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Research Paper

Involvement of Plant Growth Regulators and Varieties in the Multiplication of Cassava Planting Materials: A case study of Rwanda

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) is widely grown in tropical and sub-tropical regions of the world primarily for its starch-rich roots and, in some cases, its nutritional foliage. The overarching goal of this paper was to generate a comprehensive information on the status of the involvement of plant growth regulators and varieties in the multiplication of cassava planting materials with particular reference to Rwanda. This information will provide a platform and further strengthen the knowledge base on the application of plant growth regulators (PGRs) by stakeholders in producing clean, high-quality cassava plantlets. We demonstrated the interactive effect between different phytohormones and varieties. The study showed the classical plant response to hormones with an increase in triggering an optimal value before inhibition sets in. Phytohormone, 1-Naphthaleneacetic acid (NAA), and benzyl adenine (BAP) were reported as being crucial in root and shoot growth, respectively:- with 10 mg/l being optimal doses. The outcome is crucial in recommending users the choice of hormone and specific varietal combinations and points to the fact that various PGRs concentration levels can be conveniently utilized successfully in the multiplication of different cassava varieties. Since the hormonal response has been shown to trigger differential reactions between different varieties, adopting such technologies needs to be considered with caution in consultation with researchers and policymakers to avoid adverse effects. In conclusion, the rapid multiplication of cassava planting materials depends on the PGRs concentration levels and their admixtures, the genetic makeup of the varieties evaluated, subculture type/ number, and shoot tip/ nodal segment used.

HIGHLIGHTS

- Cassava is mainly grown primarily for its starch-rich roots and, its nutritional foliage.
- Whereas 1-Naphthaleneacetic acid plays a key role in triggering root growth, benzyl adenine is crucial in enhancing shoot growth.
- For rapid multiplication of cassava planting materials, the optimal doses for these phytohormones ought to be 10 mg L^{-1}.

Keywords: Planting medium, supplementation, propagation, varieties, hormonal concentrations, root, shoot

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Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub that belongs to the family *Euphorbiaceae* (Sessou *et al.* 2020). It is a rain-fed crop grown widely in tropical and sub-tropical countries of Latin America, Africa, and Asia. It is a staple food for over 800 million people globally, providing a cheap source of carbohydrates (Burns *et al.* 2010). The crop has gained economic importance as a raw material for the industrial processing of foods, ethanol, and starch (Kilwinger *et al.* 2021). It is also used as a cash crop in various cassava-growing regions (Spencer and Ezedinma, 2017; Munganyinka *et al.* 2018). El-Sharkawy, 2004) stated that the yield potential of cassava under optimum conditions is about 90 tons of fresh roots per hectare, equivalent to 30 tons of cassava dry matter per hectare.

In Rwanda cassava ranks 3rd after banana and sweet potato as a staple crop, with an annual production of approximately 1,124,090 MT per annum (MINAGRI, 2011). The crop is grown mainly in the southern and eastern provinces of the country and occupies 22% of the area under cultivation. More than 700, 000 households (i.e. 42% of all family farms) grow cassava annually (MINAGRI, 2011). The crop grows well on soils with relatively low fertility and is also relatively drought and acid tolerant, making it an ideal crop for smallholder farmers in unfavorable upland environments (Malik *et al*. 2020; Howeler and Aye, 2014).

Conventionally, cassava can propagate through seeds and stem cuttings. However, cassava seeds are typically diverse because of the cross-pollination nature of the crop. Generally, any particular cassava clone is highly heterozygous (Ceballos *et al.* 2004). Seed viability, dormancy, irregular blooming patterns, and poor seed set are all characteristics that restrict the use of seeds as a viable source of propagation. As a result, stem cutting is the primary mode of propagation (Leihner, 2002). However, this approach has drawbacks such as a low rate of multiplication, ten cuttings per plant every year (1:10), which is difficult and time-consuming, sluggish and resulting in delayed dissemination of new better cultivars, bulky to transport, and insufficient planting supplies for large-scale plantations (Demeke *et al.* 2014). Furthermore, disease build-up throughout the vegetative cycle, high distribution costs, and poor storage quality of the planting material are some of the drawbacks of utilizing stem cutting as a propagation material in cassava (Escobar *et al.* 2006).

Another drawback is diseases such as Cassava Mosaic Disease (CMD) and Cassava Brown Streak Disease (CBSD), which are currently the most threatening biotic stresses to cassava production in East and Central Africa (Alicia *et al.* 2007; Hillocks and Thresh, 2000; Legg and Raya, 1998; Legg *et al.* 2001; Tumwegamire *et al.* 2018). The two diseases cause devastating effects on root quantity and quality, with field and storage losses ranging from 30% to 100% (Kawuki *et al.* 2016; Okonya *et al.* 2019; Patil *et al.* 2015). Both diseases spread via a whitefly vector (*Bemisia tabaci*) and the use and exchange of infected planting material (Legg et *al.* 2011). This implies that farmers' use of local susceptible varieties and recycling of stem cuttings from the previous crop can aggravate the impact of the diseases. Therefore, the introduction of resistant varieties and the availability of clean planting material is of high importance (Night *et al.* 2011) for successful and sustainable cassava production.

Moreover, the newly released cassava varieties, and the scarcity of high-quality and true-to-type planting material are among the main limitations for their widespread commercialization and increased production (Escobar *et al.* 2006). It is, therefore, imperative to consider new technologies for the rapid multiplication of disease-free planting material, which will be a significant step towards achieving adequate, true-to-type, high-quality cassava planting materials. Santana *et al.* (2009) acknowledged the plant tissue culture technique as a powerful tool for studying and solving fundamental and applied problems of cassava production. Moreover, Loyola-Vargas *et al.* (2006) described the plant tissue culture technique as a quicker and less space-consuming technology compared to conventional methods of preparing cassava cuttings. Similarly, Le *et al*. (2007) established that the tissue culture technique is one of the most realistic and efficient means of supplying large volumes of true-to-type clean planting materials of cassava within a limited period. This is also an advantage for producing large populations as required in mutation breeding. In addition, plant tissue culture has many applications, such as clonal propagation and somatic embryogenesis, germplasm exchange, embryo rescue, genetic transformation, etc. (Rao,

1996). This work aims to generate a comprehensive information on the status of the involvement of plant growth regulators and varieties in the multiplication of cassava planting materials, with specific reference to Rwanda as a representative of other developing countries.

Methodology

To acquire information relevant to this work, we used the Google Scholar search engine to Identify relevant peer-reviewed research articles published in high-impact journals.

A total of 58 articles were used that ranged from experimental, reviews, and policy papers. Keywords used in the Google Scholar search engine were Rwanda, Plant growth regulators (PGRs), cytokinins, Kinetin, benzyl adenine (BAP), Thidiazuron (TDZ), Zeatin, 1-Naphthaleneacetic acid (NAA), varietal differences, clean planting material, the government of Rwanda, Basal supplementation, Casava, Seedling regeneration, Murashige and Skoog, varietal interactions with phytohormones. The paper is focused broadly on various methodical strategies used in cassava to improve multiplicative rates using phytohormones in different varieties and plant species in Rwanda and other parts of the world.

Basal media supplementation with plant growth regulators on cassava seedlings regeneration

Growth regulators (PGRs), especially Cytokinins are among the most critical components that play a significant role in micro shoots regeneration (Abu-Romman *et al.* 2015; Garland and Stoltz, 1981; Huy *et al.* 2019; Lane, 1979). Specifically, the use of growth regulators for growth initiation from the meristem culture of different cassava varieties is recommended by Razdan (2005). Cytokinin could interact with other growth regulators to stimulate the vegetative growth of plants (Maxwell and Keiber 2004). This is because, in the plant physiology process, cytokinin influences cell division to broaden the area of the tissues and plantlet height (Davies 2004). Kinetin, benzyl adenine (BAP), Thidiazuron (TDZ), and Zeatin have been used in cassava micropropagation (Konan *et al.* 1997; Faye *et al.* 2015; Kabir *et al.* 2015; Opabode, 2017). However, the most commonly used cytokinin for inducing shoots in cassava is either BAP alone or in combination with NAA (Cacaï *et al.* 2012; Faye *et al.* 2015; Sesay *et al.* 2012) (Fig. 1). Besides, Auxins are essential factors involved in rooting because they promote the formation of adventitious roots in most species (De Klerk, 2002).

Use of plant growth regulators to enhance shoot multiplication

Several authors maintain that BAP is the best phytohormone for shoot initiation and shoot multiplication in cassava (Opabode *et al.* 2017; Pita *et al.* 2001; Onuoch and Onwubiku, 2007; Guohua, 1998; Trigiano and Gray, 2000; Roca, 1984). In support of this, Konan *et al.* (1997) observed the effect of BAP on the formation of multiple shoots from enlarged axillary buds of cassava cultivar TMS 30555, and the variable rates of shoot proliferation were observed 5-6 weeks after culture initiation. We have shown schematically how the mechanism works (Fig. 1).

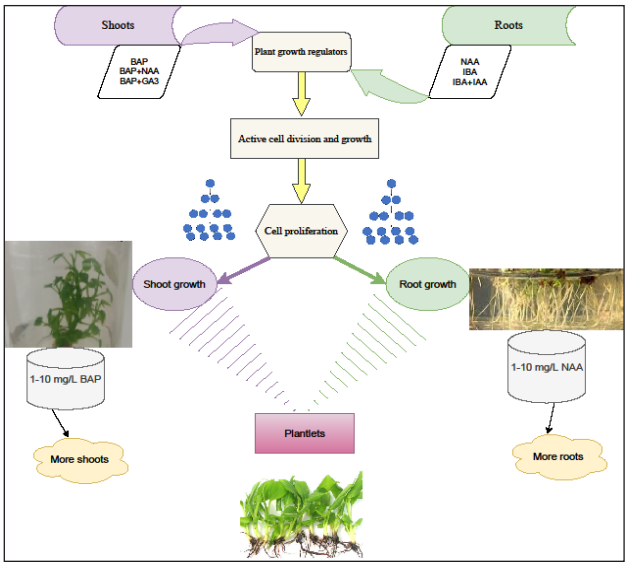

Fig. 1: Schematic representations of plant growth regulators on shoot and root growth for enhanced planting material multiplication. Different hormones have differential triggers on roots and shoots, but they work within a range with specific optimal values. In the diagram, the BAP is shown to have a better influence on shoot regeneration up to 10mg/l after which there is inhibition. A similar trend is shown for roots, though with NAA

The highest number of shoots per explant was obtained with BAP at 10 mg/1 BAP where the percentage of explants forming shoots was shown to be about seven times higher than with 15 mg/1 BAP (73% vs. 9%). On the contrary, Fan *et al.* (2011) reported that BAP 0-2.0 mg/L was effective on shoot regeneration. Furthermore, Konan *et al.* (1997)

Table 1: The response of cassava shoots cultured on Murashige and Skoog (MS) media supplemented with plant growth regulators

Cassava genotype	Country	Propagation media	Explant	Treatment that gave the best results	References
Agric-rouge, Atinwewe, Agblehoundo.	Kenya	MS	Nodes	10 uM BAP	Sessou *at al.* (2020)
Darul Hidayah, Malang-6 and Adira-4	Indonesia	MS	Auxiliary shoots	1 mg/L BAP + 0.1 mg/L TDZ	Sukmadjaja and Widhiastuti (2011)
92/0326, 95/0289, I-30572	Nigeria	MS	Stem	a) 0.01 mg/L NAA; 0.05 mg/L BAP b)0.02 mg/L NAA; 0.10 mg/L BAP	Mapayi *et al.* (2013)
MAUS 2, MAUS 4	Australia	MS and 2.5 uM IBA	Axillary buds	1.0pM BAP and 0.25 uM NAA	Smith *et al.* (1986)
4/72-NR: 44/72-NW.	Ethiopia	MS	Nodal buds	BAP and Kin each at 0.75 mg/L	Demeke *et al.* (2014)
Sree Padmanabha.	India	MS	Apical node	0.3 mg/L NAA	Shiji *et al.* (2014)
TMS 98/0379; TMS 98/0581	Nigeria	MS	Stem	0 mg/L BAP	Onuoch and Onwubiku, (2007)
Slicass 6, Slicass 11 and Cocoa	Sierra-leone	MS	Stem	1.0 mg/L NAA	Janatu *et al.* (2018)
		MS	Stem	0.1 mg/L BAP	Janatu *et al* (2018)
CM6740-7 cassava cultivar	Venezuela	MS; 2 g/L Hydro Agri´s fertilizer (12-11-18/3 (MgO-EDTA)	Stem	0.027 M NAA, 0.011 M IBA, 0.027 M IAA)	Santana *et al.* (2009)
Qulle and Kello	Ethiopia	MS	Stem	0.5 mg/L BAP; 1 mg/L GA_3; 0.01 mg/L NAA	Beyene *et al.* (2010)
Arara, Caturra, Cacau Vermelha and Roxinha.	Colorado do Oeste	MS	Shoot petiole	MS - BAP addition,	Menegazzo *et al.* (2019)
Cultivar TMS 30555, ipin Valenca, MBra 769, CMC 76, MCub 51, MCub 58, MMex 55, MPar 133, TMS 60444 Red, TMS 30395, TMS 50395, TMS 60142, TMS 83350, TMS 84537, TMS 90059, TMS 90853, and Mpira Red	—	MS	Nodes	10mg/L BAP	Konan *et al.* 1997

reported a maximum of 25 shoots per explant in a solid Murashige and Skoog (MS) medium with 10 mg/l BAP. Similarly, Sessou *et al.* (2020) showed that BAP at 10 µM contributed to the highest number of micro shoots/explant (3.60) though the response was cultivar and concentration-dependent. Although in an exciting, and contradicting report, Beyene *et al.* (2010) pointed out that increased supplementation of BAP concentration from 5 mg/L to 10 mg/L, as a multiplication medium, caused shoot dwarfing and resulted in abnormal morphological appearance of stems and leaves, which probably depended on variety.

Sessou *et al.* (2020) reported that BAP beyond 10 µM significantly reduced the number of micro shoots/explant and their lengths. They also observed that the multiple shoots regenerated from media supplemented with BAP were stunted compared to those obtained with kinetin (Table 1). This might be a result of the supra-optimal amount of the hormone. This response is in agreement with the results of Berrie (1984), Onuoch and Onwubiku (2007), who observed that high BAP concentrations had a negative effect on the plantlet's physiology, such that there was an inhibitory effect on shoot growth at high concentrations. Moreover, Shiji *et al.* (2014) observed that MS medium with NAA alone produced better results in terms of the response of

explants to shoot initiation, intermodal elongation/ shoot length, number of nodes, number of leaves and number of roots when compared to the other treatments.

Work by Beyene *et al.* (2010) revealed the highest number of shoots per explants (where 27 and 21 shoots for 'Qulle' and 'Kello' varieties, respectively) that were obtained in multiplication medium with 0.5 mg/L BAP in combination with 1 mg/L GA_3 and 0.01 mg/l NAA (Table 1). This might be due to the combined effect of the three growth regulators (Staden *et al.* 2008). Further, Konan *et al.* (2006), working with nodal explants of cassava with axillary meristems cultured on MS medium supplemented with 0.1 mg/1 NAA, 1 mg/1 BAP, and 0.1 mg/L GA_3, reported that a combination of these phytohormones was superior in producing multiple shoots. Smith *et al.* (1986) in their report from *in vitro* propagation of cassava using nodal culture, recommended the use of 1.0 mg/l BAP, supplemented with 0.25mg/L NAA-induced multiple-shoot formation (Table 1). In addition, Kabir *et al.* (2015) found that among the media components used, MS + 2.0 mg/l BAP + 0.1 mg/l NAA achieved the best for multiple shoot formation at 3rd subculture, in which 90% of explants produced multiple shoots. Consistently, the average number of shoots per explant was 6.30, the average length of the shoot was 10.30 ± 0.70 cm, and the average number of nodes per explant was 6.25 in the same medium. Furthermore, in their study, Kabir *et al.* (2015) revealed that BAP in combination with NAA performed comparatively better in terms of multiple shoot initiation, which suggests that the combination of growth regulators has a good impact on *in vitro* shoot proliferation of cassava. Similar results were observed by many authors using different concentrations and combinations of BAP + NAA in other plants, including sweet oranges (Roy and Kabir, 2006), apples (Roy and Kabir, 2006), cabbage (Munshi *et al.* (2007), sugarcane (Roy and Kabir, 2007). In addition, Alla *et al.* (2013) reported that the maximum number of performed shootlets per explants (5.67) was achieved on MS medium supplemented with 1.0 mg/L BAP plus 0.05 mg/L NAA (proliferation medium).

Murashige and Skoog medium has also been supplemented with other phytohormones such that Mapayi *et al.* (2013) reported that the best survival rate (100%) of cassava shoots was, generally, obtained in MS medium supplemented with a low concentration of NAA and BAP hormones and high concentration of sucrose (Table 1). Sukmadjaja and Widhiastuti (2011) reported that the highest number of shoots from three elite cassava cultivars were obtained on media supplemented with a combination of BAP and thidiazuron (TDZ) (Table 1). According to Lu (1993), the addition of TDZ in media containing BAP could increase the explant's ability to produce shoots. Medium supplemented with GA_3 only and in control recorded the lowest number of shoots per explant. This is because GA_3 concentration increases the shoot height rather than multiplying the number of shoots. Similar results were obtained by Acedo (2006). According to Moshkvo *et al.* (2008) this could be due to the physiological effect of the GA_3 hormone that causes stem elongation and inhibits the formation of adventitious root and shoot formation. Although Beyene *et al.* (2010) observed that combined supplementation of BAP and GA_3 resulted in shoots with very good morphological appearance (reasonable shoot height, stem thickness, and leaf structure in comparison with the other multiplication medium in combination), the effect might be related to the combined reaction of the two PGRs. In addition, Kane (2005) reported cytokinins, BAP/Kinetin (0.01-10 mg/L) as the most widely used and effective plant growth regulators for shoot multiplication. Further, Faye *et al.* (2015) found that kinetin gave better results than BAP in regenerating micro cassava shoots from nodal explants. Sessou *et al.* (2020) also observed that the multiple shoots regenerated from media supplemented with BAP were stunted compared to those obtained with kinetin. Kinetin has also been found to be superior to other cytokinins in *Tacca leontopetaloides* (Martin *et al.* 2012).

Use of plant growth regulators in root multiplication

In the work of Medina *et al.* (2007) it was apparent that 0.54 mM NAA was most effective in stimulating root formation. To support this argument, Demeke *et al.* (2014) used 0.5 mg/L NAA and reported the production of 6.14 roots within 4 weeks (Table 2), while Cacaï *et al.* (2012) used 0.1 mg/L NAA and reported the production of 5.2 roots. Furthermore, Fan *et al.* (2011) reported that NAA ranging from

Table 2: The response of cassava roots cultured on Murashige and Skoog (MS) media supplemented with plant growth regulators

Cassava genotype	Country	Propagation media	Explant	Treatment that gave the best results	References
92/0326, 95/0289, I-30572	Nigeria	MS	Stem	a) 0.01 mg/l NAA, 0.05 mg/l) BAP and b) 0.02 mg/l NAA; 0.10 mg/l BAP	Mapayi *et al.* (2013)
MAUS 2, 4, 7	Australia	MS and 2.5 Um IBA	Auxiliary bud	1.0pM BAP and 0.25 uM NAA	Smith *et al.* 1986
Sree Padmanabha.	India	MS	Apical node	0.1 mg/l NAA,	Shiji *et al.* 2014
Slicass 6, Slicass 11 and Cocoa	Sierra-leone	MS	Stem	1.0 mg/L (NAA)	Janatu *et al.* 2018

0-2.0 mg/ L was effective on root development. In addition, Kane (2005) reported that auxin NAA (0.01-10 mg/L) was the most widely used and effective plant growth regulator (PGR) for root induction. In consistence, Shiji *et al.* (2015) (Table 2) and Opabode (2017) observed that NAA was the best supplement for rooting in cassava.

There is more research on hormones and elite cassava varieties. Sessou *et al.* (2020) found that IBA performed better than NAA in rooting the micro shoots from the 3 elite cassava cultivars. Similar results were reported by Kabir *et al.* (2015), who concluded that cassava micro shoots rooted better in MS media supplemented with IBA compared to NAA and IAA. In the same breadth, the effectiveness of IBA for rooting over other auxins has also been reported by Naranjo and Fallas (2017) in cassava. Additionally, Alla *et al.* (2013) ascertained that MS medium supplemented with 2.0 mg/L IBA achieved the maximum number of root formations (10.2), with a 100% rooting percentage. Similar observations have also been made in many other *in vitro* cultured plants. For instance, Sadeghi *et al.* (2015) achieved 100% *in vitro* rooting of *Prunus empyrean* in MS medium with IBA, and Singh *et al.* (2016) reported IBA as the best auxin for rooting in *Santalum album*. Further, Acedo and Laban (2008) reported that IAA at 0.02 mg/L and IBA at 0.04 mg/L were the best for rooting short-maturing cassava genotypes, and 0.06 mg/L IBA was the best for long-maturing genotypes. Smith *et al.* (1986) also showed that the use of 2.5 mg/l of IBA effectively improves the root initiation of cassava plantlets. On the contrary, Beyene *et al.* (2010) found that half and full-strength MS at 0, 0.01, and 0.1 mg/L of IBA had less rooting frequency, and roots were long and fragile with very few secondary roots. Nevertheless, at high concentrations of IBA (0.5 and 1 mg/L IBA) roots become short and thick without secondary roots.

Furthermore, Sessou *et al.* (2020) observed that increasing the concentration of IBA from 10 to 20 µM significantly reduced the mean number of roots in plantlets derived from the media supplemented with the three cytokinins. Further, Mushiyimana *et al.* (2011), Yandia *et al.* (2018), and Faye *et al.* (2015) proved that MS medium without exogenous auxins is the best in cassava micro rooting. Rooting in a medium without growth regulators has been reported in *Yucca glauca* Bentz *et al.* (1988) and *Gentiana dinarica* Beck plant (Vinterhalter *et al.* 2012). A possible explanation could be that there is a high level of endogenous auxins.

Varieties and their interactive effect with growth regulators on the multiplication of different plantlets

Marked differences are observed among the plant varieties and their interaction with PGRs on the shoot and root rapid multiplication. A comparison of *Solanum tuberosum* varietal effect showed that Cardinal produced the highest number of shoots explant^{-1} (1.98) followed by Dheera (1.83), whereas the lowest number of shoots explant^{-1} (1.71) was obtained from Diamant (Hossain *et al.* 2013). Similarly, Nagib *et al.* (2003) reported that Cardinal was the best, and Diamant was also found to be more responsive than Multa and Lal Pakri. Comparing both studies, the Cardinal variety emerged to be the best. The result is also supported by other findings of Millar *et al.* (1987) where differential responses of different potato

varieties due to genetic makeup towards *in vitro* shoot multiplication and their development was reported. Combined effects of variety and plant growth regulator revealed that Cardinal gave the maximum number of shoots explant^{-1} (2.43) with 1.0 mg/L BAP followed by Granula with 1.5 mg/L BAP (2.40) (Hossain *et al.* 2013). Similar results were also obtained by Nagib *et al.* (2003), where Cardinal gave the maximum number of shoots explant^{-1} with 0.5 mg/L BAP than with 2.0 mg/L and without BAP.

Working with French beans under water-stressed conditions, Kalawa *et al.* (2018) demonstrated the interactive effects of growth regulars in alleviating drought stress and hence increasing yield, particularly compared to the non-stressed and also with control without phytohormones. Several researchers (Nduwimana *et al.* 2020; Ochieng *et al.* 2021; Gweyi-Onyango *et al*. 2009; Munene, 2017) working with different types of nitrogen in soils as well as in culture media were able to report varietal responses to different N forms, particularly nitrate-treated plants. In these cases, the nitrate acted through a phytohormonal transduction cascade by eliciting more root and shoot divisions and hence increased multiplicative rates and not necessarily playing a role of nutrient *per se*. Working on superior cassava clones, i.e. Darul Hidayah *et al.* (2011) revealed that the highest number of shoots were 4.93 Darul Hidayah, 4.20 Malang-6, and 7.20 Adira-4, although this was recorded from different MS medium supplementation that is 1 mg/L BAP+ 0.1 mg/L thidiazuron, 1 mg/L BAP, and 1 mg/L BAP + 0.1 mg/L thidiazuron respectively. Similarly, this was observed in root multiplication such that Darul Hidayah and Adira-4 showed an increase of both root number and length resulting from adding IAA to the nutrient media. However, no significant effect of IAA and NAA were obtained on the number and length of roots in Malang-6 variety. This difference could be attributed to naturally occurring endogenous phytohormones that differ among genotypes and are coupled with their response to the exogenous growth regulators added to the culture medium (Rahimi *et al.* 2022). Popular cultivars among farmers, namely Victoria, Kachpot 1, and Kinigi differed as influenced by a combination of GA_3 and NAA in shoot height (Nuwagira *et al.* 2015). The shoot height and the number of buds, roots, leaves, and nodes were significantly different for varieties and hormonal combinations (Awati *et al.* 2019). Furthermore, shoot multiplication of *Solanum tuberosum* L.Gudiene and Belete varieties differed as influenced by growth hormones. The combination of 1.5 mg/l BAP and 3.0 mg/LNAA proved best in Gudiene, whereas 1.0 mg/L BAP and 2.0 mg/l NAA produced more shoots in Belete (Hajare *et al.* 2021) (Fig. 1). Furthermore, Sakha *et al.* (2019) showed varietal differences in sweet potatoes' production and regeneration of planting materials.

CONCLUSION

By and large, the study showed that the multiplication of planting materials is dependent on the genotype, growth medium composition, its supplementation with individual PGRs and their mixtures, the genetic makeup of the varieties evaluated, subculture type/ number, and shoot tip/ nodal segment used. Moreso, it is 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. If used through consultation with scientists, government agencies, and policymakers, the technology can enormously contribute to the enhanced and sustainable production of clean cassava planting materials in a short period in Rwanda.

REFERENCES

Abu-Romman, S.M., Al-Hadid, K.A. and Arabiyyat, A.R. 2015. Kinetin is the most effective cytokinin on shoot multiplication from cucumber. *J. Agric. Sci.*, **7**(10): 159.

Acedo, V.Z. 2006. Improvement of *in vitro* techniques for rapid meristem development and mass propagation of Philippine cassava (*Manihot esculenta* Crantz). *J. Food, Agric. Environ.*, **4**(1): 220–224.

Acedo, V.Z. and Labana, C.U. 2008. Rapid propagation of released Philippine cassava varieties through tissue culture. *J. Root Crop*, **34**(2): 108–14.

Alicai, T., Omongo, C.A., Maruthi, M.N. *et al.* 2007. Re-emergence of cassava brown streak disease in Uganda. *Plant Disease*, **91**: 24–29.

Alla, N.A.A., Ragab, M.E., El-Miniawy, S.E.M. and Taha, H.S. 2013. *In vitro* studies on cassava plant micro propagation of cassava (*Manihot esculenta* Crantz). *J. Appl. Sci. Res.*, **9**(1): 811–820.

Awati, R., Bhattacharya, A. and Char, B. 2019. Rapid multiplication technique for production of high-quality seed potato (*Solanum tuberosum* L.) tubers. *J. Appl. Biol. Biotechnol.*, **7**(1): 1.

Bentz, S.E., Parliman, B.J., Talbott, H.J. and Ackerman, W.L. 1988. Factors affecting *in vitro* propagation of *Yucca glauca. Plant Cell Tissue Org. Cul.*, **14**(2): 111–20.

Berrie, A.M. 1984. Germination and growth regulators. In: Advanced plant physiology. Malcom, W.B. (ed.), English Language Book Society. Longman, pp. 987.

Beyene, D., Feyissa, T. and Bedada, G. 2010. Micro propagation of selected cassava (*Manihot esculenta* Crantz) varieties through meristem culture. *Ethiopian J. Biol Sci.*, **9**(2).

Burns, A., Gleadow, R., Cliff, J., Zacarias, A., Cavagnaro, T. 2010. Cassava the drought, war and famine crop in a changing world. *Sustainability*, **2**(11): 3572–607.

Cacaï, G.H.T., Ahanhanzo, C., Dangou, J.S., Houedjissin, S.S., Agbangla, C. 2012. Effets de différentes combinaisons hormonales Sur l'organogenèse *in vitro* de quelques cultivars locaux et variétés améliorées de *Manihot esculenta* Crantz (manioc-Euphorbiaceae) cultivées au Bénin. *Int. J. Bio Chem. Sci.*, **6**(4): 1593–607.

Ceballos, H., Iglesias, C. A., Perez, J.C. and Dixon, A.G. 2004. Cassava breeding: opportunities and challenges. *Plant Molec. Boil.*, **56**(4): 503–516.

Davies, P.J. 2004. The plant hormones: Their nature, occurrence and function In: Davies, P.J. (Editor) Plant Hormones Biosynthesis, Signal Transduction, Action. Kluwer Acad Press, pp. 1–15.

De Klerk, G.J. 2002. Rooting of micro cuttings; theory and practice. In Vitro Cell Development. *Bio. Plant.*, **38**(5): 415–22.

Demeke, Y., Tefera, W., Dechassa, N. and Abebie, B. 2014. Effects of plant growth regulators on *in vitro* cultured nodal explants of cassava (*Manihot esculenta* Crantz) clones. *Afr. J. Biotechnol.*, **13**(28).

El-Sharkawy, M.A. 2004. Cassava biology and physiology. *Plant Mol. Biol.*, **53**: 621–641.

Escobar, R.H., Andez, C.H., Larrahondo, N., Ospina, G., Restrepo, J., Noz, L.M. and Roca, W.M. 2006. Tissue culture for farmers: Participatory adaptation of low-input cassava propagation in Colombia. *Exp. Agric.*, **42**(1): 103–120.

Fan, M., Liu, Z., Zhou, L., Lin, T., Liu, Y. and Luo, L. 2011. Effects of plant growth regulators and saccharide on *in vitro* plant and tuberous root regeneration of cassava (*Manihot esculenta* Crantz). *J. Plant Growth Regul.* 3030303030: 11–19.

Faye, A., Sagna, M., Kane, P.M.D. and Sane, D. 2015. Effects of different hormones on organogenesis *in vitro* of some varieties of cassava (*Manihot esculenta* Crantz) grown in Senegal. *African J. Plant Sci.*, **9**(8): 305–12.

Garland, P. and Stoltz, L.P. 1981. Micro propagation of *Pissardi plum*. *Ann Bot.*, **48**(3): 387–9.

George, E.F. and Sherrington, P.D. 1984. Plant Propagation by Tissue Culture. Handbook and Directory of Commercial Laboratories. Exergetics Ltd., Eversely, UK.

Guohua, M.A. 1998. Effects of Cytokinins and Auxins on Cassava Shoot Organogenesis and Somatic Embryogenesis from Somatic Embryo Explants, *Plant Cell Tissue and Organ Culture*, **54**: 1–7.

Gweyi-Onyango, J.P., Neumann, G. and Roemheld, V. 2009. Effects of different forms of Nitrogen on relative growth rate and growth components of tomato (*Lycopersicon esculentum Mill.*). *Afr. J. Hort. Sci.*, **2**: 43–55.

Hajare, S.T., Chauhan, N.M. and Kassa, G. 2021. Effect of growth regulators on *in vitro* micropropagation of potato (*Solanum tuberosum* L.) Gudiene and Belete varieties from Ethiopia. *The Sci. World J.*, 2021: 5928769.

Hillocks, R.J. and Thresh, J.M. 2000. Cassava mosaic and cassava brown streak virus diseases in Africa: a comparative guide to symptoms and aetiologies. Roots **7** *Special Issue*: 3–8.

Hossain, M.A., Nasiruddin, K.M. and Kawochar, M.A. 2013. Effect of 6-benzyl aminopurine (BAP) on meristem culture for virus free seed production of some popular potato varieties in Bangladesh. *Afr. J. Biotechnol.*, **3**: *12*(18).

Howeler, R.H. and Aye, T.M. 2014. Sustainable Management of Cassava in Asia—From Research to Practice; International Center for Tropical Agriculture (CIAT), the Nippon Foundation: Hanoi, Vietnam, pp. 168.

Huy, N.P., Luan, V.Q., Tung, H.T., Hien, V.T., Ngan, H.T.M., Duy, P.N. *et al.* 2019. *In vitro* polyploid induction of *Paphiopedilum villosum* using colchicine. *Sci. Hort.*, **252**: 283–90.

Janatu, V.S., Nicole, G.G.Y., Joseeph, S.K. and Dan, D.Q. 2018. Development of *in vitro* propagation protocol for some recalcitrant cassava (*Manihot esculenta* Crantz) genotypes in Sierra Leone. *Afr. J. Biotechnol.*, **17**(18): 606–613.

Kabir, M.H., Mamun, A., N.K., Roy, P.K., Islam, M.R., Jahan, M.T. and Talukder, S.U. (2015). *In vitro* propagation of cassava (*Manihot esculenta* Crantz). *Nuclear Science and Applications*, **24**(1-2): 23–28.

Kalawa, I.L., Wekha, N.W., Korir, N.K. and Gweyi-Onyango, J. 2018. Interaction Effect of Growth Regulators and Irrigation Schedules on Growth and Yield of French Beans in Kiambu County, Kenya. *Asian J. Res Crop Sci.*, **1**(1): 1–8.

Kane, M.E. 2005. Shoot culture procedures. In: Plant Development and Biotechnology Trigiano, R.N. and Gray, D.J. (Eds.). CRC Press, Boca Raton; pp. 145–157.

Kawuki, S.R., Kaweesi, T., Esuma, W. *et al.* 2016. Eleven years of breeding efforts to combat cassava brown streak disease. *Breed. Sci.*, **66**(4): 560–571.

Kilwinger, F., Mugambi, S., Manners, R., Schut, M., Tumwegamire, S., Nduwumuremyi, A. and Almekinders, C. 2021. Characterizing cassava farmer typologies and their seed sourcing practices to explore opportunities for economically sustainable seed business models in Rwanda. *Outlook Agric.*, **50**(4): 441–454.

Konan, N.K., Sangwan, R.S. and Sangwan-Norreel, B.S. 2006. Efficient In Vitro Shoot regeneration Systems in Cassava (*Manihot esculenta* Crantz). *Plant breeding.*, **113**(3): 227–236.

Konan, N.K., Schopke, C., Carcamo, R., Beachy, R.N. and Fauquet, C. 1997. An efficient mass propagation system for cassava (*Manihot esculenta* Crantz) based on nodal

explants and axillary bud-derived meristems. *Plant Cell Reports*., **16**: 444–449.

Lane, D.W. 1979. *In vitro* propagation of Spirea bumalda and *Prunus cistena* from shoot apices. *Can J. Plant Sci.,* **59**(4): 1025–9.

Le, B.V., Anh, B.L., Soytong, K., Danh, N.D. and Anh, Hong, L,T. 2007. Plant regeneration of cassava (*Manihot esculenta* Crantz) plants. *J. Agric. Technol*., **3**(1): 121–127.

Legg, J. P., Jeremiah, S.C., Obiero, H.M. *et al.* 2011. Comparing the regional epidemiology of the cassava mosaic and cassava brown streak virus pandemics in Africa. *Virus Res.,* **159**(2): 161–170.

Legg, J.P. and Raya, M.D. 1998. Survey of virus diseases in Tanzania. *Int. J. Pest Manag*., **44**: 17–23.

Legg, J.P. Okao-Okuja, G. Mayala, R. *et al.* 2001. Spread into Rwanda of the severe cassava mosaic virus disease pandemic and the associated Uganda variant of East African cassava mosaic virus (EACMV-Ug). *Plant Pathol*., **50**(6): 796.

Leihner, D. 2002. Agronomy and cropping systems. Cassava: Biology, production and utilization, pp. 91–113.

Loyola-Vargas, V.M., Vázques-Flota, F. 2006. Plant cell culture protocols. In: Loyola-Vargas, V. M. and Vázquez-Flota, F. (Eds). 2nd Methods in Molecular Biology 318. Humana Press Inc., New Jersey, pp. 3–8.

Lu, C.Y. 1993. The use of thidiazuron in tissue culture. *Cell Dev. Biol*., **29**: 92-96.

Malik, A.I., Kongsil, P., Nguyễn, V.A., Ou, W., Sholihin, S.P., Sheela, M., López-Lavalle, L.A.B., Utsumi, Y. and Lu, C. 2020. Cassava breeding and agronomy in Asia: 50 years of history and future directions. *Breed. Sci*., **70**: 145–166.

Mapayi, E.F. Ojo, D.K., Oduwaye, O.A. and Porbeni, J.B.O. 2013. Optimization of *in-vitro* propagation of cassava (*Manihot esculenta* Crantz) genotypes. *J. Agr Sci.,* **5**(3): 261.

Martin, A.F., Ermayanti, T.M., Hapsari, B.W. and Rantau, D.E. 2012. Rapid micro propagation of *Tacca leontopetaloides* (L.) Kuntze: The 5th Indonesia Biotechnology Conference; Hal., pp. 240–551.

Maxwell, B., and Kieber, J. 2004. Cytokinin signal transduction. *In:* Davies PJ, ed. Plant Hormones. Biosynthesis, Signal Transduction, Action. Dordrecht, The Netherlands: Kluwer Academic Publishers, pp. 321–49.

Medina, R.D., Faloci, M.M., Gonzalez, A.M. and Mroginski, L.A. 2007. *In vitro* cultured primary roots derived from stem segments of cassava (*Manihot esculenta*) can behave like storage organs. *Ann Bot*., **99**(3): 409–23.

Menegazzo, R.F., Rickli, M.E., Menegazzo, A.W., Lopes, A.D., Manfio, C.E .Koefender, J. and Koefender, J. 2019. *In vitro* multiplication of cassava varieties. *Arquivos de Ciências Veterinárias e Zoologia da UNIPAR*; **22**(4): 101–107.

Millar, P.R., Stuchbury, L.T. and Bevan, M.W. 1987. The use of plant growth regulators in micropropagation of slow-growing potato cultivars. *Potato Res*., **28**: 479–486.

MINAGRI – Rwandan Ministry of Agriculture and Animal Resources (2011(MINAGRI). Annual Report for Year 2010/2011. 2011.

Moshkov, I.E., Novikova, G.V., Hall, M.A. and George, E.F. 2008. Plant Growth Regulators III: Gibberellins, Ethylene, Abscisic Acid, their Analogues and Inhibitors; Miscellaneous Compounds. In: Plant Propagation by Tissue Culture 3rd ed. (George, E. F., Hall, M. A. and De Klerk, G. eds.).; pp 227–281. Springer.

Munene, R., Changamu, E., Korir, N. and Gweyi-Onyango, J.P. 2017. Differential Impact of Nitrogen Forms on Selected Phytochemicals and Oxalate Contents in Three Vegetable Amaranth Varieties in Kenya. *Annals, Food Sci Technol.,* **18**(2): 238–256.

Munganyinka, E Ateka, EM Kihurani, AW Kanyange, M.C. and Tairo, F. 2018. Cassava brown streak disease in Rwanda, the associated viruses and disease phenotypes. *Plant Pathol*., **67**: 377–387.

Munshi, M.K., Roy, P.K., Kabir, M.H. and Ahmed, G. 2007. *In vitro* Regeneration of Cabbage (*Brassica oleracea* L. var. Capitata) through Hypocotyl and Cotyledon Culture, *Plant Tissue Cult. Biotechnol.,* **17**(2): 131–136.

Mushiyimana, I Hakizimana, E Gashaka, G Sallah, PYK Kalisa, S Gatunzi, F *et al.,* Micro-propagation of disease resistant cassava variety in Rwanda. *Rwanda J.,* **24**: 49–57.

Nagib, A Hossain, SA Alam, MF Hossain, M.M., Islam, R. and Sultana, R.S. 2003. Virus free potato tuber seed production through meristem culture in tropical Asia. *Asian J. Plant Sci*., **2**(8): 616–622.

Naranjo, C. and Fallas, E. 2017. Ex vitro establishment and macro propagation of cassava (*Manihot esculenta*) to obtain disease-free rooted plants. VII Int Symp Prod *Establishment Micro propagated Plants,* **12**: 217–20.

Nduwimana, D., Mochoge, B., Danga, B., Masso, C., Maitra, S. and Gitari, H. 2020. Optimizing nitrogen use efficiency and maize yield under varying fertilizer rates in Kenya. *Int. J. Biores Sci*., **7**(2): 63–73.

Night, G., Asiimwe, P., Gashaka, G. *et al.* 2011. Occurrence and distribution of cassava pests and diseases in Rwanda. *Agric., Ecosys. Environ.,* **140**(3–4): 492–497.

Nuwagira, F., Mukasa, S.B., Wagoire, W.W., Namugga, P., Kashaija, I.N. and Barekye, A. 2015. Determination of hormonal combination for increased multiplication of tissue culture potato plantlets. *Uganda Journal of Agricultural Sciences*, **16**(1): 129–137.

Ochieng', I.O., Gitari, H.I., Mochoge, B., Esmaeil, Rezaei-Chiyaneh, E.R. and Gweyi-Onyango, J.P. 2021. Optimizing Maize Yield, Nitrogen Efficacy and Grain Protein Content under Different N Forms and Rate. *J. Soil Sci. Plant Nut.,* **21**(3): 1867–1880.

Okonya, S.J., Ocimati, W., Nduwayezu, A. *et al.* 2019. Farmer reported pest and disease impacts on root, tuber, and banana crops and livelihoods in Rwanda and Burundi. *Sustainability*, **11**: 1592.

Onuoch, C.I. and Onwubiku, N.I.C. 2007. Micropropagation of Cassava (*Manihot Esculantum* Crantz.) Using Different Concentrations of Benzyaminopurin, *J. Eng. Appl Sci.,* **2**: 1229– 1231.

Opabode, J.T. 2017. Enhanced mass regeneration of pro-vitamin a cassava (*Manihot esculenta* Crantz) varieties through multiple shoot induction from enlarged axillary buds. *Bio Technologia.,* **98**(4): 305–14.

Patil, L. B., Legg, P.J., Kanju, E. *et al.* 2015. Cassava brown streak disease: A threat to food security. *Afr. J. General Virol*., **96**: 956–968.

Pita, J.S., Fondong, V.N., Sanggare, A., Kokora, R.N.N.N. and Fauquet, C.M. 2001.Genomic and Biological Diversity of The African Cassava Gemini viruses, *Euphytica*., **120**: 115– 125.

Rahimi, A., Mohammadi, M.M., Moghadam, S.S., Heydarzadeh, S. and Gitari, H. 2022. Effects of stress modifier biostimulants on vegetative growth, nutrients and antioxidants contents of garden thyme (*Thymus vulgaris* L.) under water stress. *J. Plant Growth Reg*., **41**: 2059–2072

Rasool, R., Kamili, A.N., Ganai, B.A. and Akbar, S. 2009. Effect of BAP and NAA on shoot regeneration in Prunella vulgaris. *J. Nat. Sci. Math.,* **3**(1).

Razdon, M.K. 2005. Introduction to plant tissue culture (2th ed.). New Delhi: Oxford and IBH.

Roca, W.M. 1984. Cassava, In Handbook of Plant Cell Culture, (eds. Sharp WR, Evans DA, Ammirato P and Yamada Y), New York, MacMillan, *Crop Species,* **2**: 269–301.

Roy, P.K. and Kabir, M.H. 2007. *In vitro* mass propagation of Sugarcane (*Saccharum officinarum* L.) var. Isd 32 Through Shoot Tips and Folded Leaves Culture, *Biotechnol.,* **6**(4): 588–592.

Roy, P.K. and Kabir, M.H. 2006. *In vitro* Propagation of Sweet Orange (*Citrus Sinensis*) Through Plumule and Cotyledon Culture, *Bangladesh J. Life Sci.,* **18**(2): 107– 112 .

Roy, P.K. and Kabir, M.H. 2007. Micro propagation of Apple, Malus Domestica Borkh. Through *In vitro* Culture, Bangladesh *J. Life Sci*., **19**(1): 107–113.

Sadeghi, F., Yadollahi. A., Kermani, M.J. and Eftekhari, M. 2015. Optimizing culture media for *in vitro* proliferation and rooting of tetra (*Prunus empyrean* 3) rootstock. *J. Gen. Eng. Biot.,* **13**(1): 9–23.

Sakha, M.A., Jefwa, J. and Gweyi-Onyango. J.P. 2019. Effects of Arbuscular Mycorrhizal Fungal Inoculation on Growth and Yield of Two Sweet Potato Varieties. *J. Agric. Ecol. Res. Int.,* **18**(3): 1–8.

Santana, M.A., Romay, G., Matehus, J., Vicente-Villardon, J.L. and Demey, J.R.A. 2009. simple and low cost strategy for micro propagation of cassava (*Manihot esculenta* Crantz). *Afr. J. Biotechnol.,* **8**(16): 3789–3897.

Sesay, J.V., Yamba, N.G.G., Sherman-Kamara, J. and Quee, D.D. 2018. Development of *in vitro* propagation protocol for some recalcitrant cassava (*Manihot esculenta* Crantz) genotypes in Sierra Leone. *Afr. J. Biot.,* **7**(18): 606–13.

Sessou, A.F., Kahia, J.W., Houngue, J.A., Ateka, E.M., Dadjo, C. and Ahanhanzo, C. 2020. *In vitro* propagation of three mosaic disease resistant cassava cultivars. *BMC Biotechnol*, **20**(1): 1–13.

Shiji, R., George, J., Sunitha, S. and Muthuraj, R. 2014. Micro propagation for rapid multiplication of planting material in cassava (*Manihot esculenta* Crantz). *J. Root Crop*., **40**(1): 23–30.

Singh, C.K., Raj, S.R., Jaiswal, P.S., Patil, V.R., Punwar, B.S., Chavda, J.C. *et al.* 2016. Effect of plant growth regulators on *in vitro* plant regeneration of sandalwood (*Santalum album* L.) via organogenesis. *Agrofor Syst.,* **90**(2): 281–8.

Smith, M.K. Biggs, B.J. and Scott, K.J. 1986. *In vitro* propagation of cassava (*Manihot esculenta* Crantz). *Plant Cell, Tiss. and Organ Cultu.,* **6**(3): 221-228.

Spencer, D.S.C. and Ezedinma, C. 2017. Cassava Cultivation in Sub-Saharan Africa. Cambridge: Burleigh Dodds Science Publishing Limited, pp. 123–148.

Staden, J.V., Zazimalova, E. and George, E.F. 2008. Plant Growth Regulators II: Cytokinins, their Analogues and Antagonists. *In:* Plant Propagation by Tissue Culture 3rd ed. (George, E.F., Hall, M.A. and De Klerk, G. (eds.); pp 205–226. Springer.

Sukmadjaja, D. and Widhiastuti, H. 2011. Effects of plant growth regulators on shoot multiplication and root induction of cassava varieties culture *in vitro*. *Southeast Asian J. Trop. Biol.,* **18**(1): 50–60.

Trigiano, R.N. and Gray, D.J. 2000. Plant Tissue Culture Concepts and Laboratory Exercises, CRC Press, Washington, USA, pp. 1, 454.

Tumwegamire, S., Kanju, E., Legg, J. *et al.* 2018. Exchanging and managing in-vitro elite germplasm to combat cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) in Eastern and Southern Africa. *Food Security,* **10**(2): 351–368.

Vinterhalter, B., Milošević, D.K., Janković, T., Milojević, J. and Vinterhalter, D. 2012. *In vitro* propagation of *Gentiana dinarica* Beck. *Cen. Eur. J. Biol.,* 7(4): 690–7.

Yandia, S.P., Gandonou, C.B., Silla, S., Zinga, I. and Toukourou, F. 2018. Response of four cultivars of cassava (*Manihot esculenta* Crantz) plantlets free of cassava mosaic virus to micro propagation in different media. *Afr. J. Biot.,* **17**(1): 9–16.