

Micropropagation of Cassava (*Manihot esculenta* Crantz): Review

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ABSTRACT

Cassava is a vital crop to the food security of millions of people worldwide, particularly in Sub-Saharan Africa. Because the crop produced a reasonable yield on marginal soils, it could help relieve global hunger. As a result, increasing cassava output and its quality attributes are significant. However, the low multiplication rate of this main crop has resulted in the delayed dissemination of improved varieties among farmers. As a result, tissue culture techniques may be a feasible solution for overcoming these challenges. Cassava *in vitro* propagation had done using either the shoots multiplication technique or somatic embryogenesis. However, the shoot multiplication approach is preferable since it retains clonal fidelity. Plant regeneration via somatic embryogenesis or organogenesis entailed the use of numerous basal media containing various plant growth hormones. Several studies found that each type of cassava clone required a unique protocol to achieve optimal shoot initiation, shoot multiplication, root induction, and elongation. This review describes recent research on cassava micropropagation that makes use of a variety of experimental systems. While each of these systems focuses on a different aspect of technique, they can be significant in understanding the *in vitro* production of cassava planting material.

Keywords: Acclimatization, Cassava, Micropropagation.

1 Introduction

Cassava (*Manihot esculenta* Crantz) is a perennial dicotyledonous plant in the Euphorbiaceae family. It is grown across the world's lowland tropical areas at elevations as high as 2300 meters above sea level [1]. It is a root crop grown by over 800 million people in the tropics [2]. Nigeria, Thailand, and Brazil are the top three cassava producers accounting for more than 37% of world cassava production [3]. It is the fourth most significant food crop in developing nations, next to rice, wheat, and maize [4]. It is a vital source of food calories in Sub-Saharan Africa, and it plays an essential role as a food security crop [5]. Cassava is the second most important source of starch worldwide after maize [6]. The crop also contributes to the energy sector by competing with sugar cane in Southeast Asia; the crop has become the center of ethanol production [4].

Conventionally, cassava could 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 [7]. 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 [8]. 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 delayed dissemination of new better cultivars, bulky to transport, and insufficient planting supplies for large-scale plantations [9]. Furthermore, disease buildup 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 [10]. In general, the scarcity of high-quality, true-to-type planting material for newly released varieties is the main limitation to their widespread commercialization and increased



cassava yield [10]. As a result, new technology for the rapid multiplication of disease-free planting material has been a significant step toward adequate, true-to-type, high-quality cassava planting material. The tissue culture technique enables the continuous generation of high-quality planting material in large quantities, making it one of the best and most important commercial applications of plant biotechnology technology. Its quickness, ability to produce a high amount of planting material in small space by starting from small explants and ease of transport makes it more preferable than the conventional one.

Numerous studies on cassava tissue culture are conducted, beginning with the initial report by Kartha *et al.* [11], who reported regeneration of shoots from meristems of five cassava clones. Since then, several investigations on cassava employing nodal culture, meristem culture, somatic embryogenesis, and *in vitro* blooming had conducted. Different researchers published scientific articles on successful *in vitro* culture protocol for different cassava clones. Their results showed the existence of interaction of genotype with hormonal type and concentration.

2 Botany and Taxonomy of Cassava

Cassava (*Manihot esculenta* Cranz.) is a perennial shrub that originated in Latin America, both genetically and geographically. The genus *Manihot* belongs to the Euphorbiaceae family, containing about 100 species, with *Manihot esculenta* being a notable member [12]. Cassava domestication began in the Amazon, Brazil, between 5000 and 7000 years ago [1]. It was spread throughout the world by Europeans (Henry and Hershey, 2002). In the 16th century, Portuguese navigators shipped cassava from Brazil to the West Coast of Africa [13]. It is well adapted to areas with a long dry season and unpredictable rainfall, making it an excellent crop for drought-prone areas [14]. Cassava is classified based on its morphological characteristics, leaf shape and size, plant height, stem color, petiole length and color, inflorescence and flower color, storage root shape and color, earliness, and cyanogenic glycoside content [2]. Cassava storage roots cannot be used for propagation because the plant will not regenerate from root tissue; instead, mature, woody stems are harvested and cut into short "stakes" (15-30 cm) used for planting the next crop [1]. The botanical seed has rarely use for commercial propagation. Genetically, each cassava genotype is highly heterogeneous [15], and sexual seed propagation results in a broad and unpredictable diversity of phenotypes. This diversity is appealing to breeders but creates challenges for farmers [7]. Cassava grown from botanical seeds generally develops a primary tap root system as dicotyledonous species [1, 7]. The taproot that gives rise to adventitious roots grows vertically downward into the soil [1]. Seedlings are grown from stem cuttings, on the other hand, develop adventitious roots from the cutting's basal-cut surface or the bud under the soil [16].

3 Current Production Practices

Cassava is drought tolerant and can grow in a variety of agro-ecologies, including marginally fertile soils, making it the best candidate crop for achieving food security, particularly in Africa, where climate change is reducing cereal crop yields [17]. The crop is also highly suited to intercropping with many types of crops and, its time of harvest is flexible. It has a wide variety of food, feed, and industrial uses [4]. These attributes make cassava a significant crop in food production and income generation, benefitting the poor in the tropical regions of the world [18]. Despite these positive attributes, cassava production and productivity is low. Among different factors, farm size, pests, post-harvest root deterioration, a lack of improved planting material, and poor farming practices were the main cassava production constraints [19].

Conventional breeding is effective in a steady supply of improved cultivars, resulting in a significant increase in yield for staple crops. However, cassava breeding is difficult and time-consuming due to the crop's genetic makeup. Thus, new tools and technologies were required to improve the efficiency of conventional breeding, particularly in light of climate change, shrinking resources, land scarcity, and increased food demand. Biotechnology and new genomic approaches boost cassava production and productivity while improving smallholder farmers' livelihoods [20]. Micropropagation by tissue culture is the most promising area of applied and commercial research in plant biotechnology. However, the adoption of tissue culture

technology in the sub-Saharan region had limited due to a lack of investments in agricultural technology for plant production [21].

4 Trends of Biotechnology in Africa

According to the (FAO) report year 2019, 820 million people suffered from hunger and malnutrition [22]. Farm production would need to more than double by 2050 [23]. Thus, the world must look for a new way to improve productivity in line with improving the efficiency of supply chains and reducing wastage. Increasing agricultural productivity and tackling such problems need innovation and productivity-raising technologies [24]. Genetically engineered crops may be an option to increase agricultural productivity in developing countries. However, Africa took a long time to embrace GM technology. Burkina Faso, Sudan, and South Africa are the only African countries that have allowed commercial cultivation of genetically modified (GM) crops. Only South Africa grows a genetically modified food crop, while Burkina Faso has banned the cultivation of GM cotton following a massive failure for farmers and seed companies [25]. According to Brink *et al.* [26], biotechnology development in Africa should be accomplished in stages starts from a tissue culture phase to the development of transgenic capacity. Thus, plant tissue culture technology might develop the skill required to maintain and manage a biotechnology laboratory. However, Abebaw *et al.* [27] mentioned various constraints to plant tissue culture technology. The main impediment to technology is the combination of technical, administrative, and financial problems. This technique is required to improve cassava productivity in Sub-Saharan Africa and around the world. Adoption of the technology in seedling production will increase output while also improving quality. However, to maximize its use in cassava propagation, strategies for lowering the cost of plantlet production must be identified and implemented. It will allow resource-constrained farmers to obtain healthy planting materials at a reasonable price, increasing cassava production [28]. Different researchers showed effective cost-reducing protocols for micropropagation of cassava [29-32].

5 *In vitro* Propagation of Cassava

Conventional cassava propagation suffered from low multiplication rates, expensive labor, time-consuming, and the possible transfer of diseases by stem cuttings from generation to generation, limiting the effectiveness of this approach [33]. This prolonged propagation period creates a significant bottleneck in commercial propagation and breeding programs. As a result, conventional propagation methods could not meet the rising demand for newly released cultivars. As a result, the use of plant tissue culture techniques offers an alternate option for cassava multiplication and development [18]. *In vitro*, it is feasible to generate over a million clonally uniform and disease/pathogen-free plants in a short amount of time from single explants. Newly developed cultivars can also be offered for commercial planting in a short period of time [34].

Cassava *in vitro* propagation is a study topic owing to the crop's commercial importance, and it could accomplish using either the shoots multiplication method or somatic embryogenesis. The first stage entails the differentiation of culture cells or callus tissue into organs such as shoots and roots connected with its vascular system to that of the parental tissue. Depending on the hormonal composition of the medium and the physiological condition of the explants, it can occur straight from the explants. However, Somatic embryogenesis describes the process a bipolar structure develops from a single somatic cell via several phases indicative of zygotic embryo development [35]. Somatic embryogenesis had proposed as an alternative approach for cassava mass multiplication [36]. However, because the frequency of plant regeneration from cassava somatic embryos is low, this approach has not been used for large-scale multiplication. It is the most often employed regeneration method in cassava for the recovery of transgenic events [37, 38]. Thus; the shoot multiplication method is used as the easiest and safest method for cassava *in vitro* growth [39].

6 Explant Sources

The process of micropropagation begins with plant tissues (explants) from a healthy, vigorous mother plant. Explants could generate from any parts of the plants (leaf, apical meristem, bud, and root). Micropropagation through nodal explant is a commercially feasible protocol for producing genetically uniform plantlets identical to the mother plant in a relatively short period and with a high multiplication rate [40].

6.1 Shoot Establishments

This stage is very crucial for effectiveness of any tissue culture protocol as it depends on both sterilization method and plant growth regulators used. In this stage the tissue (explant) must be surface sterilized to remove microbial contaminants and transferred into nutrient medium. In cassava different sterilization protocols had implemented, but the most commonly used disinfectants are sodium hypochlorite, calcium hypochlorite, ethanol and mercuric chloride [43]. Different research works demonstrated that Murashige and Skoog's media is the commonly used medium for cassava micropropagation. For shoot initiation in cassava, various amounts of cytokinin alone and in combination with auxins had employed [41, 42]. There is also a report on the cytokinin combination [39, 9].

Table 1. Reports of shoot induction medium for *in vitro* micropropagation of cassava

Explant	Treatments that gave the best results	Shoot induction%	References
Nodal segment	BAP at 10 μ M	100%	[43]
Nodal segment	0.5 mg/l BAP and 0.02 mg/l NAA	100%	[44]
Axillary buds	10 mg/l BAP and 0.3 mg/l NAA	84.1-93.2%	[45]
Nodal segment	1.6 mg/L GA3 and 0.01 g/L NAA	-	[46]
Axillary nodal section	MS medium containing 0.1 mg/L kinetin	-	[47]
Nodal segment	MS medium supplemented with 2.0 mg/l BA + 0.1 mg/l NAA	90%	[48]
Nodal segment	MS medium supplemented with 3.0 mg/l BAP + 1 mg/l IBA	-	[49]
Nodal segment	MS medium containing 0.3 mg/L NAA	-	[50]
Nodal segment	MS medium containing 1.6 mg/l GA ₃ + 0.01 mg/l NAA	-	[9]
Nodal segment	0.05 mg/l BAP and 0.01 mg/l NAA	-	[51]
Auxiliary shoot	30 g/L sucrose and 100 mg/L polyvinyl pirolidone (PVP)	-	[39]
Meristem	5 mg/l BAP, 1 mg/l GA3 and 0.01 mg/l NAA	100%	[42]
Meristem	0.25 mg/l GA3, 0.1 mg/l BAP and 0.2 mg/l NAA	60-80%	[52]

6.2 Adventitious Shoot Proliferation

Shoot multiplication is a critical step propagation of any species for commercial use, and the highest rates are desired. Variables such as the type and ratio of plant growth regulators, explant type, culture media composition, and genotype all influence the frequency of shoot multiplication. The requirement for growth substance varies depending on the type and source of the explants and their endogenous level [53]. In general, plant growth regulators work both alone and in combination to produce the desired effect in tissue culture [54]. A wide range of cytokinins in varying concentrations was used in cassava *in vitro* shoot proliferation. NAA, KIN, TDZ, and 6-BAP are the most widely utilized hormones. BAP proved effective for shoot proliferation alone [41, 46] or with auxins or cytokinins [55, 40].

Abdalla *et al.* [40] found that a high cytokinin-to-auxin ratio was necessary for adventitious shoots rather than cytokinin alone. The authors discovered the highest number of multiple shoots (5.67) on MS medium with 1mg/l BAP+0.05 mg/l NAA. Kabir *et al.* [48] obtained the maximum number of shoots (6.3) on MS medium+2.0mg/l BA+0.1mg/l NAA. Mahdi and Edward [56] found that the control treatment (1.0 mg/l BAP + 0.01 mg/L NAA) produced the most shoots (3.77) in the cassava Putih variety. Furthermore, Perveen and Mansuri [49] observed the most effective response of shoot length on medium with 1.5 mg/L BAP and 0.025 mg/L NAA. BAP has also had shown to be effective on its own in terms of cassava shoot proliferation. Sesay *et al.* [46] found that Warima and Munafa, SLICASS 6, Coco cassada, and SLICASS 7 genotypes multiplied best on MS with 0.1 mg/l, 1.0 mg/L, 0.02 mg/l, and 2.0 mg/l BAP, respectively. Furthermore, Faye *et al.* [47] found that medium MS + BAP 1 mg/L resulted in the most shoot and leaf proliferation. Konan *et al.* [36] reported that MS media supplemented with ten mg/L BAP induced multiple shoots (25 shoots). On the other hand, Sessou *et al.* [43] demonstrated that MS medium with BAP: 10 μ M gave the highest micro shoots/explants (3.60).

Furthermore, there are several studies on cytokinin interactions. Demeke *et al.* [9] found that combining BAP with Kinetin at 0.75 mg/L resulted in an average of 7.30 micro shoots/explant. Deden and Herni [39] evaluated the maximum number of axillary shoots and stem nodes of cassava vars Darul Hidayah, Malang-6, and Adira-4 on medium containing different concentrations of BAP and thidiazuron.

6.3 *In Vitro* Rooting

The effectiveness of *in vitro* propagation is dependent on the successful roots and subsequent adaptation of regenerated shoots. Once a sufficient number of shoots had been produced, a part of the explants containing one or more shoots might be moved to a medium with a higher auxin, resulting in the development of roots. Some species can readily initiate roots by lowering the cytokinin level or growing them on MS medium with or without additional root-promoting auxins [57]. The roles of IAA, NAA, and IBA on rooting have been proven in previous works (Shiji *et al.* 2015b)[58]. Generally, the concentration of auxins used in the culture medium varies between 0.0 to 2 mg/l [55]. Several studies on the role of auxin type and concentration indicate that each growth regulator type with its specific amount have an impact on the rooting of the *in vitro* shoots. Even though findings on IBA and IAA were reported, NAA was the most efficient auxin for cassava root initiation *in vitro* [50]

6.4 Somatic Embryogenesis

Somatic embryogenesis (SE) is a technique used by plants to reproduce bipolar structures from somatic cells [59]. It entails the co-ordination of four steps: the production of embryogenic lines, proliferation, maturation, and plant regeneration (Figure 1) [60]. This approach serves as a significant experimental model for studying the processes of plant embryogenesis and is also one of the best methods for mass multiplication of elite or genetically modified plants [61]. Somatic embryos can be initiated directly or indirectly via a callus phase intervention [62]. The first somatic embryogenesis study in cassava had published in 1982 [63]. Even though various procedures for inducing somatic embryogenesis have been established, responses to somatic embryogenesis vary considerably among genotypes [64, 62], and it is also impacted by explant type and culture medium composition.

Somatic embryogenesis in cassava begins with the induction of primary embryos from the explants by culturing on MS medium supplemented with auxins. This technique depends on the genotype, as well as the source and kind of the initial explant [65, 38]. Several authors have employed various explants for primary somatic embryo induction. Immature leaf lobes are the most often employed cassava explant. Many publications have reported on the effective use of different synthetic auxins such as 2, 4-dichlorophenoxyacetic acid, picloram (4-amino-3, 5, 6-trichloropiconic acid), and dicamba (3, 6-dichloroanistic acid) for embryogenic calli initiation [45]. Picloram, on the other hand, outperformed other auxins at inducing primary somatic embryos in cassava [66, 67].

In cyclic embryogenesis, the cotyledons of matured primary embryos are fragmented and cultured on embryo initiation medium (solid or liquid MS medium supplemented with auxin) to induce embryogenic calli. The induced embryogenic calli contain globular embryos that develop to the cotyledonary stage on embryo maturation medium, a cytokinin (0.1 mg/L BAP) [68, 69]. In micropropagation, cyclic embryogenesis could increase the frequency of embryo multiplication by a repeated subculture of matured embryos that are allowed to develop into organized somatic embryos for conversion into plants [62].

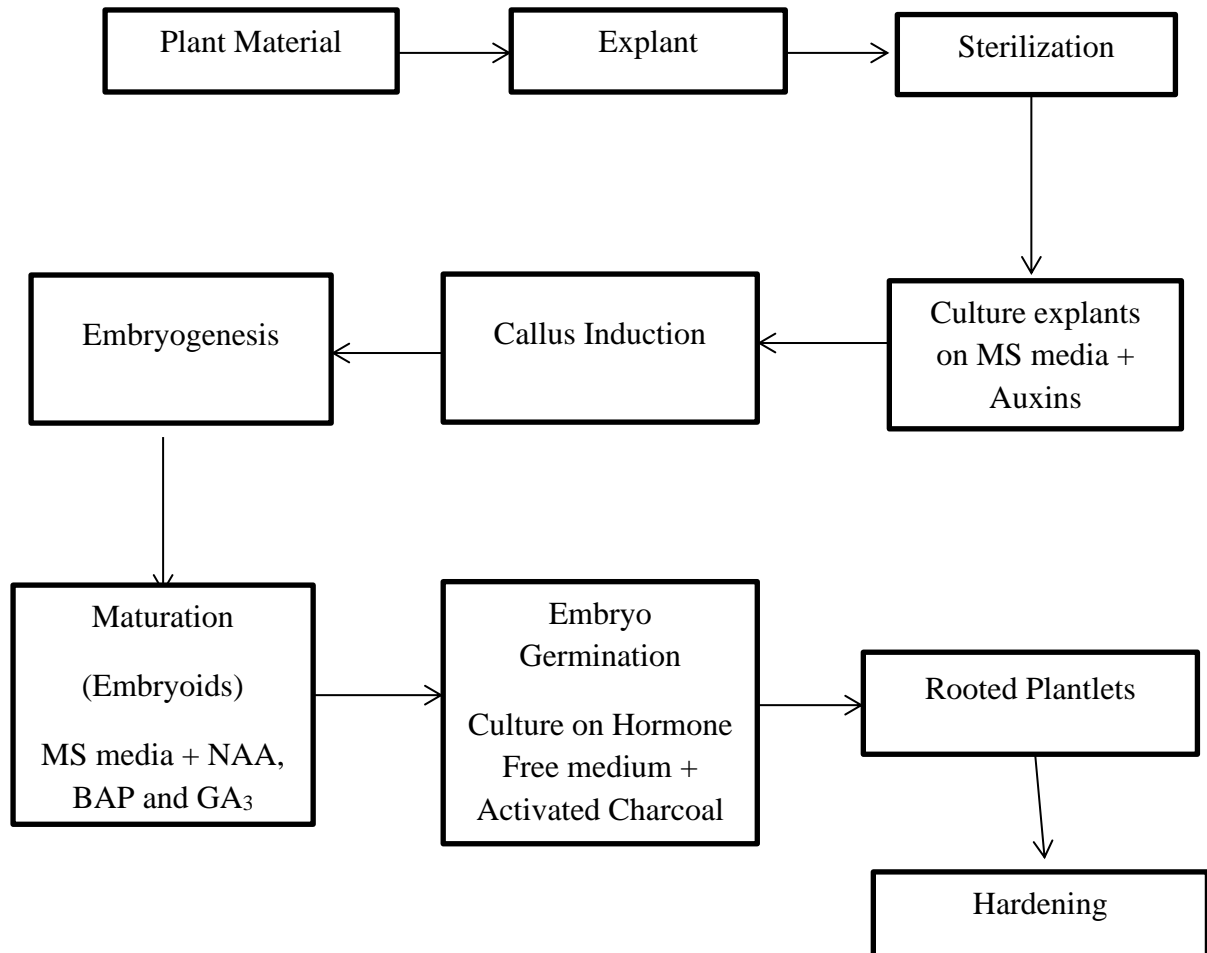


Figure 1. Flow diagram illustrating production of primary somatic embryo on cassava

6.5 Clonal Fidelity

Clonal fidelity is a necessary condition for any crop species during micropropagation. The presence of somaclonal variation is a significant limitation frequently encountered with tissue culture. This issue arises from several factors, including plant growth regulators, explant source, culture media type, and regeneration method [70]. In cassava, the somatic embryogenesis technique had somaclonal variation than the shoot organogenesis technique. It is due to the high rate of cell division during callus formation, which has induced by high auxin levels in the induction medium [69]. According to some research, the incidence of somaclonal variation in cassava regenerated by somatic embryogenesis is negligible [71]. However, this protocol may not be suitable for true-to-type mass propagation [70].

6.6 Acclimatization

Acclimatization of in vitro produced plantlets to the ex vitro environment is a main stage in the propagation process [50]. This stage is critical for the success of the in vitro multiplication process since in vitro propagated plantlets have tiny juvenile leaves with low photosynthetic capability and malfunctioning

stomata [72]. Acclimatization is challenging in cassava, and significant loss has occurred. And, more recently, several works have been done to reduce the loss. Shiji *et al.* [50] found that potting medium sand + vermiculite + soil resulted in a 100% survival rate in the greenhouse. Demeke *et al.* [9] found that sterilized potting mix of forest soil, fully decomposed coffee husk, and red sand in the ratio of 1:1:2 generated 93.3 % and 86.7 % acclimatized plantlets in two cassava varieties, 44/72-NR and 44/72- NW, respectively. Beyene [42] reported on two cassava varieties (Kello and Qulle) with 89.1 % and; 75% acclimatized plantlets, respectively, utilizing a 1:1:2 ratios of red soil, compost, and sand. Abdalla *et al.* [40] reported complete survival of acclimatized plantlets on a 1:1 combination of peat moss and sand on an American cassava clone. According to Sessou *et al.* [43], the treatment comprised of soil: sand: manure in the ratio of 3:2:1 gives the maximum survival rate of 100, 98, and 98 % for Agric-rouge, Atinwewe, and Agblehoundo cassava genotypes, respectively.

7 Conclusion

One of the significant constraints to cassava production is the slow rate of multiplication. Tissue culture techniques could be a viable solution to this problem. This technique, however, was dependent on the genotype, explant type, phytohormone type and amount, and regeneration method used. This review indicated that, Nodal segment explant was identified to be a reliable method for generating true-to-type plantlets in cassava micropropagation. A high cytokinin concentration combined with a low auxin concentration resulted in a high shoot proliferation rate, and NAA and IBA were discovered to be the most commonly used auxins for *in vitro* rooting on cassava.

8 Competing Interests

No potential conflict of interest was reported by the author.

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