

Evaluation of genetic variability in micropropagated propagules of ornamental pineapple [*Ananas comosus* var. *bracteatus* (Lindley) Coppens and Leal] using RAPD markers

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ABSTRACT. The objective of the present study was to evaluate the genetic variability in micropropagated plantlets of ornamental pineapple, after the fourth period of subculture. The basal culture medium consisted of MS salts, vitamins, 3% sucrose, liquid formulation, supplemented with 6-benzylaminopurine (BAP) at concentrations of 0.125, 0.25, 0.5, 1.0, and 2.0 mg/L. The addition of BAP influenced the occurrence of genetic variation revealed using random amplified polymorphic DNA (RAPD) markers. Of a total of 520 primers tested, 44 were selected and amplified; 402 monomorphic bands (97.2%) and 18 polymorphic bands (2.8%) resulted among regenerated plantlets. The polymorphic fragments were produced by 12 primers (OPA-01, OPA-20, OPB-01, OPB-19, OPC-19, OPF-13, OPL-17, OPM-13, OPP-16, OPT-07, OPV-19, and OPX-03). Among the primers that identified polymorphism, OPA-01, OPA-20, OPB-19, OPC-19, OPL-17, OPP-16, and OPX-3 each showed, one polymorphic band and OPF-13 amplified a maximum of three bands. In this study, the RAPD technique was effective in showing the occurrence of

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somaclonal variations that occur during the micropropagation process of ornamental pineapple cultivation in BAP-supplemented medium, and it is possible to detect the presence of genetic variation in early stages of plant development.

Key words: *Ananas comosus* var. *bracteatus*; Somaclonal variation; Genetic variation; *In vitro* culture; Mutation

INTRODUCTION

The ornamental pineapple (Ananas comosus var. bracteatus (Lindley) Coppens and Leal) is a monocotyledon plant from the Bromeliaceae family (Collins, 1960; Coppens and Leal, 2003) with great ornamental potential. The commercial propagation of this species is vegetative, using three types of propagules: crowns, suckers, and slips produced by the plant in the field. Each plant produces up to ten propagules per year, which limits its commercial cultivation (Correia et al., 2000). In addition, this kind of propagation may favor disease dissemination in the case of an unhealthy original plant. A strategy to avoid this limitation is to use in vitro culture for clonal propagation and fast growth of plants with important genotypes, theoretically, with the same genetic identity of the original material and with a high phytosanitary status. However, it has been observed that this technology may cause, in some situations, genetic modifications known as somaclonal variations (Larkin and Scowcroft, 1981) and also phenotypic modifications, called epigenetic variations (Evans and Bravo, 1986). These variations in micropropagated material, such as genetic and epigenetic modifications, need to be characterized. Genetic variations in plants from tissue culture have been cited in Lycopersicon esculentum Miller (Evans and Sharp, 1983), Nicotiana alata (Evans and Bravo, 1986), Ananas comosus (Das and Bhowmik, 1997; Gottardi et al., 2002; Feuser et al., 2003; Rodrigues et al., 2007), Musa sp (Martin et al., 2006) and Curcubita pepo (Leljak-Levanic et al., 2004), among others.

Recently, molecular markers have been utilized in the detection of variation or confirmation of genetic fidelity during micropropagation (Gupta et al., 1998; Tyagi et al., 2007). Among the polymerase chain reaction (PCR)-based markers frequently used, RAPD (random amplified polymorphic DNA) is considered to be efficient and cost effective. The technique only needs a few nanograms of DNA for a fast polymorphism analysis, does not require prior knowledge of DNA sequence, and does not involve radioactivity (Williams et al., 1990). RAPD markers have been employed to determine the clonal fidelity of micropropagated plants (Rani et al., 1995; Tang, 2001; Feuser, 2003). In the literature consulted, there is no knowledge about the genetic variability within and between plants derived from tissue culture of ornamental pineapple. The present study summarizes the application of RAPD markers to solve the problem mentioned.

MATERIAL AND METHODS

To evaluate genetic variability using RAPD, leaves were taken from *in vitro* cultures of *Ananas comosus* var. *bracteatus*. The cultures were established from axillary bud explants excised from the crown of one plant growing under field conditions. The starting medium was composed of MS salts (Murashige and Skoog, 1962), 3% sucrose, 0.7% agar and 100 mg/L myo-inositol, 0.1 mg/L thiamine-HCl, 0.05 mg/L pyridoxine-HCl, 0.05 mg/L nicotinic acid, 2.0 mg/L glycine, 1.0 mg/L 6-benzylaminopurine (BAP), 2.0 mg/L gibberellic acid (GA₃), and 0.01 mg/L α-naphthalene

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acetic acid. The cultures were illuminated 16 h per day with 32 µmol.m⁻².s⁻¹ irradiance provided by fluorescent light and exposed to a temperature of $27 \pm 2^{\circ}$ C for 90 days. After this period, regenerated propagules of 1.5-2.0 cm in length were selected and added to flasks containing growth medium composed of MS salts, vitamins and 3% sucrose, supplemented with 0.125, 0.25, 0.5, 1.0, and 2.0 mg/L BAP, in liquid formulation. The pH of the medium was adjusted to 5.7 ± 0.1 , before autoclaving. The cultures were incubated at 27°C with a 16-h photoperiod with exposure to 32 µmol.m².s⁻¹ irradiance. The propagules were subcultured monthly for 120 days. After the fourth subculture, 19 individuals for each BAP concentration were randomly selected for RAPD analysis, totaling 95 micropropagated propagules and one mother plant (control). The propagules were rooted and transferred to 3.0-cm diameter tubes containing PlantMax substrate, until they were transferred to a greenhouse where they were kept for evaluation of possible morphological changes. Every micropropagated plant characterized in this study was derived from just one axillary bud excised from the mother plant. Initially, a screening using 520 Operon Technologies Inc. primers was performed. To select the primers, the mother plant DNA and two individuals from in vitro culture were used. The primers that showed polymorphic bands and/or amplified a larger number of robust and well-defined bands were selected and afterwards evaluated to determine which ones were more informative with respect to polymorphism.

DNA extraction and RAPD analysis

DNA was extracted from fresh *in vitro* leaves of regenerated plants and the mother plant, as described in Ferreira and Grattapaglia (1998). The DNA quantification and quality evaluation was verified on 1% agarose gels stained with ethidium bromide and visualized under ultraviolet light. The DNA amplification reactions were run by PCR. Each reaction mixture contained: $3.0 \ \mu\text{L} 3.0 \ ng/\mu\text{L}$ genomic DNA, $4.92 \ \mu\text{L}$ autoclaved Milli-Q water, $1.30 \ \mu\text{L} 10X$ Taq DNA polymerase buffer, $1.04 \ \mu\text{L} d\text{NTPs}$ at 2.5 mM, $1.5 \ \mu\text{L}$ primer (Operon Technologies, USA) at 10 $ng/\mu\text{L}$ and $0.2 \ \mu\text{L}$ Taq DNA polymerase enzyme, in a 13- μ L total reaction. PCR amplification was performed in an automated thermal cycler (Perkin Elmer) programmed for 40 cycles , each consisting of an initial denaturation of 1 min at 92° C, followed by an annealing step of 1 min at 35° C and an extension step of 2 min at 72° C, followed by a final extension step at 72° C for 7 min. The amplified fragments were electrophoresed on 1.5% (w/v) agarose gels in 1X TBE buffer, visualized by staining with ethidium bromide and photographed on ultraviolet light using the EagleyeTM system (Stratagene[®]). The bands were identified visually by comparison to a 1-kb ladder (75 to 12,216 bp). The samples were scored based on presence or absence of the same size bands, where coding was 1 for presence on gel and 0 for absence. The entire analysis was repeated and only the reproducible bands were considered.

RESULTS AND DISCUSSION

Previously, a variety of experiments were conducted to select plant growth regulators to establish medium requirements for adventitious organ regeneration in ornamental pineapple tissue culture. BAP was effective for inducing *in vitro* shoot proliferation in this species (Santos, 2008). It seemed of interest to screen for the presence of somaclonal variation in regenerants obtained in medium supplemented with different BAP concentrations.

High-quality DNA was isolated from the ornamental pineapple leaves. Among the 520 RAPD OP-primers screened, 44 primers were selected based on the production of well-defined and scorable bands, which were used for fingerprint comparison between the

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mother plant and micropopagated propagules. The data in Table 1 show the primers, primer sequences, number of bands produced and total number of amplicons revealed by RAPD analysis of micropropagated plantlets.

Table 1. Primers, primer sequences, and number of amplification bands produced by 44 RAPD primers in the evaluation of micropropagated propagules of *Ananas comosus* var. *bracteatus*, in culture medium supplemented with 6-benzylaminopurine, for 120 days.

$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Primer	Sequence (5'-3')	Total number of bands	Polymorphic bands		
OPA-08 GTA GG TAG G 12 OPA-19 CAA AGG TCG G 11 OPB-19 GTT GC ATC C 7 1 OPB-19 GTT GC CT C 12 2 OPB-19 ACC CCC GAA G 9 1 OPC-19 GTG ACC GG C 7 1 OPC-19 GTC AGG CC 7 1 OPC-19 GTC AGG GC AGA G 14 3 OPC-19 GTC AGG GC AA 10 1 OPC-19 GTC AGG GCA A 10 1 OPC-19 GTC AGG GCA A 10 1 OPC-19 GTC AGG GCA A 10 1 OPL-13 GGC GG CA GA 10 1 OPL-10 CAG GCA CAG G 12 1 OPL-11 AAG GCG GCA G 10 1 OPL-13 CCA CAC TAC C 7 1 OPL-14 TGA ACG GC GGA A 6 1 OPL-17 CCA CAC TAC C 7 1 OPL-18 AGC GG TG CA A 12 2 OPN-16 AAG CGA CT G G 12	OPA-01	CAG GCC CTT C	12	1		
OPA-19CAA ACG TCG G11OPA-20GTT GCG ATC C71OPB-01GTT TCG CTC C122OPB-18CCA CAG CAG T101OPC-19GTT GCC AGC C71OPC-08TGG CA CG CG71OPC-09GT GCA CAG CG G87OPF-13GGC TCA CAG A143OPC-19GT CAG GC CAG A107OPI-01GCA GG GCA G97OPI-02GA GGA GAG G107OPI-03AAG GCG GCA G107OPI-11ACA TGC CGT G101OPI-12CCA ACC TAC C77OPI-13CCA CAC TAC C77OPI-14TGA CGG CGG T87OPI-15CCC ACC TG G101OPI-16CCA ACC TG C101OPI-17AGC CTG AGC C101OPI-18AGC AGG TGG A62OPI-19TCA GAG CTG C162OPI-16CCA AGC TGC C81OPI-07CAG GAC TGC C77OPI-08AGC AGG TGC C77OPI-16CCA AGC TGC C77OPI-16CCA AGC TGC C77OPI-17CGG CAG GT T62OPI-18CCC GTT GC T62OPI-19GG CAG GT CA G121OPI-10CCA GAC CC CT T62OPI-11CCG AGC GT G T16 <t< td=""><td>OPA-08</td><td>GTG ACG TAG G</td><td>12</td><td></td></t<>	OPA-08	GTG ACG TAG G	12			
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OPB-01GTT TCG CTC C122OPB-18CCA CAG CAG T10OPC-19GTT GCC AGC C71OPC-08TGG ACC GGT G87OPF-04GGT GC AGA A143OPG-19GTC AGG GCA A107OPF-04GGA CG GCA G97OPF-05GAA GGC GCA G97OPF-06AAG GCG GCA G107OPF-07CAG CGA CAG G107OPI-08AAG GCG GCA G101OPI-11ACA TGC CGT G101OPI-13CCA CAC TAC C77OPI-14TGA CGG CG T81OPI-15CCA CAC TAC C71OPI-16CAG CG GG TG G101OPI-17AGC CTG AGC C101OPI-18GG TG CAA G22OPI-17AGC CTG AGC C101OPI-17AGC CTG AGC C147OPI-18CCA CAC TGA C91OPI-19CTA GCA CTG A121OPI-10TCA AGA CTG C101OPI-11CCA GAC TGA C101OPI-12CGA GAT TCC C71OPI-14CTA GCA CTA G121OPI-15CCA CTC CC A81OPI-16CCA AGC TGC C101OPI-17CGC GG GG T101OPI-18CAC CCC CTT G50OPI-19GG CAG CT GT162<	OPA-20	GTT GCG ATC C	7	1		
OPB-18 CCA CAG CAG T 10 OPB-19 ACC CCC GAA G 9 1 OPC-19 GTG CC AGC C 7 1 OPC-08 TGG ACC GGT G 8	OPB-01	GTT TCG CTC C	12	2		
OPB-19ACC CCC GAA G91OPC-19GTT GCC AGC C71OPC-08TGG ACC CGT G87OPF-13GGT GAT CAG G77OPF-13GGC TGC AGG AA143OPG-19GTC AGG GCAA107OPI-02GGA GGA GA G97OPI-03AAG GCG GCA G107OPI-04CAG CGA CAA G127OPI-07CAG CGA CAA G127OPI-11ACA TGC CG TG G101OPI-13CCA CAC TAC C77OPI-14TGA CGG CGG T81OPI-17AGC CTG AGC C101OPI-18GGT GGT CAA G122OPI-19CCA CAC TAC C91OPI-17AGC CTG AGC C147OPI-18GGT GGT CAA G122OPN-19CTA GA GC GC C147OPI-10CCA GC TG C81OPO-10TCA GA GC GC C147OPI-14CGG GC GT CTT G47OPI-15CCG GT GT GC C77OPI-16CCA AGC TGC C77OPI-17CCG TAC GTA G97OPI-18CAG CAG CTG T107OPI-19CGG CAG CTG T107OPI-10CCG TG CG GT GT162OPI-11CCG GAG CTG A122OPI-11CCG GAC GT CA87OPI-10CGG AGG CTG A	OPB-18	CCA CAG CAG T	10			
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OPL-08AGC AGG TGG A6OPL-17AGC CTG AGC C101OPM-13GGT GGT CAA G122OPN-16AAG CGA CTG G167OPO-07CAG CAC TGA C97OPO-10TCA GAG CGC C147OPQ-05CCG GT CTT G47OPR-08CCC GTT GCC T67OPR-10CCA AGC TGC C77OPR-14CAG GAT TCC C77OPR-16CTC TGG GCG T162OPR-17CCG TAC GTA G97OPR-18CAC CCC CTT G52OPR-19GGC AGG CTG T162OPX-01ACG GAC GTC A127OPX-01GGC AGG CTG T62OPX-10GGC AGG CTG T162OPX-11CTC GAC AGA G77OPX-10GGA CTG CTG G87OPX-11CTC GAC AGA G57OPX-10GGA CTG GTG G122OPX-03TGG CGC AGT G121	OPK-17	CCC AGC TGT G	10			
OPL-17AGC CTG AGC C101OPM-13GGT GGT CAA G122OPN-16AAG CGA CCT G16OPO-07CAG CAC TGA C9OPO-10TCA GAG CGC C14OPP-16CCA AGC TGC C81OPQ-05CCG CGT CTT G4POR-08CCC GTT GC T6OPR-10CCA TTC CCC A8OPR-14CAG GAT TCC C7OPR-16CTC TGC GCG T10OPR-17CCG TAC GTA G9OPR-18CAC CCC CTT G5OPT-07GGC AGG CTG T162OPU-01ACG GAC GTC A12OPU-01ACG GAC GTC A12OPU-01GGA CGT CC7OPU-01GGA CGT GT G122OPV-10GGA CCT GCT G8OPV-11CTC GAC AGA G5OPV-19GGG TGT GCA G122OPX-03TGC CGC AGT G121	OPL-08	AGC AGG TGG A	6			
OPM-13GGT GGT CAA G122OPN-16AAG CGA CCT G16	OPL-17	AGC CTG AGC C	10	1		
OPN-16AAG CGA CCT G16OPO-07CAG CAC TGA C9OPO-10TCA GAG CGC C14OPP-16CCA AGC TGC C81OPQ-05CCG GT CTT G41POR-08CCC GTT GCC T61OPR-10CCA TTC CCC A81OPR-11CCA TTC CCC A81OPR-12CG GAG GT GT C710OPR-13CCG TAC GTA G91OPR-04CAC CCC CTT G52OPL-01ACG GAC GTC A122OPU-01ACG GAC GTC G71OPU-01ACG GAC GTC A22OPV-10GGA CCT GCT G82OPV-11CTC GAC AGA G52OPV-19GGG TGT GCA G122OPV-19GGG GTG GCA GT G122OPV-19GGG CAGG CG GT G121	OPM-13	GGT GGT CAA G	12	2		
OPO-07 CAG CAC TGA C 9 OPO-10 TCA GAG CGC C 14 OPP-16 CCA AGC TGC C 8 1 OPQ-05 CCG GT CTT G 4 1 POR-08 CCC GTT GC C 6 1 POR-09 TGA GCA CGA G 12 1 OPR-10 CCA TTC CCC A 8 1 OPR-16 CTC TGC GCG T 10 1 OPR-16 CTC TGC GCG T 10 1 OPR-17 CCG TAC GTA G 9 1 OPS-04 CAC CCC CTT G 5 2 OPU-01 ACG GAC GTC A 12 1 OPV-07 GAA GCC AGC C 7 1 OPV-01 ACG GAC GTC A 12 2 OPV-07 GAA GCC AGC C 7 1 1 OPV-10 GGA CT GCT G 8 1 1 OPV-10 GGA CT GCT G 8 1 1 OPV-11 CTC GAC AGA G 5 5 1 <td>OPN-16</td> <td>AAG CGA CCT G</td> <td>16</td> <td></td>	OPN-16	AAG CGA CCT G	16			
OPO-10TCA GAG CGC C14OPP-16CCA AGC TGC C81OPQ-05CCG CGT CTT G41POR-08CCC GTT GCC T61POR-09TGA GCA CGA G121OPR-10CCA TTC CCC A81OPR-14CAG GAT TCC C710OPR-17CCG TAC GTA G92OPS-04CAC CCC CTT G52OPU-01ACG GAC GTC A122OPV-07GGA GCC AGC C70OPV-10GGA CCT GCT G82OPV-11CTC GAC AGA G52OPV-19GGG TGT GCA G122OPX-03TGG CGC AGT G121	OPO-07	CAG CAC TGA C	9			
OPP-16CCA AGC TGC C81OPQ-05CCG GT CTT G41POR-08CCC GTT GCC T612OPR-09TGA GCA CGA G121OPR-10CCA TTC CCC A81OPR-16CTC TGC GCG T101OPR-17CCG TAC GTA G91OPS-04CAC CCC CTT G52OPU-01ACG GAC GTC A122OPV-07GAA GCC AGC C71OPV-10GGA CT GCT G82OPV-11CTC GAC AGA G52OPV-19GGG TGT GCA G122OPX-03TGG CGC AGT G121	OPO-10	TCA GAG CGC C	14			
OPQ-05CCG CGT CTT G4POR-08CCC GTT GCC T6POR-09TGA GCA CGA G12OPR-10CCA TTC CCC A8OPR-14CAG GAT TCC C7OPR-16CTC TGC GCG T10OPR-17CCG TAC GTA G9OPS-04CAC CCC CTT G5OPU-01ACG GAC GTC A12OPV-07GAA GCC AGC C7OPV-10GGA CCT GCT G8OPV-11CTC GAC AGA G5OPV-19GGG TGT GCA G12OPV-19GGG CGT GCA G12OPX-03TGG CGC AGT G121TGC GCC AGT G12	OPP-16	CCA AGC TGC C	8	1		
POR-08 CCC GTT GCC T 6 POR-09 TGA GCA CGA G 12 OPR-10 CCA TTC CCC A 8 OPR-14 CAG GAT TCC C 7 OPR-16 CTC TGC GCG T 10 OPR-17 CCG TAC GTA G 9 OPS-04 CAC CCC CTT G 5 OPU-01 ACG GAC GTC A 12 OPU-01 ACG GAC GTC G 7 OPV-10 GGA CCT GCT G 5 OPV-11 CTC GAC AGA G 5 OPV-11 CTC GAC AGA G 5 OPV-19 GGG TGT GCA G 12 OPX-03 TGG CGC AGT G 12 OPX-03 TGG CGC AGT G 12	OPO-05	CCG CGT CTT G	4			
POR-09TGA GCA CGA G12OPR-10CCA TTC CCC A8OPR-14CAG GAT TCC C7OPR-16CTC TGC GCG T10OPR-17CCG TAC GTA G9OPS-04CAC CCC CTT G5OPT-07GGC AGG CTG T16OPU-01ACG GAC GTC A12OPV-10GGA CCT GCT G8OPV-11CTC GAC AGA G5OPV-19GGG TGT GCA G12OPV-03TGG CGC AGT G12OPV-04CAC GCC GCT G12OPV-19GGG TGT GCA G12OPV-19GGG CCC GCT G12OPX-03TGG CGC AGT G12OPX-04CAC GCC GCT G12	POR-08	CCC GTT GCC T	6			
OPR-10CCA TTC CCC A8OPR-14CAG GAT TCC C7OPR-16CTC TGC GCG T10OPR-17CCG TAC GTA G9OPS-04CAC CCC CTT G5OPT-07GGC AGG CTG T162OPU-01ACG GAC GTC A122OPV-07GAA GCC AGC C70OPV-10GGA CCT GCT G82OPV-11CTC GAC AGA G52OPV-19GGG TGT GCA G122OPX-03TGG CGC AGT G121	POR-09	TGA GCA CGA G	12			
OPR-14 CAG GAT TCC C 7 OPR-16 CTC TGC GCG T 10 OPR-17 CCG TAC GTA G 9 OPS-04 CAC CCC CTT G 5 OPT-07 GGC AGG CTG T 16 2 OPV-01 ACG GAC GTC A 12 7 OPV-10 GGA CT GCT G 8 7 OPV-11 CTC GAC AGA G 5 5 OPV-19 GGG TGT GCA G 12 2 OPX-03 TGG CGC AGT G 12 1	OPR-10	CCA TTC CCC A	8			
OPR-16 CTC TGC GCG T 10 OPR-17 CCG TAC GTA G 9 OPS-04 CAC CCC CTT G 5 OPT-07 GGC AGG CTG T 16 2 OPU-01 ACG GAC GTC A 12 7 OPV-07 GAA GCC AGC C 7 7 OPV-10 GGA CT GCT G 8 7 OPV-11 CTC GAC AGA G 5 5 OPV-19 GGG TGT GCA G 12 2 OPX-03 TGG CGC AGT G 12 1	OPR-14	CAG GAT TCC C	7			
OPR-17 CCG TAC GTA G 9 OPS-04 CAC CCC CTT G 5 OPT-07 GGC AGG CTG T 16 2 OPU-01 ACG GAC GTC A 12 7 OPV-07 GAA GCC AGC C 7 7 OPV-10 GGA CT GCT G 8 7 OPV-11 CTC GAC AGA G 5 7 OPV-19 GGG TGT GCA G 12 2 OPX-03 TGG CGC AGT G 12 1	OPR-16	CTC TGC GCG T	10			
OPS-04 CAC CCC CTT G 5 OPT-07 GGC AGG CTG T 16 2 OPU-01 ACG GAC GTC A 12 OPV-07 GAA GCC AGC C 7 OPV-10 GGA CCT GCT G 8 OPV-11 CTC GAC AGA G 5 OPV-19 GGG TGT GCA G 12 2 OPX-03 TGG CGC AGT G 12 1	OPR-17	CCG TAC GTA G	9			
OPT-07 GGC AGG CTG T 16 2 OPU-01 ACG GAC GTC A 12 OPV-07 GAA GCC AGC C 7 OPV-10 GGA CCT GCT G 8 OPV-11 CTC GAC AGA G 5 OPV-19 GGG TGT GCA G 12 2 OPX-03 TGG CGC AGT G 12 1	OPS-04	CAC CCC CTT G	5			
OPU-01 ACG GAC GTC A 12 OPV-07 GAA GCC AGC C 7 OPV-10 GGA CCT GCT G 8 OPV-11 CTC GAC AGA G 5 OPV-19 GGG TGT GCA G 12 2 OPX-03 TGG CGC AGT G 12 1	OPT-07	GGC AGG CTG T	16	2		
OPV-07 GAA GCC AGC C 7 OPV-10 GGA CCT GCT G 8 OPV-11 CTC GAC AGA G 5 OPV-19 GGG TGT GCA G 12 2 OPX-03 TGG CGC AGT G 12 1	OPU-01	ACG GAC GTC A	12			
OPV-10 GGA CCT GCT G 8 OPV-11 CTC GAC AGA G 5 OPV-19 GGG TGT GCA G 12 2 OPX-03 TGG CGC AGT G 12 1	OPV-07	GAA GCC AGC C	7			
OPV-11 CTC GAC AGA G 5 OPV-19 GGG TGT GCA G 12 2 OPX-03 TGG CGC AGT G 12 1	OPV-10	GGA CCT GCT G	8			
OPV-19 GGG TGT GCA G 12 2 OPX-03 TGG CGC AGT G 12 1	OPV-11	CTC GAC AGA G	5			
OPX-03 TGG CGC AGT G 12 1	OPV-19	GGG TGT GCA G	12	2		
	OPX-03	TGG CGC AGT G	12	-		
OPX-19 TGG CAA GGC A 12	OPX-19	TGG CAA GGC A	12			
OPZ-08 GGG TGG GTA A 9	OPZ-08	GGG TGG GTA A	9			

The 44 primers produced 420 well-defined fragments; among them, 402 bands were monomorphic (97.2%) and 18 bands were polymorphic (2.8%) (Table 2). A total of 40,320 fragments (number of propagules x number of bands obtained) were produced with an aver-

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age of 9.5 bands per primer. The length of the amplification products was between 298 and 5090 bp. The number of bands per primer varied from 4 (OPQ-05) to 16 (OPN-16 and OPT-07) (Table 1). The polymorphic fragments were produced by 12 primers (OPA-01, OPA-20, OPB-01, OPB-19, OPC-19, OPF-13, OPL-17, OPM-13, OPP-16, OPT-07, OPV-19, and OPX-03). Among the primers that identified polymorphisms, OPA-1, OPA-20, OPB-1 and OPB-20 produced one polymorphic band and OPF-13 amplified a maximum of 3 bands (Table 1). The polymorphism generated by OPC-19 was observed in 10 samples with genetic variation (38%). Three variants were detected by the primer OPL-17; OPF-13, OPP-16, and OPT-7 were each able to identify two independent variants. OPA-20, OPA-1, OPB-1, OPB-19, OPN-13, OPV-19, OPX-3 independently identified one somaclonal variant (Table 3). Examples of RAPD profile of mother plant and micropropagated plantlets are shown in Figure 1.

Table 2. Summary of random amplified polymorphic DNA (RAPD) products from micropropagation propagules and original plant of Ananas comosus var. bracteatus used in the present study.

Description	RAPD	
Number of primers tested	520	
Number of primers selected	44	
Number of primers that showed polymorphisms	12	
Total number of amplified bands	420	
Total number of monomorphic amplicons	402	
Total number of polymorphic amplicons	18	
Percent of polymorphic bands	2.8%	
Size of amplified bands	298-5090 bp	
Average number of polymorphic bands per primer	1.5	
Average number of bands per primer	9.5	

Table 3. Presence (+) or absence (-) of polymorphic RAPD bands generated by primers in *Ananas comusus* var. *bracteatus* propagules cultured in media with different 6-benzylaminopurine (BAP) concentrations, after 120 days.

Primer	Polymorphic regenerants																								
	М	5	7	15	16	26	27	28	38	39	50	51	52	53	54	58	63	64	66	74	75	78	86	93	95
OPA-01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
OPA-20	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPB-01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
OPB-01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	$^+$	-	-	-	-	-	-	-	-	-
OPB-19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
OPC-19	-	-	-	+	+	+	$^+$	$^+$	+	+	+	+	-	-	-	-	$^+$	-	-	-	-	-	-	-	-
OPF-13	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPF-13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
OPF-13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
OPL-17	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-
OPM-13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
OPM-13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
OPP-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
OPT-07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
OPT-07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
OPV-19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
OPV-19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
OPX-03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-

M: mother plant; propagules 5, 7, 15, and 16 (0.125 mg/L BAP); propagules 26, 27, 28, 38, and 39 (0.25 mg/L BAP); propagules 50, 51, 52, 53, 54, and 58 (0.5 mg/L BAP); propagules 63, 64, 66, 74, and 75 (1.0 mg/L BAP); propagules 78, 86, 93, and 95 (2.0 mg/L BAP).

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Figure 1. Molecular pattern of micropropagated plantlets revealed by RAPD on 1.5% agarose gels, with the primers: **A.** OPC-19; **B.** OPF-13, and **C.** OPM-13. The arrows indicate polymorphic markers. The propagules were maintained in basal medium, liquid formulation, supplemented with BAP, subcultured every 30 days, for 120 days. M = mother plant. Lanes 1-96 = propagules regenerated *in vitro* in medium containing BAP.

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The percentage of polymorphism detected in Ananas comosus var. bracteatus in BAPcontaining medium was estimated to be 21 to 42%, confirming the occurrence of variation during the micropropagation process. The variation of the electrophoretic pattern found in genomic DNA of regenerated propagules, after 120 days of culture, was probably due to the medium supplementation with BAP, according to a contingency test that showed a significant chi-square value (Table 4). This finding is consistent with those of Evans et al. (1984), Rani et al. (1995), Salvi et al. (2001), Feuser et al. (2003), and Costa and Zaffari (2005). In Ananas bracteatus cv. striatus, phenotypic variations of 52% albino plants and 20.5% green plants were described by Costa and Zaffari (2005). In commercial pineapple, Feuser et al. (2003) observed a more reduced rate of somaclonal variation among plantlets regenerated from *in vitro* culture using either stationary liquid medium or a temporal immersion system. These authors utilized 10 RAPD primers and detected 7.5 and 5.0% of somaclonal variants for the stationary and temporal immersion systems, respectively. However, in this latter study, the authors did not state the period of culture in which the samples were taken for RAPD analysis. The culture period directly influences the appearance of somaclonal variation (Skirvin et al., 1994), and the range of variation of 1 to 3% is expected in the process of micropropagation. Some authors have indicated that the treatment used in tissue culture, with high growth rate, may increase the variant numbers (Bairu et al., 2006). The regeneration systems from organized meristems, such as shoot tip and axillary buds, are considered to be the most efficient methods to guarantee genetic integrity of the micropropagated material. The regeneration methods from leave explants (Kawiak and Lojkowska, 2004) and callus (Skirvin et al., 1994) are considered to be less stable permitting the occurrence of genetic variation. In Drosera binata, plantlets regenerated through shoot tip preserve the genetic integrity of micropropagated plants. In Curcuma longa, rhizome bud explants used to establish cultures show genetic homogeneity in the regenerated propagules, when comparing them with the mother plant (Tyagi et al., 2007). However, plants regenerated from leaf base callus have shown variation at the DNA level during *in vitro* culture (Salvi et al., 2001; Tyagi et al., 2007).

propugates and original plant or manus comosas val. or accurates as a result of the interopropugation process.										
BAP concentration (mg/L)	Normal plants	Variant plants	χ^2	Р						
0.125	15	4	10.3	0.001-0.005						
0.25	15	4	10.3	0.001-0.005						
0.5	11	8	55.0	< 0.001						
1.0	14	5	18.1	< 0.001						
2.0	14	5	18.1	<0.001						

Table 4. Chi-square values (χ^2) and probability (P) of contingency test in the determination of genetic variability of propagules and original plant of *Ananas comosus* var. *bracteatus* as a result of the micropropagation process.

The propagules were maintained *in vitro* in liquid medium supplemented with 6-benzylaminopurine (BAP), subcultured every 30 days and evaluated by RAPD after 120 days.

In this study, RAPD was effective in showing the variations that may occur as a result of mutations during the micropropagation process of *Ananas comosus* var. *bracteatus* in BAPsupplemented medium, and that could be useful in detecting the presence of genetic variation in the initial stages of plant development. Also, these findings could be applied in the breeding of this species. The regenerated plants are currently growing in a greenhouse, and later they will be transferred to the field and screened for morphological variation.

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