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Determination of hormonal combination for increased multiplication of tissue culture potato plantlets

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Abstract

Use of plant growth hormones either singly or in combination is vital for rapid multiplication of virus-free *in-vitro* potato (*Solanum tuberosum* L.) plantlets for the production of clean seed potato. This study was carried out at Kachwekano Zonal Agricultural Research and Development Institute, Kabale district in south-western Uganda to identify a suitable hormonal combination and optimum concentrations for production of a high number of *In-vitro* plantlets for three farmer-preferred varieties Kachpot-1, Kinigi, and Victoria. Eight hormonal combinations were formulated and tested using a completely randomized design with three replicates in the tissue culture laboratory. Ten shoot tips from *in-vitro* raised plantlets were excised and transferred to each of these hormonal combinations. The effect of hormonal combinations was variety dependant. N3 produced the highest mean number of nodes (6.42), buds (4.32) and tallest plantlets (7.38) for Kinigi. N2 produced the highest number of buds in Kachpot 1. N4 produced the tallest plantlets (7.19), highest mean number of buds (5.88) and nodes (7.66) for Victoria. Therefore, N3, N2 and N4 should be used for rapid *In-vitro* propagation of Kinigi, Kachpot 1 and Victoria respectively.

Key words: *In-vitro* propagation, *Solanum tuberosum*

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, planted on 20 million ha in the world in 2005 (FAO, 2007. In Uganda, potato is mainly grown in the higher altitude areas (>1500 m above sea level), where it serves as the main source of income. The areas 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 northern region. About 40%

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of the national harvest comes from the intensely farmed Kabale highlands (Ferris *et al*., 2001). Potatoes in these regions are grown mainly by small scale farmers. These farmers use conventional means to propagate potato through the use of tubers. This propagation is characterised by low multiplication ratios that ranges from 1:4 to 1:15 (Rabbani *et al.,* 2001). Because of this low rate of multiplication, it takes many years to generate large quantities of seed to meet the demand of the seed potato industry.

Several methods have the capacity to greatly improve the rate of seed potato production; such methods include tissue culture and aeroponics technologies (Molitor *et al.,* 1999; Ritter *et al.,* 2001). 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 (Mahmoud, 2006).

Several researchers have reported the use of MS medium, without hormones during proliferation stage but the growth was slow (Yousef *et al*., 1997; Badon and Chauhan, 2010). Addition of growth regulators to the culture media has been reported to improve the number, growth and development of shoots (Ahmed *et al.,* 1993; Rabban *et al.,* 2001), though they are genotype dependant. The use of higher concentrations of GA_3 supplemented with NAA and Vitamins has been reported to increase the number of nodes (Miller *et al.,* 1985; Yousef *et al.,* 1997). Rabbani *et al.* (2001) recommended the use of higher concentrations of GA_3, supplemented with other phytohormones like BAP and NAA, and vitamins in order to increase the multiplication capacity of potato. In Uganda, the use of tissue culture is a new technique in seed potato production, therefore, appropriate media composition for rapid multiplication of potato tissue culture plantlets has not been optimised. Thus, the objective of this study was to optimize hormonal combinations for increased multiplication of tissue culture potato plantlets.

Materials and methods

This study was carried out in the tissue culture laboratory at Kachwekano Zonal Agricultural and Research Development Institute (KaZARDI) in Kabale district, southwestern Uganda. Three popularly grown and farmer-preferred varieties, namely Victoria, Kachpot1 and Kinigi obtained from KaZARDI were used in this study. Mini-tubers, previously harvested from the aeroponics culture, were used as the starting plant materials. The sprouted mini-tubers were planted in the wooden boxes containing moist sterilised soil in a screen-house to generate mother plants.

After 14 - 21 days from planting (DAP), all the emerged plants had 5 - 8 leaves per plant. Three leaves, one each 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). The virus-free mother plants were retained, from which explants were obtained. The diseased mother plants were uprooted using a pair of forceps and destroyed by inceneration.

After four weeks, when the plants were approximately 20 -30 cm tall, with 4 - 8 nodes, they were cut from the shoot bases using sterile surgical blades into individual shoots measuring approximately 20 - 28 cm tall. The large leaves were

trimmed off and the nodal explants put in a flat bottomed cornical flask of 1000 ml capacity. They were washed under running tap-water, supplemented with 5 drops of sodium stearate (liquid soap) for 40 minutes to remove the surface contaminants. The explants were then surface-sterilised under a laminar flow hood by immersing them in 70% ethanol for five seconds. They were further immersed in 14% sodium hypochlorite solution (JIK), supplemented with three drops of Tween-20 for two minutes. The explants were washed with autoclaved distilled water, three times, at an interval of five minutes, to remove residual JIK.

The disinfected materials were placed on sterilised cut pieces of paper, ready for inoculation onto the media. The explants with more than one node were cut into single nodes and initiated in glass autoclavable test tubes (14 cm height x 2 cm diameter), containing MS (Murashige and Skoog, 1962) basal media to generate experimental plantlets. After inoculation onto the MS media, the cultured explants were transferred to a growth room at 16 hour photoperiod at 18 ^{o}C, under approximately 1000 lux light intensity. The plantlets were micro-propagated after 3 - 4 weeks to increase the number of experimental plantlets.

Four weeks after the last sub-culture level on MS media, the plantlets were ready for excision and transfer to different hormonal combinations/treatments. Hormonal combinations N1–N4 and B1–B4, composed of MS alone and MS media, supplemented with different concentrations of Giberrellic acid (GA_3), Benzyl amino purine (BAP) and Napthalene acetic acid (NAA) were formulated by adding different concentrations of growth regulators into the basal MS media. Treatment N1 was composed of MS alone), N2 of MS+3 mg l^{-1} GA_3+0.005 mg l^{-1} NAA, N3 of MS+4 mg l^{-1} GA_3+0.01 mg l^{-1} NAA) and N4, MS+5 mg l^{-1} GA_3+0.02 mg l^{-1} NAA. B1 was composed of MS alone, B2, MS + 3mg l^{-1} GA_3 + 1.5 mg l^{-1} BAP, B3, MS+4 mg l^{-1} GA_3+2.0 mg l^{-1} BAP and B4, MS+5 mg l^{-1} GA_3+2.5 mg l^{-1} BAP.

The pH of the MS media was adjusted to 5.8, solidified with 6 g l^{-1} agar and autoclaved at 121 ^{o}C for 20 minutes. When the temperature of the media decreased to about 50 ^{o}C, the growth regulators were added by filter-sterilisation and mixed thoroughly by handshaking under a lamina flow hood. About 8–10 ml of the media were dispensed in glass test tubes (14 cm height x 2 cm diameter) and left to cool. Ten shoot tips were sub-cultured to each of the hormonal combinations, in 3 replications. The experiment was laid out in a completely randomised design (CRD). The experiment was repeated three times.

Four weeks after transferring the plantlets onto different hormonal combinations, data were collected on shoot height, number of shoots, number of buds, number of visible primary roots and number of nodes. The data were subjected to analysis of variance (ANOVA), using GenStat Statistical Package, 13th Edition. The significant means were compared using the Least Significant Difference at 5% level of probability.

Results

Number of shoots per plant, variety, hormonal combinations and the interaction showed no significant effect (P>0.05). However, shoot height, and number of buds, roots, leaves and nodes were significantly different for varieties and hormonal combinations.

Variety, hormonal combinations and their interaction had a high significant effect on potato plantlet shoot height ($P<0.001$). The tallest plantlets were produced by Victoria and hormonal combinations N2 – N4 had the highest overall effect on shoot height, though not significantly different from each other. However, they were different from the rest of the combinations.

Hormonal combination N4 produced the tallest plantlets for Kachpot 1 and Victoria; yet N3 produced the tallest plantlet for Kinigi. The shortest plantlets were produced by hormonal combination B2 for both Katchpot 1 and Victoria. In each variety, N1 and B1 did not differ from each other on shoot height (Table 1).

Interactions among variety, hormonal combinations and their interaction were highly significant ($P<0.001$) for number of buds per plant (Table 2). The highest number of buds was produced by Victoria, while the lowest by Kinigi. Hormonal combination N3 had the highest overall effect on bud initiation, closely followed by N2.

Hormonal combination N2 initiated the highest number of buds for Kachpot 1, closely followed by hormonal combination N3. The lowest number of buds was initiated by hormonal combination B2. Hormonal combination N4 initiated the highest number of buds for Victoria but did not differ significantly from N3 and N2. The lowest number of buds was initiated by hormonal combination B3. Hormonal combinations N2 and N3 initiated the highest number of buds for Kinigi. They were however, not significantly different from N4. The lowest number was initiated by B3. In each variety, N1 did not differ from B1 on the number of buds initiated (Table 2).

Results regarding the hormonal combinations, varieties and their interaction indicated a very high statistical significance ($P<0.001$) on root development (Table 3). Kachpot 1 produced a significantly higher number of roots while Victoria and Kinigi did not significantly differ from each other.

Table 1. Shoot height of potato plantlet arising from the interaction between varietal and hormonal treatments

Treatment	Shoot height (cm)		
	Kachpot	Kinigi	Victoria
N1	5.81	3.74	5.96
N2	6.14	6.80	6.63
N3	6.17	7.38	7.05
N4	6.76	6.00	7.19
B1	5.81	3.74	5.96
B2	3.19	3.59	4.31
B3	3.37	2.65	4.57
B4	3.25	2.59	4.38
$LSD_{0.05}$		0.57	

Table 2. Number of buds on potato plantlet arising from the interaction between varietal and hormonal treatments

Treatment	Buds per plant		
	Kachpot	Kinigi	Victoria
N1	4.72	3.02	4.93
N2	5.36	4.32	5.70
N3	5.27	4.32	5.76
N4	4.79	4.08	5.88
B1	4.72	3.02	4.93
B2	2.32	1.97	3.07
B3	2.42	1.68	2.99
B4	2.50	1.84	3.37
$LSD_{0.05}$		0.39	

Hormonal combination N4 had the highest overall impact on root development. This was followed by hormonal combinations N2 and N3. Hormonal combination N4 initiated the highest number of roots for Victoria. The lowest number was induced by hormonal combination B4.

Hormonal combination N2 induced the highest number of roots for Kachpot 1, though it did not differ from N3 and N4. The lowest number was induced by B4. Hormonal combination N2 induced the highest number of roots for Kinigi though not statistically different from N3 and N4. B4 had the least effect on root initiation and development though not statistically different from B2 for Kinigi (Table 3).

The ANOVA table showed that there was a very high statistical significance between variety, hormonal combinations and their interaction on the development of leaves ($P<0.01$). The highest number of leaves was noticed on Kachpot 1, followed by Victoria while Kinigi produced the least number of leaves. Hormonal combination N1 and B1 had the greatest impact on the development of leaves. The least impact was produced by hormonal combinations B3 (Table 4).

Hormonal combination N1 and B1 induced the highest number of leaves in Victoria. This was followed by N3. The least number of leaves was induced by hormonal combinations B3 in Victoria. The highest number of leaves in Kachpot 1 was also induced by N1 and B1. This was followed by hormonal combination N4 that did not significantly differ from N2 and N3. The lowest number was induced by B2 for Kachpot 1. Hormonal combination N2 induced the highest number of leaves in Kinigi though it was not significantly different from N1, B1 and N3. B3 induced the least number of leaves in Kinigi (Table 4).

For both varieties, hormonal combination and their interaction were highly significant ($P<0.001$). Kachpot 1 and Victoria had similar number of nodes produced; while Kinigi produced the least number of nodes. Hormonal combination N3 induced the highest number of nodes

Table 3. Number of roots on potato plantlet arising from the interaction between varietal and hormonal treatments

Treatment	Roots per plant		
	Kachpot	Kinigi	Victoria
N1	4.68	4.28	3.56
N2	6.28	4.96	5.14
N3	5.83	4.87	5.27
N4	5.90	4.94	5.76
B1	4.68	4.28	3.56
B2	0.59	0.37	0.24
B3	1.53	0.84	1.19
B4	0.34	0.10	0.19
$LSD_{0.05}$		0.46	

Table 4. Number of leaves on potato plantlet arising from the interaction between varietal and hormonal treatments

Treatment	Leaves per plant		
	Kachpot	Kinigi	Victoria
N1	8.71	5.03	7.14
N2	6.64	5.18	6.23
N3	6.63	5.07	6.51
N4	6.70	4.63	6.43
B1	8.71	5.03	7.14
B2	4.84	3.70	5.22
B3	4.89	3.40	4.82
B4	4.87	3.51	4.87
$LSD_{0.05}$	0.45		

Table 5. Number of nodes on potato plantlet arising from the interaction between varietal and hormonal treatments

Treatment	Nodes per plant		
	Kachpot	Kinigi	Victoria
N1	8.14	4.89	6.51
N2	7.17	6.57	7.43
N3	7.19	6.42	7.72
N4	7.11	6.41	7.66
B1	8.14	4.89	6.51
B2	5.24	5.04	5.37
B3	5.01	4.46	5.28
B4	4.86	4.53	5.14
$LSD_{0.05}$		0.41	

closely followed by N2 and N4 with the same mean.

B4 induced the lowest overall number of nodes (Table 5). Hormonal combination N1 and B1 produced a significantly higher number of nodes in Kachpot 1. This was followed by hormonal combination N3. However, N2, N3 and N4 did not significantly differ from each other. The lowest number of buds was initiated by B4 in Kachpot 1. Hormonal combination N3 initiated the highest number of nodes in Victoria though it did not significantly differ from N2 and N4. The lowest number was induced by B4. N2 induced the highest number of nodes in Kinigi though it did not significantly differ from N3 and N4. The least number of nodes was initiated by hormonal combination B3. B3 however did not significantly differ from hormonal combination B4 (Table 5).

Discussion

It is often necessary to alter the composition and or concentration of growth regulators in the culture medium depending on the genotype, origin of the explants and culture conditions. It is known that organogenesis is highly dependent on the interaction between naturally occurring endogenous growth hormones and exogenous growth regulators added to the culture medium (Muhammad and Hakoomat, 2004). Regeneration through direct organogenesis (directly from organs) gives plants that do not exhibit somaclonal variation and are true to type. A combination of GA_3 and NAA increased the shoot height though there were variations among the cultivars. These results are similar to those of Badoni and Chauhan (2009) who reported that a combination of GA_3 and NAA showed best results for improving all the growth parameters. Abdul *et al.* (2003) and Zaman *et al.* (2001) obtained maximum plant height (9 cm) when NAA alone was added to the medium at 0.15 mg l^{-1}. Maximum shoot height was also obtained by Rabbani *et al.* (2001) and Ahmed *et al.* (1993) when a high concentration of GA_3 at 4 mg l^{-1} and 4.5 mg l^{-1} respectively. GA_3 is physiologically involved in cell elongation in plants (Levitt, 1974). This explains the increased shoot height for hormonal combinations N2 to N4 in the present study.

The number of shoots did not differ significantly ($P<0.001$) across all the hormonal combinations for all the varieties. However, several researchers have reported that use of BAP alone has been the most effective growth regulator in stimulating organogenesis in different solanum tuberosum cultivars (Abdul *et al.,* 2003; Molla *et al.,* 2011). The combination of GA_3 and BAP did not result in increased number of shoots. This could be due to the effect of hormonal combination (antagonistic) or the concentrations used. The results of this

study suggest that BAP should be used alone or the concentrations of the hormonal combinations be lowered in order to induce multiple shoots.

Though there are differences among the cultivars, a combination of GA_3 and NAA resulted in more number of roots. The results on root development are in agreement with those of Vinterhalter *et al*. (1997) and Khadiga *et al.* (2009) who reported that potato is an easy to root specie and may not need exogenous hormones for rooting. The plantlets in all MS supplemented with GA_3 and BAP (B1 - B4) developed very few roots. This can be attributed to the fact that BAP at a higher concentration inhibits root development and promotes shoot development. All the hormonal combinations did not result in increased number of leaves. Hormonal combination N1 may result in increased photosynthetic ability of the plantlets due the high number of leaves.

Zaman *et al.* (2001) obtained the highest number of leaves when a higher concentration of auxin alone was used. The reduced number of leaves was as a result of low auxin concentration and probably a combination of low auxin and high GA_3 concentration that resulted in narrow and elongated shoots.

The MS media, when supplemented with GA_3 and NAA resulted in increased number of nodes for Kinigi and Victoria (Table 5). These results are similar to those obtained by Miller *et al.* (1985) who reported an increased number of nodes at a higher concentration of GA_3 along with NAA (1.0 mg l^{-1}). However, when Rabbani *et al.* (2001) used a higher concentration of GA_3 alone, it did not result in increased number of nodes. Moreover, Zaman *et al.* (2001) obtained a higher number of nodes at a higher concentration of auxins alone. When MS was supplemented with a combination of GA_3 and BAP, the number of nodes did not increase. In view of these results and those obtained by Anoop and Chauhan (2009) and Miller *et al.* (2009), higher concentration of GA_3 should be supplemented with NAA.

The effect of hormonal combinations on the multiplication of tissue culture plantlets varied from variety to variety (Tables 1-5). Hormonal combination N2 increased the number of *in vitro* plantlets for Kachpot 1 variety due the increased number the initiated buds. Hormonal combination N4 increased the number of buds, shoot height and number of nodes for Victoria. Hormonal combination N3 increased the plant shoot height, buds and number of nodes for Kinigi.

In view of the results obtained in the present study, the use of GA_3 and NAA is recommended. This combination will help increase the rate of multiplication in shoot tip culture by increasing the plantlet height, the number of nodes and buds on the plantlets obtained. The concentrations of GA_3 and BAP used in this study favoured callus development and therefore they should be lowered. In addition, NAA should be included in the hormonal combination and a proper balance between BAP and NAA be observed. Though callus proliferation from the tissues of most plants is thought to require the presence of both auxin and cytokinins in the growth medium, Yousef *et al.* (1997) obtained callus-free potato plantlets when low concentrations of BAP and NAA were used.

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