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THE EFFECTIVENESS OF REPEATED SHOOT TIP CULTURE ON PATHOGENS LOAD REDUCTION IN DIFFERENT LOCAL POTATO GENOTYPES IN MALAWI

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ABSTRACT

Potato production in Malawi still remains low, estimated at 11t/ha as compared to the potential 40t/ha due to disease infection resulting from seed recycling. Shoot tip culture in vitro is one of the tissue culture techniques that can potentially eliminate pathogens from infected plantlets. A study was therefore carried out to evaluate the effectiveness of repeated shoot tip culture on pathogen load reduction of some selected local potato genotypes grown in Malawi. A factorial experiment was laid out in a Completely Randomized Design (CRD) in which five genotypes including Magalabada, Rosita, Simoki, Usiwawatha and Violet were evaluated. Results showed that the mother stock tubers for all genotypes were infected with multiple pathogens at different incidences and intensities. PVY had an incidence of 100% with a low intensity in all genotypes. PVX had an incidence of 100% in Rosita and Magalabada, 80% in Usiwawatha and Violet and 60% in Simoki and their intensity was very high. The incidence for PVM was 80% in Usiwawatha, 60% in Violet, 10% in Rosita and Magalabada and 3% in Simoki; and their intensity was high. The incidence for PVS was 60% for Rosita, Magalabada and Usiwawatha, 18% for Violet and 10% for simoki; with a very high intensity. PVA incidence was 100% for Usiwawatha, 60% for Violet, 18% for Rosita and Simoki, and 5% for Magalabada; with a very low intensity. PLRV incidence was 10% for all genotypes and had a very low intensity. Traces of Bacterial wilt in low intensities were detected. Potato plantlets obtained after repeated sub-culturing of over six times resulted in reduced pathogen load at

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(P≤0.05) for the different pathogens. The chance of reducing the pathogen load in the different genotypes was relatively high at (P≤0.05).

Keywords: Production, Low, Disease, Plantlets, Tissue culture.

Contribution/ Originality

This study contributes in the existing literature of tissue culture technique by providing a less expensive tool for the developing nations' attainment of food security; since repeated sub-culturing can reduce and/eliminate pathogens load in potato (Solanum tuberosum L) due to the cells undifferentiation principle, for enhanced yield and quality.

1. INTRODUCTION

Potato is an important source of food, employment and income in developing countries, including Malawi (Demo *et al*., 2007). In Malawi, potato is also a crop that bridges hunger months (from November to February) when most families run short of the main food crop (maize). Hence the potato crop plays an important role in food security in Malawi (Demo *et al*., 2009). The potato's high nutrient component and ease of production makes it an important component of urban agriculture industry (Food and Agriculture Organization of the United Nations, 2008; Muthoni and Nyamongo, 2009). However, production in Malawi has not increased over time due to use of recycled and diseased seed materials (Mwenye *et al*., 2011).

In Malawi, the potato crop is propagated through the conventional method such that virtually infected materials are passed on to the next generation, and eventually yields get reduced. Despite Malawi being ranked second in potato production in the Sub-Saharan Africa (SSA) (Food and Agriculture Organization of the United Nations, 2008), potato production still remains low at 11t/ha as compared to the potential yield of 40t/ha. It has been reported (Demo *et al*., 2007) that one of the challenges that lead to low production levels in the potato crop is the use of virus infected seed. The presence of virus infections has been reported by a number of researchers and some of the identified viruses include Potato virus Y, Potato virus X, Potato Virus A, Potato virus M, Potato leaf roll virus, and Potato virus S (Food and Agriculture Organization of the United Nations, 2008; Kagona, 2008). Thus, disease infestation remains a major constraint towards potato production and productivity in Malawi (Mih and Atiri, 2006).

Shoot tip culture is a disease elimination technique whereby pieces of the apical growing point are excised from a plant and cultured in a sterile growth media *((Sim and Golino, 2010)*. This technique uses a small sized growing tip about 0.5-1.0 mm in length including the apical meristem and leaf primordial (Golino *et al*., 2000). This method has the advantage of regenerating a single plant from a single, minuscule shoot within a short period of about two-four weeks (Muhammed, 2002). The technique has potential to reduce systemic diseases due to the presence and subsequent fast multiplication of the meristematic tissues (Sim, 2006).

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Shoot tip culture *in vitro* has been used for many decades to eliminate pathogens in a number of crop plants (Von Arnold *et al*., 2002; George *et al*., 2008). (Muhammed, 2002) reported that Alfalfa Mosaic Virus Free Potatoes were obtained when potato shoot tips were used as explants. On the other hand, reports (Hadidi *et al*., 2003) shows that while shoot tip culture can effectively eliminate viruses may not be effective for viroids. In addition, shoot tip culture is reported to reliably eliminate the Ag*robacterium vitis* bacterium, which causes grape crown gall disease but it does not effectively eliminate virus infections (Sim, 2006). However, shoot tip culture done repeatedly may eliminate different types of pathogens (Milošević *et al*., 2011). Thus, while repeated shoot tip culture has been found as one technique in eliminating the different pathogens, there is little information on number of culturing cycles that would ensure pathogen elimination in the different potato genotypes.

This study therefore aimed at evaluating the performance of repeated shoot tip culture on pathogen load reduction of different potato genotypes grown in Malawi. Specifically, the study objective was to determine the minimum number of sub-culturing cycles of repeated shoot tip culture that can reduce the pathogen load for the different local potato genotypes.

2. LITERATURE SURVEY

2.1. Potato Propagation Techniques

2.1.1. Conventional Method

In traditional seed potato production system, potato is mainly propagated via seed tubers (Mih and Atiri, 2006; Food and Agriculture Organization of the United Nations, 2008; Kagona, 2008). This method is sometimes called conventional propagation and it ensures uniformity of the crop in terms of growth and yield, but results in degeneration of the crop due to virus infection, the rate of degeneration varying from place to place and from cropping season to cropping season (Tadesse, 2000). The viruses are transmitted through different ways including through planting infected tubers. If the seed stock is not maintained well or frequently replaced with fresh ones, the virus infiltration can reach up to 100% in 3 - 4 successive crop seasons resulting in almost half or one third yields reduction (Khurana *et al*., 2001). As such, this method has disadvantages in terms of poor seed health and low rate of multiplication thereby providing low yields and reduced tuber quality (Naik and Karihaloo, 2007). This is the major problem faced by seed producers. In addition to this, conventional seed multiplication methods take a long time and are prone to virus attack (Biniam and Tadesse, 2008).

2.1.2. True Seed Breeding

True potato seed (TPS) produced through sexual mode is used as an alternative planting material in some parts of Bangladesh, China, India, Republic of Korea, Nepal, The Philippines, Vietnam and some countries of Central Asia. Since TPS is produced through fertilization, it is also called botanical seed and resembles seeds of other solanaceous plants like tomato, brinjal and

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chilly. A single fruit or berry contains an average 150-200 seeds. TPS has several advantages. It can be produced easily, it is not bulky and thus easy to store and transport, only about 50-125g TPS is sufficient to plant one hectare area and most viruses are eliminated during the process of sexual seed formation (Naik and Karihaloo, 2007). To add on this, it is reported (Nizamuddin *et al*., 2010) that since relatively few diseases are transmitted through the seed, the true potato seed (TPS) produces free plantlets which can be transplanted as the planting material for the ware potato or used to produce relatively disease free seed tubers. In the first system, major savings in storage of seed are achieved as a few kilograms of TPS can replace tonnes of seed tubers. TPS is used to grow commercial potato crop by two methods: (i) transplanting seedlings and (ii) use of seedling tubers. In the former method, potato seedlings are raised in nursery beds and transplanted in the field at 4-5 leaf stage. Potato crop raised from seedlings is harvested at maturity. This method requires about 125g TPS and 75m^2 nursery bed areas for growing seedlings sufficient for transplanting in one hectare area (Naik and Karihaloo, 2007; Black, 2008). In the second method, small size tubers are produced by allowing the seedlings to mature in nursery beds. Seedlings are dehaulmed (cutting of plants at ground level) at maturity and harvested in another 10-15 days when the tuber skin becomes firm and well developed. These seedling tubers are stored and used as planting material for growing the commercial crop in the following crop season. About 50-60g TPS and 250-300 m2 nursery bed area are required for producing seedling tubers sufficient for planting for one hectare (Naik and Karihaloo, 2007; Black, 2008; Nizamuddin *et al*., 2010)

TPS progeny are a collection of genotypically different individuals which show considerable diversity in many of the important quality traits of potatoes (Black, 2008). Considerable effort is being made at the International Potato Centre (CIP) to improve TPS through selection of parental lines which produce progenies with improved uniformity in plant and tuber characteristics. Attention is also being made to the factors influencing the yield of seed of tropic latitudes as sparse flowering and reduced seed set are serious limitations to TPS production in the Tropics (Black, 2008; Nizamuddin *et al*., 2010). The other disadvantages with TPS technology are that the seed are not genetically pure and exhibit high heterogeneity; the crop is late in maturity as compared to the crop grown from seed tubers; and the technology is labor intensive (Naik and Karihaloo, 2007).

2.1.3. Tissue Culture Techniques

Tissue culture is the technique through which any plant part is cultured on a nutrient medium, under sterile conditions and controlled environment, with the purpose of obtaining growth (Ahloowalia *et al*., 2002; Ilieve *et al*., 2010). In tissue culture, the term *in vitro* is very often used to mean in glass (as in a closed container) entailing aseptic conditions are always met for possible growth and the term is used in contrast to the term *in vivo* which means as in nature (Thiart, 2004; Ilieve *et al*., 2010). Potato can also be propagated rapidly in large scale by tissue

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culture techniques (Thiart, 2004; Ilieve *et al*., 2010). Tissue culture multiplication through use of explants derived from mother stocks, are cut and propagated through multiple cycles under aseptic laboratory conditions. New plantlets are regenerated from cuttings of plantlets from the previous cycle. After the final cycle of propagation, growers transplant the plantlets into a greenhouse for mini-tuber production (Black, 2008).

Microtubers are miniature tubers that are developed *in vitro* under tuber inducing conditions. Their average weight ranges from 100 to150 mg and this make them convenient for handling, storage and long distance transportation (Naik and Karihaloo, 2007). *In vitro* plantlets are usually produced from nodal cuttings and micro tubers hence can be produced all year round on complete plantlets or on plant organs. Such systems allow faster multiplication and the build-up of a large stock, which is disease-free (Thiart, 2004).

2.1.4. Tissue Culture Applications and Advantages

Some of the tissue culture applications in research and commerce include rapid propagation, pathogen elimination, storage of germplasm, embryo culture, anther and pollen culture, hybridization, gene transformation and production of secondary substances for use especially in the medical field. In this study, wider concentration has been given to the first two applications with reference to the potato industry.

2.1.5. Rapid Propagation

Through tissue culture technique, an explant can be cultured *in vitro* with the purpose of obtaining a large number of plantlets, vis a viz, rapid propagation (Naik and Karihaloo, 2007). Rapid propagation is desirable for obtaining a large number of clones as compared to conventional methods. For instance, assuming effective generation of minimum 3 nod-al cuttings by *in vitro* potato plantlet, and subculturing interval of 25 days, theoretically, 14.3 million micro plants would be obtained from a single virus free plantlet in a year (Naik, 2005). This is so because tissue culture production is independent on season (Thiart, 2004). According to some reports (Donnelly *et al*., 2008), rapid propagation is also enhanced because a small amount of space is required to maintain or multiply plants and that plant materials need very little attention between subcultures, with no requirement for watering, weeding and spraying (Naik and Karihaloo, 2007).

2.1.6. Pathogen Elimination

One of the setbacks in vegetatively propagated plants is disease infestation due to pathogen attack (Naik and Karihaloo, 2007). Plants produced through vegetative propagation are infested with bacteria, fungi, nematodes, or other pathogens such as viruses and viroids whose symptoms are hard to trace but have a significant effect on yield reduction and decrease in quality over generations (Thiart, 2004). Viruses are notoriously difficult to combat in vegetative crops because

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once vegetatively propagated plants are infected with viral diseases, the virus passes from one generation to the next and the entire population of a given plant over years, become infected with the same pathogen(Mahmoud *et al*., 2009). Tissue culture offers different techniques that have been proved to eliminate pathogens even the notorious viruses and viroids (Demo *et al*., 2007; Naik and Karihaloo, 2007). There have been reports over potato pathogens being eliminated through meristem culture, thermotherapy treatment and chemotherapy treatment of different potato varieties across the world. For instance, PVY got eliminated after meristem culture (Nascimento *et al*., 2003).

3. MATERIALS AND METHODS

3.1. Experimental Site and Duration

The experiment was conducted in Malawi at Bunda College of Agriculture and Chitedze Research station for the tissue culture work and pathological studies respectively.

3.2. Plant Materials, Explants Preparation and Sterilization

Five potato genotypes grown in Malawi namely; Rosita, Magalabada, Usiwawatha, Simoki and violet were selected for use in this study. Sprouted potato tubers were planted in the greenhouse. Three weeks after germination, shoots were collected as explants from the greenhouse. The explants were then washed under running tap water for about five minutes and then rinsed in distilled water. Surface sterilization followed by dipping in 70% ethanol for 15 seconds and thereafter dipped in 1% sodium hypochlorite with 2 drops of tween 20 solution (for every 100ml) for 20 minutes. The explants were then rinsed with sterile distilled water for three times to remove the sterilant.

3.3. Sub Culturing Cycles

Culture initiation was done in sterilized Murashige and Skoog (MS) media to obtain new shoots. Inoculation of explants into medium and subsequent sub culturing was carried out under sterile conditions in the laminar airflow chamber. The ensuing plantlets were sub cultured soon after emerging to free them from any contamination and the first sub culture was regarded as cycle number zero. Thereafter, any other subsequent sub culturing cycle was regarded as a treatment. Shoot tips segment of potato was excised and inoculated in the MS semi-solid media. The cultured explants were incubated in the growth room at a temperature range of 18-24°C under a 16 hour photoperiod with a photosynthetic photon flux density of 40µmol m^{-2} s^{-1} provided by overhead cool fluorescent lamps (Philips, India 30 Watts). The plantlets were then sub cultured 8 times at 2 weeks interval. Pathogen load assessment was done at the 2^{nd}, 4^{th}, 6^{th} and 8^{th} cycle.

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3.4. Detection of Pathogen Load

To detect the viral and bacterial wilt load in the mother stock tubers and the subcultured plantlets developed from repeated shoot tip culture, a DAS-ELISA kit was used (Appendix I and II). For the mother stock tubers, 6 tubers were used as a sample for each genotype. Whilst for the sub cultured plantlets were first grown in the green house for 5 weeks and then leaves were taken from the upper, middle and lower part of the growing plant. In total, 840 samples were tested for bacterial wilt, PLRV, PVA, PVM, PVS, PVY and PVX.

4. RESULTS

Pathogen Incidence and intensity for mother stock tubers

The results of the pathogen load of mother stock tubers using the ELISA Kit indicate pathogen incidence hereby being referred to as the percentage of absence of pathogen attack, whilst intensity as the degree of infection. The results indicated that potato tubers that were evaluated were infected with multiple potato viruses in a single stock sample (Figure 1a and 1b) regardless of the type of genotype. PVY had an incidence of 100% with a low intensity in all genotypes. PVX had an incidence of 100% in Rosita and Magalabada, 80% in Usiwawatha and Violet and 60% in Simoki and their intensity was very high. The incidence for PVM was 80% in Usiwawatha, 60% in Violet,

Figure-1. Pathogen load (incidence and intensity) for potato mother stock tuber

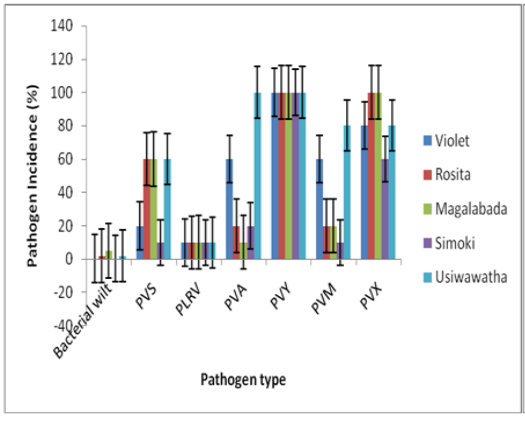

Figure-1a. Pathogen load incidence for potato mother stock tubers of different genotypes.

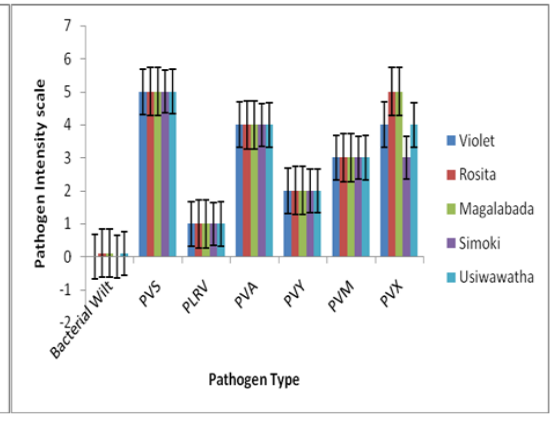

Figure-1b. Pathogen intensity in potato mother stock tubers for different genotypes. Where; 1=Very Low, 2=Low, 3=High, 4=Higher, 5=Very High (Source: CIP, 2001)

10% in Rosita and Magalabada and 3% in Simoki; and their intensity was high. The incidence for PVS was 60% for Rosita, Magalabada and Usiwawatha, 18% for Violet and 10% for simoki; with a very high intensity. PVA incidence was 100% for Usiwawatha, 60% for Violet, 18% for Rosita and Simoki, and 5% for Magalabada; with a very low intensity. PLRV incidence was 10%

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for all genotypes and had a very low intensity. There were traces of Bacterial wilt and their intensity was very low. The genotype Usiwawatha was the one that had been highly attacked with almost each type of the virus.

Pathogen (bacterial wilt and viral) load for plantlets developed under repeated shoot tip culture

The term 'antilog of estimate' on the tables of results (Tables 1 and 2) refer to the probability or the chance of success of a variable, whilst 't.pr' refers to the statistical significance of the parameter. The results showed that there were significant differences in the chance of success for the load of the different pathogens to be reduced by repeated shoot tip culture at ($P<0.05$) both for the genotypes and subculturing cycles independently but not for their interaction, with reference to genotype BP (pathogen free) and subculturing cycle 0 (plantlets that had just gone through zero subculturing cycle). The probability of reducing the pathogen load for bacterial wilt was 1 both for the genotypes and subculturing cycles and their observations showed negative reaction for all cleaned plantlets, meaning that bacterial wilt was cleaned in the process. With reference to Genotype BP (pathogen free), at ($P<0.05$) the chance of success for PLRV in the genotype *Magalabada* was 0.47, *Rosita* was 0.30, *Simoki* was 0.31, *Usiwawatha* was 0.30 and *Violet* was 0.28. With reference to the subculturing cycle 0, the probability that PLRV would be cleaned by subculturing cycle 6 was 6.7, and for cleaning cycle 8 was 6.8; whilst subculturing cycles 2 and 4 were not significantly different in their chances of success and their observations showed positive reaction .

Table-1. Pathogen load for bacterial wilt, PLRV, PVA and PVM for plantlets developed under repeated shoot tip culture

	Bacterial wilt		PLRV		PVA		PVM	
Parameter	t.pr.	antilog of estimate	t.pr.	antilog of estimate	t.pr.	antilog of estimate	t.pr.	antilog of estimate
Constant	<.001	0.2	<.001	0.06912	<.001	0.08523	<.001	0.06906
Magalabada	1	1	0.023	0.2873	0.119	0.4795	0.116	0.4733
Rosita	1	1	0.028	0.3074	0.071	0.4135	0.047	0.3647
Simoki	1	1	0.031	0.3175	0.101	0.4573	0.116	0.4733
Usiwawathat	1	1	0.028	0.3074	0.119	0.4795	0.052	0.3753
Violet	1	1	0.023	0.2873	0.049	0.3706	0.057	0.386
Sub Cycle 2	1	1	1	1	0.542	1.461	0.537	1.532
Sub Cycle 4	1	1	1	1	0.128	2.404	0.175	2.398
Sub Cycle 6	1	1	0.003	6.757	0.026	3.433	0.009	4.798
Sub Cycle 8	1	1	0.003	6.84	0.014	3.862	0.003	5.691

Parameters for factors are differences compared with the reference level:

Factor	Reference level
Sub Cycle	0
Genotype	BP

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The chance of reducing PVA load for the genotype *Violet* was 0.37, and subculturing cycles 6 and 8 had the chances of reducing PVA load by 3.4 and 3.8 respectively. The chance of reducing the PVM load in the genotype *Rosita* was 0.36, and subculturing cycles 6 and 8 had the chances of eliminating PVM by 4.7 and 5.6. The chance of reducing PVS load from the genotype *Magalabada* was 0.31, *Rosita* was 0.33, *Simoki* was 0.34, *Usiwawatha* was 0.33 and *Violet* was 0.31; whilst the chance of reducing PVS load in subculturing cycle 6 and 8 was 6.4 and 6.5 respectively. Subculturing cycles 2 and 4 were not significantly different and their observations showed positive reaction. The probability of reducing PVX load from the genotype *Magalabada* was 0.35 and in subculturing cycles 6 and 8 was 5.1 and 5.6 respectively whilst subculturing cycles 2 and 4 were not significantly different and their observations showed positive reaction. In reducing PVY load, the chance of success was 0.34 for the genotype Violet and 3.5 and 4.7 for the subculturing cycles 6 and 8 respectively, whilst subculturing cycles 2 and 4 were not significantly different and their observations showed positive reaction.

Both statistical and observational results from Elisa kit test showed that bacterial wilt reaction was negative in all the four different subculturing cycles and the genotypes, giving an impression that the causal agent for the bacterial wilt disease, *Ralstonia solanacearum*, was eliminated in the course of the experiment. On average, subculturing cycles 2 and 4 showed positive reaction to PLRV, PVA, PVM, PVS, PVX and PVY whilst subculturing cycles 6 and 8, showed negative reaction for these pathogens, giving an impression that these potato viruses were eliminated in the sixth and eighth subculturing cycles. However, the different genotypes showed different reactions for the different potato viruses.

Table-2. Pathogen load for PVS, PVX and PVY for plantlets developed under repeated shoot tip

	PVS		PVX		PVY	
Parameter	**t.pr.**	**antilog of estimate**	**t.pr.**	**antilog of estimate**	**t.pr.**	**antilog of estimate**
Constant	<.001	0.069	<.001	0.069	<.001	0.085
Magalabada	0.029	0.315	0.043	0.353	0.076	0.419
Rosita	0.035	0.336	0.052	0.374	0.099	0.453
Simoki	0.039	0.346	0.146	0.506	0.053	0.377
Usiwawatha	0.035	0.336	0.082	0.428	0.058	0.387
Violet	0.029	0.315	0.082	0.428	0.039	0.345
Sub Cycle 2	1.000	1.000	0.571	1.482	0.741	1.245
Sub Cycle 4	1.000	1.000	0.215	2.241	0.287	1.927
Sub Cycle 6	0.004	6.462	0.006	5.173	0.026	3.575
Sub Cycle 8	0.003	6.538	0.003	5.690	0.005	4.734

Parameters for factors are differences compared with the reference level:

Factor	**Reference level**
Sub Cycle	0
Genotype	BP

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5. DISCUSSION

The study's results on mother stock tubers incidence and intensity, agree with findings of (Demo *et al*., 2009), who reported that in Malawi, the common viral diseases are PVA, PVY, PVX and PLRV. However, of late PVS has been observed from Mchinji and Ntchisi districts. In addition to this, it has been reported (Mih and Atiri, 2006) that in Malawi PVA has been found in mixed infections with PVX. So the findings above give an impression that the potato industry in Malawi could be at a threat if something is not going to be done over eradication of viruses.

(Khurana, 2000) reported that *Potato leaf-roll virus* (PLRV) genus *Luteovirus*, *Potato virus* X (PVX) genus *Potexvirus*, and *Potato virus Y* (PVY) genus *Potyvirus* are the main viruses that are of economic importance to the potato industry in Africa, whilst (Salazar, 2003) pointed out that potato leaf roll (PLRV), potato virus Y (PVY), potato virus A (PVA), potato virus X (PVX), potato virus M (PVM) and potato virus S (PVS) are the most important potato viruses when their distribution and effect on yield is combined world widely. (Khurana, 2000) reported that infections of PVY and PLRV have the potential to reduce yields up to 80% while mild viruses, such as PVX, PVS, and PVM, also depress yields by as much as 30% in infected plants. Whilst (Salazar, 2003) reported that PLRV, PVY and PVA reduce yield by 90%, PVX by 40% and PVS and PVM reduce yields by 20%. There have been some schools of thought that a combination of viral infection is the one that is more dangerous to the potato industry. For instance, (Wangai and Legult, 2006) reported that PVS is the latent virus that occurs in seeds up to 90% level but causes a significant reduction in tuber yield only when combined with PVX and/or PVY. To concur with this claim, (Mih and Atiri, 2006) reported that disease symptoms are often severe when the viruses occur in mixed infections and the yield losses can be up to 80% as has been found in combinations involving PVX, PVS and PLRV.

The study's findings on pathogen load for plantlets developed through repeated subculturing mean that the pathogen load was getting reduced with an increase in the subculturing cycles, because the phloem system that carries the cellular molecules such as RNA responsible for the genetic information, also carries pathogens such as viruses that multiply through the cells. This concurs with (Banarjee *et al*., 2006), who reported that plants move RNA and proteins from the cell of origin through the phloem to target sites to activate environmentally regulated and developmental processes, and this is the reason that plants such as potato that are propagated by vegetative means get infected by pathogens by passing them on to the next generation. To support this further, there is another school of thought that the shoot tips phloem system is not well differentiated, that it can function normally as compared to a mature and well differentiated phloem system (Zhang *et al*., 2009). As such, this could be the reason that culturing the shoot tips repeatedly over time results in the pathogen particles getting reduced and in some instances eliminated. To concur with this, (Muhammed, 2002) proved that it is possible to eliminate Alfalfa Mosaic Virus (AMV) from infected potato by shoot tip culture alone without any additional treatment; and (Mahmoud *et al*., 2009) reported of eliminating potato virus X in potatoes using

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shoot tip culture. Similarly, grape vines have been eradicated off their viruses (Constable and Drew, 2004).

6. CONCLUSIONS

Results from this study indicate that subculturing potato plantlets from sixth to the 8th cycles of repeated shoot tip culture; there are chances of reducing pathogen load for the different potato pathogens i.e. Bacterial wilt, PLRV, PVA, PVM, PVS, PVX and PVY at different probability levels. However, this is dependent on the different pathogen load in the potato genotype mother stocks. In addition to this, the study has also revealed that in the potato mother stock tubers, there were very few traces of bacterial wilt in the potato genotypes which even got eliminated in all different subculturing cycles.

7. RECOMMENDATIONS

From the study results, it can be recommended that the sixth and eighth subculturing cycle can be used for eliminating potato pathogens for different potato genotypes.

7.1. Recommendations

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