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Ploidy Variation in Hybrids from Interploid 3x X 2x Crosses in *Musa*

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Summary

Hybrids were obtained after in vitro *germination of embryos from interploid crosses between triploid 'French' plantain cultivars (*Musa *spp. AAB group) 'Ntanga 2' and 'Bobby Tannap' with diploid banana (*Musa acuminata *subsp.* burmannicoides*) 'Calcutta 4'. Cross-pollinated bunches were harvested at full maturity and ripened with acetylene in a room for 4 days. Seeds were extracted from peeled ripe fruits by squashing. Embryos from the seeds were excised aseptically after 2 days and germinated* in vitro. *Seedlings were subsequently planted in early evaluation trials after acclimatising in the greenhouse. Chromosome counts were carried out on root tips of mature and maiden suckers to determine ploidy levels using a modified squashing technique. Counts showed that two of the hybrids were aneuploids (trisomics) with somatic chromosome number of 2n=2x+1 =23, one hybrid was diploid while the other two were tetraploids. Tetraploids are the most promising hybrids for the genetic improvement of plantains. Diploids are valuable material for further improvement of the plantain genome at this ploidy level. Trisomics provide means for further characterisation of the* Musa *genome and physical gene mapping in plantain and banana.*

Résumé

Des hybrides ont été obtenus à partir d'embryons propagés in vitro *de croisements interploïdes impliquant des cultivars triploïdes de plantain French (*Musa *spp. groupe AAB) 'Ntango 2' et 'Bobby Tannap' avec la banane diploïde (*Musa acuminata *subsp.* burmannicoides*) Calcutta 4. Des régimes issus de pollinisation croisée ont été récoltés à pleine maturité et mûris à l'acétylène en chambre pendant 4 jours. Des fruits mûrs épluchés ont été écrasés pour l'extraction des graines. Deux jours après, les embryons ont été excisés des graines, en condition aseptique, et mis en germination* in vitro. *Les plantules ainsi obtenues ont été subséquemment plantées dans des essais d'évaluation précoce, après leur acclimatation en serre. Le comptage de chromosomes a été effectué sur des extrémités de racines de souches matures et de souches non fructifiées, à l'aide de la technique de* squashing *modifiée. Les comptages ont révélé que deux des hybrides étaient aneuploïdes (trisomie) avec un nombre 2n=2x+1=23 de chromosomes somatiques, un hybride était diploïde avec un nombre 2n=2x=22, tandis que les deux autres étaient tétraploïdes avec un nombre 2n=4x=44.*

Introduction

The tropical monocotyledonous genus *Musa* belongs to the family Musaceae and order Zingiberales (syn. Scitaminae) and comprises giant perennial monoecious herbs (9). Out of the four sections of this genus namely *Rhodoclamis, Australimusa, Calimusa* and *Eumusa,* only *Eumusa* comprises edible crops such as plantains and bananas. These crops originated from two diploid parents *M. acuminata* (A genome) and *M. balbisiana* (B genome) through hybridisation and parthenocarpy.

Aneuploid plants are used for cytogenetic research to determine the localisation of genes within chromosomes, and to separate chromosomes from distinct genomes (2, 5). Aneuploid plants in *Musa* are mostly derived from triploid by diploid crosses (11). These aneuploid plants may be of value for *Musa* breeding and genetics as shown earlier in wheat (7).

In addition to aneuploids, various levels of euploidy have also been observed to arise in embryos from triploid by diploid crosses (1, 11). Diploids, triploids and tetraploids are observed after *in vitro* germination of hybrid seeds. It is evident, however, that the frequency of triploids from such crosses after *in vitro* germination is least while the frequency of diploids is highest (11).

Despite the economic importance of plantain and banana, there have been very few investigations on the *Musa* genome. This is not the case with other crop plants such as tobacco *(Nicotiana tabacum)* (3), maize *(Zea mays)* (4, 8), wheat *(Triticum ocitiorum)* (7), or potato *(Solanum tuberosum)* (13). Recovery of

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aneuploids from products of *Musa* breeding will pave the way to further genome analysis of the *Musa genus*. Aneuploids will help to determine the relationship between the A and B genomes, and can be useful for physical mapping of important *Musa* genes.

Material and Methods

Mature triploid 'French' plantain cultivars 'Ntanga 2' 'Bobby Tannap' (*Musa* spp. AAB group) in anthesis were cross-pollinated with *M. acuminata* subsp. *burmannicoides* (Calcutta 4). Mature pollinated bunches were harvested and ripened at room temperature with acetylene in a room for 4 days. Ripe fruits were peeled and their seeds extracted by squashing. The collected seeds were air-dried. After 2 days, the seeds were sterilised in 0.5-0.75 % (w/v) sodium hypochlorite (NaOCl) for 15 min. The embryos, which are small (0.7 mm - 1 mm), were excised from the seeds with the aid of a stereomicroscope following procedures reported by Vuylsteke et al. (10). Excised embryos were cultured aseptically on a modified MS medium with macro- and micro-nutrients at half-concentration, but FeEDTA at full strength (10). This medium was supplemented with 3% sucrose, 2 mg l - l glycine, 0.5 mg l - l nicotinic acid, 0.5 mg l - l pyrodoxin.HCl, 0.4 mg l - l thiamine.HCl, and 20 mg l - l ascorbic acid. The medium was solidified with 1.5 g l - l Gelrite (R). Aliquots of medium (20 ml) were dispensed in 15 x 2.5 cm glass test tubes, capped with cellulose stoppers, and autoclaved for 15 min at 121° C (1.05 kg cm^{-2}).

Viable embryos germinated into seedlings that were acclimatised in the greenhouse and later planted in the field to allow normal plant development. Root tips were collected and immediately pre-treated for three hours with 0.002 M solution of 8-hydroxyquinoline at room temperature (27° C). This pre-treatment was followed by fixation of root samples in 1:3 acetic ethanol) for 12-18 hours at low temperature (4° C-8° C). Root tips were squashed under a drop of FLP orcein according to the method of Osuji et al. (6). Chromosome counts were carried out on a sample of 10 cells for each hybrid at magnifications of X 400 and X 1000 (oil immersion) using a Leitz Diaplan binocular microscope.

Results and Discussion

There was variation in the chromosome number of the different hybrids. Seedling 1518-4 was a diploid with somatic number 2n=2x=22. Seedlings 1605-1 and 9722-1 were trisomics with the somatic chromosome number 2n=2x+1 =23 while seedlings 1187-8 and 8223-1 were tetraploids with the chromosome number 2n=4x=44 (Figure 1 and Table 1).

Table 1. Ploidy levels of some Tropical *Musa* Plantain hybrids

Clone	Female parent	Male parent	Ploidy
1187-8	'Bobby Tannap'	Calcutta 4	2n=4x=44
1518-4	'Bobby Tannap'	Calcutta 4	2n=2x =22
1605-1	'Ntanga 2'	Calcutta 4	2n=2x+ 1 =23
8223-1	'Bobby Tannap'	Calcutta 4	2n=4x=44
9722-1	'Bobby Tannap'	Calcutta 4	2n=2x+ 1 =23

Calcutta 4 is *Musa acuminata* subsp. *burmannicoides*.

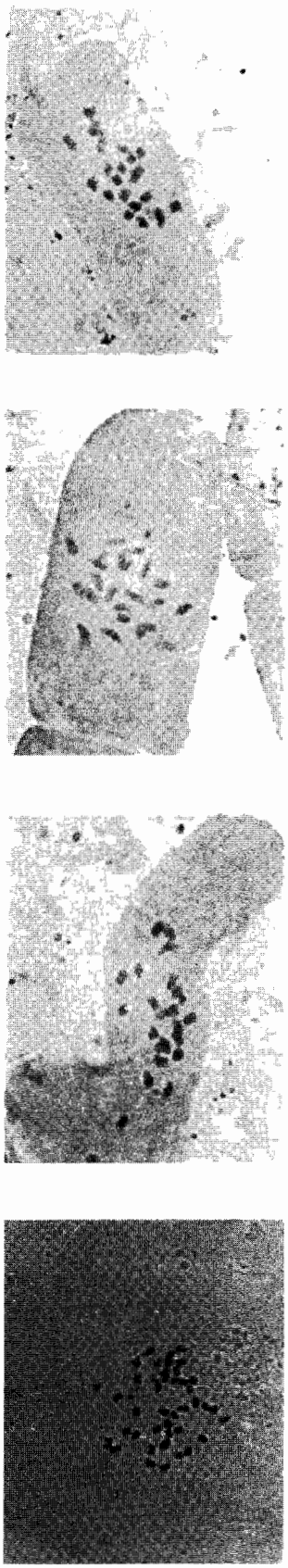

Figure 1. Chromosomes of plantain-banana hybrids obtained after triploid-diploid crosses: diploid 1518-4 with 22 chromosomes (a), trisomics 1605-1 (b) and 9722-1 (c) with 23 chromosomes, and tetraploid 8223-1 with 44 chromosomes (d). Scale bar = 10.2 µm.

Many aneuploids, which resulted from the crosses reported in this paper, were destroyed earlier because the primary objective of the breeding program was to produce hybrids that are resistant to black sigatoka disease (12). For example, from 1988 to 1990 about 23,600 hybrid seeds were obtained from 3x X 2x crosses between plantains and bananas, and 550 hybrid embryos were germinated and transferred to the breeding nursery. Most seedlings were aneuploids as shown by their gross abnormal foliage and stunted growth. Only 250 diploids, triploids and tetraploids were established in the field for agronomic evaluation (11). Thus, a high frequency of aneuploid *Musa* plants can be produced by interploid 3x X 2x crosses.

Aneuploids may contribute significantly to the cytogenetic characterisation of the *Musa* chromosomes and genomes. Likewise, aneuploids will be valuable tools for physical mapping of genes in plantain and banana chromosomes. Knowledge on chromosome number, structure and behaviour may facilitate the manipulation of the *Musa* genome for its further improvement using conventional and new breeding tools.

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