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Full Length Research Paper

Determination of ploidy level by flow cytometry and autopolyploid induction in cocoyam (*Xanthosoma sagittifolium*)

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A procedure for chromosome doubling of the white cocoyam (*Xanthosoma sagittifolium*) type was established by using colchicine and oryzalin treatments to *in vitro* plantlets. Flow cytometry was successfully used for analyzing ploidy levels within three cocoyam types and regenerated plants. Treating *in vitro* white cocoyam with 0.05% colchicine for 7 days produced tetraploids. The oryzalin treatment (0.05% for 3 days) produced only mixoploids, but it has in turn, a lower rate of mortality. Therefore, in white cocoyam, oryzalin could constitute an alternative to the use of colchicine, whenever the selection and stabilization of polyploids induced by this reagent will be achieved.

Key words: colchicine, oryzalin, flow cytometry, ploidy level, tetraploid, mixoploid, cocoyam (*Xanthosoma sagittifolium*).

INTRODUCTION

Cocoyams (*Xanthosoma sagittifolium*) are among the most important edible tuber and leafy crops for millions of people in tropics and sub-tropics. They are generally grouped into three types white, red and yellow on the base of the tuber flesh colour. Earlier studies using conventional chromosome counting have revealed that the white and red cocoyam types are diploid ($2n=2x=26$) whereas the yellow type is tetraploid ($2n=4x=52$) (Ngouo, 1988).

The white type is widely preferred because of its taste and high yield. However, it is particularly susceptible to the root rot disease caused by an Oomycete soil-borne pathogen, *Pythium myriotylum* (Boudjeko et al., 2005), while the yellow tetraploid cocoyam shows some level of resistance to cocoyam root rot disease (CRRD) (Boudjeko et al., 2005). So far, attempts to breed the resistant yellow tetraploid cocoyam with the highly preferred diploid one using conventional techniques have not been successful, probably due to ploidy incompatibility.

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Polyploidy induction has often been used to develop new cultivars (Peng et al., 2008). Increased vegetative growth and plant vigour always associated with tetraploid yellow cocoyam suggests that resistance of diploid white cocoyam may be improved by doubling its chromosome numbers. The induced tetraploid plants could also be used for crossing to selected resistant yellow cocoyam. Doubling of chromosome number may be achieved when some antimitotic agents are applied to meristematic regions of plants or young seedling plants. In earlier studies tetraploid plants were induced in some cocoyam cultivars using colchicine (Esnard et al., 1993; Tambong et al., 1998). However, this antimitotic agent is very toxic for human beings and also shows undesirable mutagenic activity on plants (Van Tuyl et al., 1990). Another compound used for doubling the chromosome number in plants is oryzalin (4-(dipropylamino)-3, 5- dinitro-benzenesulfonamide), which was developed as an herbicide. Oryzalin has been found to be less toxic and more efficient in inducing polyploidy in some plants (Yemets and Blume, 2008). The double objective of this study was therefore to determine the ploidy level of the three cocoyam types using flow cytometry, and to assess the potential of oryzalin for polyploidy induction in white

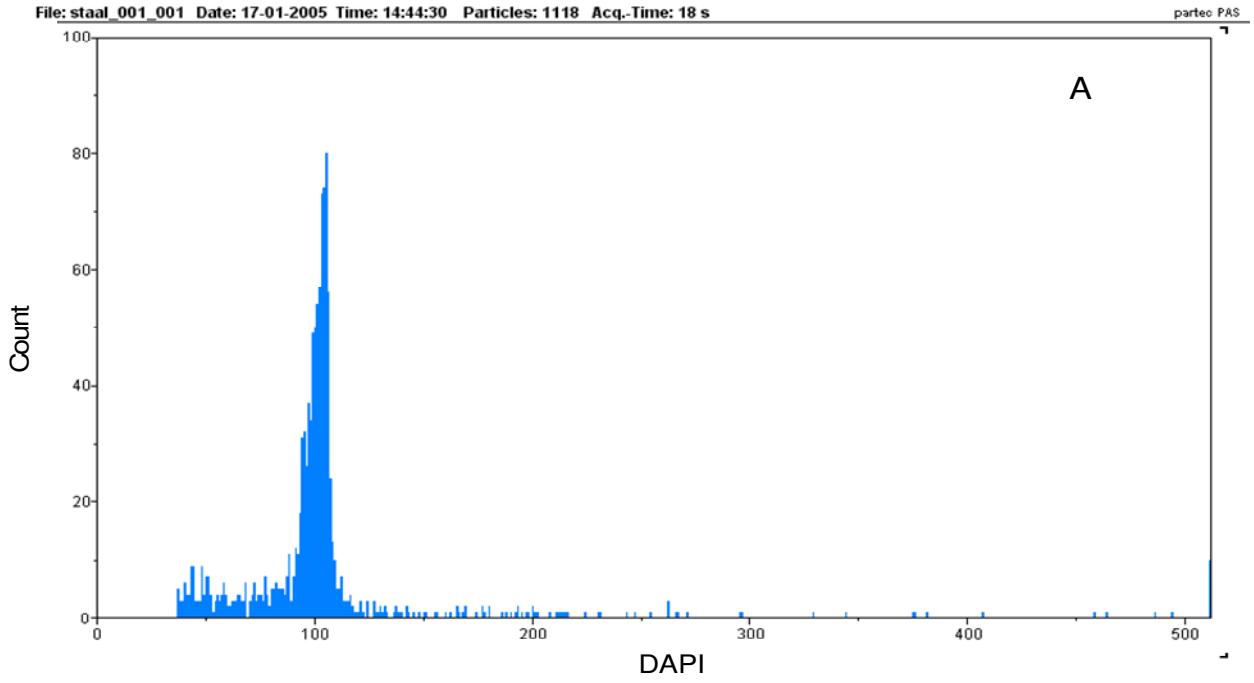

Figure 1. Histograms showing different ploidy levels for the standard diploid (A) white and red cocoyam cultivar, (B) the induced tetraploid and (C) mixoploid.

cocoyam.

MATERIALS AND METHODS

In vitro production of plantlets and colchicine or oryzalin application

In vitro cocoyam plantlets were produced as described by Zok et al. (1998). For the antimitotic treatment, 4 week old *in vitro* plants were pre-treated by incubating at 4°C for 96 h to minimise cell division. The *in vitro* plants were returned to 25°C with light for 8 h prior to colchicine or oryzalin application. Filter sterilised colchicine or oryzalin dissolved in 2% dimethylsulfoxide (DMSO) was added to liquid B5 medium to obtain the following concentrations: 0.01; 0.05 and 0.25%. The pre-treated plantlets were then aseptically transferred into fresh B5 medium supplemented with various colchicine or oryzalin concentrations and treated for 3 and 7 days. Non-treated *in vitro* plants served as a control. The set up was incubated in the plant growth chamber at 25°C in a 16 h photoperiod. 15 plantlets were used for each treatment. After each treatment duration, roots and leaves of the plantlets were removed. The remaining shoots were rinsed in sterile distilled water and aseptically transferred to solid B5 medium without antimitotic compounds (growth conditions as mentioned above) for 2 months. Youngest leaves were systematically collected for leaf squashes and flow cytometric analyses. For the determination of the ploidy levels of the three cocoyam types, leaves were collected from 2 month *in vitro* plants grown on B5 medium.

Flow cytometric analysis

Leaves chosen from each surviving individual were crushed slightly using a sharp razor blade in a nuclei extraction buffer (solution A of the High Resolution Kit for Plant DNA). After filtration through a 30 µm nylon sieve, a staining solution containing the dye 4, 6-diamino-2-phenylindole (solution B of the kit) was added. The mixture was analysed using a Partec flow cytometer (Partec Gmbh-Munster, Germany). To estimate the ploidy level, the fluorescent intensity peak of the sample on a histogram was compared to that of a standard plant with a known ploidy level. Leaves of the same plantlet were analysed a second time for confirmation one month after the first analysis.

RESULTS AND DISCUSSION

Flow cytometry analysis indicated that the diploid white and red cocoyam types showed a peak at the position of channel 100 (Figure 1A), whereas the tetraploid yellow type showed a peak at channel 200 (Figure 1B). This result is consistent with the results previously reported on the base of chromosome count (Ngouo, 1988; Tambong et al., 1998). Therefore, flow cytometry may constitute an alternative method for attributing ploidy levels in cocoyam, which would be faster than the chromosome counting.

The ploidy level of the treated plantlets was affected by the concentration of colchicine and oryzalin and the duration of treatment. When plantlets were treated with 0.05% colchicine for 3 days, plantlets did not exhibit increases in number of chromosomes as shown in the control plants. At the same concentration of colchicine (0.05%) but when the time of exposure was increased to 7 days, 30% tetraploids (Figure 1B) and 60% mixoploids (4x+8x) were found among tested plantlets. Tetraploid

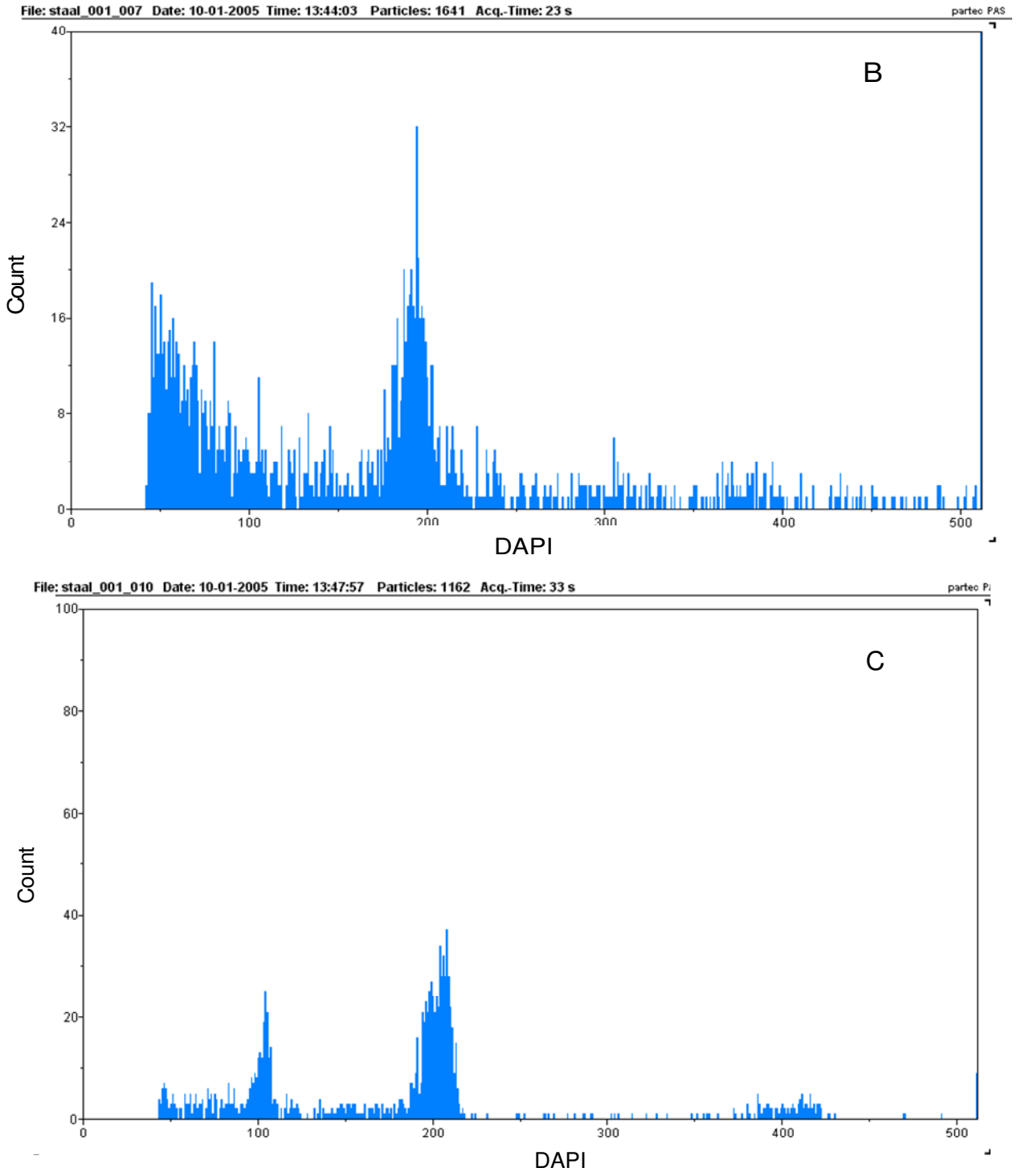

Figure 1. Contd.

plants were not induced with increasing colchicine concentration (Table 1).

When plantlets were treated with 0.01% oryzalin, plantlets exhibit only diploid cells. No overall difference between 3 and 7 days exposure was obvious, but oryzalin concentration above 0.01% were necessary for mixoploid induction. Among plantlets tested with 0.05% oryzalin, 80% mixoploids (2x+4x) were found (Table 1). However, in all mixoploid plantlets, 4x cells (Figure 1C) seemed to dominate and probably resulted due to differential absorption of antimitotic agents by different cells. After proliferation, tetraploids could be sub-

Table 1. Ploidy level was analyzed two months after antimitotic treatment.

Treatment		Ploidy level (%)			
Concentration (%)	**Duration (days)**	**2x**	**4x**	**2x + 4x**	**4x + 8x**
Control	0	100	0	0	0
0.05 Col.	3	100	0	0	0
0.05 Col.	7	10	30	0	60
0.25 Col.	3	16	0	84	0
0.01 Ory.	3	100	0	0	0
0.01 Ory.	7	100	0	0	0
0.05 Ory.	3	20	0	80	0

Ploidy level was analyze 2 months after antimitotic treatment, Col: colchicines; Ory: oryzalin

sequently selected from these mixoploids as indicated by Kadota and Niimi (2002) in the Japanese pear cultivar.

Tetraploids were not found in the plantlets exposed to oryzalin, showing that colchicine was more successful in inducing tetraploidy in cocoyam than oryzalin. Madon et al. (2005) and Omidbaigi et al. (2010) also found that colchicine effectively induced chromosome doubling in oil palm and basil, respectively.

The *in vitro* chromosome doubling technique has been used to produce polyploidy in several species, but only little research has been applied to cocoyam. As far as we know, no report exists on the induction of polyploids in oryzalin-treated cocoyam plantlets. Although, oryzalin has been successful in inducing tetraploidy in *Miscanthus sinensis* (Petersen et al., 2002) and *Rosa rugosa* (Allum et al., 2007), it was ineffective in the production of tetraploid white cocoyam cultivars.

Most *in vitro* plantlets survived all combination of oryzalin and exposure time. Colchicine showed a higher mortality rate than oryzalin treatment (data not shown). This may be due to chromosome losses or re-arrangements and gene mutation (Luckett, 1989). Though tetraploid plants were not directly induced with oryzalin in this study, this chemical can still be exploited in chromosome doubling of white cocoyam due to the fact that it induces mixoploid. Further research testing higher oryzalin concentration and exposure time is useful.

Conclusion

This study shows that flow cytometry can be used as an efficient method for rapid detection of ploidy level in cocoyam. This ploidy level can be increased in white cocoyam using colchicine and oryzalin treatments. Then the stability of the induced polyploids needs to be monitored along with that of physiological, morphological and agronomic traits.

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