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*Full Length Research Paper*

# Agronomic performance of locally adapted sweet potato (*Ipomoea batatas* (L) Lam.) cultivars derived from tissue culture regenerated plants

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**Tissue culture techniques have opened a new frontier in agricultural science by addressing food security and agricultural production issues. A study was conducted to compare growth and yield characteristics between the tissue culture regenerated and conventionally propagated sweet potato cultivars. Five locally adapted sweet potato cultivars *Mugande, SPK004, Kemb10, Japon tresmesino* and *Zapallo* were regenerated *in vitro* by the methods of indirect and direct embryogenesis and grown under field conditions in a RCBD replicated three times. Significant (P<0.05) interaction was detected between the test cultivars and regeneration method for the growth and yield variables. The highest tuber numbers and marketable yield was recorded with *Zapallo*. Conventional propagation method gave highest growth rates however the difference in yield between the conventional propagation and tissue culture regenerated plants did not vary significantly (P<0.05). Likewise, virus detection for SPFMV by ELISA established that field plants had a higher virus titre compared to the tissue culture regenerated plants.**

**Key words:** Sweet potato (*Ipomoea batatas*), tissue culture, regeneration, embryogenesis, propagation method.

## INTRODUCTION

The sweet potato (*Ipomoea batatas* (L.) Lam.) is a native American plant belonging to the family Convolvulaceae, order Polemoniales (Burden, 2005) and is ranked third most important tuber after potato (*Solanum tuberosum*) and cassava (*Manihot esculentum*) (FAO, 2003). In Africa, extensive cultivation of sweet potato has been practised in regions surrounding Lake Victoria in Uganda, Northwest Tanzania (Onueme, 1978); Central and Western Province in Kenya where the crop occupies a national status as a food security crop (Munga et al., 2000). Sweet potato has received increased attention because the crop can adapt to a wide range of environmental conditions and grow on marginal areas with poor soils of limited fertility and inadequate moisture (Bioethics, 2004).

Despite sweet potato having a high potential, yield in Kenya has been declining over the years resulting in low production. The greatest danger has been identified as the susceptibility of sweet potato to virus diseases which has caused substantial yield reduction of up to 80% (Karyeija et al., 2000; Odame et al., 2002). The greatest challenge is SPFMV (genus *Potyvirus*; family *Potyviridae*) because symptoms of infected plants are transient and farmers are unable to recognize them (Wambugu, 1995).

Clean planting material has been produced in tissue culture and virus indexed by Enzyme Linked Immuno-Sorbent Assay (ELISA) which has been the most effective method for virus and pathogen detection in plants (Mukasa et al., 2003). However, in tissue culture micro propagation, the health status of the donor mother plant

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**Abbreviations:** SPFMV, Sweet potato feathery mottle virus; RCBD, random complete block design, TC, tissue culture; cvs cultivars.

and of the plants multiplied from it are among the most critical factors, which determine the success of a tissue culture operation (Nowak and Pruski, 2002). Tissue-cultured propagules are produced under controlled environment and the nutrients in the growing media are far in excess of those in the soil (Doods and Roberts 1995). Such an environment causes developmental distortions and may repress or modulate several metabolic pathways (Jeong, 1995). In addition, *in vitro* plantlets have small juvenile leaves with reduced photosynthetic capacity and malfunctioning stomata (Pierik, 1993); hence these plantlets need to be hardened gradually in order to improve survival upon transfer to soil (Lineberger, 2006).

Nevertheless, the production of high quality and vigorous plants through *in vitro* culture requires enhancement of post transplanting ability for water management, efficiency of photosynthesis and resistance to diseases (Nowak and Pruski, 2002). The ability of the propagules to withstand transplanting stress very often determines the success or failure of tissue culture operations and determines the final performance of the propagules (Ahloowalia, 2002). Good quality propagules with well developed roots and leaves are easy to acclimatize to the external environment (Nowak and Pruski, 2002). Any successful tissue culture regeneration and acclimatization protocol must ensure that the plants maintain active growth during the entire hardening period (Schmidt et al., 2004).

Low cost tissue culture technology has high priority in agriculture, horticulture, forestry, and floriculture as it contributes significantly to the improvement of agricultural productivity and food security (Odame et al., 2002). However, the challenge remains its integration into existing production systems in a cost effective manner (Ahloowalia, 2002) that will ensure improved yield and high quality plants from locally adapted cultivars in an effort to build confidence of the farmers or end-users to *in vitro* produced tissue culture regenerated planting material. The objective of this study was aimed at assessing the agronomic performance of local sweet potato cultivars regenerated in tissue culture and comparing plant growth and yield with conventionally propagated plants.

## MATERIALS AND METHODS

### Study site

The study was carried out at Njoro (36° E 0°20'S) in Nakuru District, Kenya, which lies at an altitude of 2160m above sea level, located in the agro-ecological zone $LH_2$ low highlands. The soils are vintric mollic andosols that are well drained, deep to dark reddish brown friable and silt clay soils with humic top soil. The rainfall is bimodal and well distributed (886 mm/yr) with mean temperatures ranging from 7 –10°C (Jaetzold and Schmidt, 1983).

### Plant material

Five sweet potato *cvs* were sourced from Centre Internationale de Potato (C.I.P) Nairobi, Kenya and included *SPK004, Kemb 10, Mugande, Japon tresmesino* and *Zapallo* chosen based on their popularity, preferred taste and their growth performance under local conditions.

### Plant establishment *ex vitro*

Plants were obtained from indirect embryogenesis by culturing leaf explants on MS basal media (Murashige and Skoog, 1962) media supplemented with 40 $gL^{-1}$ Sucrose, 2 $mL^{-1}$ Thiamine HCL, 100 $mgL^{-1}$ Myo-inositol, 0.5 $mgL^{-1}$ 2,4-D and 2.5 $gL^{-1}$ Phytagel as reported by Chee et al. (1990) and Zheng et al. (1996), and placed in the dark for 4 weeks. The cultures were transferred whole to light conditions and on fresh embryo initiation media containing 2 $mL^{-1}$ ABA. Green plants were regenerated after plants were cultured on the above media but devoid of hormones (Figure 1). With direct embryogenesis, somatic embryos were initiated from auxiliary buds cultured on MS basal media (Murashige and Skoog, 1962) supplemented with 30 $gL^{-1}$ Sucrose, 2 $mL^{-1}$ Thiamine HCL, 0.5 $mgL^{-1}$ 2,4-D, 2.235 $gL^{-1}$ Potassium chloride and Phytagel as reported by Newell et al. (1995) and Zhange et al. (1998). Each regenerated plant from *in vitro* grown sweet potato cvs was soaked in distilled water for one hour to rinse off culture media from roots and transferred to soil that was sieved and autoclaved to destroy soil borne pathogens. Conventional planting material was obtained from the initial mother plant. Plantlets were grown in plastic polythene bags, covered with transparent bags to prevent moisture loss for 7 days in the green house after which the plants were transplanted to the field. The plants were planted in ridges on an area of 16 x 4m with 15 plots each measuring 4 x 3m. Inter row and Inter row spacing were 0.65 and 0.5m with one seedling per ridge and 0.75m path between the replicates.

### Data collected

The number of plants that survived after hardening *in vitro* plants, plant stand count, plant height, numbers of leaves, number of branches and the leaf area (LA) of the most fully expanded leaf. The marketable tuber numbers and weight of TC regenerated plants and the conventional stem cuttings were evaluated for their agronomic performance. Leaf samples representing each treatment showing disease symptoms were collected and virus indexed for SPFMV using a three day Double Antibody Sandwich ELISA procedure as described by Jericho and Thompson (2000).

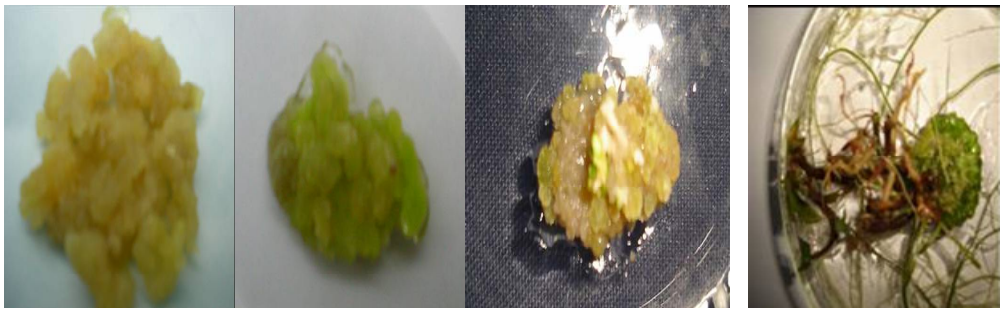
### Statistics

The TC and conventional propagated sweet potato cultivars were planted in a RCBD replicated three times. Treatments were randomly assigned after which data was subjected to ANOVA using Statistical Analysis Systems (SAS) (Proc: anova) and where F test was significant at 5% level mean separation was done using LSD (SAS Institute, 2001).

## RESULTS

### Plant survival *ex vitro*

Generally, conventionally propagated sweet potato adapted faster to the external environment than TC regenerated plants hence had a higher plant survival. Of the two TC methods plant survival was higher with indirect embryogenesis than with direct embryogenesis (Figure 2). Plant survival varied among the local sweet potato cvs though the difference was not significant. The highest percentage plant survival was recorded with cv. Zapallo



a) Plants regenerated *in vitro* by indirect embryogenesis from the c*v. Zapallo*.

b) Plants regenerated *in vitro* by direct somatic embryogenesis from the c*v. Mugande*.

c) Hardening regenerated plants *ex vitro* and potting plants in the soil (*cv Zapallo* and *Mugande* respectively) before transfer to the field.

**Figure 1.** Plants regenerated by indirect and direct embryogenesis in tissue culture, hardening *ex vitro* in the greenhouse before transfer to field conditions at KARI- Njoro.

with propagules obtained by direct somatic embryogenesis followed by when conventional cuttings were used. SPK004 followed with planting material obtained from indirect embryogenesis followed by direct somatic embryogenesis propagules. The lowest percent plant survival was scored with Mugande using regenerated TC planting material (Figure 2).

## Plant stand count

Significant ($P<0.05$) interaction was established between the sweet potato cultivars, growth in days after transplanting and propagation method for plant stand count. A general stable stand count was observed after 28 days. Plants from conventional propagated method had the highest plant stand count with *Mugande,* while the lowest stand count was attained in the *cv. J. tresmesino* followed by *Kemb 10*. (Figure 3). There was significant difference in plant stand count between direct and indirect tissue culture regeneration methods only with the cvs *Mugande* and *Zapallo*. With indirect embryogenesis plants, stand count was highest with *Mugande* and lowest with *SPK004*. Plants regenerated from direct somatic embryogenesis gave lowest stand count with the cvs *Mugande* and *J. tresmesino* (Figure 3).

## Plant height

The ANOVA detected significant ($P<0.05$) three way interaction between test cultivars, propagation method and plant growth days after transplanting. *SPK004* had the tallest plants followed by *cvs. J. tresmesino*, *Zapallo,*

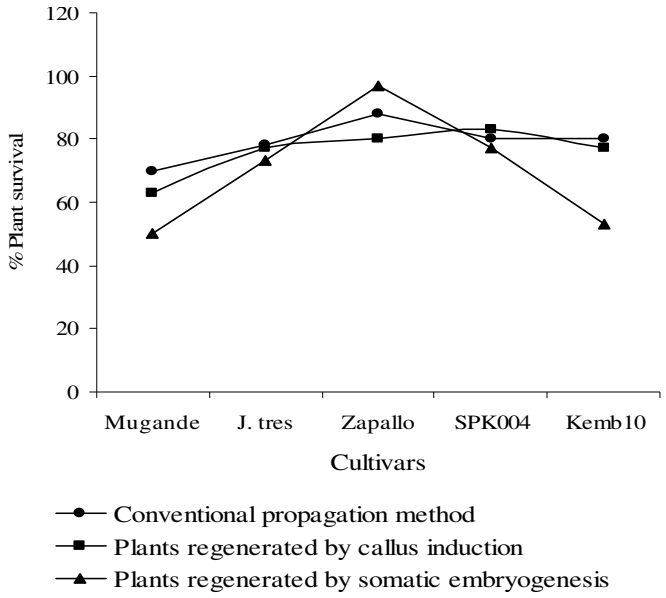



**Figure 2.** Plant survival (%) comparisons between tissue culture cultured regenerated plants and conventional stem cutting grown under green house conditions at KARI- Njoro, 2006.

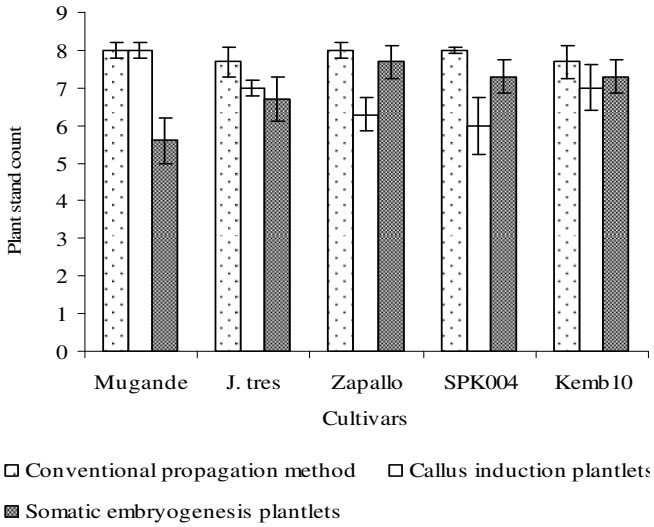



**Figure 3.** Effect of cultivar and regeneration method on plant stand count of sweet potatoes grown under field conditions at KARI- Njoro, 2006.

*Kemb 10* and finally *Mugande* with conventional propagated plants, recorded at harvest time (Table 1). The data identified significant variation in plant heights attributed to the propagation method. Treatments involving TC regenerated propagules had a tendency to be shorter than the uncultured conventional propagated propagules. A comparison of the two methods revealed that propagules regenerated through direct embryogenesis gave the tallest plants compared to indirect embryogenesis with the exception of *Mugande* (Table 1).

## Number of branches

Conventional propagation plants had higher numbers of branches with the *cv Mugande* followed by *SPK004* and *Kemb10* at harvest time.followed by plants obtained by indirect embryogenesis and lastly plants from direct somatic embryogenesis. The lowest branch numbers was recorded with the *cv J. tresmesino* (Table 2).

## Leaf area

Conventional grown sweet potato plants attained the highest leaf area followed by direct embryogenesis and lastly indirect embryogenesis method (Table 2) though the difference in leaf area among the two TC regeneration methods was not significant. Significant cultivar differences were however detected with *cv Mugande* attaining the largest leaf area (92.2 $cm^2$) followed by *Kemb 10* (76.7 $cm^2$) while the lowest leaf area was with *Zapallo* (64.1 $cm^2$). With propagation using indirect embryogenesis, leaf area was largest also with *Mugande* (90.5 $cm^2$) followed by *Zapallo* (69.3 $cm^2$), while with direct embryogenesis *Kemb 10* had largest leaf area. It was observed across all treatments in this study that maximum leaf canopy was achieved 56 days after transplanting while the lowest leaf area was at 28 days after transplanting (Table 2).

## Yield and yield components

No significant ($P<0.05$) interaction was established between test *cvs* and propagation method for tuber numbers. However, significant interaction occurred for marketable tuber weight of the local sweet potato cvs. There were differences in response to the propagation method among the locally adapted sweet potato *cvs* for tuber numbers. It was noted that highest overall tuber numbers were recorded with tubers obtained by indirect embryogenesis with *cvs Zapallo*, *Mugande,* and *J. tresmesino.* Plants obtained by conventional propagation method gave higher tubers numbers relative to the TC regenerated plants with the *cvs SPK004* and *Kemb 10* (Table 3). Likewise these method gave the highest marketable tuber weights (4.8 tonnes/ha) followed by propagules from indirect embryogenesis (3.7 tonnes/ha) with *cv J. tresmesino* and lastly direct embryogenesis propagules (3.3 tonnes/ha) with *Zapallo* followed by *J. tresmesino* and lowest weight recorded with the *cv Mugande* (Table 3). Marginal differences between the conventional stem cuttings and TC regenerated sweet potatoes were observed during this field study.

**Table 1.** Effect of cultivar, propagation method and growth days after transplanting on plant height of 5 locally adapted sweet potato grown at KARI- Njoro, 2006.

| Cultivar | Method | Growth (days after transplanting) | | | | Mean |
|---|---|---|---|---|---|---|
| | | 28 DAE | 42 DAE | 56 DAE | 70 DAE | |
| Mugande | Conv | 13.4 a | 24.0 a | 30.7 b | 102.0 a | 42.5 a |
| | I.E | 8.7 ab | 13.6 b | 17.2 ab | 66.3 b | 26.5 ab |
| | D.E | 2.8 b | 4.8 c | 6.5 b | 57.3 b | 17.9 b |
| | Mean | 8.3 b | 14.2 b | 18.1 b | 75.2 a | |
| J. tres | Conv | 28.6 a | 33.4 a | 38.4 a | 105.7a | 51.5 a |
| | I.E | 19.4 b | 26.5 a | 31.3 a | 72.3 b | 37.4 a |
| | D.E | 21.0 ab | 24.9 a | 37.2 a | 91.9 a | 43.8 a |
| | Mean | 23.0 b | 28.3 b | 35.6 b | 89.9 a | |
| Zapallo | Conv | 22.2 a | 29.1 a | 41.7 a | 121.7 a | 53.7 a |
| | I.E | 9.8 b | 15.6 b | 22.3 b | 69.7 b | 29.3 b |
| | D.E | 12.2 b | 17.5 b | 24.7 b | 74.7 b | 32.3 b |
| | Mean | 14.7 b | 20.7 b | 29.6 b | 88.6 a | |
| SPK004 | Conv | 25.1 a | 48.6 a | 85.9 a | 200.0 b | 89.9 a |
| | I.E | 7.7 b | 16.6 b | 24.6 b | 121.7 c | 42.7 b |
| | D.E | 10.7 b | 22.0 b | 37.1 b | 249.0 a | 76.7 a |
| | Mean | 14.5 c | 29.1 bc | 49.2 b | 190.2 a | |
| Kemb 10 | Conv | 13.5 a | 19.8 a | 25.8 a | 157.7 a | 54.2 a |
| | I.E | 7.1 a | 12.4 a | 20.5 a | 67.3 b | 26.8 b |
| | D.E | 10.8 b | 18.5 a | 23.1 a | 78.3 b | 32.7 b |
| | Mean | 10.5 b | 16.9 b | 23.1 b | 101.1 a | |

Conv, I.E and D.E denote conventional, indirect embryogenesis, direct embryogenesis plants.
Means within a column followed by the same letter are not significantly ($P < 0.05$) different based on Least Significant Differences (LSD).

**Table 2.** Effect of cultivar, propagation method and growth in days after transplanting on mean number of branches (†) and leaf area (††) formed in sweet potatoes.

| Parameter | Method | Cultivar | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mugande | | J.tres | | Zapallo | | SPK004 | | Kemb 10 | |
| | | † | †† | † | †† | † | †† | † | †† | † | †† |
| Propagation method | Conv | 7.0a | 92.2a | 4.0 a | 65.7a | 5.0 a | 64.1a | 6.8 a | 65.1a | 4.9 a | 76.7a |
| | I.E | 7.1a | 90.5a | 2.8b | 64.8a | 4.4ab | 69.3a | 4.5 b | 57.2a | 4.6 a | 62.1b |
| | D.E | 4.0b | 75.8b | 4.3a | 64.2a | 3.2 b | 57.9a | 4.0 b | 65.1a | 5.3 a | 79.7a |
| Growth days after transplanting | Conv | 2.8c | 38.4c | 1.1c | 24.2d | 2.0 b | 26.1a | 1.8 b | 24.4a | 1.7 c | 28.3c |
| | I.E | 4.6b | 80.3b | 1.9bc | 73.8b | 2.4 b | 66.1b | 2.6 b | 68.0b | 3.3 c | 79.6b |
| | D.E | 7.6a | 119.4a | 3.4b | 102.5a | 3.2 b | 90.6a | 8.1 a | 90.6a | 5.8 b | 108.2a |

Conv, I.E and D.E denote conventional, indirect embryogenesis, direct embryogenesis plants.
Means within a column followed by the same letter are not significantly ($P<0.05$) different based on Least Significant Differences (LSD).

**Table 3.** Effect of cultivar and propagation method on marketable tuber numbers (□) and weight (□□) in tonnes /ha in sweet potato grown under field conditions at KARI- Njoro.

| Propagation method | Cultivar | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mugande | | J. tres | | Zapallo | | SPK004 | | Kemb 10 | |
| | □ | □□ | □ | □□ | □ | □□ | □ | □□ | □ | □□ |
| Conv | 10.3a | 5.1 a | 5.7 a | 3.8 b | 7.3 b | 9.6 a | 14.7a | 2.9 a | 12.7a | 2.7 b |
| I.E | 12.3a | 4.9 a | 7.3 a | 6.0 a | 12.7a | 3.6 b | 3.7 c | 1.6 b | 6.3 b | 2.2 a |
| D.E | 7.3b | 2.1 b | 6.7 a | 4.2 b | 11.7a | 5.1 a | 7.0 b | 2.4 a | 9.7 a | 2.9 a |
| Mean | 10.0a | 4.0 ab | 6.6 a | 4.7 ab | 10.6a | 6.1 a | 8.4 a | 2.3 b | 9.5 a | 2.6 b |
| CV (%) | 51.3 | 82.6 | | | | | | | | |
| LSD (5%) | 3.47 | 1.22 | | | | | | | | |

Conv, I.E and D.E denote conventional, indirect embryogenesis, direct embryogenesis plants.
Means within a column followed by the same letter are not significantly (P<0.05) different based on Least Significant Differences (LSD).

**Table 4.** Detection of sweet potato viruses by DAS-ELISA in tissue culture regenerated plants and field plants in locally adapted sweet potato cultivars.

| Cultivar | Readings at | | | | | |
|---|---|---|---|---|---|---|
| | Regenerated plants | | | Field plants | | |
| | 30/60 min | 120 min | Mean | At 30/60 min | 120 min | Mean |
| Mugande | - (0.10) | - (0.10) | - (0.10) | + (0.15) | + (0.16) | + (0.16) |
| J. tres | - (0.13) | - (0.13) | - (0.13) | - (0.14) | - (0.15) | + (0.15) |
| Zapallo | - (0.10) | - (0.10) | - (0.10) | + (0.15) | + (0.16) | + (0.16) |
| SPK004 | - (0.13) | - (0.13) | - (0.13) | + (0.15) | + (0.16) | + (0.17) |
| Kemb 10 | - (0.14) | + (0.16) | + (0.15) | + (0.16) | + (0.16) | + (0.16) |
| Healthy control | - (0.14) | | | | | |

-, +, represents negative and positive for SPFMV, respectively.
Values in parenthesis represent mean absorbance values indicating disease incidence.

**Table 5.** Detection of sweet potato feathery mottle virus by DAS- ELISA using different propagation methods at harvesting.

| Propagation method | Overall disease score | | | |
|---|---|---|---|---|
| | Ist reading | 2nd reading | 3rd reading | Mean |
| Conv | + (0.16 a) | + (0.17 a) | + (0.21a) | + (0.18 a) |
| I.E | - (0.13 b) | -/+ (0.14 b) | + (0.2 a) | + (0.16 b) |
| D.E | -/+ (0.14 ab) | + (0.15 b) | + (0.2 a) | + (0.16 b) |

-, +, -/+ represents negative, positive and limited reaction for SPFMV, respectively.
Values in parenthesis represent mean absorbance values indicating disease incidence.

### Disease detection through ELISA

The ELISA results revealed that leaf samples obtained from symptomatic conventional plants had higher photometer readings than plants obtained from the TC regenerated propagules and the negative control (Table 4). The results further revealed that under field conditions there was a high virus titre in the conventional propagated plants relative to leaf samples obtained from TC regenerated plants (Table 5).

## DISCUSSION

The results from this study revealed that conventional propagated plants had a higher % plant stand count and survival than TC regenerated plants indicating that TC derived sweet potato plants were extremely sensitive to acclimatization *ex vitro*. The regenerated plants were very small and were transferred to the soil for the first time unlike conventional propagated plants. Lyndsey and Alderson (1986) reported that this performance was ex-

pected because regenerated plants had a delicate shoot and root system hence resulting in greater *ex vitro* plant mortality. The TC regenerated plants were sensitive to the external environment conditions therefore established at a slower rate in the face of intense competition for water and minerals, light and physical conditions reported by Lineberger (2006).

Similarly, the mean plant height, number of branches and leaf area of TC regenerated plants were lower than those of the uncultured conventional propagated plants. With conventional propagated plants, planting material was by stem cuttings which were larger in size and had an energy reserve making them have a rapid sustained growth (CVPG, 2003). TC regenerated plants rooted much later due to their sensitivity to the external local environmental conditions. Plant height and branching was cultivar dependant and it was observed that highest recording was at harvest time which was expected as this data was taken after a longer period of time. C*v. Mugande* had the lowest plant height owed to the morphological differences since this cultivar contrary to other *cvs* as *J. tresmesino, Zapallo* and *SPK004* had a creeping stem while *Mugande* grew upright as genetically determined. Furthermore, leaf area was genotype dependant therefore for a cv with broad leaves as *Mugande*, light interception was higher than a *cv SPK004* with narrow leaves an observation similar with findings by Annes (2005). Canola (2005) further reported that growth rate of a crop was closely related to the amount of solar radiation captured by the leaves hence rapid leaf development encouraged root growth and more dry matter production.

The difference in number of tubers produced and subsequent yield across the treatments was probably due to variation among the cultivars, their maturity and their response to the external environment. Conventional propagated plants acclimatized faster to the field conditions hence contributing to the better yield performance. High tuber numbers and weight with conventional propagated plants was because these plants were also less sensitive to the physical environment hence allowed for assimilate partitioning for tuber development earlier and to continue over a longer period of time (Ali et al., 2003).

The ELISA results confirmed that disease incidence based on the symptoms was cultivar dependant. The mean absorbance values from leaf samples of TC regenerated plants with the exception of *cv. SPK004* were lower than those of the healthy control indicating that these cultivars were free from the SPFMV. A higher virus titre in the field plants could have been caused by these plants having been exposed to environmental conditions prone to SPFMV vector namely aphids. ELISA tests were influenced by factors such as the ability of the plant to release the virus during crushing and the thickness of the sap produced Wangai et al. (2001) thereby being more pronounced after carrying out ELISA test. Under field conditions SPFMV incidence was higher with conventional plants than with TC derived plants and as reported by Kartha (1986) conventional propagated plants were from donor plants hence may have harboured pathogens therefore resulting in faster transmission to areas of renewed growth. Generally, regenerated sweet potato cultivars had lower photometer readings hence a low virus titre probably because they lacked a vascular system therefore the movement of the virus through their plasmodematal cells occurred at a slower pace during the invasion of the rapidly dividing meristematic cells (Kartha (1986). This study concluded that it was beneficial to plant tissue culture clean material as they showed suitable agronomic performance as conventional plants and resulted in increased tuber numbers and subsequently marketable tuber yields.

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