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VIRUS FREE PLANTLETS PRODUCTION OF SWEET POTATO *(Ipomea batata (L.) Lam)* THROUGH TISSUE CULTIVATION AND MERISTEM CULTURE

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ABSTRACT

Virus free sweet potato plantlet production were investigated through meristem culture of sweet potato plantlets meristem was isolated from field grown runner tip explants and cultured on modified Murashige and Skoog (1962) medium containing auxin (2,4- D) and cytokinins (KN, BA, BAP) was used to cultivate meristem culture and tissue culture of sweet potato *(Ipomea batata (L.) Lam)* in vitro. Concentration of sucrose was at 3% w/v level. The pH media was adjusted to 5.7 before the addition of agar 8% w/v, meristems with 2-3 leaves primordial were dissected out aseptically and inoculated to the media (various levels of hormones and in different combinations), then incubated at 27 + 2^0C under light intensity of 2000-3000 lux in the culture room. Treatments include 2,4-D and BA at levels of 1mg/L and 0.25 mg/L, respectively with GA3, (0.1 mg/L) produced better plantlet, but higher levels of 2,4-D with KN (2.5 mg/L) induced the meristems to form callus. After having DAS- ELISA test the in vitro grown plantlets were being used for massive micro propagation. Visual evaluation of the morphological trails of the meristem culture derived plants showed normal and free from various diseases. Gross yield was obtained in meristem derived plants over their source plants.

Keywords: Meristem Culture, Virus Free, Plantlets Production, Sweet Potato and Tissue Culture.

INTRODUCTION

The sweet potato *(Ipomea batata (L) Lam)* is widely grown around the world. Consumed fresh, cooked or processed to starch, alcohol, liquor, colouring agents, juice, high beta-carotene content or high alpha-tocopherol (Vitamin E) content. Sweet potato and many other vegetable propagated crops are frequently characterized by their inability to produce seed due to presence of one or more factors, such as incompatibility, dichogamy, abnormal seed and seedling development, seed dormancy and environmental conditions, which affect flowering and seed setting. Presence of these factors poses some limitation on the use of environmental techniques for improvement of these crops. Therefore, as it has been exploited for many other crops, tissue culture technology could offer a very valuable tool for improvement of root crops. Root crops (including sweet potato) are found to be infected by one or more virus. Virus infection causes a significant reduction in yield and tuber quality and therefore, susceptible varieties are becoming less popular and less productive. Though heat treatment can be used as a therapy, it is always not successful and it is effective only against some specific virus (Merja, D. and A. Stasa, 1997). Sweet Potato Feathery Mottle Virus (SPFMV) is the most common virus affecting this crop worldwide. The most important virus in Africa (particularly in Nigeria) are those that form the African virus complex, also known as Sweet Potato Virus Disease (SPVD). This complex, which includes SPFMV and Sweet Potato Chlorotic Stunt Virus (SPCSV), affects the crop from Nigerian to South Africa, via much of east Africa. To ensure the planting materials are virus free, the tissue culture process described below.

MATERIAL AND METHOD

The nutrient media contain inorganic and organic constituent according to Murashige and Skoog (1962). The inorganic constituents include; KNO_3 (1900ml), $MnSO_4.7H_2O$ (22.3ml), KI (0.83ml), $CaCl_2.6H_2O$ (0.025ml) and $ZnSO_4.2H_2O$, (0.25ml). The organic constituents include: glycine 2ml, myo-inositol 100ml, thiamine-HCl (0.1ml), pyridoxine-HCl (0.5ml), nicotinic acid (0.5ml), agar (8% w/v) and sucrose (3% w/v). Auxins, Kinetin and gibberillic acid were added to the Murashige and Skoog medium in different concentrations and combinations. The pH of the medium was adjusted to 5.7 with either 1N KOH or 1N HCl. The culture media were autoclaved at 1ib/in2 at 121^0C for 15 minutes, then maintained at 27 + 2^0C in quickly rinsed in 70% alcohol for 1 – 2 minutes in a sterilized Erlenmeyer flask. They were sterilized with 10% commercial chlorox for another ten (10)

minutes and then rinsed three (3) times with sterile. Entire tools required for dissection were previously sterilized out into small pieces (0.5 – 0.8mm) and placed separately, aseptically in culture bottle containing 10ml of Murashige and Skoog medium with various growth regulators. All operations were carried out before a laminar flow cabinet. The culture bottle containing the explants was maintained in the laboratory at 27 + 2^0C. They were exposed to artificial illumination of 2000-3000 lux by placing them at 25-30m below fluorescent light 126h photoperiod. Before shoot multiplication, a serological identification was conducted in the cultured plants and to detected virus. In this multiplication, a double antibody sandwich enzyme linked immuno absorbent assay (DAS_ELISA) methods were followed. Virus free plantlets were used for mass propagation in Murashige and Skoog with hormone supplements were used. The mass propagation was evaluated by number of shoot and root formation, shoots length and root formation frequency.

Table 1: Below Show Hormones in Different Concentration and Combinations in the Modified M & S Medium

Concentration of Growth Regulators(Mg/l)			
Treatments	**2,4d**	**BA**	**GA3**
1	0.5	0.10	0.1
2	1.0	0.25	0.1
3	1.5	0.40	0.1
4	2.0	1.00	0.1
5	2.0	1.00	0.1
6	2.5	1.50	0.1
7	1.o	0.10	0.2
8	1.0	0.20	0.2
9	1.5	0.40	0.2
10	2.5	1.50	0.2
11	0.5	0.10	0.1
12	1.0	0.20	0.1
13	1.5	0.30	0.1
14	2.0	0.60	0.1
15	2.5	1.50	0.1

2,4-D-Dichlorophenoxy acetic acid.
BA: Benzyl adenine
GA3: Gibberellic acid.
KN: Kinine.
BAP: 6-Benzyl amino purine.

Table 2: Growth of Storage Meristem Culture of Five (5) Sweet Potato Cultivars

Treatment	TIS-87/0087	TIS-8164	TIS-25320P.1.13	TIS-8164	TIS-2532 OP.1.13
1	*	*	*	*	+
2	+++	+++	+++	+++	+++
3	++	++	++	++	++
4	*	*	*	*	*
5	**	**	+	+	**
6	+	+	+	+	+
7	_	_	_	_	_
8	_	_	_	_	-
9	*	*	*	*	*
10	*	*	*	*	*
11	_	_	_	_	_
12	_	_	_	_	_
13	_	_	_	_	_
14	**	**	**	**	**
15	***	***	***	***	***

++ Good plantlets formation
+ Poor plantlet formation
***** Best callus formation**
**** Good callus formation**
*** Poor callus formation**
- No callus formation

Table 3: Days of Formation of Shoots and Roots from Meristem Cultured Tips of Five (5) Sweet Potato Cultivars

Varieties	Formation	Well formed plantlet	
	Shoot	Root	
Tis-87/0087	33	42	85
Tis-8164	32	41	82
Tis-2532.op.1.13	26	38	70
Tis-8164	32	40	78
Tis-25-2532	31	40	76
Op.1.3	31	40	76

RESULT AND DISCUTION

Primary Establishment of Meristem

Runner tips were used as explants for meristem isolation. It was collected from field grown plant and was quickly rinsed in 70% alcohol for 1-2 minute(s) in a sterilized Erlenmeyer flask. Among the different time period used for, five minutes was found healthy and free of contamination. As shown in Table 2, some of the treatment induced the formation of either plantlets or callus while others did not induce any plantlets or callus formation totally. Early appearance of root and shoots was observed Tis-2532 O.P.I.13 followed by TIS-2532OP.I.13, Tis-8 164 and Tis-8164 and Tis-87/0087.

Serially (Table 3)

When the hormones supplements were considered low level of 2,4-D, BA with GA3 produce plantlets was 1mg/L 2,4-D,0.235mg/L BA and 0.1mg/L GA3. The medium did not induce organ formation from sweet potato callus cultures at any given level of hormone combination. All through previous studies indicated that the potential of organogenesis could be enhance, in the latter part of the same subculture organ; formation was not observed even when the culture was maintained for a longer period in the medium when fortified with a mixture of amino acid and vitamins. Some workers have noted that callus remained undifferentiated by showing proliferation regardless of the hormones and metabolite to which they exposed. Organogenesis has been related to the size of the explants culture too. Murashige and Skoog medium showed quickly establishment of meristem in culture media. In liquid culture, method for tissue culture has been reported (7-5). Meristem culture is a unique technique used to be free from various pathogens including viruses, viroides, mycoplasma, bacteria, and fungi confirmation of virus elimination in meristem derive plant sample by DAS-S-ELISA test: Before mass micro propagation meristems derive plantlets were tested for virus detection by DAS-S-ELISA technique. Virus free plantlets were used for mass micro propagation.

Field Performance of Meristem Derived Plantlets: The field performance of meristem derived plant shows, morphological characters of meristem derived plants were found normal, varietals stability was also reported among the meristem derived regenerated plant (Quak, F., 1977).

CONCLUSION

In conclusion, visual observation of the plants shows that, no symptoms of viral disease were notice in fruit (tuber) yield of meristem derive plant

was more than that of the derive plant since good plant health is a major advantage in the development of elite mother (stock material) production of virus free/disease free micro propagation offers many advantage over conventional method of plant.

RECOMMENDATION

In recommendation, sweet potato produce through micro propagation (artificial seed) can be easily exploited on a large scale, generating millions of plants in few days, and these may become a profitable multibillion rupees industry in near future, if this technique is employed as a means of free/disease free sweet potato production in the study area.

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