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Initiation of Cassava Callus Culture and Its Prospect for Starch Production: A Systematic Mapping Study

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ABSTRACT

Mass propagation of cassava on several hectares of arable land due to increasing demand for its starch is not feasible due to land availability, pests and disease invasion, and long cultivation period. Plant cell culture technology is a promising solution despite the scarcity of cassava callus culture for starch production applications. Therefore, a systematic mapping study (SMS) was performed to identify the applications of cassava tissue culture and its prospects in starch production and investigate the important parameters for cassava callus culture initiation. The SMS began with formulating research questions (RQs), conducting searches on various databases, collecting and screening related articles, and extracting and mapping the selected articles. A total of 56 of 589 articles in the initial searching phase were chosen to be used as references to answer each RQ. The extracted data indicates that cassava tissue culture was mostly used for micropropagation, while starch production from its tissue culture is still limited. Basal medium and plant growth regulators influence cassava callus culture initiation most. The findings of the SMS offer a better understanding of cassava tissue culture and the prospects of producing cassava starch.

Keywords: Cassava, *Manihot esculenta* Crantz, plant tissue culture, starch, systematic mapping study

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INTRODUCTION

Manihot esculenta Crantz, known as cassava, tapioca, yuca, and mandioca, is a tuberous edible plant that possesses many desirable characteristics, such as able to withstand climate change, could be propagated on low fertility soils and tolerant against major diseases and pests (Tokunaga et al., 2020). It is the third most important source of calories in the tropics and is deemed the sixth most important crop after sugar cane, maize, rice, wheat, and potato (Parmar et al., 2017). Cassava is a woody plant with a rigid upright stem and spirally arranged in lobes whose tapered edible roots usually have brown, white, and reddish hues (Lim, 2016). The roots contain 80% dry weight of starch, which is usually utilized as raw materials in manufacturing industries for the production of bioethanol, bioplastics, dextrin, glucose, fructose, lactic acids, and baker's yeast (Parmar et al., 2017). Starch is a natural biopolymer comprising two types of polymer chains: amylose and amylopectin (Robyt, 2008). It is considered the most important polysaccharide, with beneficial characteristics such as being renewable, biodegradable, low-cost, and obtainable from abundant plant sources. It is also a good oxygen barrier, rendering it suitable for integration into raw materials for the plastic industry (Adewale et al., 2022). Starch has been widely used in various industries, such as in the food industries as a thickening, smoothening, and clarifying agent, and in the paper industries as a flocculant, retention, bonding, and binding agent (Vamadevan & Bertoft, 2015).

Although cassava starch is in high demand due to its various applications, there are limitations in conducting large-scale or mass-propagation of cassava, such as the availability of lands and long cultivating period, besides pest and disease invasion (Howeler et al., 2012). These challenges could be potentially solved using plant cell culture technology. The term refers to the aseptic culture of cells, tissues, organs, or whole plants under controlled nutritional and environmental conditions (Hussain et al., 2012). *In vitro* cultivation of cassava is advantageous to its mass propagation through a higher rate of multiplication, protection against infection during propagation, and the ability to produce disease-free cassava plants.

However, reports regarding the application of cassava tissue culture for starch production are limited. Therefore, this systematic mapping study (SMS) aims to identify the recent applications of cassava tissue culture and their prospects in starch production. In addition, this study also investigates the important parameters for the initiation of cassava callus culture. Data acquired from various databases (Academia, Academic Journals, African Journal Online, ResearchGate, SpringerLink, Taylor and Francis Online, and Wiley Online Library) were extracted to answer all constructed research questions (RQs) based on the study's objectives. This SMS can be a platform for further experimental studies on initiating cassava callus culture and open new possibilities to produce starch using plant cell culture technology.

METHODS

SMS is the research method used in this study to map and classify previous research studies based on the research area, patterns of evaluation, and research gap to recommend more areas of study (Petersen et al., 2008). All existing evidence on specific topics is screened and selected through a detailed search strategy to meet the eligibility criteria to answer the RQs and achieve the targeted objectives. The procedures in SMS involve the identification of RQs according to the study's objectives, followed by the development of a search strategy, execution of the screening stage, and data extraction and mapping. Figure 1 illustrates the steps in conducting SMS, where the first and second screening stages were done using Parsifal software (version 1.0).

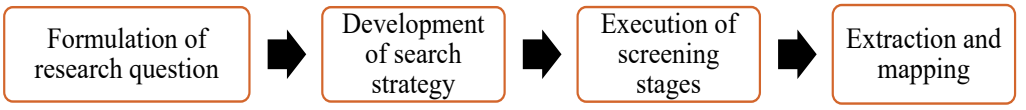

Figure 1. Flow chart of systematic mapping study

Formulation of RQs

One important step of SMS is formulating the RQs since they guide the search process. Clear and defined questions are necessary before executing all SMS procedures. Several research questions were constructed using the population, concept, and context (PCC) method to achieve this aim and purpose, as shown in Table 1 (Petersen et al., 2008).

Table 1
Research questions formulated using the population, concept, and context method

Research question (RQ) 1	
RQ 1	What applications of cassava cultures have been reported so far?
Sub-RQ 1	What are the types of cultures used for *in vitr*o propagation of cassava?
Sub-RQ 2	What are the varieties of cassava chosen in the articles?
Sub-RQ 3	What are the countries or the origin of the reported articles?
Research question 2	
RQ 2	What are the important parameters for initiating cassava callus culture?
Sub-RQ 1	What type of explants were used to study cassava callus culture?
Sub-RQ 2	What is the preparation method to surface-sterilize the explants to avoid contamination in the cassava callus culture?
Sub-RQ 3	What are the analyses used to quantify the growth of callus?
Sub- RQ 4	What statistical analysis is used to find the most significant parameters/ plant growth regulator (PGR) concentration reported in the articles?

Development of Search Strategy

The primary search process in this SMS involved various online databases, such as Academia, Academic Journals, African Journal Online, ResearchGate, ScienceDirect, SpringerLink, Taylor and Francis Online, and Wiley Online Library. A comprehensive search strategy ensures that the constructed search strings cover both objectives. Thus, the search strategy from Zein et al. (2016) was adopted to construct the search strings as follows:

- The search was conducted based on synonyms and other alternative words suitable for use as keywords.
- The use of Boolean OR to integrate the alternative keywords and suitable synonyms.
- The use of Boolean AND to connect the important terms.
- Double quotations are used for the composite words.
- Paratheses are used to separate keywords and synonyms.

Several trials were conducted, and the final search strings chosen are as follows: ("tissue culture" OR "*in vitro* propagation") and cassava.

Execution of Screening Stages

All articles went through three screening stages, which were iterative and incremental. In the first screening stage, advanced search features provided by the online databases were utilized by applying the search string. All articles were screened and selected according to the study's title and uploaded to Parsifal. Duplicated articles from the databases were removed with the help of Parsifal. The second screening stage was done by going through the abstract, where articles that did not mention the use of tissue culture for propagating cassava were removed. The selected articles eligible for selection criteria were passed to the third screening stage, i.e., reading the full-text articles, which includes the introduction, methodology, and conclusion. Toward the end of the SMS, all important information from the selected articles was extracted to unravel the RQs and achieve the targeted objectives. Table 2 lists the essential selection criteria for screening and selecting related articles.

Table 2
Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Studies must be published between 2011 and 2021	Studies published before the year 2011
Studies must be in English	Non-English articles
Studies must describe the use of plant tissue culture for *in vitro* propagation of cassava	All systematic review and meta-analysis articles
The study must mention using cassava or *M. esculenta* Crantz as an explant for tissue culture	All book chapters and Doctoral and Master's theses
Studies must be journal articles or conference papers only	Studies do not properly describe the use of tissue culture in propagating cassava

RESULTS AND DISCUSSION

Search Result

About 589 articles were initially retrieved from databases such as Academia, Academic Journals, African Journal Online, ResearchGate, ScienceDirect, SpringerLink, Taylor and Francis Online, and Wiley Online Library. Since there is the possibility of duplicate articles in all databases, it is crucial to filter them out first. A total of 32 duplicates were removed using Parsif.al. Table 3 summarizes the number of articles accepted in the first, second, and third screening stages of this SMS, while Figure 2 illustrates the results of the first screening stages in percentage.

Table 3
List of sources and results of the screening stages

Online databases	Stage 1	Stage 2	Stage 3
Academia	8	6	5
Academic Journal	14	13	12
African Journal Online	19	12	8
ResearchGate	47	25	20
ScienceDirect	12	5	2
SpringerLink	18	10	4
Taylor and Francis Online	8	5	4
Wiley Online Library	6	2	1
Total	132	78	36

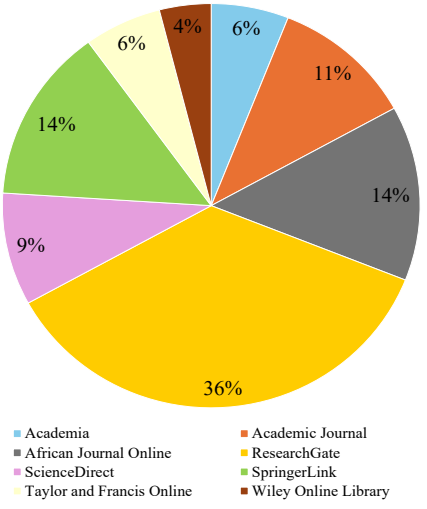

Figure 2. Stage 1 screening result distribution in percentage

From the recorded number of articles in Table 2, 557 were screened in the first screening stage after removing duplicates in the initial search result. A total of 132 articles were selected in the first screening phase, excluding articles with titles unrelated to the study. Figure 2 illustrates the overall result from the first screening stage, where ResearchGate contributed the highest percentage (36%), followed by African Journal Online and SpringerLink at 14%. From the second screening stage, 78 articles were accepted after going through their respective abstracts. At this stage, any articles that did not mention the use of plant tissue culture in propagating cassava were excluded from the SMS. Finally, after the third screening phase, only 56 articles were selected and chosen to answer the constructed RQs as they met all requirements listed in the inclusion criteria (Table 2).

The second objective involved further screening to choose the studies that

utilize callus culture for *in vitro* cassava propagation. Articles in the third screening stage must clearly describe the protocols and important parameters needed to initiate cassava callus culture. Only 11 articles were selected at the third screening stage, which exhibits all the information needed to complete the second objective of this SMS (Figure 3). Five articles were obtained from the Academic Journal online database, followed by the African Journal Online, with three. None of the articles from ScienceDirect, SpringerLink, Taylor and Francis Online, and Wiley Online

Library discussed the protocols and important parameters to initiate cassava callus culture. All selected articles in the SMS are listed in Table A1 in the Appendix.

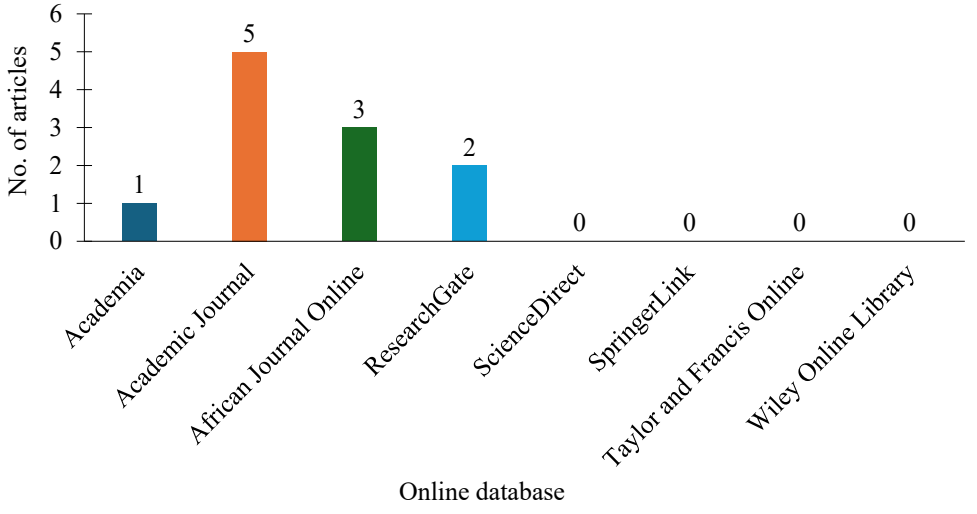

Figure 3. The number of articles selected to achieve Objective 2 after the third screening stage

Answering the RQs

All RQs constructed at the beginning of the SMS are answered according to the information extracted from the 56 chosen articles.

RQ 1: What are the Applications that Have Been Reported So Far?

The applications of cassava cultures are illustrated in Figure 4 from the final selection of 56 articles.

Figure 4 depicts that *in vitro* cultivation of cassava is mostly used for micropropagation purposes, as indicated by the 75% distribution in the pie chart. Micropropagation refers to the rapid production of plantlets, resulting in uniform genetic characteristics and free from disease and pests as they grow in a controlled environment (Mahdi & Edward, 2018). Most of the objectives in the studies involving micropropagation of cassava were to investigate the best media formulation and culture conditions for a high cassava yield.

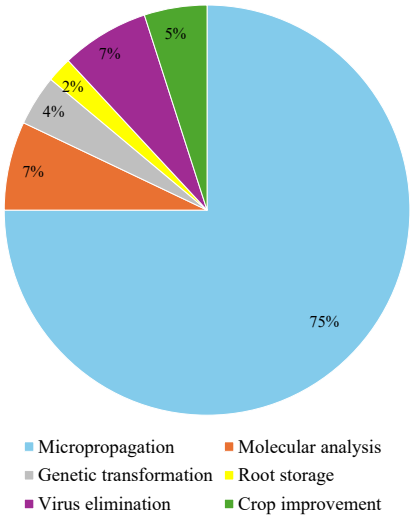

Figure 4. Applications of *in vitro* cultivation of cassava-based on 56 studied articles

Meanwhile, about 7% of the articles (Figure 4) refer to using plant tissue culture in cassava for molecular analysis and virus elimination, corresponding to 4 articles. Cassava is usually infected with viral diseases such as cassava mosaic disease (CMD), which can cause a yield loss from 20 up to 95% (Sessou et al., 2019). Pest problems in cassava plantations, especially whitefly species (*Bemisia tabaci*), can become a weed host on more than 600 crops, which can cause the spread of *Sri Lankan cassava mosaic virus* (SLCMV) and *Indian cassava mosaic virus* (ICMW; Duraisamy et al., 2013). Thus, various studies on cassava tissue cultures were geared to eliminate these viruses, and molecular analysis were conducted to help alleviate the issue.

Finally, the cassava root storage formation study using cassava tissue culture has the lowest distribution, i.e., 2% (Figure 4). According to the study by Yao et al. (2013), calcium significantly influences the *in vitro* growth of cassava root formation and its starch accumulation. Calcium is important in cell wall synthesis, membrane function, and cell signaling; thus, it needs to be supplemented in the culture medium to support *in vitro* growth of plant cultures (Smith, 2013). Results showed that although the addition of calcium positively affects the diameter of *in vitro* cassava storage roots, it is detrimental to the induction rate and starch content. The observation of *in vitro* cassava roots and field cultivation of cassava roots through the scanning electron microscope (SEM) showed that starch grains formed by these two different ways were similar in size and shape, indicating that the study of starch synthesis from *in vitro* induction of cassava roots is feasible (Yao et al., 2013).

SUB 1 RQ 1: What Types of Cultures are Used for *In Vitro* Propagation of Cassava? The SMS successfully highlights six types of plant tissue culture that have been used for *in vitro* cassava propagation, and the statistics are illustrated in Figure 5. The shoot and meristem cultures have the highest distribution among all cultures, i.e., 61%, with 36 articles. Shoot culture refers to *the in vitro* cultivation of shoot tips to produce shoot clumps from axillary or adventitious buds. It is also widely used for clonal propagation (Smith, 2013). The shoot culture contributed the highest in generating cassava *in vitro* because it has proven very effective in plant micropropagation (Gusain et al., 2021). Besides, the risk of getting bacterial or fungal contamination is very low in shoot culture, and the cultivation time is shorter compared to other types of culture.

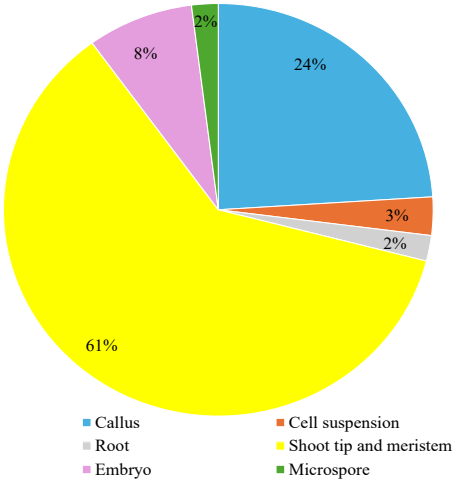

Figure 5. Types of cassava cultures for *in vitro* propagation of cassava

Callus culture refers to the growth of an unorganized, growing, and dividing mass of cells with the supply of auxin and cytokinin, where any part of the plant can be used as an explant (Smith, 2013). This type of cassava culture has the second highest distribution (24%), with 14 articles. Callus culture has been widely used as an experimental system to solve a wide range of basic research problems regarding plant cytology, morphology, physiology, anatomy, pathology, biochemistry, and genetics. It has also been used to solve research problems related to the propagation of horticultural and agronomic plants, especially in organogenesis and embryogenesis sections (Abdalla et al., 2013). Microspore culture and root culture are the least utilized, i.e., at 2%, with 1 article each. Microspore culture refers to cultivating cassava haploid tissue using pollen or anthers as an explant (Smith, 2013). More than 250 plant species have been propagated using microspore culture; however, only a handful of studies have reported developing *in vitro* double haploid protocols in cassava (Perera et al., 2014).

SUB 2 RQ 1: What are the Varieties of Cassava Chosen in the Articles?

Cassava has a broad range of different types of cultivars, which are distinguished or grouped by certain characteristics in terms of their morphology or physiology. These characteristics are important in horticulture, agriculture, or forestry applications. Researchers and farmers require plants with a particular characteristic or adaptation to their environment and cultivation practices to suit the purposes of cultivating the plant either *in vitro* or traditionally.

Detailed information on cassava varieties used in the 56 articles can be found in Table A1 (Appendix). All varieties used in the experimental studies were well established either directly from the greenhouse, farm fields, or experimental fields or obtained from research institutes. Generally, before the cassava plants were used as the explant for *in vitro* cultivation, they were well petted and grown under almost perfect conditions at 28°C under fluorescent lights with 70 $\mu mol.m^{-2}.s^{-1}$ and 12 hr light/12 hr dark of photoperiod regime (Tokunaga et al., 2020). Some researchers described the reasons for choosing specific cassava cultivars for their studies. The most common factor in cassava variety selection is due to the farmer's preference genotype that exhibits good performance, such as early maturity, high productivity,

palatability, and resistance to disease and pest (Fletcher et al., 2011; Maruthi et al., 2019; Sesay et al., 2018).

SUB 3 RQ 1: What are the Countries or the Origin of the Reported Articles? The origin or country of all 56 articles obtained in this SMS is depicted in Figure 6. Africa contributes the highest to the publication of studies on the *in vitro* cultivation of cassava, at 55%, with 31 articles. It reflects that cassava is the major staple food for millions in East and Central Africa, especially in rural areas. The tuberous roots of cassava, rich in carbohydrates, are suitable for human consumption after being processed and cooked (Osena et al., 2017). Besides, cassava can be utilized for bioenergy generation, biomaterial production, and animal feed because of its quality and quantity of starch content and high biomass productivity. Thus, any efforts to increase the production of cassava crops in Africa are supported by the government, and millions of dollars are allocated annually for cassava research and development to combat diseases and pests that affect crop yield (Fotso et al., 2014). Africa is actively involved in studies to produce cultivars that are resistant to the *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV), where these viruses are transmitted by whiteflies (*B. tabaci* Gennadius) to the planting materials and can cause 100% yield losses (Kidulile et al., 2018).

Meanwhile, China is the second highest contributor to the study of *in vitro*

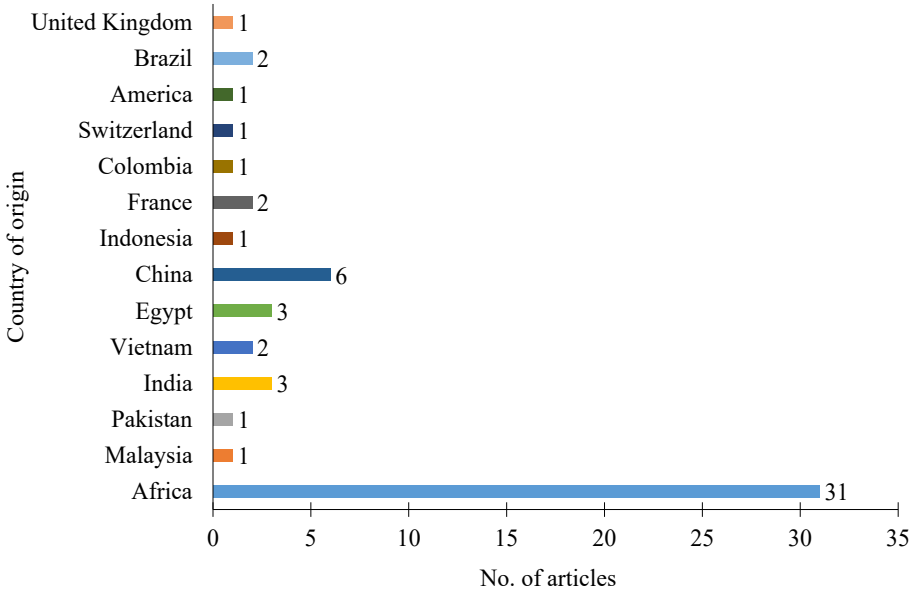

Figure 6. Origin of the published articles on *in vitro* propagation of cassava

propagation of cassava, at 11%, with six articles (Figure 6). Cassava research in China is mainly focused on the storage capacities of starchy cassava roots, and most of the articles found for this SMS were more focused on developing its tuber roots and storage capacity. For instance, Wu et al. (2014) concluded that sucrose plays a crucial role in cassava tuberous root formation and saccharide accumulation. Likewise, Yao et al. (2013) reported that calcium concentration in the medium influenced *in vitro* growth of cassava root formation and its starch accumulation (Yao et al., 2013). Figure 6 depicts almost similar distribution in the studies of cassava using plant tissue culture in other remaining countries, at about 3–5%, with a range of 1–3 published articles.

RQ 2: What are the Important Parameters to Initiate Cassava Callus Culture?

The important parameters to initiate cassava callus culture are divided into three categories: (1) the basal medium, (2) the PGRs, and (3) the growth condition and cultivation periods. Plant culture media used in the *in vitro* cultivation of cassava comprises essential elements or mineral ions (supplied as a complex mixture of salts), an organic supplement (supplied as vitamins and/or amino acids), and a carbon source (usually supplied as sucrose). Basal medium refers to the basic media without supplementation, which aims to support the growth of plant cell culture. From 11 articles extracted from SMS for the second objective, ten articles used the Murashige and Skoog (MS) basal medium, while another opted for the Gresshoff and Doy (GD) basal medium. Both MS and GD basal media comprise microelements, macroelements, iron sources, and organic supplements needed for the growth of plant cell culture, albeit with slight differences in the concentration of each element inside the basal medium. Moreover, some elements are only available in the MS medium but not in the GD medium and *vice versa*. MS medium is regarded as the most commonly used basal medium and is a basis for other media formulations (Smith, 2013).

PGRs are important in determining the pathway of plant cell development; they stimulate cell division and regulate the growth and differentiation of shoots and roots of cultured explants. For instance, the optimum type and PGR concentration for callus induction and growth for a particular plant do not necessarily have a similar impact on the development of shoots or roots (Gusain et al., 2021). Five main PGRs are used in plant culture systems: auxins, cytokinin, gibberellins, abscisic acid, and ethylene. The most common PGRs mentioned in this study's articles are auxins and cytokinin.

Auxins, which are 2,4-dichlorophenoxyacetic acid (2,4-D), 1-naphthaleneacetic acid (NAA), and picloram, are commonly used in studies of cassava callus culture initiation to promote cell division and growth. 2,4-D is the most used and effective auxin in the induction of plant cell culture (Smith, 2013). In a comparison between 2,4-D and picloram, the former exhibited a better response in the induction of callus culture, giving a

slightly higher frequency of callus formation (%). However, the highest frequency of callus formation for both PGRs was achieved at the same concentration, i.e., 8 mg/L (Ngugi et al., 2015). In a study to determine the best 2,4-D concentration that produces a high frequency of callus formation, a higher level of 2,4-D (12–16 mg/L) proved to be the best callus inducer (Elibariki et al., 2014). Meanwhile, Abdalla et al. (2013) demonstrated that the best 2,4-D concentration for inducing cassava callus culture is 15 mg/L, with 100% callus formation frequency. In addition, an experimental study on the effect of three types of cytokinin, i.e., thidiazuron (TDZ), 6-benzylaminopurine (BAP), and kinetin, showed that TDZ with a 1.5-µM concentration produced the highest frequency of callus formation (%; Sessou et al., 2019). It is not feasible to generalize the optimum PGRs for cassava callus culture due to the difficulty in predicting its effects on plant cell culture due to the differences in culture response between species, cultivars, and plants grown under different conditions. Further studies are required to confirm the optimum type and concentration of PGR for callus induction.

Table 4
Types of explants used to initiate cassava callus culture

Type of explant	No. of articles
Shoot	2
Leaf (immature leaf lobes, young leaf lobes, meristematic leaf lobes)	7
Stem internodes	4
Root	1
Axillary buds	1

Finally, the cultural environment is important to promote the growth of cassava callus culture. From the chosen articles in this SMS, the optimum temperature to cultivate the cassava callus culture is 22 to 28ºC, and most articles reported the incubation of callus culture in a dark room with a photoperiod of mainly 12 to 16 hr. The main benefit of using plant tissue culture in cultivating cassava is the ability to control the culture condition, which could prevent factors such as drought, rain, diseases, and pests from affecting the cultures.

SUB 1 RQ 2: What is the Type of Explants Used to Study Cassava Callus Culture?

Explants refer to small pieces of plant parts or tissues being cut aseptically and used to initiate a culture in a nutrient medium (Putri et al., 2019). Explants can be taken from different plant parts as long as they can be differentiated into totipotent cells. The types of explants used to initiate cassava callus culture extracted from the articles are listed in Table 4.

From Table 4, leaf explants, including immature leaf lobes (ILL), young leaf lobes, and meristematic leaf lobes, are widely used to initiate cassava callus culture. ILL and stem explants demonstrated different abilities to initiate callus culture in terms of the duration, characteristics, and frequency of callus formation (%), where ILL can generate callus on an average of 10 days while stem explant took up to 15 days (Syombua et al., 2019). The callus culture formed from the ILL explant is more translucent, gelatinous, and highly

embryonic than the stem explant, giving rise to mainly loose, friable, and non-embryonic callus. Syombua et al. (2019) further mentioned that the leaf explant gave higher frequencies of callus formation (%) than the stem explant. This notion is similar to the report by Abdalla et al. (2013).

Stem nodes are the second most frequently used type of explant to initiate callus culture, followed by shoot, root, and axillary bud explants (Table 4). The correct selection of explant material is important to successfully establish a tissue culture. The factor in choosing an explant depends on the type of culture to be initiated, the application of the proposed culture, and the plant species to be used.

SUB 2 RQ 2: What is the Preparation Method to Surface-sterilize the Explants to Avoid Contamination in the Cassava Callus Culture? Microbial contamination in plant tissue culture has become a constant problem compromising the *in vitro* culture development, especially when the explant is originally from field-grown plants (Putri et al., 2019). Surface sterilization is a pre-treatment method to prepare an explant before being cultured in the growth media. It refers to the process of immersing the explant in an appropriate concentration of chemical disinfectant(s) or sterilant(s) for a specific duration, resulting in the establishment of a contamination-free culture (Bello et al., 2018). This method is the most crucial step in developing plant tissue culture protocol, where inappropriate concentrations of chemical disinfectant or sterilization duration can have a lethal effect on the division of cells and restrict plant growth and development. Thus, it is necessary to take heed of the chemical sterilant's concentration, combination, and exposure duration to ensure a successful *in vitro* culture establishment.

Most of the studies required first cleaning the explants under running tap water and cleaning with soap to remove any physical dirt and surface debris attached to the explants (Elibariki et al., 2014; Faye et al., 2015; Fotso et al., 2014; Sessou et al., 2019). Next, explants are immersed in 70% ethanol, i.e., the concentration that effectively kills microbes, bacteria, and other microorganisms on the surface of the explant. The duration of explant immersion in 70% ethanol should not exceed 5 minutes as it can destroy its cells (Elibariki et al., 2014; Fotso et al., 2014; Sessou et al., 2019).

The immersion of explants in bleach is also crucial to further ensure the removal of contaminants from the explants, especially microorganisms. Sodium hypochlorite, commercially known as laundry bleach, is the most frequent choice, where it will be diluted to 25% for surface sterilization purposes. A balanced concentration and duration of immersion must be determined correctly to prevent phytotoxicity on the explant, which usually takes between 20 s and 20 min, depending on the concentration and type of explant (Elibariki et al., 2014; Fletcher et al., 2011; Sessou et al., 2019). The addition of Tween 20 in the bleach solution reduces the surface tension of the explant and allows better contact of the

surface with the surfactant(s). Calcium hypochlorite has also been reported (Faye et al., 2015). It is highlighted that this kind of bleach may cause less injury to plant tissues than sodium hypochlorite. Finally, the most important step in the surface sterilization method is to rinse the sterilized explant thoroughly with sterile distilled water, usually three to four times within each step.

SUB 3 RQ 2: What Analyses are Used for Quantifying Callus Growth? The growth of cassava callus, but Table 5 lists the most used procedures or analyses for observing, monitoring, and evaluating the success of initiating cassava callus culture.

The most common way to quantify the growth of callus culture is by determining the frequency of callus formation (%, Table 5). The frequency of callus formation is expressed as the percentage of the calluses produced by the explant per total number of explants being used in the experiment (Sessou et al., 2019), as presented in Eq. (1):

No additional procedures are required for this analysis compared to the fresh weight (FW) or dry weight (DW) analyses,

$$\text{Frequency of callus formation (\%)} = \frac{\text{Explant produced callus}}{\text{Total cultured explants}} \times 100\% \quad [1]$$

whereby specific and additional procedures must be conducted to determine the results. Besides, the callus formation method frequency is non-destructive and very convenient, considering that in the early stage of callus initiation, its amount is limited. Other quantifying methods, i.e., the size and weight of the callus, are the least preferred by researchers (Table 5). In the FW technique, the jar and media will be pre-weighed on the analytical weighing balance, and the measurement will be denoted as A before inoculating the explant. The data will be collected after four weeks of cultivation. The callus will be taken out from the glass jar and dabbed with filter paper to remove any moisture on the surface of the callus (Fotso et al., 2014). The callus, the jar and the medium will be weighed using the analytical weighing balance. The measurement will be recorded and denoted as B. Thus, the FW of the callus will be denoted as C, and the calculation will be made according to Eq. (2):

Table 5
Quantifying analysis for the growth of cassava callus culture

Quantifying analysis	No. of articles
Frequency of callus formation (%)	8
Size of callus	3
Fresh and dry weight	3

$$\text{Fresh weight of callus (g)}, C = B - A \quad [2]$$

For the DW, after determining the callus FW, the sample will be dried in the oven at 40°C for 24 hr in the Petri dish. The callus sample is left to cool to room temperature in a desiccator containing silica gels to prevent the cells from absorbing any moisture in the surroundings. The sample will be then weighed on the analytical weighing balance, and the measurement will be recorded.

Then, the callus sample will be returned to the drying oven for another 4 hr, and the step will be repeated until the sample reaches a constant weight (Abdalla et al., 2013). The constant weight is denoted as D, whereas the pre-weighed jar and medium are denoted as A. The FW of the callus culture is calculated based on Eq. (3):

$$\text{Dry wright of callus (g), } E = D - A \quad [3]$$

The FW and DW methods of callus growth quantification hinder the callus from being used for plant regeneration since the culture is exposed to high temperatures and contamination during the procedures. This disadvantage is especially evident when the amount of callus established is small, specifically when the research is at the beginning stage (initiation of callus culture).

SUB 4 RQ 2: What Statistical Analysis was Used to Find the Most Significant Parameters/PGR Concentration Reported in the Articles? Statistical analysis refers to collecting and evaluating data to identify patterns and trends. Table 6 summarizes the statistical analyses and tests conducted by the researchers to investigate the most optimum variables or parameters to induce the highest frequency of calluses (%).

Table 6 lists that all articles used ANOVA to determine any statistically significant differences between the means of two or more independent groups. ANOVA can be done through various programs, such as Minitab, SAS, SPSS, and PRISM software. Tukey analysis, also known as Tukey's range test, is used to find significantly different means. Meanwhile, Newman-Keuls, also known as Student-Newman-Keuls, can be run once ANOVA has given statistically significant results to see which specific pairs of means are different. *F*-test is the most often used to compare statistical models fitted to a data set to identify the model that best fits the population from which data were sampled. All the tests were done based on the number of variables manipulated in the experimental studies.

Table 6
Statistical analyses used in cassava callus culture

Test	Analysis	Software
Tukey analysis	ANOVA	Minitab
Student, Newman, and Keuls analysis	ANOVA	CoStat, PRISM
F-test	ANOVA	SAS
Least significant difference test	ANOVA	SAS
Duncan's multiplication range test	ANOVA	SPSS

CONCLUSION

This paper reviews cassava tissue cultures focusing on their applications and prospects for starch production, as well as the important parameters for initiating cassava callus culture using SMS. A total of 589 research articles published between 2011 and 2021 obtained from various databases were analyzed. After passing through three screening stages, which involved the implementation of the exclusion and

inclusion criteria, only 56 articles were used to extract important information to answer all RQs. About 42 articles discussed the micropropagation of cassava plants using tissue culture with various objectives, such as media optimization and protocol development. However, only one article highlighted *in vitro* cassava root formation and its starch accumulation. It demonstrates that studies of cassava tissue cultures on starch production are still scarce. Interestingly, using cassava tissue cultures, starch production is possible by supplementing specific nutrients to the growth medium. Therefore, the prospect of using cassava tissue cultures for starch production will grow based on the current research trend. The type of basal medium, PGRs, and culture environment play important roles in initiating cassava callus culture. Other than that, surface-sterilization methods and the choice of explant are crucial to ensure the success of cassava callus culture initiation. It is best to use 70% ethanol and 5% sodium hypochlorite in the surface-sterilization steps and the leaf as the explant source. All formulations, media, and techniques have been proven to produce the highest frequency of callus formation (%). To conclude, the information from this SMS can serve as a future reference for practical experimentation relating to the initiation of cassava callus culture and starch production.

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SUPPLEMENTARY DATA

Table A1

List of articles used in this systematic mapping study

No.	Title
1	Evaluation of regeneration potentials of farmer-preferred cassava (*Manihot esculenta* Crantz) landraces to unlock cassava transformation barriers
2	*In vitro* propagation of Malaysian cassava (*Manihot esculenta* Crantz) variety through low-cost tissue culture media
3	Rapid propagation of a biodiesel plant cassava (*Manihot esculenta* Crantz) through tissue culture
4	Optimization of *in vitro* propagation of cassava (*Manihot esculenta* Crantz) genotypes
5	Molecular studies on the transmission of *Sri Lankan cassava mosaic virus* (SLCMV) in cassava by *Bermisia tabaci* collected from cassava and cassava breed crops
6	Callus induction in three mosaic disease-resistant cassava cultivars in Benin and genetic stability of the induced calli using simple sequence repeat (SSR) and sequence-characterized amplified region (SCAR) markers
7	Cost-effective medium for *in vitro* propagation of Tanzanian cassava landraces
8	Development of *in vitro* propagation protocol for some recalcitrant cassava (*Manihot esculenta* Crantz) genotypes in Sierra Leone
9	Effects of different hormones on organogenesis *in vitro* of some varieties of cassava (*Manihot esculenta* Crantz) grown in Senegal
10	Explant type and hormone regime influence somatic embryogenesis and regeneration in cassava
11	Exploring the induction of doubled haploids in cassava through gynogenesis
12	Generating virus-free cassava plants by *in vitro* propagation with chemical and heat treatment
13	*In vitro* embryo rescue and plant regeneration following self-pollination with irradiated pollen in cassava (*Manihot esculenta* Crantz)
14	Response of four cassava cultivars (*Manihot esculenta* Crantz) plantlets free of cassava mosaic virus to micropropagation in different media
15	Shoot nodal culture and virus indexing of selected local and improved cassava genotypes (*Manihot esculenta*) from Sierra Leone
16	Somatic embryogenesis in two Nigerian cassava cultivars (Sandpaper and TMS 60444)
17	Use of multivariate analysis to evaluate the effect of sucrose on *in vitro* cassava conservation
18	An efficient protocol for *Agrobacterium*-mediated transformation of β-glucuronidase (*Gus/Gusplus*) gene into cassava plants (*Manihot esculenta* Crantz)
19	Callus induction, regeneration, and molecular characterization of cassava (*Manihot esculenta* Crantz)

Table A1 *(Continue)*

No.	Title
20	The effects of calcium in the *in vitro* cassava storage root formation
21	Somatic embryogenesis and organogenesis of biofuel plant cassava (*Manihot esculenta* Crantz) Chinese cultivars
22	Effect of 1-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) on *cassava's in vitro* regeneration and hardening (*Manihot esculenta* Crantz)
23	Differing sucrose requirements for *in vitro* conservation of cassava genotypes
24	Effects of plant growth regulators on shoot multiplication and root induction of cassava varieties *in vitro* culture
25	Regeneration of Kenyan cassava (*Manihot esculenta* Crantz) genotypes
26	*In vitro* micropropagation of cassava through low-cost tissue culture
27	Effects of sucrose on tuberous root formation and saccharide accumulation in *Manihot esculenta* Crantz *in vitro*
28	Elimination of *Cassava brown streak virus* from infected cassava
29	Shoot induction from the axillary bud of β-carotene enriched *Manihot esculenta* Crantz and molecular stability of regenerants
30	Efficacy of chemotherapy and thermotherapy in eliminating cassava mosaic virus from Tanzanian cassava landrace
31	An efficient method of propagating cassava plants using aeroponic culture
32	Somatic embryogenesis and regeneration of five multipurpose cassava landraces extensively integrated into the African cropping system
33	Somatic embryogenesis and plant regeneration of cassava (*Manihot esculenta* Crantz) landraces from Cameroon
34	Effect of 2,4-D, explants type and cultivar on the callogenesis expression of cassava (*Manihot esculenta* Crantz) in Ghana
35	Responses of cassava (*Manihot esculenta* Crantz) varieties to *in vitro* mannitol-induced drought stress
36	Production of virus-free cassava through hot water therapy and two rounds of meristem tip culture
37	The comparatively proteomic analysis in response to cold stress in cassava plantlets
38	Evaluation of *Agrobacterium*-mediated transformation of two Nigerian cassava (Manihot *esculenta* Crantz) cultivars TME 419 and “Okwuoto"
39	A milestone in the doubled haploid pathway of cassava
40	Robust transformation procedure to produce transgenic farmer-preferred cassava landraces
41	Evaluation of cassava plants generated by somatic embryogenesis in different stages of development using molecular markers

Table A1 *(Continue)*

No.	Title
42	*In vitro* selection and characterization of salt tolerant cell lines in cassava plant (*Manihot esculenta* Crantz)
43	Genetic stability of cassava plants regenerated through organogenesis using microsatellite markers
44	*In vitro* clonal propagation of cassava plant
45	*In vitro* multiplication of cassava varieties
46	Inducing autotetraploids in cassava using oryzalin and colchicine and their *in vitro* morphophysiological affects
47	Micropropagation for rapidly multiplying planting material in cassava (*Manihot esculenta* Crantz)
48	A method for generating virus-free cassava plants to combat viral disease epidemics in Africa
49	Effect of types and concentrations of auxins on callus induction and primary somatic embryogenesis in low cyanide cassava cultivars (*Manihot esculenta* Crantz)
50	Cost-effective nutrient sources for tissue culture of cassava (*Manihot esculenta* Crantz)
51	Fruit, seed, and embryo development of different cassava (*Manihot esculenta* Crantz) genotypes and embryo rescue
52	Plant regeneration via protoplast electrofusion in cassava
53	Effects of plant growth regulators on *in vitro* cultured nodal explants of cassava (*Manihot esculenta* Crantz) clones
54	The effect of exogenous phytohormones and sucrose on the micropropagation and microtuberisation of *Manihot esculenta* Crantz var. TMS 96/0023
55	Effects of cytokinin on secondary somatic embryogenesis of selected clone Rayong 9 of *Manihot esculenta* Crantz for ethanol production
56	Micropropagation of disease-resistant cassava variety in Rwanda