Journal of Bio-Science
https://doi.org/10.3329/jbs.v33i2.87444
pISSN: 1023-8654 | eISSN: 2408-8595
Volume 33 | Issue 02 2025

RESEACH ARTICLE

In Vitro Mass Propagation Systems of Cassava (*Manihot esculenta* Crantz) in Bangladesh

Mst. Mahbuba Aktar, Md. Abu Sayem Azad and S. M. Shahinul Islam*

Plant Biotechnology and Genetic Engineering Lab., Institute of Biological Sciences, University of Rajshahi-6205, Bangladesh.

***Correspondence:**
Email: shahinul68@gmail.com

How to Cite the Article:
Aktar, M. M., Azad, M. A. S. and Islam, S. M. S. (2025). *In Vitro* Mass Propagation Systems of Cassava (*Manihot esculenta* Crantz) in BangladesH. Journal of Bio-Science 33(2): 67-76.

Peer Review Process:
The Journal abides by a double-blind peer review process such that the journal does not disclose the identity of the reviewer(s) to the author(s) and does not disclose the identity of the author(s) to the reviewer(s).

Abstract

Cassava, a key tropical crop for food and starch, faces constraints in genetic improvement due to stem cutting propagation. The present study aimed to develop an efficient *in vitro* micropropagation protocol for cassava through callus culture for the production of disease-free planting materials derived from nodal segments. Under this study, nodal explants were cultured on Murashige and Skoog (MS) medium supplemented with various concentrations of auxins 2,4-D, NAA and IAA for callus induction. The highest callus induction (95%) occurred on MS medium containing 15 mg/L 2,4-D. Here calli were transferred to regeneration medium (MS) that was supplemented with BAP, NAA and IAA. Callus proliferation and greenish colour comes in medium where BAP and Kin were used singly or in combination with 0.5 mg/L NAA, 0.1mg/L respectively. Maximum shoots (8.9 ± 0.5) per explant (average length 6.1 ± 0.4) cm) was found on MS medium that supplemented with 2.0 mg/L BAP and 0.5 mg/L NAA. Root induction occurred on half-strength MS medium supplemented with 2.5 mg/L NAA, resulting in 97.3% rooting, with the highest number (10.5) and length (6.2 cm). Regenerated plantlets were successfully acclimatized in pots containing 2:1:1 mixture of autoclaved soil, cocopeat and sand with high survival rate in the field condition.

Keywords: Acclimatization, Callus, Cassava, *In vitro* micropropagation, Nodal segments, Plant growth regulators.

Copyright: © 2025 by the author(s). This is an open-access article distributed under the terms of the **Creative Commons Attribution 4.0 International License (CC BY-4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

Received: 02 August 2025 | **Revised**: 12 October 2025 | **Accepted**: 06 November 2025 | **Published**: 31 December 2025

Introduction

Cassava (*Manihot esculenta* Crantz), a member of the Euphorbiaceae family, is the most significant of the genus *Manihot*, which comprises over 200 species. It serves as the primary carbohydrate source for more than 800 million people worldwide, particularly in tropical regions of Africa, Asia, and Latin America (Taye 2009, Rosenthal and Ort 2012). Globally, cassava is cultivated on 19.64 million hectares, producing 252.2 million tones with an average productivity of 12.8 t ha^{-1} (FAO 2011). In Africa, it ranks as the third most important staple after maize and rice (Otekunrin 2024). Cassava is drought tolerant plants and can easily survive in less fertile soil with uncertain rainfall patterns. Globally, the production reached 315 million tones in 2021, marking a 9% increase from 2017 (FAOSTAT 2011). Cassava roots are rich in carbohydrates, mainly starch, making up 80-90% of their dry weight, while its leaves are edible, providing protein, vitamins, and minerals. Its drought tolerance, adaptability to poor soils, and ability to remain unharvested for extended periods make it a vital crop for food security (FDRE 2002, Mulualem 2012). Despite its significance, it has historically received limited attention in national agricultural research, though countries like Ethiopia have recently prioritized its expansion in drought-prone regions (FDRE 2002).

Conventional propagation through stem cuttings is slow, with a multiplication ratio of ~1:10, limiting the rapid dissemination of improved varieties. Production faces challenges including low multiplication rates, susceptibility to pests and diseases, short shelf life of cuttings, and restricted availability of improved cultivars (Escobar et al. 2006). Rising demand for biofuel and industrial starch has further increased the need for high-quality planting material (Best and Henry 1994). Over the past five decades, tissue culture techniques have emerged as powerful tools for cassava propagation and improvement (Robert and Dennis 2000, Adane 2009, Santana et al. 2009) and since then, they have been recognized as powerful tools for studying and solving basic and applied problems of cassava production and productivity like many other crops (Robert and Dennis 2000, Adane 2009).

Micropropagation enables rapid clonal multiplication of disease-free planting material, while callus-mediated regeneration supports genetic transformation, somaclonal variation, and polyploid induction (Rahman and Islam 2024, Paul et al. 2013, Islam N et al. 2017). Numerous studies have demonstrated the effectiveness of *in vitro* techniques in cassava, including somatic embryogenesis and nodal culture (Konan et al. 1994, Sofiari et al. 1997, Sultana et al. 2019). Although somatic embryogenesis is widely explored, indirect organogenesis via callus culture remains less reported yet equally valuable for crop improvement (Konan et al. 2006, Medina et al. 2006). This technique is faster and requires less space than that required for conventional methods of preparing cuttings (Loyola-Vargas and Vazques-Flota 2006). Cassava is highly susceptible to viral diseases, especially cassava mosaic disease (CMD), which severely reduces tuber yield (Alabi et al. 2011). *In vitro* micropropagation and meristem culture provide a feasible approach for producing large volumes of disease-free planting material within a short period (Smith et al. 1986).

In this regard, available research findings (Le et al. 2007) have long proved the tissue culture techniques to be the only realistic and efficient means for supplying large volumes of fresh planting materials of any new high-value crop variety like cassava within short period. However, successful tissue multiplication requires the optimization of plant growth regulators (PGRs) concentrations in the MS medium that plays a crucial role in directing plant growth and development (Staden et al. 2008, IITA 2009, Rahman and Islam 2024). Beyond its role as a staple food, cassava provides nutritional, medicinal, and industrial value. Its leaves are rich in proteins, vitamins, and bioactive compounds with antioxidant, anti-inflammatory, and analgesic properties (Adeyemi et al. 2008, Boukhers et al. 2022). Cassava starch, being gluten-free, has wide applications in food processing, textiles, adhesives, pharmaceuticals, and bioethanol production. Growing global demand highlights cassava as a strategic, locally viable starch source in countries like Bangladesh. In this context, *in vitro* techniques such as micropropagation, callus culture, and somatic embryogenesis offer promising avenues for cassava improvement. While micropropagation enables rapid clonal multiplication of disease-free material, callus-mediated regeneration provides a platform for genetic transformation, somaclonal variation, and polyploid induction. However, optimizing PGRs concentrations helps to develop a reliable system for large-scale propagation, conservation, genetic improvement and further advance biotechnological works.

Materials and Methods

Healthy explants of cassava were collected from the research field of the Institute of Biological Sciences, University of Rajshahi. Immature leaves and nodal segments of 1-2 cm length containing axillary buds were excised from 2-month-old plants and used as explants. Explants were cultured on Murashige and Skoog (1962) medium supplemented with different concentrations of 2,4-D (5.0 - 20.0 mg/L), IAA (5.0-20.0 mg/L) and NAA (5.0-20.0 mg/L) for callus induction. The percentage of callus induction, callus morphology (friable, compact, or nodular), and fresh weight were recorded after three weeks. The medium was supplemented with 30 g/L sucrose and solidified with 7.0 g/L agar, and adjusted the pH from 5.6 - 5.8 before autoclaving at 121°C (15 psi) for 20 minutes. All the above operations were performed under aseptic conditions in laminar airflow cabinet. Cultures were incubated at 25 $\pm$ 2°C under for 16 h photoperiod with cool white fluorescent light.

For shoot regeneration, white friable calli were transferred to MS medium containing different concentrations of BAP (0.5-4.0 mg/L), NAA (0.5-4.0 mg/L) or Kin (0.1-1.0 mg/L). Regenerated shoots were further sub-cultured onto MS medium with BAP or NAA or Kin or IBA in different concentrations range for evaluation of shoot multiplication and elongation. Regeneration efficiency was evaluated after four weeks by recording the number of shoots per explant and average shoot length.

For rooting elongated micro-shoots measuring about 5-6 cm in length were excised from the culture tube and transferred to MS medium supplemented with different concentrations of IBA, NAA and IAA (0.5 - 3.0 mg/L) either individually or in combinations. Percentage of roots, number per explants and average root length were recorded after four weeks of culture initiation. Plantlets with well-developed roots were carefully removed from culture vessels, washed under running tap water to remove agar, and transplanted into earthen pots containing sterilized mixture of 2:1:1 autoclaved soil, cocopeat and sand. Plantlets were covered with transparent polyethylene bags to maintain humidity for 7-10 days and gradually acclimatized in a shade house. Survival percentage was recorded after four weeks.

The experiments were arranged in a completely randomized design (CRD). Each treatment consisted of 10 culture vessels, and all experiments were repeated three times. Data were analyzed using analysis of variance (ANOVA) in Microsoft Excel and treatment means were compared using the least significant difference (LSD) test at the 5% significance level ($p<0.05$). Values are presented as mean ± standard error (SE).

Results and Discussion

Callus induction

Callus induction was observed within 3-4 weeks of inoculating cassava nodal explants on MS medium supplemented with different concentrations of IBA, NAA, and 2,4-D (5.0-20.0 mg/L). Among all treatments, 2,4-D proven the most effective, with 15.0 mg/L inducing profuse, cream-white, friable callus suitable for regeneration (Table 1). In contrast, NAA (15.0 mg/L) produced a less amount (45–65%) of grayish, compact callus that became non-regenerable upon sub-culture. Lower concentrations of 2,4-D or combinations with MS basal medium resulted in poor or no callus formation. These findings suggest that cassava requires relatively high 2,4-D concentrations for effective callus induction (Table 1). The results align with previous reports (Khan et al. 1998, Khatri et al. 2002), confirming that elevated 2,4-D levels promote somatic embryogenesis and callus proliferation in cassava (Fig. 5B-C).

Table 1: Effect of 2,4-D, NAA and IBA on callus induction of cassava after culture initiation.

PGRs	Treat.	PGRs concentration (mg/L)	Types of explants	No. of explants showed callusing	% of explants with callus induction	Quality of calli	Time required (weeks)
2,4-D	T_1	5	IML	16	40	LC	4
			NS	22	55	CC	
	T_2	10	IML	24	60	CC	
			NS	28	70	CC	
	T_3	15	IML	30	75	CC	
			NS	38	95	IC	
	T_4	20	IML	20	50	LC	
			NS	24	60	CC	
NAA	T_5	5	IML	16	40	LC	3-4
			NS	18	45	LC	
	T_6	10	IML	22	55	CC	
			NS	24	60	CC	
	T_7	15	IML	18	45	LC	
			NS	26	65	CC	
	T_8	20	IML	24	60	CC	
			NS	16	40	LC	
IBA	T_9	5	IML	02	5	NC	3-4
			NS	04	10	NC	
	T_{10}	10	IML	08	20	NC	
			NS	10	25	NC	
	T_{11}	15	IML	06	15	NC	
			NS	08	20	NC	
	T_{12}	20	IML	12	30	LC	
			NS	14	35	LC	

NC = No callusing (0-25%), LC = Little callusing (26-50%), CC = Considerable callusing (51-75%), IC = Intensive callusing (76-100%), IML = Immature leaf, and NS = Nodal segment.

Callus weight is widely used as a quantitative parameter in plant tissue culture, including cassava, to evaluate the effectiveness of plant growth regulators (PGRs) for further regeneration and compare the responsiveness. It reflects the growth rate and biomass accumulation, indicates better cell proliferation and indicate more suitable hormonal balance. Callus growth (fresh weight) was measured and determine the best conditions for friable embryogenic callus (Sofiari et al. 1997) using 2, 4-D.

In this study, callus fresh weight varied depending on the type of auxin used. The highest callus weight was obtained with 2,4-D (15.0 mg/L), followed by NAA (15.0 mg/L), and the lowest with IBA (20.0 mg/L). The callus fresh weight was: NS = 467 mg 2,4-D: IML = 290 mg, NAA: NS = 355 mg, IML = 210 mg, IBA: NS = 180 mg, IML = 85 mg respectively (Fig. 1).

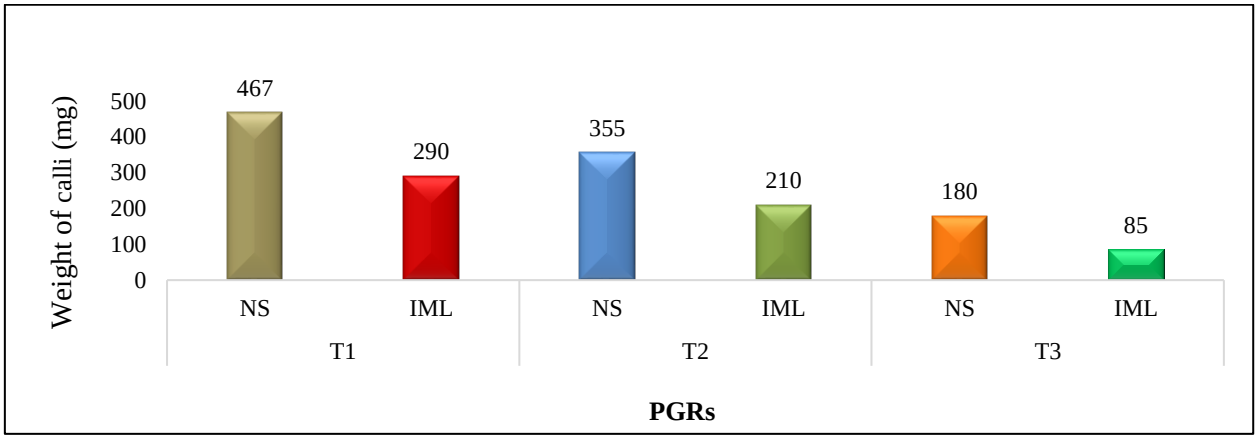

Fig. 1: Effects of 2,4-D, NAA and IBA in different concentrations on creating callus (weight). Here, T_1 = 2,4-D (15.0 mg/L), T_2 = NAA (15.0 mg/L), T_3 = IAA (20.0 mg/L), IML = Immature Leaf and NS = Nodal segment.

Shoot regeneration

Different concentrations and combinations of cytokinins (BAP, kinetin) and auxins (IBA, NAA) were evaluated for shoot regeneration from cassava callus. Shoot formation was strongly influenced by both the type and concentration of growth regulators. The highest regeneration efficiency (91.6%) was achieved on MS medium supplemented with BAP (2.0 mg/L) and NAA (0.5 mg/L), producing an average of 8.9 ± 0.53 shoots per explant with a mean shoot length of 6.1±0.48 cm (Fig. 2, Table 2). In this study, (BAP+NAA) combinedly promoted greater elongation (Fig. 3). The second-best response was obtained with BAP (2.0 mg/L) + IBA (0.5 mg/L), yielding 5.8 ± 0.39 shoots of 4.1±0.4 cm average length (Table 2). These findings agree with Islam et al. (1982), who also reported enhanced shoot formation with the BAP + NAA combination (Smith et al. 1986).

A high cytokinin-to-auxin ratio proved essential for efficient adventitious shoot differentiation, as single cytokinin treatments were less effective. Regeneration potential varied among genotypes and depended on hormonal composition, consistent with earlier reports (Maretzki and Nickell 1973, Maretzki 1987). Calli induced by different auxins exhibited variable regenerative capacities, supporting the synchrony between callus induction, proliferation, and regeneration noted in other species (Geetha and Padmanadhan 2001). Overall, BAP combined with a low concentration of NAA showed the most synergistic effect, where BAP promoted shoot induction and NAA supported elongation and differentiation, in agreement with previous findings in cassava and related crops (Konan et al. 1994, Sofiari et al. 1997).

Table 2: Effect of different concentrations and combinations of BAP, NAA, IBA and Kin on shoot proliferation and increasing of their lengths after culture initiation.

PGRs	PGRs concentration (mg/L)	% of explants produced shoots	No. of shoots/explants (Mean±SE)	Average length of shoots (cm) (Mean±SE)	Time required (weeks)
BAP	2.5	67.8	5.1±0.23	4.1±0.21	2-3
Kin	2.5	55.5	3.2±0.28	4.1±0.31	3-4
BAP + NAA	0.5+0.2	66.6	4.3±0.26	3.1±0.29	3-5
	1.0+0.2	70.8	4.9±0.16	4.4±0.27	
	2.0+0.5	91.6	8.9±0.53	6.1±0.48	
BAP + IBA	0.5+1.0	60.4	4.2±0.29	2.1±0.10	2-3
	1.0+0.1	39.5	4.5±0.27	3.6±0.37	
	2.0+0.5	75.1	5.8±0.39	4.1±0.40	

The production of plants from axillary buds has proved to be the most applicable and reliable method of *in vitro* propagation (Fig. 5D-E). According to George and Sherrington (1984) shoot tip/nodal segment culture depended on stimulating axillary shoot growth by the incorporation of growth regulators into the medium. Fan et al. (2011) reported that the cytokinin, BAP (0-2.0 mg/L) was effective on shoot regeneration and the auxin, NAA (0-2.0 mg /L) proved to be effective on root development in cassava. Konan et al. (2006) and Acedo (2008) revealed that shoot multiplication of many crops including cassava could be enhanced with a relatively higher concentration of cytokinins; while rooting is promoted by the use of auxins. Kane (2005) also reported cytokinins, BAP/Kinetin (0.01-10 mg/L) and auxin, NAA (0.01-10 mg/L) as the most widely used and effective plant growth regulators for shoot multiplication and root induction, respectively.

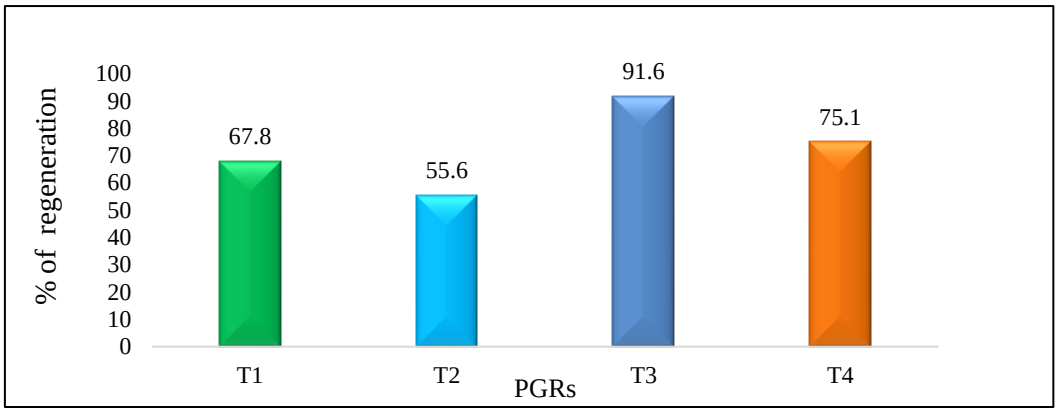

Fig. 2: Regeneration of plantlets of cassava form callus on MS medium with different concentrations of BAP and Kin (mg/L). T_1 = BAP (2.5), T_2 = Kin (2.5), T_3 = BAP (2.0) + NAA (0.5), T_4 = BAP (2.0) + IBA (0.5).

In this study, interestingly, while higher BAP + NAA concentrations enhanced shoot elongation, they did not significantly increase shoot number, reflecting the commonly observed trade-off between proliferation and elongation under elevated cytokinin levels (Mousavi et al. 2012, Erfani et al. 2017, Sessou et al. 2020).

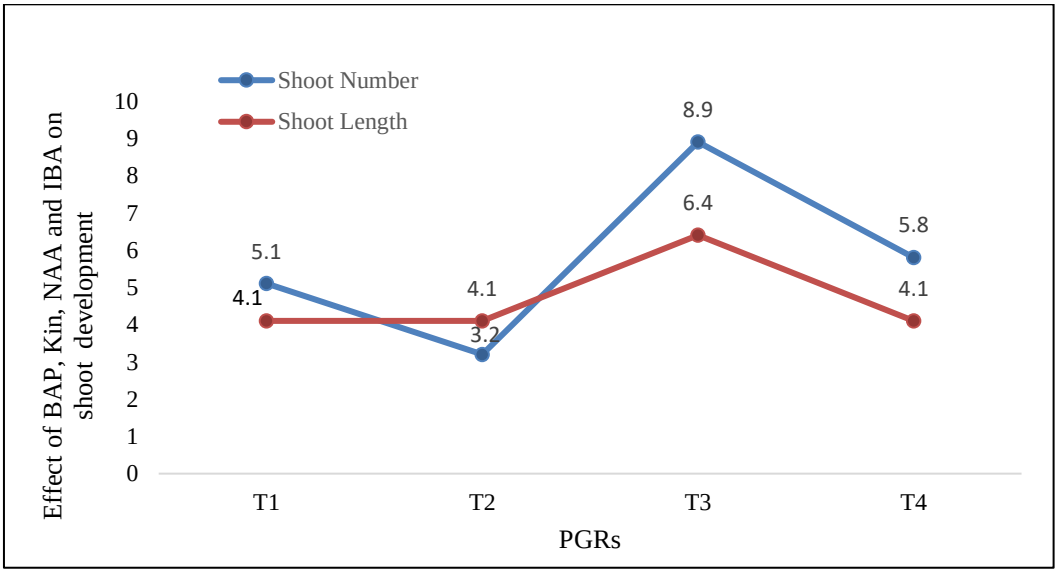

Fig. 3: Effect of BAP and kinetin (Kin) on shoot number and shoot length of cassava cultured on MS medium supplemented with different concentrations of plant growth regulators (PGRs) either singly or in combination (mg/L). Treatments: T_1 = BAP (2.5), T_2 = Kin (2.5), T_3 = BAP (2.0) + NAA (0.5) , T_4 = BAP (2.0) + IBA (0.5).

Root induction and development

Different types of auxins were used with different concentrations and combinations to regenerate adventitious roots. Among them NAA and IBA showed better response than IAA for profuse rooting. NAA+IBA combination showed positive result. Best rooting was observed in ½MS medium supplemented with 2.5 mg/l NAA (Table 3) and the highest number roots per micro shoots were 10.5 ±0.70, which take only 8-12 days for initiation of root primordial with average root length 6.2 ± 0.65 cm (Table 3). It was most effective with 2.5 mg/L NAA, confirming its superiority among auxins for cassava because it promoted root number and root length at same time (Fig. 4). According to Lal and Singh (1994) root can be easily induced on culture shoots by their transfer to another medium with or without NAA ,where optimal growth were observed with ½MS medium. Baksha et al. (2002) used 5.0 mg/l NAA for best rooting response in half strength of MS medium. Sabaz et al. (2008) used 1.0 mg/l IBA as the best root initiating growth hormone with highest number of roots. Baksha et al. (2002) also got rooting response at 0.1 - 0.5 mg/l IBA along with 0.5 - 2.0 mg/l BAP but these were of poor quality. Mamun et al. (2004) obtained best results of rooting on MS medium supplemented with auxins (NAA + IBA) 0.5 mg/l for each one. It was observed that 0.5 mg/l NAA+2.5 mg/l IBA showed second best feasible rooting response with 6.01±0.5 number of roots and 3.9± 0.04 cm of root length. The use of ½MS medium minimized salt stress and enhanced root elongation.

Table 3: Effect of different concentrations and combinations of NAA, IBA and IAA on root formation of the *in vitro* grown micro-shoots cultured on MS medium.

PGRs	PGRs concentration (mg/L)	% of explants produced roots	No. of roots/explants (Mean±SE)	Average length of roots (cm) (Mean±SE)	Time required (weeks)
NAA	2.5	97.3	10.5±0.70	6.2±0.65	2
IBA	2.5	72	4.8±1.5	3.7±0.94	3
IAA	2.5	30	2.5±0.16	2.2±0.47	2
NAA+IBA	0.5+2.5	78	6.01±1.08	3.9±0.47	2

The plantlets with well-developed shoot and roots after acclimatization were successfully transplanted in soil with 85% acclimatization of survivability potential (Fig. 5 A-H). The high acclimatization success demonstrates the robustness of the developed micropropagation protocol, providing a reliable means for year-round production of disease-free, true-to-type cassava planting materials.

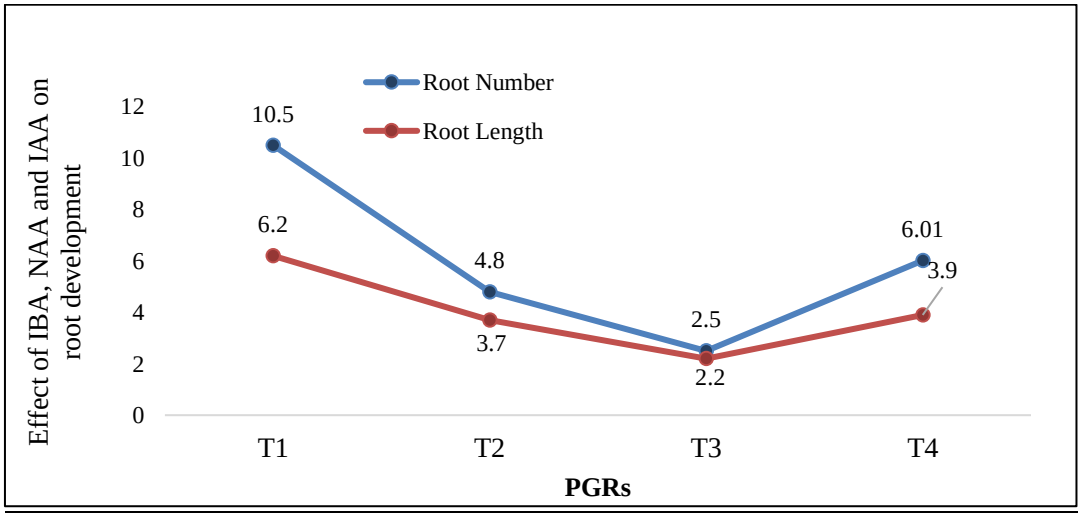

Fig. 4: Rooting on ½MS medium with IAA, IBA and NAA showing numbers of roots and its length using various concentration of PGRs. Treatments (mg/L): T_1 = NAA (2.5), T_2 = IBA (2.5), T_3 = IAA (2.5), and T_4 = NAA (0.5) + IBA (2.5).

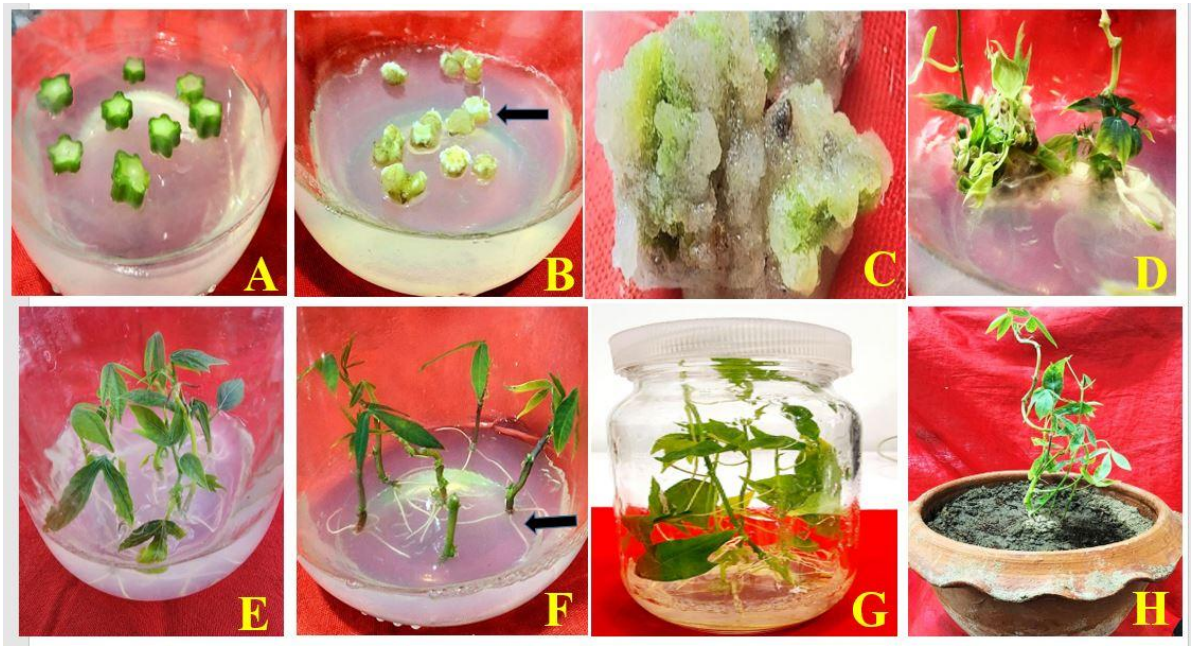

Fig. 5(A-H): *In vitro* regeneration of cassava. (A) Nodal segments inoculation (Day 1), (B) Calli initiation (7–10 days), (C) Calli proliferation (2–4 weeks), (D) Shoot induction (4–6 weeks), (E) Shoot multiplication and elongation (6–8 weeks), (F) Root induction (8–10 weeks), (G) Complete plantlet formation (10–12 weeks), (H) Transfer of plants to pot after acclimatization (12–14 weeks).

Conclusion

This study established a reproducible *in vitro* micropropagation protocol for cassava. Here we optimized the plant growth regulators for callus induction, shoot regeneration, rooting, and acclimatization. Among auxins, 15.0 mg/L 2,4-D was most effective for friable callus induction, while BAP outperformed NAA, kinetin and IBA in shoot regeneration. A combination of 2.0 mg/L BAP with 0.5 mg/L NAA achieved 91.6% regeneration with enhanced shoot proliferation. For rooting, half-strength MS medium with 2.5 mg/L IBA yielded vigorous roots and 97.3 % response. Regenerated plantlets acclimatized successfully in cocopeat-soil-sand with high survival rates, confirming the protocol's practical utility. Overall, this optimized system provides a reliable platform for large-scale clonal propagation of cassava, supporting rapid multiplication and year-round supply of disease-free planting material. It also offers a basis for advanced applications, including somatic embryogenesis, genetic transformation and trait improvement, contributing to cassava breeding, conservation, and food security.

Acknowledgements

The authors are very much grateful to the University Grant Commission (UGC) of Bangladesh for providing fellowship and for study leave permission to the Ministry of Education (MoE); Special allocation by the Ministry of Science and Technology and Plant Biotechnology and Genetic Engineering Lab., at the Institute of Biological Sciences, University of Rajshahi, for offering fellowship support and various resources for this research.

Conflict of interest: The authors declare no conflict of interest regarding the publication of this article.

Author's contribution: SMSI designed the experiment, supervised the study, and corrected the manuscript. MMA and MASA conducted experiments, collected data statistical analysis procedure and completed the draft of the article.

Funding sources: No funding.

Data availability: Data generated in the study are reported in the manuscript, and unprocessed data is with the corresponding author and available upon request.

References

Acedo VZ and Labana CU (2008). Rapid propagation of released Philippine cassava varieties through tissue culture. Journal of Root Crops 34 (34): 108-114.

Adane A (2009). A Review on agricultural biotechnology research and development in Ethiopia. African Journal of Biotechnology (8): 7196-7204.

Adeyemi OO, Yemitan OK and Afolabi L (2008). Inhibition of chemically induced inflammation and pain by orally and topically administered leaf extract of *Manihot esculenta* Crantz in rodents. Journal of Ethnopharmacology 119(1): 6-11.

Alabi OJ, Kumar P and Naidu RA (2011). Cassava mosaic disease: A curse to food security in sub-Saharan Africa doi:10.1094/APSnetFeature-2011-0701.

Baksha R, Alam R, Karim MZ, Paul SK, Hossain MA, Miah MAS and Rahman ABMM (2002). *In vitro* shoot tip culture of sugarcane (*Saccharum officinarum*) variety LSD28. Biotechnology 1(24): 67-72.

Best R and Henry G (1994). Cassava: towards the year 2000. *In*: International Network for Cassava Genetic Resources. International Crop Network Series No. 10, IPGRI, Rome, Italy.

Boukhers I, Boudard F, Morel S, Servent A, Portet K, Guzman C, Vitou M, Kongolo J, Michel A and Poucheret P (2022). Nutrition healthcare benefits and phytochemical properties of cassava *Manihot esculenta* (Crantz.) leaves sourced from three countries (Reunion, Guinea, and Costa Rica). Foods 11(14): 2027.

Erfani M, Miri SM and Imani A (2017). *In vitro* shoot proliferation and rooting of Garnem rootstock as influenced by basal media, plant growth regulators and carbon sources. Plant Cell Biotechnology and Molecular Biology 18(3&4):101-9.

Escobar RH, Andez CH, Larrahondo N, Ospina G, Restrepo J, Noz LM and Roca WM (2006). Tissue culture for farmers: Participatory adaptation of low-input cassava propagation in Colombia. Experimental Agriculture 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). Journal of Plant Growth Regulation (30): 11-19.

FAOSTAT (2011). Food and Agriculture Organization of the United Nations. faostat.fao.org/site/567/default.asp

FDRE (2002). Industrial Development Strategy. Micropropagation of cassava suspension culture derived from nodal explants. Journal of Plant Tissue Culture (27): 185-189.

Geetha S and Padmanadhan D (2001). Effect of hormones on direct somatic embryogenesis in sugarcane. Sugar Tech (3): 120-121.

George EF and Sherrington PD (1984). Plant propagation by tissue culture. Handbook and directory of commercial laboratories. Exegenetics Ltd., Basingstoke, Hants, England, pp. 444-447.

IITA (2009). Cassava Processing and Gene Banking Manual. (Online) Available from: www.iita.org. (Accessed on 19 February, 2010). Journal of Plant Diseases and Protection (88): 17-22.

Islam N, Islam T, Hossain MM, Bhattacharjee B, Hossain MM and Islam SMS (2017). Embryogenic callus induction and efficient plant regeneration in three varieties of soybean (*Glycine max*). Plant Tissue Cult. & Biotech. 27(1): 41-50.

Kane ME (2005). Shoot culture procedure in plant development and biotechnology. *In*: RN Trigiano and DJ Gray (eds): Chemical Rubber Company Press.

Khan IA, Khatri A, Ahmed M, Siddiqui SH, Nizamani GS, Khanzada MH, Dahar NA and Khan R(1998). *In vitro* mutagenesis in sugarcane. Pakistan Journal of Botany (30): 253-261.

Khatri A, Khan IA, Javed MA, Siddiqui MA, Khan MKR, Khanzada MH, Dahar NA and Khan R (2002). Studies on callusing and regeneration potential of indigenous and exotic sugarcane clones. Asian Journal of Plant Science (1): 41-43.

Konan NK, Sangwan RS and Sangwan NBS (2006). Efficient *in vitro* shoot regeneration system in cassava (*Manihot esculenta* Crantz). Journal of Plant Breeding (113): 227-236.

Konan NK, Schöpke C, Carcamo R, Beachy RN and Fauquet CM (1994). An efficient mass propagation system for cassava (*Manihot esculenta* Crantz) based on nodal explants and somatic embryogenesis. Plant Cell Reports (13): 312–316.

Lal, N and Singh HN (1994). Rapid clonal multiplication of sugarcane through tissue culture. Journal of Plant Tissue Culture (4): 1-7.

Le BV, Anh BL, Soytong K, Danh ND and Anh Hong LT (2007). Regeneration of cassava (*Manihot esculenta* Crantz) plants. Journal Agricultural Technology (3): 123-127.

Loyola-Vargas VM, Vazquez-Flota F (2006). Plant Cell Culture Protocols (2nd ed.). Methods in Molecular Biology (318): 3-8.

Mamun MA, Skidar MBH, Paul DK, Rehman MM and Islam M (2004). *In vitro* micropropagation of some important sugarcane varieties of Bangladesh. Asian Journal of Plant Sciences 3(6): 666-669.

Maretzki A (1987). Tissue culture: Its prospects and problems *In*: Sugarcane Improvement through breeding. (Ed.) Heinz DJ. Elsevier Science Publisher B.V, pp. 343-384.

Maretzki A and Nickell LG(1973). Formation of protoplasts from sugarcane cell suspensions and the regeneration of cell cultures from protoplasts. *In*: Protoplastes et Fusion de Cellules Somatiques Vegetales. International Colloquium of the Center for Natural Resource Studies (212): 51-63.

Medina RD, Faloci MM, Gonzalez AM and Mroginski LA (2006). *In vitro* cultured primary roots derived from stem segments of cassava (*Manihot escuenta*) can behave like storage organs. Annals of Botany Annals of Botany (209): 444-449.

Mousavi ES, Behbahani M, Hadavi E and Miri SM (2012). Callus induction and plant regeneration in lisianthus (*Eustoma grandiflorium*). Trakia Journal of Sciences 10(1): 22-25.

Mulualem, T and Ayenew B (2012). Cassava (*Mannihot esculenta* Cranz) varieties and harvesting stages influenced yield and yield related components. Journal of Natural Sciences Research 2(10): 122-8.

Murashige T and Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum (15): 473-497. doi: 10.1111/ j.1399-3054. 1962. tb08052.

Otekunrin OA (2024). Cassava (*Manihot esculenta* Crantz): A global scientific footprint-production, trade, and bibliometric insights. Discover Agriculture 2(1): 94.

Paul KK, Bari MA, Islam SMS and Debnath SC (2013). *In vitro* shoot regeneration in elephant foot yam, *Amorphophallus campanulatus* Blume. Plant Tissue Culture and Biotechnology 20(1): 55-61.

Rahman MS and Islam SMS (2024). Rapid multiplication by nodal segment and shoot tips through *in vitro* micropropagation of six mulberry (*Morus alba*) genotypes. Plant Tissue Culture and Biotechnology 34(2): 105-114.

Robert N and Dennis J (2000). Plant Tissue culture Concepts and Laboratory Exercises (2nd ed.). CRC press. p. 454.

Rosenthal DM and Ort DR (2012). Examining cassava's potential to enhance food security under climate change.Tropical Plant Biology (5): 30-38.doi: 10.1007/s12042 011-9086-1.

Sabaz AK, Rashid H, Fayyaz CM, Chaudhry Z and Afroz A (2008). Rapid micropropagation of three elite Sugarcane (*Saccharum officinarum* L.) varieties by shoot tip culture. African Journal of Biotechnology 7(13): 2174-2180.

Santana MA, Romay G, Matehus J, Villardón JL and Demey JR (2009) Simple and low-cost strategy for micropropagation of cassava *Manihot esculenta* (Crantz.). African Journal of Biotechnology 8(16): 3789-3897.

Sessou AF, Kahia JW, Houngue JA, Atek, EM, Dadjo C and Ahanhanzo C (2020). *In vitro* propagation of three mosaic disease resistant cassava cultivars. BMC Biotechnology 20(1): 51.

Smith MK, Biggs BJ and Scott KJ (1986). *In vitro* propagation of cassava (*Manihot esculenta* Crantz). Plant Cell, Tissue and Organ Culture 6(3): 221-228.

Sofiari E, Raemaker C, Kanju E, Danso K, Van Lammeren AM, Jacobsen E and Visser RGF (1997). Comparison of NAA and 2,4-D induced somatic embryogenesis in cassava. Plant Cell, Tissue and Organ Culture (50): 45-56.

Staden JV, Zazimalova *E* and George EF (2008). Plant Growth Regulators II: Cytokinin, their Analogues and Antagonists. *In*: George EE, Hall MA, and De Klerk G (3rd edition), pp. 205-226.

Sultana MS, Frazier TP, Millwood RJ, Lenaghan SC and Stewart Jr CN (2019). Development and validation of a novel and robust cell culture system in soybean (*Glycine max* (L.) Merr.) for promoter screening. Plant Cell Reports 38(10): 1329-1345.

Taye B (2009). Cassava, Africa's Food Security Crop. http://www.worldbank.org/htm/cigar/newsletter/Mar96/4cas2.htm.