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Artículo Científico

Genetic fidelity analysis in pineapple vitroplants obtained from Venezuelan Amazonian ecotype using RAPD markers

Análisis de fidelidad genética en vitroplantas de piña obtenidas de un ecotipo amazónico venezolano mediante marcadores RAPD

Héctor A. Blanco Flores[1], Teresa E. Vargas Cedeño[1], Carmen C. García García[2], Eva C. García de García[1*]

[1]Professor and Researcher. Plant Biotechnology Laboratory. Institute of Experimental Biology. Faculty of Sciences, Universidad Central de Venezuela (UCV), Suapure Street, Colinas de Bello Monte. Caracas, Venezuela. [2]Professor in General Pathology and Physiopathology. Luis Razetti School, Faculty of Medicine, Universidad Central de Venezuela (UCV). University City, Postal code: 1050. Caracas, Venezuela.

*Corresponding author: eva.cristina.garcia@gmail.com

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Abstract

The present study aims to evaluate the genetic variability of vitro plants obtained from a pineapple ecotype (*Ananas comosus*) from the Venezuelan Amazon, known as Gobernadora through Randomly Amplified Polymorphic DNA (RAPDs markers). Vitroplans were obtained via three different protocols: Bud culture (BC), somatic embryogenesis (ES) and adventitious organogenesis (OA). Genomic DNA was obtained from leaves sections from different groups of vitroplants and the mother plant. Fourteen decameric primers of arbitrary sequence were tested in the analysis. The electrophoresis revealed 77 bands: 70 were common both to the mother plant and to the vitroplants obtained by the three morphogenic processes, (90.9 monomorphic bands) and 7 were not common (9.1% polymorphic bands). The initiators OPA-01, OPB-01, OPF-13, and OPV-19 detected polymorphism in a plant obtained through bud culture, which was named Gob B6. This plant had a different leaf anatomical structure than that of the mother plant and that of the population of in vitro plants obtained through bud culture. The genetic instability presented in the in vitro plants obtained through bud culture leads us to conclude that the processes of somatic embryogenesis and adventitious organogenesis are more reliable systems for the mass clonal propagation of these plants. This study may provide information on how different morphogenic pathways, induced in the *in vitro* environment, can affect the genetic stability of *in vitro* pineapple plants.

Keywords: Clonal propagation, organogenesis, somatic embryogenesis, buds culture, clonal stability, molecular markers.

Resumen

El presente estudio tuvo como objetivo evaluar la variabilidad genética de vitroplantas obtenidas de un ecotipo de piña (*Ananas comosus*) de la Amazonía venezolana, conocido como Gobernadora a través de marcadores de ADN Polimórfico Amplificado Aleatoriamente (RAPDs). Las vitroplantas se obtuvieron a

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través de tres diferentes protocolos: cultivo de yemas (CY), embriogénesis somática (SE) y organogénesis adventicia (OA). El ADN genomico se obtuvo de secciones de hojas de las vitroplantas y de la planta madre. El análisis se realizó con catorce iniciadores decaméricos de secuencia arbitraria. La electroforesis reveló 77 bandas: 70 fueron comunes a la planta madre y a las vitroplantas obtenidas mediante los tres procesos morfogenéticos (99 bandas monomorfas), y 7 bandas que no eran comunes (9,1 % de bandas polimorfas). Los iniciadores OPA-01, OPB-01, OPF-13 and OPV-19 detectaron polimorfismo en una planta obtenida a través del cultivo de yemas, la cual se denominó Gob B6. Esta planta tuvo una estructura anatómica de la hoja diferente a la de la planta madre y a la de la población de plantas in vitro obtenidas mediante cultivo de yemas. La inestabilidad genética presentada en las plantas in vitro obtenidas mediante el cultivo de yemas, nos lleva a concluir que los procesos de embriogénesis somática y organogénesis adventicia son sistemas más fiables para la propagación clonal masiva de estas plantas. Este estudio puede proporcionar información sobre cómo las diferentes vías morfogénicas, inducidas en el ambiente *in vitro*, pueden afectar a la estabilidad genética de las vitro plantas de piña.

Palabras clave: propagación clonal, organogénesis, embriogénesis somática, cultivo de brotes, estabilidad clonal, marcadores moleculares.

Introduction

Pineapple (*Ananas comosus* L.) Merr.) is one of the most commercially important tropical fruits due to its pleasant taste and its high content of antioxidants, enzymes, nutrients and compounds beneficial to the body. In Venezuela, 'Española Roja' constitutes the main commercial pineapple cultivar (d'Eeckenbrugge *et al.*, 2011). In the south of the country there are different endogenous pineapple ecotypes which are of local importance, nonetheless their market is restricted to several localities of the Amazonas state and are cultivated mainly by aboriginals of the Piaroa ethnic group (Betancourt, 2006). They cultivate pineapples throughout the year mainly by use of vegetative propagules like crowns, or suckers. The time from planting to harvest depends on the type of planting material used, approximately 15 to18 months and for shoots, and 22-24 months for crowns (d'Eeckenbrugge *et al.*, 2011).

Thus, plant tissue culture has been used as an alternative for massive *in vitro* clonal propagation of different cultivars of A. comosus (Sripaoraya *et al.,* 2003; Mogollón *et al*., 2004; Firoozabady and Moy, 2004; Saucedo Aguiar *et al.*, 2008; Pineda *et al.*, 2012), Venezuelan Amazonian ecotypes (Blanco *et al.*, 2011; Blanco Flores *et al*., 2017) and Mexican creole pineapple (Torres Ruiz *et al.*, 2023). The use of these vitroplants makes it possible to obtain great quantity of plantlets in a timely fashion thought the whole year, regardless of seasonal changes.

Obtaining whole plants from plant cells or tissues is an asexual process that involves only mitotic division and, in theory, should not cause genetic alterations (Bairu *et al.*, 2011). However, it has been observed that this biotechnological tool can, in some cases, generate variants product of somaclonal variation (Larkin and Scowcroft, 1981). These changes may be a consequence of both mutations and mechanisms that alter the pattern of genetic expression, epigenesis. Somaclonal variation has been reported in different commercial cultivars of *A. comosus* (Perez *et al.*, 2012) also in the ornamental pineapple var. Bracteatus (Santos *et al*., 2008).

Molecular techniques have been used for the evaluation of the genetic stability of plants derived from different in vitro clonal propagation systems (Giménez *et al.*, 2001; Vargas *et al.*, 2008; Bairu *et al.*, 2011; Nandhakumar *et al*., 2017). The set of molecular techniques, used for this purpose, are modifications or are coupled

to ex situ amplification of the DNA by means of the Polymerase Chain Reaction **(PCR)** (Bardakci, 2001). Among these, Randomly Amplified Polymorphic DNA **(RAPD)** has been widely used because it represents an efficient and inexpensive technique to detect different variants or to confirm the genetic fidelity of plants of different species derived from plant tissue culture (Sebastiani and Ficcadenti, 2016; Stanišić *et al*., 2015; Shair *et al.,* 2016; Kumar *et al*., 2016; Thorat *et al.,* 2016).

The present study aims to genetically characterize vitroplants of Amazonian ecotype of A. comosus, called Gobernadora through the use RAPD markers. The vitroplants were obtained by three different *in vitro* processes: bud culture, somatic embryogenesis and adventitious organogenesis.

Material and Methods

Gobernadora pineapple plants obtained through axillary buds culture (Blanco *et al*., 2011) were used in this research. This material is kept in the *in vitro* and *ex situ* germplasms of the Laboratorio de Biotecnología Vegetal of the Instituto de Biología Experimental, Universidad Central de Venezuela.

These vitroplants were multiplied using three different in vitro protocols: a) bud culture **(BC)**, somatic embryogenesis **(SE)**, and adventitious organogenesis **(AO)**, and the new plants were analyzed for assessment genetic fidelity (table 1). Foliar sections of Gobernadora's mother plants, growing in soil, were used as control.

Genomic DNA isolation

Genomic DNA was extracted from pineapple leaf tissue using a modified CTAB protocol based on Michiels *et al.* (2003), optimized for samples with high polysaccharide content. The main steps of the extraction procedure are summarized in table 2.

Integrity and quantification purified DNA

The integrity of the genomic DNA was evaluated by 0.8% agarose gel, in horizontal electrophoresis prepared with 0.5 X Tris-Borate EDTA buffer solution (0.5 X TBE) and stained with 1 μg.mL^{-1} ethidium bromide. The DNA bands were visualized by ultraviolet irradiation supplied by a GEL-DOC 2000 BIO RAD brand transilluminator. For the electrophoretic analysis, a kit of molecular mass markers (phage DNA fragments λ formed by the action of the Hind III endonuclease) was

Table 1

Summary of in vitro propagation protocols for Ananas comosus (Gobernadora).

Protocol	Explants	Culture Medium (Key Additives)	Duration & Conditions
Bud Culture (BC)	Axillary buds from vitroplants	MS + BAP 0.5–1.0 mg.L^{-1}, NAA 0.01 mg.L^{-1}, Thiamine, Myo-inositol, 30 g.L^{-1} sucrose.	4 weeks in liquid, then semi-solid; RITA system (Blanco *et al.*, 2011).
Somatic Embryogenesis (SE) (Figure 1).	Leaf base sections	MS + Picloram 5 mg.L^{-1} + Thidiazuron 1 mg.L^{-1} (induction), hormone-free for maturation.	10 weeks (dark) + 6 weeks (light, 16 h photoperiod). (Blanco Flores *et al., 2017).*
Adventitious Organogenesis (AO)	Leaf base sections	MS + NAA 2 mg.L^{-1} + BAP 5 mg.L^{-1} (induction), hormone-free for development.	10 weeks (light) + 6 weeks (light). (Blanco Flores *et al.*, 2017).

Note. MS= Murashige and Skoog medium.

Figure 1

Acclimatized Gobernadora plants obtained though somatic embryogenesis.

Table 2

Summary of genomic DNA isolation protocol (modified from Michiels et al., 2003).

Step	Procedure / Reagents	Conditions
Tissue preparation	0.1–0.2 g leaf sections macerated in liquid nitrogen.	
Cell lysis	Add 700 µL 4% CTAB buffer + 5 µL β-Mercaptoethanol (preheated 65°C).	Incubate at 65°C, 1–2 h; invert every 10 min.
Phase separation	Add 700 µL chloroform:isoamyl alcohol (24:1), mix gently.	RT, invert 10 min; centrifuge 13,000 RPM, 10 min.
Aqueous phase recovery	Carefully transfer upper phase to new tube.	
DNA precipitation	Add 100 µL cold 7.5 M ammonium acetate + 700 µL cold absolute ethanol.	Incubate –20°C, 30 min; centrifuge 13,000 RPM, 15 min.
DNA washing	Wash pellet with 1 mL 70% ethanol.	Centrifuge 13,000 RPM, 5 min.
Final resuspension	Resuspend DNA in 10 mM Tris-HCl-EDTA (TE), pH 8.0.	Store at –20°C.

used. Two methods were used to estimate the yield of the extraction (µg DNA.g leaf tissue^{-1}): a) measurement of the OD of the samples at 260 nm using Pharmacia brand spectrophotometer model Ultrospect III. This method has the disadvantage of overestimating the amount of DNA by the hyperchromic effect. For that reason, an additional method (visual analysis of the intensity of Agarose gel bands) was used. Serial dilutions were made to the DNA plants samples with TE buffer, and then subjected to electrophoresis. Dilutions were done in order to obtain band intensities that were comparable to the intensity of any of the MW (molecular weight marker) whose mass is known. Information on the amount of DNA (ng) per µL of load for each MW is supplied by the manufacturers. With this semi-quantitative method, it was possible to estimate the concentration of the extracted DNA ([ADNext] = [ng band DNA sample µL^{-1} load] x Dilution factor) and the Yield ([Rend] = µg AD Next g^{-1} leaf tissue). Concentrated and diluted samples were stored at -20°C until used in PCR-RAPD amplification reactions.

PCR-RAPD analysis

The genetic stability of vitroplants was evaluated by RAPD primers-PCR analysis. The amplification reactions were carried out in a final volume of 25 µL containing approximately 9 ng of genomic DNA, 2.5 µL of 10 X buffer solution, 2.5 µL of each 5 µM RAPD initiator, 0.5 µL of the mixture of 40 mM DNTPs, 1U of GoTaq® DNA Polymerase (Promega, Madison, USA) and the reaction volume was completed with miliQ water previously autoclaved. Fourteen RAPD initiators (Bioneer, South Korea) (table 3) selected by previous studies on genetic stability in micropropagated pineapple plants were tested (Soneji *et al*., 2002; Santos *et al*., 2008). For each initiator a control reaction was prepared to rule out contamination from another source of DNA (all reaction components except the DNA sample).

Once the reaction mixture was prepared, these were placed in the BIORAD Thermocycler programmed with the following physical conditions: Initial denaturation, 94°C (10 min); 40 cycles with denaturation 94ºC (1 min), alignment 36ºC (1 min) And extension 72ºC (2 min) and final extension at 72ºC (10 min). The amplification products (amplicons) were separated on 1.5% agarose gel by horizontal electrophoresis, stained with ethidium bromide, visualized on the Transilluminator and the image exported and stored in TIFF format on the computer.

Bands analysis

The size of the amplicons was estimated by comparison with the running pattern of 1Kb molecular mass

Table 3

Decameric arbitrary sequence oligonucleotides (initiators) used in the RAPD analysis of the plants obtained by in vitro culture of the Gobernadora pineapple.

Primers	Sequence (5′- 3′)	Primers	Sequence (5′- 3′)
OPA-01	CAG GCC CTT C	**OPF-13**	GGC TGC AGA A
OPA-02	TGC CGA GCT G	**OPL-17**	AGC CTG AGC C
OPA-03	AGT CAG CCA C	**OPM-13**	GGT GGT CAA G
OPA-20	GTT GCG ATC C	**OPP-16**	CCA AGC TGC C
OPB-01	GTT TCG CTC C	**OPT-07**	GGC AGG CTG T
OPB-19	ACC CCC GAA G	**OPV-19**	GGG TGT GCA G
OPC-19	GTT GCC AGC C	**OPX-03**	TGG CGC AGT G

markers in the Bioneer ladder, South Korea. Data were recorded as presence (+) or absence (-) of bands. The RAPD profile of the plants obtained by *in vitro* culture was contrasted with the amplification pattern of the mother plant. All amplifications were performed in duplicate and only those reproducible bands were considered for analysis. In case of variation, in some cases, a third amplification was necessary for confirmation. The total number of amplified bands was recorded, those that were mono and polymorphic were identified, and an estimate of Polymorphism (Number of polymorphic bands divided by total number of bands) was performed on plant samples obtained by different *in vitro* culture methods and the mother plant. For the analysis, 9 vitroplants propagated by BC, 5 obtained via SE, 5 obtained via AO and 5 mother plants of the ecotype Gobernadora, were analyzed.

Anatomical patter

Leaf anatomical patterns of the new pineapple variant Gob B6 and its mother plant were analyzed. Foliar sections of Gobernadora's mother plants growing in soil, were used as control, to determine changes in the leave structure. Leaves from 5 mother Gobernadora plants and leaves from 5 Gob B6, were cut in small sections, dehydrated with ethanol series, stained with Toluidine blue, fixed with glycerin (30%) and mounted in glad slides. The were observed through Nikon 14 MLAB-2 and Nikon OPTIPHOT optical microscopes, the latter with a built-in light polarizer. Photographs of anatomical sections were taken with a camera SONY, Ciber-shot, DSC-brand digital camera, 10.1 Mega pixels.

Results and Discussion

Monitoring the genetic identity of plants obtained through in vitro culture is crucial, as interactions between intrinsic tissue factors and the microenvironment can induce genetic variation. To this end, high-molecular-mass DNA (approximately 23,130 bp) was isolated from Gobernadora plants of the A. comosus ecotype.

The estimated average yield according to the spectrophotometric analysis was 251.9 ± 42.13 $\mu g.g^{-1}$ leaf tissue. However, it was necessary to utilize an additional determination method since spectrophotometry overestimates said parameter (hyperchromic effect, Voet *et al*. (2009). At λ = 260 nm, not only does the integrated double-stranded DNA absorb energy, so do single-stranded nucleic acids and free nucleotides. These last two have a higher molar extinction coefficient and, therefore, have a greater capacity for UV light absorption than the double-chain molecule. The alternative method was a visual analysis of the intensity of bands at different dilutions in the agarose gel. This last method allowed to determine the amount of whole DNA extracted from the plants (semi-quantitative estimation). The average yield according to this alternative methodology was 2.35 ± 0.08 $\mu g\ g^{-1}$ leaf tissue.

In the PCR-RAPD analysis, fourteen arbitrary sequence decameric initiators **(ID)** were tested for the genetic stability analysis of Gobernadora pineapple plants obtained by *in vitro* culture (table 1). Of the 14 RAPD initiators only 8 gave DNA amplification (table 4). Electrophoretic analysis revealed a total of 77 bands; of which 70 were common both for the mother plant and for the vitroplants obtained by the 3 morphogenic pathways (90.9% monomorphic bands) and 7 not common (9.1% polymorphic bands). Polymorphism, in pineapple plants derived from tissue culture, was detected both by absence of existing bands, and by the presence of new bands with respect to the mother plant.

The above can be compared with the results of Santos *et al*. (2008), who evaluated genetic variability in micropropagated Ananas comosus var. Bracteatus plants after four subculture cycles using varying concentrations of benzyladenine **(BA)**. They observed a 2.8% polymorphism and concluded that BA can influence the

Table 4

RAPD analysis of A. comosus (Gobernadora) plants obtained in vitro culture.

Initiators	Number of total bands	Number of polymorphic bands
OPA-01	10	2
OPA-03	15	0
OPA-20	8	0
OPB-01	10	1
OPF-13	8	1
OPT-07	10	0
OPV-19	11	3
OPX-03	5	0
Total	**77**	**7**

occurrence of genetic variation. In our study, although different morphogenetic protocols and cytokinin concentrations were employed, off-type plants only emerged in the group propagated by axillary buds grown on media containing a relatively low concentration of BA (0.5 mg L^{-1}), notably lower than the 5 mg L^{-1} commonly used to induce organogenesis. This suggests that while plant growth regulators, such as cytokinins, can induce genetic changes, other factors, such as prolonged cultivation duration and endogenous cytokinin levels, also contribute to somaclonal variation.

From eight IDs which amplified plant DNA, four of them (OPA-01, OPB-01, OPF-13 and OPV-19), detected different genetic variations in the vitroplant population. These four IDs detected polymorphism in a plant obtained by bud culture, called Gob B6 (figures 2 to 5).

IDs OPA-01 and OPF-13 detected variation only in the Gob b6 vitroplant (figures 2 and 3), OPA-01 detected the absence of two bands, one approximately to 2000 bp and another located between 1610 and 982 bp (figure 2), and the OPF-13 ID detected the absence of these latest band between 1610bp and 982bp (figure 3).

OPV-19 revealed the same genetic variation in two vitroplants obtained by bud culture, Gob B1 and Gob B6, the amplification pattern shows the absence of 3 bands with molecular masses between 2961 and 1610 bp (figure 4). IDs OPA-01, OPF-13 and OPV-19 did not reveal polymorphism in the vitroplants obtained by both SE and AO.

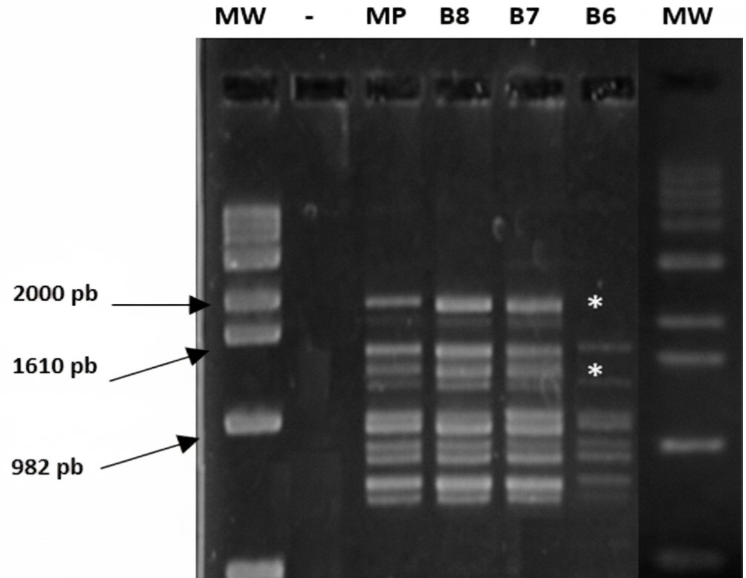
The OPB-01 initiator revealed an additional band (<500 bp) in all the Gobernadora's sampled vitroplants obtained by the 3 morphogenic pathways (BC, SE and AO) that is not present in the mother plant (figure 5). Additionally, the pattern generated by Gob B6 is different from those generated by the others plants tested.

The pattern generated by OPB-01 in all the vitroplants is the same when they are obtained by somatic embryogenesis or adventitious organogenesis (figure 6).

Interestingly, primer OPB01 revealed a polymorphic band in all tested Gobernadora vitroplants. This echoes the results of Santos *et al*. (2008), who detected a similar band across various micropropagated *A. comosus* var. Bracteatus samples, irrespective of culture medium. Lee (2019) further emphasized the central role of plant hormones and their regulatory interactions in

Figure 2

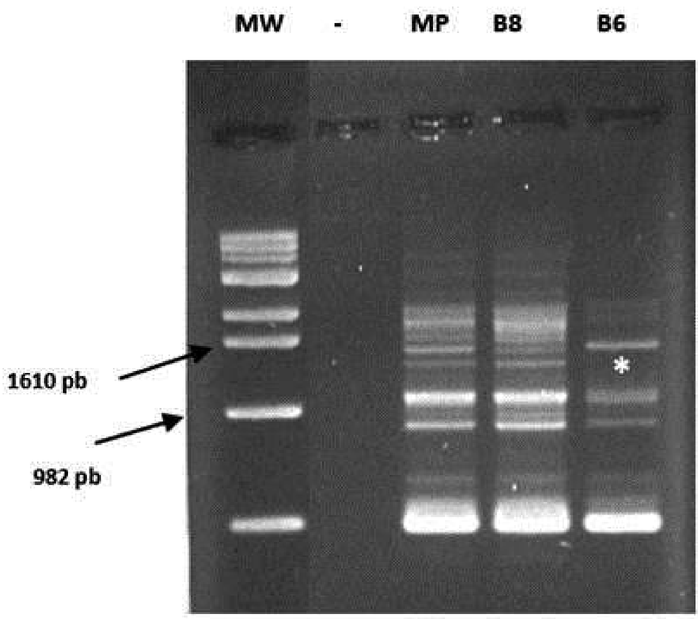
Amplification pattern generated by OPA-01 in the Gobernadora ecotype plants.



Note. **MW:** molecular mass marker, **(-):** the initiator, **MP:** control mother plant. B6, B7 and B8 are Gobernadora plants obtained by BC. **(*):** indicate the absence in Gob B6 pattern, of two bands: one close to 2000 bp, and other between 1610 bp and 982 bp.

Figure 3

Amplification pattern generated by OPF-13 in pineapple Gobernadora plants ecotypes B6 and B8 obtained by BC.



Note. **(*):** indicate the absence one band, close to 1610 bp, in Gob B6 pattern.

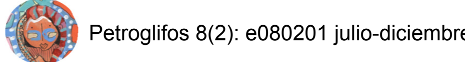
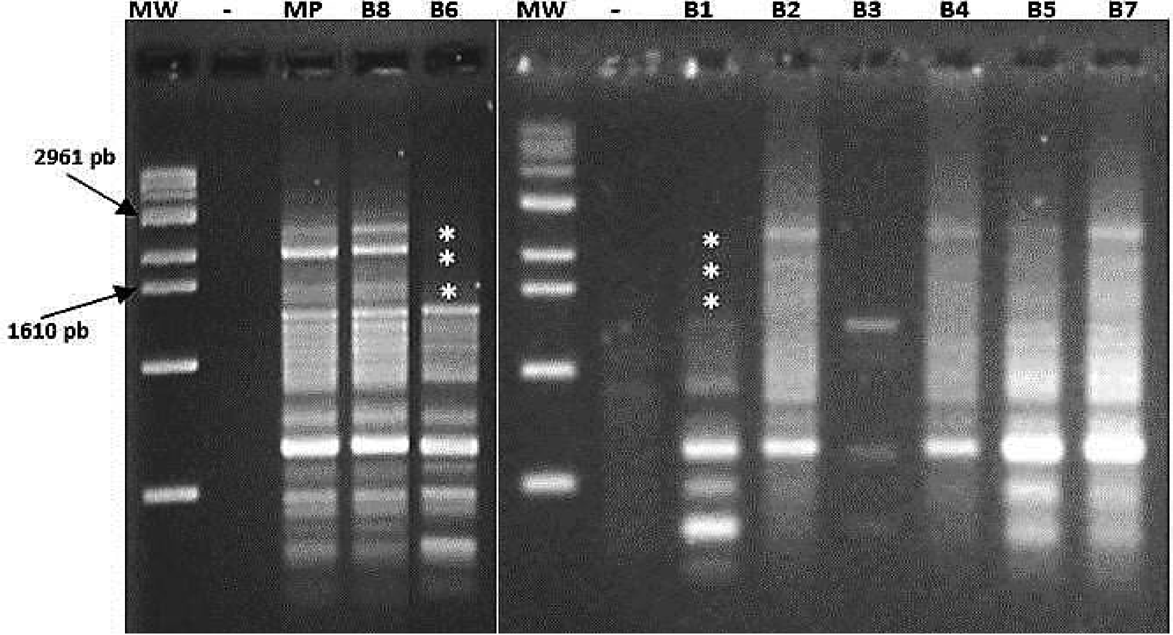


Figure 4

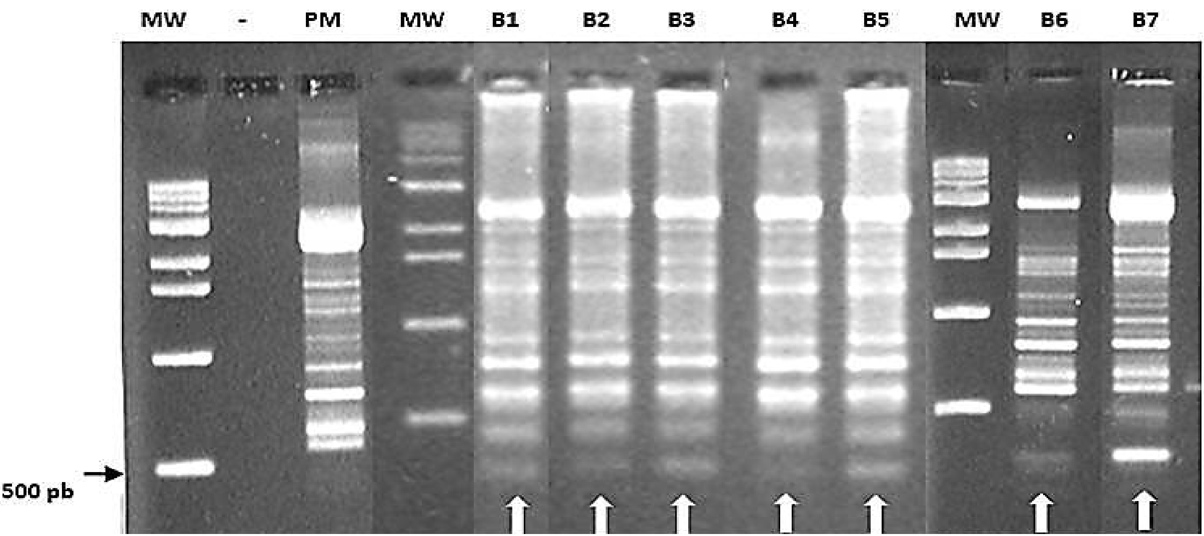
Amplification pattern generated by OPV-19 in pineapple Gobernadora ecotypes B1, B2, B3, B4, B5, B6, B7 and B8, obtained by BC.



Note. **MW:** molecular mass marker. **(*):** indicate the absence of 3 bands between 1610 and 2961 base pairs, in the patterns generated by Gob B6 and Gob B1.

Figure 5

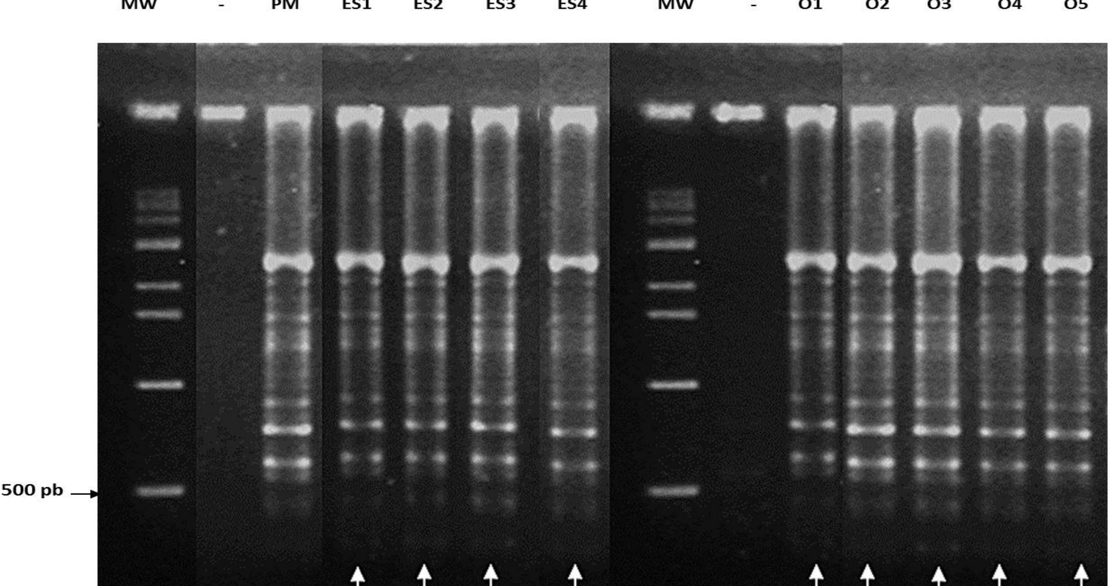
Amplification pattern generated by OPB-01 in plants de Gobernadora ecotype plants: B1, B2, B3, B4, B5, B6 and B7 obtain by BC.



Note. The arrows indicate the presence of an additional band <500 base pairs, that is not present in the mother plant (MP). The pattern generated by Gob B6 is different from those generated by the others plants tested.

Figure 6

Amplification pattern generated by OPB01 in vitroplants of Gobernadora ecotype obtained by somatic embryogenesis (ES1, ES2, ES3, and ES4).



Note. This picture also shows the amplification pattern generated by OPB01 in vitroplants of Gobernadora originated through adventitious organogenesis induced in the base of leaves (O1, O2, O3, O4, O5). The arrows indicate the presence of an additional band <500 base pairs, that is not present in the mother plant.

determining meristematic activity. Given the consistent presence of this polymorphic band across multiple bud-derived subcultures, we hypothesize that this genetic mutation likely occurred early in the multiplication phase.

However, Stanišić *et al.* (2015), when assessing genetic stability in Iris sibirica plants regenerated using SE and OA with 2,4-D and thidiazuron, found no variation using RAPD, supporting the idea that certain regeneration protocols are less likely to induce mutations. Our findings align with this, as pineapple plants regenerated from SE and AO using leaf base explants demonstrated superior genetic stability compared to those derived from axillary buds. These results suggest that morphogenetic induction from leaf base explants is more reliable for maintaining clonal fidelity, whereas bud culture may subject tissues to stress, increasing the risk of somaclonal variation.

In their study, Soneji *et al.* (2002) used RAPD analysis to differentiate between normal (spined) and variant (spineless) A. comosus ‘Queen’ regenerants. Interestingly, spineless variants were derived from axillary buds of crowns with normal leaves. Among 58 arbitrary primers used, only a few distinguished between phenotypes. Likewise, in our study, OPA01 detected variation in the Gob B6 vitroplant derived from bud culture. Meanwhile, OPA03 again showed no polymorphism, and OPA02 did not amplify.

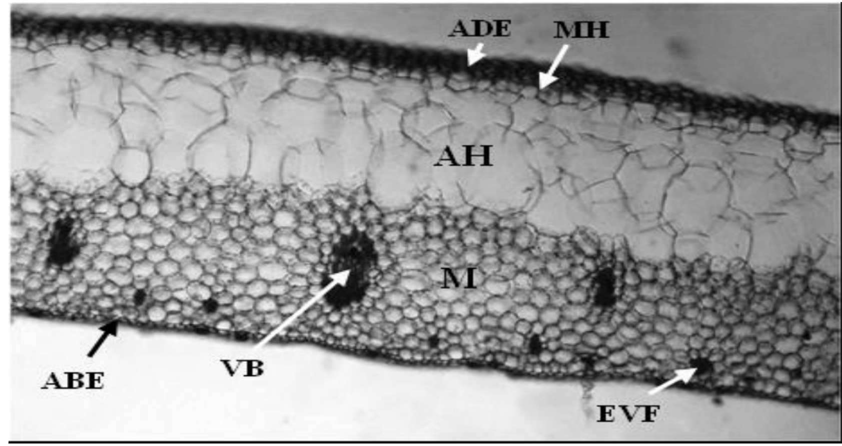
Roostika *et al.* (2016), working with the Simadu pineapple clone, identified extended culture duration as a major contributor to somaclonal variation, though the authors also recognized the role of regeneration method and plant growth regulators. In our study, all plant material originated from Gobernadora pineapple germplasm established via axillary bud culture (Blanco *et al.*, 2011) and maintained for over ten years. Despite

their common origin, the plants responded differently under alternative regeneration protocols. Notably, plants regenerated through SE and AO from leaf-base explants displayed greater genetic stability than those derived from bud culture.

Gob B6 vitroplant, which was obtained throughout bud culture, was detected as off-type plant by four independent initiators. It is interesting to note that the anatomical leaf structure of this vitroplant is also different from that of the population of vitroplants obtained through bud culture. The structural analysis of the leaf demonstrated a decrease in the thickness of the epidermis, reduction of cell layers in the aquifer hypodermis, and a mesophyll practically occupied by large chlorenchymal cells (figures 7 and 8). Based on these results, Gob B6

Figure 7

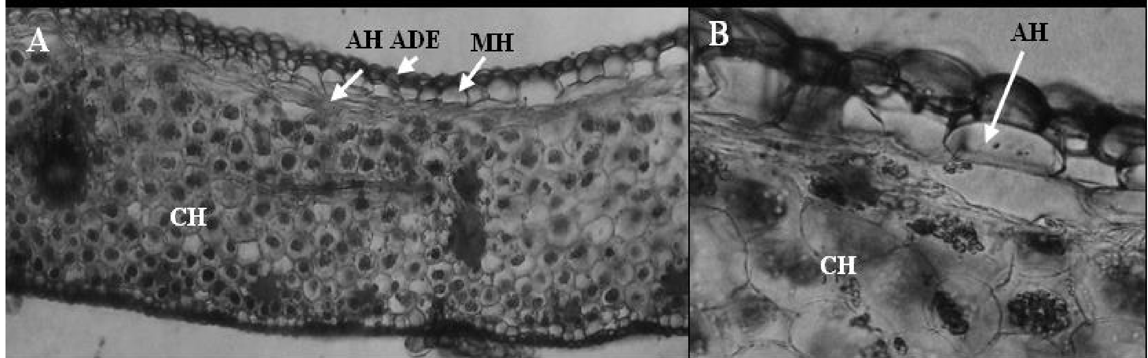
Leaf anatomical pattern of mother plant of Gobernadora ecotype.



Note. **ADE:** adaxial epidermis, **MH:** mechanical hypodermis, **AH:** aquifer hypodermis, **ABE:** abaxial epidermis, **VB:** vascular bundle, **EVB:** extravascular fiber bundle.

Figure 8

Leaf anatomical pattern of Gob B6 variant obtain through adventitious organogenesis.



Note. **A:** General aspect (100X) **ADE:** Adaxial epidermis, **AH:** aquifer hypodermis, obliterate cells are observed between the chlorenchyma **(CH)** and the poorly differentiated mechanical hypodermis **(MH)** (100X). The mesophyll is dominated by the chlorenchyma. **B:** Details of the aquifer hypodermis show that it is formed by a layer of obliterated flattened cells (400X).

could be considered a somaclonal variant.

Pendi *et al.* (2022) studied in vitro-propagated MD2 pineapples using both RAPD and ISSR markers and concluded that morphological traits did not always correlate with underlying genetic structure. Consistent with this, our study found that four primers detected genetic variation specifically in the Gob B6 plant obtained via bud micropropagation. This variant condition was also validated through foliar anatomical analysis, which revealed structural alterations including chlorenchymatous mesophyll, a poorly differentiated mechanical hypodermis, and a single-layer aquifer hypodermis with obliterated cells. These findings confirm that Gob B6 represents a somaclonal variant.

Conclusions

The present study demonstrates that the morphogenic induction (adventitious organogenesis or somatic embryogenesis) in sections of leaf base explants, produces genetically stable pineapple vitroplants. On the contrary, axillary buds were more susceptible to the stress generated by the in vitro culture and therefore, are more prone to give rise to genetic variability.

Based on the results of the RAPD analysis we concluded that OPA-01, OPB-01, OPF-13 y OPV-19 primers were adequate and sufficient for the assessment of clonal fidelity of *A. comosus* (ecotype Gobernadora) vitroplants.

The instability associated with *in vitro* bud culture for *A. comosus* 'Gobernadora' points toward somatic embryogenesis and adventitious organogenesis as more robust methods for large-scale clonal propagation of genetically stable pineapple plants.

Amplification patterns generated by OPA-01, OPF-13 and OPV-19 primers showed polymorphism in a vitroplant obtained through bud culture (Gob B6). OPV-19 RAPD pattern showed polymorphism in another vitroplant, (Gob B1) that was also obtained through bud culture. The statement that Gob B6 is a variant is corroborated also by the leaf anatomical analysis. The anatomical leaf structure is different from that of the population of vitroplants obtained through bud culture. It is characterized by a decrease in the thickness of the epidermis, reduction of cell layers in the aquifer hypodermis, and mesophyll practically occupied by large chlorenchyma cells. Based on its distinct genetical and anatomical patterns we conclude that Gob B6 is a somaclonal variant. The next step will be to grow Gob B6 in the field to assess productivity and quality to evaluate if it meets commercial standards.

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