Evidence of spontaneous triploidy in cassava *Manihot esculenta* Crantz


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Spontaneous polyploidy has never been reported in strictly *Manihot esculenta* individuals although artificial polyploids are currently created in breeding programs using colchicine-induced tetraploids (Sreekumari et al. 1999) and interspecific triploids (*M. esculenta x M. glaziovii*) have been reported (Hahn et al. 1990). We here describe two *M. esculenta* spontaneous triploid clones that were selected, conserved and propagated by farmers in Vanuatu (Oceania, South Pacific). These two clones, exhibiting typical triploid morphology, are grown in traditional home-gardens and are highly appreciated for domestic uses. The first clone, biskit, has already spread throughout the archipelago and is now cultivated throughout the country. The second clone, mariango red, was collected in the village of Lamlu in the island of Tanna but has not spread yet to the other islands despite its outstanding characteristics. We present hereafter, cytological and molecular evidence of their triploid level.

### Flow cytometry

Flow cytometry analysis shows in both cases that the peak of the control diploid individual of *M. esculenta* is on the channel 50 and that the peaks for the triploid clones are on the channel 75.

We used for this analysis a 2X individual as a control. Preparation procedure was conducted following Partec ready-to-use reagent kits and protocols: 0.5 cm² of young leaves were chopped in 0.5 ml of buffer of CysStain® UV Ploidy (5001) and then filtered at 30 µ. The nuclei suspensions were incubated 1 mn, 1.5 ml of buffer 5001 were added and the analysis were conducted with the UV excitation flow cytometer Partec II.

### Chromosome counting

The chromosome counts in metaphase at mitosis confirmed the triploid level of biskit and mariango red: we counted 54 chromosomes for both samples and 36 chromosomes for the diploid control individual of *M. esculenta*.

Root tips were treated in 0.04% hydroxyquinoline for 4 h in the dark at room temperature. Then they were rinsed twice with a fixative solution (ethanol : glacial acetic acid, 3 : 1 v/v) and stored in 70% ethanol. Then, chromosome preparations were made according to the method described by D’Hont et al. (1995). After staining with 4,6-diamino-2-phenylindole / Vectashield, chromosomes were visualized and counted under fluorescent light with a microscope Leica DMRAX 2.

### Genotyping

Biskit and mariango red exhibited 3 alleles at two SSR loci each (respectively NS 376 – SSRY 19 and SSRY 179 - SSRY 19).

For each clone and a 2X control individual, DNA was extracted from 150 mg of dried leaves following the method described by Risterucci et al. (2000). They were genotyped with 11 SSR markers developed by Mba et al. (2001) that were already used to assess the extent of the genetic base of cassava in the country (Sardos et al. Unpublished).

Biskit and mariango red shared their alleles with the *M. esculenta* national sample (77 genotypes) and didn’t present any specific allele. Moreover, we genotyped a hybrid between *M. esculenta* and one of its wild relative, probably *M. glaziovii*, collected in Vanuatu. This hybrid exhibited 41% of specific alleles that were not present in the national germplasm neither in biskit nor mariango red.

### Conclusion

This is the first time that spontaneous and intra-specific triploid clones of cassava are documented at the cytological and molecular level. High yields (50-60 tonnes/ha in 9 months, with no inputs) were obtained after the first agronomic evaluations of these two clones. It is now necessary to characterize the physico-chemical characteristics of their roots.

### References


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