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In Vitro Agronomic Performance and Mini-Tuber Production of Potato Varieties

Namugga Prossy, Niwagaba Peter, Mateeka Benon and Barekye Alex
National Agricultural Research Organisation, Uganda

Abstract: Establishment of variety performance *in vitro* is paramount in routine and successful micro propagation of potato. The purpose of this study was to establish the *in vitro* agronomic performance and mini-tuber production of potato varieties. Sixty plantlets of four farmer preferred potato varieties (Victoria, Kachpot1, Kinigi and Rutuku) were evaluated on MS media and growth characteristics were monitored on a weekly basis between 2013 and 2014. Mini-tuber production was achieved on sterile soil media in the screen house for three seasons. Victoria performed better than other varieties *in vitro* and as a result produced more mini-tubers (12.49) on average. The results of this study indicate that some varieties require more time in culture to achieve maximum growth while others need supplementation of MS media with pyhtohormones for particular agronomic characters.

Key words: micro propagation, tissue culture, potato, mini-tuber

1. Introduction

Potato (*Solanum tuberosum L.*) is an annual crop belonging to the family *Solanaceae* and genus *Solanum*. Potato is a crop of global importance, ranking as the fourth most important food crop in the world after wheat, rice and maize [1]. In Uganda, potato is mainly grown in the higher altitude areas (>1500 m above sea level) where it serves as the main source of food and income. Potato is mainly cultivated in highland areas of Uganda which include the Kigezi highland districts of Kabale and Kisoro in the southwest, Mbale and Kapchorwa districts on the slopes of Mount Elgon in the eastern and Nebbi district in the North-west. About 40% of the national harvest comes from the intensely farmed Kabale highlands [2]. In all these districts, potato is both a staple food and a major source of household income and compared to men, more women and children are involved in the field activities for potato production.

Potato is grown by about 300,000 smallholder households in Uganda that produce 154,437 metric tons on 101,000 hectares per year [3]. These farmers use conventional means to propagate potato through the use of tubers. This propagation is characterized by low multiplication ratio that ranges from 1:4 to 1:15 [4], the aassociated risks of seed borne diseases and the eventual degeneration in seed quality [5]. Because of the low rate of multiplication, it takes a number of years to generate large quantities of seed to meet the demand of the seed potato industry. A number of different methods such as tissue culture and aeroponics technologies have the capacity to greatly improve the rate and quality of seed potato production [6, 7].

Tissue culture is an excellent technology that can allow rapid multiplication of potato. Micro-propagated potatoes establish more quickly, grow more vigorously and produce higher yield than those propagated by conventional means [8]. Tissue culture is vital in mass propagation of clonal materials

Corresponding author: Namugga Prossy, Master of Science in Plant Breeding and Seed Systems, research areas/interest: plant tissue culture, breeding and seed systems. E-mail: namuggak@gmail.com.

in disease-free environment, a large number of plantlets can be obtained; it is faster and requires a relatively small amount of space to multiply the plantlets. Tissue culture materials can be conveniently stored, transported and production is possible throughout the year [9]. Among factors limiting multiplication rates for *in vitro* potato micro propagation are short heights of plantlets, low number of nodes on the plant let [10]. *In vitro* optimization is key if routine propagation of different varieties is to be carried out. This study was conducted to establish the *in vitro* performance of four potato varieties and the eventual number of mini-tubers. The varieties used in the study are preferred by farmers and thus this would accelerate provision of clean plant materials… Understanding the performance of these varieties would ease routine and commercial propagation of different potato varieties; and also provide establish which varieties require more nutrients and supplements for optimal *in vitro* growth. Owing to the fact that potato tissue culture for production of clean planting materials has just been established in Uganda, there is need to optimize protocols for routine propagation. Additionally, information on the length of culture period to generate plantlets for a particular variety and frequency of subculture are all determined and influenced by varietal performance. Several researchers have reported the use of MS medium without hormones during proliferation stage but the growth was slow [11, 12]. However, most potato varieties can do well without hormones [4]. It is against this background that this study was conducted to establish *in vitro* performance of four farmer preferred potato varieties on basal MS media.

2. Materials and Methods

2.1 Study Site and Plant Materials

This study was carried out in the tissue culture laboratory based at Kachwekano Zonal Agricultural and Research Development Institute (KaZARDI) in Kabale district, south western Uganda 01° 16’S 29° 57’E at 2200 m a.s.l. Four popularly grown and farmer preferred varieties namely Victoria, Kachpot1, Kinigi and Rutuku obtained from KaZARDI were used in this study (Table 1). Starter materials were obtained from mother plants grown in a protective shade, 30 days after planting. Three leaves, one from the top, middle and bottom were picked from each plant and screened for the presence of potato viruses namely PVS, PVX, PVY, PLRV, PVM and PVA using Double Antibody Sandwich ELISA kit (DAS-ELISA) [13, 14]. Shoot cuttings with 3-6 nodes were made from virus free mother plants using sterile surgical blades.

2.2 Explant Preparation and Inoculation

The large leaves were trimmed off the nodal explants and surface sterilization was accomplished by washing the explants under running water with liquid detergent for one hour and shaking at intervals of 30 minutes. Under a laminar flow hood, the explants were immersed in 70% ethanol for five seconds and rinsed with sterile water. This was followed by a solution of 14% sodium hypochlorite supplemented with two drops of tweenty-20 for two minutes and rinsing to remove all traces of the disinfectant.

The pH of the media was adjusted to 5.8 and agar was added at 5 g/L as a gelling agent. Afterwards the media was autoclaved at 121°C for 15 minutes for sterilization.

Explants were then excised into nodal fragments and inoculated on to basal MS media [15] (Table 2) in test tubes and cultured in a growth room at 16 hour photoperiod at 18-20°C under approximately 1000 lux light intensity. The plantlets were sub-cultured two times at an interval of 3-4 weeks to generate the required number of experimental plantlets.

2.3 Experimental Design and Data Collection

The experiment was factorial with two factors that is, varieties and weeks laid out in a completely randomised design (CRD). Sixty plantlets were studied per variety for three times.

Table 1 Characteristics of the Varieties Used

Variety/Characteristics	Victoria	Kachpot 1	Rutuku	Kinigi
Resistance to late blight	Susceptible	Moderately tolerant	Tolerant	Tolerant
Resistance to bacterial wilt	Susceptible	Tolerant	Susceptible	Susceptible
Days to maturity	80-90 days	90-100 days	110-120 days	110-120 days
Dormancy	60-70 days	70-80 days	90-120 days	90-120 days
Skin colour	Light pink	Pink	Red	Purple
Flesh colour	Cream	Cream	Cream/ yellow	Cream/ Yellow
Flower colour	Light pink	Pink	Purple	Purple
Growth habit	Erect	Semi- erect	Erect	Erect

Table 2 Composition of MS Media Used

Composition	Concentration
Media premix with vitamins	3 grams/litre
Sucrose	25 grams/litre
Gelrite	3 grams/litre
PH	5.8

Data was collected on in vitro agronomic characters such as shoot height and number, number of leaves, number of roots and number of nodes on a weekly basis (every after 7 days) for four weeks. Four weeks old plantlets were transferred to sterile soil media in a screen house (2400 m.a.s.l) to study minituber production between 2013 and 2014 where season A is planting between (March and June) while season B is between (September and December). Plantlets were sprayed with a fungicide immediately after plantings and NPK was side dressed 30 days after planting. Recommended agronomic practices were observed. The produced mini-tubers per plant of each variety were counted and recorded. Data analysis was done using Genstat 14th software [22]. Significant means were compared using fishers protected LSD at 5% level of probability.

3. Results and Discussion

Variance analysis of growth characteristics showed highly significant differences among varieties ($P > 0.001$) for number of leaves, nodes and roots. This was true for the four varieties per week and their interaction. While varieties had the same number of shoots, significant weekly differences were observed for shoot height (Table 3).

The mean number of leaves per plantlet was 6.15. Victoria produced more leaves (8.1) compared to other varieties followed by Rutuku and Kachpot1 (5.2) the least mean number of leaves. In the first week after inoculation, the mean number of leaves for Victoria was significantly different from the three varieties whose mean number was the same. In week 2, Kachpot 1 and Kinigi did not show significant differences for number of leaves while Rutuku and Victoria varied. In week 3 and 4, the mean number of leaves varied greatly among the test varieties. From week 1 to 3 all the test varieties showed a progressive increase in the mean number of leaves with significant differences among them. In the fourth week, these numbers did not differ significantly from week three for varieties Kachpot 1 and Rutuku while variations were observed for Victoria and Kinigi (Table 4).

Varietal differences for *in vitro* performance were reported by Moradi-Payam et al. (2014) [9]. These results indicate that all test varieties were able to produce sufficient number of leaves to support the photosynthetic process and eventual establishment in the soil media. More leaves per plantlet can be obtained with the use of growth regulators [16, 17] however, this may not be necessary depending on the size of the culture vessel and the number of plantlets per vessel.

Table 3 Mean Squares for Growth Parameters

Mean squares						
Source of variation	d.f.	No. of leaves	No. of nodes	No. of roots	No. of shoots	Shoot height (cm)
Variety	3	452.281***	398.473***	584.068***	0.009	498.505***
WEEK	3	338.145***	276.431***	30.162***	0.001	232.711***
Variety. WEEK	9	11.537***	18.162***	5.301***	0.003	13.699**
Residual	944	2.119	2.859	1.414	0.003	5.210
Total	959					

*, **, and *** represent significance levels at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Table 4 Mean Number of Leaves per Plantlet of Each Variety

Variety	Week 1	Week 2	Week 3	Week 4	Varieties mean
Kachpot	3.97a	5.38bc	5.62bcd	5.71bcd	5.17
Kinigi	3.85a	5.22b	5.82cd	5.97de	5.21
Rutuku	4.33a	6.34e	6.87f	6.98f	6.13
Victoria	5.62bcd	8.19g	8.93h	9.67i	8.10
Weeks mean	4.44	6.28	6.83	7.06	6.15

Therefore MS media may not require supplementation of plant growth hormones to achieve more leaves per plantlet.

The mean number of nodes was 6.04. The maximum number of nodes per plantlet was 7.76 for Victoria, followed by Rutuku, Kachpot 1 and least for Kinigi. In the first week of inoculation, varieties Rutuku and Kachpot 1 had the same number of nodes. During the consecutive weeks, the number of nodes per plantlet varied significantly for each variety with the exception of Kachpot in weeks three and four. For all the varieties, the highest number of nodes was recorded in the fourth week (Table 5).

Nuwagira (2013), however, reported different results on MS media for varieties Kachpot1, Victoria and Kinigi. This slight difference could be attributed to changes in culture conditions and part of explant used. This is because axillary buds and apical meristems establish faster in culture compared to other plant parts. Different results on number of nodes per plantlet have also been reported by other scholars; Rabanni et al. (2001) [4] obtained 5.20 nodes on basal MS for variety Desiree and after addition of GA3 at varying concentrations, the highest mean number of nodes was 6.20 for the same variety; 15.00 nodes were recorded at 0.25 mg/L of GA3 for potato variety Agria [9] and 7.30 was the maximum number of nodes for Desiree with 0.5 mg/L of NAA [11, 16]. These differing results indicate that some varieties can produce more nodes on basal MS media while others need to be supplemented in order to achieve maximum growth. The highest number of nodes recorded in week four for all the varieties implies that for continued multiplication and

Table 5 Mean Number of Nodes per Plantlet of Each Variety

Variety	Week 1	Week 2	Week 3	Week 4	Varieties mean
Kachpot	4.62bc	5.31d	5.58def	5.67def	5.29
Kinigi	3.65a	4.48b	5.13ce	6.10ef	4.84
Rutuku	4.62bc	6.18f	7.02g	7.17gh	6.25
Victoria	5.49de	7.69h	8.33i	9.52j	7.76
Weeks mean	4.59	5.92	6.54	7.09	6.04

sub culturing, plantlets should be kept in culture at least for four weeks if higher numbers are to be obtained. However, planting may be done after two or three weeks.

Mean number of roots was 4.22. More roots were recorded for Victoria (mean = 5.65) and least for Kinigi (mean = 2.08). On a weekly basis, no significant differences were observed on number of roots per plantlet for Kachpot 1 throughout the culture period. For Kinigi, significant variations were observed only in the fourth week. The number of roots per plantlet varied for Rutuku in the first two weeks while for Victoria variation was observed over the four weeks (Table 6).

All varieties were able to produce roots in culture and hence could all establish in the soil. However, higher numbers have been reported by other researchers when growth regulators were used with GA3 [9]; with IBA and NAA [16]. More root numbers were recorded by other researchers with application of plant hormones mainly IBA and NAA [16]. However, for establishment in soil media, an *in vitro* plantlet will establish even with the least number of roots. The same number of roots for Kachpot 1 throughout the culture and study period indicated that, for Kachpot1 roots establish early in the first week meaning that plantlets can be conveniently transplanted 2-3 weeks after inoculation. This is vital in places where *in vitro* space is limiting and a large number of plantlets is required for a target no of minitubers. Kinigi with fewer roots requires more time in culture if plantlets are to establish well in the soil.

Table 6 Mean Number of Roots per Plantlet of Each Variety

Variety	Week 1	Week 2	Week 3	Week 4	Varieties mean
Kachpot	3.93c	4.17c	4.21c	4.21c	4.13
Kinigi	1.82a	1.83a	2.10a	2.57b	2.08
Rutuku	4.17c	5.04d	5.47e	5.47e	5.04
Victoria	4.96d	5.63cd	5.91fg	6.09g	5.65
Weeks mean	3.72	4.24	4.47	4.47	4.22

All plantlets for the test varieties gave the same number of shoots, no significant differences were observed amongst them (Table 7). Single shoots were reported by Nuwagira (2013) on MS media and different hormonal combinations. However, multiple shoots have been reported with the use of growth regulators mainly BAP and GA3 [4]. Multiple shoots are unfavorable when the plantlets are very tall on the other hand.

Mean shoot height was 3.88 cm. Plantlets of Kachpot1 were relatively taller than (4.66 cm) other varieties while Kinigi registered the shortest (mean = 1.72 cm). Significant differences were observed progressively for Kinigi plantlets over the culture period while for other varieties it was between the first two weeks (Table 8). Plantlets in this study were shorter compared to those in other studies on MS media (6.50 cm [4] and 5.20 cm [18]). This could be attributed to variations in culture conditions. Conversely, taller plantlets have been produced in other studies where plant hormones were used [19, 20]. Based on the results of this study, plantlets of varieties Kachpot 1, Rutuku and Victoria can be transplanted after the second week once roots are well developed, however, for Kinigi, three to four weeks are required in culture before transplanting. Also when relatively taller plantlets are required, MS media should be supplanted with the relevant growth regulators. The results of this study are in tandem with the findings of Nuwagira (2013) [18] where poor *in vitro* performance of Kinigi on all the growth parameters on different hormonal combinations compared to other varieties was reported.

Table 7 Mean Number of Shoots per Plantlet of Each Variety

Variety	Week 1	Week 2	Week 3	Week 4	Varieties mean
Kachpot	1.02a	1.02a	1.02a	1.00a	1.01
Kinigi	1.00a	1.00a	1.00a	1.00a	1.00
Rutuku	1.00a	1.01a	1.01a	1.00a	1.00
Victoria	1.02a	1.01a	1.00a	1.02a	1.01
Weeks mean	1.01	1.01	1.01	1.00	1.01

Table 8 Mean Shoot Height per Plantlet of Each Variety

Variety	Week 1	Week 2	Week 3	Week 4	Varieties mean
Kachpot	3.00d	5.33ef	5.26ef	5.07ef	4.66
Kinigi	0.98a	1.53ab	1.84bc	2.54cd	1.72
Rutuku	3.03d	4.90e	5.55ef	4.79e	4.57
Victoria	2.74d	4.82e	5.86f	4.89e	4.58
Weeks mean	2.44	4.15	4.63	4.32	3.88

** Mean values followed by the same letter (s) in respective category are nor significantly different from each other at 5% level of probability.

Table 9 Analysis of Variance for Minituber Production

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	3	3679.29	1226.43	103.92	< .001
Season	2	137.15	68.58	5.81	0.003
Variety Season	6	780.73	130.12	11.03	< .001
Residual	708	8355.77	11.80		
Total	719	12952.95			

Variations were observed in the number of mini-tubers for each variety and in seasonal production at $P > 0.001$ and $P > 0.003$ respectively. Also varieties interacted significantly with the season ($P > 0.001$) (Table 9). The mean number of minitubers was 8.74. On average Victoria yielded more than other varieties (12.49) followed by Rutuku (8.36) and Kinigi (7.6) and Kachpot1 (6.52). More mini-tubers per plantlet were realised in 2014B (9.34) followed by 2013B (8.56) and 2014A (8.32) (Table 10).

Differential performance of varieties in each season may be attributed to changes in the amount of rainfall where the long rains during the B season positively affected tuberization and hence the number of mini-tubers per plant. This stems from the fact that

Table 10 Mean Comparison of Minituber Number per Variety in Each Season

	Season			
Variety	**2013B**	**2014A**	**2014B**	**Variety means**
Kachpot1	7.55bc	6.60ab	5.40a	6.52
Kinigi	7.95c	7.45bc	7.40bc	7.60
Rutuku	6.57ab	7.51bc	11.00d	8.36
Victoria	12.17d	11.70d	13.57e	12.49
Season means	9.34	8.56	8.32	8.74

cool temperatures of about 18°C are required for tuberization and as soil temperatures rise above 20°C both tuber formation and development are slowed down [21]. The poor performance of Kachpot1 in 2014B remains unexplained in this study while it mini-tuber production in 2014A may be attributed to its ability to stand increasing temperatures. Much as the test varieties were grown in the screen house where watering was done, outside temperatures are believed to have had effect on variety performance.

Additionally, Victoria compared to other varieties in this study is early maturing (80-90 days). This could have contributed to its fast *in vitro* establishment and better agronomic performance.

4. Conclusion

Results from this study indicate that Victoria exhibited better *in vitro* growth compared to others even with mini-tuber production. With this Victoria may not require hormonal supplementation while other varieties may depending on the purpose of micro propagation. Additionally routine micro propagation of potato may not require regular use of plant growth hormones with the exception of varieties which are heavy feeders and also in case of use in virus elimination. More mini-tubers can still be obtained per plantlet with good management in the screen house.

Also different varieties require varying culture periods to show maximum in vitro growth and this may affect the rate and interval of sub culturing. We recommend that tissue culture protocols be optimized for the different varieties and new varieties should be studied for their performance *in vitro* for successful routine and commercial rapid multiplication.

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