Short communication

Ploidy investigation of bananas (\textit{Musa} spp.) from the National Banana Germplasm Collection at Rubona–Rwanda by flow cytometry

A. Nsabimana, J. van Staden*

Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

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Abstract

Bananas (\textit{Musa} spp.) are important staple and income-generating crops for millions of people in the highlands of East and Central Africa. This region is considered as a secondary center of banana diversity. The determination of ploidy level in different germplasms is important for better management of genetic diversity and is required before starting concerted breeding programmes. The use of reliable and high-resolution methods such as flow cytometry is recommended. In our study, flow cytometry was used on 89 frozen leaf accessions from the National Banana Germplasm collection at Rubona–Rwanda. Our results indicate that the ploidy level of 65 Highland banana clones analysed, is triploid, as was previously reported using morphological characteristics. However, the clones \textit{Pomme}, \textit{Kamaramasenge}, \textit{Gisubi kayinja}, \textit{Gisubi kagongo}, and \textit{Dibis} previously classified in National Banana Germplasm collection at Rubona as diploid, diploid, diploid, triploid, and tetraploid, respectively, were found to be triploid, triploid, triploid, diploid and triploid. These results should therefore, have great significance in banana germplasm management, for breeding programmes as well as for the \textit{Musa} Germplasm Information System (MGIS).

Keywords: Flow cytometry; Germplasm; \textit{Musa} spp.; Ploidy

Banana and plantain (\textit{Musa} spp.) are important staple and income-generating fruit crops for millions of people in the tropical and subtropical regions of the world (Robinson, 1996; Ssebuliba et al., 2005). The main focus of breeding programmes is to improve the quality of bananas and plantains for consumption. Biotechnological advances have provided information in addition to that obtained through conventional methods, such as screening for seed fertility, interspecific hybridization and ploidy manipulations (Wong et al., 2001; Pillay et al., 2002; Ssebuliba et al., 2005). Exact knowledge of the ploidy of a variety is important to breeders when attempting to manipulate a multi-ploidy crop such as banana (Pillay et al., 2003). Crossing of a triploid banana with a diploid variety generates diploid, triploid, tetraploid, aneuploid and hyperploid progeny (Vuylsteke et al., 1993). In the genus \textit{Musa}, accurate determination of the ploidy by chromosome counting is laborious. A variety of phenotypic traits, including stomatal size, density, and pollen size, are used as alternative approaches to estimate ploidy (Hamill, 1992; Dolezel, 2004). These techniques do not always provide consistent data, mainly due to strong genotypic influences (Vandenhout et al., 1995; Van Duren et al., 1996). Dolezel et al. (1997) demonstrated that rapid and reliable ploidy screening in \textit{Musa} could be performed using DNA flow cytometry.

Flow cytometry has frequently been used in ploidy analysis (Awoleye et al., 1994; Dolezel et al., 1997; Johnson et al., 1998; Egesi et al., 2002; Emshwiller, 2002; Stacy et al., 2002; Beatson et al., 2003; Walker et al., 2005) as it has an advantage over the traditional chromosome counting technique in that it can be used to screen many plants in a short time and can be applied to any plant tissue (Roux et al., 2003; Dolezel and Batros, 2005).

Sgorbati et al. (1986) used fixed tissues of plants for analysis of nuclear DNA content and the cell cycle and found reliable results when compared with flow cytometry of fresh isolated nuclei. As far as can be ascertained from the literature, ploidy determination using frozen banana plant material has not previously been attempted.

* Corresponding author.

E-mail address: vanstadenj@ukzn.ac.za (J. van Staden).

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The objectives of this study were to examine the feasibility of using banana leaves stored at \( -70 \) \(^\circ\)C for ploidy determination to investigate the ploidy level of banana germplasm from Rwanda.

In vitro-grown banana plantlets obtained from the Musa Germplasm Transit Center, International Network for Improvement of Banana and Plantain (INIBAP), Catholic University of Leuven, Belgium, were used for internal calibration of the flow cytometer. The fresh banana leaf material from National Banana Collection at Rubona was swirled in 95\% ethanol for 1 min followed by decontamination in commercial bleach (JIK, 3.5\% [m/v] NaOCl) for 5 min. Samples were then re-immersed in fresh 95\% ethanol for 30 s and stored in plastic bags in a refrigerator at \( 4 \) \(^\circ\)C. The following day, the leaves were transported in a vacuum flask to the Research Centre for Plant Growth and Development (RCPGD) at the University of KwaZulu-Natal, South Africa where they were stored at \(-70\) \(^\circ\)C until used.

The flow cytometric analysis started with the preparation of the samples using a modified Galbraith’s (Galbraith et al., 1983) buffer (45 mM MgCl\(_2\); 36 mM of trisodium–citrate; 22 mM MOPS; 0.1\% of Triton X-100, pH 7.14). Approximately 40 mg of frozen leaves were chopped with a sharp razor blade in a plastic Petri dish containing 2 ml buffer and 10 mM dithiotreitol (DTT) and incubated for 30 min on ice. The suspension was filtered through a 40 \(\mu\)m nylon filter and 500 \(\mu\)l was placed into a plastic tube. After that, the nuclear DNA of the samples was stained by adding 500 \(\mu\)l of 0.02 mg ml\(^{-1}\) propidium iodide. The sample was mixed gently and incubated

<table>
<thead>
<tr>
<th>Name</th>
<th>Genomic group</th>
<th>Origin/donor</th>
<th>Expected ploidy</th>
<th>FC-determined ploidy</th>
<th>CV, %</th>
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<tbody>
<tr>
<td>1. Gisubi Kagongo</td>
<td>?</td>
<td>X</td>
<td>3x</td>
<td>2x</td>
<td>1.1</td>
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<tr>
<td>2. Dibis</td>
<td>?</td>
<td>Burundi</td>
<td>4x</td>
<td>3x</td>
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<tr>
<td>3. Kamaramasenge</td>
<td>AB</td>
<td>X</td>
<td>2x</td>
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<td>2.3</td>
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<td>4. Pommesc</td>
<td>AB</td>
<td>X</td>
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<td>3x</td>
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<tr>
<td>5. Gisubi kayinja</td>
<td>AB</td>
<td>X</td>
<td>2x</td>
<td>3x</td>
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</tbody>
</table>

?: unknown genomic group  
X: unknown origin/donor

INIBAP: International Network for Improvement of Banana and Plantain.

In all cases the coefficients of variation (CV) was less than 5\%. Shown here are only those accessions where the determined ploidy differed from the expected.

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briefly on ice before mixing again. Prior to standardisation of flow cytometry using a known banana diploid from the Musa Germplasm Transit Centre, we used flow check fluorospheres (Beckman Coulter) and controlled the half-peak coefficient of variance (HPCV) to less than 2%. Relative fluorescence intensity of stained nuclei was analysed using a Beckman Coulter Epics XL-MCL Flow Cytometer with a 488-nm laser. The ploidy analyser was calibrated so that the G1 peak of stained nuclei from Musa acuminata subspecies malecensis, diploid, (fresh material) was set at channel 200 and the flow was given a stop time of 300 s, allowing a total of 5000–10 000 nuclei to be analyzed per sample.

A total of 91 accessions were analysed (Table 1). Of these, ITC 0249 and ITC 0084, with known ploidy level, were fresh samples, received from INIBAP. The other 89 were from the National Banana Germplasm collection at Rubona station — Rwanda, of which, 18 accessions; ‘Poyo’, ‘Gisubi kagongo’, ‘Kivuvu’, ‘Dibis’, ‘Gisubi kayinja’, ‘Kamaramasenge’, ‘Pommes’, ‘Petite Naine’, Guindi, ‘Pelipita 2’, ‘Saba’, ‘Americani’, ‘Prata’, ‘Ice cream’, ‘Lacatan’, ‘Grande Naine’, ‘Naasuna’ and ‘Yangambi km5’, were exotic. The histograms of the flow cytometric analysis of mixed nuclei from fresh material of diploid Musa acuminata subspecies burmannicoides (ITC 0249) and triploid ‘Mbwaizirume’ (ITC 0084) are shown in Fig. 1. The dominant peak corresponding to the G1 nuclei reflects the ploidy of each plant. The peak of the diploid ITC 0249 was approximately on channel 200, while the peak for the triploid ‘Mbwaizirume’ (ITC0084) was at channel 300. There was no peak at channels 400 and 600 which would represent the G2 and triploid ploidy of each plant. The peak of the diploid ITC 0249 was approximately on channel 200, while the peak for the triploid ‘Mbwaizirume’ (ITC0084) was at channel 300. There was no peak at channels 400 and 600 which would represent the G2 phase for the diploid and triploid phases, respectively. This suggests that there was no further cell division related to the leaf position of samples.

The histograms of the frozen leaves are shown in Figs. 2 and 3. Most histograms revealed a coefficient of variation (CV) of less than 5% (Table 1). The histograms of the fresh material showed less debris than those for the frozen samples and the nuclei count was higher (Figs. 1–3). The low nuclear count, the presence of debris and the presence of a high CV are presumably due to mechanical damage to the cells caused by storage at −70 °C and the brief storage in liquid nitrogen before the tissues were chopped. However, this debris did not affect the determination of ploidy levels of the bananas.

Our study indicated that the accession ‘Dibis’, previously reported as a tetraploid hybrid received from Burundi, is in fact a triploid (Fig. 2), whilst ‘Pomme’, ‘Kamaramasenge’, ‘Gisubi kayinja’, and ‘Gisubi kagongo’ reported as diploid, diploid, diploid and triploid, respectively, turned out to be triploid, triploid and diploid, respectively (Fig. 3). Our results concerning the ploidy level were identical to those reported recently by Dolezelová et al. (2005) for fresh material of ‘Kamaramasenge’.

All 65 East African Highland bananas analysed were triploid (Table 1). These Highland bananas are phenotypically triploid with the A genome and belong to the subgroup Lujugira–Mutika (Simmonds, 1966). Our results confirm their ploidy level using high-resolution methods. This study showed the feasibility of using banana leaves stored at −70 °C for analysis of ploidy using flow cytometry. These results should be valuable for the National Banana Germplasm collection, and specifically for banana breeders, as some Highland banana cultivars can be used as female parents for a crossing programme in the improvement of East African Highland bananas (Ssebuliba et al., 2005), as well as for the International Network for the Improvement of Banana and Plantain (INIBAP) for the Musa Germplasm Information System (MGIS) project.

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References


