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Effects of Sucrose and Gibberellic Acid on Growth and Survival of Local Potato (*Solanum tuberosum* L.) Varieties *in vitro* in Kenya

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ABSTRACT

Tissue culture techniques' have become useful technologies for producing disease & pest free seed potato (*Solanum tuberosum* L.) in the developed world. However, these techniques have yet to be standardized for locally produced potato varieties in Kenya. Developing countries can also use these innovations for rapid multiplication of popular local seed material through rooted apical cuttings generated from either plantlets or micro tubers. *In vitro* experiments were therefore conducted to determine the optimum concentration of sucrose and gibberellic acid for growth and survival of local potato varieties, namely, *Shangi, Unica* and *Wanjiku*. The explants were cultured in Murashige and Skoog (MS) media supplemented with sucrose at a concentration of 20, 30 and 40 gL^{-1}, while gibberellic acid was applied at a concentration of 0.2, 0.5 and 1.0 mgL^{-1}. The study was laid out in a completely randomized design (CRD). MS Medium with sucrose 40 $gL^{-1}$and gibberellic acid 0.5 mgL^{-1} significantly enhanced shoot length, with the longest shoot (10.3 cm) being recorded for *Wanjiku*. The same treatment also gave the highest plant survival of 90%. Murashige and Skoog media, added with 0.5 mgL^{-1} gibberellic acid along with 40 gL^{-1} sucrose is recommended for generating *wanjiku, unica and shangi* apical rooted cuttings because it gave the best improvement of *in-vitro* clonal growth.

Keywords: Gibberellic acid, *in-vitro*, growth, nutrient, potato, sucrose, tissue culture.

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I. Introduction

Potato (*Solanum tuberosum*) is the third most important food crop in the world after rice and wheat in terms of human consumption. In developing countries, production of the crop has increased with rising urban population where food security is a major problem (Muthoni *et al*., 2010). Potato plays a major role in food security in Kenya and contributes to poverty relief through income generation and employment creation (Muthoni *et al*., 2013).

As a result of limited supply of certified seed, most farmers recycle seed tubers for many seasons causing an overall decline in seed quality due to accumulation of seed borne diseases through seed degeneration (Thomas-Sharma *et al*., 2015). The informal system has led to the use of poor-quality seeds planting material that hastens the spread of seed-borne diseases such as bacterial wilt (Kinyua *et al*., 2001). To mitigate this, there is need for the use of rapid multiplication techniques such as *in vitro* propagation (Chindi *et al*., 2014). Several seed production techniques are currently used worldwide to address seed production problems (Otieno *et al*., 2017). These include use of micro propagation to produce plants for hydroponics and aeroponics systems (Chindi *et al*., 2014).

Osmotically active solutes has shown that sucrose acts as a carbon source and as osmotic regulators. Sucrose and their concentration is important factor on potato microtuberization and has a profound effects on the tuber growth (Azar *et al*., 2013). It acts as an energy for growth and biosynthetic processes, and may influence growth of *in vitro* (Ferreira *et al*., 2011). Sucrose is also closely related to stomatal density and photosynthetic pigment content, as well as induction development in some plant tissues, such as vascular and support tissues (Mohamed & Alsadon, 2010; Iarema *et al*., 2012). Increase in sucrose concentration in medium can enhance the microtuber production up to some extent (Khan *et al*., 2018). However, high sucrose concentrations in the

medium may decrease the photosynthetic ability of *in vitro* potato plants (Fuentes *et al*., 2005).

The plant growth regulators (PGRs) modulate plant growth and development and mediate responses to both biotic and abiotic stress. They are of importance in regulating shoot and root development in seed potatoes *in vitro* (Badoni & Chauhan, 2010; Hoque, 2010). Previous studies show that micro propagation of seed potatoes depends on the biological value of cultivars, explant type (leaf, node, shoot tip, etc.), type of culture medium, season, temperature, photoperiod, and a balanced combination of plant growth regulators (PGRs) in the culture media (Akhtar *et al.*, 2006; Dhital *et al*., 2010). GA_3 is involved in cell elongation and its addition in MS medium enhances shoot growth (Camara *et al.*, 2018; Rizza *et al*., 2017). The basic Murashige &Skoog (MS) medium (Murashige & Skoog, 1962) is the most widely used media in production of potato (Solanum tuberosum L.). However, the amounts of various ingredients in the medium vary for cultures of different species. The objective of this experiment was to determine the nutrient concentration for survival and growth of three potato varieties grown *in vitro.*

II. Materials And Methods

A. Varieties

Three local potato varieties, Shangi, Unica and Wanjiku, obtained from the Agricultural Development Corporation (ADC) Molo laboratory were used in this experiment. They were selected on the basis of their popularity among farmers due to their marketability and high yield.

B. Sterilization

All glassware, culture vessels, test tubes, petri dishes, pipettes and small instruments (forceps, scalpel) needed for sterile dissection were autoclaved at 121 °C for 45 minutes. Surface Sterilization was carried out by first switching on the laminar air flow cabinet for 15 minutes. Laminar air flow cabinet surfaces were wiped with 70% ethanol. Apparatus were sanitized using 70% ethanol and kept inside the cabinet. The UV light in the laminar air flow was switched on for 20 minutes after which it was switched off. The laminar air flow cabinet remained on until culturing was completed. The conical flasks, test tubes and glass jars containing prepared media were sterilized by autoclaving at 121 °C for 20 minutes.

C. Stock Solution in In vitro Culture

Murashige and Skoog (MS) (Murashige and Skoog, 1962) medium was prepared by dissolving the appropriate amount of macro and micro nutrients and organic supplements in sterile distilled water. Single node cuttings of each potato variety were cultured *in vitro* on different media containing 3 different concentrations of sucrose (20, 30 and 40 gL^{-1}) and GA_3 (0.2, 0.5 and 1.0 mgL^{-1}) solidified with 7gL^{-1} of agar. One single node cutting was cultured in each test tube and sealed with parafilm. Test tubes were incubated and maintained at 22±2 °C under photoperiod 16 h light/ 8 h dark.

D. Experimental Layout

The study was laid out in a 3×3×3 factorial arrangement in completely randomized design (CRD) with three replications. The treatments were standardized MS media with three levels of sucrose (20, 30 and 40 gL^{-1}) and three levels of GA_3 (0.1, 0.5 and 1.0 mgL^{-1}). Petioles with leaf from each variety were used as explants.

E. Data Collection

The following parameters were measured; Days to shoot initiation was counted from the day of culturing in the media to the appearance of the first shoots. Number of shoots were counted from four samples and their average were recorded. Length of shoots (cm) were measured after one week of culturing in the media from the point of emergence to the tip using a ruler and the average length from four samples were used for analysis. Number of leaves from four samples were counted and the average leaf numbers were recorded. Days to root initiation was done by counting the days from the day of culturing in the media to the emergence of first initial roots. Number of roots were counted from four samples and the average root number were recorded. Percentage plants survival was determined as reported by (Parveen, 2011).

F. Data Analysis

Data collected was subjected to Shapiro wilk test at probability P ≤0.05 for normality test using SAS software. Data collected was subjected to general linear model (GLM) to partition the variance component using SAS software version 9.0 and means separated using Tukey's Honestly Significant Difference Test (HSD) at P ≤ 0.05. Pearson's Correlation test at P ≤0.05 was also performed to determine the strength of linear relationships among the response variables. The research model was as given below:

$Yijk = \mu + vari + G3j + Suk + VarG3ij + VarSuik + G3Sujk + VarG3Suijk + \varepsilon ijk$

Where: Yijk: observed responses, μ= Overall mean level, var= Effect due to ith level of variety, G3= Effect due to jth level of GA_3, Su= Effect due to kth level of sucrose, VarG3= Interaction between ith level of variety and jth level of GA_3,VarSu= Interaction between ith level of variety and kth level of sucrose, G3Su= Interaction between jth level of GA_3 and kth level of sucrose, VarG3Su= Interaction between variety, GA_3 and sucrose, Ɛijk= Random error term.

III. Results and Discussion

A. Days to Shoot Initiation

The interaction effects on days to shoot initiation due to sucrose, GA_3 and varieties were highly significant at P≤0.001. The minimum days to shoot induction was observed in *Wanjiku* (5.11 d) at a concentration of 0.2 mgL^{-1}, GA_3 while in *Shangi* (5.56 d) and *Unica* (6.22d) it was observed at the concentration of 1.0 mgL^{-1} GA_3. Increasing GA3 concentration resulted in delayed shoot induction in respect to *Wanjiku*. Ahmet, (2014) reported that the minimum days to shoot induction were observed on cv. Granola (4.25 d) compared to other two varieties of seed potato on 1.0 × MS medium containing 0.25 mgL^{-1} GA_3 + 1.0 mgL^{-1} NAA. (Fig 1). The minimum days to shoot induction was observed in *Shangi* (5.11 d) followed by *Wanjiku* (5.56d) and *Unica* (5.67 d) on MS Medium containing 30 gL^{-1} sucrose (Fig 2). Optimum level of sucrose for days to shoot initiation was observed at a concentration of 30 gL^{-1} and at a higher concentration (40 gL^{-1}), shoot induction was delayed.

However, these results contradicted those of Usman *et al*., (2012) who reported that 45 gL^{-1} of sucrose as the optimum level for faster growth and development of guava plants *in vitro*. This may be due to differences in varietal variations responding differently to different treatment levels.

B. Days to Root Induction

The minimum days to root induction was observed in *Unica* (11.3 d) and *Shangi* (19.9 d) at a concentration of 1.0 mgL^{-1} GA_3, while in *Wanjiku* (14.8 d) at 0.2 mgL^{-1} GA_3 (Fig. 1). This was as a result of differences in genotypes responding to different treatment levels. These results were in agreement with those of Ullah *et al*., (2012) and Ahmet, (2014) who observed that GA_3 at a concentration of 0.25 mgL^{-1} resulted in minimum days to root initiation in Desiree compared to Pasinler potato varieties. Root induction was delayed the most in *Wanjiku* (29.3 d), followed by *Unica* (24.6 d) at a concentration of 0.5 mgL^{-1} GA_3 and *Shangi* (21.9 d) at 0.2 mgL^{-1} GA_3 (Fig. 1). Minimum days to root induction was recorded on *Unica* (7 d) at 0.2 mgL^{-1} GA_3 + 30 gL^{-1} sucrose, followed by *Shangi* (10 d) and *Wanjiku* (12 d) at 0.5 mgL^{-1} GA_3 + 40 gL^{-1} sucrose. Days to root induction were minimum at low concentrations of GA_3 (0.2 mgL^{-1}) and optimum concentration of sucrose (30 gL^{-1}). High sucrose concentration may not determine root induction, but it may induce better growth. However, high sucrose concentrations may limit growth, due to an increase in the osmotic potential in the medium caused by sucrose. Similar results were reported by Martins *et al*., (2015) who recorded that high sucrose concentration (41 gL^{-1}) inhibited roots growth.

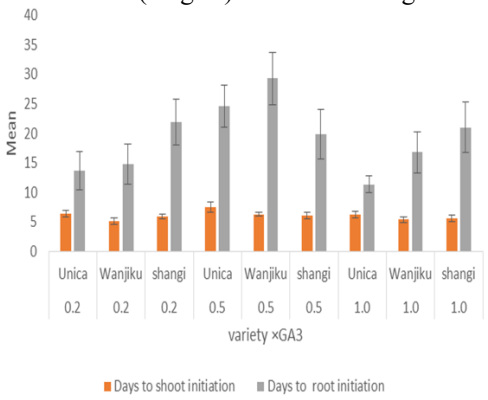

Fig.1. Effects of GA_3 by variety on shoot and root initiation of Shangi, Wanjiku and Unica varieties in Kenya.

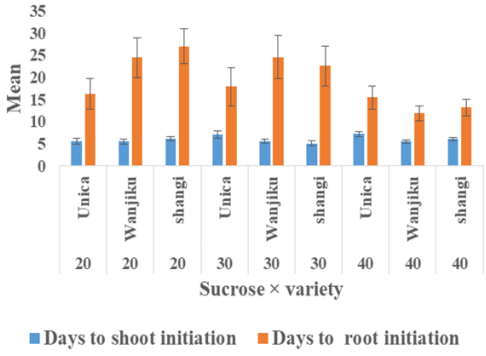

Fig. 2. Effects of sucrose by variety on shoot and root initiation of Shangi, Wanjiku and Unica varieties in Kenya.

C. Effects of GA_3 and Sucrose on the Survival of In vitro

High plant survival was observed on materials treated with 0.5 mgL^{-1} GA_3 + 40 gL^{-1} sucrose (90%) and this was significantly different from other treatments (Table II). *Shangi* showed the highest survival (75.56%) at 0.5 mgL^{-1} GA_3 but this was not significantly different from *Wanjiku* and *Unica* (68.89%) at the same GA_3 level (Table III). Lowest survival was observed for those entries treated with 0.5 mgL^{-1} GA_3 + 20 mgL^{-1} sucrose (61.11%). However, this was not significantly different from those treated with 0.2 mgL^{-1} GA_3 + 20 mgL^{-1} sucrose (62.22%). *Wanjiku* showed the highest survival (77.8%) followed by *Shangi* (73.3%) and *Unica* (73.3%) at 40 gL^{-1} of sucrose (Table II). Optimum concentration of sucrose (40 gL^{-1}) and GA_3 (0.5 mg mgL^{-1}) gave the highest survival of *in vitro*. Sucrose at 40 gL^{-1} increased the osmotic pressure and therefore nutrients and water retention forces in the medium decreased thus higher survival recorded compared to other treatments. In addition, number of leaves decreased with an increase in GA_3 concentration thus reducing the photosynthetic area of the plant. These results are in agreement with that of Mazri (2014) who reported that explants cultured MS medium supplemented with 40 gL^{-1} sucrose showed the highest survival rate.

D. Number of Leaves

The sucrose × GA_3 × variety interaction effects on number of leaves was highly significant at P≤0.001. The maximum number of leaves (8.5) was recorded in *Shangi*, followed by *Wanjiku* (8.0) and *Unica* after treatment with 40 gL^{-1} sucrose (Table I). The highest number of leaves was observed on 0.5 gL^{-1} GA_3 + 40 gL^{-1} (Table II). The highest number of leaves was observed in *Shangi* (8.2) at a concentration of 0.5 mgL^{-1} GA_3, followed by *Wanjiku* (7.9) at a concentration of 0.2 mgL^{-1} GA_3 and *unica* (7.2) at 1.0 mgL^{-1} GA_3 (Table III). Genotypes were found detrimental for *in vitro* growth responses; it is not possible to micro-propagate both cultivars on the same combination of GA_3. However, the optimized concentration of GA_3 gave different responses into different varieties in this study. Similar responses of GA_3 concentration were observed by Farhatullah *et al*., (2007) and Fatima *et al*., (2005) who reported that different concentration of treatments varied among varieties.

E. Number of Roots

The interaction effects of GA_3 by sucrose by variety were highly significant on the number of roots at P≤0.01. The maximum number of roots were observed in Unica (4.6/plantlet), followed by Shangi (4.4) and Wanjiku (3.4) at 1 mgL^{-1} GA_3 (Table IV). Sucrose at 40 gL^{-1} produced the maximum number of roots in Shangi (8.5), followed by Wanjiku (8.0) and Unica (7.2) (Table I). Minimum number of roots were observed in Wanjiku (2.0) at 20 gL^{-1} sucrose. At higher levels of sucrose, the osmolarity of the medium increased enabling plants to undergo stress that resulted to production of more roots. These results were in agreement with the findings of Mazri (2014) who reported that plantlets of date palm (Phoenix dactylifera L.) cultured on MS medium containing 40 gL^{-1} sucrose had the best rooting response (4.8roots/plantlet). He also observed that lower concentrations of sucrose (10, 20, or 30 gL^{-1}) resulted in fewer roots. The highest number of roots (8.8) was observed at a concentration of 0.5 mgL^{-1} GA3 +40 gL^{-1} sucrose (Table II).

TABLE I: INTERACTION EFFECTS OF SUCROSE BY VARIETY ON THE NUMBER OF LEAVES, NUMBER OF ROOTS, SHOOT LENGTH (CM), NUMBER OF SHOOTS, LEAF FRESH WEIGHT AND LEAF DRY WEIGHT (GRAMS) (MEAN ±SE) OF SEED POTATO IN KENYA

Level of Sucrose	Level of Variety	Survival (%)	Leaves number	Roots number	Shoots length	Shoots number	Leaf Fresh Weight	Leaf Dry Weight
20	shangi	62.22±3.85ab	6.93±0.43b	2.77±0.36bc	5.53±0.42a	1.44±0.12b	0.65±0.11cd	0.62±0.11c
20	Unica	63.33±6.45ab	7.18±0.38b	3.37±0.31d	6.18±0.43b	1.16±0.05a	0.76±0.05de	0.74±0.04d
20	wanjiku	60.00±5.00a	6.96±0.37b	1.99±0.21a	5.73±0.39a	1.58±0.12c	0.38±0.13b	0.35±0.13b
30	shangi	73.33±5.77c	7.49±0.38c	3.86±0.45e	7.36±0.54c	1.49±0.11bc	0.65±0.10c	0.62±0.10c
30	Unica	65.56±5.85b	6.36±0.37a	2.90±0.27c	5.31±0.47a	1.44±0.10b	0.83±0.13f	0.82±0.12e
30	wanjiku	61.11±3.47a	7.00±0.44b	2.61±0.28b	5.56±0.42a	1.47±0.09b	0.30±0.08ab	0.27±0.08ab
40	shangi	73.33±10.00c	8.51±0.52e	5.43±0.46g	8.11±0.59d	1.98±0.15e	0.81±0.22ef	0.78±0.22de
40	Unica	73.33±8.66c	7.24±0.42bc	4.83±0.40f	6.49±0.46b	1.49±0.08b	0.54±0.16c	0.52±0.16c
40	Wanjiku	77.78±5.61c	8.02±0.44d	4.54±0.32f	6.98±0.48c	1.82±0.11d	0.28±0.07a	0.25±0.07a

Means within a column followed by the same letters are not significantly different (P≤0.05) according to Tukey's HSD test

TABLE II: INTERACTION EFFECTS OF GA3 BY SUCROSE ON THE NUMBER OF LEAVES, NUMBER OF ROOTS, SHOOT LENGTH (CM), NUMBER OF SHOOTS, LEAF FRESH AND LEAF DRY WEIGHT (GRAMS) (MEAN ±SE) OF SEED POTATO IN KENYA

Level of GA_3	Level of Sucrose	Survival (%)	Leaves number	Roots number	Shoot length	Shoots number	Leaf Fresh Weight	Leaf Dry Weight
0.2	20	62.22±3.85a	7.20±0.45b	2.30±0.22a	6.00±0.36b	1.73±0.14e	0.66±0.10d	0.64±0.10d
0.2	30	67.78±6.31bc	7.24±0.45b	4.12±0.31d	7.04±0.51c	1.49±0.11cd	0.44±0.05b	0.41±0.05b
0.2	40	68.89±4.51c	7.64±0.48b	3.92±0.32cd	6.78±0.45c	1.84±0.13e	0.13±0.05a	0.11±0.05a
0.5	20	61.11±5.36a	6.58±0.39a	2.12±0.34a	5.31±0.43a	1.29±0.08b	0.50±0.12c	0.47±0.12c
0.5	30	62.22±5.61ab	6.22±0.36a	2.37±0.40a	5.20±0.49a	1.49±0.11cd	0.63±0.16d	0.61±0.16d
0.5	40	90.00±4.08d	8.78±0.53c	5.06±0.40e	8.04±0.62d	1.87±0.15e	0.52±0.06c	0.50±0.05c
1.0	20	62.22±6.31ab	7.29±0.33b	3.70±0.31c	6.13±0.43b	1.16±0.05a	0.63±0.12d	0.60±0.12d
1.0	30	70.00±4.50c	7.38±0.37b	2.88±0.28b	5.98±0.45b	1.42±0.07c	0.72±0.13d	0.69±0.13d
1.0	40	65.56±6.53abc	7.36±0.33b	5.83±0.43f	6.76±0.45c	1.58±0.07d	0.97±0.21e	0.95±0.21e

Means within a column followed by the same letters are not significantly different (P≤0.05) according to Tukey's HSD test

TABLE III: INTERACTION EFFECTS OF GA3 LEVELS BY VARIETY ON THE NUMBER OF LEAVES, NUMBER OF SHOOTS, SHOOTS LENGTH (CM), NUMBER OF SHOOTS, LEAF FRESH AND LEAF DRY WEIGHT (GRAMS) (MEAN ±SE) OF SEED POTATO IN KENYA

Level of GA_3	Level of Variety	Survival (%)	Leaves Number	Roots Number	Shoots Length	Shoots Number	Leaf Fresh Weight	Leaf Dry Weight
0.2	Shangi	68.89±5.36b	7.22±0.50b	3.50±0.41c	6.76±0.53c	1.89±0.14f	0.49±0.10cd	0.46±0.10cd
0.2	Unica	67.78±3.85ab	6.96±0.39ab	3.41±0.25bc	6.24±0.40b	1.38±0.10bc	0.41±0.09bc	0.40±0.09bc
0.2	Wanjiku	62.22±5.61a	7.91±0.48d	3.43±0.25bc	6.82±0.40c	1.80±0.13ef	0.33±0.11ab	0.30±0.11ab
0.5	Shangi	75.56±9.18c	8.16±0.53d	4.17±0.50d	7.42±0.63d	1.71±0.16de	0.54±0.10d	0.51±0.11d
0.5	Unica	68.89±10.58bc	6.64±0.44a	3.10±0.38b	5.78±0.52b	1.29±0.08a	0.77±0.10e	0.76±0.10e
0.5	Wanjiku	68.89±8.39bc	6.78±0.39a	2.28±0.34a	5.36±0.46a	1.64±0.11d	0.34±0.11ab	0.31±0.11ab
1.0	Shangi	64.44±6.53ab	7.56±0.29c	4.39±0.44d	6.82±0.45c	1.31±0.07ab	1.07±0.16g	1.04±0.16g
1.0	Unica	65.56±6.53ab	7.18±0.36b	4.59±0.38d	5.96±0.44b	1.42±0.07c	0.95±0.10f	0.93±0.10f
1.0	Wanjiku	67.78±5.61ab	7.29±0.37bc	3.43±0.33bc	6.09±0.44b	1.42±0.07c	0.30±0.07a	0.27±0.07a

Means within a column followed by the same letters are not significantly different (P≤0.05) according to Tukey's HSD test

F. Shoot length

The sucrose × GA_3 × variety interaction effects on the shoot length were highly significant for at P≤0.001. A longer shoot length was observed in *Shangi* (8.1 cm), followed by *Wanjiku* (7.0 cm) and *Unica* (6.5) at 40 gL^{-1} sucrose (Table I). Increasing the sucrose concentration promoted an increase in shoot length. These results were in agreement with the findings of Mazri (2014) who reported that plantlets of date palm (Phoenix dactylifera L.) cultured on MS medium containing 40 gL^{-1} sucrose produced the longest shoots (average = 14.9 cm). The longest shoot (8.0 cm) was observed in 0.5 mgL^{-1} GA_3 +40 gL^{-1} sucrose (Table II). *Shangi* produced the longest shoot length (7.4 cm) at 0.5 mgL^{-1} GA_3, followed by *Wanjiku* (6.8 cm) and *Unica* (6.2 cm) at 0.2 mgL^{-1} GA_3 (Table III). GA_3 at 0.5 mgL^{-1} and 0.2 mgL^{-1} increased shoot length by extending the internodes of growing shoots. These results were in agreement with the findings of Yildirim (2019) who reported that GA_3 at 0.5 mgL^{-1} gave the best results in terms of shoot length and shoot number on micro propagation of lentisk (Pistacia lentiscus L.).

G. Number of Shoots

The interaction effects of GA_3 by Sucrose by variety significantly influenced the shoots number P≤0.001. The

maximum number of shoots were observed on *Shangi* (1.9), followed by *Wanjiku* (1.8) at 0.2 mgL^{-1} GA_3 and finally *Unica* (1.4) at 1.0 mgL^{-1} GA_3 (Table III). Number of shoots tends to decrease as GA_3 concentration increases in the culture media. GA_3 at high concentration prevented shoot formation. These results also may have occurred due to genotypic variations interacting with different treatment levels. These results were in agreement with the findings of Padron *et al*., (2020) who observed that increasing the supply of GA_3 in the medium reduced the number of shoots of Alpinia purpurata (Zingiberaceae). *Shangi* recorded a maximum number of shoots (2.0), followed by *Wanjiku* (1.8) and *Unica* (1.5) at. 40 gL^{-1} sucrose (Table I). Shoot induction increased with increase in sucrose concentration resulting to an increase in osmotic pressure that hastens shoot induction. Usman *et al*. (2012) and Doaa and Mokhtar (2015) also observed that shoot induction increased with increase in sucrose concentration 45 gL^{-1} and 50 gL^{-1} in guava plants *in vitro* and fig (Ficus carica L.) respectively. However, these results contradicted the findings of Taha *et al.,* (2001) who reported more shoot bud formation on medium containing 30 gL^{-1} sucrose than on media containing 10, 20 or 40 gL^{-1} sucrose in the date palm cultivar 'Zaghlool'. This shows that treatment levels varies among the plant species. The highest number of shoots (1.9) was observed on 0.5 mgL^{-1} GA_3 +40 gL^{-1} sucrose (Table II).

H. Leaf Fresh Weight and Dry Weight

The interaction effects of GA3 by Sucrose by variety significantly influenced the leaf fresh weight at $P \leq 0.05$. The highest leaf fresh weight was observed on 1.0 mgL^{-1} GA_3 + 40 gL^{-1} sucrose, whereas the lowest was observed on 0.2 mgL^{-1} GA_3 +40 gL^{-1} sucrose (Table II). *Unica* recorded the highest leaf fresh weight (0.83 g) from the treatment with 30 gL^{-1}, followed by *Shangi* (0.81 g) at 40 gL^{-1} and *Wanjiku* (0.38) at 20 gL^{-1}. However, results obtained in *unica* for leaf fresh weight were not significantly different from that of *shangi*. This was due to difference in genotypic variations. Similar results were reported by Haida *et al*., (2020) who recorded that treatment of 30 gL^{-1} sucrose in clinacanthus nutans (Sabah Snake Grass) produced the highest leaf fresh weight. Although the highest number of leaves were recorded in unica and wanjiku at 40 gL^{-1} sucrose, the leaves produced were smaller than that of the sucrose concentration at 30 gL^{-1} and 20 gL^{-1} for *unica* and *wanjiku* respectively.

I. Correlation Analysis

There was strong positive correlation between number of leaves and length of shoots (r=0.76***), number of leaves and number of roots (r=0.65***) and number of roots and length of shoots (r=0.67***). An increase in root numbers resulted in an increase in shoot length and leaf numbers. Also, increase in shoot length and shoot numbers led to an increase in the number of leaves (Table IV). Number of roots and number of shoots showed weak correlation of 0.45***. This may have been due to the limited space for growth in the test tubes that led to fewer number of roots thus leading to fewer number of shoots. There was also strong positive correlation between leaf fresh weight and leaf dry weight (r=0.99***), days to root initiation and days to shoot initiation (r=0.71*), days to root initiation and leaf dry weight (r=0.69*) and days to root initiation and leaf fresh weight (r=0.66*). Leaf dry weight increased with increase in leaf fresh weight. An increase in leaf fresh and dry weight as a result of early root initiation can be explained by earlier root development which led to increased nutrient uptake, increased shoot growth and maturity of the shoots/leaves (Table V). Similar results were reported by Nasir & Toth (2021) who recorded that above ground biomass positively correlated with plant height, number of leaves, leaf area index and foliage dry matter.

TABLE IV: PEARSON CORRELATION COEFFICIENTS ON THE RESPONSE VARIABLES OF NUMBER OF LEAVES, NUMBER OF ROOTS, LENGTH OF SHOOTS, NUMBER OF SHOOTS

Response variables	Number of Leaves	Number of roots	Length of shoots
Number of roots	0.65***	-	-
Length of shoots	0.76***	0.67***	-
Number of shoots	0.58***	0.45***	0.57***

*** Significant at P<0.001

TABLE V: PEARSON CORRELATION COEFFICIENTS ON THE RESPONSE VARIABLES OF DAYS TO SHOOT INITIATION, DAYS TO ROOT INITIATION, LEAF FRESH WEIGHT AND LEAF DRY WEIGHT

Response variables	Days to shoot initiation	Days to root initiation	Leaf fresh weight
Days to root initiation	0.71*	-	-
Leaf fresh weight	0.51ns	0.66*	-
Leaf dry weight	0.49ns	0.69*	0.99***

* Significant at P<0.05, ***significant at P<0.001

IV. CONCLUSION

Using MS Medium in growth of *in vitro* and sucrose as a carbon source at the rate of 40 gL^{-1} gave better growth in terms of leaves number, number of shoots, shoot length and the number of roots. The supply of gibberellic acid (GA3) at a concentration of 0.5 mgL^{-1} combined with sucrose at a concentration of 40 gL^{-1} significantly recorded a better shoot length, the number of shoots, number of roots, number of leaves, root initiation, shoots fresh weight and shoots dry weight on local potato varieties cultured *in vitro*. Potato varieties responds differently to different treatment levels. These results showed that 0.5 mgL^{-1} GA3 along with 40 gL^{-1} sucrose had better effect on survival and improvement of shoot growth.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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