Salt and sugar levels for effective plant regeneration from in vitro cultured tissues of cocoyam[1]

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ABSTRACT

Tips from apical and axillary meristems of cocoyam were grown in modified Murashige and Skoog's (M S) medium with a range of concentrations of salts and sucrose. Sixteen treatments were evaluated for their effect on root and shoot formation and plantlet development. The whole regeneration cycle from meristem tip to plantlet was obtained in a single medium (one step) system. Multiple regeneration was favored by high sugar-reduced salt combinations. The best results were obtained with combinations containing 133% of the sugar and 80% of the salt concentration in the unmodified M S medium. Better regeneration was obtained from axillary meristems than from apical.

RESUMEN

Se establecieron cultivos *in vitro* de meristemos apicales y de yemas laterales de cormos de yautía. Los meristemos se cultivaron en el medio de Murashige y Skoog (M S) modificado. Las modificaciones incluyeron varias concentraciones de sales y sacarosa. Se evaluaron 16 tratamientos con respecto a la formación de raíces y tallos y al desarrollo de plántulas. Solo en un medio se obtuvo el ciclo completo de regeneración desde el meristemo hasta el desarrollo de la plántula completa. Las combinaciones con concentraciones altas en azúcar y bajas en sales favorecieron la regeneración de plantas múltiples. Los mejores resultados se obtuvieron en combinaciones que contenían un 133% de azúcar y 75% de sales al compararlas con el medio M S modificado. Se obtuvo mejor regeneración de plántulas de las yemas laterales que de las yemas apicales.

INTRODUCTION

Edible aroids such as *Xanthosoma* and *Colocasia* are usually propagated by vegetative methods requiring the use of relatively large portions of the main corm or whole cormels. The large pieces of edible tissues which are used for propagation represent salable food resources. In addition, the high labor requirement of the propagation practice itself contributes to decreasing returns in the economic exploitation of these crops. Also these propagation methods have been associated with the

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spread of pathogens responsible for the principal diseases affecting these crops.

Plant regeneration from tissue and cell cultures have been suggested as an alternative to these problems. Efforts in this direction have been encouraging since successful propagation via tissue culture has been obtained in several countries (2,3,4,7). One of the problems still unsolved associated with tissue culture methods is production of offtype plants resulting from somaclonal variation. These offtypes are frequent, particularly when plant regeneration is obtained from calli. Genetically uniform plants would be required for large scale applications of these methods and in species preservation efforts. An added difficulty in plant regeneration has been the utilization of cumbersome systems in which two or more media are required for successful results. This article describes efforts to maximize the regeneration of genetically uniform material obtained by a one-step regeneration system from axillary and apical buds by adjusting the salt and sugar content of the growth medium. Similar modifications have proved to be succesful in other crops (8).

MATERIALS AND METHODS

Apical and axillary buds of corms of the white-fleshed cocoyam "Alela" variety (*Xanthosoma* spp) were excised from the corm and carefully washed in tap water to remove dirt and other contaminants. These buds were then transferred to an aseptic chamber. The excised apical buds were then submerged for 10 min in a 10% commercial Clorox solution; the axillary buds, in a 0.1% mercuric chloride solution. Then they were washed three times with sterilized distilled water for further sectioning.

Sectioning was performed under a dissecting microscope to produce meristematic apices approximately 0.5 mm long with some leaf primordia attached. These explants were transferred into culture tubes containing 10 ml of modified Murashige and Skoog's (MS) medium (6) with different concentrations of salt, sugar and hormones. Salt and sugar concentrations were modified to obtain 100, 75, 50, and 25% of the salt with 133, 100, 67 and 33% of the sucrose used in the basic MS medium. Hormone concentrations selected on the basis of preliminary results were adjusted to 1 mg/l kinetin and 6 mg/l naphthaleneacitic acid. The culture tubes containing the explants were placed in a growth room at 28° C and 85% relative humidity under continuous illumination by fluorescent light providing an intensity of approximately 15μE m^{-2} s^{-1}. Cultures were transferred to a fresh medium on a monthly basis.

The experiment included 16 combinations of salts and sugars with at least 15 replications of each treatment. Observations on survival, callus formation and root and shoot development were collected every 2 weeks.

The experiment was repeated twice; the data shown represent the average values obtained.

RESULTS AND DISCUSSION

Table 1 presents plant regeneration after 12 weeks of observation for the different treatments. Regeneration was obtained in slighty over 50% of all the replicates and was favored by high sugar concentration. The best results were obtained in the treatments containing from 133% to 75% of the sugar concentration present in the MS medium. No clear-cut picture of salt effects was observed except that the two best treatments corresponded to 75% to 100% salt concentrations in the MS medium. It is difficult to compare our results with other findings inasmuch as other researchers have concentrated their attention on hormone modification of the medium with little or no attention to sugar and salt effects. Gupta (4) used three different media without apparent significant effects on plant regeneration.

Better regeneration at high sugar concentration was apparently independant of osmotic effects and of the type of meristem (apical versus axillary) used. The percentage of plant regeneration obtained in the best treatments is comparable to those reported by Acheampong and Henshaw (1), who obtained over 80% regeneration in some varieties.

Table 2 presents data on average number of plantlets regenerated after 12 weeks of observation. Plantlet formation in various degrees was observed in all treatments. Better regeneration was obtained at high levels of sugar, particularly with explants obtained from axillary meristems. In general terms, the fewest plants were regenerated from treatments combining low salt and low sugar concentrations. Cultures with

TABLE 1.—*Effect of salt and sugar concentration on percentage of replicates with more than one plantlet*[1]

Sucrose concentration	Salt Concentration								
	1		*0.75*		*0.50*		*0.25*		
	Percentage replicates with one or more plantlets[2]								
	A	B	A	B	A	B	A	B	Mean
1.33	62	50	70	81	52	66	50	64	61.8
1.00	83	43	57	69	53	62	57	31	56.8
0.67	59	22	55	35	38	23	36	55	41.6
0.33	53	42	36	53	30	41	53	51	44.8
Mean	64.25	39.25	57.00	59.50	43.25	48.00	49.00	50.25	51.3

[1]Concentration ranges were determined by means of the modified MS medium as standard.

[2]A = Apical meristems; B = Axillary meristems.

TABLE 2.—*Effect of salt and sugar concentration on average number of plantlets regenerated from in vitro cultured meristems of cocoyam*[1]

Sucrose concentration	Salt concentration									
	1		0.75		0.50		0.25			
	Number of plantlets[2]								*Mean*	
	A	B	A	B	A	B	A	B	A	B
1.37	1.23	2.43	1.53	2.83	1.17	1.94	1.11	1.00	1.26	1.92
1.00	1.13	1.00	1.25	1.81	1.36	2.83	1.33	1.00	1.27	1.66
0.67	1.00	1.00	1.15	1.0	1.14	1.20	1.33	1.40	1.16	1.15
0.33	1.00	1.00	1.00	3.5	1.00	1.00	1.56	2.00	1.14	1.88
Mean	1.09	1.35	1.23	2.28	1.17	1.74	1.33	1.35		

[1]Concentration ranges were determined by means of the modified MS medium as standard.

[2]A = Plants regenerated from apical meristems; B = Plants regenerated from axillary meristems.

the most plants also had the most vigorous plants. Results are similar to those reported by Acheampong and Henshaw (1), who obtained an average of 1.5 plants per replicate. The lower degree of regeneration obtained from apical buds has been observed previously and probably results from the activity conditions of the meristems (4).

Typical response of the best treatments consisted of tissue swelling occurring around 4 weeks after culturing, followed by shoot and root formation. Fully developed plantlets were observable at approximately 8 weeks of culture. Subculturing of plants in MS medium containing 0.1 mg/L and 1 mg/L. NAA stimulated further development of axillary shoots which eventually became plantlets in this same medium.

When plantlets obtained from primary cultures or from subcultures were transferred to pots containing sterilized Promix mixture, they developed into normal plants and were ready for transplanting to the field within 3 months. Field-grown plants produced by this procedure showed no significant variation in leaf morphological character. Working with the same variety, Gupta (4) reported 18% variants for leaf morphology when plants were generated from calli; this percentage is considered large as compared to results obtained in other crops (5).

Figure 1 shows a frequency distribution of number of plantlets in the salt sugar combination regenerating more plantlets than those in the salt sugar concentration specified in the modified MS medium. Plant regeneration in the MS medium was poor with none of the replicates producing more than one plantlet, and only 7 out of 16, or 43% of replicates, producing one plant. This percentage contrasts with results in the high-sugar reduced-salt medium in which 17 out of 21 or 80% of the replicates pro-

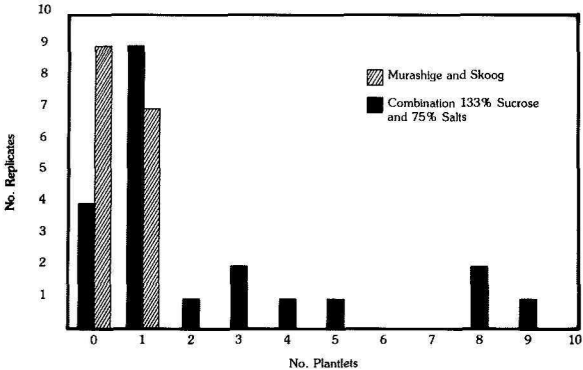

FIG. 1.—Frequency distribution of plantlets in a selected HSRS combination as compared to distribution in unmodified MS medium.

duced plantlets, and approximately 48% produced more than one shoot. Results demonstrate that multiple plant regeneration can be accomplished in a single medium system by modifying and optimizing salt and sugar levels. This finding represents an improvement over recent reports in which two media regeneration systems were used (4,7). In those reports plant regeneration has been indirect; that is, callus or protocorm formation has preceded plant regeneration. In contrast direct regeneration from the meristem was obtained in the present study.

Results reported herein demonstrate the feasibility of obtaining rapid multiplication of cocoyam by optimizing the salt and sucrose concentration of the culture medium. This procedure, coupled to efficient plantlet subculturing, should permit rapid and inexpensive in vitro multiplication of cocoyam.

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