# Micro-Propagation of Disease Resistant Cassava Variety in Rwanda

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***Abstract***

*Cassava* ***(Manihot esculenta)*** *belongs to the* ***Euphorbiaceae*** *family and has about 100 species. In Rwanda, it plays a key role as food security and income generating crop. It is described as "classic food security crop" because it offers the advantage of a harvest even in situations of erratic rainfall and infertile soils. Cassava is a major staple food in Rwanda but production has been drastically declining in the last decade due mainly to diseases, pests and lack of disease resistant varieties. Among the major diseases, viral diseases are the most important in tropical Africa and Cassava Brown Streak Disease (CBSD) is the most damaging, causing over 50% yield loss and threatening the livelihoods of farmers. Recently, the Rwanda Agriculture Board (RAB) acquired some elite cassava varieties that are resistant to Cassava Mosaic Disease (CMD) and CBSD. The objective of this study was to develop an efficient, rapid tissue culture protocol for propagating the elite varieties. Nodal explants harvested from* ***in vitro****–grown plantlets were cultured on different Murashige and Skoog (MS) formulations. In a separate trial, full, half and one-quarter MS media were supplemented with 5, 10, 20 and 40 µM/l Giberellic acid ($GA_3$). The Tukey test showed that there were highly (p=0.0027) significant differences among the different $GA_3$ levels for shoot elongation in cassava. The best regeneration media was full MS media supplemented with 40 µM/l $GA_3$ that gave the highest mean shoot length of 8.93 ± 2.67 mm. Plantlets were successfully transferred to sterile soil mixture (soil: sand: manure in the ratio of 3:2:1) and acclimatized in the greenhouse. The new protocol developed in this research will enhance rapid production of high quality cassava planting materials for increased food security in Rwanda.*

***Key words****: Cassava,* ***Manihot esculenta****, tissue culture, microshoot regeneration*

## 1. Introduction

Cassava (*Manihot esculenta* Crantz) has been traditionally considered a subsistence crop, but nowadays there is evidence from Africa, Asia and Latin America that proves its global emergence as a cash crop. The main value of root and tuber crops resides in the

production of more edible energy per hectare per day than other crops and the capacity to generate high yields under conditions where other crops might fail (Konan *et al*., 1997). Cassava is normally propagated by means of stem cuttings, which are known as 'stakes'. This mode of propagation has many disadvantages, including the low multiplication rates and accumulation of viral and bacterial diseases which reduce productivity of the crop. There is, therefore, a need to look for more efficient methods of propagating the crop, and tissue culture offers such alternative.

Tissue culture is one of the most successful, commercially exploited components of biotechnology and has been used for rapid clonal multiplication (micropropagation) of selected genotypes of diverse groups of plant species (Rani and Raina, 2000). The first study on tissue culture of cassava was by Kartha *et al*. (1974) who reported regeneration of shoots from meristems of five cassava varieties cultured on MS medium supplemented with 0.1 mg/l Benzylamino purine (BAP), 0.04 mg/l Gibberellic acid ($GA_3$) and 0.2 mg/l Naphylacetic acid (NAA). Bhagwat *et al*. (1996) reported regeneration of multiple shoots from nodal explants of cassava using 0.11 to 0.22 µM/l thidiazuron (TDZ), 2.2 µM/l BAP and 1.6 µM/l $GA_3$, and Groll *et al*. (2001) regeneration of cassava secondary somatic embryos in a media supplemented with picloram.

A frequent *in vitro* culture manipulation for cassava involves standard media such as the Murashige and Skoog (1962) (MS medium), but with altered macro-and/or micronutrient concentrations. This manipulation was initially restricted to embryo culture and nodal micro propagation, but was later extended to somatic embryogenesis (Taylor et al., 1996). Propagation on half-strength MS medium was used for multiple shoot induction from nodal explants by Smith et al. (1986), and one-third strength MS medium was used for rooting through to hardening stages for culture from meristem explants (Roca, 1984). A medium low in salts was similarly used for the recovery of cassava plants following culture of zygotic embryos, with concentrations varying from one-third-strength MS medium (Fregene et al., 1999) to half-strength MS medium (Biggs et al., 1986). Due to the importance of this culture factor, the current study was conducted to

determine the optimal MS medium concentration for regeneration of MM06/0138, an elite cassava variety and the effect of supplementing MS media with GA3 on plant regeneration.

## 2. Materials and Methods

Nodal explants of MM06/0138 cassava variety were harvested from *in vitro*–grown plantlets and cultured on different Murashige and Skoog (MS) formulations of Full MS (FMS), Half MS (HMS) and Quarter MS (QMS) supplemented with 0.5 µM/l ascorbic acid, 0.05 µM/l biotin, 0.5 µM/l calcium pathonate, 1.0 µM/l cystein, 3% sucrose and gelled with 0.3% phytagel. In a second trial, the above media were supplemented with 5, 10, 20 and 40 µM/l $GA_3$.

The cultures were incubated in a growth-room maintained at 26°C and 16 hour photoperiod using a completely randomized design with eight replications. The data was analyzed using the one way analysis of variance procedure for number of microshoots per explant, microshoot length, number of roots per explant, and root length per explant. The data was analyzed using one way ANOVA. Means separated using Tukey test (0.05) (SAS version 9.1).

## 3. Results

The results of the different MS media formulations on plantlet regeneration are presented in Table 1. There was no significant difference among the three MS media formulations for number of microshoots per explant, averaging 1.0 over the three media. Full MS differed from Half MS in terms of microshoot length, number of roots per explant, and root length per explant; and from Quarter MS in microshoot length. Quarter MS was not significant different from Half MS in number and length of roots per explant. Quarter MS was the best media with 1.18±0.19 number of shoots per explants and full Ms was the best in terms of mean microshoot length with 4.83±0.78 mm shoot length. During the current study, a one single step protocol for regenerating cassava plantlets was developed. The step involves culturing the nodal explant in an MS media (Plate 1a) and regeneration of roots and shoots after one week (Plate 1b, c). The plantlets were then taken out of the culture vessels and the agar washed of (Plate 1d) and this was followed by placing them in a

beaker containing 2% Benlate fungicide for one hour (Plate 1e). The plantlets were then planted in a sterilized substrate for weaning (Plate 1f).

The effect of FMS supplemented with $GA_3$ on shoots and roots regeneration is presented in Table 2. The different $GA_3$ concentrations did not differ significantly in number of microshoots and roots per explant, but differed in terms of mean microshoot and root lengths per explant. $GA_3$ at 40 µM/l produced significantly longer microshoots and longer roots than the other concentrations evaluated. Increasing concentration of $GA_3$ from 5 to 40 µM/l increased the mean root and microshoot length.

The effects of half MS supplemented with $GA_3$ on shoots and roots regeneration is presented in Table 3. No significant differences were observed among the $GA_3$ concentrations for number of microshoots per explant. $GA_3$ at 40µM/l produced the longest roots per explant with a mean of 36.29±8.15mm, followed by 5 with 36.00±7.87mm, 20 µM/l with 35.50±6.42mm, respectively. $GA_3$ at 10 µM/l was less effective than all the other $GA_3$ concentrations in microshoot and root growth in explants.

The effects of quarter MS supplemented with $GA_3$ on shoot and root regeneration are presented in Table 4. $GA_3$ at 40 µM/l gave the highest mean number (1.71±0.74) of shoots per explant while $GA_3$ at 10 µM/l gave the highest (3.32±0.77mm) mean shoot length, and $GA_3$ at 5 µM/l gave the highest mean 3.32±0.77mm root length. In general, the lower $GA_3$ concentrations produced better root and shoot proliferation than the higher concentrations.


**Plate 1:** Plantlet regeneration from cassava nodal explants. a. Freshly inoculated cassava node, b: Regenereted microshoot and roots after two weeks, c: Regenerated microshoot and roots after three weeks, d: Rooted Cassava plantlets, e: Cassava plantlets in fungicide before weaning, f: Plantlets being weaned.

**Table 1:** Effects of different MS media formulations on microshoot and root proliferation in Cassava.

| **Media** | **Mean number of microshoots per explant (±SE)** | **Mean length of microshoots (mm) (±SE)** | **Mean number of roots per explant (±SE)** | **Mean root length per explant (±SE)** |
|---|---|---|---|---|
| Full MS | 1.07±0.10[a] | 4.83±0.78[a] | 1.00±0.11[a] | 25.43±1.77[a] |
| Half MS | 1.00±0.09[a] | 2.42±0.44[b] | 0.77±0.10[a] | 27.66±3.72[a] |
| Quarter MS | 1.18±0.19[a] | 2.60±0.27[b] | 0.95±0.14[a] | 28.23±2.54[a] |
| P value | 0.6453 | 0.0027 | 0.3655 | 0.7553 |

Values represent means ± SE. Means within a column followed by different letters are significantly different at $P = 0.05$ (Tukey test), N = 56.

**Table 2:** Effects of full MS media supplemented with $GA_3$ on microshoot and root proliferation in Cassava.

| Concentration (μM/l $GA_3$) | Mean number of microshoots per explant (±SE) | Mean length of microshoots (mm) (±SE) | Mean number of roots per explant (±SE) | Mean root length per explant (±SE) |
|---|---|---|---|---|
| 5 | $1.07 \pm 0.13^{a}$ | $3.39 \pm 1.00^{a}$ | $1.00 \pm 0.33^{a}$ | $22.79 \pm 3.31^{a}$ |
| 10 | $1.14 \pm 0.21^{a}$ | $4.36 \pm 0.52^{ba}$ | $1.00 \pm 0.21^{a}$ | $19.64 \pm 3.18^{a}$ |
| 20 | $1.00 \pm 0.23^{a}$ | $2.64 \pm 0.39^{b}$ | $1.00 \pm 0.18^{a}$ | $28.07 \pm 4.15^{b}$ |
| 40 | $1.07 \pm 0.22^{a}$ | $8.93 \pm 2.67^{b}$ | $1.00 \pm 0.18^{a}$ | $31.21 \pm 2.91^{b}$ |
| P value | 0.9686 | 0.0168 | 1.0000 | 0.0876 |

Values represent means ± SE. Means within a column followed by different letters are significantly different at $P = 0.05$ (Tukey test), N= 14.

**Table 3:** Effects of half MS media supplemented with $GA_3$ on microshoot and root proliferation in Cassava.

| Concentration (μM/l $GA_3$) | Mean number of microshoots per explant (±SE) | Mean length of microshoots (mm) (±SE) | Mean number of roots per explant (±SE) | Mean root length per explant (±SE) |
|---|---|---|---|---|
| 5 | $1.00 \pm 0.18^{a}$ | $1.96 \pm 0.29^{a}$ | $0.93 \pm 0.20^{a}$ | $36.00 \pm 7.87^{a}$ |
| 10 | $1.00 \pm 0.15^{a}$ | $1.61 \pm 0.21^{a}$ | $0.43 \pm 0.13^{a}$ | $2.86 \pm 1.08^{b}$ |
| 20 | $1.00 \pm 0.21^{a}$ | $3.79 \pm 1.67^{a}$ | $0.86 \pm 0.20^{a}$ | $35.50 \pm 6.42^{a}$ |
| 40 | $1.00 \pm 0.21^{a}$ | $2.32 \pm 0.40^{a}$ | $0.86 \pm 0.25^{a}$ | $36.29 \pm 8.15^{a}$ |
| P value | 1.0000 | 0.3219 | 0.2916 | 0.0009 |

Values represent means ± SE. Means within a column followed by different letters are significantly different at $P = 0.05$ (Tukey test), N = 14.

**Table 4:** Effects of quarter MS media supplemented with $GA_3$ on microshoot and root proliferation in Cassava.

| Concentration (μM/l $GA_3$) | Mean number of microshoots per explant (±SE) | Mean length of microshoots (mm) (±SE) | Mean number of roots per explant (±SE) | Mean root length per explant (±SE) |
|---|---|---|---|---|
| 5 | $1.00 \pm 0.10^{a}$ | $2.71 \pm 0.32^{a}$ | $1.00 \pm 0.31^{a}$ | $3.32 \pm 0.77^{a}$ |
| 10 | $1.00 \pm 0.20^{a}$ | $3.32 \pm 0.77^{a}$ | $1.00 \pm 0.33^{a}$ | $33.36 \pm 4.09^{ba}$ |
| 20 | $1.00 \pm 0.10^{a}$ | $2.00 \pm 0.19^{a}$ | $0.93 \pm 0.27^{a}$ | $23.36 \pm 6.20^{ba}$ |
| 40 | $1.71 \pm 0.74^{b}$ | $2.36 \pm 0.66^{a}$ | $0.86 \pm 0.25^{a}$ | $16.29 \pm 4.57^{b}$ |
| P value | 0.4714 | 0.3665 | 0.9831 | 0.0031 |

Values represent means ± SE. Means within a column followed by different letters are significantly different at $P = 0.05$ (Tukey test), N = 14.

## 4. Discussion

It had previously been observed that the concentration of inorganic constituents in the MS medium was suboptimal for cassava growth (Meyer and van Staden, 1986). Specific research on somatic embryogenesis yielded contradictory information in this regard and several researchers tested alternative media and/or MS medium at different strengths and with alternative nitrogen sources (Konan et al., 1994). Taylor et al. (1996) observed that half□strength MS medium was superior to full□strength MS medium for the induction of a friable embryogenic callus in cassava. The viability of meristems excised from expanded axillary buds of cassava on media containing half and quarter of the concentration of MS salts was also enhanced relative to that of buds on full□strength MS medium (Konan et al., 1997). Most of the studies on optimal MS salts have been on somatic embryogenesis. However, there are no reports on the effect of MS salts concentration on regeneration of plantlets from nodal explants. The current study was conducted to determine the optimal medium salt concentration for the regeneration of plantlets from nodal explants in cassava. In this study, full MS media was found to be superior to half and quarter MS media and this contradicts the report of Smith et al. (1986) who reported that regeneration of plantlets from cassava nodal explants was better on Half MS media. Probably, genotypic differences of cassava in the two studies accounted for this disparity and needs to be investigated.

Nodal explants are occasionally cultured on media supplemented with GA3 to increase the length of shoots during multiplication or prior to rooting (Moshkov et al., 2008). The most characteristic effects of GA3 on shoot growth are increased inter-node extension, increased leaf-growth and enhance apical dominance. The elongated shoots are then subdivided to serve as starting mother stock culture for another multiplication cycle. In the current study, incorporating GA3 in the media significantly increased the mean shoot length and full MS supplemented with40 μM/l $GA_3$ that gave the highest mean shoot length of 8.93 ± 2.67 mm. The results of this study contradict the work of Villaluz (2005) who reported that when used singly, GA3 did not elicit any growth response in cassava shoot apical meristem. It is hoped that the results of this study will enhance provision of quality cassava planting materials to farmers in Rwanda.

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