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Protocol Optimization and Mass Propagation of Virus Free Sweet Potato[Ipomoea Batatas L. (Lam) to Enhance production in Southern, Ethiopia

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Abstract
Sweet potato is one of the most produced and utilized root crops in the world. It is the second most cultivated root crops in southern part of Ethiopia next to enset(CSA, 2016/17). Its productivity nowadays is hardly decreased due to sweet potato virus disease which is transmitted and distributed through infected plantlets and low quality planting materials. Plant tissue culture through meristem shoot tip culture followed by virus indexing and mass propagation is a crucial technique to get healthy planting materials and hence restore the production and productivity. The experiment was designed to get in vitro shootlets and test to confirm the efficiency of the technique in cleaning the virus from plants. Explants from two varieties kulfo and beletsech were used as experimental materials and NCM-ELISA package protocol was used to test regenerated plants for virus elimination. The result of plantlets regeneration showed that 77% and 70% of kulfo and beletsech explants were initiated as contamination free where as 6 and 5.3 nod numbers were recorded for kulfo and beletsech plantlets in MS media supplied with 1mg/l BAP media. In NCM-ELISA test 98.5% of plantlets were found to be virus free and 1.5 % were suspected for SFMV virus from 96 sample plants tested.
Keywords: Sweetpotato, Virus, explants, ELISA

Introduction
Sweet potato [*Ipomoea batatas* L. (Lam)] is a dicotyledonous, perennial plant, and belongs to the family convolvulacea (Austin, 1987). It is an important tuberous root crop cultivated throughout the tropical and warm temperate regions wherever there is sufficient water to support growth Demissew, 2006 and Vincent, 2009).
Sweet potato is a dry-land crop that is tolerant of a wide range of edaphic and climatic conditions. It is widely cultivated in developing countries from tropical to temperate regions, such as Asia, Africa and Latin America. Among the major starch staple crops, sweet potato produces relatively better yield per unit area (Woolfe, 1992). In Ethiopia, sweet potato is grown around a densely populated area in the South, Southwestern and Eastern parts of the country and is one of the most important crops for at least 20 million Ethiopians (Assefa *et al*., 2007). Productivity of sweet potato is greatly constrained by pests and diseases, the most important one being viral diseases. Twenty viruses have been recently reported to infect sweet potato (Fuglie, 2007). These include the Sweet potato feathery mottle virus (SPFMV), Sweet potato chlorotic stunt virus (SPCSV), Sweet potato virus G (SPVG), Sweet potato mild mottle virus (SPMMV), Sweet potato chlorotic fleck virus (SPCFV), Sweet potato latent virus (SPLV), Sweet potato caulimo-like virus (SPCaLV), Cucumber mosaic virus (CMV) and Sweet potato leaf curl virus (SPLCV). Depending on cultivar, infecting virus, stage of infection and whether the crop is infected with a single or multiple viruses, viral diseases may cause up to 100% yield loss (Gibson *et al*., 1997). Production of virus-free planting materials and virus indexing of the regenerated plants is one of the viable disease controlling strategies in vegetative propagated crops like sweet potato (Geleta, 2010). Meristem culture, meristem culture after thermotherapy treatment and shoot thermotherapy are important biotechnology tools for the production of virus free sweet potato micro cutting (Panta *et al.*, 2007; El Far and Ashoub, 2009). Hence this experiment was conducted to reproduce virus free planting materials using shoot tip explants and test before further propagation and distribution.

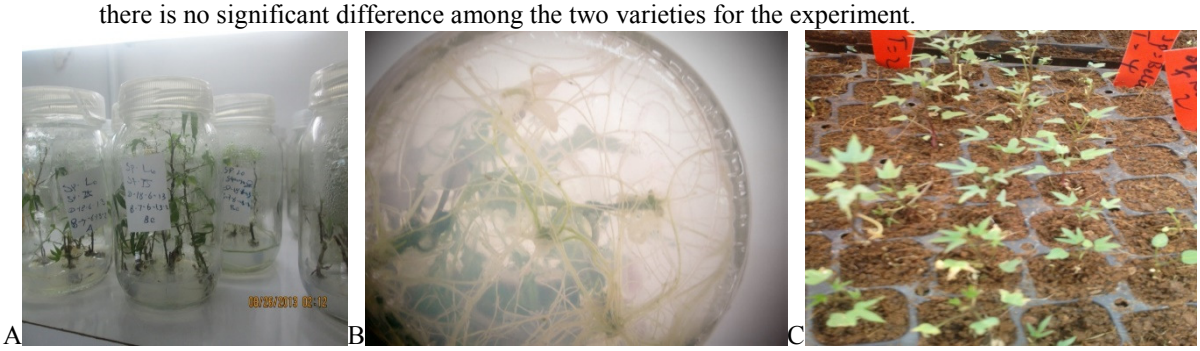
Materials and Methods:
Shoot induction and Regeneration
Shoot tip explants of varieties: LO-323(Kulfo) and Beltsech manipulated to 0.5-1cm length were initiated on MS media enriched with 1mg BAP for initiation and multiplication and 1mg of IBA in rooting stage.
Virus Indexing
Number of varieties tested: Two varieties LO-323(Kulfo) from orange flashed type and Beletseh from white flashed type
Sample sources: *in vitro* plants, green house acclimatized plants, vine cuttings in multiplication from Hawassa net tunnels, vine cuttings in multiplication fields of Hawassa and Areka initially from tissue culture.

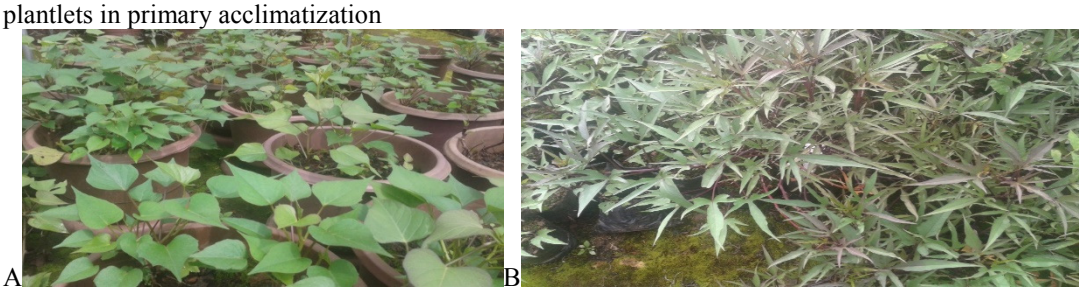
Total Sample Tested: 92
Testing Method used: NCM-ELISA(Nitrocellulose Membrane- Enzyme Linked Immune Sorbet Assay) virus indexing technique was used by taking 46 sample from both varieties
Data collected : Percent of explants survival, days to shoot induction, shoot length, Node number, leaf number, shoot length, Root number, root length percent of sample cleaned from virus.
Data management: all parameter recordings except percent of explants survival were analyzed by ANOVA 9.2 version as mean at probability of 5% and tested for significance by LSD.

Results:
Table 1 Initiation and plantlets regeneration performance

variety	% succ.	DSI	SL	LN	NODN	RN
Beletech	70	3.66	**5.9**[b]	5.5	5.3	3
Lo 323	77	3.5	7[a]	6	6	3.3
Mean	73.5	3.58	6.45	5.75	5.65	3.15
LSD(5%)	-	NS	0.64	NS	NS	NS
CV(%)	-	15	4.38	15.38	14.4	13

1. Percent of explants survival: initiation of shoots from explants in recommended media after surface sterilization showed difference in percentage (70%) for beletsech and 77% for Lo 323 of explants initiated to give cleean shoots with no contaminating microbes.
2. Days to shoot induction: Effect of variety on days to new shoot induction after cultured to media and let in growth room with 16/8 hr light and dark photoperiod under +27+(-) ^{0}C temperature and up to 10,000 lux light through cool light releasing fluorescent ampoules showed no significance difference.
3. Shoot length: there is significant difference among the two varieties used in which larger shoots were obtained for variety Lo 323(7cm) where as lower shoots were observed for Beletsech(5.9cm).
4. For shoot regeneration parameters leaf number, number of nods and root number per shoots regenerated there is no significant difference among the two varieties for the experiment.


A B C
Fig.1 In vitro regeneration and Acclimatization.A Shootsregenerated B, roots from bottom of growth jars. C. plantlets in primary acclimatization


A B
Fig. 2 Growth preformance of virus tested plantlets in propagation. A. Beletsech variety and B. Lo 323 Variety

Virus Indexing
All samples tested for all the ten viruses were found to be negative except two samples of Beletsech variety from Areka green house and one Lo-323 sample from the same source was suspected to SPFMV virus since they showed light purple color unlike dark purple for positive control(Fig.3D). But all samples taken from *in vitro*, Hawassa net tunnels and from multiplication field of Areka and Hawassa showed no reaction to all antigen –antibody-enzyme link reaction that shows they are free from all ten potential viruses tested(Fig 3D and annex 1).

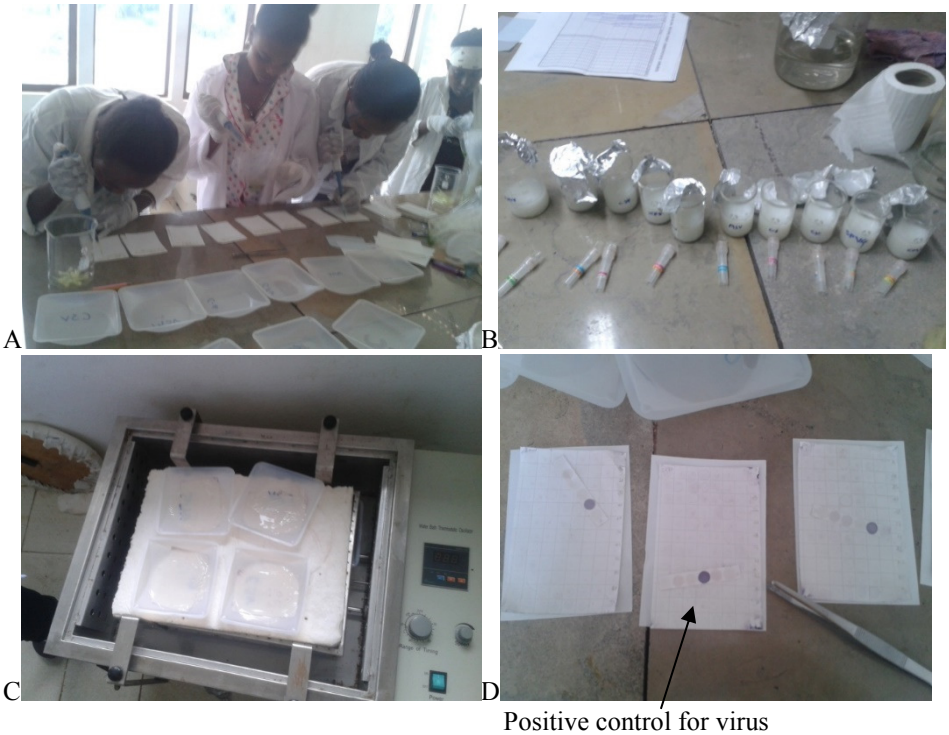


Fig.3: Partial pictures of the experiment. A. placing sample on membrane, B. Preparation of specific antibodies. C. shaking of antigen-antibody-enzyme for color development. D. test result.

Discussion

Plantlets regenaretion was successfully conducted by collecting 3-4 cm explants from shoot tip of two month old vines grown in green house as mother plants. The explants were washed thoroughly by tap water and home cleaning detergent largo before taking to laminar hood where it was surface sterilized by 75% of ethanol for 2 minutes followed by 1.25% Sodium chloride for ten minutes in which more than 70% of contamination free explants were initiated.

The initiation ,multiplication and rooting was done in MS media supplied with 1mg/l BAP growth regulating hormone promoting shoot cell division and 1mg/l IBA hormone enhancing root proliferation and growth *in vitro*. The result of shoot growth recorded average shoot length of up to 6.45 cm and node number 5.65 recorded after four weeks of sub-culturing date. This indicates that more than five new shoots can be transferred to fresh media for miro-propagation in one month interval was achieved at Areka tissue culture laboratory condition which is consistent to different study results reported(Geleta D., 2011)

The virus indexing test showed that 100 % of plantlets regenerated through tissue culture technique at Areka in micro propagation and 98.5% for those in propagation by cutting under green house which is inconsistent to report from (Geleta D., 2010) who used meristem culture accompanied by thermotherapy and tested by the same indexing technique NCM-ELISA.

Conclusion and recommendations

From the protocol optimization experiment conducted at Areka tissue culture laboratory and result achieved it is possible to conclude that virus free sweet potato planting materials production *in vitro* is possible using shoot tip culture on MS +1mg/l BAP for shoot initiation and growth.

In NCM-ELISA test to check the cleaneness of plantlets befor further propagation and distribution all materials in vitro were found negative from all ten potential viruses tested.

Recommendation

Shoot tip culture followed by virus indexing is a better solution to restore sweet potato production potential in SNNPr Ethiopia

It is better to use other indexing techniques in addition to NCM-ELISA to confirm absence of all viruses including those for which antibodies were not developed like begomovirus.

It is also better to recommend further investigation on frequency of sub-culturing and vine cuttings to get true to type and high quality sweet potato planting materials.

References

Demissew, S. (2006). Convolvulaceae In: Flora of Ethiopia. pp 161-231. (Hedberge, I. and Edward, S. Ensermu Kelbessa, Edwards. S., Sebsebe Demissew ,Persson. E., eds.), Gentianaceae to Cyclocheilaceae. Addis Ababa/Uppsala, Ethiopia/Sweden, The National Herbarium, Vol. 5.

El Far, M. M. M., El Mangoury, K. and Elazab, H. E. M. (2009). Novel plant regeneration for Egyptian sweet potato (Ipomoea batatas (L.) Lam.) Abees cultivar via indirect organogenesis stimulated by initiation medium and cytokinin effects. Aust. J. Basic & Appl. Sci. 3(2):543-551.

Fuglie KO, 2007. Priorities for Sweet Potato Research in Developing Countries: Results of a Survey. HortScience 42:1200–1206.

Gibson RW, Mwanga ROM, Kasule S, Mpembe I, Carey EE, 1997. Apparent Absence of Viruses in the Most Symptomless Field-Grown Sweet Potato in Uganda. Annals Applied Biology 130:481-490.

Panta, A., Espinoza, C. and Roca, W. (2007). Health status testing and virus elimination in sweet potato, CIP, pp. 1-3.

Pozzer, L., Dusi, A. N., Silva, J. B. C. and Kitajima, E. W. (1994). The rate of Tofu A, Anshebo T, Tsegaye E, Tadesse T, 2007. Summary of Progress on Orange-Fleshed Sweet Potato Research and Development in Ethiopia. Proceedings of the 13th ISTRC Symposium. pp. 728–731.

Annex 1

Table: NCM-ELISA Test Result

No.	Variety name	Sample Source	Sample code	Result of Sweet potato Virus (SPV)Types Tested								
				SPFMV	MMV	LV	CFV	C-6	MSV	CaLV	SPVG	CMV
1	Lo-323	GH Aw	01	-	-	-	-	-	-	-	-	-
2	Lo-323	,,	02	-	-	-	-	-	-	-	--	-
3	Lo-323	,,	03	-	-	-	-	-	-	-	-	-
4	Lo-323	,,	04	-	-	-	-	-	-	-	--	-
5	Lo-323	,,	05	-	-	-	-	-	-	-	-	-
6	Lo-323	,,	06	-	-	-	-	-	-	-	--	-
7	Lo-323	,,	07	-	-	-	-	-	-	-	--	-
8	Lo-323	,,	08	-	-	-	-	-	-	-	-	-
9	Lo-323	,,	09	-	-	-	-	-	-	-	-	-
10	Lo-323	,,	10	-	-	-	-	-	-	-	--	-
11	Lo-323	,,	11	-	-	-	-	-	-	-	-	-
12	Lo-323	,,	12	-	-	-	-	-	-	-	--	-
13	Lo-323	,,	13	-	-	-	-	-	-	-	-	-
14	Lo-323	,,	14	-	-	-	-	-	-	-	--	-
15	Lo-323	,,	15	-	-	-	-	-	-	-	--	-
16	Lo-323	,,	16	-	-	-	-	-	-	-	-	-
17	Lo-323	,,	17	-	-	-	-	-	-	-	--	-
18	Lo-323	,,	18	-	-	-	-	-	-	-	-	-
19	Lo-323	,,	19	-	-	-	-	-	-	-	--	-
20	Lo-323-FAw-1	AW field	20	-	-	-	-	-	-	-	-	-
21	Lo-323-FAw-2	,,	21	-	-	-	-	-	-	-	--	-
22	Lo-323-FAw-3	,,	22	-	-	-	-	-	-	-	--	-
23	Lo-323	,,	23	-	-	-	-	-	-	-	-	-
24	Lo-323	,,	24	-	-	-	-	-	-	-	--	-
25	Lo-323	,,	25	-	-	-	-	-	-	-	-	-
26	Lo-323	,,	26	+	-	-	-	-	-	-	--	-
27	Lo-323	,,	27	-	-	-	-	-	-	-	-	-
28	Lo-323	,,	28	-	-	-	-	-	-	-	--	-
29	Lo-323	,,	29	-	-	-	-	-	-	-	--	-
30	Lo-323	,,	30	-	-	-	-	-	-	-	-	-
31	Lo-323	,,	31	-	-	-	-	-	-	-	-	-
32	Lo-323	,,	32	-	-	-	-	-	-	-	-	-
33	Lo-323	,,	33	-	-	-	-	-	-	-	--	-
34	Lo-323	,,	34	-	-	-	-	-	-	-	-	-
35	Lo-323	,,	35	-	-	-	-	-	-	-	--	-
36	Lo-323	,,	36	-	-	-	-	-	-	-	--	-
37	Lo-323	,,	37	-	-	-	-	-	-	-	-	-
38	Lo-323	,,	38	-	-	-	-	-	-	-	--	-
39	Lo-323	,,	39	-	-	-	-	-	-	-	-	-
40	Lo-323	,,	40	-	-	-	-	-	-	-	--	-
41	Lo-323-Lab-1	TC lab.	41	-	-	-	-	-	-	-	-	-
42	Lo-323- Lab-1	,,	42	-	-	-	-	-	-	-	--	-
43	Lo-323- Lab-1	,,	43	-	-	-	-	-	-	-	--	-
44	Lo-323 -Lab-1	,,	44	-	-	-	-	-	-	-	-	-
45	Lo-323- Lab-1	,,	45	-	-	-	-	-	-	-	--	-

continuation

No.	Variety name	Sample Source	Sample code	Result of Sweet potato Virus (SPV)Types Tested								
				SPFMV	MMV	LV	CFV	C-6	MSV	CaLV	SPVG	CMV
46	Lo-323- GH-1	GH Are.	46	-	-	-	-	-	-	-	-	-
47	Lo-323- GH-2	,,	47	-	-	-	-	-	-	-	--	-
48	Lo-323- GH-3	,,	48	-	-	-	-	-	-	-	-	-
49	Lo-323- GH-4	,,	49	-	-	-	-	-	-	-	--	-
50	Lo-323- GH-5	,,	50	-	-	-	-	-	-	-	-	-
51	Lo-323- GH-6	,,	51	-	-	-	-	-	-	-	--	-
52	Lo-323- GH-7	,,	52	-	-	-	-	-	-	-	--	-
53	Lo-323- GH-8	,,	53	-	-	-	-	-	-	-	-	-
54	Lo-323- GH-9	,,	54	+	-	-	-	-	-	-	-	-
55	Beletech Lab-1	,,	55	-	-	-	-	-	-	-	--	-
56	Beletech Lab -2	,,	56	-	-	-	-	-	-	-	-	-
57	Beletech Lab -3	,,	57	-	-	-	-	-	-	-	--	-
58	Beletech Lab -4	,,	58	+	-	-	-	-	-	-	-	-
59	Beletech Lab -5	,,	59	-	-	-	-	-	-	-	--	-
60	Beletech Lab -6	,,	60	-	-	-	-	-	-	-	--	-
61	Beletech Lab -7	,,	61	-	-	-	-	-	-	-	-	-
62	Beletech Lab -8	,,	62	-	-	-	-	-	-	-	--	-
63	Beletech Lab -9	,,	63	-	-	-	-	-	-	-	-	-
64	BeletechGH-1	,,	64	-	-	-	-	-	-	-	--	-
65	BeletechGH-2	,,	65	-	-	-	-	-	-	-	-	-
66	BeletechGH-3	,,	66	-	-	-	-	-	-	-	--	-
67	BeletechGH-4	,,	67	-	-	-	-	-	-	-	--	-
68	BeletechGH-5	,,	68	-	-	-	-	-	-	-	-	-
69	BeletechGH-6	,,	69	-	-	-	-	-	-	-	--	-
70	BeletechGH-7	,,	70	-	-	-	-	-	-	-	-	-
71	BeletechGH-8	,,	71	-	-	-	-	-	-	-	--	-
72	BeletechGH-9	,,	72	-	-	-	-	-	-	-	-	-
73	Lo-323-Far-1	Ar.Field	73	-	-	-	-	-	-	-	--	-
74	Lo-323-Far-2	,,	74	-	-	-	-	-	-	-	--	-
75	Lo-323-FAr-3	,,	75	-	-	-	-	-	-	-	-	-
76	Lo-323-FAr-4	,,	76	-	-	-	-	-	-	-	-	-
78	Lo-323-FAr-5	,,	78	-	-	-	-	-	-	-	-	-
79	Lo-323-FAr-6	,,	79	-	-	-	-	-	-	-	--	-
80	Lo-323-FAr-7	,,	80	-	-	-	-	-	-	-	-	-
81	Lo-323-FAr-8	,,	81	+	-	-	-	-	-	-	--	-
82	Lo-323-FAr-9	,,	82	-	-	-	-	-	-	-	--	-
83	Lo-323-FAr-10	,,	83	-	-	-	-	-	-	-	-	-
83	Beletech-FAr.-1	,,	83	-	-	-	-	-	-	-	--	-
84	Beletech-FAr.-2	,,	84	-	-	-	-	-	-	-	-	-
85	Beletech-FAr.-3	,,	85	-	-	-	-	-	-	-	--	-
86	Beletech-FAr.-4	,,	86	-	-	-	-	-	-	-	--	-
87	Beletech-FAr.-5	,,	87	-	-	-	-	-	-	-	--	-
88	Beletech-FAr.-6	,,	88	-	-	-	-	-	-	-	--	-
89	Beletech-FAr.-7	,,	89	-	-	-	-	-	-	-	-	-
90	Beletech-FAr.-8	,,	90	-	-	-	-	-	-	-	--	-
91	Beletech-FAr.-9	,,	91	-	-	-	-	-	-	-	--	-
92	Beletech-FAr.-10	,,	92	-	-	-	-	-	-	-	-	-

Key:- *Ar- Areka, AW- Awassa, FAr- Farm of Awassa/Areka, GH- Green house, Lab-laboratory, +(Positive), -(Negative)*