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 and 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 g L⁻¹, while gibberellic acid was applied at a concentration of 0.2, 0.5 and 1.0 mg L⁻¹. The study was laid out in a completely randomized design (CRD). MS Medium with sucrose 40 g L⁻¹ and gibberellic acid 0.5 mg L⁻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 mg L⁻¹ gibberellic acid along with 40 g L⁻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.

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). GA3 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 in the media to the emergence of first initial roots. The UV light in the laminar air flow cabinet for 15 minutes. Laminar air flow cabinet surfaces were autoclaved at 121 °C for 45 minutes.

C. Stock Solution in In vitro Culture

Murashige and Skoog (MS) (Murashige & 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 GA3 (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 3x3 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 GA3 (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:

\[ Y_{ijk} = \mu + \text{vari} + G_{3j} + S_{uk} + \text{VarG}_{3ij} + \text{VarSu}_{ik} + G_{3S_{ujk}} + \epsilon_{ijk} \]

Where: Yijk: observed responses, \( \mu \): Overall mean level, vari: Effect due to ith level of variety, G3= Effect due to jth level of GA3, Su= Effect due to kth level of sucrose, VarG3= Interaction between ith level of variety and jth level of GA3, VarSu= Interaction between ith level of variety and kth level of sucrose, G3Su= Interaction between jth level of GA3 and kth level of sucrose, VarG3Su= Interaction between variety, GA3 and sucrose, \( \epsilon_{ijk} \): Random error term.

III. RESULTS AND DISCUSSION

A. Days to Shoot Initiation

The interaction effects on days to shoot initiation due to sucrose, GA3 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, GA3 while in Shangi (5.56 d) and Unica (6.22d) it was observed at the concentration of 1.0 mgL⁻¹ GA3. 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 GA3 + 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 g L\(^{-1}\) of sucrose was 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.

**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\(^{-1}\) GA\(_3\) +40 gL\(^{-1}\) sucrose (Table II).
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 g L$^{-1}$ 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 g L$^{-1}$ sucrose produced the longest shoots (average = 14.9 cm). The longest shoot (8.0 cm) was observed in 0.5 mg L$^{-1}$ GA$_3$ + 40 g L$^{-1}$ sucrose (Table II). Shangi produced the longest shoot length (7.4 cm) at 0.5 mg L$^{-1}$ GA$_3$, followed by Wanjiku (6.8 cm) and Unica (6.2 cm) at 0.2 mg L$^{-1}$ GA$_3$ (Table III). GA$_3$ at 0.5 mg L$^{-1}$ and 0.2 mg L$^{-1}$ 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 mg L$^{-1}$ gave the best results in terms of shoot length and shoot number on micro propagation of lentisk (Pistacia lentiscus L.).

### F. Shoot length

The interaction effects of GA$_3$ by Sucrose by variety significantly influenced the shoots number P≤0.001. The
The maximum number of shoots was observed on *Shangi* (1.9), followed by *Wanjiku* (1.8) at 0.2 mg L\(^{-1}\) GA\(_3\) and finally *Unica* (1.4) at 1.0 mg L\(^{-1}\) 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 g L\(^{-1}\) 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 g L\(^{-1}\) and 50 g L\(^{-1}\) 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 g L\(^{-1}\) sucrose than on media containing 10, 20 or 40 g L\(^{-1}\) sucrose in the date palm cultivar ‘Zaghlool’. This shows that treatment levels varies in agreement with the findings of Padron et al., (2020) who also observed that *Unica* and *Shangi* for leaf fresh weight and shoot length, the number of shoots, 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 mg L\(^{-1}\) GA\(_3\) + 40 g L\(^{-1}\) sucrose (Table II).

### 2. Leaf Fresh Weight and Dry Weight

The interaction effects of GA\(_3\) by Sucrose by variety significantly influenced the leaf fresh weight at P≤0.05. The highest leaf fresh weight was observed on 1.0 mg L\(^{-1}\) GA\(_3\) + 40 g L\(^{-1}\) sucrose, whereas the lowest was observed on 0.2 mg L\(^{-1}\) GA\(_3\) +40 g L\(^{-1}\) sucrose (Table II). *Unica* recorded the highest leaf fresh weight (0.83 g) from the treatment with 30 g L\(^{-1}\), followed by *Shangi* (0.81 g) at 40 g L\(^{-1}\) and *Wanjiku* (0.38) at 20 g L\(^{-1}\). 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 Haïda et al., (2020) who recorded that treatment of 30 g L\(^{-1}\) 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 g L\(^{-1}\) sucrose, the leaves produced were smaller than that of the sucrose concentration at 30 g L\(^{-1}\) and 20 g L\(^{-1}\) for *unica* and *wanjiku* respectively.

### 3. 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 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 g L\(^{-1}\) gave better growth in terms of leaves number, number of shoots, shoot length and the number of roots. The supply of gibberellic acid (GA\(_3\)) at a concentration of 0.5 mg L\(^{-1}\) combined with sucrose at a concentration of 40 g L\(^{-1}\) 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 mg L\(^{-1}\) GA\(_3\) along with 40 g L\(^{-1}\) sucrose had better effect on survival and improvement of shoot growth.

### Acknowledgement

The research was partially funded by CARP+ and Kenya Climate Smart Agriculture (KCSAP) Seed potato projects at Egerton University. The authors acknowledge the Agricultural Development Corporation (ADC), Molo for permitting the use of their tissue culture laboratory.

### Conflict of interest

Authors declare that they do not have any conflict of interest.
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DOI: http://dx.doi.org/10.24018/ejbio.2022.3.4.372