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Ploidy estimation and pre-selection of banana autotetraploids after in vitro polyploidy induction

Estimativa da ploidia e pré-seleção de autotetraploides de bananeira após indução da poliploidia *in vitro*

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ABSTRACT - This study aimed to evaluate the efficiency of the parameters fresh leaf mass and stomatal density in the estimation of ploidy and pre-selection of putative banana autotetraploids. Young plants of the diploid cultivar Ouro (AA), previously subjected to in vitro polyploidization with the antimitotic amiprofos-methyl – APM (0, 10, 20, 30, 40, and 60 µM L^{-1}) and colchicine (2.5 mM L^{-1}) were evaluated for survival, height, number of leaves, pseudostem diameter, leaf disc fresh mass, and stomatal density. Ploidy was determined by flow cytometry in a random sample of 200 plants to find the relationship between the genomic content and the analyzed variables. Spearman's correlation analysis showed a strong correlation (0.84**) between leaf disc fresh mass and tetraploid plants, as well as an absence of significant correlation between stomatal density and ploidy levels. Thus, the pre-selection of plants was carried out using a reference value of leaf disc fresh mass and resulted in 688 plants pre-selected as putative tetraploids. Among them, 318 were confirmed as tetraploids, 291 as diploids, and 79 as mixoploids after flow cytometry analysis. The pre-selection efficiency was 46.2% of tetraploid plants from the total pre-selected. The results indicate that the use of leaf disc fresh mass is a simple, practical, and promising method to estimate ploidy when a high number of plants is obtained in in vitro banana polyploidization studies, reducing the total number of plants to have confirmed ploidy.

Keywords: *Musa acuminata*. Leaf fresh mass. Stomatal density. Amiprofos-methyl (APM). Colchicine.

RESUMO - Este estudo objetivou avaliar a eficiência dos parâmetros massa fresca foliar e densidade estomática na estimativa da ploidia e pré-seleção de autotetraploides putativos de bananeira. Plantas jovens da cultivar diploide Ouro (AA), previamente submetidas à poliploidização in vitro com os antimitóticos amiprofos -metil - APM (0, 10, 20, 30, 40 e 60 µM L^{-1}) e colchicina (2,5 mM L^{-1}) foram avaliadas quanto à sobrevivência, altura, número de folhas, diâmetro do pseudocaule, massa fresca de disco foliar e densidade estomática. Determinou-se a ploidia, via citometria de fluxo, numa amostra aleatória de 200 plantas, a fim de encontrar relação entre o conteúdo genômico e as variáveis analisadas. Por meio da análise de correlação de Spearman, verificou-se forte correlação (0,84**) entre a massa fresca de disco foliar e as plantas tetraploides, bem como ausência de correlação significativa entre a densidade estomática e os níveis de ploidia. Assim, a pré-seleção das plantas foi feita mediante um valor de referência de massa fresca de disco foliar e resultou em 688 plantas pré-selecionadas como tetraploides putativos. Destas, 318 foram confirmadas como tetraploides, 291 como diploides e 79 mixoploides, após análise de citometria de fluxo. A eficiência de pré-seleção foi de 46,2% de plantas tetraploides do total pré-selecionado. Os resultados indicam que o uso da massa fresca de disco foliar é um método simples, prático e promissor para estimar a ploidia quando é obtido um elevado número de plantas em trabalhos de poliploidização in vitro da bananeira, reduzindo o total de plantas a ter a ploidia confirmada.

Palavras-chave: *Musa acuminata*. Massa fresca foliar. Densidade estomática. Amiprofós-metil (APM). Colchicina.

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INTRODUCTION

Banana (*Musa* spp.) varieties are classified according to their ploidy level as diploid (2n=2x), triploid (2n=3x), and tetraploid (2n=4x). Triploids are the most cultivated because they have higher productivity and fruit quality, but they have a narrow genetic base, different degrees of sterility, and susceptibility to diseases such as black and yellow Sigatoka and Panama disease (SILVA et al., 2013). Strategies for genetic improvement of banana involve sexual hybridization between genotypes with different ploidy levels and phenotypic selection of viable offspring. However, factors such as parthenocarpy, low fertility, aneuploidy, and low seed viability due to meiosis abnormalities make improvement difficult, especially in triploid cultivars (BROWN et al., 2017; AMAH et al., 2019; SANTOS et al., 2019).

Polyploidy induction is an alternative approach to conventional banana improvement. It consists of inducing chromosome duplication in promising diploids through the application of antimitotic agents (inhibitors of mitotic spindle formation), followed by obtaining fertile autotetraploid plants that can be used as parents in crosses with improved diploids, finally originating a secondary triploid.

Thus, it allows introducing resistance to diseases and obtaining hybrids with fruit characteristics similar to those of varieties of interest (BAKRY et al., 2007; BORGES et al., 2016).

At least three subcultures are indicated for in vitro polyploidization, after the application of the antimitotic agent and establishment of treated explants, in order to reduce the occurrence of mixoploidy (plant cells or organs with different ploidy levels) in regenerated plants and increase cell proliferation with the number of chromosomes doubled. Then, ex vitro acclimatization and ploidy verification are performed by counting the number of chromosomes or using flow cytometry (DHOOGHE et al., 2011; OLIVEIRA et al., 2013).

However, time and resources can be optimized using indirect methods to estimate ploidy and pre-select possible polyploids when dealing with hundreds and even thousands of obtained plants. Morphological and anatomical traits are associated with polyploid plants because they have larger cells and organs and are usually more robust than diploid ones (COSTA et al., 2011; JESUS et al., 2016; MANDAIL et al., 2022). Parameters such as leaf blade thickness, which can be indirectly measured by leaf disc mass, cell size, and stomata size and density (GANGA; CHEZHIYAN, 2002; FOSCHI et al., 2013; TOMÉ et al., 2016), can be used to considerably reduce the number of plants to be analyzed for ploidy determination, facilitating polyploidization research.

Considering the lack of studies verifying the applicability of morphological parameters in the identification of putative polyploids within the set of treated plants, this study aimed to evaluate the efficiency of leaf fresh mass and stomatal density in estimating ploidy and pre-selection of autotetraploid plants of banana of the cultivar Ouro (AA).

MATERIAL AND METHODS

The experiment was carried out at Embrapa Cassava & Fruits (12°40′9″ S and 39°06′22″ W, at 220 m altitude), in Cruz das Almas, BA. The diploid (AA) cultivar Ouro (*Musa acuminata* Colla), which presents resistance to black Sigatoka, rusticity, and small fruits, but high sensory quality for fresh consumption, was evaluated (SILVA et al., 2016).

Polyploidization

Shoot apexes (1 cm) were treated with the antimitotics amiprofos-methyl – APM (0, 10, 20, 30, 40, and 60 μM L^{-1}) and colchicine (2.5 mM L^{-1}) in two exposure periods: 24 and 48 hours. This dose of colchicine showed better results in polyploidization studies with banana (GANGA; CHEZHIYAN, 2002; RODRIGUES et al., 2011) and was used as a comparison with APM. For the application of these substances, 30 explants per treatment were immersed in liquid MS culture medium, added with antimitotic solutions, and kept under mechanical stirring at 120 rpm for 24 and 48 hours in a growth room with a 16-hour photoperiod and temperature of 25±2 °C. Then, they were maintained under stirring in sterile water for 24 hours and subsequently established in vitro.

After establishment, three subcultures, or multiplication cycles, were carried out at 45-day intervals. MS culture medium gelled with 2.4 g L^{-1} of Phytagel (Sigma-Aldrich, St. Louis, MO, USA), supplemented with 2.5 mg L^{-1} of 6-benzylaminopurine (BAP) and pH adjusted to 5.8±0.1, was used in all these steps. The characterization of responses to polyploidy induction during in vitro establishment and subcultures is described in Borges et al. (2016).

Acclimatization and early growth

A total of 3,526 obtained seedlings were transferred to a greenhouse after the in vitro steps and planted in tubes containing a mixture of soil, commercial substrate, vermiculite, and sand. After 20 days of planting, the plants were transferred to a screen house with 50% shading. The variables survival (%), plant height (cm), pseudostem diameter (cm), number of expanded leaves, the fresh mass of two leaf discs (g), and stomatal density (mm^2) were measured at 150 days after acclimatization.

The data were subjected to analysis of variance ($p<0.05$) and polynomial regression models were adjusted for the mean doses of antimitotics. Analyses were performed using the statistical software R (R DEVELOPMENT CORE TEAM, 2020).

Pre-selection: Leaf mass and stomatal density

A random sample of 200 plants was evaluated for leaf fresh mass, stomatal density, and ploidy level using a flow cytometer to indicate the pre-selection method of putative autotetraploids. The third expanded leaf of each plant was collected early in the morning and maintained in closed styrofoam until weighing to quantify the leaf parameters. Two leaf discs per leaf per plant were extracted from the median region of the leaf blade using a 1.75 cm diameter punch and weighed on a precision scale (0.001 g) to quantify the leaf fresh mass.

The epidermis printing technique, in which three random fragments of the abaxial portion of the same leaf used to determine the leaf mass were placed on a glass slide containing a drop of universal instant adhesive (cyanoacrylate ester) for three minutes, was used to determine the stomatal density. The stomata were counted under an optical microscope in three fields of view for each impression in the pre-established area of 0.25 mm^2, totaling nine counts per slide per plant. Thus, the density per mm^2 was determined by multiplying the mean counts by four.

The collected data were subjected to analysis of variance ($p<0.05$) and the means were compared by Tukey's test at 5% probability. Additionally, Spearman's correlation coefficient was determined to verify the degree of association between leaf fresh mass and stomatal density with ploidy levels. All assessments were performed using the R statistical software (R DEVELOPMENT CORE TEAM, 2020). The pre-selected plants were transferred to 3-L polyethylene bags filled with soil, vermiculite, and commercial substrate for

further confirmation of ploidy by flow cytometry.

Flow cytometry

The flow cytometry technique was used to determine the ploidy of the evaluated plants to guide the pre-selection and, subsequently, the pre-selected plants. For this purpose, approximately 60 mg of leaf tissue from the middle region of young banana leaves and the internal reference standard *Citrus sunki* hort. ex Tanaka (2C = 0.745 pg) (CARVALHO et al., 2016) were ground in a Petri dish with 1 mL of ice-cold LB01 buffer for the release of nuclei. The suspension was filtered through a 30-µm membrane of the Partec CellTrics® filter and the nuclei were stained by adding 25 µL of 1 mg mL^{-1} propidium iodide solution. At least 10,000 events (nuclei) were counted per sample in an Attune® Acoustic Focusing cytometer (Life Technologies). Histograms were obtained with the Attune Cytometric software version 1.2.5. The nuclear DNA content (pg) of the plants was estimated using the ratio between the fluorescence intensities of the G1 nuclei of the reference standard (*C. sunki*) and the G1 nuclei of the sample, multiplying this ratio by the amount of DNA of the reference standard.

RESULTS AND DISCUSSION

During acclimatization, all plants obtained after polyploidy induction were evaluated for survival, growth, leaf mass, and stomatal density. The variables survival, leaf disc fresh mass, and stomatal density in the treatments with the antimitotic APM showed significant differences only for the isolated factor antimitotic concentration (Figure 1). Plant survival was reduced to below 50% at 60 µM L^{-1} with increasing APM concentration. Regression equations with biological significance could not be adjusted for all variables due to data dispersion, a common fact in studies involving in vitro cultivation.

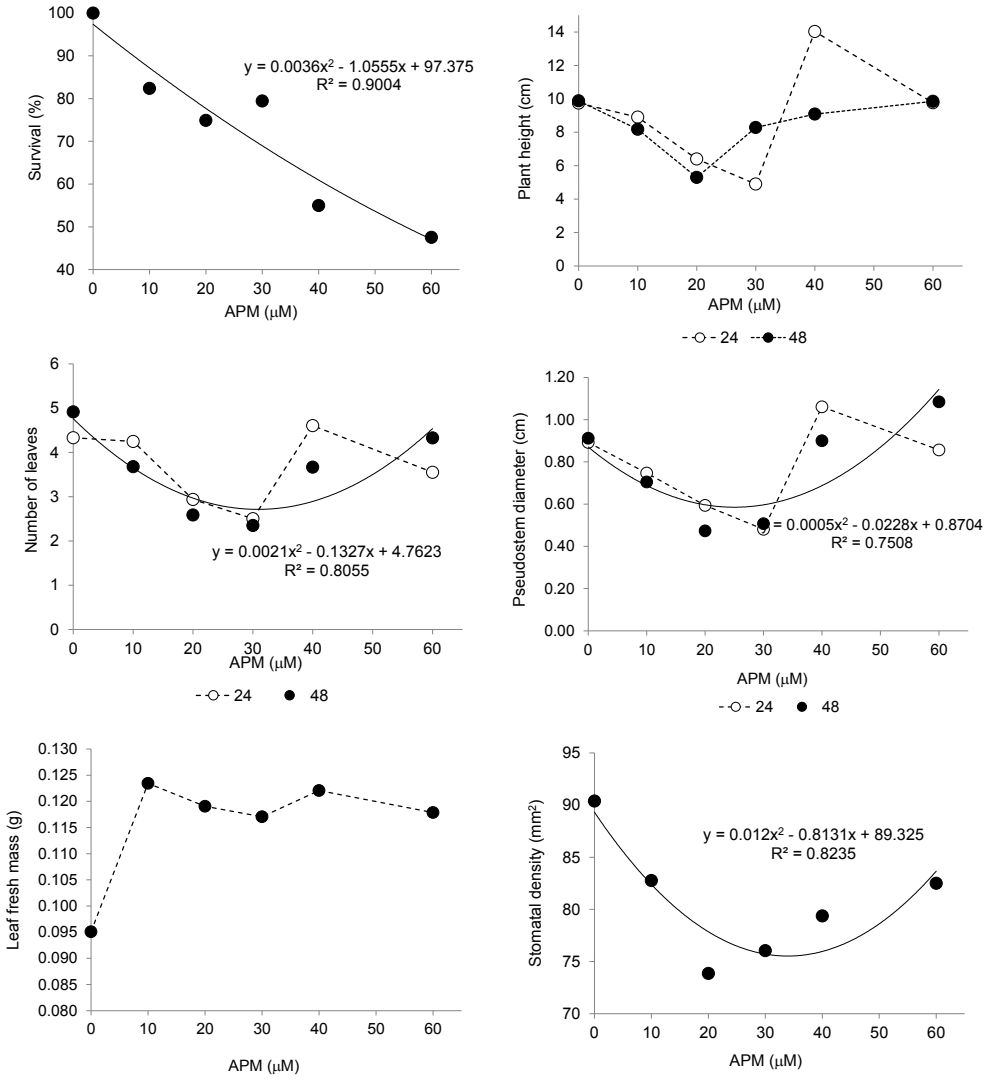

Figure 1. Percentage of survival, plant height (cm), number of leaves, pseudostem diameter (cm), leaf fresh mass (g), and stomatal density (mm^2) of banana cv. Ouro submitted to in vitro polyploidization with amiprofos-methyl (APM) after 150 days of acclimatization.

The dimension of the effects caused by antimitotics is widely variable and directly proportional to their degree of toxicity due to their way of acting. Colchicine is the most used antimitotic agent for inducing polyploidy (SATTLER; CARVALHO; CLARINDO, 2016), and is commonly used in the polyploidization of crops. However, it causes side effects such as sterility, abnormal growth, and mutations, in addition to being highly toxic to humans due to its affinity with animal cell microtubules (DHOOGHE et al., 2011; MIRI, 2020). These disadvantages encouraged research in search of less toxic cell cycle inhibitors, such as some herbicides, including oryzalin, trifluralin, and amiprofos-methyl (RODRIGUES et al., 2011; MELCHINGER et al., 2016; HOOGHVORST; RIBAS; NOGUÉS, 2020; WANG et al., 2020; KONDO et al., 2020; ALAVI; MAROUFI; MIRZAGHADERI, 2022).

The concentration and time of exposure to the antimitotic are the most important parameters in chromosome duplication protocols (ENG; HO, 2019), requiring tests to determine the best combination for each crop, including variety/cultivar. Low doses are generally not efficient to induce polyploidy, while high doses cause toxic effects, causing abnormal growth, reduced viability, and high mortality (TROJAK-GOLUCH et al., 2021). Kondo et al. (2020) induced chromosome duplication in an interspecific hybrid of *Dendrobium* and reported a reduction in the survival and growth of regenerated seedlings as APM concentration increased, with no significant variation in terms of exposure time. Foschi et al. (2013) also found a reduction in the percentage of survival in *Allium cepa*, obtaining around 50% survival in the treatment with the highest APM concentration, as in the present study.

Similar values were obtained for the number of leaves and pseudostem diameter in the two periods of exposure, with an increase in these variables being observed at concentrations of 40 and 60 µM L^{-1}, mainly at 48 h of exposure to APM. Considering that these treatments caused higher lethality, probably the plants that managed to survive benefited from the fact that mitosis-inhibiting herbicides can stimulate plant growth when used at low concentrations (GANGA; CHEZHIYAN, 2002).

Polyploidy confers changes to plants, such as an increase in cell nuclei, cells, and organs (SATTLER; CARVALHO; CLARINDO, 2016). Thus, the trend of control plants, which were entirely made up of diploid plants, to present a lower mean value of leaf fresh mass was confirmed (Figure 1). Higher stomatal density was also verified in these plants since one of the nucleotide effects associated with increased ploidy is the increase in stomatal size and, therefore, polyploid plants tend to have lower stomatal density than diploid ones (MANDAIL et al., 2022). However, a parabolic behavior is observed in this variable as a function of antimitotic doses.

The 24-hour variation in the time of exposure to colchicine did not provide significant differences in the analyzed variables during the initial plant growth (Table 1). However, the treatment with 48 hours of exposure to the antimitotic resulted in a high number of plants with distinct morphological characteristics, such as reduced growth, markedly oblong leaves, leaves with a leathery texture, and differentiated architecture.

The mechanism of induction of polyploidy occurs through the ability of antimitotic agents to interrupt the cell cycle, especially during the late stage of metaphase, preventing the normal polymerization of microtubules through the formation of complexes with tubulin, the protein that constitutes them. The mitotic spindle is not formed without the polymerization of microtubules and, consequently, the separation of metaphase chromosomes does not occur, resulting in an increased ploidy level (SALMA; KUNDU; MANDAL, 2017). Thus, the disorders that occur during mitosis result in some plants with duplicated genetic content but there is also the formation of plants that present various abnormalities during growth due to chromosomal mutations.

Table 1. Leaf and initial growth parameters in plants of banana cv. Ouro submitted to polyploidization with colchicine at exposure times of 24 and 48 hours after 150 days of acclimatization.

Colchicine (2.5 mM L^{-1})	24 h	48 h
Survival (%)	58.36 a	64.17 a
Plant height (cm)	9.2 a	8.1 a
Number of leaves	3.3 a	3.8 a
Pseudostem diameter (cm)	0.98 a	0.87 a
Leaf fresh mass (g)	0.1224 a	0.1196 a
Stomatal density (mm^2)	68.44 a	65.09 a

Means followed by the same lowercase letter in the row do not differ statistically from each other by Tukey's test at a 5% significance.

Ploidy was determined by estimating the DNA content via flow cytometry in 200 randomly sampled plants aiming to indicate a practical and effective way of carrying out the pre-selection of putative autotetraploids. Figure 2 shows the histograms that represent the obtained ploidy levels. The peaks on the left correspond to the reference standard used to calculate the DNA content of the evaluated samples, and those on the right correspond to the samples of banana plants. The

results of the flow cytometry analyses were diploid plants, with 2C DNA content (Figure 2A), tetraploid plants with 4C DNA (Figure 2B), and mixoploid plants with two peaks, corresponding to the 2C and 4C DNA contents (Figure 2C). Among the analyzed plants, 114 were identified as diploid (2x), 74 were tetraploid (4x), and 12 were mixoploid (2x+4x).

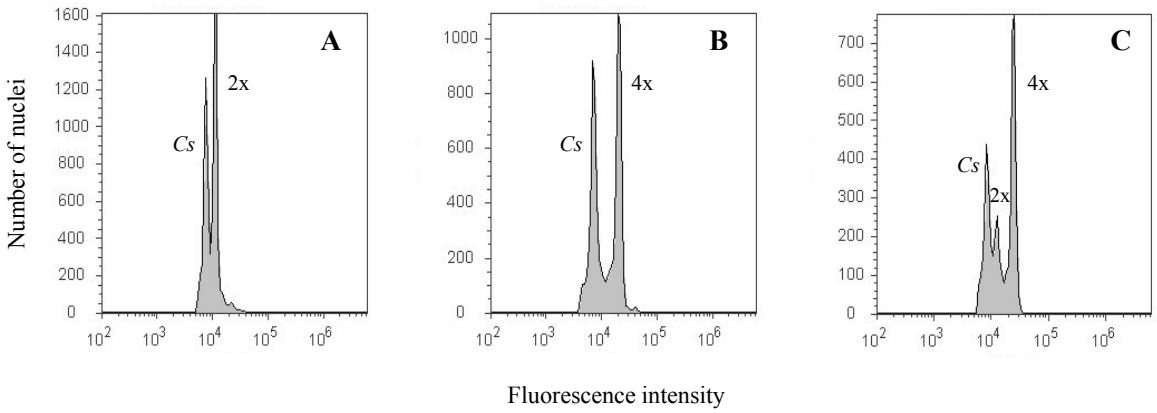

Figure 2. Histograms obtained after flow cytometry for quantification of the nuclear DNA content of leaves of banana cv. Ouro submitted to polyploidy induction, using *Citrus sunki* (Cs) as the reference standard. A – diploid plants (2x); B – autotetraploid plants (4x); C – mixoploid plants (2x+4x).

The evaluation of leaf and growth characteristics as a function of ploidy showed significant variation only for leaf disc fresh mass (LDFM) and stomatal density (SD) (Table 2). Diploid plants presented lower leaf disc fresh mass (LDFM) compared to tetraploid and mixoploid plants, in addition to higher stomatal density (SD). Tetraploid and mixoploid plants showed no difference in terms of leaf mass. However, mixoploids had lower stomatal density than tetraploids, demonstrating that SD has higher oscillation than LDFM.

Table 2. Leaf and initial growth parameters as a function of ploidy level in plants of banana cv. Ouro submitted to in vitro polyploidization after 150 days of acclimatization.

	HGT	NL	DIAM	LDFM	SD
Diploid	10.39 a	3.43 a	0.66 a	0.0974 b	86.73 a
Tetraploid	7.68 a	3.35 a	0.61 a	0.1275 a	76.80 b
Mixoploid	7.25 a	3.25 a	0.48 a	0.1162 a	62.36 c
CV%	37.17	21.35	25.39	10.2	10.24
Overall average	8.44	3.34	0.58	0.1137	75.30

Means followed by the same lowercase letter in the columns do not differ statistically from each other by Tukey's test at a 5% significance. HGT: plant height; NL: number of leaves; DIAM: pseudostem diameter; LDFM: leaf disc fresh mass; SD: stomatal density.

Considering that both LDFM and SD differ between plants as a function of ploidy, Spearman's correlation coefficient was applied to determine the degree of association between these variables (Table 3). A strong positive correlation with high significance (0.85**) was observed between LDFM and tetraploid plants, and a highly significant but negative correlation was found with diploid plants (−0.79). This result shows that the higher the LDFM, the higher the probability that the plant is tetraploid and the lower the LDFM, the higher the probability that the plant is diploid.

Table 3. Spearman's correlation coefficients between the probability of occurrence of diploid, tetraploid, and mixoploid plants of banana cv. Ouro versus stomatal density (SD) and leaf disc fresh mass (LDFM).

	LDFM	SD
Diploid	−0.7884**	0.3053^{ns}
Tetraploid	0.8481**	-0.2084^{ns}
Mixoploid	-0.1500^{ns}	-0.3061^{ns}

**Significant at 1% probability by Student's t-test. ^{ns}Not significant.

No correlation was established between stomatal density and ploidy, according to Spearman's coefficient. This result demonstrates that there is no linear association between these two variables, that is, the reduction in stomatal density is not linearly associated with the occurrence of tetraploid individuals, as well as the relationship that the higher the stomatal density, the higher the probability of the plant to be diploid was also not significant.

Polyploid plants usually show phenotypic changes due to an increase in the size of cells and organs, the so-called giga effect, as a consequence of gene duplication (SATTLER; CARVALHO; CLARINDO, 2016; AMAH et al., 2019). Polyploid banana plants usually present arched leaves with a thicker blade, a higher ratio between leaf width and length, larger fruits, and robust, more compact plants with slow growth (BAKRY et al., 2009; KANCHANAPOOM; KOARAPATCHAIKUL, 2012; AMARAL et al., 2015).

Several authors have mentioned stomatal density as one of the morphological parameters that can interfere with the ploidy level in banana, in which polyploid plants are among those with the lowest number of stomata per area (ENG; HO, 2019). Pio et al. (2014) evaluated the size and stomatal density in the estimation of ploidy in banana genotypes submitted to chromosomal duplication and found a great variation in the values and an absence of pattern in the association with ploidy, in which diploids presented 2.6 to 60.6 stomata mm^{-2} and tetraploid had 19.8 to 58.0 stomata mm^{-2}.

Vandenhout et al. (1995) analyzed the correlation between stomatal density and ploidy of banana hybrids and found a significant negative association but with a low correlation coefficient (r=−0.49). The authors also found that the variability of stomata (size and density) is influenced by the genotype, even at the same ploidy level, a result corroborated by Mandail et al. (2022). Thus, there are no reports of the use of stomatal density as a method to effectively pre-select presumably tetraploid plants.

Due to the absence of a linear association between stomatal density and ploidy, a reference value that would allow its use in the pre-selection of plants could not be established. Thus, given the strong correlation found between leaf disc fresh mass and tetraploid plants, as well as its ease of measurement, this variable was used as a pre-selection method for putative autotetraploids. Considering that in the plants analyzed by flow cytometry to guide the pre-selection, the lowest fresh mass value of two leaf discs in those identified as tetraploid was 0.1046 g and the average was 0.1275 g (Table 2), we used 0.105 g as the cut-off point for screening. Thus, all plants that presented LDFM higher than or equal to 0.105 g were pre-selected.

A total of 688 plants were pre-selected as putative tetraploids at the end of flow cytometry analyses, taking leaf fresh mass as a reference, corresponding to 19.51% of the total of 3,526 evaluated plants. This reduction in the number of plants to be examined after acclimatization optimized time and resources, enabling confirmation of ploidy by flow cytometry.

Flow cytometry is a technique that involves analyzing the optical properties (light scattering and fluorescence) of particles (cells, nuclei, chromosomes, and organelles) flowing in a liquid suspension. This principle allows estimating the amount of DNA through comparison with samples of a species used as an internal reference standard (DOLEŽEL; BARTOŠ, 2005). Regarding the chromosome count, it has as its main advantage the detection of mixoploidy and aneuploidy, besides not being necessary to evaluate cells in division.

The analysis of the pre-selected plants showed a total of 318 confirmed as tetraploids, 79 mixoploids, and 291 diploids In the present study, we considered the efficiency of pre-selection based on the number of tetraploid plants relative to the total number of surviving plants after in vitro cultivation. The reliability of the method was tested using a random sample of 100 plants among those that were not pre-selected by leaf fresh mass, which was assessed for ploidy and the result showed 100% of diploid plants.

Table 4 shows the pre-selection efficiency based on leaf disc fresh mass. The percentage of plants selected as possible autotetraploids relative to the total number of plants evaluated per treatment ranged from 11.1% (10 µM of APM for 48 hours of exposure) to 36.6% (2.5 mM of colchicine for 48 hours of exposure). The percentage of tetraploid plants among those pre-selected ranged between 11.1% (20 µM of APM for 48 hours of exposure) and 90.4% (2.5 mM of colchicine for 24 hours of exposure).

The success rate in chromosome duplication studies is generally low. According to Dhooghe et al. (2011), it varies between 15 and 55% and this range is influenced by the variables used in the calculation, such as accounting for obtaining mixoploid plants or only effectively polyploid ones. Azhar, Mak, and Mohd Nazir (1998) screened banana plants by means of stomatal size and density and found 37.5% of autopolyploid plants after flow cytometry but only 7.8% of them consisted of tetraploid and octoploid plants, while the vast majority were mixoploid plants.

Only the number of tetraploid plants was considered relative to the pre-selected total to estimate the efficiency of the method used in this research, and the result was 46.2%. This is an expressive value for studies of this nature and demonstrates that the use of leaf disc fresh mass as a pre-selection parameter for autotetraploid plants after an initial evaluation and establishment of a reference value is a promising method due to its practicality, no need for sophisticated equipment, the immediate result, and, mainly, the verified effectiveness.

Table 4. Efficiency of pre-selection of putative autotetraploids of banana cv. Ouro after in vitro polyploidization with the antimitotics amiprofos -methyl (APM) and colchicine using leaf disc fresh mass as a method of estimating ploidy.

Concentration	24 h exposure			48 h exposure		
APM (µM)	TPSP	%TA	%TETRA	TPSP	%TA	%TETRA
10	53	19.8	47.2	50	11.1	46.0
20	37	19.9	27.0	9	31.5	11.1
30	32	14.2	25.0	13	13.9	15.4
40	132	12.1	53.8	26	21.8	65.4
60	10	17.1	40.0	51	25.9	13.7
Colchicine (mM)						
2.5	21	18.3	90.4	254	36.6	51.6

TPSP: total pre-selected plants; %TA: percentage corresponding to the total acclimatized; %TETRA: percentage of tetraploid plants.

CONCLUSION

Stomatal density does not present an effective result for the pre-selection of putative autotetraploids of banana cv. Ouro because it does not correlate with ploidy level.

Leaf disc fresh mass has a high correlation with ploidy, which allows its use in the pre-selection of autotetraploid plants of banana cv. Ouro after defining a reference value.

Pre-selection efficiency based on leaf disc fresh mass is 46.2% of tetraploid plants relative to the total pre-selected.

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