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In vitro cleaning of sugarcane (*Saccharum officinarum*) explants against the viral infections by application of the viral inhibitors (DHHT and virazol): a preliminary study

Asseng Charles Carnot[1]*, Ambang Zachée[2], Pomozkov Youri Ivanovich[3] and Keuldeuch Marina Alexandrovna[3]

[1]University of Douala, Department of Biology of the Vegetal Organism P. O. Box 24157, Cameroon.
[2]University of Yaounde I, Department of Plant Biology and Physiology P. O. Box 812, Yaounde, Cameroon.
[3]Russian University of the People Friendship, 117198 Moscow, Miklouxo-Maklaya street, N°8/1, Russia Federation.

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The objective of this work was to cleanse explants of *Saccharum officinarum* cultivated *in vitro* using the viral inhibitors DHHT and virazol. The basic vegetal material constituted seeds of 4 varieties of sugarcane, 99-055, 97-029, X99-071 and X97-035, obtained from the CIRAD of Guadeloupe. The explants of foliar, stem and root origin size 0.7 - 1.5 mm were cultivated on modified medium of Mourassigue and Skougou. The viral inhibitors introduced in the culture medium to proportions of 1%. The diagnosis of the viruses were made by symtomatologic tests confirmed by enzyme linked immunosorbent assay (ELISA). 5 viruses (SCMV, CMV, RLMV and YDBV) were screened. The use of the virazol and DHHT makes possible to obtain 40, 55, 35 and 60 % explants respectively free from SCMV, CMV, RLMV and YDBV on the X97-029 variety for explants size 1.2 - 1.5 mm. The results show that it is possible to obtain a seed-bearer material of sugarcane free from some viruses by the method of cleaning using the viral inhibitors on explants of larger cuts (1.2 - 1.5 mm).

Key words: *Saccharum officinarum*, explants, viral inhibitors.

INTRODUCTION

Sugarcane has been cultivated for a long time and represent the principal sugar producing culture in several countries. It is the only means by which one obtains food sugar in several countries. The productivity varies from country to country Brazil (73 tons/ha), India (64.5 tons/ha), China (64 tons/ha), Cuba (22.5 tons/ha) . In Africa, the highest productivity is obtained in Egypt (13 tons/ha) and South Africa (49.5 tons/ha) (FAOSTAT, 2006). Sugar consumption in the developing world remains higher than their production. According to Africon-*seil* Cameroon, sector sweeter (2000), Cameroon produced 80000 T of sugar in 1998 but consumed 110000 tons; the surplus was imported at a cost of 8.1 billion FCFA. In spite of favorable climatic conditions as regard to sugar production, Cameroon productivity is low (10 tons/ha), compared for example to Senegal (11.7 tons/ha) with more unfavorable production conditions. This is generally caused by the limited technical agro-resources and the considerable losses resulting from devastating diseases and insects.

The only most effective way to increase sugarcane production consists of raising its output, by reducing the maximum losses due to diseases and devastating insects during the various stages of production. Nowadays, more than 30 diseases caused by viruses, bacteria and fungi

*Corresponding author. E-mail: carnotass@yahoo.fr. Tel.: (237) 99 981197.

Table 1. Viability of explants according to its origin

Varieties	Alive explants in % /day						
	Sheets	Stems				Roots	
	10	10	18	25	28	10	18
X99-055	0	98 ± 2	20 ± 1	3 ± 0	0	21 ± 1	0
X97-029	0	80 ± 2	15 ± 1	4 ± 0	0	14 ± 1	0
X97-035	0	81 ± 2	5 ± 1	1 ± 0	0	3 ± 0	0
X99-071	0	85 ± 2	10 ± 1	0 ± 0	0	4 ± 0	0

infect sugarcane (Pomazkov, 1989), among these diseases, viral diseases are often more represented due to the ecological requirements of sugarcane. The effective protection of sugarcane against these diseases must include all the prophylactic and therapeutic methods. The development of this project must be based on the effective use of healthy seedling materials for the creation of the new plantations (Shmeuglia, 1979). *In vitro,* culture of the meristem with an aim of obtain the unscathed seeds of the viruses, aims at as much as possible reducing the explants size without compromising its proliferate capacity.

For the cleansing of the sugarcane, the method of meristem culture *in vitro* is insufficiently studied (Hendre et al., 1975). That is caused, primarily by the difficulties of rooting of sugarcane explants in particular and of *Poaceae* in general due to their short life duration. As conesquence, these difficulty lead to the impossibility healthy seed-bearer material (cultivars) in large scale. *In vitro* culture fabrics being the only method of acquisition a healthy seed- bearer material to great number deserves an integration of protection means against the viral infections in order to produce virus free sugarcane planting material. It is accordingly that one considered to be necessary to lead the present study on the sensitivity of the biotechnological operations in the cleansing of sugarcane explants of various dimensions, by the use of the viral inhibitors on modified medium of Mourassigue and Skougou (1962).

MATERIALS AND METHODS

The experiments were carried out in the laboratory of phytopathology in the department of plant protection, faculty of agriculture in the Russian people friendship university and recovery of the department of vegetal biology and physiology in the university of Yaounde I and in the laboratory of vegetal biotechnologies.

Seedlings origin and artificial infection

The seedlings of sugarcane is used for taking away explants raised in greenhouse starting from seeds in a mini greenhouse were arranged at the laboratory. The samples of seeds of the sugarcane varieties (X99-055, X99-071, X97-029 and X97-035) used in this study came from the foundation of CIRAD (Centre de Cooperation International en Recherche Agronomie), Guadeloupe. The seeds (caryopses), before their pregermination in petri dishes (90 mm of diameter), on blotting paper wet before hand with distilled water and sterilized and soaked for 3 min in a sodium permanganate solution (0.1 g/l). Limp containing seeds were deposited in the mini greenhouse in the presence of 12 h of light (5000 - 10000 lux) and 12 h of darkness at the temperature of 20 - 22°C. As of the appearance of a radicle approximately 2 - 3 mm the pre germinates seeds were transferred on a liquid medium containing distilled water with saccharose (30 g/l) inside the test tubes. The seedlings thus 10 days old were infected mechanically by isolates of the most regular viruses sugarcane mosaic virus (SCMV), Tobacco mosaic virus (TMV), cucumber mosaic virus (CMV), yellow dwarf barley virus (YDBV) and peanut clumpvirus (RLMV). The seedlings were maintained in this state infected during 14 days before carrying out and taking away explants.

Explants culture

The culture of explants was done under the aseptic conditions on a culture medium made up of macro and micronutrients according to Mourassigue and Skougou (1962). In this culture medium, one added iron chelate, thiamin, pyridoxine, nicotine acid (0,5 mg/l), ascorbic acid (1,0 mg/l), saccharose (30 g/l) and agar (7 g/l). -indolylpentanoïque acid (IPA) with 0,1 mg/l, combination 6-benzoaminopurine (BAP) and the gibberellic acid (GA) in the concentrations of 0,1 and 2,5 mg/l were used like stimulants of growth. The explants were isolated in their active growth phase according to Siriram (1998). Explants dimension 0.7 - 1.0 and 1.2 - 1.5 mm were cut out with scalpel and deposited aseptically on the culture medium. Since the viability of explants depends on their origin (Table 1), only the explants stems were used.

Experimental design

Experimental design was composed of 3 treatments, in 3 repetitions. The treatments were as follows, To (control) explants of the seedlings infected and cultivated without application of inhibitor T1- explants of the infected seedlings cultivated with application of the virazol, T2- explants of the infected seedlings then cultivated with application of the DHHT. The viral inhibitors were incubated in the culture medium (0.1 g/l) after the sterilization, at the same moment as the growth hormones and the vitamins.

Measure parameters

The explants size was given using the objectives graduated on magnifying glasses for explants size old less than 6 days. With more than 6 days, explants size were done using a scale. The taking away were carried out until the 25th day after sowings and 6 taking away were carried out in the 4 days intervals after the 6th day. The viability was expressed by the % of alive explants according to the formula:

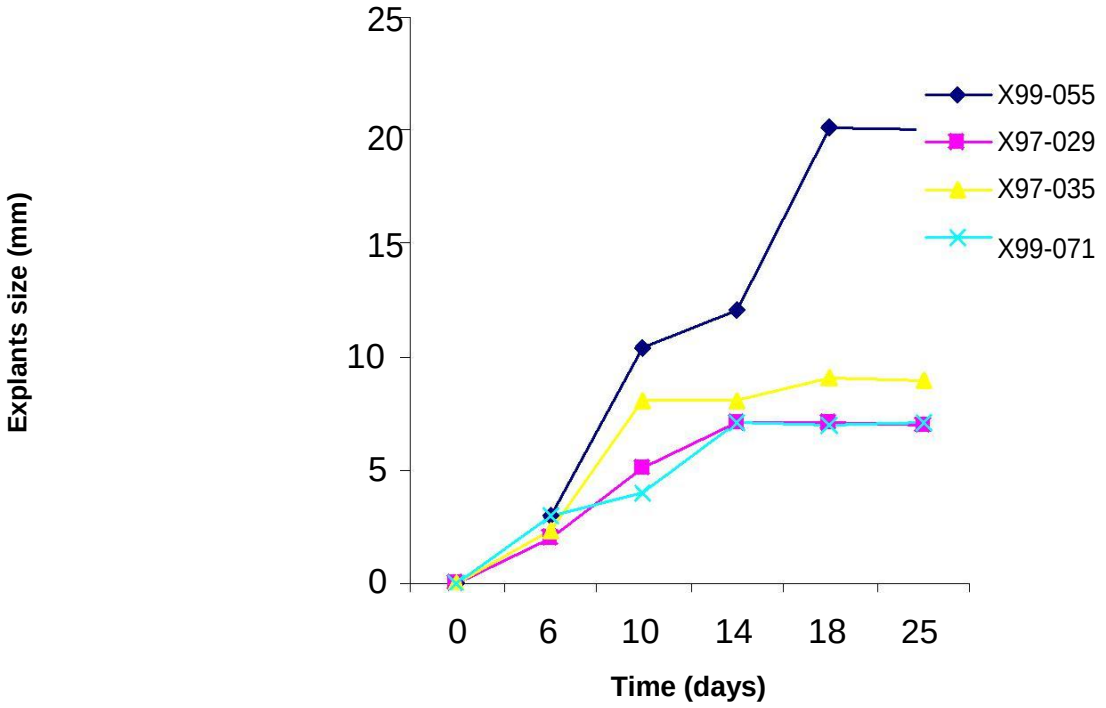

Figure 1. Growth rate and explants viability of some varieties of sugar cane (initial size 2 mm).

viability = (Nv/Nt) 100

Nv- number of alive explants
Nt - total number of sown explants

Evaluation of the viral infections

The evaluation of the viral infections was made with ELISA method (Clark and Adams, 1977). This method gives a qualitative and quantitative analysis of viruses. The wavelength used was 490 Nm. The test was made on 2 moths old the seedlings treated with the antiviral products.

Statistical analysis

Results obtained were subjected to the averages separated according to the test from Despekhov (1979) to the threshold from 5%.

RESULTS

Growth and explants viability

The explants growth depended primarily on the genetic potentialities of the various varieties tested. The data obtained (Figure 1) shows that the X99-055 variety has a higher growth rate than the 3 other varieties. It was noticed that the variety X99- 071 and X97- 029 have similar growth rate, particularly as from 14 days after the sowing sowing. The size big explants (20 mm) were recorded on the 25th day on X99-055, whereas at this same period we recorded 6.5 and 80.5 mm explants respectively with the varieties of X99-071 (X97-029) and X97-035.

The capacity to survive in the culture medium varies according to time (Figure 2) for varieties there is likelyhood of no statistical difference. The recorded data showed that 50% explants remain alive until the 14th day after setting in culture for all the varieties except for X99-071 where we recorded a viability of 30%. After 25 days of setting in culture, the viability of explants fell enormously and stabilized to 4% for X99-071, 3% for X99-055, 1% for X97-035 and 0% for X97-029.

Evolution of the survival of explants after the 18th day (Figure 2), indicate that X99-055 and X97-029 varieties seem to resist better in the medium with a respective % survivor rate of 22.5 and 21%.

Effect of the viral inhibitors on the cleaning sugarcane against viruses

ELISA tests carried out on treated explants with inhibitors and those of the control showed that cleansing explants was not compete (Table 2).

Taking into consideration the result of this Table, one can notice that, low dimensions explants (0.7 - 1.0 mm)

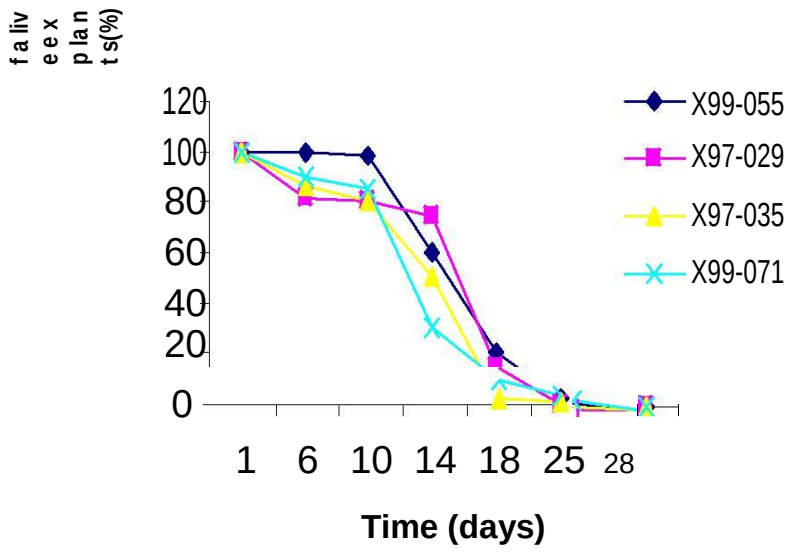

Figure 2. Explant viability of some varieties of sugar cane.

Table 2. Inhibitors influences on the cleansing sugarcane on the complex of viruses.

Treatments	Varieties	Size of explants (mm)	Percentage of healthy explants after ELISA test on 20 treated samples				
			SC MV	TMV	CMV	RLMVYDBV	
T_o (Control)	X99-055	0.7 - 1.0	0	0	0	0	0
		1.2 - 1.5	0	0	0	0	0
	X97-029	0.7 - 1.0	0	0	0	0	0
		1.2 - 1.5	0	0	0	0	0
T_1 (Virazol)	X99-055	0.7 - 1.0	35	10	45	30	55
		1.2 - 1.5	30	0	45	20	55
	X97-029	0.7 - 1.0	40	5	60	30	70
		1.2 - 1.5	40	0	50	25	60
T_2 (DHHT)	X99-055	0.7 - 1.0	30	0	55	35	65
		1.2 - 1.5	20	0	50	35	55
	X97-029	0.7 - 1.0	35	0	65	40	60
		1.2 - 1.5	25	0	55	35	60

are likely to give more clean material released completely of the viral infections. The virazol applied to each variety for small size explants, gave better % of the completely cleansed seedlings of the viruses as compared to those subjected to the DHHT. However big size explants cleansed with the 2 antiviral products also gave interesting % of seedlings completely released of viral infections, for example 60% of seedlings treated with the DHHT and with the virazol were completely released from virus YDBV with the X99-029 variety (Table 2).

DISCUSSION

The study made on growth rate and explants viability of 4 varieties of the sugarcanes (Figure 1 and 2), shows that the explants regeneration on culture *in vitro* depends to a great part on the genetic potentialities of these varieties. The decreasing viability of explants which would be cancelled after the 25th day would be certainly due to progressive impoverishment in nutritive elements in the culture medium (Boutenko, 1983; Chriki and Al, 2003). It is also important to consider the assumption according to which the extracted explants fabrics already differentiated (roots, stems and sheets) would require specific culture medium, composed of suitable hormones as suggested Siriram (1998).

The study of viability made possible to sift out some varieties among the studied varieties in order to carry out

an adequate cleansing. The varieties X99-055 and X97-029, which were selected thanks to their capacity of adaptation under the conditions of the study, confirms the results of Boutenko (1983), which in similar studies had dismounted that the genetic capacities of the vegetal material to overcome the stress, constitute a paramount factor in the culture of *in vitro* fabrics.

The results obtained on the explants cleaning shows that used antiviral products DHHT and virazol, have a potential inhibitory capacity. These results corroborate those of Bobeure (1978) and Zirka (1984), which working on similar effects showed that the use of certain chemical bonds causes inhibition, the replication and the reduction of the biological activity of the viruses.

The high number of material cleansed in culture obtained *in vitro* starting from explants of small size (0.7 - 1.0 mm) compared to those of larger size (1.2 - 1.5 mm), would be explained by the fact why the material of reduced size would contain less virus, thus confirming the results of Abramenko (1982), Hayami and al. (1984). However, the disadvantage of small size explants lies in the complexity of their regeneration like it had been underlined by Donets (1990), Hendre et al. (1975). Otherwise, the method of cleansing applied in this study made possible to obtain certain number of completely cleansed material released of the viral infections, starting from the explants of big size (1.2 - 1.5 mm) and which preserved their regeneration capacity until obtaining the seedlings. Although the use of big size explants was contradictory with several former research, the results of this study show that one can increase the number of cleaned material of virus by using small size (0.7 - 1.0 mm) and big size (1.2 - 1.5 mm) explants.

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