be necessary to evaluate the heritability of traits that showed significant changes in several successive generations, to identify chromosomal changes through the development of karyotypes and study changes at the DNA level using molecular markers (Sánchez-Chiang and Jiménez, 2009). The occurrence of somaclonal variation at very high rates in potato is influenced by several factors such as type of explant, culture medium, age of the donor plant, number of subcultures and tissue culture system among all the factors described in the literature, the genotype is probably the most important (Etienne and Bertrand, 2003; Bordallo *et al*., 2004; Ooms *et al*., 1987) This suggests that the variety Parda pastusa may be less susceptible to somaclonal variation.

Conclutions

The results obtained in this study constitute a step forward in the process of a possible commercial release of these transgenic potato varieties with resistance to *Tecia solanivora*. However, the likelihood of hybridization with commercial varieties of potato requires consideration, as a possible biological risk involved in the release of transgenic varieties, particularly considering that Colombia is a center of origin and spread of potato species.

In the present study all lines of the variety Diacol Capiro presented morphological abnormalities, which are probably related to the occurrence of somaclonal variation. The line PP 40.a proved to be the clone with better morphology, it would be advisable to continue with this line for further studies, molecular testing, safety and substantial equivalence.

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