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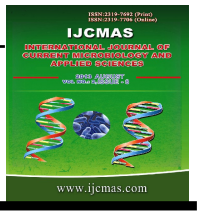

Original Research Article

Effect of different concentration of sterilant and exposure time on sweet potato (*Ipomoea batatas* Lam) explants

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A B S T R A C T

Investigation on the effect of different levels of concentration of sterilant (Sodium hypochlorite or bleach) and exposure time on sweet potato explants was conducted at Tissue Culture Laboratory of National Root Crop Research Institute Umudike, to determine the appropriate concentration of sterilant and exposure time to produce clean culture. The experiment was a 5x5 factorial in a Completely Randomized Design (CRD) and the data collected were analyzed with Genstat Release VS 2008. Ipomea 87/00087 collected directly from the field was used in the study and was incubated in a culture room at 28^{o}C ± 2 and exposed to artificial illumination of 2000 – 2500 lux for 16 hours daily for seven days. The result showed that Sodium hypochlorite is not a very good sterilant as very high percentage of the explants was contaminated in all the experiments conducted. However a low percentage (40%) of clean culture was observed at 20% sterilant concentration and 20 minutes exposure time.

Keywords

Sterilant (Sodium hypochlorite) concentration; exposure time, sweet potato explants.

Introduction

The production of sweet potato in Nigeria in recent years is on the increase as the crop is now grown in almost all the agroecological zones in the country (Tewe *et al.*, 2001), unlike in the past the cultivation of the crop was confined within its traditional central and riverine areas where it was grown as "backyard" crop or "gap filler" (Nwauzor *et al.*,2006). Although Nigeria is the second largest producer of sweet potato in Africa and the third in the World after China and Uganda, with annual output of 2.15 million MT (FAO, 2004), the country is yet to achieve its potential in the production of sweet potato. The problems of pests like sweet potato weevil (*Cylas* species), vine borer (*Omphisa anastomosalis*) and diseases like sweet potato virus, soft rot and stem rot in the production of sweet potato has been reported (Uguru, 1996; Anikwe *et al.*, 2005).

Worse still the improvement of the crop through convectional breeding method is difficult as many cultivars of sweet potato

not only produce defective pollen, but also are self and cross incompatible.

Tissue culture technique has been found valuable in the development of crops that provide better yield, more nutritious foods, tolerate drought, resist pests and disease causing organism, and survive in hash environments (Onwubiko and Mbanaso, 2006). Its successful application in the improvement of potato and other crops like cassava, garlic and ginger has been reported (Ketchum *et al.*, 1987; Pierik, 1987). However this technique is faced with the problem of infection. Contamination is one universal problem in tissue culture studies and it is experienced in all tissue culture laboratories and workers. Generally, there are four possible sources of contaminants; the plant which may be internal or external, the nutrient media that may be insufficiently sterilized, the air and the research worker who may be inaccurate. The adverse effect of contaminants in tissue culture experiments include; loss of valuable products, time, efforts, money, inaccurate or erroneous experimental results due to alteration of growth and characteristics of culture, personal embarrassment, etc. While some contaminants are visible which include bacteria, yeast and fungi, others are hidden or cryptic and are often difficult to detect, causing most serious problems. In the latter group are toxic chemicals, viruses, parasites, insects, mycoplasma and other cell lines. Again, culture contaminants may be categorized into biological or chemical.

Maintaining an aseptic or sterile condition has been identified as essential in successful tissue culture procedure (Chawla, 2003; Badoni and Chauhan, 2010). The desire of every researcher in tissue culture studies is to eliminate or prevent contaminations. Unfortunately contamination cannot be eliminated totally but can only be managed to reduce both the frequency of occurrence and the seriousness of its consequences, and this can be achieved through chemical sterilization (Pierik, 1987). Chemicals used in sterilization of materials for tissue culture should be effective, cheap, available and non-toxic. An effective chemical (or sterilant) is one that is strong enough to inhibit the growth of disease causing microorganisms even in small quantity and at the same time will not injure the explants. Hence it is important to determine the appropriate concentration of the sterilant and exposure time inorder to standardize the sequence of using sterilants to minimize explants injury for better result. Therefore this study was set up to determine the appropriate concentration of sodium hypochlorite (commonly known as bleach) and exposure time for best sterilization protocol in *in vitro* culture of sweet potato explants.

Materials and Methods

This study was carried out in the Tissue Culture Laboratory of National Root Crop Research Institute, Umudike in Abia State located on latitude 050 29' north and longitude 07 0 33' east at 122 m above sea level. The experimental design was a Complete Randomized Design (CRD) in a 5 x 5 factorial replicated 5 times. The treatment combinations were derived from five different levels of concentrations of sterilant (0 %,5 %,10 %,15 %,20 %,25 % and 30 %) and exposure time (0,10,15,20,and 25 minutes), giving a total of 25 treatment combinations.

The sweet potato explants used in the study were collected from the mother plant

of *Ipomoea batatas* 87/0087. This hybrid is characterized by its high starch content, dry matter and root yield. They were collected from the sweet potato nursery farm in a water filled beaker and were kept under running water prior to sterilization, after which they were cut with their nodes and taken to the aseptic manipulation room, where they were subjected to a sterile condition in a laminar air flow cabinet.

The explants were distributed into sterile bottles and 70 % ethanol was added to remove impurities. A wetting agent, Tween 20 was first added to the sterilant (bleach which consists of 10% NaClO as its active constituents), before adding the sterilizing fluid to each sterile bottle according to its concentration and left to be exposed respectively after decanting the ethanol. The sterilant was decanted from the explants and the explants were washed thoroughly with sterile water.

The initiation properly commenced by using the surface sterilized explants and dipping directly into culture medium vessels containing macro and micro salts according to Murashige and Skoog (1962), iron salts, vitamins, myo-inositol, sucrose and phytagel or gelrite. They were carefully labeled and incubated into the culture room at 28 ^{o}C ± 2 where they received artificial illumination at 2000 – 2500 lux for sixteen hours daily. 5 explants were used in each treatment and the rate of contamination was determined 3 days after establishment.

Data on the effect of different concentrations of sterilant and exposure time on clean, moderately, slightly and heavily contaminated culture (see plate 1) were collected and analyzed using Genstat Release 2008. (where clean culture is one without contaminants or zero level of contamination, moderately contaminated culture is one with less than or equal to ¼ of the total explants showing contamination, slightly contaminated culture is one with contaminated explants that are above ¼ but less than or equal to ¾, and heavily contaminated culture is one with above ¾ of its total explants showing contamination).

Result and Discussion

The results on the effect of different concentration of sterilant and exposure time on clean, slightly, moderately and heavily contaminated culture of potato explants are displayed in Tables 1 to 4. On clean culture, the different levels of sterilant concentrations and time of exposure did not significantly affect the inhibition of microbes. Furthermore the effect of the interaction between the different levels of sterilant concentrations and exposure time did not also significantly affect the number of clean culture (Table 1).

Similar to the result on clean culture, there was also no significant difference on the effect of different levels of sterilant concentrations and exposure time on slightly contaminated culture. Again the effect of the interaction of different sterilant concentrations and exposure time on slightly contaminated culture was also insignificant (Table 2)

Table.3 below shows the result on moderately contaminated culture. There was no significant difference on the effect of different levels of sterilant concentrations and exposure time on moderately contaminated culture. Again the interaction between different sterilant concentrations and exposure time was not significantly different.

Plate.1 Picture of (A) clean (B) moderately (C) slightly (D) heavily contaminated culture.

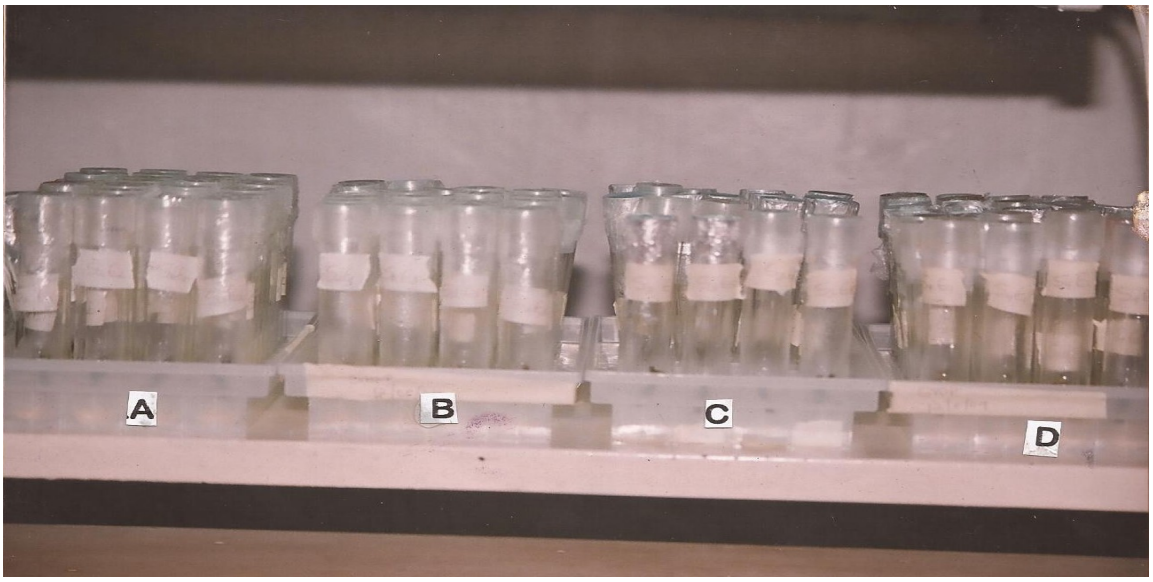

Table.1 The effect of sterilant concentration and exposure time on clean culture

	Sterilant concentration (%)					
Exposure time (mins)	0	15	20	25	30	Mean time
0	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.33	0.66
15	0.00	2.67	0.67	0.00	0.00	0.67
20	0.00	0.33	0.67	0.00	0.33	0.32
25	0.00	0.67	6.67	0.00	0.00	0.27
Mean conc.	0.00	0.73	0.40	0.00	0.13	

LSD $_{0.05}$ =0.743 for conc., 0.734 for time, 0.1662 for conc. and exposure time

Table.2 The effect of sterilant concentration and exposure time on slightly contaminated culture

	Sterilant concentration (%)					
Exposure time (mins)	0	15	20	25	30	Mean time
0	0.33	0.33	0.00	0.33	1.00	0.40
10	0.00	1.33	2.33	0.33	3.00	1.68
15	0.67	3.33	1.33	1.33	1.67	1.67
20	0.33	1.67	2.00	3.00	1.67	1.73
25	1.33	0.67	2.67	3.33	1.33	1.87
Mean conc.	0.53	1.47	1.67	1.67	1.73	

LSD $_{0.05}$ =1.120 for conc., 1.120 for time, 2.55 for conc. and exposure time

Table.3 The effect of different sterilant concentrations and exposure time on moderately contaminated culture

Sterilant concentration (%)						
Exposure time(mins)	0	15	20	25	30	Mean time
0	0.67	0.33	0.67	2.67	1.00	1.07
10	0.00	0.67	1.00	1.67	0.67	0.80
15	0.00	2.33	1.33	1.67	0.67	1.20
20	0.33	1.33	1.33	1.67	0.67	1.07
25	1.00	1.33	1.00	0.67	0.67	0.93
Mean conc.	0.40	1.20	1.06	1.67	1.74	

$LSD_{0.05}$ = 0.29 for conc., 0.29 for time, 0.65 for conc. and exposure time

Table.4 The effect of different sterilant concentrations and exposure time on heavily contaminated culture

Sterilant concentration (%)						
Exposure time (mins)	0	15	20	25	30	Mean time
0	4.00	4.33	3.33	0.00	0.00	2.33
10	5.00	3.00	1.67	0.00	0.33	2.00
15	4.33	2.00	1.67	0.00	0.00	0.67
20	4.33	1.67	1.00	0.33	2.33	1.93
25	2.67	2.33	0.67	1.00	3.00	2.20
Mean conc.	4.07	2.67	1.67	0.26	1.13	

$LSD_{0.05}$ =1.11 for conc., 1.11for time, 2.484 for conc. and exposure time

Contrary to the result on clean, slightly and moderately contaminated cultures, the different levels of sterilant concentrations significantly affected the number of heavily contaminated culture. At no (or zero) sterilant concentration, more contaminated cultures (4.07) were observed. However, the mean separation using LSD shows that sterilant concentrations of 15 %, 20 %, 25 % and 30 % were not different in their effect on the number of heavily contaminated culture. The effect of the interaction between different sterilant concentrations and exposure time was not significantly different (Table 4).

In this investigation, the different levels of concentration of Sodium hypochlorite and exposure time did not inhibit the growth of microbes in all the culture evaluated including the control, inferring that Sodium hypochlorite is not a good sterilant in sweet potato tissue culture procedure. Furthermore, the result of this experiment has some implication on the microbial load of the crop. The fact that all the cultures evaluated; clean, slightly, moderately and heavily contaminated cultures performed badly even at high level concentration of the sterilant suggests that the crop has a high microbial load. This observation is in agreement with several reports from many workers on high microbial load of sweet potato (Rao, 1982; Brant and Blake, 1983; Sawyer, 1984; Collins, 1984; Crowmo *et al*, 1986; Dusing, 1988; Clerk, 1988).

The fact that Sodium hypochlorite could not inhibit the growth of microbes in this experiment could be due to its strength which depends on its constituents. Sodium hypochlorite or bleach consists of 10 % active ingredient of NaClO. Invariably, only 1 % of this active constituent is used. This means that a solution of Sodium hypochlorite contains 1 part active ingredient (NaClO) and 9 parts tap water. Consequently at low level of this sterilant concentration, the observed high level of contamination was inevitable as the strength of Sodium hypochlorite is small. This explains why even at higher concentration of Sodium hypochlorite (30 %), eradication of micro-organisms could not be achieved in all the cultures evaluated. In addition, it has been observed that the response to a particular hormonal message did not only depend on its content or strength (Pierik, 1987), but also upon how it is interpreted by its recipient (Curtis *et al*, 1985). Hence the observed high microbial load of this crop may not only depend on the strength of this sterilant but also on its interpretation by the potato explants.

In the four cultures used in the study; clean, slightly, moderately and heavily contaminated cultures, the effect of the sterilizing effect of Sodium hypochlorite was only significant on heavily contaminated culture. It was observed that more cultures (4.07) were contaminated at no sterilant application. Invariably this sterilant is not effective in potato tissue culture studies. From this result the sensitivity of Sodium hypochlorite in inhibition of microbes in potato tissue culture studies is extremely low, reemphasizing that the strength of the sterilant in this study is low. Hence the choice of sterilant to be used in chemical sterilization of plants for tissue culture studies should be based on the strength of the sterilant (Pierik, 1987).

Generally, explants are sensitive to sterilant concentration especially at higher level. In fact it has been observed that the surface layers of explants are destroyed by high concentration of sterilants (Pierik, 1987). This detrimental effect of high sterilant concentration also encourages the growth of microbes. Another important factor is sterilization time, as lengthy sterilization could have some lethal effect on the explants and this as well favours the growth of microbes. In this study, this fact was considered in the choice of levels of sterilant concentration bearing in mind the bleaching effect of the sterilant. Higher levels of sterilant concentrations of 35 % and beyond were avoided.

Sodium hypochlorite in this study was not efficacious in the inhibition of microbes and therefore is not a very good sterilant in tissue culture studies of sweet potato explants, although little clean and less contaminated culture was observed in some results. Subsequently, it is recommended that other sterilants be used in chemical sterilization of potato explants for tissue culture studies.

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References

Anikwe, M.A.N., V.N. Onyia, O.E. Ngwu and Mba, C.N. 2005. Ecophysiology and cultivation practices of arable crops, New generation book, Enugu.

Badoni, A., and Chauhan, J.S. 2010. *In vitro* sterilization protocol for micropropagation of *solanum tuberosum* cv 'Kufri Himalini', Academia Arena. 2(4).

Brant, R., and Blake, J., 1983. A lovely clone of cocomits. New Scientist (London). 98 554-557.

Chawla, H.S., 2003.Plant biotechnology: laboratory manual for plant biotechnology. Oxford & IBH Publishing Co Pvt. Ltd, New Delhi.

Clerk, C.A., and Moyer J.W., 1988.Compendium of sweet potato disease. St Paul M.M. American Phytophthalogcal.

Collins, G.B., 1984. Biotechnology and agriculture: importance for developing countries. In Sawyer WO, editor. Biotechnology in the Americans: Prospect for the developing countries, Washinton DC Intecience Association.

Curtis, H., and Barnes, N., 1985. Invitation to Biology, worth publishers. New York, 4th edition, 356 -373.

Crocomo, O.J., W.R. Sharp, O.A. Evans, J.E. Bravo, C.A. Tavares and Paddock, E.F. 1986. Biotechnology of plants and microorganisms. Columbus, Ohio State University.; pp.473 .(In Press).

Dusing, A.M., 1988. Recently published papers on the application of biotechnology to certain tropical crops (annotated bibliography) Ede the Netherlands Technical Centre for Agriculture and Rural Cooperation(CTA)pp. 79.

Gason, J.P., 1982. Les culture tropicales invitro. Intertropigues, 00:121-125.

Ketchum, J., O.L. Gamborg, G.E. Hanmining and Nebors, M.W. 1987.Tissue Culture of Crops-forth Colorado State University.

Nwauzor, E.C., S.O. Afuape, D.S. Korieocha and Ezuike, T.O. 2006. Studies on the use of neem leaf preparations for the control of cylas puncticollis damage of sweet potato root tubers in sweet potato production. In: Asumugha, G.N., Olojede, A.O., Ikeorgu, J.G., Ano, A.O.,and Herbert, U. editors. Repositioning agriculture for sustainable millennium development goals in Nigeria. Proc. of the 40th Annual Conf. of Agricultueal Society of Nigeria, 16th -20th Oct.

Onwubiko, O., and Mbanaso, E.N.A., 2006. Millennium Development Goal. In: Asumugha, GN, Olojede AO, Ikeorgu, JG, Ano AO, Herbert U. editors. Repositioning agriculture for sustainable millennium development goals in Nigeria. Proc. of the 40th Annual conf of Agric soc of Nig held in Umudike. Abia state, 16th-20th October.

Pierik, R.I.M., 1987.In-vitro culture of higher plants. 3rd edition Dordirectit, Martinus Nishoff publishers, kluwer academic publishers groups.

Sawyer, W.O., 1984. Biotechnology in the Americans: Prospect for the developing countries, proceedings of a symposium held in San Jose, Costa Rica, 3-6 May Washington DC Intecience Association (1776 Massachusetts Avenue, N.W. Washington DC,20036).pp. 76.

Tewel, O.O., O.O. Abu, E.F. Ojeniyi and Nwokocha, H.N. 2001.Status of sweet potato production,ultilization and marketing in Nigeria. In: Akoroda MO, Ngeve JM. Root crops in 21st century. pp.65-74.

Uguru, M.I., 1996.Crop Production: tools, techniques and practice. Fulladu Publishing Company.