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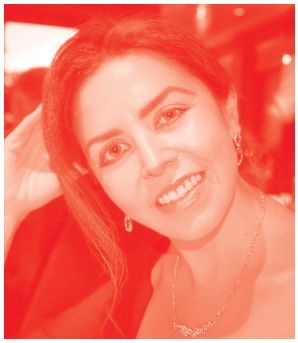

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Meet the editor

Dr. rer. nat. Andri Frediansyah is a researcher in the Research Division for Natural Product Technology (BPTBA), the Indonesian Institute of Sciences (LIPI), where he has been since 2010. His research interests span from basic to applied sciences of natural products connected to food and health. Dr. Frediansyah earned a BSc in Biology from Gadjah Mada University, Indonesia, in 2010 and an MSc in Applied Biological Sciences from Chulabhorn Graduate Institute, Thailand, in 2015. He earned his 3.5-year-Ph.D. (magna cum laude) in Pharmaceutics from the University of Tuebingen, Germany, Additionally, he is an accomplished writer with numerous scientific articles to his credit. He also received numerous accolades throughout his career as a scientist.

Contents

Preface

Cassava (*Manihot esculanta* Crantz) is a life-sustaining source of carbohydrates for more than a billion people worldwide, primarily in the tropics. Human consumption accounts for more than two-thirds of this crop's total output, with the remainder going to animal feed and industrial use. When compared to other starchy staples, cassava yields more than rice and maize. Cassava is well known for being the most productive crop under marginal conditions, in addition to its enormous potential for agronomic and genetic development. It has spread extensively throughout Latin America, Asia's tropical regions, and Sub-Saharan Africa. Given these characteristics, it is an important crop for supplying energy and nutrition as well as ensuring food security in a number of countries where it is widely grown.

This book discusses four main topics: (1) cassava diversity and its microbiome; (2) cassava disease; (3) enhancement of cassava production; and (4) cassava post-harvest processing. These are critical factors in determining cassava production and agricultural value in the future. The biological and technological aspects of this endeavour have been the primary focus. This is because such actions are critical in ensuring sufficient cassava output to meet customer demand. Harvesting is a critical step in the cassava value chain as well. The development of labour-saving cassava harvesting technologies has emerged as the most pressing issue confronting cassava transformation globally.

I would like to express my heartfelt gratitude to the authors who generously contributed chapters to this book. This project would not have been a success without their contributions. Furthermore, I'd like to express my heartfelt appreciation to IntechOpen. I have gained valuable experience and I look forward to working on additional projects in the future. I'd also like to express my appreciation to Author Service Manager Ms. Maja Bozicevic for her unwavering assistance in compiling materials.

Finally, I hope that this book will assist both scientists and non-scientists in making informed decisions about cassava, including its diversity, biological characteristics, disease, production, and post-harvest processing.

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Section 1

Cassava Diversity and Its Microbiome

Chapter 1

Identification of Cassava Varieties in Ex-Situ Collections and Global Farmer's Fields: An Update from 1990 to 2020

Luis Augusto Becerra Lopez-Lavalle, Adriana Bohorquez-Chaux and Xiaofei Zhang

Abstract

The identification of cassava cultivars is important for understanding the crop's production system, enabling crop improvement practitioners to design and deliver tailored solutions with which farmers can secure high yields and sustainable production. Across the lowland tropics today, a large number improved varieties and landraces of cassava are under cultivation, making it inefficient for breeders and geneticists to set improvement goals for the crop. The identification and characterization of cassava genotypes is currently based on either morphological characters or molecular features. The major aim of cultivar identification is to catalog the crop's genetic diversity, but a consensus approach has still not been established. Of the two approaches to the identification of variety, morphological characters seem to account for most of the genetic variability reported in cassava. However, these characters must be treated with caution, as phenotypic changes can be due to environmental and climatic conditions as well as to the segregation of new highly heterozygous populations, thus, making the accurate identification of varieties difficult. The use of molecular markers has allowed researchers to establish accurate relationships between genotypes, and to measure and track their heterozygous status. Since the early 1990's, molecular geneticists working with cassava have been developing and deploying DNA-based tools for the identification and characterization of landraces or improved varieties. Hence, in the last five years, economists and social scientists have adopted DNA-based variety identification to measure the adoption rates of varieties, and to support the legal protection of breeder's rights. Despite the advances made in the deployment of molecular markers for cassava, multiple platform adoption, as well as their costs and variable throughput, has limited their use by practitioners of crop improvement of cassava. The post-genomic era has produced a large number of genome and transcriptome sequencing tools, and has increased our capacity to develop and deploy genome-based tools to account for the crop's genetic variability by accurately measuring and tracking allele diversity. These technologies allow the creation of haplotype catalogs that can be widely shared across the cassava crop improvement community. Low-density genome-wide SNP markers might be the solution for the wide adoption of molecular tools for the identification of cultivars or varieties of cassava. In this review we survey the efforts made in the past 30 years to establish the tools for cultivar

identification of cassava in farmer's fields and gene banks. We also emphasize the need for a global picture of the genetic diversity of this crop, at its center of origin in South America.

Keywords: cassava, genotype, varieties, SNPs, identification

1. Introduction

Cassava (*Manihot esculenta*) is a key food commodity in the tropics, being the second most important food staple in the least-developed countries, and the fourth highest source of calories in developing countries [1, 2]. Due to cassava's efficient use of soil nutrients and water resources, poor farmers can still expect reasonable harvests in areas where many other crops will fail to be productive. Thus cassava, as an agricultural commodity, has the potential to have significant global impact on nearly all of the United Nations Sustainable Development Goals (SDGs), with emphasis on SDG-1 (no poverty), SDG-2 (zero hunger), SDG-3 (good health and well-being), SDG-12 (responsible production and consumption), SDG-13 (climate action), and SDG-15 (life on land) [3].

Today, a large number of the varieties of cassava which are under cultivation have persisted from pre-Columbian times, having been perpetuated through vegetative propagation, particularly at its center of origin in South America [4, 5]. From South America, this crop spread to sub-Saharan Africa in the 16th century [6], and from South and Southeast Asia (SEA) in the late 18th and early 19th century to Asia [7]. Crop improvement, led by International Institute of Tropical Agriculture (IITA) in Africa and the International Centre for Tropical Agriculture (CIAT) in Latin America and the Caribbean (LAC), as well as in South Asia and SEA, has made improved varieties more common in farmer's fields [2, 8, 9]. For instance, CIAT and Kasetsart University in Thailand developed what is considered to be the most successful variety ever breed, KU50, which has a notably high fresh root yield and dry matter content [10, 11]. Since its official release in Thailand, this variety has spread throughout SEA. In Vietnam, KU50 was released in 1995 as KM94, and was later introduced in Cambodia as Malay [12–14]. It covers nearly one million hectares today. In cassava, it is quite common for the same variety to be re-named when it is introduced to a new area, leading to the existence of synonymous varieties. The opposite situation also occurs, where different varieties are identified under the same name (homonyms) [15–17].

There is currently little understanding of the number of cassava varieties grown throughout the lowland tropics, but, this number is likely to be in the order of thousands, based on the results obtained by Rabbi et al. [2] and Floro et al. [4]. This number can also be estimated from the total number of the crop accessions (genotypes) kept under conservation in different ex-situ gene banks. In 2010, CIAT commissioned a survey of the status of germplasm conservation of cassava across 50 cassava gene banks [18]. Out of the 50 gene banks surveyed, 34 provided information that allowed the estimation that as many as 14,791 distinct landraces were under conservation in gene banks [18]. The real number, however, is likely be significantly lower, once all varieties are characterized using DNA-based molecular markers [2, 4, 12].

In the past 30 years, a body of knowledge about the varietal identification and genetic diversity of cassava has been developed for genetic materials found in ex-situ collections, experimental field trials, and farmers' fields, using morphological descriptors [17, 19–48], morphological descriptors and molecular markers [16, 49–57], and molecular markers alone [2, 4, 12, 15, 25, 58–126]. The morphological

descriptors were first defined by CIAT in the late 70's and early 80's [47, 48], and were later revised by Fukuda et al. [21, 46]. Approximately 75 morphological descriptors, also known as traits, have been defined, and 199 alleles have been made available for distinguishing cassava varieties under ex-situ conservation or to catalog the local varietal inventory of farmers, In more than half of these studies, a measure of their genetic diversity was included [19, 23, 25, 29, 30, 33–37, 40, 41, 43]. Additional efforts to identify cassava varieties have been undertaken, combining morphological descriptors and molecular markers, under the assumption that combining knowledge of farmers with DNA-based genetic profiles should more accurately account for the large genetic differentiation observed among cassava varieties in gene banks, breeding programs, and in famers' fields [16, 49–57].

Across the scientific community investigating cassava, the most widely used methods for identification of varieties, and the estimation of its genetic diversity, have involved molecular markers [127]. Since the advent of DNA-based molecular marker technologies, cassava scientists have adopted nearly all of the most popular techniques to elucidate and describe the crop's varietal identities, diversity, domestication, and ancestry [2, 4, 64, 72–74, 78, 126]. These molecular approaches have focused on two primary objectives: (1) to access an adequate number of highly informative DNA-based molecular markers across the cultivated species; and (2) to assess the crop's global ex-situ germplasm, and that of populations produced at publicly funded breeding programs. Thus, the use of molecular markers could allow building a global varietal haplotype catalog, containing the molecular descriptions of the most common varieties of cassava grown during the last 50 years across sub-Saharan Africa, South and SEA, and LAC. This information will facilitate the development, registration, and release of varieties that will effectively replace old varieties with the latest modern cultivars.

Access to a global catalog of the crop's molecular haplotypes will enable the conducting of studies on the adoption of improved varieties [4]. DNA-based marker technology must be cost-effective, easy to use, and reproducible across laboratories. The reproducibility of molecular marker techniques is extremely important in cassava, due to the presence of fixed somatic mutations, which are potentially caused by clonal propagation, although evidence for this phenomenon is limited [36, 68, 95, 128, 129].

A robust set of highly informative DNA-based markers could be used for variety identification, quality control, and the measurement of genetic diversity, with a potential use in variety registration. Thus, cassava breeders will be able to trace infringements of Plant Breeder's Rights, particularly when the cassava variety is licensed for exclusive commercial use.

2. Morphological descriptors

The need to improve cassava varieties, to fight hunger, malnutrition, and poverty in the tropics, has led to the identification of the problem of discriminating between *M. esculenta* cultigens, particular between landraces and improved types. CIAT and IITA's publicly funded breeding programs have introduced new varieties and cultivars in tropical countries, increasing the number of crop varieties available to farmers in Africa (IITA), Asia, LAC, and SEA (CIAT) [130]. In the late 1970s, CIAT established and evaluated its cassava germplasm collection, developing and using 54 basic morphological descriptors [21], aimed at the efficient selection of parental lines for breeding. In the 2000's, Fukuda et al. [21, 46] revisited the morphological descriptor list by defining 75 descriptors, with the objective of standardizing the characterization data and improving the selection of new exotic

parental lines for breeding in Africa, The aim of this work was to reduce unavoidably subjective interpretations generated using the morphological method in light of the genetic variability of cassava.

Cassava has large phenotypic variance in the field, with a wide eco-geographic adaptation range, suggesting that there is a significant amount of genetic diversity available for breeding. Thus, the identification and differentiation of commercial and landrace cultivars is very important. It has been necessary until recently to rely on the morphological characteristics of the vegetative parts of cassava. Consequently, a range of vegetative descriptors has been used to distinguish cassava varieties from each other in Africa, Asia, LAC, SEA, and Oceania (**Table 1**). The resolution achieved by Fukuda's et al. morphological descriptors can account for cassava's genetic differentiation between accessions, facilitating the understanding of the crop's genetic resources. Among the 28 morphology-based cassava varietal identification and genetic differentiation studies used in this review, the ranges of qualitative (6–44) and quantitative (0–28) morphological descriptors are significantly different (**Table 1**). Approximately one-third of these studies jointly evaluated qualitative and quantitative morphological descriptors, producing a noticeable increase in the number of genetic targets sampled, and thus improving the assessment of genetic diversity in both natural and segregating populations, allowing for the selection of contrasting parents for breeding.

Although cassava displays strikingly high levels of heterozygosity, clonal propagation has permitted the spread of a small set of superior clones, increasing their frequency of occurrence across different regions. This set of clones is grown under large number of different names. A single genotype cultivated in a given geographical region might be found under different names, resulting in the unintentional presence of duplicated genotypes in any one collection. The results of variety identification based on morphological descriptors in cassava has not revealed the presence of these duplicated entries in the ex-situ collections or under cultivation (**Table 1**), although 20 to 25% genotypic redundancy is expected. This review covers a total of 4,285 cassava accessions from Africa, Asia, LAC, SEA, and the Pacific Islands, but the number of duplicate cassava accessions reported is extremely low (1.4%) (**Table 1**). This result might be explained by the high morphological variability reported in cassava due to changes in soil, climatic, and biotic factors, making it difficult to precisely describe the morphological characteristics of this crop. The inability to identify genetic duplicates in a germplasm collection has profound implication for cost-effective germplasm conservation, as well as for germplasm use by breeding programs. Thus, the accurate and reliable identification and elimination of duplicates within a germplasm collection will facilitate genetic resource management and use, while reducing maintenance costs.

These studies have revealed an important heterogeneity within cassava cultivars, particularly those held by farmers [31, 33, 37, 39, 43]. The use of morphological descriptors in the early characterization and identification of cassava varieties is useful to identify new genetic variability, but it can be a lengthy process, taking more than a year to obtain and analyze this type of data. The number of potentially uncharacterized varieties still used in traditional farming is estimated to be as high as 15,000 [18]. Thus, it is likely that the available number of morphological descriptors is inadequate to account for the crop's large genetic variability, as well as the number of cassava cultigens which are affected by environmental factors that influence their phenotypes. This situation highlights the need to develop a method to measure the crop's genetic variability, reducing or eliminating the need to use morphological descriptors. Molecular markers, due to their nature, could provide an immense advantage in the identification of varieties and the characterization of genetic variability, by providing more detailed information about its

Region	Location	Cassava *(M. esculenta)*		Morphological Descriptors				Rev. Ref.
				Scoring Schedule	No. of variables		No. of Dupl.	
		Source	No.		QLT	QNT		
Africa	Côte d'Ivoire	Collection maintained at CNRA's research station at Bouaké	340	5–12 MAP	14	0	35	[42]
	Nigeria	Collection maintained at IITA, Ibadan, Nigeria	1766	NP	32	8	0	[39]
		Collection maintained at IITA, Ubiaja	1890	NP	28	8	0	
	Benin	Field collected in 55 villages surveyed in the southern region	125	NP	20	0	0	[37]
	Côte d'Ivoire	Field collected across 26 villages in the Centre-west, South-west and West region	159	5–12 MAP	14	0	16	[19]
	Chad	Field collected in Mandoul, Moyen Chari, Tandjilé, Logone Occidental and Oriental region	59	3, 6, 9 & 12 MAP	32	13	3	[35]
	Cameroon	Field collected across de Humid Forest & Guinea Savannah Agroecologies	89	3, 6, 9 & 12 MAP	35	14	0	[29]
	Côte d'Ivoire	Field collected in the forest zone of the Ivory Coast	44	NP	20	4	0	[30]
	Angola	Collection maintained at the Agronomic Investigation Institute	40	12 MAP	12	10	0	[25]
LAC	Brazil	Collection maintained at Embrapa Mandioca e Fruticultura, Cruz das Almas	14	8 MAP	10	4	0	[43]
	Brazil	Collection maintained at Mandioca do Cerrado (BGMC) - Embrapa	16	12 MAP	33	0	0	[41]
	Costa Rica	Collection maintained at Centro Agronómico Tropical de Investigación y Enseñanza (CATIE)	37	NP	44	28	0	[40]
	Brazil	Collection maintained at Embrapa Mandioca e Fruticultura, Cruz das Almas	200	NP	19	16	0	[26]
	Brazil	Collection maintained at Embrapa Mandioca e Fruticultura, Cruz das Almas	95	11–12 MAP	32	0	0	[38]
	Brazil	Collection maintained at Embrapa Mandioca e Fruticultura, Cruz das Almas	95	11–12 MAP	35	13	0	[32]
	Brazil	Regional Germplasm Bank of Eastern Amazon, situated in Belém, Pará, Brazil,	262	11–12 MAP	21	0	0	[28]
	Brazil	Collection maintained at Mato Grosso's State University (UNEMAT - Cáceres) and Embrapa Agrossilvipastoril)	158	6–8 & 12 MAP	29	9	0	[23]
	Brazil	Field collected in the Brazilian Middle North Regions, Viçosa-MG	10	8 MAP	24	0	0	[31]

Region	Location	Cassava *(M. esculenta)*		Morphological Descriptors				Rev. Ref.
				Scoring Schedule	No. of variables		No. of Dupl.	
		Source	No.		QLT	QNT		
Asia	India	Western Ghats region of Tamil Nadu, covering 32 villages in the southern region of Western Ghats with altitude ranging from 250 to 2552 feet above MSL	56	NP	6	2	0	[34]
SEA	Indonesia	Field collected in Java, Sumatera, Kalimantan, Sulawesi, Maluku, Nusa Tenggara Timur and Papua Islands	181	12 MAP	10	0	0	[33]
	Vietnam	Collection maintained at the Root Crop Research and Development Center (RCRDC), and Field Crops Research Institute, located in Chuong My, Hanoi	7	4–8 MAP	20	0	0	[20]
Pacific Islands (Oceania)	Vanuatu	Collection maintained at Vanuatu Agricultural Research and Training Centre (VARTC)	145	12MAP	12	0	4	[36]

The number of cassava (M. esculenta) varieties identified in Africa, LAC, Asia, SEA, and Oceania, as well as the number of qualitative (QLT) and quantitative (QNT) descriptors used for the morphological identification. NP = not provided, MAP = months after planting.

Table 1.
The literature pertaining to cassava (rev. ref.) on agro-morphological characterization reviewed in this paper.

polymorphisms, independent of the physiological status of the plant or the environmental conditions in which it grows.

3. Molecular markers deployed in cassava

Since the mid 80's, molecular markers have been used in cassava for a large number of genetic diversity and variety identification studies (**Table 2**). The scientific community working on cassava is therefore well acquainted with the development and use of these markers [64, 71–74, 78, 81, 126, 127, 131, 135–140]. The first attempt to use molecular markers for variety identification in crops was undertaken at CIAT by Hussain et al. [131], Ramirez et al. [139], and Ocampo et al. [122], using isozymes. In 1992, Ocampo et al. [122], using αβ-esterase isozymes, analyzed 86% of the global cassava collection of 4,034 *M. esculenta* accessions maintained at CIAT, and found 2,158 accessions (50%) with 2 to 39 clones sharing the same banding pattern. These results highlight the need to analyze morphological and molecular data together, to gradually eliminate duplicates from the germplasm collection.

In 1995, Ocampo et al. [121] implemented a DNA fingerprinting method for genetic analysis called restriction fragment length polymorphisms (RFLPs). RFLPs allowed Ocampo et al. [121] to estimate the number of duplicates in the CIAT collection; of the 5500 genotype approximately 1,000 could be duplicates indicating an approximate 18% redundancy in the global germplasm collection (**Table 2**). Therefore, the RFLP marker system is an attractive approach because they are inherited in a co-dominant mode, allowing homozygotes to be distinguished from heterozygotes, and are locus-specific and highly informative, targeting specific sites on the genome, due to restriction-site specificity [123]. However, the use of RFLPs can be challenging, as their use is laborious, costly, and can only resolve mutations at the enzyme cut site, limiting their use in phylogenetic reconstruction [123]. Nevertheless, these efforts demonstrate that the identification of genetic variety can be achieved using molecular genetic tools, and used for germplasm management, including quality control of experimental lines across breeding programs.

The polymerase chain reaction (PCR) technique, published in 1986 by Mullis et al. [141], allowed cassava scientists at CIAT to investigate genetic differences using minute amounts of DNA, coupled with random primer amplification to produce random amplified polymorphic DNA (RAPD) [70, 83, 86, 126, 142, 143]. RFLPs were therefore superseded by PCR-based markers [127]. Since then, other PCR-based molecular markers tools have been adapted and deployed, such as amplified fragment length polymorphisms (AFLPs) [72, 73], inter-simple sequence repeats (ISSRs) [56, 115, 117], single sequences repeats (SSRs) [71, 73, 74, 78], sequence-related amplified polymorphisms (SRAPs) [94], inter-sequence tagged repeats (ISTRs) [86], and diversity arrays technology (DArT) [67]. Over the past 30 years, SSRs have been the molecular marker approach most widely used in cassava, both for variety identification and to estimate the genetic diversity of the crop (**Table 2**). Chavarriaga-Aguirre et al. [73], used this approach to search for duplicates in the CIAT's core collection, but reported a lower frequency of duplicates than was reported by Ocampo and co-workers [121].

The release of the cassava reference genome by Prochnik et al. [144] allowed cassava geneticists in Africa and LAC to identify tens of thousands genome-wide sequence variations across multiple landraces and improved cultigens [2, 145, 146]. These genomic variations were unraveled by re-sequencing using restriction-site associated DNA-sequencing (RAD-seq) [145] or genotyping by sequencing (GBS) [147]. These two methods can detect small genetic differences between individuals, and therefore may be useful for studying organisms with reduced genetic

Region	Location	Source	No. of cassava samples	Morphological Descriptors	Isozymes	RFLPs	RAPDs	SSRs	ISSRs	SRAPs	ISTR	AFLPs	DaRTs	SNPs	No. of Duplicates	Rev. Ref.
Global	A	1	19		X										0	[131]
	A	1	4304		X										2158	[122]
	A	1	88			X									51	[121]
	A	1	7			X	X								0	[70]
	A	1	105	X								X			0	[72]
	A	1	552					X							0	[74]
	A	1	521	X	X			X				X			19	[73]
	A	1	38					X							0	[71]
	B	1&2	74											X	0	[89]

Region	Location	Source	No. of cassava samples	Morphological Descriptors	Isozymes	RFLPs	RAPDs	SSRs	ISSRs	SRAPs	ISTR	AFLPs	DaRTs	SNPs	No. of Duplicates	Rev. Ref.
Africa	C	3	365		X										181	[124]
	D	2	29									X			10	[66]
	D&E	4	283					X							0	[81]
	E	5	24				X				X				0	[86]
	F	6	28				X								1	[83]
	G	7	288	X				X							0	[53]
	D	2	63					X				X			0	[91]
	H	8	93	X								X			5	[50]
	B	1&2	53											X		[59]
	D	2	24					X							0	[101]
	I	9	43	X				X							10	[49]
	E	10	21					X							1	[58]
	I	11	320					X							0	[106]
	B	12	10					X							1	[104]
	J	13	12	X					X						0	[56]
	C	2&14	327		X										163	[15]
	I	15	981											X	629	[2]
	D	16	7376											X	2594	[63]
	K	17	96	X				X							22	[16]
	I	1, 2&11	89					X							0	[118]
	I	2&11	87	X										X	9	[119]
	G	18	547											X	461	[116]
	L	19	102	X										X	0	[112]

Region	Location	Source	No. of cassava samples	Morphological Descriptors	Isozymes	RFLPs	RAPDs	SSRs	ISSRs	SRAPs	ISTR	AFLPs	DaRTs	SNPs	No. of Duplicates	Rev. Ref.
Asia	M	20	218	X	X										62	[62]
	M	21	58	X				X							2	[52]
	M	20	110	X				X							0	[132]
	M	20	45					X							0	[93]
	N	21	18							X					0	[94]
	M	20	6						X						0	[117]
	M	20	14					X	X						0	[115]
EU	O	3	80			X									0	[123]
	O	3	20				X								2	[126]
LAC	P	22	32	X			X								1	[64]
	Q	23	31									X			0	[76]
	P	24	54				X	X				X			0	[133]
	P	1&25	118				X	X							0	[78]
	Q	26	29	X								X			0	[68]
	P	27	28		X										0	[80]
	P	28	117					X							0	[82]
	P	29	20		X										0	[85]
	R	30	66		X										0	[84]
	A	1	33										X		0	[67]
	P	31	137	X				X							42	[69]
	R	30	145					X							0	[88]
	P	32	42					X							2	[90]
	P	33	83					X							1	[98]

Region	Location	Source	No. of cassava samples	Morphological Descriptors	Isozymes	RFLPs	RAPDs	SSRs	ISSRs	SRAPs	ISTR	AFLPs	DaRTs	SNPs	No. of Duplicates	Rev. Ref.
LAC	P	34	14	X			X								0	[51]
	S	35	185					X							2	[95]
	P	25	93					X							4	[96]
	P	33&41	20					X							0	[99]
	P	36	16	X				X							0	[97]
	P	37	36					X							19	[61]
	P	38	419	X				X							0	[54]
	P	36	16	X			X								0	[55]
	P	25	1280											X	0	[103]
	A	39	173	X										X	20	[105]
	T	1&40	163					X							0	[108]
	P	41	60					X							1	[125]
	P	42	121					X							5	[109]
	A	43	436											X	316	[4]
	P	44	51					X							0	[110]
	P	25	2731											X	614	[134]
	P	45	303					X							18	[120]
	P	46	106					X							4	[113]
	P	47	144					X							0	[114]
	P	25	3354											X	1818	[65]

Region	Location	Source	No. of cassava samples	Morphological Descriptors	Isozymes	RFLPs	RAPDs	SSRs	ISSRs	SRAPs	ISTR	AFLPs	DaRTs	SNPs	No. of Duplicates	Rev. Ref.
SEA	U	48	10	**X**	**X**		**X**								0	[111]
	V	49	1570											**X**	1535	[12]
Total			29730	21	10	3	10	37	3	1	1	8	1	13	10783	

Plant materials used: [1] Collection maintained at CIAT's GRU, [2] Collection maintained at IITA's GRU, [3] Collection maintained at ORSTOM (IRD-France), [4] Field collection in Tanzania and Nigeria, [5] Field collected in Kibaha and Ikiriguru, [6] Collection maintained at National Agricultural Research Institute, Maputo, [7] Field collected in Hoima, Kumi, and Luwero districts, [8] Field collected in Baka, Mkondezi, Chitala, Chitedze, and Makoka Agricultural Research Stations, [9] Collection maintained at Plant Genetic Resource Research Institute at Bunso in the Eastern Region of Ghana and the University of Cape Coast, [10] Collection maintained at MARI, [11] Collection maintained at CSIR-Crops Research Institute (CRI), [12] Collection maintained at Kenya Agricultural Research Institute (KARI), [13] Collection maintained at University of Kinshasa, [14] Collection maintained at CNRA, [15] Field collected in Ghana Brong Ahafo, Ashanti, and Eastern, [16] Field collected in North Central followed by South–South, Southwest, and Southeast regions, [17] Field collected in Southern and Central Benin, [18] Field collected in Apac, Arua, Kibaale, and Masindi, [19] Collection maintained at Njala Agricultural Research Centre (NARC), [20] Collection maintained at ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvanathapuram, [21] Tapioca and Castor Research Station (T&CRS) GRU, [22] Field collected in Campinas, [23] Field collected in Makushi Village, [24] Field Collected in Sao Paolo state, [25] Embrapa Cassava and Fruit Crops, Cruz das Almas, BA, [26] Field collection in Rewa, a Makushi community, [27] Field collection in Pernanbuco, [28] Field collection in Amazonian region, [29] Field collected in Maringá, [30] Field collection in Talamanca and Coto Brus, [31] Southeastern part of the Brazilian Atlantic Forest, [32] Genetics Department of ESALQ/USP, [33] Instituto Agronómico at Campinas, Sao Paul, [34] Field collected in Maringá, Paraná, [35] Field collected across the island and gene bank, [36] Banco Regional de Germoplasma de Mandioca do Cerrado (BGMC), [37] Embrapa's germplasm collection Belém, Pará, [38] Escola Superior deAgricultura Luiz de Queiroz, Sao Paulo University, [39] Field collected by SINCHI, [40] Cuban Cassava Germplasm Collection, [41] Field collected in Campo Grande, Mato Grosso do Sul, [42] Field collected in Maringá, Cianorte, and Toledo, Paraná, [43] Field Collected in Cauca, [44] Field collected in Southern of Minas Gerais State, [45] Field collected in Paraná and Santa Catarina (South), Mato Grosso do Sul (Midwest), and Minas Gerais (Southeast), [46] Embrapa - Acre, Rio Branco, [47] Germplasm Bank (BGM) of Maringá State University (UEM) Paraná state, [48] West Bangka District, Bangka District, and South Bangka District, [49] Field collected across 32 villages and HLARC, RCRDC, and AGI's GRUs.
Countries where the studies took place: [A] Colombia, [B] Kenya, [C] Ivory Coast, [D] Nigeria, [E] Tanzania, [F] Mozambique, [G] Uganda, [H] Malawi, [I] Ghana, [J] Congo, [K] Benin, [L] Sierra Leone, [M] India, [N] China, [O] France, [P] Brazil, [Q] Guyana, [R] Costa Rica, [S] Puerto Rico, [T] Cuba, [U] Indonesia, [V] Vietnam.

Table 2.
Literature reviewed on the molecular characterization of cassava (rev. ref.).

variation, such as those found in clonal lineages, such as cassava, or highly inbred organisms, such as maize. However, one has to ask whether the large number of SNPs resolved with or without prior knowledge of the genome are more reliable than SSRs or SNP arrays built from expressed sequence tag databases with a high frequency of heterozygous loci in the population. Two SNP arrays have been built for cassava: the Illumina GoldenGate 1,190SNPs-assay by Ferguson et al. [59], and the Fluidigm® Dynamic 96 SNP Array™ SNPY-Chip by Becerra Lopez-Lavalle and co-workers at CIAT [4, 12, 105].

4. Current status of PCR-based DNA analysis for variety identification

PCR-based DNA molecular markers have been used to assess the genetic diversity of cassava, and to establish the relationships among genotypes (**Table 2**). In 1994, Marmey et al. [126] showed the value of RADPs for analyzing the crop's genetic diversity, as well as for detecting duplicated accessions (10%) among collections. In 1996, Angel et al. [70] showed that RADPs give comparable results to RFLPs, offering a cost- and time-effective alternative to restriction and hybridization DNA analysis. Another powerful PCR-based molecular marker tool used in cassava [72] is the AFLP method used by Vos et al. [148], in which selected restriction fragments from the digestion of total DNA are reduced in complexity by PCR and resolved with 1 to 2 bp difference. Roa et al. [72] concluded that AFLPs were an effective and efficient molecular methodology with which to estimate genetic similarities in the genetic variability of cassava, and among other Manihot species.

Of the 77 studies listed in **Table 2**, 13% used RAPDs, and 12% used AFLPs, including studies incorporating morphological descriptors [50, 51, 55, 64, 68, 72, 73, 111]. Both molecular marker methods have been shown to be powerful and able to provide genetic data that reflects the observed phenotypic differences, geographic origins, and pedigree background of the plants. The AFLP fingerprinting technique detected a larger number of duplicates in the African and LAC cassava landraces than RAPDs, suggesting that AFLPs are a suitable for estimating genetic similarity and dissimilarity [72]. The identification of duplicates across these studies ranged from 4 to 35%. AFLP data indicated that cassava varieties can become widespread and adopted by farmers under different names, leading germplasm curators to consider them to be different varieties.

SSRs, which were used in 47% of the studies reviewed here (**Table 2**), and their use has been favored over that of RADPs or AFLPs in cassava. SSR markers are abundant and evenly distributed across the cassava genome, are co-dominant, highly polymorphic, and are not influenced by the environment [149]. Compared with AFLPs, SSRs are less technically challenging to implement. These marker system data can easily be shared across different laboratories, particularly if fingerprinting data is generated with fluorescently labeled SSR markers and resolved in capillary DNA-sequencing instruments. Overall, the authors consulted for this review agreed that SSR profiles generated for improved and landrace genomes were extremely useful in the conservation of diversity in Africa, Asia, LAC, and SEA, as well as for guiding the best crop improvement strategy. Studies involving the development of molecular tools to accelerate the introgression of observed phenotypic differences on disease resistance, such as cassava mosaic disease (CMD) have been extremely successful in identifying the SSRs that will best guide this effort. CMD resistance has been efficiently introgressed into LAC's breeding lines, and successfully transferred to Africa [150–152].

Over the last decade, we have witnessed an important shift in the cassava research community in Africa and LAC, led by IITA and CIAT, toward

sequence-based nucleotide variation mining. In sub-Sahara Africa, Ferguson et al. [59] characterized and validated 1,190 SNPs using the V4.1 of the cassava genome [144] and Illumina's GoldenGate assay. They demonstrated that SNP markers could successfully measure the genetic variability of cassava, while accurately detecting duplicates in the IITA's gene bank collection. The SNP data of Ferguson et al. [59] allowed, the comparison of the genetic diversity between cassava varieties from the Americas and Africa, and showed that cassava from the Americas displayed greater genetic diversity than their counterparts in Africa. These researchers showed that the levels of genetic diversity in west, southern, eastern, and central Africa were similar. These two observations suggested a massive adoption by IITA of improved varieties developed for African farmers.

In 2015, Rabbi et al. [2] undertook a large varietal identification survey on 917 accessions using 56,489 SNP loci generated by next-generation sequencing [147], compared against 64 released cassava varieties and popular landraces in Ghana. Rabbi et al. [2] accomplished variety identification and ancestry estimation through two complementary cluster methods: distance-based hierarchical clustering, and model-based maximum likelihood admixture analysis. They found that 30% of the identified accessions from farmers' fields matched specific released varieties. A hierarchical clustering analysis revealed that the number of major varieties was 11, and 69% of the accessions belonged to one of the 11 groups, while the remaining accessions had two or more ancestries. Rabbi et al. [2] demonstrated that reduced subsets of SNP markers could reproduce the results obtained from the full set of markers, concluding that GBS can be performed at higher DNA multiplexing. However, these results, as well as those by Ferguson et al. [59], indicated that a large numbers of SNPs may not be needed to achieve accurate identification of cassava varieties, whether in farmers' fields or in formal germplasm collections.

Concurrently, CIAT and the Beijing Genome Institute (BGI) [153] committed to developing genomic resources in the post-genomic era, with the aim of increasing scientists' understanding of the evolution and distribution of cassava from its origin in the Americas to Africa and Asia. Next-generation sequence information from both wild and domesticated species offered cassava researchers the opportunity to investigate individual genes which have played a role in the domestication of cassava. Whole genome sequences allow researchers to exploit genomic variations associated with resistance to pests such as whiteflies or mites, and diseases such as frog skin disease and cassava brown streak disease, as well as to improve the nutritional value of the crops, such as by increasing the pro-vitamin A content. In 2013, CIAT's geneticists and bioinformaticians explored the genetic variation present in 150 LAC accessions, and identified a panel of 180 highly informative single nucleotide variants (SNVs, MAF > 0.25), with high discriminative power and a uniform genome distribution of 5 to 10 SNP per chromosome. These SNVs were transferred to a SNPtype™ allele-specific PCR assay and validated on the same set of samples (Fluidigm® Dynamic Array™, USA) (Becerra Lopez-Lavalle, personal communication).

Of the 180 SNVs identified by CIAT, a 96 SNPs Fluidigm® Dynamic Array™ (referred to as an "SNPY-CHIP") was first assembled and used by Peña-Venegas et al. [105], who aimed to validate the identity of 173 Amazonian cassava landraces classified as unique by indigenous growers. The cassava SNPY-CHIP allowed the classification of 44 genotypes into 21 duplicate-genotype clusters, confirming the uniqueness of 150 (87%) of the 173 materials identified as unique by indigenous people of the Colombian Amazon. The SNPY-CHIP array also allowed the exploration of the diversity and population structure of these materials. When the 150 unique genotypes characterized in this study were compared with genotypes from the CIAT core collection, the cassava genotypes from the Tikuna community of San Martín de Amacayacu (AMA) appeared to be closely related to Peruvian manioc

genotypes (PER). CIAT scientist demonstrated that these SNP markers have a very low genotyping error rate, and are easy to store and share in genotype databases. The information generated from the 99 accessions evaluated along with the 150 from the Peña-Venegas et al. [105] study allow us to assess the value of each SNP with a high MAF, indicating a genotyping success. The 99 cassava genotypes represents a good sampling of the global cassava germplasm collection. Of the 99 genotypes, 71 were from the Americas: five from Argentina, two from Bolivia, four from Brazil, 26 from Colombia, three from Costa Rica, three from Cuba, three from Ecuador, five from Guatemala, five from Mexico, two from Panama, two from Paraguay, five from Peru, one from Puerto Rico, and five from Venezuela; nine from Asia: two from China, three from Thailand, two from Indonesia, and two from Malaysia; three hybrids from ICA-CIAT (Colombia), three genotypes from Africa (TMS60444, C18 and TME3) and 13 samples of unknown origin (AM206-5, AM560-2, FLA 21, FLA61, FLA 19, GLA8, GM905-52, GM905-57, GM905-60, SM301-3, SG107-35, GUT64, and JAC3). These 99 genotypes exhibit good phenotypic differentiation and are likely to be of ancient origin in the Americas. Of the 272 genotypes analyzed, 249 (91%) were unique genotypes, showing the effectiveness of these SNPs for varietal identification and the identification of duplicates (9%). The SNP results unequivocally identified all accessions, including those nominated as morphological duplicates.

The accurate identification of cassava varieties in the Colombian Amazon using DNA-based SNPY-CHIP provided the opportunity to undertake large variety adoption studies using SNP-based DNA fingerprinting. This approach established the basis for the methodology of a multidisciplinary approach and for synergy of efforts between agricultural scientists and economists [4]. Floro et al. [4] estimated the level and determinants of adoption of improved varieties in the Cauca department of Colombia, using the SNPY-CHIP. They collected cassava samples from each variety identified by cassava growers, and interviewed 217 households in Cauca, Colombia. Four hundred and thirty six cassava samples were collected, and DNA fingerprinting was undertaken using the SNPY-CHIP. The genetic analysis allowed the identification of duplicated genetic material, as well as the improved hybrids developed by CIAT, thus reducing the 117 named varieties by farmers to 60 true genetic types found in CIAT's germplasm collection or its global cassava breeding program (**Figure 1**). A set of 60 unique genotypes was identified showing this set of genotypes are missing at CIAT's germplasm collection (**Figure 1**). DNA fingerprinting was therefore shown to be important in the procurement of new germplasm to introduce into breeding programs or furnish publicly funded gene banks with the most diverse and complete set of accessions. The cassava genetics research team at CIAT reorganized the 436 stem samples collected, and planted them back in the Cauca region in the Morales Municipality, to assess their morphological features. The morphology displayed by each of the 120 varietal plots confirmed the results obtained by SNP fingerprinting (**Figure 2**).

An ambitious variety adoption study using DNA fingerprinting (SNPY-CHIP) and socioeconomic approaches was undertake by CIAT scientists in Vietnam [154]. The cassava germplasm found in Vietnam has very limited morphological description and molecular information, limiting its use for breeding. However, farming communities in Vietnam have maintained traditional knowledge about this genetic diversity through the vernacular names given to varieties. Depending on the context, however, informal naming of varieties can lead to either overestimates or underestimates of crop diversity. Ocampo et al. [12] studied the varietal composition found in Vietnamese cassava production regions using SNP markers. They procured 97 different varieties based on farmer identification, from a total of 1,570 cassava genotypes collected across six agro-ecological zones. Vietnamese farmers distinguished the different varieties mainly by the morphology of the vegetative parts, such as Bamboo Leaf, Long Leaf, Purple Bud, Red Bud, and Red

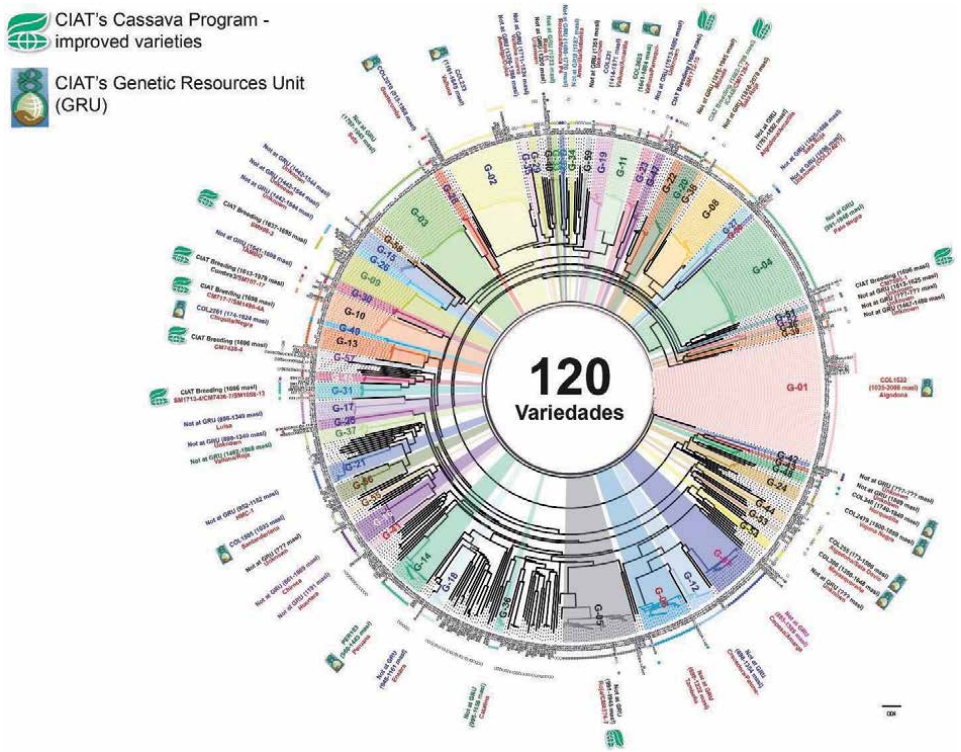

Figure 1.
Cluster analysis of 436 accessions constructed with the neighbor joining (NJ) method using shared alleles to define genetic distances.

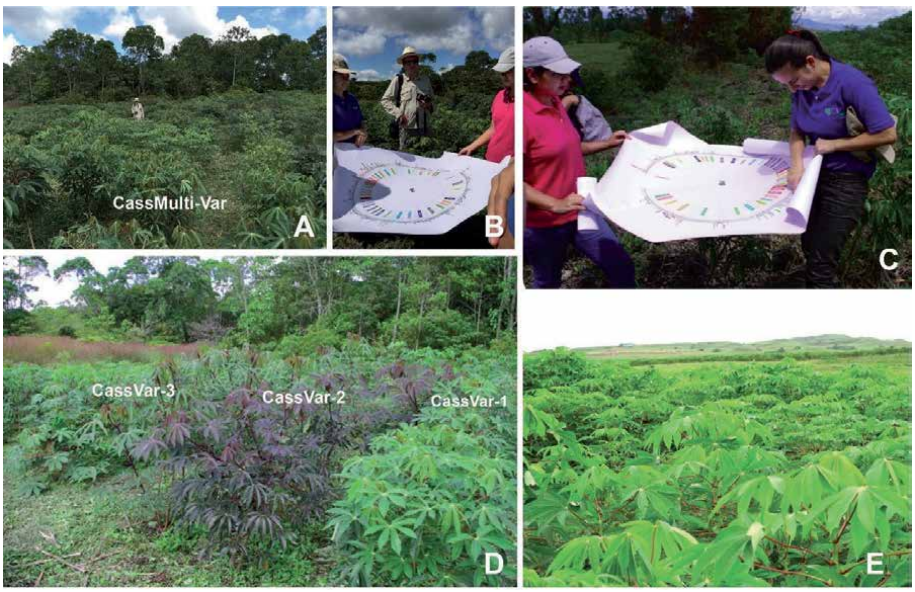

Figure 2.
(A) The process of field-based varieties collection in Cauca, Colombia, (B&C) molecular-based selection, (D) cassava variety [CassVar] clustering for morphological validation based on molecular information and (E) agronomic performance evalaution of variety clusters.

Branch. CIAT's SNPY-CHIP allowed for the characterization of 85 distinct genetic groups out of the 1570 genotypes collected, and indicated a 12.4% overestimation of varietal differences based on vernacular names given by local farmers. When compared against CIAT's global germplasm reference set, the allele diversity contained in 85 genetically distinct varieties represents a rich and diverse collection. Hence, a set of ten major varieties grown across Vietnam, named KM94, KM419, BRA1305, KM101, KM140, PER262, KM60, KM57, and two unidentified varieties with a high accounted for 82% of the frequency distribution, of which KM94 (KU50) and KM419 represented 48% of the genotypes investigated.

5. Conclusions: challenges and future perspective for the varietal and cultivar identification of cassava

This review has highlighted the potential of SNP-based variety identification in cassava, as a means to assess the rate of variety adoption, acquisition of novel genetic resources, and quality control of breeding products. Further progress toward a full characterization of varieties across all cassava growing regions, using SNP-based approaches, can be anticipated. Among the 21 morphological and 77 molecular-based variety identification studies used in this review (**Tables 1** and **2**), those based on morphological descriptors are lengthy, time consuming, labor intensive, and space demanding. As the number of varieties to be evaluated increases, the number of morphological descriptors available for the identification of new genotypes is limited.

The basic principles of molecular marker technologies focus on the detection of polymorphisms, from protein or ribonucleic acid information. For cassava isozymes, RFLPs, RAPDs, SSRs, ISSRs, SRAPs, ISTR, AFLPs, DaRTs, and SNPs have been successfully used for detecting genetic variation in the crop (**Table 2**). Of these markers, SSRs are by far the most popular molecular method used by cassava scientists order to describe the differentiate among varieties and to measure the crop's genetic diversity. Nearly 17% of the 4,950 materials that underwent varietal identification were fingerprinted using SSR markers. However, SSR-based fingerprinting data has limited use outside the discrete experimental units evaluated in this review, thus limiting the opportunity to consolidate and globally use SSR genotyping information into a general database, which could enable a global variety identification system.

Unlike SSRs, SNP alleles have been recommended for the construction of shared DNA fingerprinting databases [155]. CIAT's newly designed SNPY-CHIP has been used to genetically characterized approximately 2,100 cassava genotypes, collected from both farmers' fields and in ex-situ collections (**Table 2**). This set of 96 single SNPs are well-distributed throughout the cassava genome. These SNP markers have proven to be stable and repeatable, and have a high power of discrimination. The SNPY-CHIP alleles initially deployed in the Fluidigm® Dynamic Array™ technology (San Francisco, CA, USA) should be transferable across platforms, allowing for direct global data analysis, with SNP information coming from next-generation sequencing performed by other laboratories or research groups. CIAT, through CGIAR, has a collaborative agreement with Intertek-AgriTech (https://www.intertek.com/agriculture/agritech/) to access genotyping services, thus ensuring high quality, cost-effective data production. The emphasis on high quality breeding products stresses the need for quality control at all levels of the variety development pipeline, ensuring traceability and preservation of identity. This is the first step toward building a robust identification platform for the global conservation and use of cassava, as well as standardizing the administration and management of plant varieties. Considering the effectiveness the 96 SNPY-CHIP

markers, in 2021, we transferred them to the Intertek genotyping platform with the support from the Excellence in Breeding (EiB, https://excellenceinbreeding.org/toolbox/collection/236), and 93 markers passed the validation stage with 345 diverse accessions from the genebank and breeding progenitors. These markers are publicly available in the EiB low-density genotyping platform for quality control and variety identification for the cassava community. A databased with more than 2,100 accessions genotyped using these 96 SNP markers has been developed and maintained in the cassava program at CIAT, which will enhance the variety identification and genetic diversity analysis for the global cassava community.

As growing emphasis is placed on quality, at all levels, and on traceability and the preservation of the identity of varieties, accurate identification of the varieties of cassava grown by farmers will improve its management and production, and facilitate tracking and replacing specific varieties. Breeders can replace varieties susceptible to pests and diseases with more tolerant or resistant varieties. Knowledge of the distribution of susceptible varieties will help policy makers to target breeding for the development of resistant of tolerant varieties for full varietal replacement and seed system development.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 2

The Microbiome of Cassava (*Manihot esculanta*)

Andri Frediansyah

Abstract

The plant microbiome, like the plant, influences the processes that lead to plant development, health, and crop productivity. Cassava is a perennial herbaceous plant native to South America that has been cultivated for centuries as a staple food throughout the world. Not only is cassava a good source of carbohydrates, but it also has a high tolerance for a variety of phenotypic conditions, and the majority of cassava plants are susceptible to a variety of diseases. Thus, using cassava as a model, this chapter discusses the plant microbiome. We discuss the structure and function of the microbiome, as well as the technique for studying microbiomes. Additionally, we conducted a systematic review of references pertaining to the microbiome of the cassava plant using cultivation-dependent or cultivation-independent methods. Numerous significant genera of bacteria and fungi are found in cassava's phyllosphere and rhizosphere, including groups of gram-negative bacteria, gram-positive Actinobacteria, and gram-positive non Actinobacteria. Additionally, we identified critical organisms in the phyllosphere and rhizosphere. Cassava endophytes also produce antifungal secondary metabolites such as pumilacidins and surfactin. The investigation of their phenotypes and interactions with the cassava plant will aid in increasing productivity.

Keywords: cassava microbiome, metagenomic, plant microbiome, staple crop, phyllosphere, rhizosphere

1. Introduction

The microbiome was defined for the first time as the ecological niche within the human body where symbionts, pathogens, and commensal or neutral microorganisms coexist [1]. It is then widely used in a variety of habitats infested with microorganisms, including plants and their microbes. As with the plant itself, the plant microbiome influences the various processes that contribute to plant development, health, and crop productivity [2]. These connections have an effect on both nutrient absorption and susceptibility to biotic and abiotic stress [3]. Furthermore, factors such as regional landscape, plant species and cultivars, genotypes, soil, soil-borne microorganisms, climate and other environmental factors, farming management practices, and crop safety all influence the microbiome's dynamic and distribution [4–6]. Moreover, microbes associated with plants colonized both the plant's surface and internal tissue. They are frequently referred to as the plant's second genome due to their presence in the inner plant bodies as well [7]. Additionally, the complexity of nearly all plant microbiomes including its rhizosphere is still unknown [8].

Additionally, there is still a knowledge gap regarding plant-colonizing microbes, their interactions, and the microbiome's structure.

Cassava (*Manihot esculenta* Crantz) is an herbaceous perennial plant native to South America that is a member of the *Euphorbiaceae* family [9]. It is widely grown in tropical and subtropical regions [10]. Cassava was grown on a global scale of up to 201 million hectares in 2017, with Africa accounting for more than 60% of the total [11]. Furthermore, Nigeria was the largest producer of cassava, followed by Thailand and Indonesia [9, 11]. Cassava's tuberous roots contain an unexpected amount of starch, making it an extremely valuable food source, particularly in developing countries. As a result, cassava has developed into a staple food for roughly 800 million people worldwide [11]. Crop management and fertilization [12], food process development and fermentation [9, 13–17], component functional status [18], cassava disease [19, 20], and raw material and product quality control [21, 22], are just a few of the cassava-related studies published worldwide.

Cassava plants, like other plants, support a diverse range of microorganisms and plant-microbial interactions that enable the crop to perform a variety of task [23]. As illustrated in **Figure 1**, the cassava microbiome is distributed throughout the plant's body, including the portion of the upper and lower leaf surface (phyllosphere) that contains stems (caulosphere) and leaves (phylloplane), as well as the portion of the bellow grounds that contains roots and a trace of associated soil (rhizosphere). Within compartments, fungal and bacterial (and, to a lesser extent, archaeal) communities can be classified as epiphytes, which colonize the exterior surface of plant tissues, and endophytes, which penetrate the outermost plant cell layer (epidermis) and colonize the internal intercellular and intracellular sections of plant tissues.

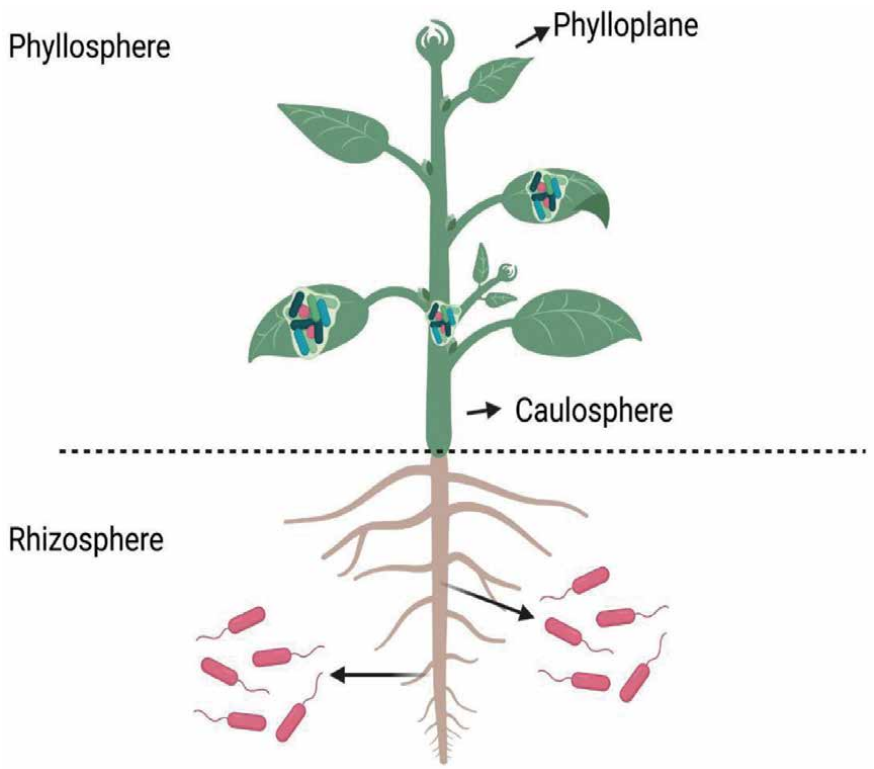

Figure 1.
The distribution of microbiome in cassava plant.

Cassava is not only a good source of carbohydrates, but it also has a high tolerance for a variety of phenotypic conditions, including heat, nutrient deficiency, and drought [24, 25]. Additionally, the majority of cassava plants are susceptible to a variety of diseases, including cassava brown streak disease, cassava mosaic disease, and cassava bacterial blight [26–28]. As a result, our understanding of these correlations with the vastness of the microbiome is still limited at the moment. Thus, in this chapter, we will use cassava as a model plant to investigate its microbiome. Each cassava plant compartment is thoroughly examined. Additionally, we discuss the microbiome's structure and function, as well as the data collection methods used. Finally, we investigate the possibility of manipulating the microbiome to increase cassava crop productivity.

2. The technique to study cassava microbiome

There are two approaches to studying the cassava microbiome: cultivation-dependent and cultivation-independent approaches. Historically, the cultivation-dependent method was used to evaluate microbial communities. This strategy is based on the technique of microbial isolation. However, this is debatable given that only 1% of microbes can be cultured in the laboratory [29]. This is because a variety of factors affect microbes' cultivability, including nutrients, oxygen levels, temperature, salinity, pH, and growth factor [30, 31]. This technique has numerous advantages, including the ability to cultivate culturable microbes, the ability to quantify the cell, and the ability to identify viable cells in samples. Therefore, as consequence, using this approach results in a low level of taxonomic diversity, contamination, the requirement of time and resources, and the reliance on phenotypical biochemical characterization.

Without cultivating the bacteria, a molecular technique utilizing unprecedented amounts of 16S RNA or ITS data, such as denaturing and temperature gradient gel electrophoresis [32] and single-strand conformation polymorphism [33]. Additionally, polymorphisms in the terminal restriction fragment length [34], restriction analysis of amplified ribosomal DNA [35], random amplified polymorphic DNA [36], and sequencing of SSU ribosomal DNA [37], can be used to determine the microbial composition of the sample.

Moreover, recent advances in high-throughput sequencing, combined with a variety of omics techniques [38–41], have enabled researchers to gain a new level of understanding of the microbiome's structure and dynamics, as well as host-microbiome interactions. It also can provide a wealth of information about the microbial partners of a plant, including their identity and relative abundance [42, 43]. Therefore, employing this cultivation-independent approach, using sequencing technology, may result in an avalanche of data, which must be mitigated by using an experimental design and technique that are appropriate for the scientific question at hand [44–46]. It is critical to have a thorough understanding of the various types of biases and errors that can occur when selecting the system.

In plant microbiome research, including cassava, high-throughput sequencing of marker gene amplicons is increasingly being used to elucidate the structure, organization, and spatial distribution of microbial communities [5, 47–49]. Amplicon sequencing has the distinct advantage of being able to target specific microbe classes or even functional genes. Although the high specificity of amplicon sequencing enables it to positively classify unusual organisms, it is susceptible to contamination due to its sensitive nature [50]. Thus, any experiment involving a significant amount of amplicon sequencing should include both positive and negative controls [51].

When it comes to confirming the existence of rare organisms, shotgun metagenomics is less robust than amplicon sequencing [52–54]. The abundances measured, on the other hand, are less skewed, and the data can be binned into draft genome sequences [54–56]. These enable us to connect taxonomic identity to essential plant functions like nitrogen fixation, or to determine whether symbionts can communicate with plants via secretion systems or effectors. Metagenomic approaches also can supplement other high-throughput molecular methods such as transcriptomics, proteomics, and metabolomics [57–59].

In general, these techniques provide access to a microbial genetic pool that cultivation-dependent techniques do not provide, which means that microbial isolates do not need to be cultured because sequences are generated directly from environmental samples. High specificity and the ability to freeze samples for later use are also advantages. However, we were unable to obtain colonies for further research. Furthermore, there is a high risk of contamination with this technique, and the researchers are unable to distinguish between living and dead cells. Last but not least, the method is dependent on a well-designed primer plate, precise sequence identification, and a high-quality cell lysis process.

3. The phyllosphere and its microbiome

The phyllosphere is the first compartment in the microbiome of the cassava plant. This compartment is the visible portion of the leaf surface on both the upper and lower leaf surfaces [60]. Cover only the area above the ground, however. Microbial cells can colonize arial plant surfaces such as leaves (phylloplane) and stems in this environment (caulosphere) [61]. Leaves may be one of the largest microbial habitats on the planet, with an estimated global terrestrial leaf surface area of 10^8 km^2 [62]. Along with bacteria, filamentous fungi, archaea, viruses, yeast, bryophytes, lichens, protozoa, and nematodes thrive in this environment. Bacteria, on the other hand, have been found to be the most abundant cell type in the phyllosphere, with up to 10^7 cells cm^{-2} of leaf tissues present [63]. Another type of microorganism, filamentous fungi, appears to be more prevalent [63]. For all of these leaves' living things, water and food are scarce resources.

Special consideration will be given to endophytes when it comes to the cassava microbial community. Endophytic microorganisms are microorganisms that live inside the tissues of plants without harming the host [64]. The majority of endophytes spread systemically via the xylem to various plant compartments such as the stem, leaves, and fruits. They maintain the plant's viability throughout or part of its life cycle by colonizing the internal leaf tissues (endophyllosphere) and internal plant reproductive tissue [65]. Due to the fact that they live within the tissue, their nutritional requirements are also reduced [66]. As consequences, they multiply and grow rapidly within the plant tissue. They defend themselves by producing toxins and enzymes that aid them in colonizing the plant and competing with other microorganisms. Additionally, several of them produce beneficial secondary metabolites such as antibiotics, antifungals, anti-inflammatory agents, and biological control agents as part of the host's development and physiological process [67].

Melo, Fiore [68] successfully cultured several endophytes bacteria from the cassava phyllosphere using a cultivation-dependent approach. They were able to grow bacteria from cassava stems (23 strains) and leaves (17 strains). The 16S rRNA coupled with fatty acid methyl ester (FAME) assay could only be used to examine a small number of bacteria. *Bacillus* was found to be the most prevalent bacteria in this study [68]. *Bacillus anthracis, Bacillus pumilus, Brachybacterium paraconglomeratum,* and *Brevibacillus brevi* were discovered in the cassava stem, as well as

gram-negative bacteria *Enterobacter aerogenes, E. cancerogenus, Salmonella enteritidis, S. bongori, S. choleraesus, Escherichia coli,* and *Serratia rubidae* [68]. Furthermore, *Bacillus cereus, Clavibacter michiganensis, Curtobacterium luteum, Microbacterium aerborescens, Microbacterium imperial,* and *Ochrobactrum antropi* were the predominant bacteria in cassava leaves, followed by gram-negative bacteria such as *Pseudomonas rhodesiae* and *Enterobacter cloacae* [68], as shown in **Table 1**. They also demonstrated that environmental factors largely determined the phyllosphere's microbial composition.

Interestingly, *Bacillus pumilus* isolated from stem cassava was considered as a biocontrol agent with anti-fungal activity in a detailed study conducted by Melo, Fiore [68]. This rod bacteria produces pumilacidins A–E, as shown in **Figure 2**. The molecular formulas of pumilacidin A, B, and C are $C_{54}H_{95}N_7O_{13}$, $C_{53}H_{93}N_7O_{13}$, and $C_{56}H_{99}N_7O_{13}$, respectively. Moreover, pumilacidin D and E share a molecular formula of $C_{55}H_{97}N_7O_{13}$. However, the amino acid valine was substituted for ileusin in pumilacidin D, resulting in pumilacidin E.

Another study from Canova, Petta [70] discovered that *Paenibacillus* sp. IIRAC-30 from cassava could produce a major surfactin C (**Figure 2**) compound with the molecular formula $C_{53}H_{93}N_7O_{13}$ and a $[M + H]^+$ on peak at m/z 1037.0. This strain also produces surfactin A ($C_{51}H_{89}N_7O_{13}$, the $[M + H]^+$ on peak is at m/z 1036.9) and surfactin B ($C_{52}H_{91}N_7O_{13}$, the [M + H] + on peak is at m/z 1022.9) as shown in **Figure 2**. These three secondary metabolites showed antifungal activity.

In addition, using a similar approach, Leite, Pereira [71] discovered 24 bacterial endophytes in cassava stems. According to Leite, Pereira [71] the most common genera discovered in this study were *Achromobacter, Bacillus, Burkholderia, Enterobacter, Pantoea, and Pseudomonas*. The majority of them demonstrated a variety of biological activities related to cassava plant growth and productivity [71]. In studies conducted by Teixeira and Vieira [72] and Teixeira, Melo [73], several

Genera	Stems	Leaves
Bullera	V	V
Fusarium	V	V
Alternaria	V	V
Cryptococcus	V	V
Saitoella	V	V
Pseudpzyma	V	—
Ramichloridium	V	—
Aeurobasidium	V	—
Colletotrichum	V	—
Hannaella	V	—
Phaeosphaeriopsis	—	V
Pseudocercospora	—	V
Nigrospora	—	V
Aureobasidium	—	V
Pyrenochaetopsis	—	V
Sphaerulina	—	V

Table 1.
Bacterial genera in cassava stems and leaves [69].

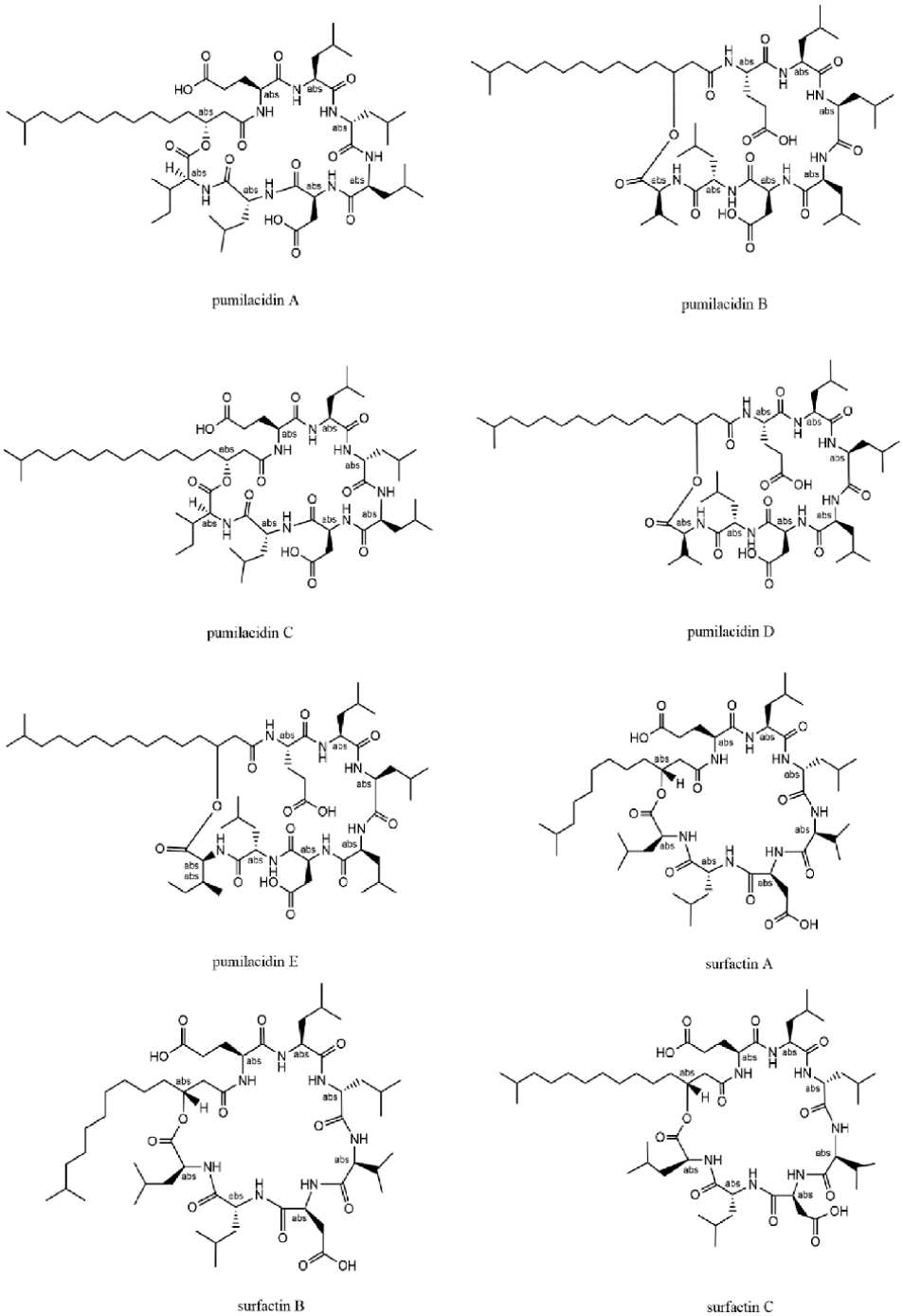

Figure 2.
Natural products produce by endophytic bacteria in cassava.

endophytic bacteria from cassava were identified, including *Bacillus, Burkholderia, Enterobacter, Escherichia, Salmonella, Serratia,* and *Stenotropomonas*.

Using a cultivation-dependent approach, Hartanti, Susanti [74] successfully cultured 14 endophyte fungi from cassava plants. All of them were examined using the ITS rDNA primers ITS 5 (forward: 5'–TCCTCCGCTTATTGATATGC–3′) and ITS 4 (reverse: 5'–TCCGTAGGTGAACCTGCGC–3). *Aspergillus* sp., *Aspergillus fumigatus, Fusarium*

Genera	Stems	Leaves
Enterobacter	V	V
Pantoea	V	V
Pseudomonas	V	V
Escherichia	V	V
Stenotrophomonas	V	—
Aeromonas	V	V
Chloroplast	V	V
Klebsiella	V	V
Paenibacillus	V	—
Shigella	V	V
Lelliottia	V	—
Acinetobacter	V	V
Exiguobacterium	V	—
Erwinia	V	V
Methylobacterium	—	V

Table 2.
Fungal genera in cassava stems and leaves [69].

falciforme, Fusarium lichenicola, Fusarium oxysporum, Fusarium solani, Lasiodiplodia sp., Nectria pseudotrichia, Penicillium citrinum, and *Schizophyllum commune* were discovered in this study [74]. Using similar approach, Suciatmih and Supriyati [75] successfully discovered *Guignardia endophyllicola*, an endophytic fungus, in cassava stems.

Zhang, Zhang [76] used a cultivation-independent approach of shotgun metagenome sequencing to determine the microbiome composition of cassava stems and leaves. The cassava phyllosphere's key bacterial genera have been identified as a result of this research. Gram-negative bacteria *Lelliottia* and *Stenotrophomonas* were isolated from cassava stems, gram-positive bacteria *Exiguobacterium* were isolated from leaves, and gram-negative bacteria [76], as shown in **Table 1**, the most prevalent genera were *Methylobacterium* from leaves. Therefore, the major fungi genera appear to be more complex than previously believed. Zhang, Zhang [69] discovered *Pseudpzyma, Ramichloridium, Aeurobasidium, Colletotrichum*, and *Hannaella* were among the key fungal genera identified from cassava stems as shown in **Table 2**. Six fungal genera were discovered in the casava leaves, including *Phaeosphaeriopsis, Pseudocercospora, Nigrospora, Aureobasidium, Pyrenochaetopsis, and Sphaerulina* (**Table 2**). In-depth analysis showed that *Bullera, Alternaria, Fusarium, Cryptococcus,* and *Saitolla* were identified in both phyllosphere samples [69].

In general, bacteria, fungi, and other microbes migrate into the plant phyllosphere via rain water, air, seeds, pollution, and animal sources [77]. Additionally, research indicates that some of these microbes are passed down from generation to generation [78]. The distribution of microbiomes in the phyllosphere may vary due to nutritional heterogeneity, such as carbon source uptake [79].

4. The rhizosphere and its microbiome

The soil ecosystem is one of the most complex and diverse on the planet. The soil contains a complex microcosm that interacts with the roots of plants [80].

This category includes archaea, bacteria, filamentous fungi, yeast, bryophytes, lichens, and protozoa. This soil organism significantly aids in the growth of various plants. Complex biochemical processes, such as the release of essential substances from organic matter, enable plants to access nutrients such as nitrogen, sulfur, and phosphorus, as well as essential growth hormones and toxic degradation products [81]. Furthermore, by providing pathogen protection, non-pathogenic microbes can alter plant immune responses [82].

In general, when plants live in a composite environment, they interact with specific soil microorganisms that live in the rhizosphere, the region around their roots [83]. This compartment is the narrow area of soil immediately surrounding the root system where the plant and microbes interact. It is defined by biological, chemical, and physical gradients that vary radially and longitudinally along the roots. The plant microbiome beneath the ground may be constructed in two stages: first, the rhizosphere may be colonized by a subset of bulk microbial communities, and then the rhizoplane (root surface) and root endosphere may be colonized by a subset of the rhizosphere communities [84].

Thousands of distinct microbial communities, including pathogens, mutualists, and commensals, coexist in the rhizosphere of cassava roots, just as they do in other plants. Their connection to the rhizosphere is complex and dynamic. However, it may be facilitated by the root exudate produced by the plant. Exudates play a critical role in plant–soil feedback by regulating plant survival in the face of antibiotic and biotic stress [85]. To the detriment of neighboring plants, plants regulate the rhizosphere via root-secreted metabolites [86]. Additionally, it is a critical mechanism of communication between plants and soil microbes [87]. The majority of root exudation takes place at the root's tip [88]. The root tip is the first part of the plant to investigate a new soil environment, and it plays a critical role in root responses to environmental stimuli [88]. Roots secrete a diverse array of primary metabolites, including amino acids, growth factors, vitamins, fatty acids, hormones, and antimicrobial compounds, which are believed to be lost passively from the root and utilized by rhizosphere-dwelling microbes [89].

Additionally, via a complex mechanism, exudates play a critical role in shaping microbial diversity [90]. However, no specific research on the microbial shaping of cassava plants in response to exudate has been conducted. However, research on other plants may explain this discrepancy.

Bacillus, a genus bacteria, in tomatoes produce systemic exudates of acylsugar metabolites, as demonstrated in a study of Korenblum, Dong [91]. Additionally, the metabolomes and transcriptomes of tomato leaves and systemic roots change in response to the rhizosphere's microbial community structure [91]. In-depth analyses of the systemic root metabolome suggest that glycosylated azelaic acid may function as a signaling molecule that is induced by the microbiome and then excreted as free azelaic acid [91]. The results indicate that the rhizosphere microbiome assembly plays a molecular and chemical role in systemically induced root metabolite exudation and soil conditioning.

Another study by Strehmel, Böttcher [92] reported that when *Arabidopsis thaliana* was grown hydroponically, it produced over a hundred distinct metabolites belonging to a variety of chemical classes. This metabolic diversity suggests that plants have developed a sophisticated chemical language for mediating an infinite number of rhizosphere interactions [93]. In conclusion, these studies indicated that structural changes in microbial communities have the potential to significantly alter host phenotypes. Additionally, root exudates have the potential to act as messengers between roots and soil organisms, triggering biological and physical interactions.

Melo, Fiore [68] used a cultivation-dependent approach to successfully cultivate 27 endophyte bacteria from cassava root. According to this study, *Bacillus*

predominates in cassava root [68]. Gram-negative bacteria (*Kluyvera cryocrescens, Stenotrophomonas maltophilia, Enterobacter aerogenes, Klebsiella pneumoniae,* and *Acidovorax avenae*), gram-positive non-Actinobacteria (*Bacillus cereus, Bukrhoklderia cepacia, Bradyrhizobium japonicum*, and *Microbacterium homonis*) and gram-positive Actinobacteria (*Streptomyces olivaceus*) were found in cassava root [68]. Another study by Leite, Pereira [71] used a similar approach to discover 28 bacterial endophytes in the root. The most prevalent bacteria found in cassava root were *Bacillus, Burkholderia, Enterobacter,* and *Pantoea.* The majority of them possessed biological properties, including the ability to solubilize inorganic phosphate and the capacity to synthesize Indole acetic acid [71].

Zhang, Zhang [76] successfully identified a variety of bacterial genera in the cassava root. Gram-negative *Enterobacter, Pantoea, Pseudomonas, Escherichia, Aeromonas, Chloroplast, Shigella,* and *Klebsiella* were discovered in cassava roots, as were gram-positive *Lactococcus* and *Paenibacillus* [69]. Therefore, the only major bacterial genera found in root cassava were gram-positive cocci *Lactococcus*. Additionally, using a cultivation-dependent technique, Ilyas [94] isolated endophytic fungi *Fusarium* sp. and *Penicilium* sp. from cassava roots.

A recent study took a non-cultivation-dependent approach. Zhang, Zhang [76] discovered 11 fungal genera in the cassava root, including *Bullera, Fusarium*, and *Alternaria*, as well as eight key fungal genera that were not found in the cassava stems and leaves, including *Humicola, Penicillium, Nigrospora, Beauveria, Thozetella, Codinaeopsis, Paraphaeosphaeria,* and *Dinemasporium* [76]. Additionally, *Ascomycota* have been described as domination endophyte assemblages. According to a study conducted by Li, Yan [95], *Stephanonectaria, Cutaneotrichosporon, Pleurotus, Wallemia, Aspergillus, Gibberella, Lachancea, Yamadazyma, Neurospora, Cladosporium, Wickerhamomyces, Penicillium, Diaporthe, Fusarium,* and *Lasiodioplodia* were successfully detected in cassava root. Therefore, *Lasiodioplodia* was genus-level dominant [95].

5. The effect of plant genotypes and genetic background on plant microbiome

Plants live harmoniously with a diverse array of microorganisms. These microbes, which include bacteria, archaea, filamentous fungi, and nematodes, can live as endophytes or epiphytes, as well as in any plant organ or tissue, including cassava. A rapidly growing body of literature has documented the influence of the microbiome on critical plant traits such as disease resistance [96], nutrient acquisition and growth [97], and abiotic stress tolerance [98]. Thus, the microbiome can be viewed as an extended phenotype of the plant genome that can assist plants in dealing with environmental stressors.

Li, Yan [95] investigated the microbiome of various cassava cultivars in a study. They examined four cassava cultivars, two of which were resistant to rot (SC124 and SC205) and two of which were susceptible to rot (SC124 and SC205) (SC10 and SC5). Surprisingly, both groups were dominated by gram-positive *Weissella* (family *Leuconostaceae*) close behind with gram-negative *Serratia* (family *Enterobacteriaceae*). At the phylum level, the most prevalent phyla were *Proteobacteria* and *Firmicutes* [95]. Thus, *Lasidiplodia* (family *Botryosphaeraceae*) was the most prevalent fungus in the susceptible and tolerant groups, followed by *Fusarium* from family *Nectriaceae* and *Diaporthe* from family *Diaporthaceae* [95]. Thus, susceptible cultivars have been found to harbor bacteria such as *Paenalcaligenes, Parapusillomonas, Corticicoccus,* and *Lachinoclostridium* that have not been detected in tolerant cultivars [95]. On the other hand,

Phascolarctobacterium, *Olivibacter*, and *Citrobacter* were key genera found exclusively in the tolerant group [95]. *Culvularia* was the most frequently encountered fungus among vulnerable groups. *Hortaea* and *Agaricostilbomyctes* were significantly more abundant in the tolerant cultivar, indicating the importance of relative abundance [95].

Zhang, Zhang [69] is also investigating the microbiome of cassava plants that is associated with disease resistance. Interestingly, several microorganisms involved in disease resistance include *Lactococcus* sp., *Pantoea dispersa*, and *Saccharomyces cerevisiae* [69]. Additionally, the presence of nisin-related genes in *Lactococcus* was positively associated with disease resistance in cassava plants [69].

6. Manipulation of cassava microbiome to improve the yield

Like in other plant, manipulation of the plant microbiome may aid in increasing cassava productivity [99]. By increasing soil bioavailability and plant tolerance to biotic and abiotic stresses, good soil management practices such as the use of beneficial microbes in the Rhizosphere can be achieved, thereby reducing reliance on agricultural chemicals. Crop rotation is also an option for increasing the diversity of soil microbes, which contributes to plant pathogen resistance [100]. A stimulating biofertilizer as shown in **Figure 3**, which includes co-inoculation of several beneficial strains, including endophytes, will enhance microbial root colonization capability and establish a useful niche for plant pathogens to compete. *Bacillus pumilus* and *Paenibacillus* spp. inoculation will improve fungal pathogen suppression on cassava plants as a biofertilizer agent capable of producing pumilacidines and surfactins. Additionally, inoculants containing microorganisms and microbial phosphorus solubilizers capable of producing active indole acetic acid promote the growth of manicured plants (as shown in **Figure 3**).

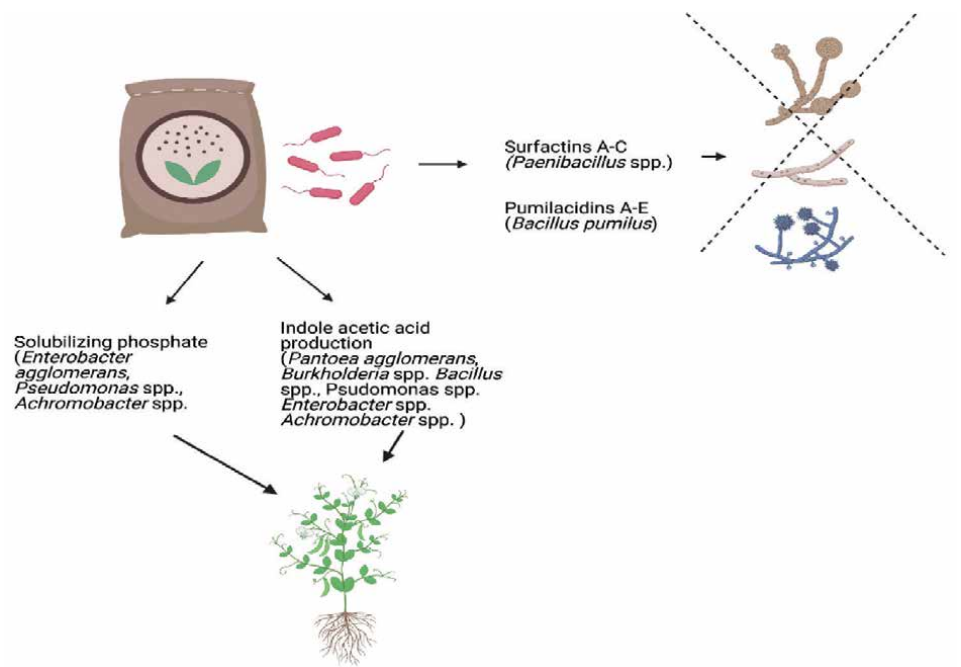

Figure 3.
Consortium's inoculant strategy for improving the cassava microbiome and production.

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Section 2

Cassava Disease

Chapter 3

Seasonal Variation on the Incidence and Severity of Major Foliar Diseases of Cassava in Sierra Leone

Alusaine Edward Samura, Sahr N. Fomba, Augustine Mansarsy, Musa D. Saffa, Dan D. Quee and Johnny E. Norman

Abstract

A diagnostic survey was conducted in the rainy and dry seasons from 2014 to 2015 to determine the incidence and severity of major diseases of cassava in Sierra Leone. At least three chiefdoms and five villages per district were targeted. The survey was carried out in fourteen districts of the country with geo references using a GPS. On the spot assessment was conducted in all fields. Prevalence, severity and incidence were calculated. The most dominant diseases included the cassava mosaic disease and the cassava bacterial blight. The prevalence of cassava mosaic disease was high with 69.1% and 61.5% in the rainy season and dry season, respectively. The prevalence of cassava bacterial blight was 100% and 92% in the rainy season and dry season, respectively. Diseases of less importance included white spot and brown spot diseases as well as cassava anthracnose disease. The study provides bases for the deployment of improved varieties and provides information on the seasonal prevalence, incidence and severity of cassava diseases in Sierra Leone.

Keywords: Cassava Diseases, Incidence, severity and distribution

1. Introduction

Cassava (*Manihot esculenta* Crantz, family: Euphorbiaceae synonyms: yucca, manioc, and mandioca), a native to South America, is believed to have been introduced into Sierra Leone during the period of slave trade proliferated by Portuguese traders during the 16th century [1]. Cassava is grown over a range of climates and altitudes and on a wide variety of soils and ecologies. These includes the lowlands and uplands as commonly practised in Sierra Leone. Cassava is tolerant to drought; it is productive in poor soil where other staple crops cannot grow without intensive inputs [2]. The crop has long been recognized as an important source of carbohydrate for over 500 million people in Africa as well as feed for livestock, providing higher food energy production density (1045 kJ/hectare) than other root crops, such as maize grain (836 kJ/hectare) and fresh sweet potato root (752 kJ/hectare) [3]. The flexibility in growth conditions entertained by the cassava crop enables it

to grow successfully under a wide range of agro-ecological zones where cereals and other crops cannot thrive, making it a suitable crop for resource-poor farmers to cultivate under marginal environments in Africa. The other attraction for farmers to grow cassava is that it produces higher yields per unit of land than other crops such as rice, sweet potato, wheat, sorghum, maize and banana fruit [3]. The pivotal role of cassava in the lives of Africans is evident from many documents [4, 5]. In Sierra Leone, cassava is the second most important food crop after rice the country's staple. It is also the most important root and tuber crop [6]. Cassava is also grown all over the country which has shown remarkable progress in cassava processing at both domestic and commercial scales, although to varying degrees. Some common cassava products processed in the country include *gari*, foo-foo, gbodor, dried chips, starch and boiled cassava with beans. Cassava-based products such as raw tubers, *gari* and cassava bread (very thin, small, flat, round pieces) are traded mainly in Sierra Leone [7]. Cassava leaves provide a source of income for women. The leaves are used to prepare a very popular national cassava leaf sauce [6]. Much of this success may be attributed to its adaptability, its capacity to provide acceptable yields under marginal farming conditions and its tolerance to drought [8].

According to the Food and Agriculture Organization of the United Nations (FAO) statistics, Sierra Leone is one of the lowest cassava producing countries in the world with an estimated yield of 13.2 t/ha [9]. A substantial increase in production was observed from 95,000mt in the '80s to 241,000mt in 2000 and is now estimated at 4,588,612 tons with 346,266 ha harvested area [7, 9].

As part of the World Bank's strategy for increasing the productivity of cassava in West Africa, the West Africa Agricultural Productivity Programme introduced new varieties of cassava, intensified production in terms of land area and processing of cassava countrywide. This situation if not monitored could lead to a high incidence of pests and diseases as the crop is grown more intensively over larger areas and planted throughout the year for industrial processing [10].

Cassava is affected by the following principal diseases: Cassava mosaic disease (CMD) is perhaps the most important diseases of cassava. Cassava brown streak virus disease associated with tuber rot and other virus diseases [4]. Two virus diseases, CMD and cassava brown streak disease (CBSD) are both thought to have risen from infection of cassava by viruses already present in the indigenous Africa flora [8].

It has been estimated that cassava bacterial blight (Xanthomonas *axonopodis pv. manihotis*) can account for 30% loss of yield in one growing cycle, and up to 80% by the third cycle if no control measures are taken [11]. Other diseases of importance include Cassava Anthracnose Disease (CAD) caused by *Colletotrichum gloeosporioides* f.sp. *manihotis* Henn, also introduced from South America in the 1970s, and to a lesser degree cercosporiosis caused by *Cercospordium henningsii* [8, 12].

Following the success of the cassava mealybug and cassava green mite biological control programs, CMD and CBSD have become prominent in research and management initiatives, and CMD is now commonly considered to be the most damaging pest or disease constraint to cassava production in Africa. Cassava mosaic disease (CMD) caused by cassava mosaic geminiviruses (CMGs) (family Geminiviridae; genus *Begomovirus*) is widely distributed throughout Sierra Leone [13]. Cassava bacteria blight is emerging as an economic disease especially for the cassava leaf producers and markets. The disease has become more important due to an increase in symptom expression which can be attributed to changing climatic condition [14].

There is however a dearth of knowledge on the incidence, severity and distribution of cassava diseases in Sierra Leone. As a one-year duration crop, there is also limited information on the incidence and severity of diseases during the rainy and dry season. Knowledge of the incidence, severity and distribution of major diseases is critical for the deployment of germplasm in the management of these biotic

stresses. Potential threats include the re-emergence of new viruses, higher incidence and severity of diseases due to variation in weather and to a greater extent the lack of response due to lack of information on the level and location of biotic threats which may lead to a huge economic loss.

2. Description of field sampling methodology and analysis

Two surveys were conducted in 2014 and 2015 to assess the seasonal variability of major diseases associated with cassava in farmers' field across Sierra Leone. The survey routes were determined using the road maps of Sierra Leone and such routes included highways, secondary roads and feeder roads. The routes were selected to target major cassava growing areas within the geopolitical districts as well as the major agro-ecologies in Sierra Leone. The distance between farms ranged between 2 and 3 Km in small chiefdoms and 3–5 Km in big chiefdoms. In each district, both primary (3–6 months) and secondary fields (more than 10 months) were sampled. In both primary and secondary fields, the predominant cassava variety was sampled although other varieties were noted. In the primary fields, 30 cassava plants were sampled at regular intervals using a diagonal transect, while in the secondary field, 10 plants were sampled at regular intervals along the field's diagonals. Three chiefdoms per district were targeted. The rainy season survey was conducted for twenty (20) days, July 8 to 28, 2014; and covered thirteen out of fourteen districts (including Bo, Bonthe, Pujehun, Moyamba, Port Loko, Koinadugu, Kambia, Tonkolili, Bombali, Kono, Western area (Urban and Peri-Urban) and Kenema). Kailahun district was not sampled because of the Ebola threat. The dry season survey was conducted in the dry season from 11th February to the 2nd Marc 2015. The survey was carried out in all fourteen districts of the country. A total of one hundred and seventy-two (172) and one hundred and ninety-five (195) cassava farms were visited countrywide in the rainy and dry seasons, respectively.

At each site data were taken on location including coordinates (altitude, latitude and longitude) using GPS sets GARMIN e Trex Legend 1200 E 151st Street, Olathe, Kansaa 66062 U.S.A) from which GIS maps were generated for cassava diseases assessed.

The age and size of each field were noted; information on crop age was obtained from the farmers and the size of the field determined from visual estimation. The cropping systems, names of each cassava variety and whether it was local or improved was also noted. Interviews were also conducted to capture the views of men, women and youths on their knowledge on, insect pests' diseases, weeds identification and control.

On the spot assessment was conducted in all fields in the Western Area and 12 districts and 5 agro-ecologies which include the rain forest, savannah lowland, savannah highland, coastal plains and the peninsular mountain. Major diseases assessed included the African Cassava Mosaic Disease, Cassava Bacterial Blight and Cassava Anthracnose Disease. Prevalence was calculated as the number of sites infected over the total number of sites visited expressed in percentages. The severity of diseases was assessed using the five-point scale where 1 represents no visible symptom and 5 severe symptom expression [15]. Percent incidence was calculated by expressing in percent the total number of infected plants over the total number of plants sampled.

3. Key findings

The most dominant diseases included the cassava mosaic disease and the cassava bacterial blight. Cassava white spot and brown spot diseases were considered minor

but affect the esthetic value of cassava leaf. Most of the cassava mosaic infected varieties were local varieties while cassava bacterial blight infected both local and improved varieties.

3.1 Field, crop and environmental characteristics of cassava farms in Sierra Leone

The national survey, supported by the West African Agricultural Productivity Program (WAAPP) recorded the names of 25 cassava varieties of which, 88% were local varieties while 12% were improved varieties. Likewise, sixty-four percent of the farms visited had local cassava varieties while thirty-six percent of the farms had improved varieties. The most common local varieties grown were Warima, Cooking soon, Cocoa, Gendemeh, Munafa, Three-month cassava, Ndiamonyamawo, rubber and kandagboi, Nikanyeyea, Kandabendue, Cotton tree, Monobia, Mawola, Minikit, Shortman, Sameteteh, Sweet cassava, and Yakanu Pa Jalloh [13]. The improved varieties recorded were Slicass 1, Slicass 4, Slicass 6 and Slicass 3.

At the district level, more improved varieties were grown in Bonthe (100%), Moyamba (60.00%), Pujehun (60.00%) and Kambia (46.67%) (**Table 1**). On the other hand, local varieties were grown predominantly in Kailahun (100%), Kono (93.33%), Bo (93.33%), Port Loko (93.33%) Kenema (86.66%), Koinadugu (86.67%), Tonkolili (86.67%), Bombali (86.67%) and Western Area (76.92%), respectively (**Table 1**).

Mean cassava field size ranged from 0.22 hectare in Western Area to 2.70 hectares in the Bonthe district (**Table 1**). The mean age of the cassava fields ranged from 3.86 months in Bombali district to 12.80 months in Moyamba district. The mean altitudes of the fields in the thirteen locations ranged

District	Average farm size (Ha)	Average altitude (M)	Average age of farm (Month)	Variety grown by Farmers	
				Local (%)	Improved (%)
Bo	1.12	98.93	11.60	93.33	6.67
Pujehun	1.41	59.33	11.73	40.00	60.00
Moyamba	1.25	73.13	12.80	40.0	60.00
Bonthe	2.70	47.0	12.0	0	100.00
Kenema	0.73	168.13	11.73	86.66	13.34
Kono	0.69	369.40	9.80	93.33	6.77
Kailahun	0.51	200.53	12.00	100.00	0.00
Bombali	1.06	90.53	3.86	86.66	13.34
Koinadugu	0.23	382.26	4.33	86.67	13.33
Kambia	0.52	53.80	7.06	53.33	46.67
Port Loko	0.65	56.33	9.40	93.33	6.67
Tonkolili	0.82	148.93	9.00	86.67	13.33
Western Area	0.22	107.23	8.92	76.92	23.08

Source: Cassava pest and disease survey conducted in 2014, supported by the West African Agricultural Productivity Program (WAAPP).

Table 1.
Field crop and environmental characteristic.

from 47.00 m above sea level in Bonthe district to 382.26 m above sea level in Koinadugu district [14].

3.2 Seasonal prevalence of major diseases of cassava in Sierra Leone

The prevalence of cassava mosaic disease in the rainy season was 69.1% out of one hundred and seventy-two (172) farms sampled. During the dry season, the prevalence was recorded at 61.5% out of one hundred and ninety-five (195) farms sampled (**Table 2**).

Figure 1a–d shows the expression of diseases infected cassava and an improve variety.

Major foliar cassava diseases	Prevalence	
	Rainy season	Dry season
Cassava Mosaic disease	69.1	61.5
Cassava Bacterial blight	100	92
Cassava anthracnose disease	6.9	29.7
Cassava brown spot disease	1.16	22.5
Cassava White spot disease	5.2	36.9

Table 2.
Prevalence of major diseases of cassava in 2014/2015.

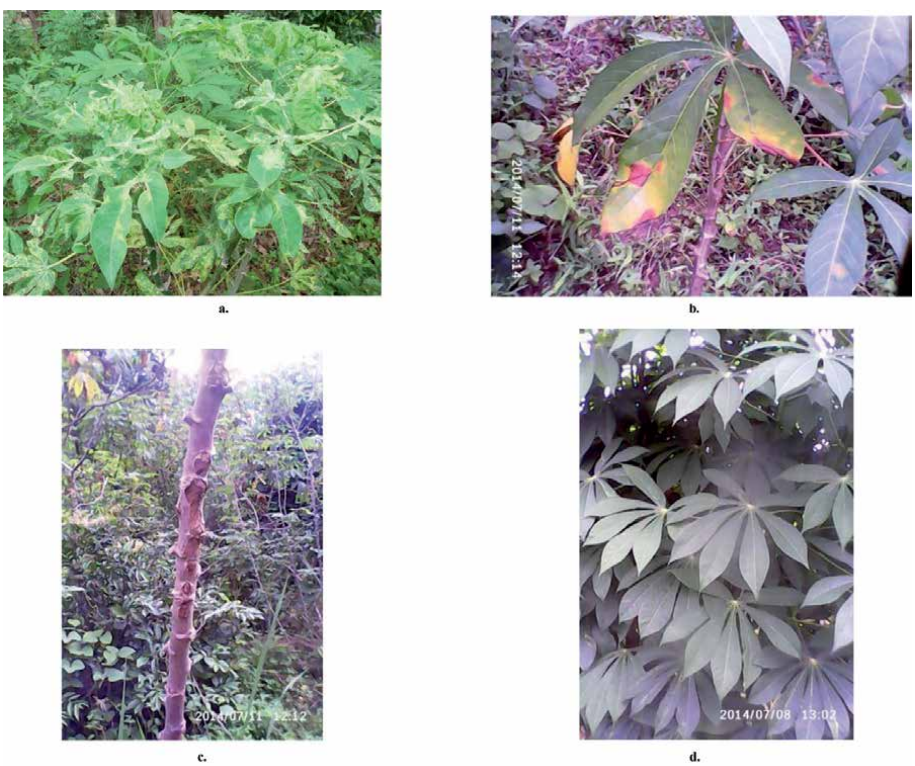

Figure 1.
a. Cassava plant infected cassava mosaic disease observed in 2014/2015; b. cassava plant infected cassava bacteria blight 2014/2015; c. cassava plant infected cassava anthracnose disease observed in 2014/2015; d. healthy cassava variety observed in 2014/2015.

3.3 Incidence and severity of cassava mosaic disease in the rainy and dry season

In 2014/2015, the incidence and severity of Cassava Mosaic disease in the rainy season was significantly the highest in the Kono ($P < 0.001$) (94.3%), Koinadugu (84.3%) and Kenema (80.2%) districts. Bonthe district had the lowest incidence of 10.3% an occurrence attributed to the break down in resistance of the popular released variety SLICASS 4 commonly referred to as blue boat. The Western Area had a disease incidence of 24.7% followed by Pujehun and Moyamba with an incidence of 32% and 37% respectively. Port Loko, Kambia and Tokolili districts had diseases incidence ranging between 56.3% to 66.7%.

The severity of the cassava mosaic disease across districts was generally considered to be moderately infected with a significant ($p < 0.001$) difference between districts. Kenema had the highest severity score of 2.8 followed by Kono, Tonkolili and Bombali with severity scores of 2.6, 2.3 and 2.2 respectively. Bonthe district had the lowest severity score of 1.1 followed by Moyamba, Pujehun and Bo with a severity score of 1.7, 1.7.and 1.8 respectively (**Table 3**).

In the dry season, the incidence of cassava mosaic disease was significantly different across districts. The highest incidence of 86.7% was observed in Kono. Bombali and Tonkolili had incidence74.7% and 73.3% respectively. Bonthe district had the lowest incidence of 0% followed by Kailahun 29.8, Bo 41.9%, Kambia 46.7% and Moyamba 48.1%.

The severity of the cassava mosaic disease across districts was generally considered to be moderately infected with a significant difference between districts. Kono, Koinadugu, Bombali and Kambia had the highest severity score of 1.9, 1.85, 1.82 and

District	White Spot		Cassava Mosaic Disease		Cassava Anthracnose Disease		Cassava Bacteria Blight	
	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity
Bo	5	1.1	43.7	1.8	8.7	1	94.3	2
Bombali	0	1	57	2.2	3	1	97.7	2.1
Bonthe	12	1.1	10.3	1.1	2	1	90.7	1.9
Kambia	0	1	65	2.2	0	1	96	2.1
Kenema	1.1	1	80.3	2.8	0	1	99.7	2
Koinadugu	0	1	84.3	2.3	0	1	100	2
Kono	0	1	94.3	2.6	2	1	100	2.2
Moyamba	0	1	36	1.7	0	1	100	2
Port Loko	0	1.1	56.3	1.9	0	1.2	98	2.3
Pujehun	6.7	1	32	1.7	5	1.2	88	2
Tonkolili	0	1	66.7	2.3	0	1	100	2.2
Western Area	0	1	23.7	1.4	1	1	100	2.1
SE±	2.39	0.04	10.2	0.3	2.6	0.05	4.1	0.09
CV(%)	448.7	11.9	73	45	565.8	20.3	11.7	17.7

Table 3.
Incidence and severity of major disease of cassava in the rainy season in Sierra Leone in 2014/2015.

District	White Spot		Cassava Mosaic Disease		Cassava Anthracnose Disease		Cassava Bacteria Blight		Brown Spot	
	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	severity
Bo	32.5	1.35	41.9	1.48	26.5	1.37	68.8	1.71	0	1
Bombali	8.3	1.14	78.7	1.82	0	1	49.7	1.5	0	1
Bonthe	52.9	1.52	0	1	42.9	1.38	59.6	1.7	0	1
Kailahun	29.4	1.34	29.9	1.3	13.8	1.17	79.9	1.85	0	1
Kambia	33.3	1.33	46.7	1.8	6.7	1.1	100	2.54	50	1.5
Kenema	13.6	1.13	45.9	1.47	0.5	1.1	84.7	3.35	0	1
Koinadugu	5	1.05	74.7	1.85	0	1.1	42	1.51	3	1
Kono	33.7	1.35	86.7	1.9	2.3	1	75	1.94	2.3	1
Moyamba	38.1	1.38	48.1	1.49	31.3	2	76.2	1.79	9.1	1.1
Port Loko	0	1	57	1.58	20.3	1.2	77.7	1.93	15.7	1.2
Pujehun	37	1.4	30.3	1.35	44.7	1.5	71.7	1.69	7	1.1
Tonkolili	25	1.2	73.3	1.81	31.3	1.3	82.3	1.98	33.3	1.3
Western Area	0	1	20.8	1.22	11.97	1.1	51.1	1.53	0	1
SE±	13.48	0.14	16.04	0.195	8.46	0.28	14.49	0.53	8.75	0.08
CV(%)	155.9	31.6	91	34.8	188	61.4	56.1	75.8	273.6	22.5

Table 4.
Incidence and severity of major disease of cassava in the dry season in Sierra Leone in 2014/2015.

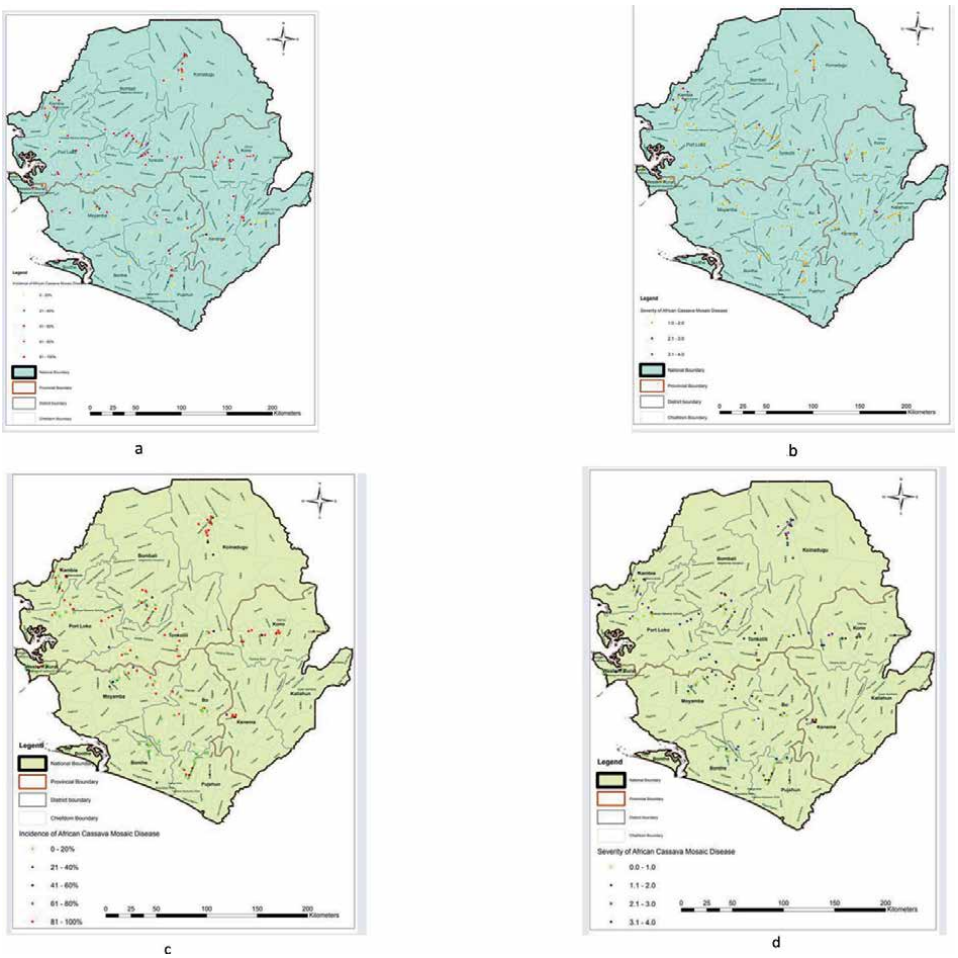

Figure 2.
a. Incidence of cassava mosaic disease dry season 2014/2015; b. severity of cassava mosaic disease in the dry season 2014/2015; c. incidence of cassava mosaic disease rainy season; d. severity of cassava mosaic disease in the rainy season 2014/2015.

1.8 respectively. Bonthe district and the western area had the lowest severity score of 1.0 and 1.2 followed by Kailahun (1.3) and Pujehun (1.35) **Table 4**.

The distribution of cassava mosaic disease across the country was represented in spatial distribution in (**Figure 2**).

3.4 Incidence and severity of cassava bacterial blight disease in the rainy and dry season

In the rainy season, the incidence of cassava bacterial blight was high with a significant difference across districts. Koinadugu, Kono, Moyamba, Tonkolili and the Western Area districts had an incidence of 100%. The lowest disease incidence of 88% was observed in the Pujehun district. All other districts recorded an incidence of 90% and above. The severity of cassava bacterial blight was low despite the high incidence recorded. Severity ranged between 2.0 and 2.3 with no significant difference across districts (**Table 3**).

Incidence and severity of cassava bacterial blight disease in the dry season was high with a significant difference across districts. The highest incidence of 100%

was observed in Kambia, followed by Kenema (84.7%) and Kailahum 79.9. The lowest disease incidence of 49.7% was observed in the Bombali district. All other districts recorded an incidence of 90% and above. The severity of cassava bacterial blight was low despite the high incidence recorded. Severity ranged between 2.0 and 2.3 with no significant difference across districts (**Table 4**). The distribution of cassava bacterial blight disease across the country was represented in spatial distribution in (**Figure 3**).

3.5 Incidence and severity of cassava anthracnose disease

The incidence of cassava anthracnose disease in the rainy season was low across districts with no significant difference. In most districts including Kambia, Kemema, Koinadugu, Moyamba, Port Loko and Tonkolili no symptom of cassava anthracnose disease was observed in the rainy season, However, Bo, Bombali, Bonthe, Kono and Pujehun had an incidence of 8.7%, 3%, 2%, 2%, and 5% respectively. The severity of Cassava anthracnose was also low and, in most cases, did not exceed a severity score of 1.2 **Table 3**.

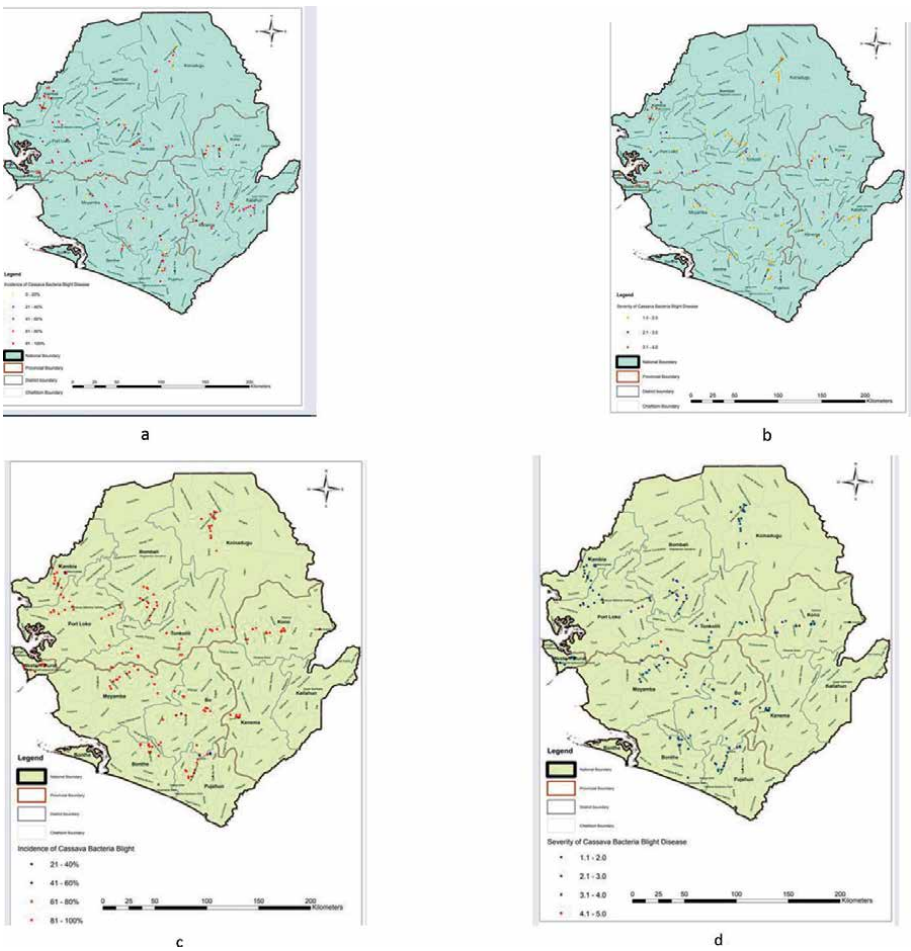

Figure 3.
a. Incidence of cassava bacterial blight in the dry season 2014/2015; b. severity of cassava bacterial blight in the dry season 2014/2015; c. incidence of cassava bacterial blight in the rainy season 2014/2015; d. severity of cassava bacterial blight in the rainy season 2014/2015.

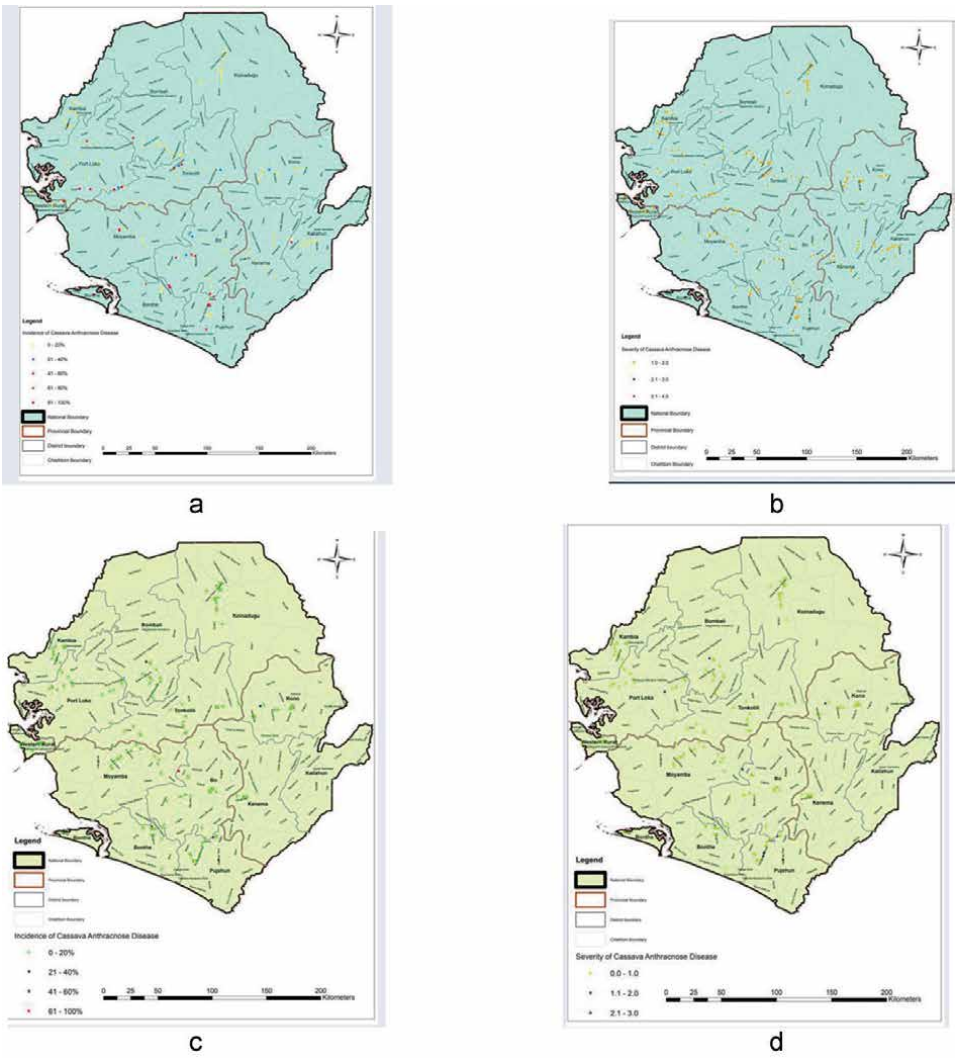

Figure 4.
a. Incidence of cassava anthracnose disease in the dry season 2014/2015; b. severity of cassava anthracnose disease in the dry season 2014/2015; c. incidence of cassava anthracnose disease in the rainy season 2014/2015; d. severity of cassava anthracnose disease in the rainy season 2014/2015.

The incidence of cassava anthracnose disease was also low in the dry season with a significant difference across districts. Pujehun and Bonthe districts had the highest incidence of 44.7 and 42.9% respectively. Bombali and Koinadugu recorded a disease incidence of 0%. The severity of Cassava anthracnose was also low. Pujehum had the highest disease severity score of 1.46 followed by Bonthe with 1.38 **Table 4**. The distribution of cassava anthracnose disease (CAD) across the country was represented in spatial distribution in (**Figure 4**).

3.6 Incidence of cassava brown spot disease

The incidence of cassava brown spot disease was low and was observed only in the dry season. The highest incidence of 50% was observed in Kambia followed by Tonkololi with 33.3%. Except for Koinadugu, Kono, Moyamba, Port Loko and Pujehun districts with incidence ranging between 2–7%, all other districts recorded an incidence of 0%. The severity score was low and did not exceed 1.5 across all

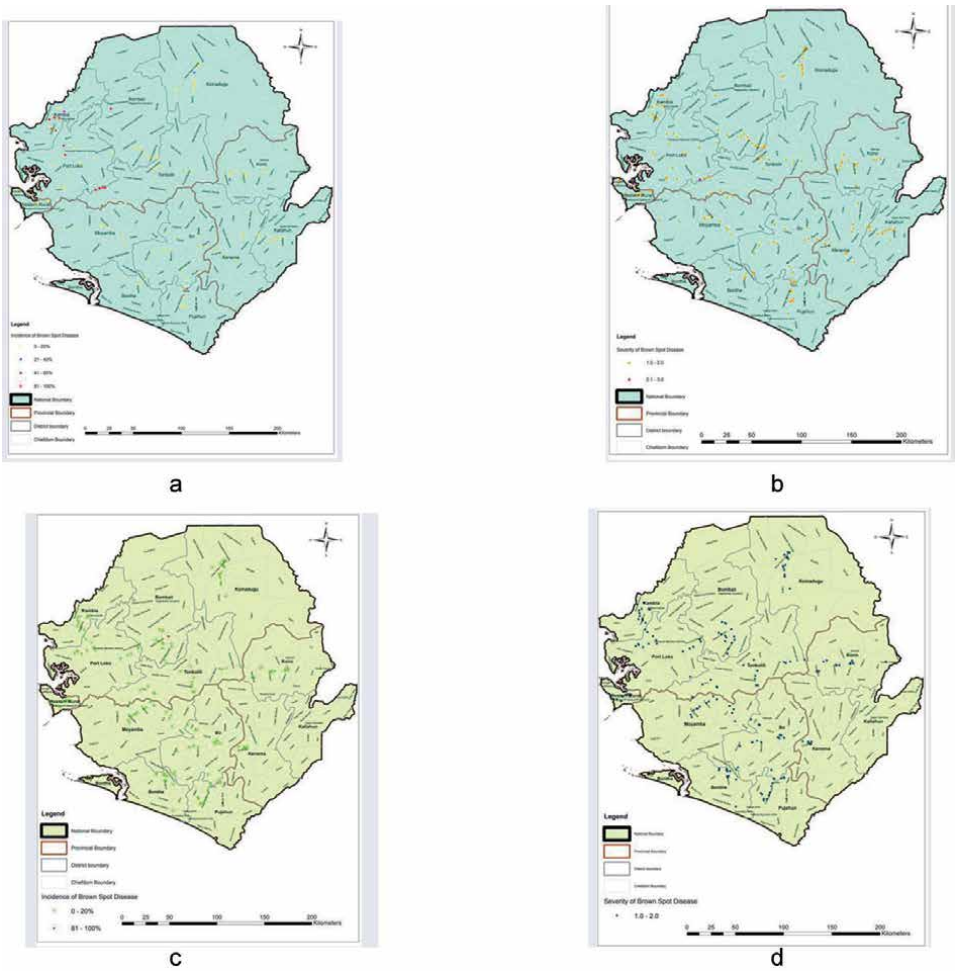

Figure 5.
a. Incidence of brown spot in the dry season 2014/2015; b. severity of brown spot in the dry season 2014/2015; c. incidence of brown spot in the rainy season 2014/2015; d. severity of brown spot in the rainy season 2014/2015.

districts **Table 4**. The distribution of cassava brown spot disease across the country was represented in spatial distribution in (**Figure 5**).

3.7 Incidence and severity of white spot disease

Incidence of cassava white spot disease was low in the rainy season. Most districts had an incidence of 0% except Bo, Bonthe, Kenema, Pujehun with disease incidence ranging between 1–612% (**Table 3**). In the dry season, the incidence of cassava white spot disease was similarly low. Port Loko and Western Area districts had no disease symptoms while Bonthe, Moyamba, Pujehun and Kono recorded diseases incidence of 52.9%, 38.1%, 37% and 33.7% respectively (**Table 4**). The distribution of the disease was represented in **Figure 6**.

3.8 Incidence and severity of major Foliar diseases of cassava across agro-ecologies

In the rainy season, the incidence of cassava mosaic disease across ecologies was significantly different. Peninsular Mountain in the western area had the

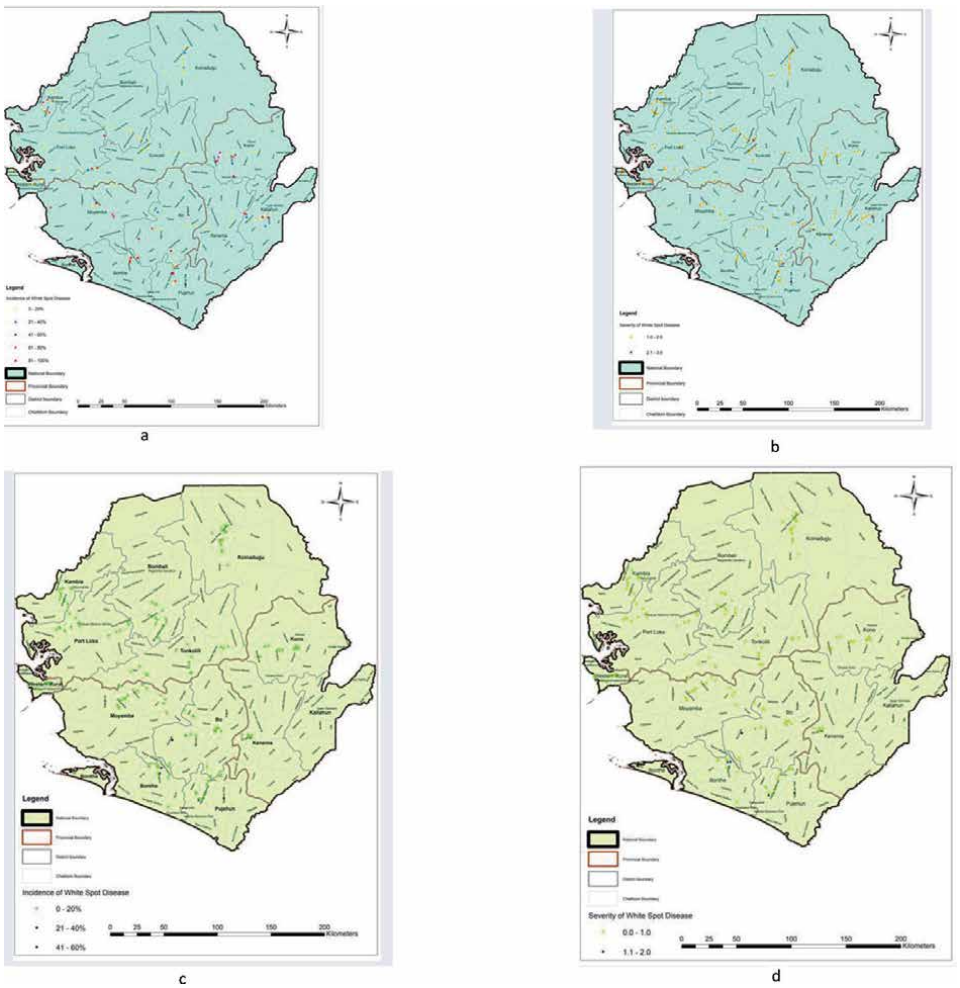

Figure 6.
a. Incidence of white spot disease in the dry season 2014/2015; b. severity of white spot disease in the dry season 2014/2015; c. incidence of white spot disease in the rainy season 2014/2015; d. severity of white spot disease in the rainy season 2014/2015.

lowest incidence of 23.7% followed by the coastal plain with 33.3%. The rain forest zone, consisting of Kenema, Kono, Kailahum, had the highest incidence of 90.1%. This was followed by the savannah highland which had an incidence of 84.3%, in the Koinadugu district. The savannah lowland, comprising the districts of Bo, Moyamba, Tonkolili, Kambia, Bombali and Pujehun had an incidence of 50.1%. The severity of the cassava mosaic disease was mild and not significantly different across agro-ecologies. The highest severity score of 2.64 was observed in the rain forest while the lowest severity score of 1.44 was observed in the Peninsular Mountain followed by the coastal plain with a severity score of 1.52 (**Table 5**).

In the dry season, however, Peninsular Mountain in the Western Area had the lowest incidence of 20.8% followed by the coastal plain with 37.4%. The savannah highland had the highest incidence of 74.7%, in the Koinadugu district. The rain forest zone, consisting of Kenema, Kono, Kailahum, an incidence of 57.0%. The savannah lowland, comprising the districts of Bo, Moyamba, Tonkolili, Kambia, Bombali and Pujehun had an incidence of 53.5%.

The severity of the cassava mosaic disease was mild with significantly different scores across agro-ecologies. The highest severity score of 1.85 was observed in

Agro ecology	White Spot		Cassava Mosaic Disease		Cassava Anthracnose Disease		Cassava Bacteria Blight	
	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity
Coastal Plain	6	1.1	33.3	1.52	1	1.05	94.3	2.1
Peninsular Mountain	0	1	23.7	1.44	0.7	1.01	100	2.1
Rain Forest	0	1	90.1	2.64	1.6	1,02	99.9	2.1
Savannah Highland	0	1	84.3	2.3	0	1	100	2
Savannah Lowland	0	1	50.1	2	2.75	1.04	96	2.1
SE±	2.6	0.03	11.53	0.25	2.86	0.06	3.25	0.1
LSD	5.3	0.06	29.8	0.58	5.6	0.11	6.42	0.2
CV (%)	476	12.2	75	46.2	549	20.4	12	1.8
		NS					NS	NS

Table 5.
Incidence and severity of major diseases of cassava across agro ecologies in the rainy season in 2014/2015.

the savannah highland while the lowest severity score of 1.2 was observed in the Peninsular Mountain followed by the Coastal Plain with a severity score of 1.38 (**Table 6**).

The incidence of cassava bacterial blight was high across agro-ecologies in the rainy season. The peninsular mountain and the savannah highland area had a 100% incidence. The rain forest had a 99.9% incidence and the lowest incidence of 94.3%was observed in the coastal plains. The severity of cassava bacterial blight was low and ranged between 2 to 2.1 with no significant difference across ecologies (**Table 5**).

A similar result was observed in the dry season. The rain forest had the highest incidence of 79.5% followed by the savannah lowland with 74.9% and the coastal plain with 72%. The savannah highland and the Peninsular Mountain had the lowest incidence of 42% and 51.2% respectively. 94.3%was observed in the coastal plains.

The severity of cassava bacterial blight was low and ranged between 1.5 to 2.5 with no significant difference across ecologies (**Table 6**).

The incidence of cassava anthracnose disease was low in all agro-ecologies with no significant difference among them. Incidence did not exceed 3%. The severity of cassava anthracnose disease was also very low. In the dry season, the incidence of cassava anthracnose disease was slightly higher than the rainy season result but low in all agro-ecologies with significant differences among agro-ecologies. The coastal plain and the savannah low land accounted for the highest incidence of 27.4% and 23.3% respectively. The savannah highland had 0% while the rain forest had 5.3%.

The severity of cassava anthracnose disease was also very low and did not exceed 1.35 for all agro-ecologies (**Table 6**).

During the dry season, the incidence of cassava brown spot disease was low in all agro-ecologies but not significantly different. The savannah lowland had the highest incidence of 17% followed by the coastal plain with 9.6% and the savannah highland with only 3%. The lowest incidence of 0% was observed in the peninsular mountain and the rain forest zone. The severity of cassava brown spot disease was also very low and did not exceed 1.2 (**Table 6**) The rainy season account shows that

Agro ecology	White Spot		Cassava Mosaic Disease		Cassava Anthracnose Disease		Cassava Bacteria Blight		Brown Spot	
	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity
Coastal Plain	16.7	1.16	37.4	1.38	27.4	1.26	72	1.9	9.6	1.7
Penisular Mountain	0	1	20.8	1.21	6.1	1.07	51.2	1.5	0	1
Rain Forest	26.2	1.28	57	1.59	5.3	1.08	79.5	2.3	0	1.3
Savannah Highland	5	1.05	74.7	1.85	0.5	1.07	42	1.5	3	1.1
Savannah Lowland	28.8	1.31	53.5	1.63	23.2	1.35	74.9	1.9	17	1.3
SE±	10.63	0.11	13.28	0.15	9.57	0.21	11.29	0.413	7.6	0.11
LSD	20.99	0.22	26.24	0.31	18.91	0.43	22.3	0.81	15.1	0.22
CV (%)	172	32.7	93.5	36.1			56.9	76.9	283.9	32.7

Table 6.
Incidence and severity of major diseases across agro ecology in the dry season in 2014/2015.

the incidence of cassava white spot disease was also low in all agro-ecologies with no significant difference among them. Incidence did not exceed 6%. The severity of cassava anthracnose disease was also very low and did not exceed 1.1.

The incidence of cassava white spot disease was low in all agro-ecologies but significantly different. The expression of the white spot disease was observed mostly in the dry season. The savannah lowland had the highest incidence of 28.8% followed by the rain forest with 26.2% and the coastal plain with 16.7%. The lowest incidence of 0% was observed in the peninsular mountain and the savannah highland with a 5% incidence. The severity of cassava white spot disease was also very low and did not exceed 1.3 (**Table 6**).

4. Discussion of the findings

The survey results showed that the Cassava Mosaic Disease (CMD) and the Cassava bacteria blight (CBB) were the most important diseases affecting cassava production [16] . The High prevalence of Cassava Mosaic Disease (CMD) in the rainy season could be attributed more to the use of susceptible local varieties. Time of planting and environmental conditions was also major factors that influence the seasonal variation observed in the expression of the diseases [17]. Higher incidence of cassava mosaic disease recorded in the dry season in Bombali, Port Loko, Kono and Tonkololi could be attributed to late planting in September to December. The project dissemination of new agricultural technologies in Africa (DONATA) highly influenced the adoption of recommended planting date (May to June) observed mostly in the southern province which consequently had lower diseases incidence as manifested in Bonthe district. The early manifestation of the disease was also attributed to the planting of infected cuttings and whitefly population [18]. This implies that the search for adaptable varieties that are high yielding and tolerant to cassava mosaic disease remains a requirement for the mass propagation of planting materials. Deployment of resistant genotypes remains the most feasible approach however consideration should be given to traits desirable to farmers. A trait such as good cooking ability fits better into the food utilization pattern a characteristic dominant among local varieties susceptible to the cassava mosaic disease.

Thomas [19] had reported a 100% incidence of the diseases in the Western Area, a situation which was reflective of the trend country-wide before this survey. The intensity however differs mostly across agro-ecologies. The much lower disease incidence and severity rating observed in this study can be attributed to the adoption of improved cassava varieties promoted by the Government of Sierra Leone and development partners. The contrasting results for the Bonthe district can best be explained by associating farmers with the cultivation of improved cassava varieties and processing machines for gari and foo-foo production, which is the major source of livelihood in the district. No clear hot spot for the Cassava Mosaic Disease was could be identified. This could be explained based on the diversity of the cassava genotypes both local and improved cultivated in Sierra Leone.

In the case of cassava bacterial blight, the high prevalence in the rainy season could also be attributed more to the use of susceptible local varieties. Time of planting and environmental conditions were considered to be the major factor that influenced seasonal variation observed in the expression of the disease.

The search for adaptable varieties that are high yielding and tolerant to cassava bacterial blight disease remain a requirement for mass propagation of planting materials. In the absence of resistant genotypes, training farmers in the identification and control of cassava bacterial blight disease within the early warning systems perspective is critical to avoid disease outbreak.

The Cassava bacterial blight disease (CBB) has not been studied extensively in Sierra Leone in terms of yield loss and loss associated with the esthetic value of the marketable leaves. This is important because cassava leaf is highly consumed as a vegetable sauce among millions of people countrywide [14]. An increase in the incidence and severity of cassava bacteria blight will affect the cassava leaf market as well as the livelihood of smallholder farmers especially women.

Cassava anthracnose disease (CAD) and leaf spot disease (LSD) were the least important in terms of threat in cassava production. Higher incidence of cassava anthracnose disease and leaf spot disease recorded in the dry season in Bombali, Port Loko, Kono and Tonkololi remains a concern.

5. Conclusion and recommendation

From the research the following conclusion can be drawn:

Cassava Mosaic Disease (CMD) was considered to be more prominent in the rainy season than the dry season. In district terms, Kono, Koinadugu and Tonkolili districts had the highest incidence and severity of Cassava Mosaic Disease (CMD) in the dry season. Koinadugu, Kenema and Kambia had the highest incidence in the rainy season. Generally, Bonthe and Western Area were among the lowest in terms of incidence and severity of the Cassava Mosaic Disease (CMD). No clear hot spot was identified.

In agroecological terms, the rain forest had the highest incidence and severity of the cassava mosaic disease while the coastal plains and the Peninsular Mountains were among the lowest. Spatial distribution map using the geographic information system mapping revealed that CMD was widely distributed across the country.

Based on the current level of expansion in cassava production in Sierra Leone for use as feed, food and industries, it is recommended that routine and comprehensive standardized surveys of cassava diseases be conducted to determine the epidemiology of the diseases. A multidisciplinary approach has to be adapted and should involve entomologists, agronomists, weed scientists and more related disciplines, this integrated approach could be used to design model for diseases forecast in different agro-ecological zones, and avert indiscriminate use of insecticides in the control of most disease vectors should the need arise.

Cassava bacterial blight disease (CBB) was considered to be more prominent in the rainy season than the dry season. At the district level, Kono, Koinadugu and Tonkolili districts had the highest incidence and severity of cassava bacterial blight disease (CBB) in the dry season. Koinadugu, Kenema and Kambia had the highest incidence in the rainy season. Generally, Bonthe and Western Area were among the lowest in terms of incidence and severity of the disease. No clear hot spot was identified.

In agroecological terms, the rain forest had the highest incidence and severity of the cassava bacterial blight disease while the coastal plains and the Peninsular Mountains were among the lowest. Spatial distribution map using the geographic information system mapping revealed that CBB was widely distributed across the country. Despite the high incidences, severity was considered low. From the study, it is recommended that the search for resistant genotypes and farmer education on disease identification and control should be prioritized.

Cassava anthracnose disease (CAD) and Cassava leaf spot disease was considered to be more prominent in the dry season than the rainy season. However, disease incidence and severity were low and therefore considered cassava diseases of less importance in Sierra Leone.

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Section 3

Enhancement of Cassava Production

Chapter 4

Research and Development for Improved Cassava Varieties in Ghana: Farmers' Adoption and Effects on Livelihoods

Patricia Pinamang Acheampong, Eric Owusu Danquah, Kennedy Agyeman, Kwame Obeng Dankwa and Monica Addison

Abstract

The importance of Cassava in the food systems of Ghanaians cannot be underestimated. As a main staple crop, Cassava contributes about 22% and 30% to the Agricultural Gross Domestic Product (AGDP) and daily calories intake respectively. Per capita consumption of 152 kg makes it the highest among all food crops. Due to Cassava's importance, there have been lots of attention paid to it by the Government and Donor agencies towards its improvement. This has yielded substantial results in terms of the development of cassava varieties and good agronomic practices. This chapter reviewed cassava technologies development in Ghana, adoption of these technologies by smallholder farmers, and livelihood implications. Results generated showed that Research and Development since 1993 has developed, released, and disseminated 25 new cassava varieties to smallholder farmers. Average cassava yields have increased from about 14 t/ha in 2009 to 21 t/ha in 2018. Partial budget analysis showed that smallholder farmers' profitability has increased over the years from GH₵644.32 (about US$ 111) in 2009 to GH₵5243.27 (about US$ 904) in 2018. Again, the crop is gradually gaining attention as an industrial crop for flour, starch, and alcohol production, a drive that would further improve on returns to farmers. It is a food security crop because it is robust, produces more per unit area, and versatile for multiple usages in household foods and derivatives. It is recommended that continuous policy consideration on cassava in national agricultural agenda setting is essential.

Keywords: food system, per capita consumption, productivity, Policy, technologies

1. Introduction

Cassava (*Manihot esculenta Crantz*) is an important food security and income-generating crop cultivated by many smallholder farmers globally, mainly in developing countries [1, 2]. Cassava came to Africa in the 16th century from Brazil through Portuguese traders and was adopted for home consumption as a famine-reserve crop during drought seasons [3]. Cultivation of cassava in Ghana started

around trading ports, castles, and forts as a major food for the Portuguese and their slaves. By mid of the 18th century, its cultivation had spread along the coastlines of Ghana. The serious drought in Ghana in 1982–1983 cropping seasons failed resulting in the failure of many staple food crops accounted for the wild spread of cassava from the coastal area to other areas of the country [4]. Despite the important role of Cassava in Ghanaian food security, productivity is below potential. The continuous efforts by Research and development to increase productivity are necessary to improve the current situation.

The contribution of agricultural technology, such as improved seeds to agricultural productivity and increases in rural incomes, cannot be underestimated. Improved agricultural technology is believed to lead to poverty alleviation through positive effects on consumers' food prices, producers' incomes, and labourers' wage incomes [5, 6]. The impacts of agricultural technologies on poverty alleviation could be direct and indirect; nonetheless, the direct effects are far more significant than the indirect effects, as evident from many countries [7]. The main goal of developing agricultural technologies such as high-yielding crop varieties is to reduce hunger, malnutrition, and poverty in rural and urban areas [5]. [5] opined that a percentage increase in agricultural productivity could decrease the percentage of poor people living on less than $1 a day by 0.6% and 2%. There had not been any known economic activity that has generated that kind of effect for the poor.

Ghana's agriculture sector, compared to other sectors, is dominated by staple crops and has a greater impact on poverty reduction as it employs the majority of the working force [8, 9]. Cassava is particularly important amongst the Ghanaian staple crops, as it guarantees good yields even in harsh conditions. It also offers flexibility to resource-poor farmers because it could serve as either subsistence or as a cash crop. As a cash crop, cassava generates cash income for the largest number of households with other staples. Due to cassava's importance, a lot of attention has been paid to it. Research and development have released and disseminated 25 new varieties [10] over the years. Many studies [11–13] have reported on their adoption by farming households. The current theme looks at cassava research, cassava varieties developed, improved cassava variety adoption, and their impact on smallholder farmers livelihood.

2. Cassava production, area harvested, and productivity

As a main staple food crop, cassava contributes about 22% and 30% to the Agricultural Gross Domestic Product (AGDP) and daily calories intake of Ghanaians respectively. Ghana since 2005 has ranked 6th globally in terms of value [9, 14]. It is the most widely cultivated and consumed root and tuber crop followed by yam and cocoyam. As presented in **Figures 1**–**3**, between 2009 and 2018 cassava production, the area planted, and per capita consumption averaged 16,190,210 Mt, 897,230 ha, and 152.9 respectively. Within the same period the production, area planted, and per capita consumption averaged 6,870,810 Mt, 424,080 ha, and 125 respectively for yam and 1,348,230 Mt, 203880 ha, and 40, respectively for cocoyam [9, 14, 15].

The forest-Savannah transition and the forest agro-ecological zones consisting of Eastern, Brong-Ahafo, Ashanti, Central, and Volta regions are the major cassava producing areas contributing about 86% of the total national production. Nation-wide area planted to cassava and production increased by 1.13% and 5% respectively in 2016–2018 [9]. Although productivity increase has been observed,

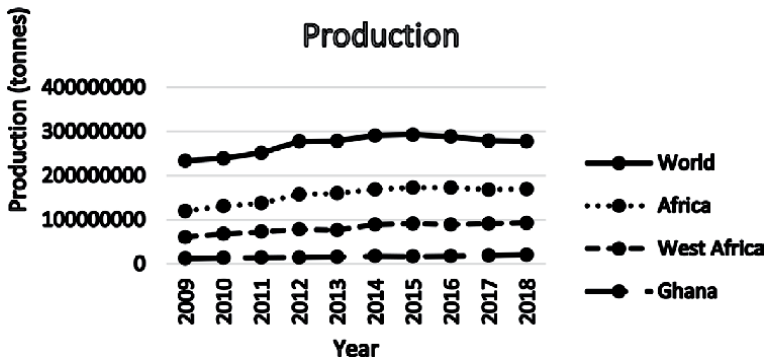

Figure 1.
Production of cassava in Ghana compared with the world, Africa and West Africa. Source: FAOSTAT [15].

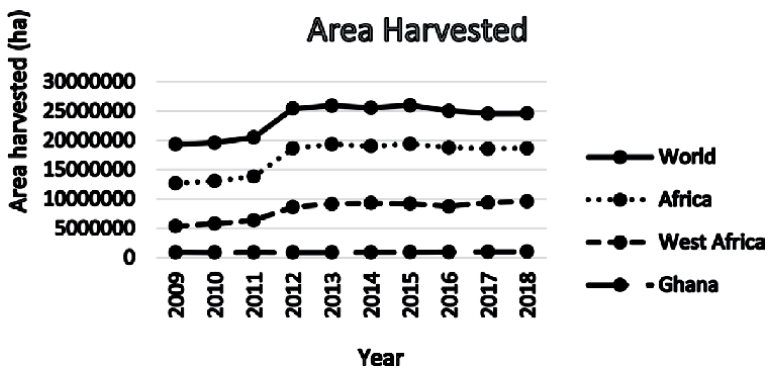

Figure 2.
Cassava area harvested in Ghana and compared with world, Africa and West Africa. Source: FAOSTAT [15].

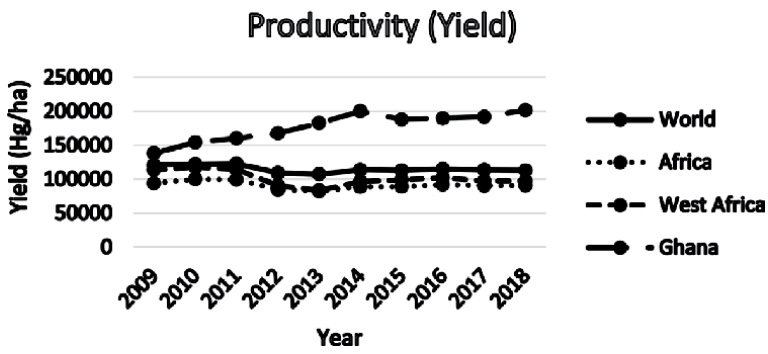

Figure 3.
Cassava yield trend (2009–2018) in the world, Africa and West Africa compared to Ghana. Source: FAOSTAT [15].

production expansion may be due to area expansion as only 47.40% (21.33 Mt/ha) of the potential yield of 45 Mt/ha is achieved currently [9].

3. Cassava improvement in Ghana

Originally, farmers selected or collected cassava stems from previous cultivation to establish new fields with little to no knowledge of disease transfer and trait heritability.

In the '1930's Cassava Mosaic Virus Disease (CMVD) was first reported in Ghana [3]. The disease was highly destructive and severely affected all existing local varieties, especially at the early stages [16]. The government of Ghana's attention was drawn, and the first intervention towards the development of improved cassava varieties began.

The first intervention from the government of Ghana (GoG) toward developing improved cassava varieties was in 1930 when the Cassava Mosaic Virus Disease (CMVD) was first reported in Ghana [3]. The disease was highly destructive enough to merit attention, as all existing local varieties were severely affected significantly at the early stages [17]. This intervention involved introducing superior cassava varieties from other countries in West Africa, East Africa, and the Caribbean. Several crosses were made between these superior varieties and their local counterparts followed by selections for desirable traits resulting in the release of four outstanding cassava varieties namely "Queen", "Gari", "Williams" and "Ankrah" in 1935. These crops were high-yielding (7–10 t/ha), of good taste, highly resistant to CMVD, and were grown extensively across the country [18]. In the late 1950s, the newly released varieties except "Ankrah" became highly vulnerable to CMD, blaming it on either increased virulence of the virus or breakdown in resistance or purity, compelling a second breeding intervention for CMVD resistance [18].

The second breeding intervention involved crosses between the local varieties and four other species closely related to *M. esculenta* since no resistance could be found in any *M. esculenta* varieties. These inter-species crosses were carried out all through the mid-1950s to mid-1960. Out of these crosses four selected progenies K357, K162, K680, and K491 were released to farmers [18]. The best of these varieties, K680, yielded around 19 t/ha, had moderate resistance to CMVD with good palatability and cooking quality. These varieties were cultivated widely and their good characteristics were maintained until the late 1970s and early 1980s. A third intervention had to be sought to obtain varieties resistant to two new pests (cassava green spider mite and cassava mealybug) and a new disease, Cassava Bacterial Blight (CBB), in addition to CMVD [18].

Ghana entered into a bilateral agreement with IFAD, leading to the implementation of the Ghana Smallholder Rehabilitation and Development Programme (SRDP). The National Root and Tuber Crops Improvement Project (NRTCIP), which commenced in 1988, was a component of SRDP [19]. Among the chief aims of the NRTCIP was giving support to root crop adaptive trials, starting a program of biological control of cassava mealybug and cassava green mite, and supporting human resources development for root and tuber crops research and biological control of the pest.

An appreciable number of research works were carried out on Cassava in the late 90s, including; mealybug and green spider mite biological control programme. The SRDP project ended in 1995, but the NRTCIP continued to receive some funding under the succeeding project, the Smallholder Agricultural Development Project (SADEP) born out of SRDP. With the assistance of the World Bank, the Government of Ghana In 1991 launched a National Agricultural Research Project (NARP) as a long-term process to strengthen Ghana's agricultural research system. The project generated improved technologies to contribute to national development objectives and growth in the agricultural sector.

The severe drought and famine experienced during the 1980s in Ghana intensified factors limiting agricultural development, resulting in agricultural production decline [20]. To augment the stumpy growth in the national economy, the government of Ghana (GOG), under the guidance of the World Bank and IMF, established an Economic Recovery Programme (ERP) in 1983 to stabilize the deteriorating economy. Effective policies introduced by the ERP resulted in the decline in

Variety	Year Released	Maturity Period (Months)	Mean Root Yield (T/ha)	Total Dry Matter (%)	Uses	CMV Resistance
Afisiafi	1993	12-15	28-35	32	Starch, flour, gari	Tolerant
Abasafitaa	1993	12-15	29-35	35	Starch, flour, gari	Tolerant
Tekbankye	1997	12-15	30-40	30	fufu, ampesi, gari	Susceptible
Dokuduade	2005	12	35-40	30	Starch, gari	Resistant
Agbelifia	2005	12	40-45	33	Starch, gari	Resistant
Essam bankye	2005	12	40-50	35	Flour, gari	Resistant
Bankyehemaa	2005	9-12	40-50	32	Flour, gari, fufu	Resistant
Capevars bankye	2005	9-12	30-35	30	Flour, gari, fufu, starch	Resistant
Bankyebotan	2005	12-15	25-30	28	Flour, gari, starch	Tolerant
Eskamaye	2005	15-18	16-23	25	Tuo, konkonte	Tolerant
Filindiakong	2005	15-18	16-20	28	Tuo, konkonte	Tolerant
Nyerikobga	2005	15-18	17-29	30	Tuo, konkonte	Tolerant
Nkabom	2005	12-15	28-32	32	Starch, fufu	Tolerant
IFAD	2005	12-15	30-35	30	Starch, fufu	Tolerant
Ampong	2010	12	40-50	36	Flour, Starch, fufu	Resistant
Broni Bankye	2010	12	40-45	33	Flour, bakery products	Resistant
Sika bankye	2010	12	40-45	36	Flour, Starch	Tolerant
Otuhia	2010	12	35-40	39	Flour, Starch	Resistant
CRI-Duade Kpakpa	2015	12-15	60	37	Poundable, Flour, starch	Resistant
CRI-Amansan bankye	2015	12	57	38	Flour and bakery products	Resistant
CRI-AGRA bankye	2015	12	63	32	Starch, flour	Resistant
CRI-Dudzi	2015	12	49	38	Starch, Flour	Resistant
CRI-Abrabopa	2015	12-15	46	40	Hi-starch	Resistant
CRI-Lamesese	2015	12	50	39	Poundable, Beta-Carotene, Flour	Tolerant

Note: CMV is cassava mosaic virus. Source: CSIR - Crops Research Institute's Annual reports (various).

Table 1.
Improved Cassava Varieties Released in Ghana and Their Characteristics.

the production of major food crops, including cassava [21]. Under the ERP, an Agricultural Services Rehabilitation Project (ASRP) was launched in 1987 to expand agricultural production through research, extension services, irrigation, policy planning, monitoring, and coordination [21].

In 2000, the Agricultural Services Sub-sector Investment Programme (AgSSIP) [22, 23] was also introduced, followed by the Root and Tuber Improvement and Marketing Program (RTIMP). In 2008 the West Africa Agricultural Productivity Program (WAAPP) was also initiated to develop improved technologies for roots and tubers in collaboration with the Root and Tuber Improvement and Marketing Program (RTIMP) [23]. Over the years, these programmes and projects have brought about the development and release of 25 new cassava varieties (**Table 1**).

All these varieties have moderate resistance to the cassava mealybug pests and tolerant to the cassava mosaic virus. Without fertilizer application, the new cassava varieties have 40% higher yields than the local varieties on farmers' fields [1]. In terms of various post-harvest attributes and for intercropping these new varieties are as good as the local varieties.

4. Good agronomic practices

The performance of any released improved cassava or crop varieties is significantly influenced by the agronomic package that goes with it. For example, cassava farmers who adopted the Root and Tuber Improvement and Marketing Programme (RTIMP) Technology of improved planting materials and agronomic packages increased productivity [24]. Thus, apart from developing the improved varieties, the research systems have developed improved agronomic technologies that are complementary. They include minimum tillage, spacing, plant density, fertilizer application, use cover and manure of green, weed control, and disease control. The practice of minimum tillage in cassava production is strongly recommended. Farmers are advised to slash but not burn their whole farm but rather practice spot burning when necessary. It is recommended that minimum tillage is practiced in sandy soils to conserve soil moisture and reduce soil erosion. Infertile soils, recommended plant population of 10,000 plants/ha is used. Stem cuttings must be planted at 1.0x1.0 m for sole crop cassava or at wider row spacing (up to 2 m between rows) and closer in-the-row spacing (down to 0.5 m) for intercropping [25–28].

While Cassava can grow better than most other crops in impoverished soils, the crop does respond well to chemical fertilizers or animal manures. A fertilizer application rate of 200 kg of N-P_2O_5-K_2O 15-15-15/ha or 20 g of fertilizer per plant is recommended. Leguminous intercrops and green manures can improve the N status of soil through N fixation. In areas where ploughing is used, farmers are advised to plough leguminous cover crops such as Mucuna to improve the soil's physical and chemical properties. They are also advised to add manure such as cow dung or poultry droppings at land preparation [25, 26].

Cassava is a poor competitor and may suffer severe yield losses if weeds are not adequately controlled during the early growth stages. Generally, weeds should be cleared 2-3 times during the first three months or until canopy closure. Weeding is most often done manually by hoe or with herbicides. Weed competition is lessened by adequately applying fertilizer to speed up canopy closure, intercropping, and planting in the early dry season when weed growth is less vigorous. When herbicides are used, it is recommended to apply glyphosate (roundup)/ paraquat (gramoxone) as a pre-planting herbicide to kill fallow vegetation. For post-emergence control of weeds, a shield should be used to keep chemicals off the crop [25, 26].

The primary diseases affecting cassava are bacterial blight, mosaic disease, root rot, and anthracnose. The means of controlling the mosaic disease are not yet known. Planting tolerant varieties, planting clean stem cuttings, roguing out disease plants and burn to reduce diseases' spread are some of the recommendations [25].

Ghana's agro-ecological zones have annual rainfall ranging between 800 mm and 2200 mm with a soil pH of 3.5–7.8. The Forest, Forest-Savannah transition and Coastal agro-ecological zones have bimodal rainfall whiles the Guinea and Sudan savannahs have unimodal rainfall. These suitable conditions for cassava production give Ghana a competitive advantage for cassava production in the entire world [9].

5. Adoption of improved varieties

The major reasons for the development of improved technologies and the release of high-yielding varieties are to reduce hunger, malnutrition and poverty. Also, it is expected to result in improved income and livelihood of poor people living in marginal areas [5]. Interestingly, cassava production has been increasing in the past five years since 2007. In 2007, cassava's total production was a little over 10.2 million tons (MT). Currently, cassava production is estimated at 19.2 million tonnes, the highest among all food crops [9].

The increase in production can be associated with the adoption of improved cassava varieties. In Ghana, farmers' preference for the variety they choose for cultivation is based on; yield, in-soil storage (longevity) and disease resistance [29].

Variable	All zones	Forest	Transition	Costal savannah	Guinea Savannah
All improved Cassava	41.22	47.85	48.39	41.03	43.85
Afisiafi	15.69	10.84	18.71	19.23	11.63
Filindiakong	0.25	0.00	0.00	0.00	1.00
Tech bankye	0.34	0.41	1.29	0.00	0.34
IFAD	0.08	0.00	0.65	0.00	0.00
Nkabom	0.34	0.61	0.65	0.00	0.00
Capevarse	0.25	0.61	0.00	0.00	0.00
Bankyehemaa	11.62	13.70	15.48	7.26	9.63
Esambankye	1.36	2.45	1.94	0.00	0.66
Agbelifia	0.34	0.20	1.29	0.00	0.00
Abasafita	0.59	0.41	0.00	0.43	1.33
Ampong	1.53	1.23	2.58	1.14	1.00
Sika	3.73	4.91	0.00	1.28	5.98
Otuhia	0.93	0.00	0.00	4.70	0.00
Bronibankye	0.17	0.00	0.00	0.00	0.33
Agric/MoFA	4.66	7.77	2.58	2.14	6.97
Indigenous	58.86	52.15	49.68	58.97	56.15

Source: Acheampong et al. (2007).

Table 2.
Adoption rates of improved varieties by agro-ecological zones, Ghana.

For example, [30] reported that some farmers in the Brong-Ahafo and the Ashanti Regions have testified that the improved varieties of cassava yield three times more than the local varieties. Poverty alleviation is possible with the use of improved technologies [31]. The adoption of improved cassava varieties in Ghana is essential since the crop is cultivated by about 90% of the farming population in Ghana [32], making it the right target crop for reducing poverty in the country [33]. A recent study by [12] put the overall adoption rate of improved cassava varieties at 40%, indicating the need to do more to encourage adoption. Adoption of improved cassava varieties is very crucial to improving productivity. [34] observed that higher adoptions can be achieved through the availability and distribution of planting materials and farmer participatory demonstrations. **Table 2** presents adoption rates of improved varieties by agro-ecological zones in Ghana.

6. Livelihoods improvements

Ghana's national food security is time and again attached to the availability of root and tuber crops especially cassava. The food security role of Cassava is widely attributed to its availability during times of food shortages. Because cassava can provide multiple opportunities for poverty reduction and nourishment for poor people in Ghana, lots of research efforts have gone into the development and dissemination of it for increased production to meet increasing demand. To increase food production, policy objective and research emphasis have been on increased production and adaptability to diverse production systems and environments [24, 35]. The rapid increase in cassava production will undoubtedly have significant implications on food security, employment creation, living conditions, and economic growth [31, 36, 37]. Food security is attained once the total available physical supplies of food are adequate and households have ample access to those food supplies through either their production, the market, or other sources, and the utilization of those food supplies is appropriate to meet the specific dietary needs of individuals [38].

[39] found many uses of cassava in Ghana and other West African Countries. Cassava tubers can be eaten as a vegetable after boiling or roasting. They can also be boiled and pounded into a paste and then added to soups and stews ("Fufu" in Nigeria and Ghana). The fresh tubers can be preserved as sundried chips ("Kokonte" in West Africa) and consumed after cooking or ground into flour [40]. Cassava can also be eaten as coarse flour form known as "Gari". Apart from fresh consumption of Cassava, the crop can also be processed into chips for animal feed and into starch for either food or non-food industries. Cassava flour is used to prepare bread, biscuits, confectionery, pasta, and couscous-like products and the production of adhesives. It is used in the textile and paper industries and plywood and veneer adhesives manufacture. In pharmaceuticals, it is used in the production of glucose and dextrin syrups. Cassava root extract can be fermented to produce alcohol. As a waste material, it can be processed into biogas.

Cassava is also an income generation crop for many farming households in Ghana. Cassava yields improved from 13 to 16 t/ha to 18–20.1 t/ha between 2009 and 2012 and 2013–2018, respectively [14]. This resulted in an average benefit–cost ratio of 1: 0.59 and 1: 1.54 for 2009–2012 and 2013–2018, respectively. Thus, a profit or returns of about Gh ₵ 0.54 would be accrued in addition to the Gh ₵ 1.00 invested capital for cassava production after the 2012 cropping season. This is compared to early seasons before 2012 when losses would have been incurred (**Table 3**). Improvement in the adoption of improved cassava varieties by farmers might increase productivity (yield) after 2012.

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Year	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Average yield (ton/ha)	13.81	15.43	16.01	16.75	18.27	20.02	18.78	18.96	19.19	20.18
Average rural wholesale price (Gh₵/ton)	171.96	202.88	200.27	305.64	422.55	399.61	478.09	453.40	511.87	449.36
Gross benefit (₵/ha)	2374.32	3130.99	3206.84	5119.05	7720.16	8001.75	8978.66	8596.52	9824.72	9068.27
land clearing(₵/ha)	400.00	450.00	510.00	600.00	690.00	715.00	780.00	810.00	830.00	860.00
Cost of planting materials(₵)	300.00	350.00	380.00	500.00	640.00	650.00	690.00	710.00	740.00	780.00
Labor cost for planting(₵/ha)	280.00	330.00	360.00	410.00	430.00	460.00	490.00	615.00	640.00	655.00
Cost of weeding 2 times till harvest (₵/ha)	450.00	480.00	510.00	560.00	645.00	690.00	705.00	735.00	760.00	780.00
Harvesting cost(₵/ha)	300.00	390.00	450.00	510.00	590.00	640.00	690.00	710.00	740.00	750.00
Total cost of production	1730	2000	2210	2580	2995	3155	3355	3580	3710	3825
Net benefit	644.32	1130.99	996.84	2539.05	4725.16	4846.75	5623.66	5016.52	6114.72	5243.27
Benefit cost/Ratio	0.37	0.57	0.45	0.98	1.58	1.54	1.68	1.40	1.65	1.37

Note: Average rural price of cassava from 2012 to 2018. Source; FAOSTAT [15] & MoFA (2018).

Table 3.
Partial budgeting and profitability of cassava production in Ghana from 2009 to 2018.

A higher adoption rate of improved cassava varieties is associated with increased income, poverty reduction, and household food security. [41] showed that improved cassava varieties had significant poverty-reducing impacts in Zambia and [42] in their impact assessment of the adoption of improved cassava varieties in Ghana, revealing an increase in income of cassava farmers where the impact is more realised in female farmers than male farmers. Their Average treatment effect (ATT) estimates suggested that participation in improved cassava varieties increased total crop incomes of women by ₵3,173 (USD 1,823) whilst that of men was increased by ₵149 (USD 86) per hectare. In stimulating agricultural growth by using improved cassava varieties; household food security is also ensured as most farmers can use food from their production rather than food purchases [43–45]. Cassava provides farmers with additional income-earning opportunities and enhances their ability to contribute to household food security [46, 47]. This is beneficial to alleviating poverty as [48] reiterated that the adoption of agricultural technology by women is significant as it can generate large gains in alleviating poverty. Also, the increase in its production through the adoption of improved cassava varieties of high-yielding and disease-resistant characteristics can improve rural welfare [12]. [49] opined that cassava consumption aids in the nutrition of its consumers due to its nutrient traits. In the study, he stated that cassava produces remarkable energy quantities per day, even compared to cereals.

The rising demand for cassava starch at both the local and international market presents a great opportunity for Ghana to enhance foreign exchange revenue through export and improve farmers' livelihood through improved income. In light of this, the Government of Ghana in 2001 introduced the Presidential Special Initiative (PSI) on cassava, which aimed at industrialising the cassava sector for job creation and livelihood improvement through starch production export. The Ayensu starch company at Bawjiase was established for this purpose [50]. The general expected impact was that Ghana would improve cassava value chain and take advantage of the rising global demand for cassava-starch which stood at about 222 million metric tonnes in 2002 [51]. Cassava starch has a competitive advantage for ethanol production over other materials [44, 45] Gradually, cassava is becoming an urban food as cassava flour is reported to be a excellent supplement to wheat flour up to 20% for the production of bread and other pastries [50, 51].

7. Conclusions and future directions

This chapter has reviewed cassava varieties development, adoption, and livelihood indicators in Ghana. Generally, improved cassava technologies play critical roles in agricultural transformation and livelihood improvements of smallholder farmers and other value chain actors in Ghana. Research and development have generated many new varieties and good agronomic practices. Since 1993, 25 new cassava varieties have been developed, released, and disseminated to smallholder farmers. The average yields of cassava over the years have been encouraging. The average yield of cassava has increased from about 14 t/ha in 2009 to 21 t/ha in 2018. Partial budget analysis showed that smallholder farmers' profitability increased over the years from GH₵644.32 in 2009 to GH₵5243.27 in 2018.

Adoption of improved cassava technologies such as improved varieties and good agronomic practices should, ceteris paribus, increase cassava productivity and provide additional income for smallholder farmers. The suggestion is that a demand-driven approach should be adopted to promote and develop cassava-based industries identifying opportunities and constraints of cassava at each stage of

the commodity chain. This is achieved by individuals interested in developing the cassava industry; producers, processors, and consumers of cassava, and associated national and non-governmental organisations. Government and non-governmental organisations could support to establish a cassava seed system. Commercial seed producers could then be encouraged to adopt it through training and demonstrations and setting up sales points across the country.

Cassava serves as a major staple as in calories consumed and as a source of raw material for starch-based industries. It is now regarded as a major food security crop. It can change the livelihood of various actors along the value chain making it essential not only as a food crop but also as a significant source of income for rural households. Research on alternative food forms and breeding to improve the shelf-life of the tubers would be required.

To achieve food security through the production and supply of staple food as cassava, to meet the population's demands, the government and private sector should increase support to the development and dissemination of improved cassava technologies that improve cassava farmers' resilience to climate change. Also, smallholder farmers should be incentivised in terms of credit and other production inputs to adopt improved technologies for increased productivity and improved livelihoods.

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Conflict of interest

The authors declare no conflict of interest.

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Chapter 5

Assessing Mating Designs Utilized in Cassava Population Improvement

Prince Emmanuel Norman, Daniel K. Dzidzienyo and Kumba Yannah Karim

Abstract

Cassava breeders are curious about appropriate breeding strategies utilized to generate elite genotypes with desired complimentary traits or genes from parents used in crossing. Use of appropriate mating design is influenced by a good understanding of the flower biology of the putative parent plants, type of pollination, crossing technique, pollen dissemination, the presence of male-sterility system, the purpose of the project (that is either breeding or genetic studies), and the size of population needed. The objective of this book chapter is to assess the current knowledge on mating designs, their applications and limitations in cassava improvement. This book chapter discusses the floral biology, genetic improvement, breeding procedures and mating designs in cassava. The information utilized in this study were obtained from various sources including documentary search of the journals, books and websites of relevant stakeholder organizations. Empirical findings of selected mating designs in cassava and their impacts were discussed. Findings serve as a good guide for selection of appropriate mating arrangement to obtain useful information on parents and progenies. Findings are relevant to scientists, researchers, scholars, lecturers and other relevant stakeholders.

Keywords: cassava, breeding, genetics, mating designs, gene action

1. Introduction

Breeders and geneticists have used different mating designs for development of improved genotypes of plants [1]. Mating designs are principles involved in arranging different cross combinations and altering the genetics of plants to satisfy human needs [1, 2]. The original intent of developing these designs was to estimate additive- and/or dominance- variance genetic parameters. Successful plant breeding involves selection of appropriate mating design and parents [1, 3]. However, selection of appropriate mating designs is influenced by the type of crossing used (artificial or natural), type of pollination (self or cross pollinated), type of pollen dissemination (insect or wind), the purpose of project (genetic or breeding studies), the presence of male sterility system and the size of population desired. Selection of mating design for the estimation of genetic diversity depends on the objective of study, time, space and biological limiting factors. A suitable mating arrangement is imperative for successful plant breeding programme [4].

Mating and experimental designs are critically important in plant breeding. The four main significance of mating designs include (i) generation of information on genetic control of character, (ii) serves as basis for selection and development of elite genotypes in breeding population, (iii) estimation of genetic gain, and (iv) generation of information for evaluation of parents in breeding program [5–8]. Use of any particular mating design lies in its ability to adequately address research questions of plant breeders such as: Are genetic variabilities significant? How much of the variation is heritable or due to environment? And what types of gene(s) influence significance? Comparison of variances of both segregating and the non-segregating generations provides a good resolve of the above questions [9]. Generally, for a given number of parents, the mating design that permits larger number of crossings will produce smaller sampling variance. Path coefficient analysis, generation mean analysis, stability analysis, heritability and genetic advance, combing ability, heterosis and inbreeding depression, gene action in plant breeding, triple test cross analysis, correlation and regression analyses are also used to answer various research questions [10, 11]. The statistical components and interpretations should match the mating and experimental designs used in plant breeding experiments [6].

This book chapter focuses on the floral biology, genetic improvement, breeding procedures and mating designs in cassava. The objective was to provide updated information on breeding procedures and mating designs in cassava. This information would contribute to effectively guide scientists, researchers, students and research and development partners in the selection of adequate design for improved efficiency in cassava breeding.

2. Floral biology of cassava

Cassava belongs the genus Manihot of the family Euphorbiaceae. The genus comprises two sections, viz., the Fructicosae, which contains low-growing shrubs adapted to savannah grassland or desert conditions and the Arborae, containing tree species [12, 13]. There are about 98 species in the genus Manihot, all of which are confined to the tropical Americas [14]. Cultivated cassava belongs to the section Fructicosae, often considered as an unknown cultigen in the wild [14]. All known species of Manihot are polyploids with 2n = 36 chromosomes, although they exhibit regular bivalent pairing, thereby behaving as diploids [15].

Cassava is monoecious with both male (pistillate) and female (staminate) flowers borne on the same plant [16]. The female cassava flowers are usually borne on the lower part of the inflorescence and are fewer than the male flowers, which are borne on the upper part of the inflorescence [17, 18]. The female and male flowers on the same inflorescence open at different times, a condition known as protogyny. The female flowers open one to two weeks before the male flowers. However, female and male flowers on different branches of the same plant can open at the same time. Cassava is a cross–pollinating root crop producing highly heterozygous plants [13, 17]. Self-pollination has been noted, although the proportions of self- and cross–pollinated seeds generated depends on the genotype, the type of pollinating insect present and the plant design [13]. Inbreeding and inbreeding depression have been reported in cassava [19, 20].

Cassava exhibits varying flowering patterns ranging from those with frequent flowering pattern to types that do not flower [21]. Flowering may begin at six weeks after planting in the early types, depending on the genotype and the environment used [13]. Flowering initiation is preceded by reproductive branching known as forking. The time of forking depends on the genotype and agroecological conditions. Optimum flowering occurs at moderate temperatures of about 24 °C [17].

Since cassava does not flower during a long dry season, Kawano [18] suggested that cassava crossing blocks should be irrigated during the dry season. A good understanding of the optimum flowering period and duration of flowering is important for successful crossing.

Flowering in cassava is controlled by the complex interaction of a range of genetic and environmental factors [17]. Moreover, the control of flowering and flowering itself are major challenges in cassava breeding. For instance, genotype(s) possessing valuable complementary traits of interest may not be used in breeding due to its shyness and non-synchronized flowering [22]. Flowering synchronization of genotypes for crossing may present a problem. However, flowering on a single plant usually lasts for more than two months [13]. Since male flowers are more numerous than female flowers, the limiting factor for large production of hybrid cassava seeds is lack of sufficient female flowers [17]. Thus, genotypes intended to be used as female parents should be planted sequentially and in higher numbers than the males [18]. The number of seeds set per female flower significantly differ per genotype. This necessitates the selection of highly fertile genotypes as female parents.

3. Genetic improvement in cassava

3.1 Parental selection and hybridization in cassava

Genetic improvement in root and tuber crops begin with the assembly and evaluation of broad-based germplasm or source populations [23]. Genotypes with high frequencies of genes associated with desirable complementary traits are selected from the source populations and utilized as parents for the generation of new recombinant genotypes [20, 23]. Since cassava is highly heterozygous, selection of suitable parents for hybridization is among the most critical steps in a breeding programme. Parental selection for a cassava hybridization programme is often based on known performances of putative genotypes. However, parental selection based on phenotypic performances alone is not a sound procedure, since phenotypically superior clones may produce poor recombinants in the segregating population [24]. This led to the suggestion of selecting elite parents on the basis of genetic value [25]. Genotypic performances in hybridization programmes depend on their combining ability and the effectiveness in transmitting heredity traits to their progeny [26]. In a breeding programme where general combing ability (GCA) is more important, a small number of parents with good GCA should be utilized. If the specific combining ability (SCA) is more important, a large number of parents should be used to produce a large number of the first filial generation (F_1) families [25]. A good understanding of existing profiled parental traits is imperative to guide effective parental selection and genetic improvement of current breeding populations. Genetic improvement through hybridization consists of selection of parents, generation of F_1 progeny and selection of superior clones [25]. Clonally propagated crops such as cassava, yam, sweetpotato, etc. are generally improved by crossing two or more clones possessing desirable complementary traits, followed by selection in the F_1 progeny. Controlled pairwise-crossing could be done manually to produce full-sib families, or naturally in polycross nurseries that allow open pollination to produce half-sib families [23, 27]. The breeding procedure of clonally propagated crops is known as clonal selection [28].

3.2 Breeding procedures

The main objective of breeding is to improve the characteristic of plants to obtain new genotypes that are more desirable agronomically and economically

[25, 29]. However, improving some specific traits of certain crops may be among key priority objectives for various agronomic and/or economic reasons. Some of the common breeding methods in cassava include: clonal selection, recurrent selection and backcross breeding. Backcross breeding has been used to incorporate genes for disease resistance [23, 30].

3.3 Clonal selection

A clone is a group of genetically identical vegetative propagule obtained from a single plant [31]. Hybridization of parental clones with superior traits produces a source population that may be utilized for the selection of new clones [32]. Hybridization creates genetic variability in progeny obtained through gene recombination of parental clones used. Botanical seeds obtained from crosses are grown in seedling nurseries. The growing seedlings are exposed to major diseases and pests such as cassava mosaic disease (CMD), cassava brown-streak disease (CBSD), and cassava bacterial blight (CBB) for selection against susceptible seedlings [30]. The clonal selection is based on visual observations and on breeders' judgment of the genetic value of the clones [25]. The replicated preliminary yield trial (PYT) should involve both the selected clones and suitable standard check(s). A few clones with desirable superior performances are selected for the yield trial stages. Replicated yield trials should be conducted in several locations along with suitable standard check(s). The best clones with desirable high yield, quality and disease resistance are then multiplied and released as a new variety [28, 30]. Alternatively, the superior clones may be isolated and grown as a variety from a genetically mixed population of an asexually propagated crop. The demerit of this technique is that genetic progress is limited to the isolation of the best genotype present [28]. Moreover, the phenotypic values the clones are contingent upon include the effects of the genotype, the environment and the genotype $\times$ environment interactions [33]. During the early stages of clonal selection, single plants or single plots are used with the emphasis of eliminating weak and undesirable plants [30]. The later clonal selection stages involving replicated yield trials, yield and yield attributes are considered, with the emphasis of identifying and selecting superior clones [16].

3.4 Recurrent selection

Recurrent selection involves the selection of a number of plants with desirable phenotype, growing, evaluating and selecting seedling-derived clones obtained from seeds, and intercrossing the progenies in all possible combinations [25, 32]. At IITA, recurrent selection has been utilized for the genetic improvement of cassava breeding populations for CMD resistance and other agronomic traits [34]. Genetic improvement for CMD resistance alone was accomplished in one cycle (1–2 years). However, combination of CMD resistance with high yield potential trait took about 4–5 years. The introgression of exotic sources from other continents, especially Latin America, into IITA breeding populations was achieved after obtaining adequate resistance to CMD [34].

3.5 Backcross breeding

Backcross breeding involves repeated backcrossing of the hybrid and the progenies in subsequent generations to one of the parents [28]. The progenies obtained are increasingly similar to the recurrent parent. Interspecific hybridization between cultivated cassava (*M. esculenta*) and other related Manihot species such as *M. glaziovii* has been reported [35]. Backcrossing of progenies to cultivated cassava (the

recurrent parent) to recover positive agronomic traits and resistance to CMD produced variable results regarding fertility. However, the second backcross generation had improved fertility. Controlled back-crossing of progenies to cultivated female cassava parents successfully produced F_3 generation [15, 35].

Transfer of high protein content of tuberous roots of cassava from wild species (*M. tristis* subsp. saxiola) was achieved through backcrossing of hybrids obtained between cassava and *M. tristis* subsp. saxiola to the recurrent parent [35, 36]. However, breeding efforts to increase favorable alleles protein content in cassava roots were unsuccessful since the high protein contents in the initial hybrids were not maintained in the backcross progenies [37].

3.6 Inbreeding in cassava

Inbreeding reduces the genetic load of deleterious genes thereby increasing the frequency of desirable genes [23] and improving selection efficiency [38]. In sweetpotato, inbreeding depression was noted for storage root yield, storage root number and vine length [39]. To develop new genotypes with high dry matter content and high storage root yield, it has been suggested to develop high dry matter inbred lines for crossing among themselves or with superior cultivars. Development of genotypes with high starch content requires concentration of genes controlling starch content in the genotype [39]. In cassava breeding, inbreeding is seldom practiced due to the lengthy duration required to obtain high levels of inbreeding (9–10 years) and high level of inbreeding depression [23]. Tolerance to inbreeding depression can be bred into crops. Fifth generation inbred lines of cassava were developed at IITA [40]. In India, Easwari-Amma et al. [38] reported reasonable homozygosity in successive generations of inbred lines of cassava in four generations.

3.7 Gene action and inheritance

Gene is known as the basic unit of inheritance. Genes are responsible for the transmission of characteristics from one generation to the next [29]. Gene action and gene inheritance are involved in crop improvement process. From physiology perspective, gene action is the reflection of gene differences that provide the basis for the selection of desirable genotypes in plant breeding [41]. Gene action also determines the expression of characteristics of plants including its morphology, response to environments, and yielding ability. Gene inheritance is the transmission of genetic material or information to succeeding generations [42]. According to Klug and Cummings [43] the classical Mendelian genetic pattern of inheritance reveals the expression of one dominant, when two contrasting characters are brought together in a cross and one recessive (latent) in F_1 and in F_2 the two characters segregate and express themselves phenotypically. Knowledge of gene inheritance is relevant for the efficient recovery and maintenance of desirable genes transmitted from selected parents to their progeny [26].

Multiple genes are known to influence the phenotypic expression of a quantitative trait. The four gene actions in the phenotypic expression of traits are: additive, dominance, epistatic and overdominance. Additive genes are genes that act additively or cumulatively to a quantitative character, whereas dominance gene effects are deviations from additive effects [44]. Epistatic gene effects are non-allelic gene interactions; and over-dominance gene effects occur when the contribution of the combined alleles is greater than the separate effect of either allele [26].

Most of the physiological characters such as yield, dry matter content and disease resistance are inherited quantitatively [32]. Quantitatively inherited

characters are controlled by polygenes with small individual effects and environmental effect [42]. The varied expression of quantitatively inherited characters is continuous and measurable [45].

Different mating designs possess different genetic parameters that can be estimated. For instance, the diallel or North Carolina II mating design permits for the estimation of two key genetic parameters for the set of genotypes used [42]. The two genetic parameters include: the average performance of parents in crosses, which estimates the breeding value of a given genotype due to additive gene effects, also known as general combining ability (GCA); and the deviation of individual crosses from the mean performance of parents, due to specific allelic combinations or dominance effects, also referred to as specific combining ability (SCA). A high GCA/SCA variance ratio indicates the importance of additive gene action effects, whereas a low ratio suggests the presence of dominant and/or epistatic gene effects [46]. If the SCA is smaller than the GCA, the performance of the single cross progeny is affirmed based on the GCA of the parents.

3.8 Estimation of genetic variances

The phenotype is a combined expression of the genotype and environment effects. The breeder is mainly interested in determining the proportion of the phenotypic expression that is due to genotypic and environmental effects [24]. The genotypic effect of a genotype is the difference between the mean of all the phenotypes with that genotype and the mean of all the phenotypes in the population [42]. Prediction of genetic improvement expected from crossing and inbreeding requires estimation of variances between crosses. Thus, selection of a mating design for the development of progenies and their evaluation depends on its efficiency in estimating variance components [42].

The main purposes of using mating designs are: to inform breeders with vital information on the genetic control of the character under investigation; and to generate breeding population(s) that can be utilized as a source population for the selection and development of potential genotypes [47]. These purposes enable the breeder to choose an appropriate breeding strategy, thereby evaluating the genetic progress that can be expected for a given selection intensity [48].

3.9 Selection and evaluation stages in cassava

Early cassava breeding efforts mostly focused on improvement of the crop for staple food for humans [13]. During this period, intensive selection was done for disease resistance and root yield potential, thereby restricting the availability of genetic variability for starch and dry matter content [49]. The general breeding objectives considered were high yield, root characteristics, adaptability to a wide range of environmental conditions, early maturity resistance to major insect pests and diseases [23, 30]. Early generation testing is a pre-requisite in self- and cross-pollinated crops to permit the estimation of the genetic potential of an individual [50]. The early generation testing and selection in cassava involves seedling and clonal (single row) stages on the basis of high heritability obtained in traits such as plant type, branching habits and reaction to certain diseases [51] and harvest index [52]. Selection can also be done on the basis of single plant performance [23]. Seedlings are usually exposed to key cassava diseases such as CMD, CBSD and CBB using spreader varieties, and susceptible seedlings are eliminated.

The selection criteria for the seedlings includes short neck (1–3 cm), fat roots that are uniform, short, and compact as well as low cyanide content [49, 53]. Seedlings that are low branching (50–100 cm) are also discarded since they are

associated with high branching habit that tends to produce low harvest index and yield [20]. Sometimes root dry matter is also used as a selection criterion [49]. The relevance of root dry matter was observed by Byrne [21] that significant correlations ($r = 0.48^{**}$) exist between dry matter content in seedling and single row trials, and suggested that evaluation for this trait was feasible at the F_1 stage. The selected seedlings are harvested at 12 months after planting and screened for conformation and root characteristics. Visual evaluation with few data collection is often used at the early stages of selection involving large number of materials for easy handling at reduced costs [23]. Low planting densities have been suggested for seedling population establishment to allow equal opportunity of all plants to express their genetic potential and to minimize the effects of intergenotypic competition [54]. At the clonal evaluation trial stage, competition between neighboring genotypes may favor more vigorous plant architectures [55].

At the later stages of selection, the emphasis of selection shifts from highly heritable traits to those of low heritability such as yield. At the preliminary yield trial (PYT), advanced yield trial (AYT) and regional trial (RT) stages of selection, each plot is replicated at least twice [23]. The selection is done on the basis of yield per plot, dry matter content, CNP levels, consumer acceptance, and adaptation of the crop [30]. Multi-environments trials (METs) covering a wide range of environments are done to identify clones with reasonable stability for desired traits across METs. Superior clones are evaluated on-farm for farm level testing and farmer evaluation. The clones that are the most popular with farmers are recommended for rapid multiplication, distribution and release.

4. Mating or breeding designs in cassava

Many mating designs have been developed to enable breeders and geneticists to extract more genetic information from parents and progenies. These mating designs are broadly categorized into two [56]. The first is based on control over parents involved in a cross. This group is sub-divided into two: (i) control over seed parents only. Examples include top cross and polycross designs; and (ii) control over both parents and examples include diallel, partial diallel, line × tester, North Carolina and bi-parental designs. The second broad category is based on control over parents per progeny. This is sub-divided into four including (i) one-factor designs such as top cross and polycross; (ii) two-factors designs such as bi-parental, diallel, partial diallel, line × tester designs and generations; (iii) three factors design such as triallel design; and (iv) four factors design such as quadriallel design. Mating designs offer different hierarchical structures, such as half-, full-sib family, and individuals within family, in the progeny population. Of the mating designs highlighted above, the key ones that are often utilized in cassava breeding are discussed below.

4.1 Biparental mating design

Bi-parental mating design involves selection and paired random mating of a large number of parents (n) to obtain $\frac{1}{2n}$ full-sib families [5]. The features of the design include (i) it is viewed as the simplest mating design [57]; (ii) it involves F_2, P_1 and P_2 generations of a single cross; (iii) it requires 3 cropping seasons for generation of materials and the fourth season for evaluation; (iv) analysis is based on second order statistics and (v) it consists of full sibs or unrelated progeny. The genetic assumptions of bi-parental design are (i) random distribution of genotypes in relation to variation; (ii) random selection of plants for mating; (iii) regular

diploid segregation; (iii) absence of epistasis; (iv) equal survival of all genotypes; (v) absence of linkage; (vi) absence of maternal effects; and (vii) lack of multiple allelism.

The merits of bi-parental design are (i) it provides information on additive and dominance components of genetic variance; (ii) it is useful in selecting breeding procedure for genetic improvement of polygenic characters. The demerits of this design include: (i) it lacks the ability of providing information for estimation of all genetic parameters; (ii) use of unjustifiable assumption where critical genetic and environmental parameters are needed [48]; and (iii) overestimation of genetic component compared to environmental component where unjustified assumptions are used. Although family plots may be allotted by randomization of individual plants in the experiment to increase precision of overestimates, it is however, practically more expensive. The simpler alternative is direct estimation of VEC from families $\times$ replicates interaction mean square in an adequately replicated trial. The layout, statistical model and analysis of variance of the bi-parental mating design are reviewed in Nduwumuremyi et al. [6].

4.2 Diallel mating design

Diallel mating design is a mating arrangement used in both plant and animal breeding to investigate the genetics of qualitative and quantitative trait inheritance [58]. Diallel design is the most widely used and abused of all mating designs in estimating genetic parameters [58]. The abuse is possibly related to the presence of random and fixed models used in diallel analysis [59]. In the random model, parents are random members of a random mating population, and is useful to estimate the general combining ability (GCA) and the specific combining ability (SCA) variances. The GCA estimates the mean performance of a line in hybrid combinations and is due to additive gene action. It is also the deviation of progeny mean from the mean of all lines in the experiment [5]. The variations between maternal groups are essentially due to variations in their general combining ability. The SCA measures specific hybrid crosses that perform relatively better or worse than expected mean performance of lines involved and is due to non-additive gene action [5]. It measures the dominance deviation from the additive model. Information of GCA and SCA (the types of gene action influencing various traits) enables evaluation of parental entries and selection of the best breeding system for maximum improvement of trait [60]. In this mating scheme, the general linear models are commonly used for identification of heterotic groups [59], estimation of general or specific combining ability [61], interactions with environments and years, or estimation of additive, dominance and epistatic genetic effects and genetic correlations [62].

There are essentially four main types of diallel mating design that have been reported [60]. These include: Full diallel involving parents and reciprocal crosses along with F_1, half diallel with parent and without reciprocal crosses, Full diallel without inclusion of parents, and Half diallel without parents and reciprocal crosses. In full diallel mating design, all parents, reciprocals and F_1s are mated in all possible combinations to generate hybrids [63]. The features of this design include (i) it requires twice as many crosses and entries in experiments; (ii) it permits testing for maternal and paternal effects [60]; (iii) it is required for Hardy–Weinberg equilibrium in a population [5]. The half diallel without reciprocals is effectively applied where reciprocal effects are assumed to be negligible in a diallel mating system. Other forms of diallel mating design include partial or fractional diallel mating design and disconnected half diallel mating design.

The partial diallel or fractional diallel mating design is a technique in which part of all possible crosses are made from a diallel analysis [1]. It is usually utilized in

plant breeding to evaluate parents for their combining ability [64]. Partial diallel mating design involves omission of certain crosses thereby reducing number of crosses per parent ($n - 1$) without losing much precision. In the symmetric partial diallel mating, s number of crosses per parent (P) are sampled from n number of crossing parents ($P = 1, \ldots .., n$). The value of s is: $2 < s < n$-1, where s and n are number of parents and sample crosses, respectively. The s and n differentially odd and even (i.e. s is even where n is odd and vice versa, but $s \neq n$-1). The number of crosses generated for selected s and n is: $C = \frac{ns}{2}$. The C is developed based on the sampling constant $K = \frac{n+1-s}{2}$, which is an absolute number variance between one parent and other parents to be crossed with the former [56]. The features of partial diallel analysis are (i) it uses one type of analysis (ii) it uses one method of combining ability (iii) it involves direct crosses; (iv) it involves sampling of crosses (v) it evaluates more parents than diallel; and (vi) helps in selection of parents and breeding procedures [64].

The merits of partial diallel analysis include (i) it estimates heritability, genetic advance and heterosis; (ii) it is also utilized in open pollinated species with problem of male sterility [64]; (iii) the GCA of the parents are estimated with less precision, but larger gains may result from intense selection among a larger number of parents; (iv) selection can be done among crosses from many parents; and (v) where the parents represent a population, the variance for general combining ability can be estimated more accurately [65]. Some demerits of partial diallel analysis are: (i) each parent equal chance of mating and recombining with every other parent [64]; (ii) its analysis is complex; (iii) it does not estimate difference between each pair of GCA effects with same precision; and (iv) an unsuccessful cross further creates imbalance in partial diallel mating, whereas such missing cross can be tolerated without significant loss of balance in diallel design [56].

4.3 Polycross mating designs

Polycross is the natural intermating or crossing among group of genotypes in isolated block [6]. It was first used by Tysdal, Kiesselbach and Westover in 1942, in relation to progenies obtained from outcrossed plants in a nursery [48]. Polycross design has been used for various plants that are obligate crossers such as tree crops, forage grasses, sugarcane, legumes and especially roots and tubers that are propagated by vegetative means, i.e. cassava, sweet potato and yam [5]. Polycross design accords equal chance of natural intermating among plants thereby eliminating the possibility of selfing and hand pollination [56]. Although polycross design in cassava breeding does not prevent self-pollination, however, it produces more cross-bred botanical seeds than controlled pairwise pollination techniques [13]. Self-pollination can be minimized in polycross blocks by emasculating plants located near an intercrossing population [13]. The genetic basis of polycross is similar to topcross, but differing with its characteristic wider genetic background of pollen source.

The features of a polycross nursery include: (i) it is adequately isolated to safeguard against foreign pollen grains; (ii) it consists of small plot size and highly replicated compact blocks to permit large number of test genotypes; (iii) plots and blocks are arranged in all directions that permit random pollination by wind; and (iv) two to three border rows of seed mixture of test inbreds.

The mating arrangement of putative candidates or entries is critical for successful random mating in the polycross block [26]. Latin square design has been considered most appropriate for equal random intermating among entries in a polycross nursery [66], however, where entries are more than 10, completely randomized block design could be used [5]. About 20–30 replications are used in the crossing block of the two designs. Ideal criteria for use of polycross is practically

hard to fulfill due to several limitations such as cross incompatibility, male sterility, irregular- and non-flowering and lack of flowering synchronization, nonrandom dispersal of pollen, among others cause deviation from random mating. Plant breeders often use sequential planting of genotypes with different flowering dates for synchronization of flowering and crossing.

The progenies of each of the test genotypes are half-sib families. They are collected separately and mixed over replications and evaluated. For instance, if six inbreds will produce six polycross bulk seed samples, these samples and parental seeds are grown in replicated randomized complete blocks design. Data are collected in the same fashion as the top cross mating trial [56]. The covariance within the families is estimated as: $Cov(HS) = \frac{1+F}{4}\sigma^2{}_A$ where F is the inbreeding coefficient of the genotypes being tested. The statistical models for r_{OP}, b_{OP} and b_{AP} are similar to those of top cross design. In polycross, $b_{AP} = \frac{Cov\ (AP)}{2\ var\ (P)}$ since only one parent is known [56].

The merits of polycross design lie in its applications such as in the development of synthetic cultivars, recombination of selected entries in recurrent selection breeding, or evaluation of GCA of entries [5]. It is used for identification of maternal parents with superior genotypes based on general combining ability reflected in the performances of the progenies [6]. Conventionally, half sibs are developed from individual maternal parent. However, paternal parents could be identified using DNA fingerprinting and parentage analysis. Polycross is useful in determining variance components, GCA and heritability. The variance components and GCA are determined from the average performance of the progenies of individual maternal parents. Variations measured in a progeny are partitioned into within and between maternal parents [26]. The estimated heritability obtained reveals the usefulness of polycross and guides the choice of progenies in breeding programme [56].

The demerits of polycross include insufficient statistics to estimate all genetic parameters, expected genetic gains are reduced by half since the components of variance are estimated from maternal half sibs; information about the males is lost, there is no control over the pollen source; nonrandom mating may occur due to lack of flowering synchronization, unequal pollen production and/or position effects within crossing block [6]. Environmental variability greatly influences flowering and performances of the parents and progenies possibly due to their diverse origins and heritability of traits [6, 66]. These factors could affect the accuracy of GCA and heritability estimates suggesting a cautionary note on their use.

4.4 Line × tester mating design

The line × tester mating design involves mating between lines (f) and testers to generate 'fm' hybrids [56]. It is the simplest design that simultaneously provides full-sibs and half-sibs compared to topcross which only exhibits half-sibs. The line × tester technique was introduced by Kempthorne [67] as one of the powerful tools used to estimate the combining ability effects and assist selection of desirable parents and crosses for pedigree breeding [68].

The main features of the lines × tester design are (i) mf crosses are needed in which 'm' is male and 'f' is female; (ii) it provides information on germplasm lines; (iii) it consists of simple analysis compared to complex designs; and (iv) it follows both first and second order statistics [56].

The main features of a good tester include (i) broad genetic base; (ii) wider adaptability; (iii) low yield potential; and (iv) low performance of other traits [56].

The linear model for line × tester design is: $Y_{ijk} = \mu + g_i + g_j + S_{ij} + r_k + e_{ijkl}$ where: Y_{ijk} = observed value of the cross i × j in the k^{th} replication; μ = population

mean effect; g_i = GCA effect of i^{th} tester; g_j = GCA effect of j^{th} line; S_{ij} = SCA effect of the cross i × j; r_k = effect of the k^{th} block; e_{ijkl} = experimental error due to $(ijk)^{th}$ individual. Detailed explanation of the model is reported in Karim et al. [69].

Some merits of line × tester design are (i) it facilitates selection of desirable parents and breeding procedure via measurement of genetic components of variance; (ii) it is good for estimation of genetic gains from both V_A and V_D and evaluation of germplasm; (iii) it provides heterosis, heritability and genetic advance estimates [56]; (iv) it is useful in determining types of gene actions involved in the expression of quantitative traits [70]. Some demerits of the line × tester design are (i) it does not estimate epistasis variance, (ii) it lacks equal change of random mating [56]; (iii) limited selection intensity; and (iv) high cost.

4.5 North Carolina mating designs

The North Carolina designs were developed to improve the efficiency and save time that restrained diallel mating design. The North Carolina designs I, II, and III were developed in 1952 by Comstock and Robinson.

The general features of the NC designs include:

i. Effective in breaking undesirable linkages – mating randomly selected plants in segregating population.

ii. Selection of suitable breeding procedure for polygenic traits.

iii. Useful for self- and cross-pollinated species.

iv. Used in the generation of variability – creating heterozygosity.

v. The bi-parental mating permits evaluation of segregating (F_2 or later generation) population of individual cross made between two inbred lines.

vi. It provides information on two components of genetic variance i.e. additive and dominance variance.

vii. It is useful in the selection of suitable breeding procedures.

Despite these applications, the North Carolina designs are (i) not applicable to the segregating populations of three way, double and multiple crosses; (ii) does not permit several simultaneous segregating crosses; (iii) does not provide information on the epistatic variance; and (iv) analysis is difficult as it is based on second order statistics.

The specific characteristics and applications of the various NC designs are summarized in **Table 1**.

Of the three North Carolina designs summarized in **Table 1**, NC I and II are often utilized in root and tuber breeding programmes. The North Carolina I mating design (nested design) is a hierarchical design that involves mating between each common male and different sets of female parents. It is widely applicable in theoretical and practical plant breeding [5]. The statistical model for NC I design is: $Y_{ijk} = \mu + m_l + bf_{ij} + r_k + \mathcal{E}_{ijk}$ where Y_{ijkl} = observed value from each experimental unit; μ = population mean; m_i = effect of the i^{th}; f_{ij} = effect of the j^{th} female mated to the i^{th} male, r_k = replication effect, and $\mathcal{E}_{ijk}$ = experimental error [58].

The merits of this design include (i) it is useful for both self- and cross-pollinated species; (ii) it is utilized for estimation of additive and dominance variances; (iii) it is

No.	North Carolina Design I (NC I)	North Carolina Design II (NC II)	North Carolina Design III (NC III)
1	Each male is mated to a different group of females.	Each male is mated to the same group of females.	Each male is mated to both inbred parents of original cross.
2	'f' crosses are obtained.	'mf' crosses are obtained.	'2 m'crosses are obtained.
3	Variance is divided into 2 parts: males and females.	Variance is divided into 3 parts: males, females and male × female.	Variance is divided into 2 parts: male and male × female.
4	Variance due to male provides an estimate of additive variance (V_A).	Variance due to male and female provides an estimate of additive variance (V_A).	Variance due to male provides an estimate of additive variance (V_A).
5	Variance due to female provides an estimate of additive (V_A) and dominance variance (V_D).	Variance due to male × female provides an estimate of dominance variance (V_D).	Variance due to male × female provides an estimate of dominance variance (V_D).
6	It requires 10–12 times more area than design 3.	It requires 2–3 times more area than design 3.	It requires much less area than designs 1 and 2.
7	It is influenced by the presence of maternal effects.	It is influenced by the presence of maternal effects.	It is not affected by the presence of maternal effects.
8.	It involves F_2 plants in crossing.	It involves F_2 plants in crossing.	It involves F_2, P_1 and P_2 plants in crossing.
9.	It is least powerful design.	It is intermediate design.	It is most powerful design.

Source: Acquaah [5].

Table 1.
Characteristics of North Carolina designs.

used to evaluate full- and half-sibs in recurrent selection; (iv) it is widely used in animal studies, as well as maize breeding for determination of genetic variances [5]; (v) its merit over biparental and polycross designs, is that it gives three statistics, whereas biparental and polycross designs give two statistics [71].

The demerits of NC I are (i) it is practically inapplicable in breeding species that produce few amounts of seed, since the technique requires sufficient seeds for replicated evaluation trials; and (ii) it does not account for epistatic variance.

The North Carolina II (NC II) mating design is a factorial design that has been modified from NC I design by Comstock and Robinson [72]. In the NC II design, each progeny family exhibits half-sib relationships through common male and common female. It is most adapted to plants with multiple flowering that facilitate repeated use of each as male and female parents.

The NC II is used to estimate genetic variances and to assess inbred lines for combining ability [73]. In NC II, an equal number of different sets of females and males is randomly selected from an F_2 population, with each male mated with a female to create female half-sib (HS) groups and male HS groups [46]. This is achieved through systematic crossing of n_1 male and n_2 female in all possible mating combinations to give n_1n_2 progeny families [5]. Reciprocal crosses are if the objective is to analyze for maternal effects [48]. The mean squares for the female and male sets produce separate and independent estimates of the additive component of variation. The additive variance estimates obtained include variance due to males, and variance due to females. This technique also produces an estimate of non-additive genetic variance (dominance variance) from the interaction mean squares obtained between the males and females.

The difference between the mean performance of the progeny of a given male and the mean of the progeny of all the males used in the crosses is the GCA. The GCA gives an indication of how well the genes combine, on average, to produce the best progeny when crossed to a random sample of females in the population. Th GCA is often obtained from the mean square (MS) between HS family groups. The occurrence of significant deviation from the mean performance of the progeny is attributable to dominance or epistatic effects. The deviations of specific individual crosses are estimated from the 'male × females' MS in the ANOVA of the NC II [46].

If the number of males and females are the same ($n_1 = n_2$), the magnitude of the maternal effects is estimated from the variance ratio MSF/MSM [46]. The NC II design provides additive and dominance variance estimates (V_A and V_D) and a test of significance, thereby permitting the estimation of heritability [46].

The merits of NC I mating design are: (i) the NC II is useful for estimation of both GCA and SCA [5]; (ii) there is possibility of analyzing for maternal effects using reciprocal crosses [48]; (iii) the design is useful for evaluation of inbred lines for their combining abilities; (iv) it provides estimates of genetic gains from both V_A and V_D; and (v) it provides good information for parents and full-sib families. The demerits of this design include: (i) it lacks estimate of epistasis or G × E interaction effect [46]; (ii) limited selection intensity; and (iii) high cost.

5. Conclusions and future prospects

This chapter articulates features, applications, analysis, merits, demerits, the role and progressive modifications of mating designs in explaining the nature of quantitative variation for their use in plant breeding and genetics. The choice of appropriate parents and mating design depends on critical factors of genetic, environmental, interaction between the genotype and environment, etc. A good knowledge of the flower and pollination biology, total genetic variance due to genetic and environmental influence guides meaningful decision on resource allocation and expected response to selection. Moreover, the use of appropriate experimental design and statistical technique would help plant breeders to estimate and explain the type of gene action existing in parents and progenies. Half-sibs generated in some of the designs can be resolved using DNA fingerprinting or parentage analysis. The use of integrated conventional-molecular techniques increases precision and efficiency in plant breeding. However, as efficient molecular techniques emerge, economic phenotyping of mega trials still remains a challenge.

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Conflict of interest

The author declares no conflict of interest.

Appendices

Appendix 1. Analysis of variance (ANOVA) for bi-parental mating design

Source of variation	Degree of freedom	Mean square	Expected mean square
Between families	$\left(\frac{a}{b}\mathrm{n}\right)-1$	M_1	$\sigma^2{}_w + r\,\sigma^2{}_\mathrm{b}$
Within families	$\frac{a}{b}n(r-1)$	M_2	$\sigma^2{}_w$
Total	$\frac{a}{b}\mathrm{nr}-1$		

Source: Acquaah [5].

Appendix 2. Analysis of variance (ANOVA) for full diallel mating design (Method I)

Source of variation	Degree of freedom	Sum of squares	Mean squares	Expected mean squares	
				Model I	Model II
GCA	$(p-1)$	S_g	M_g	$\sigma^2+2p\left(\frac{1}{p-1}\right)\sum g_i{}^2$	$\sigma^2+\frac{p(p-1)}{2}$ $\sigma_s{}^2 + 2p\sigma^2_g$
SCA	$\frac{p(p-1)}{2}$	S_s	M_s	$\sigma^2+\frac{2}{p(p-1)}\sum\sum S_{ij}{}^2$	$\sigma^2+\frac{2(p^2-p+1)}{p^2}\sigma_s{}^2$
Reciprocal eff.	$\frac{p(p-1)}{2}$	S_r	M_r	$\sigma^2+2\left(\frac{2}{p(p-1)}\right)$ $\sum_{i<j} r_{ij}{}^2$	$\sigma^2 + 2\sigma^2 r$
Error	m	S_e	M_e	σ^2	

Source: Griffing [59].

Appendix 3. Analysis of variance (ANOVA) for half diallel with parents and without reciprocal crosses mating design (Method II)

Source of variation	Degree of freedom	Sum of squares	Mean squares	Expected mean squares	
				Model I	Model II
GCA	$(p-1)$	S_g	M_g	$\sigma^2+(2+p)\left(\frac{1}{p-1}\right)$ $\sum g_i{}^2$	$\sigma^2 + (p + 2)$ σ^2_g
SCA	$\frac{p(p-1)}{2}$	S_s	M_s	$\sigma^2+\frac{2}{p(p-1)}\sum\sum S_{ij}{}^2$	$\sigma^2+\sigma_s{}^2$
Error	M	S_e	M_e	σ^2	

Source: Griffing [59].

Appendix 4. Analysis of variance (ANOVA) for full diallel without inclusion of parents mating design (Method III)

Source of variation	Degree of freedom	Sum of squares	Mean squares	Expected mean squares	
				Model I	Model II
GCA	$(p-1)$	S_g	M_g	$\sigma^2+2p(p-2)\left(\frac{1}{p-1}\right)\sum g_i^2$	$\sigma^2 + 2(p-2)\sigma^2_g$
SCA	$\frac{p(p-3)}{2}$	S_s	M_s	$\sigma^2+\frac{2}{p(p-3)}\sum\sum S_{ij}^2$	$\sigma^2+ 2\sigma_s^2$
Reciprocal eff.	$\frac{p(p-1)}{2}$	S_r	M_r	$\sigma^2+2\left(\frac{2}{p(p-1)}\right)\sum_{i<j} r_{ij}^2$	$\sigma^2 + 2\sigma^2 r$
Error	M	S_e	$M_{e'}$	σ^2	σ^2

Source: Griffing [59].

Appendix 5. Analysis of variance (ANOVA) for half diallel without parents and reciprocal crosses mating design (Method IV)

Source of variation	Degree of freedom	Sum of squares	Mean squares	Expected mean squares	
				Model I	Model II
GCA	$(p-1)$	S_g	M_g	$\sigma^2+(p-2)\left(\frac{1}{p-1}\right)\sum g_i^2$	$\sigma^2+\frac{p(p-1)}{2}\sigma^2_g + 2p\sigma^2_g$
SCA	$\frac{p(p-3)}{2}$	S_s	M_s	$\sigma^2+\frac{2}{p(p-3)}\sum\sum S_{ij}^2$	$\sigma^2+ \sigma_s^2$
Error	M	S_e	$M_{e'}$	σ^2	σ^2

Source: Griffing [59].

Appendix 6. Skeleton Analysis of variance (ANOVA) for partial or fractional diallel mating design

Source of variation	Degree of freedom	Sum of square	Mean square	Expected mean square
Replication (r)	$(r-1)$	*rSS*	*rMS*	—
Crosses (c)	$(c-1)$	*cSS*	*cMS*	—
GCA (n)	$(n-1)$	*gSS*	*gMS*	$\sigma^2_r + \mathrm{r}\,\sigma^2_s + \frac{rs(n-2)}{(n-1)}\mathrm{r}\,\sigma^2_s$
SCA (s)	$(c-\mathrm{n})$	*sSS*	*sMS*	$\sigma^2_r + \mathrm{r}\,\sigma^2_s$
Error (e)	$(c-1)(\mathrm{r}-1)$	*eSS*	*eMS*	σ^2_r
Total	$(cr-1)$	*TSS*		

Source: Sharma [56].

Appendix 7. Analysis of variance (ANOVA) for polycross mating design

Source of variation	Degree of freedom	Mean square	Expected mean square	Variance components
Progenies	$(g-1)$	M_1	$\sigma^2_e + r\,\sigma^2_{prog}$	$\sigma^2_{prog} = Cov(HS) = \frac{1+F}{4}\sigma^2_A$
Blocks	$(r-1)$	M_2	—	—
Error	$(g-1)(r-1)$	M_3	σ^2_e	$\sigma^2_e = \sigma^2$

Source: Nduwumuremyi et al. [6]; Wricke and Weber [74].

Appendix 8. Analysis of variance (ANOVA) for line × tester mating design

Source of variation	Degree of freedom	Mean square	Expected mean square	
			Model I	Model II
Replication (r)	$(r-1)$			
Genotype (g)	$(g-1)$	MS_2		
Parents (p)	$(p-1)$			
Crosses (c)	$(c-1)$			
Lines (m)	$(m-1)$	M_m	$\sigma^2 + rf\left(\frac{1}{m-1}\right) + \sum g_i^2$	$\sigma^2 + v_{sca} + rf_{gca(m)}$
Testers (f)	$(f-1)$	M_f	$\sigma^2 + rm\left(\frac{1}{f-1}\right) + \sum_{\varphi} g_j^2$	$\sigma^2 + rv_{sca} + rm_{gca(f)}$
Lines × testers	$(m-1)(f-1)$	M_{mxf}	$\sigma^2 + r\left[\frac{1}{(m-1)(f-1)}\right] + \sum_{\iota}\sum_{\varphi} s_{ij}^2$	$\sigma^2 + rv_{sca}$
Error	$(r-1)(mf-1)$	MS_1	σ^2	σ^2

Where MS_2 = genotypic mean square, M_m = line mean square, M_f = tester mean square, M_{mxf} = line × tester mean square, and error MS_1 = mean square. Adapted from Nduwumuremyi et al. [6] and Fellahi et al. [75].

Appendix 9. Analysis of variance (ANOVA) for North Carolina design II

Source of variation	Degree of freedom	Mean square	Expected mean square
Replication	$(r-1)$		
Males	$(m-1)$	M_1	$\sigma^2_w + r\,\sigma^2_{mf} + rf\,\sigma^2_m$
Females	$(f-1)$	M_2	$\sigma^2_w + r\,\sigma^2_{mf} + rm\sigma^2_f$
Males × females	$(m-1)(f-1)$	M_3	$\sigma^2_w + r\,\sigma^2_{mf}$
Within progenies	$mf(r-1)$	M_4	σ^2
Error	$(mf-1)(r-1)$	M_5	σ^2_w
Total	$rmf-1$		

Source: Kearsey and Pooni [52].

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Chapter 6

Improvement in Cassava Yield per Area by Fertilizer Application

John Okoth Omondi and Uri Yermiyahu

Abstract

Cassava is a source of carbohydrates to more than 200 million people in Sub-Saharan Africa, even though its production is 6–8 t ha^{-1}, which is below the highest world production of 36.4 t ha^{-1} in India. To address this yield gap and increase cassava's availability, affordability, and adequacy, intensive but sustainable production is important. Additionally, being an emerging raw material in the animal feeds, pharmaceutical, beer industries etc., only increases its demand, however the current production levels cannot effectively sustain this. Therefore, this paper reviews: improvement in cassava yields per area under fertigation and banding of fertilizers, a common practice among many farmers; the advantage of fertilizer application on starch of the storage roots, which is the fundamental ingredient in most industries using cassava as a raw material; and the climate smart technologies for intensive sustainable cassava production. In the end, this review enhances knowledge about fertilizer application to cassava, both banding and fertigation, and expounds on effective intensive sustainable climate-smart production strategies.

Keywords: storage roots, irrigation, fertilizer, sustainability, climate smart, macro-nutrients

1. Introduction

Cassava is a root crop which provides starch to over 500 million people in the tropics and is the sixth most important crop in the world [1]. Its importance is gradually increasing in the beer and pharmaceutical industries due to demand for its starch [2]. Yet, its world production is only 262.6 million tonnes [3], in which the highest yield per hectare was achieved in India (36.4 t ha^{-1}), while Sub-Saharan Africa (SSA) produced 6–8 t ha^{-1} [3]. Also, increase in population is not parallel to food production in sub-Saharan Africa leading to deficits that can only be filled by imports. This lack of synchrony between population growth and food output is attributed to an inability of crops to achieve their potential – the yield gap. Hillocks [4] cautiously reported that there was a 46% yield gap for cassava in Africa, while globally it was 36%. He linked such to a myriad of factors: from unpredictable rainfall distribution to poor adoption of technologies, scarcity of inputs, minimum usage of inorganic fertilizers and poor agronomic practices etc. Even though these factors contribute to the yield gap, poor soil quality or lack of fertilizer application [5] are key and hence require urgent solution [6]. In order to address this, Giller et al. [7] suggested that best-fit technologies that are compatible with farm practices are essential. Such technologies include integrated soil fertility management (ISFM) and conservation agriculture (CA).

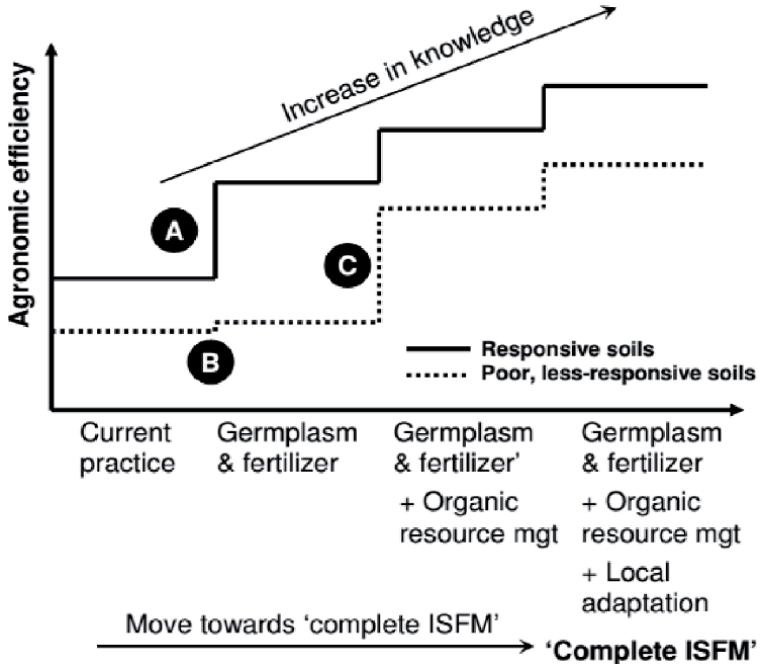

Figure 1.
Relationship between the agronomic efficiency (AE) and various components of ISFM. Source: [8].

After developing integrated soil fertility management (ISFM) concept [8], Vanlauwe et al. [9] further proposed the inclusion of appropriate fertilizer application as a principle of conservation agriculture (CA), to which Sommer et al. [10] offered a rebuttal stating that it should just be a practice rather than a pillar of CA since in Sub-Saharan Africa (SSA) low fertilizer application is a common problem not only linked to CA. Despite their divergent views, they however agreed that fertilizer usage is fundamental in SSA agricultural systems to close the yield gaps. Interestingly, it has been observed that cassava root yield increase with fertilizer application even in Sub-Saharan Africa [11–13]. Recent studies on the effects of fertigation on growth and root yield of cassava, Omondi et al. [14] established fertigation concentrations at which maximum storage root yields were achieved in the field for three cassava varieties (Mweru, Nalumino and Kampolombo).

Looking at Vanlauwe et al.'s [10] **Figure 1** on ISFM, the jump in agronomic efficiency from the current practice to germplasm and fertilizer is greater than all the other ISFM practices. However, while reinforcing the importance of appropriate fertilizer application for intensive sustainable production of cassava to close the yield gap, this review does not negate the need for improved varieties, better agronomic practices/management, adaptation to local environment and usage organic fertilizers. Thus, this paper aims to evaluate and reinforce the clarion call that appropriate application of fertilizers through the 4R-Nutrient-Stewardship (right fertilizer source, right rate, right time, right placement) [15] through fertigation or banding, proper agronomic management and right management of the ISFM traits improve cassavas' yields.

2. Cassava root yield under fertigation and banding

Table 1 shows that irrespective of the amount of fertilizer applied, there is an advantage of storage root yield against non-application. Of course, there

Site	N-P-K (kg ha^{-1})	% Fertilizer advantage on root yield against 0–0-0 NPK (kg ha^{-1})	Source
Banding			
Kabangwe (Zambia)	100–22-83	31.0	[16]
Mansa (Zambia)	100–22-83	32.9	[16]
Akure (Nigeria)	60–60-60	37.0	[17]
Kwang'amor (Kenya)	100–22-83	60.5***	[18]
Mungatsi (Kenya)	100–22-83	68.3***	[18]
Ugunja (Kenya)	100–22-83	68.3***	[18]
Kisiro (Uganda)	100–22-83	64.2***	[18]
Kerala (India)	100–300-100	56.2	[19]
Lopburi (Thailand)	250–62.5-125	23.4	[20]
Supanburi (Thailand)	250–62.5-125	19.6	[20]
Chonburi (Thailand)	250–62.5-125	23.3	[20]
Thai Nguyen (Vietnam)	160–80-160	88.7**	[21]
Fertigation			
Lusaka (Zambia)	155–23-155	24.6* (Mweru variety)	[14]
Lusaka (Zambia)	76–8-76	37.7* (Kampolombo variety)	[14]

**These varieties were harvested at eight months after planting.*
***Data used are a mean of nine years.*
****NPK application compared with the average farmer practice.*

Table 1.
Fertilizer application advantage on storage root yield of cassava.

are variations in the levels of advantage due to different factors such as variety response, agro-ecological zones characteristics, agronomic and crop management practices etc. For example, under fertigation (application of fertilizers or other soil amendments intended to improve soil fertility through an irrigation system) in [14] study, all the cassava varieties receive similar treatments yet the fertilizer advantage is different – Kamplombo variety responding better to fertilizer application.

The highest fertilizer advantage is obtained under continuous cassava cultivation (**Table 1**), for example, [21]'s long term trials indicated decline in root yield under both non – and - fertilizer applications (**Table 1**). Although, under no fertilizer application, the decline was huge perhaps due high nutrient depletion without replenishment. This is an indication that continuous cassava cultivation requires continuous application of NPK including the other elements. Here [21], the fertilizer was banded, a placement of fertilizers in bands/rings/strips near the roots, often 5 cm to the side of the plant and 5 cm deep.

Also, in their long-term nine-years study of fertilization of cassava, [21] observed a decline in storage root yield regardless of fertilizer rate or individual nutrient rates, however, they concluded that highest cassava root yield were obtained at N-P-K of 160–80-160 kg ha^{-1} (**Table 1**). Such decline in cassava's response to continuous nutrient application, especially K, on the same piece of land for five years was also observed by [22] in fourteen varieties. In both instances, [22] and [21] attributed the decline over the years to depletion of other elements such as Ca and Mg, which were not applied in their experiments. This indicates the importance of other nutrients to cassava even as many studies are focused on

NPK– adhering to Justus von Liebig Law of the Minimum - a limit in one nutrient limits the uptake of the others and hence decline in growth and yield [23].

3. Fertilizer influence on starch qualities

The importance of cassava storage roots as food and animal feed cannot be understated, especially among smallholder farmers. To enhance cassava's ability as an industrial cash crop, focus needs to shift to starch in its storage roots. However, there are many starch characteristics that are considered by various industries such as particle size, solubility, gelatinisation, purity etc. These require extensive study.

Cassava starch is being used in beer making, ethanol production [24, 25], pharmaceuticals, paper manufacturing, textile etc. [26]. In addition, it has been tested as a substitute for agar material in micropropagation in tissue culture studies with minimum success [27]. Therefore, as the usage of starch from cassava storage roots expands, factors that influence the starch suitability for various industries are of importance. Factors that influence crop growth and development like climate, soil fertility, abiotic and biotic incidences and the variety [28] are vital. Those that impact postharvest and processing are important too [29]. **Table 2** illustrates the effect of fertilizers and soil amendments on the characteristics of starch of cassava storage roots.

The response of cassava storage-root-starch varies under fertilizer application (**Table 2**). Some varieties increase storage root starch content while others decline, for example, four of the varieties tested by [32] had a decline in starch content within the storage roots. Despite these observations from [32], other studies have indicated an increase of 9–14% starch content in the storage roots on fertilizer application to cassava (**Table 2**). Remarkably, fertigating medium (Kampolombo) and long duration (Nalumino) cassava varieties improved starch content of storage

Variety	N-P-K (kg ha^{-1})	%Fertilizer advantage on starch content against 0–0-0 NPK (kg ha^{-1})	Source
Banding			
TMS 30572	22.5–22.5-22.5	10.7	[30]
TMS 419	22.5–22.5-22.5	10.2	[30]
—	60–60-150	5.6	[31]
M98/0040	24–24-24	9.6	[32]
98/0002	24–24-24	−1.3	[32]
99/6012	24–24-24	−1.7	[32]
92b/0061	24–24-24	−6.8	[32]
82/00058	24–24-24	−7.5	[32]
Fertigation			
Mweru	155–23-155	9.7	[14]
Kampolombo	76–8-76	14.0	[14]
Nalumino	54–5-54	12.5	[14]

- Variety not indicated from the source.
A negative in the % fertilizer advantage indicates that starch content under no fertilizer application was higher than under application.

Table 2.
The advantage of applying fertilizers on the starch content of storage roots of cassava.

roots than banding in other varieties (**Table 2**). It is important to note that these varieties were not tested under banding, neither were the other varieties tested under fertigation. Therefore, a direct comparison of the two fertilizer application methods (fertigation and banding) is weak, even though the observations on response of each variety are insightful.

4. Future of intensive sustainable climate-smart production of cassava

Climate is changing, and human population is growing. Cassava is one of the crops that have been observed to be tolerant to the vagaries of climate, such as increase in atmospheric temperature, CO_2 [33] and drought [34]. Furthermore, as stated in the introduction, its demand both as food and raw material for industries is increasing, to reduce the yield gap and meet the growing demand, its yield per area must improve. However, that intensive production must be sustainable and climate smart. To make it a climate-smart and a cash crop for smallholder farmers, intensive sustainable production approaches are required. Tweaking the [8]'s ISFM **Figure 1** with best of the 4R-Nutrient-Stewardship [15] of fertilizer application and other agronomic practices such as right planting time, population, pattern and proper management biotic and abiotic stresses would optimize intensive sustainable climate-smart production (**Figure 2**). Such modifications to the ISFM concept will encourage increased sustainable production not only of cassava, but other crops too and consequently feed the bulging world's population effectively.

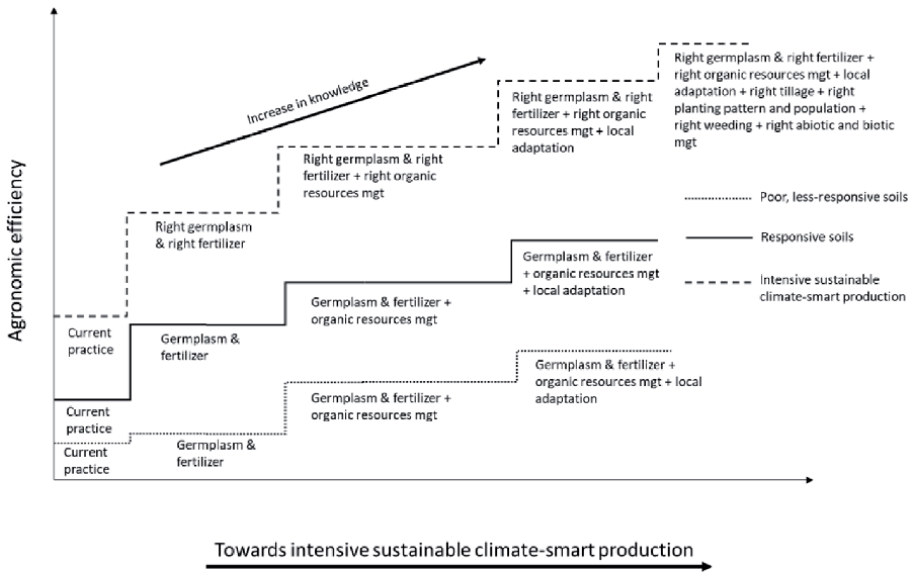

Figure 2.
The components leading to intensive sustainable climate-smart production of cassava. Mgt – Management. A modified ISFM concept from [8] to achieve intensive sustainable climate-smart production.

5. Conclusion

This review has elucidated the advantage of fertilizers to cassava storage root yield and starch content. However, this advantage is only valuable to most smallholder farmers if markets are available to absorb their produce. To improve cassava's status as a cash crop through starch production: extensive evaluations of

fertilizers effect on the characteristics of starch of storage roots of best performing improved varieties is required. There should be a concerted effort to match the high root yields obtained from fertilized cassava fields with the starch requirements of industries. This should be the next major frontier of research if cassava-producing-smallholder farmers' financial earnings is to increase exponentially.

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Section 4

Cassava Post-Harvest Processing

Chapter 7

Cassava Biomaterial Innovations for Industry Applications

Kashub Steven Tumwesigye, Jorge C. Oliveira, Sheila Namuwaya and Maria Jose Sousa-Gallagher

Abstract

Breakthrough innovations can spur growth in the modern era industry to realise sustainability and high returns on investments. Nowadays, biobased innovations for application in diverse industry sectors are considered as future pillars to counter resource depletion and ensure positive environmental impacts. Cassava is a strong flagship biomaterial promoting solution for resource-efficient use and green environment. Innovative industrial application of cassava biomaterials enriches literature, presenting cassava as a versatile and unrivalled crop that is cardinal for more sustainable environment and biodegradable industrial products. Work on novel cassava biomaterials, which are low-cost, unexploited and with zero competition for food supply, are included. Using an integrated sustainable process, it shows how to indirectly reduce waste streams, through their effective use, guaranteeing zero carbon footprints and acting as a non-traditional strategy for equilibrium atmosphere and active packaging systems. Applications of Cassava biomaterial in food, as food supplements and in packaging systems are also covered in this chapter.

Keywords: Cassava biomaterial, waste, integrated process, biodegradable products, sustainable environment, packaging system

1. Introduction

"Breakthrough innovations" is a hot topic aimed at stimulating industry research towards realisation of bioeconomy, sustainability, and high returns on investments. Eco-friendly inequities exist due to rapid population explosion, continuous resource exhaustion, industry biomaterial supply concerns and linear model of produce-consume-dispose [1]. Ultimately, innovative research solutions have been sought to ensure sustainability along the entire food system [2] to address the above challenges. The research solutions have been mainly in biobased industries, notably renewable resources, a component of sustainable biobased industries. However, bio-industries are subjected to considerable technological innovations and sustainable alternative challenges. Thus, the trend is geared towards developing integrated biorefineries with the goal of achieving availability and flexibility of multiple feedstocks' low inputs and maximum outputs [3]. There are several waste resources that can be valorised to produce unmatched feedstocks for the sustainable biorefinery developments. Examples of current integrated biorefinery feedstocks and products include vegetable oils, high value-added bio-lubricants, cosmetics and bioplastics

obtained from low input and under-utilised oil crops, which are not in competition with food and feed supply [4]. Others include green, clean, post-use biodegradable, compostable and efficient alternative supplies.

Cassava resources are versatile biomass supply chains that are bio-transformed into industrial feedstocks to replace fossil oil product streams [5]. Their biopolymers' processing and products' development using traditional techniques is accompanied by significant wastes with negative environmental impacts [6]. Cassava is a higher producer of significant wastes (peel pulp, wastewater, and leaves) during postharvest processing. Nonetheless, a comprehensive impression of cassava biomaterials, covering a wide spectrum of novel processing technologies, and underutilised and low-cost biomass, is evident.

This chapter presents a thorough discussion of bitter cassava biomaterial innovations and novel processes for bio-transforming this low-cost underexploited crop. Use of an integrated sustainable process to indirectly reduce waste streams is demonstrated. A special focus is dedicated to production of biodegradable products from intact bitter cassava waste streams of nascent sector as promising feedstocks for application in food, supplement, and packaging systems. Ultimately, concretising the concept of innovative application of cassava biomaterials can be a useful resource for academia, industry, bioeconomy, and policy.

2. Bitter cassava

2.1 A versatile, unique, unrivalled, and resilient crop for biomaterials

Bitter cassava is an equivalent of sweet cassava. While sweet cassava is edible and safe for instant use in fresh and processed forms, bitter cassava is only safe for usage after intricate processing and is regarded as a staple food [6]. Bitter cassava roots contain high toxic hydrogen cyanide (HCN) levels above 100 mg/kg on fresh basis, even going beyond 900 mg/kg in tropical regions: with the minimum reference limit of 0.02 mg/kg on dry weight [7]. Increasing region-specific sweet cassava profiles as biomaterials for foods, feeds, pharmaceuticals, and confectionery industries have greatly augmented unparalleled investment into non-traditional underutilised crops [4, 8]. In East and Central Africa, bitter cassava varieties (such as Karangwa/Tongolo) have existed for decades. Anecdotal evidence points to the advantages of bitter cassava as a food and industrial crop with superior product (e.g., Flour and crude alcohol) qualities. Bitter cassava is highly preferred due to: i) its potential to be grown organically than sweet varieties because of their more toxicity levels deterring foraging rodents and pests from feasting on the crop; ii) imposing the need to process roots directly after they are harvested deters thieving from the field; and iii) as the processing adds value in terms of time invested, the social obligation of sharing cassava with neighbours is reduced [9].

2.2 Inherent challenges present unique opportunities for its exploitation

Due to high potential cyanide content in bitter cassava, the code of practice allows adequate postharvest processing [10]. The appropriate postharvest processing, in particular fermentation, is effective in reducing HCN to minimum concentrations. Conversely, inadequate, notably using rudimentary techniques, leads to high HCN residuals in the final products. The peel (cortex) contains more HCN than edible portion (parenchyma) (**Figure 1**). As such the peels are frequently detached from the edible portion and discarded. This underutilised waste, estimated at 30% represents a great loss of feedstock and energy resources as well as potential

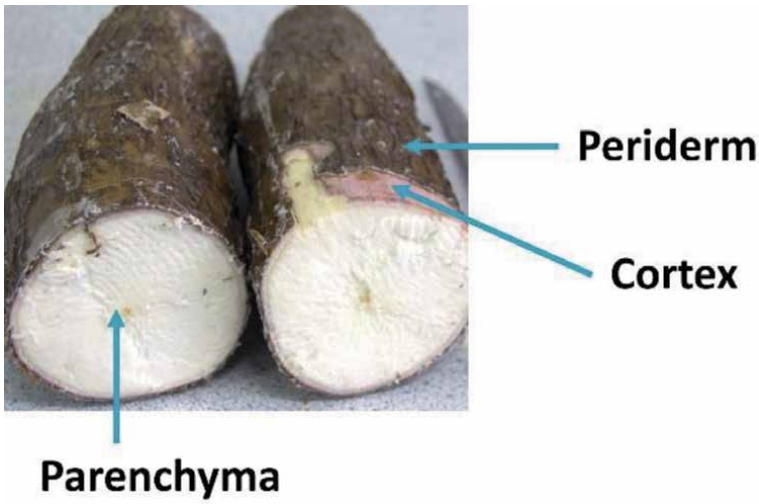


Figure 1.
Intact root showing different components.

source of eco-system contamination [4, 11, 12]. In major bitter cassava growing regions, it is transformed into primary, intermediary, and tertiary products using rudimentary fermentation processes. Traditionally, different detoxification processes, such as solid-state fermentation and retting in river ponds are used minimise HCN in bitter cassava [13]. This uncontrolled large-scale upstream and downstream process-fermentation into food and beverages contributes significantly to unsustainability, thus requiring valorisation [11, 14]. As the peel is disposed-off and its utilisation limited due to high HCN content, high fibre, and low protein; this presents exceptional opportunities to biotransformation into innovative biomaterials such as biodegradable plastics for diverse applications [4, 11, 15].

3. Bitter cassava biomaterial innovations

Cassava biomaterials are not restricted to only those produced with sweet cassava (such as food, pharmaceutical, beverage, coatings, civil works, textile industry), but emphasise biomaterials that are developed innovatively from bitter cassava wastes. Investments in bitter cassava biomaterial innovations are based on: i) the need to solve the increased environmental waste, which is caused by a linear and irreversible behavioural pattern that follows a produce-consume-dispose model; ii) innovations in biomaterial development, spurred by bitter cassava superior end-product qualities, that force nascent communities and processors to invest in the staple crop sustainably; iii) the need to reduce waste in environment and develop industrial products, in tandem, for a more competitive resource economy; and iv) solving issues of finite natural material sources and competition for food supply. Precisely, bitter cassava is a renewable resource with no competition for food supply, its valorisation minimises waste and environmental impact and is a cost-effective option.

To this end, a circular utilisation model is explored in tackling cassava biomaterial innovations. This strategy ensures that bitter cassava waste is transformed into value-added resources that later biodegrades into environment post-use, in a process of eco-designing of biomaterials for food and non-food applications.

3.1 A robust inventive process for sustainable cassava biomaterial production

Converting bitter cassava wastes into high premium resources demands an unusual approach, entailing departure from traditional cassava processing methods to robust processing methodologies. As such, a new systematic improved downstream processing methodology, known as "Simultaneous release recovery cyanogenesis (SRRC)" has been developed and piloted with success to ease downstream production of bitter cassava biomaterial as a template for diverse use [4]. The SRRC constitutes two main stages and unique procedures to produce a biopolymer derivatives biomaterial (**Figure 2**). The term "biopolymer derivatives" refers to the product recoveries from the intact root of bitter cassava, and these mainly consist of different proportions of starch, cellulose, hemicellulose, holocellulose and lignin [11, 16]. Waste derivatives are the product derivatives of waste solids and wastewaters.

3.1.1 Production of biopolymer derivatives biomaterial

Smart sourcing and preparation of starting materials is an indispensable step in the production of good quality, safe and adequate volumes of biomaterials. Bitter cassava is best sourced at maturity of 12–18 months after planting and should be designated as right harvesting time of adequate biopolymers. Using intact root (IR) is an indirect and logic approach of preventing wastes finding their way into the environment. The IR is a whole root of cassava that is composed of residues (peel, cambium, phloem, central xylem fibre) and edible parenchyma. The derivative wastes consist of the peel, internal root centre fibre (xylem bundles), unwanted trimmed solids and wastewaters. Using the SRRC methodology, intact roots are subjected to mechanical tissue rupture, biopolymer release and cyanide toxin loss. The processes typically involve feeding intact roots into automated grating machine

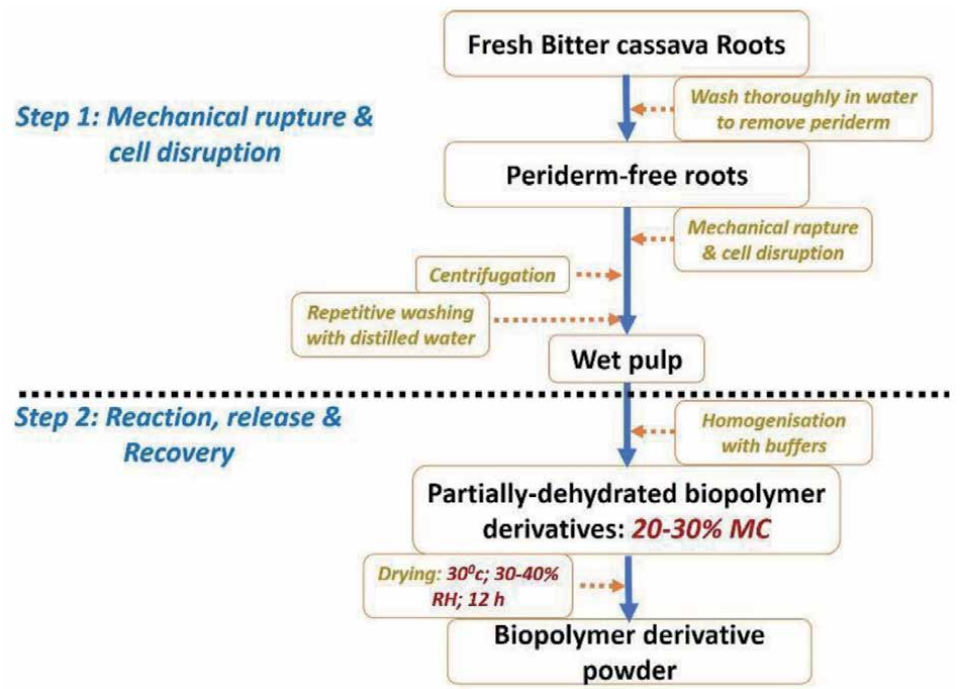


Figure 2.
Schematic flow of SRRC methodology for production of biomaterials.

and the resulting pulp mass obtained after mechanical tissue rupture and cell disruption (MTRCD). The MTRCD serves the twin role of actuating total cyanogens hydrolysis into release of volatile hydrogen cyanide and bringing together different biopolymer components for possible modification. In effect, the process involves crushing the intact root into finest pulp using high shear rate pulpers, which is also intended to improve the pulp texture and decrease the extraction time in the subsequent processes of release and recovery [16]. The biopolymer derivatives release is often achieved through homogenisation under the influence of extraction buffers (food grade NaCl and $NaHSO_3$), followed by filtration, centrifugation and washing in distilled water [4]. The derivatives recovery is realised by filtration, centrifugation, washing in distilled water, wastewater recycling and drying in a convectional laminar flow dryer. Resulting biomaterial powder is cooled and stored in airtight bags to prevent post-processing absorption of moisture as the derivatives powder is highly hygroscopic. Processing intact root using SRRC produces fibre-rich derivatives, which has been confirmed to offer better mechanical and barrier properties in the biomaterials [4]. Whether to use intact root or derived wastes depends on the envisioned final product; with edible bio-products, intact root is preferred while non-edible products (e.g., goods carrier bag, derived root residues) are the choice. During SRRC, an efficient mechanical pulping is crucial to achieve good quality (finer fibrous, 30–50 μm) and non-toxic (HCN threshold levels, near 0 ppm) biomaterials. Thus, a pulping efficiency (PE) of ≥90% was found to be sufficient when applied in pulping process using a time-dependent model (Eq. (1)) [16]. The PE of this magnitude is required because of the recalcitrant nature of most cellulosic fibre mass, which succumbs at higher shear rates. The achieved finer biomaterial powder (30–50 μm) is usually a result of softer cellulosic mass when compared to other woody plants.

$$S = 36144.36 + 33.11v - 875.74 \in + 0.01v^2 + 4.97 \in^2 \left(R^2 = 0.95\right) \qquad (1)$$

S, pulping time; V, pulper velocity; Ɛ, pulping efficiency.

3.1.2 The SRRC unique outcomes

The SRRC concept restricts between 16 and 30% wastes disposed directly into the environment. This is achieved by processing fresh bitter cassava roots, and thus avoiding underlying costs, energy, time, intended and unintended disposal efforts, of additional alternative processes for waste management. The most economic SRRC design for sustainable valorisation of bitter cassava waste into value added biomaterials avoids the need to dispose indirectly wastes into environment leading to sustainability. The SRRC processing is a unique methodology concentrated at the early stage of the design (pulping, reaction, and release) to enhance biomaterial modifications, and therefore it can be applied in processes of sweet cassava waste and most crops' residues. The SRRC reduces the extraction time and improves biomaterial texture with nominal size of 30–50 μm (finer matrices) with potential for extensive application. The ≥90% PE confers total cells breakdown and disruption, ensuring that enzyme linamarase hydrolyses linamarin (precursor of cyanide related compounds) into HCN. Cyanogenesis process is proportional to PE, only achieved in SRRC and not common with traditional methods, ensuring the safety of biomaterials (HCN near zero) [11]. The total HCN loss can be attributed to the functionalised ionic buffers and bisulphite in solution (pH 5.0–5.5) during the reaction and release stage. The affinity of bisulphites for the ketones makes them unavailable. This creates the desired gradient leading to fast HCN loss and might explain the significant detoxification of biomaterials. Ketones are released together

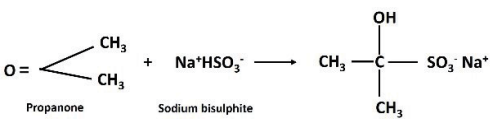
with HCN during linamarin hydrolysis. Concurrently, residual sulphur of the bisulphite forms complexes with HCN to form a non-toxic thiocyanate compound. This is a significant outcome of SRRC; in the absence of this process in traditional processing, there is partial detoxification with production of unsafe biomaterials that cannot be applied in industry. Strikingly, SRRC ensures better productivity of the biomaterials as illustrated by the optimised model [11]. The high yield (45.8% w/w) of biopolymer derivatives is attributed to ionic buffers converting nearly all root biomass into biomaterials.

3.1.3 Biomaterial (biopolymer derivative) properties

Biopolymer derivatives, a main biomaterial of SRRC and bitter cassava outcomes, can be used as a main ingredient in food, bioplastic, and packaging industries due to its compatible, biodegradable, polysaccharide-rich (starch, cellulosic fibrous, lignin), safety (0.4–2.5 ppm HCN), colourless (white) and particle size uniformity (30–50 μm and free-flowing) properties [4, 11, 15–17]. The biopolymer derivative is made available in powder form (**Figure 3**). The biomaterial colour is an impurity and often removed using rudimentary means in traditional processes. Nonetheless, the colourless characteristic of the biomaterial is achieved by reaction additives (sodium salts of bisulphites and ionic buffers) (Eqs. (2) and (3)), acting as bleaching agents. As indicated previously, compatibility was realised by combination and modification of different polysaccharides in the root during pulping and reaction processes. Biodegradable, polysaccharide-rich, toxin-free, and particle size uniformity are accomplished by using intact root and SRRC downstream processing. The amylose content of the biomaterial ranges between 18 and 24%, and higher corresponding amylopectin is attributed to inclusion of the peel waste and impact of SRRC on the peel structure [4].

The biomaterial powder is highly stable at moisture content of ≤5% but instability is often encountered when the powder is stored and handled under moisture content ≤10% because the powder is highly hygroscopic.

$$\text{Bisulphite } (HSO_3^-) \rightarrow HSO_4^- \tag{2}$$



(3)

The biomaterial has homogeneous particle sizes with round and polygonal shapes, and with slightly bigger round granule size [11].

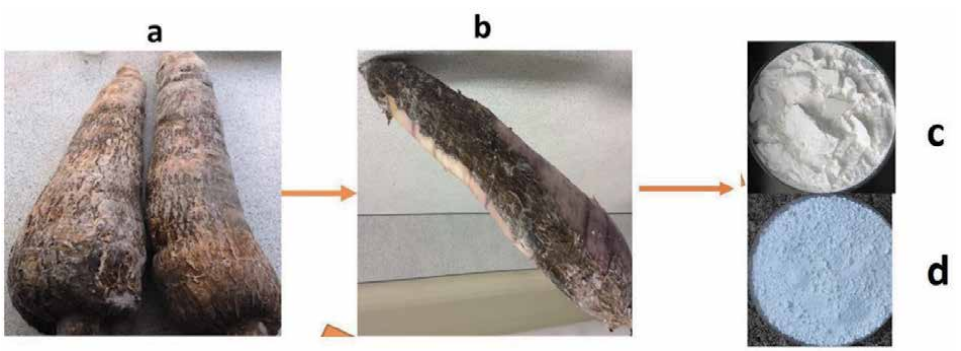


Figure 3.
Biomaterial route showing (a) intact bitter cassava root; (b) periderm-free intact root; (c & d) colourless biopolymer derivatives.

3.1.4 Production of polymeric film sheet biomaterial

Filmogenic solutions are prepared by using different proportions of the bitter cassava biomaterial powder, glycerol solution and distilled water (**Figure 4**). The resulting mixture is heated while agitated continuously until a gel is formed and turns clear. It is important that the gel is free from bubbles as they change the microstructure of the film sheet. Immediately, a known volume of the gel is cast onto glass plates and held shortly at ambient conditions to allow them to stabilise, concurrently bubble bleeding occurs. The stabilised gel casts are heated and maintained at known temperatures. The films are peeled off the plates and stabilised under environmental conditions of temperature and relative humidity.

3.1.5 Biomaterial (polymeric film sheet) properties

The physico-chemical characteristics of films (**Table 1**) perform a critical part in diverse end use systems, and knowledge of their properties is important in assessing package perform along the distribution chain. Intact bitter cassava-based films (BCFs) are transparent, with values as low as 3.6% than those obtained from starch of all botanical origin which posts 11.9% [4]. They are comparable to most commercial NatureFlex (4.6%) and Polylactide (3.9%), and much lower than polypropylene (13.6%). The low values tending to zero and higher values leaning to 100%, determined by spectrophotometric transmission and chroma lightness index respectively, indicate more transparency (**Figure 5a**). It can be confirmed that intrinsic modification of the intact bitter cassava root by SRRC produces more transparent films. Transparent films are important in many applications,

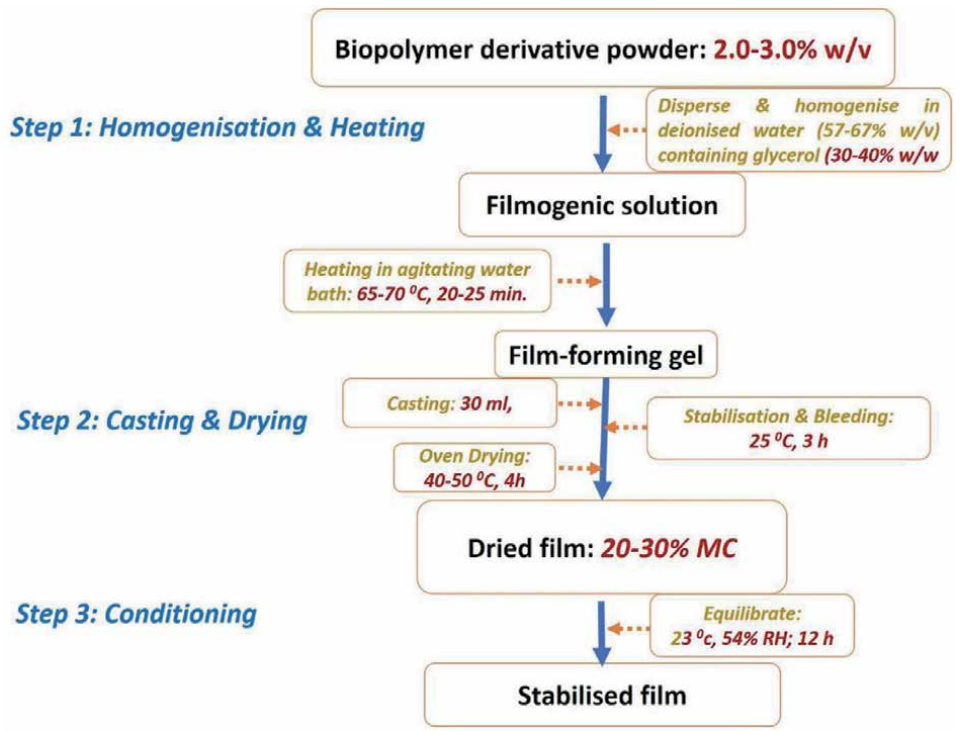


Figure 4.
Schematic flow of film fabrication process.

Property	Bitter cassava film	Commercial PLA film
Moisture (%)	0.19–0.45	0.10–0.20
Optical (%)	3.43–5.29	1.45–1.50
Solubility (%)	15.52–30.54	0.00
Water vapour permeability (gmm/(M^2.24h,kPa)	3.19–4.50	180.0–190.0
Glass transitional temperature (°C)	44.05–56.23	55.0–60.0
Melting temperature (°C)	193.57–213.63	130.0–180.0
Tensile strength (MPa)	3.71–48.44	40.1–49.5
Heat of fusion (J/g)	64.0–70.5	21.5–25.4
Degradation temperature (°C)	370.0–380.0	350.0–400.0
Glass transition temperature (°C)	50.0–60.0	60.0–65.0
Melting temperature (°C)	200.0–220.0	170.0–230.0
Crystallinity (%)	50.5–59.5	10.0–15.0
Elongation at break (%)	17.3–18.7	33.4–35.0
Elastic modulus (MPa)	0.11–15.95	2000–2300
Transparency (%)	3.0–5.0	3.0–5.0
Seal strength [N/25 mm]	305.0–325.5	25.5–30.5
Contact angle (^{0})	70–105 ^{0}	60.0–95.0
Biodegradability (days)	20–100	40–50

Table 1.
Characteristics of bitter cassava films in kin to commercial PLA bio-film.

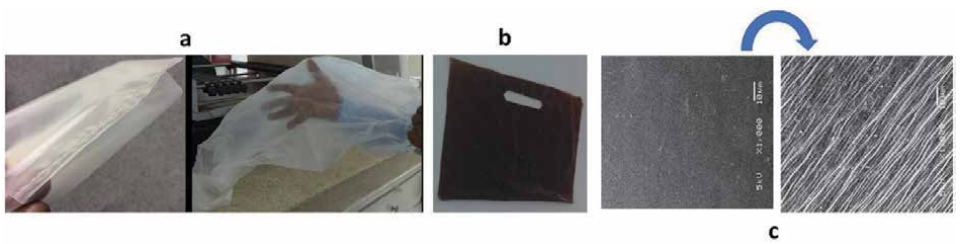


Figure 5.
Bitter cassava film depicting a) transparent nature; b) carrier bag; and c) scanning electron microscope revealed mesh structure.

particularly in packaging where products in the distribution chain are required to be viewed by buyers.

BCFs are fairly water resistant after 30 days, which is explained by the relatively stable network components in the film structure imparted by the root waste and SRRC [4, 15, 17]. Intact bitter cassava biopolymer derivatives is able to produce both water soluble and resistant films which can find application in packaging fresh foods and acting as a goods carrier bag used in an environment whereby high water resistance is high (**Figure 5b**).

BCFs possess homogeneous surfaces, which can be attributed to complete solubilisation of biopolymer derivatives in the polymer matrix, near zero solvent migration at the interface and strong and uniform adhesion of ingredients culminating into homogeneous mesh network structures in the film matrix (**Figure 5c**) [4].

BCFs are either hydrophilic or hydrophobic; those characterised with contact angles (CA) $700 \leq CA < 90^0$ contain polar functional groups that render them hydrophilic. The CA is the measure of wettability of solids and gives an indication of the extent liquids spread over solid surface [18]. The surface energy is essential in assurance of printability, adhesion, and transparency of flexible films. The BCFs have contact angles $<90^0$ (hydrophilic) and $> 90^0$ with printability features and can be used as goods carrier bags (**Figure 5b**) respectively. Nonetheless, BCFs are observed to swell differently at diverse RH; lower and higher swelling is clear at higher and lower RH respectively due to differences in plasticisation.

The fluid barrier properties of biomaterials are essential for prediction of the product-package shelf-life. The BCFs accurate performance veracity is a function of their flexibility in extremely demanding distribution situations, a role of their ability to respond timely and achieve fluid barrier appropriately. The BCFs have suitable permeability to water vapour (WVP) like commercial films polylactic acid (PLA) and Natureflex (NVS) currently applied in packaging fresh foods. This is fundamentally due to their widespread pore size distributions that contribute to fluid pathways, which are tortuous and exceedingly variable [19]. Depending on the nature of the films and their intended use, their equilibrium moisture contents (EMC) increase correspondingly with relative humidity (RH) at constant temperatures. This is caused by advanced amounts of moisture resulting into augmented mobility and dissociating. By contrast, the films EMC reduces when exposed to higher temperatures at constant RH due to film adsorption behaviour [19]. At high EMC, films moisture attraction is high with enhanced capacity adsorption and faster mobility of water causing a reduction in intermolecular attractive forces. The exponential increase of films WVP at higher temperatures is linked to higher activation energy for moisture permeation but also it is due to molecular initiation triggering film section crusade with creation of hollows that ease solvents motion through permeable films.

The permeability to oxygen (OP) of BCFs is higher than those of commercial NVS films. By contrast, permeability to carbon dioxide (CDP) by films is lower than the commercial ones. This is a good indication that the BCFs are adequate to be used in packaging fresh foods that are not highly respiring. When placed in distribution chain, BCFs under highly variable temperature and RH, the OP and CDP experience slight decreases due to antagonistic nature of RH on diffusion [19]. The interference of OP at higher RH and temperature is caused by increased molecular kinetics resulting into water molecules interfering with film voids but also on chain mobility. At higher RH and temperature, crystalline films are transformed into amorphous films (due to raised glass transition temperature and crystallinity) causing decreases in OP. The ability of these films to regulate barrier properties to gas and water, implies that they can be applied as breathable films, and is important for the choice of using them in commercial applications.

BCFs are permeable to organic and inorganic solvents differently [19] Toluene and paraffin, which are common organic solvents in the distribution chain, behave differently towards films. The higher interaction of paraffin is due to forming complexes with the film but also clinging to film surfaces. The information about the behaviour of solvents in contact with films is vital for their safe handling in the distribution chain. For example, toluene permeation makes film brittle and more crystalline with reduced molecular relaxation [19].

BCFs produce strong films that can have wide-ranging applications. Experiments have shown that BCFs tensile strength (TS) compares with commercial PLA and lies in the range of NVS and orientated polypropylene (OPP). BCFs flexibility is comparable to commercial PLA [4]. Similarly, it has also been shown that BCFs can be produced as weak films when the end use is targeted.

The seal integrity plays a vital role in packages and laminations in commercial setting. The BCFs have stand-alone self-sealing abilities compared to most commercial films that require an extra coating to enhance their sealing capacity. BCFs demonstrate comparable sealing strength with NVS, PLA and OPP that have supported sealing abilities [4]. BCFs exhibit last sealing strength for 12 h under environmental conditions (15–20°C and 50–60%RH), implying that films adhered firmly naturally [4].

BCFs are thermally stable under the influence of high temperatures. Their glass transition and melting temperatures, heat of fusion and crystallinity fall within the range of commercial PLA and LLDPE [4]. They are thermally stable than commercial films with the onset of total degradation occurring at 373°C, which is higher than most polymer networks degrading at 340–360°C [19].

BCFs are highly biodegradable in varying environmental conditions, decomposing in composite pits (within 21 days), open environment during wet conditions (maximum 45 days) and open environment during dry conditions (maximum 90 days). In all disposal environments, the bio-decomposition process uses naturally occurring bacterial/fungi to biodegrade the film into carbon dioxide, water, and compost. This is important for clean environment and sustainability, in contrast with fossil-based films that take more than 1000 years to decompose. When these biofilms are kept at room temperature and away from direct sunlight and humidity, they can biodegrade beyond 365 days. This is important when they are used as goods carrier bags and reused again.

3.2 Integrated sustainable system: a strategy to advance biodegradable products and ensuring green environment

A key part of sustainability is the minimisation of wastes during the biomaterial recovery from bitter cassava environmental waste. A sustainable system is an integrated and key strategy to realise green environment and value-added biodegradable products, and thus contributing to universal sustainability perception. The approach focuses on exploring individual process and model synergies and facilitating SRRC downstream process transition to advance cassava waste feedstocks for biodegradable product innovations. The approach emphasises developing and optimising an integrated process design based on optimising the structure of SRRC with efficient production of packaging materials and sustainable utilisation of cassava waste biomass feedstocks (waste solids and waste waters), meanwhile unlocking indirect and sustainable valorisation of cassava wastes. Apart from integrating individual processes, the strategy is intended to bring them, exclusively, into better efficient levels, through modelling and optimisations, and offer increased productivity of biodegradable packaging materials, thus creating a sustainable utilisation pull to reinforce the exploitation and competitiveness of bitter cassava crop. Standardisation, though process optimisation, of producing biomaterials eases the choice and cost of processes, by defining the design space, process parameters and biomaterial functional properties. In effect, robust production processes provide standard/optimal approaches for leveraging desired biomaterials with marginal costs and maximum functionality [20]. Besides, the effort is to bring the processing technology of small to medium enterprises (SMPs) to maturity through innovations in indirect waste disposal routes; and upgrading the development of simple, convenience and attractive substitute process designs that address cassava wastes accruing using SMPs rudimentary processing technologies.

Bitter cassava wastes that are generated by independent processes are being traditionally minimised in the environment by valorisation of bagasse into organic acids,

ethanol, aroma and biocomposites [20, 21]. Although the above processes are popular approaches, they have disadvantages of their fundamental high production costs, energy, and time. Optimal design models of individual processes are used as a solution, which gives best interface leverages in a sustainable cassava minimisation approach. In designing an integrated process, process modelling is used to ensure a holistic design for efficient utilisation of cassava wastes without compromising competition for food supply. Thus, to ensure efficient production of biomaterials, an integrated process design is used [16]. In this design, processes are well-defined and conceptualised before they are used in the integrated downstream processing model. In effect, only processes which add value in minimising waste at low cost, are energy efficient and time saving are selected and analysed in the integration design. Primarily, to increase the efficiency and functionality, the design is partitioned into unit operations whereby optimisations are focused. The source includes but not limited to recovering biomaterials from: i) the whole root of bitter cassava; ii) detached residue portions (peel, fibre, trimmings); and iii) wastewater streams.

The innovations to process design integration fall into three optimised and pooled processes to maximise recovery of safe biomaterials, i.e., efficient mechanical pulping; reaction and release; and recovery.

3.2.1 Maximise efficiency of intact root pulping

Efficiency of mechanical pulping is well explained in subsections 3.11–3.12. Precisely, the yield of biomaterials and loss of total cyanide certainly need to be augmented in optimising efficiency.

3.2.2 Modelling and optimisation for effective reaction and release of biomaterials

Reaction and release process is a key stage due to the need to free fully the biomaterials at minimum costs, taking into consideration protection of the environment due to released hydrogen cyanide. There are several variables to aid release, the processing conditions, and the desired biomaterial properties but their levels are highly variable. Resultantly, key buffers and bisulphates are preferred in order to infer release of biomaterials. Based on this approach, the research is done for purposes of not only releasing the biomaterials but also consider their yield, safety and customised for multiple functions.

Regarding reaction and release step, desirability function approach is used to optimise multiple response processes, which exploits optimal processing conditions and parameters and obtain the most desired yield, safety, and functionality of biomaterials. According to [16, 22], joint Pareto front and multi-objective desirability (MOD) approaches is used in the standardisation of the reaction and release process. In Pareto front/solutions, distribution to parameter choices is made in such a way that trade-offs ensure unequal distribution in which some factors are constrained in place of alternatives in order to find feasible choices that lie on the Pareto font [22]. In this case, choices are efficient and not dominated by any other choice. On the other hand, the MOD approach is used in target desirability optimisation due to its capacity predict desirables within anticipated ranges [16].

3.2.3 Optimisation for effective recovery of biomaterials

In the recovery step important processes take place, and they include: removing cyanogens and bisulphite residuals remaining in the wet biomaterials released; dehydrating released biomaterials in a safe and economic way through serial washing and recycling and optimal drying (**Figure 6**). In traditional washing and

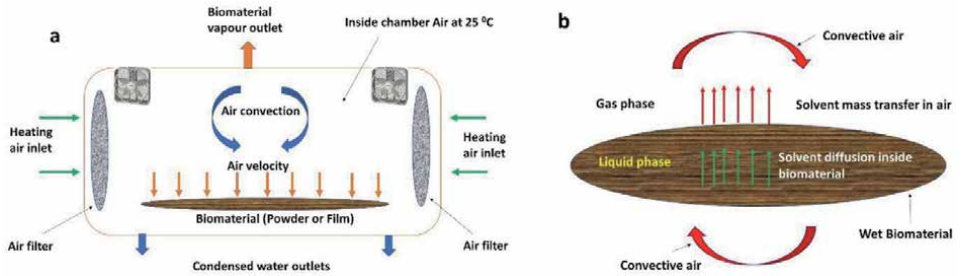


Figure 6.
Illustration of biomaterial drying process: (a) cross-sectional design of recirculating laminar flow chamber for studying optimisation and (b) mass and energy transfer.

clarification, a lot of purified water is deployed in serial washing with implications of energy costs in purification and biomaterials carried in wastewaters. In the current innovative designs, waste solvent is recycled thus reducing greatly on the costs of purification while ensuring zero contaminated biomaterials free from unwanted solvent and other clarifying-induced defects.

Conventional drying involves a lot of energy spent in the process, usually involving several hours (25°C, 12–16 h) of laminar flow drying without causing defects to the biomaterials in contrast to dying in ovens [16]. During the optimal recovery stage, energy is significantly minimised by optimised conditions in the designed recirculating laminar flow chamber (**Figure 6**). The purpose is to minimise the resident time of the biomaterial in the chamber while attaining the required residual solvent and drying process efficiency (productivity). As such operating conditions (air temperature, heat transfer coefficient, air flow rate, solvent partial pressure and velocity distributions) of the recirculating chamber are modelled and optimised to minimise significantly the polymer-solvent concentration and biomaterial resident time. Optimal conditions for drying polymer-solvent biomaterials is an outcome of a trade-off between minimising residual solvent dose producing gradients for fast dying without changing biomaterial quality. Thus, an adequate chamber with recirculating laminar flow is designed and deployed (**Figure 6**) for trial studies using computational fluid dynamics and mass and energy transport modelling [23]. In trying to attain optimal drying, interactions between biomaterial properties, dying conditions, biomaterial (polymer) solvent transport and mass/energy transport are managed. The fans enable to obtain uniform air flowing through the biomaterial. For a known uniform thickness (30 microns), temperature, time and polymer solvent amount profiles evolve as air (20–30°C, 30–40%RH) circulates through chamber containing biomaterials.

3.2.4 Characteristics of optimal drying process and dried biomaterials

Biomaterials from optimised recirculating laminar flow chamber processes are recovered and dried efficiently (high recovery, very low moisture, near zero contamination, low energy usage). The mechanism of drying biomaterial involves heat transfer from the convective air provided by external heat source and mass transfer in the biomaterial involving adsorption, diffusion, and desorption. At all stages of the drying process, drying rate is a function of solvent transport from the inside and surface of the biomaterial to the gas phase. Desorption (i.e., solvent transport from biomaterial surface) is characterised by mass transfer coefficients, gas temperature, velocity, and partial pressure, while diffusion (i.e., solvent transport within biomaterial) depends on temperature and solvent concentration [23]. Important scenarios above are modelled, optimised and outcomes presented (**Table 2**). The optimised

Parameters	Laminar flow	Recirculating airflow
Wet Biomaterial thickness, μm	30	30
Dry biomaterial thickness, μm	24	20
Initial air temperature, ^{0}C	25	25
Final air temperature, ^{0}C	30	30
Residence time, min.	360	240
Chamber partial pressure, kPa	102	102
Air Velocity, m/s	1.5	2.1
Initial solvent (moisture) content, mg	60	60
Final solvent content, mg at 240 min	32	5
Maximum weight loss, %	47	92
Heat transfer coefficient, cals^{-1} cm^{-2} °C^{-1}	0.0033	0.0024

Table 2.
Parameters at optimal drying of biomaterials in Laminar flow and recirculating laminar flow.

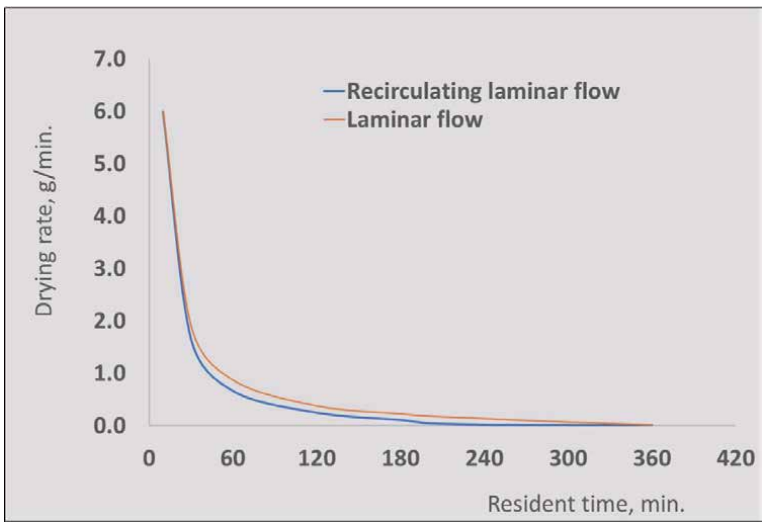


Figure 7.
Drying rates of biomaterials in recirculating laminar and laminar flows.

drying rate in recirculating laminar flow ensures short resident time compared to laminar flow (**Table 2**, **Figure 7**), implying reduced energy in drying.

The biomaterials' optimisation in recirculation laminar flow (RLF) chamber ensure that they are minimally degraded by heat and are more permeable to water vapour. Conversely, the RLF-based dying does not influence the structural and physico-chemical changes; this is also true for drying in laminar flow (LF) chamber [16]. By contrast, biomaterial thermal degradation increases in LF drying.

4. Cassava biomaterials as efficient matrices for key applications

4.1 Cassava waste powder in food supplements development

SRRC-produced biomaterials have benefits of a relatively low cost; particularly those from bitter cassava possess secondary metabolites with unrivalled properties

and varied uses, and thus have an edge over commercially available starch [4]. As a multifunctional, safe and dynamic matrix, bitter cassava biomaterials have potential application in pharmaceuticals, nutraceuticals and food supplements [15, 24]. SRRC-produced bitter cassava biomaterial is used as suitable matrix in the development of novel oral tablet excipient in iron and zinc supplements [11].

4.1.1 Fabrication of bitter cassava-based iron and zinc excipient tablets

In the production of iron and zinc tablets, the goal is to have a sustainable delivery system through chasing a carrier and delivery process that is inexpensive, green, and user-friendly. The manufacture follows a two-step process. Firstly, is to identify key bitter cassava biomaterial properties suitable for the development of self-sustaining excipient with important functionalities [11]. Next, based on the results from the first step, optimise the functionalities using granulation, formulate iron and zinc tablets and conduct dissolution tests.

In the preparation of biomaterial for excipient manufacture and dissolution tests, intact bitter cassava root biomass and SRRC methodology are explored [4]. In the conventional methods of producing biomaterials, peeled roots are the choice starting materials. For current innovation, tablets formation is done by exploring the capacity of biomaterial powder to produce strong tablets and is done by using both intact and peeled roots-derived biomaterials (**Figure 8**). Detailed procedure for tablets fabrication and dissolution tests can be found in the works of [11]. Iron and zinc are included prior to tablet formation and after granulation process. The quantities of iron and zinc included in the tablets are based on the dietary requirements of men (11 mg/day) and women (8 mg/day) while correcting for experimental loss [11, 25]. A suitable design is important if the minerals are to be distributed evenly in the tablets and an effective dissolution is needed. The design is defined based on three tablet sizes (100, 250, 500 mg), which corresponds with common pharmaceutical tablets in the market (**Table 2**) and the in vitro dissolution is accomplished using the US Pharmacopoeia (USP) method [11, 26]. For better

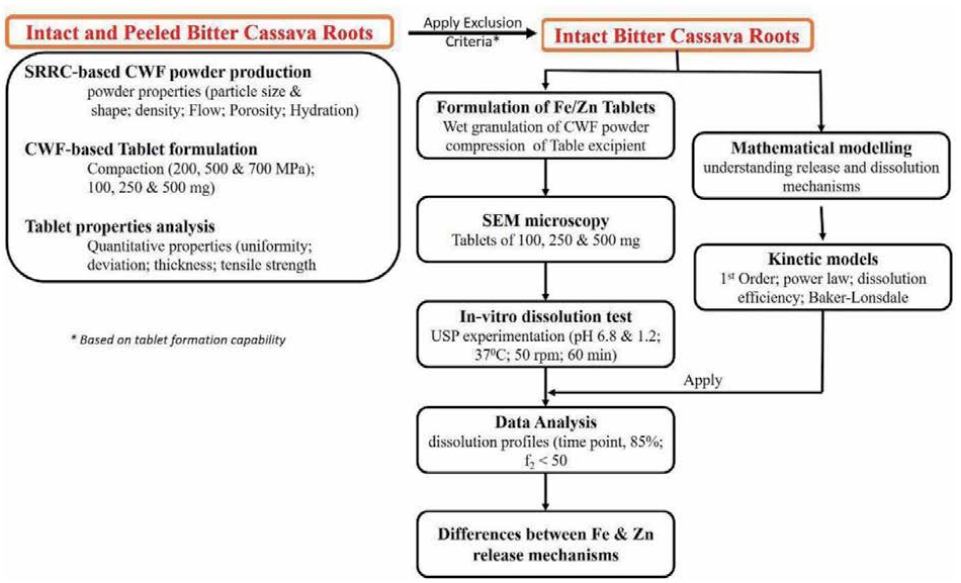


Figure 8.
Procedural investigation of excipient tablet production.

elucidation of the dissolution mechanisms (behaviour, release type, application angle of tablets), mathematical models are applied [11].

4.1.2 Physical properties of biomaterials for making iron-zinc excipient tablets

The physico-chemical properties of biomaterials play a vital role in tablet manufacture where flowability and compaction are the key properties. The biomaterials have uniform particle size and shape distribution of <3 mm, low bulk, and true densities, high tapped density and increased interparticle voids [11]. The uniform particle size is crucial in effective compaction/compression and regulated delivery matrices [11]. High tapped density is due to higher contract surface area, which is a benefit to tablet filling, higher solubility and dispersibility. Biomaterial flow properties and compressibility and solid excipient durability (desired strength, porosity and dissolution) are a function of bulk, tapped and true densities. The bulk and tapped density of the biomaterial are close to each other, which implies that it has better flow properties [11]. The biomaterial inter-particulate and intra-particulate interaction is low because of the low Carr's index (CI) and Hausner ratio (HR). The CI indicates compressibility or flowability of a powder, while HR is the number that relates to the flowability of a powder or granular materials. It has a low angle of repose and low porosity, an indication of particle uniformity and excellent flowability, which implies that the biomaterial powder cannot cake. The angle of repose of a powder or granular material is the sharpest angle of descent or dip relative to the horizontal plane to which a material can be heaped without dropping and is between 0 and 90^0. The biomaterials have low water retention capacity (WRC), water holding capacity (WHC) and swelling capacity (SC) although their WRC increases and WHC and SC decreases with time. The biomaterials have higher hydration capacity due to higher surface energy. The implication is the physical structural changes and hydration properties of the biomaterials fibres and their hydrophilic nature allows maximum moisture uptake offering a hint of better disintegrating of excipients [11].

4.1.3 Physical properties of iron-zinc excipient tablets

The novel Iron-Zinc excipient tablet (**Figure 9**) can be used extensively in developing food supplements, and as a pharmaceutical tablet with other active compounds due to its compatible, biodegradable, safe and fast dissolution

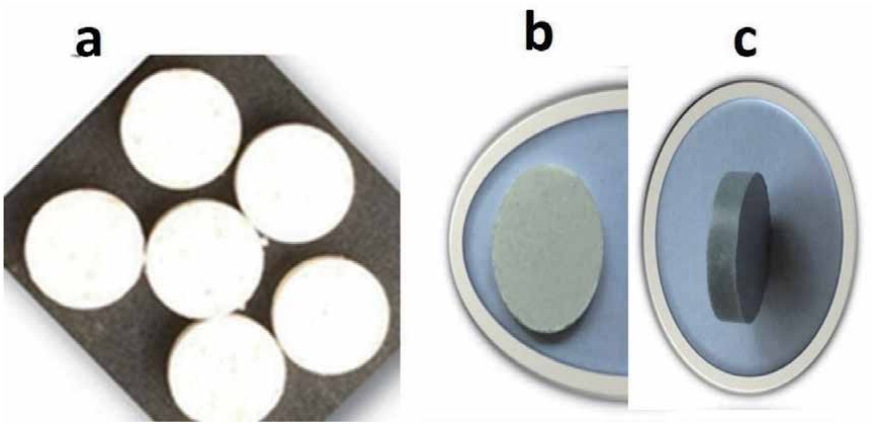


Figure 9.
The tablet prototypes containing biomaterial with: (a) no mineral; (b) iron; and (c) zinc.

Parameter	Biomaterial type		
	Derivatives powder	Tablet matrix	
Bulk density (g/cm^3)	0.38±0.0		
Tapped density (g/cm^3)	0.43±0.0		
True density (g/cm^3)	1.49±0.2		
Carr's Index (%)	9.38±0.0		
Hausner's ratio	1.13		
Flow rate (g/s^2)	20.91±0.8		
Angle of repose (^{0})	28.52±0.2		
Porosity (%)	68.87±0.9		
	CP200	**CP500**	**CP700**
Hardness (KG)	4.32±0.8	4.42±0.1	4.64±1.0
Diameter (mm)	13.09±0.0	13.08±0.0	13.07±0.0
Thickness (mm)	3.16±0.0	3.14±0.1	3.11±0.0
Weight (mg)	546.36±2.8	551.48±9.0	542.62 ±4.7
Tensile strength (MPa)	0.35±0.0	0.37±0.0	0.41±0.0
Disintegration time (s)	903±2.3	895±1.3	878±2.5
Friability	0.67±0.1	0.56±0.0	0.51±0.0

**Tablet size of 500 mg analysed at compaction pressures of 200, 500 and 700 MPa.*
CP, compression pressure.

Table 3.
Physical properties of bitter cassava biomaterial and excipient.*

properties [11]. Tablets have uniform weights (av. weight 484 ± 0.68 mg (**Table 3**), which is far lower than the recommended limit of 0.05. Uniformity and thickness of tablets are indication of good packing of tablets. The tablets exhibit reduced thickness corresponding to increases in compression force [11]. Tablets display adequate mechanical properties (hardness and tensile strength). The bitter cassava tablets are weak binders and strong disintegrants; these properties are crucial to the developments of fast release excipients where iron and zinc deliveries are fast demanded and in adequate amounts [24]. Tablet friability is compared to the USP standards implying that they can resist mechanical stress in the distribution chain. Tablet matrix porosity decreases as compression force is increased. Porosity is a function of particle size and shape; regular shaped particles become less and can fill up void spaces between large particles. In the low porosity tablets, small particle sizes allow flexibility for the tablets to pack more efficiently. However, the medium to high porosity biomaterials of bitter cassava does not permit flexibility in tablet packing suggesting a fast dissolution rate. Tablets exhibit higher disintegration time (DT) due to relatively medium to higher porosity, which facilitates rapid water penetration into the tablet resulting into bond rupture and disintegration [11]. The DT is the measure of time required for the tablet to disintegrate into particles under a given set of conditions.

The tablet matrix morphology and microstructure are considerably homogeneous, non-aggregated and uniformly blended with iron and zinc [11]. These patterns provide hope for tablets as inert excipients for oral dosage solid forms. Nutrient analysis has shown that zinc is released faster than iron in the tablet matrix, which seems to indicate that the matrix has minor resistance to zinc release than do for iron [11]. Tablet excipients release iron and zinc better in acidic

conditions than alkaline conditions within 45 minutes; this has implications for these tablets in the gastrointestinal movements and safe delivery in human body. This confirms that the nutrients have faster absorption in the stomach that in intestines. Furthermore, within 45 minutes of tablet disintegration suggests that they have one of the fastest release rates of iron and zinc nutrients and is attributed to easy tablet matrix erosion. Besides, high erosion rates of the tablet matrices are explained by high gelling, swelling and release of nutrients as fast as possible [11]. Noticeably, the low weight tablets release nutrient from the matrix faster because nutrients diffuse quicker from matrix surfaces.

4.2 Cassava waste film in equilibrium modified atmosphere packaging systems

Food industry packaging challenges created by the failure to maintain quality and safety of fresh and minimally processed foods in distribution have been mitigated mainly by modified atmosphere packaging (MAP). The MAP is a widely established system for the preservation of quality and managing shelf-life of fresh foods driven by the need and legislation to replace chemical preservatives. In the MAP system, the in-package environment is modified to match the requirements for storing fresh foods. While MAP is a popular packaging system, it has outstanding flaws such as design errors, which are corrected by active (gas flushing) and passive (equilibrium MAP) techniques [27]. Noticeably, Equilibrium MAP (EMAP) is universally used system for fresh respiring foods [15]. Notably, an EMAP is established inside the package when gas transmission rate matches product gas consumption rate [15]. Other current extenuation actions to the design errors include use low-cost biodegradable biomaterials for EMAP of fresh fruits and vegetables and cherry tomatoes [15, 28, 29] and joint plasma treatment and EMAP for cherry tomatoes [30]. A more robust package design was achieved using an ultimate EMAP across package distribution conditions [15]. This has an advantage of using the biomaterial film with heat sealing, heat resistance, relatively water resistance, good barrier, transparent and good mechanical properties in addition to their cost-effective, less competition with food supply biodegradability in all environments, ability to make pouches and bags, printing capacity, and non-perforation needs [4, 15, 16, 20].

4.2.1 Design of an EMAP system

When planning to design an EMAP for fresh foods, the fresh product respiration and transpiration behaviour and mass transfer of the package are important considerations and must be fully explored and understood [15]. The design trial includes defining the design requirements of bitter cassava biomaterial film EMAP that is stable in distribution chain characterised by low conditions (10°C, 75% RH). This is affected by knowing the impact of packaging parameters (perforation, RH, temperature) on gas (oxygen, carbon dioxide) composition, the optimal design parameters and gas composition and validated optimal EMAP [15]. The EMAP design follows the conceptual flow depicted in **Figure 10**. To design an integrated package, an active coated product is factored in the EMAP evaluation.

4.2.2 EMAP characteristics

The dynamics of an In-package headspace gas plays an important role in attainment of EMAP for food products. When cherry tomatoes are used in the EMAP trials and stored using BCFs, the headspace oxygen reached equilibrium (2–3%) after 180 h at 10°C for 75% RH (**Figure 11**). The recommended headspace oxygen is 3–5% for safe storage of tomatoes [30].

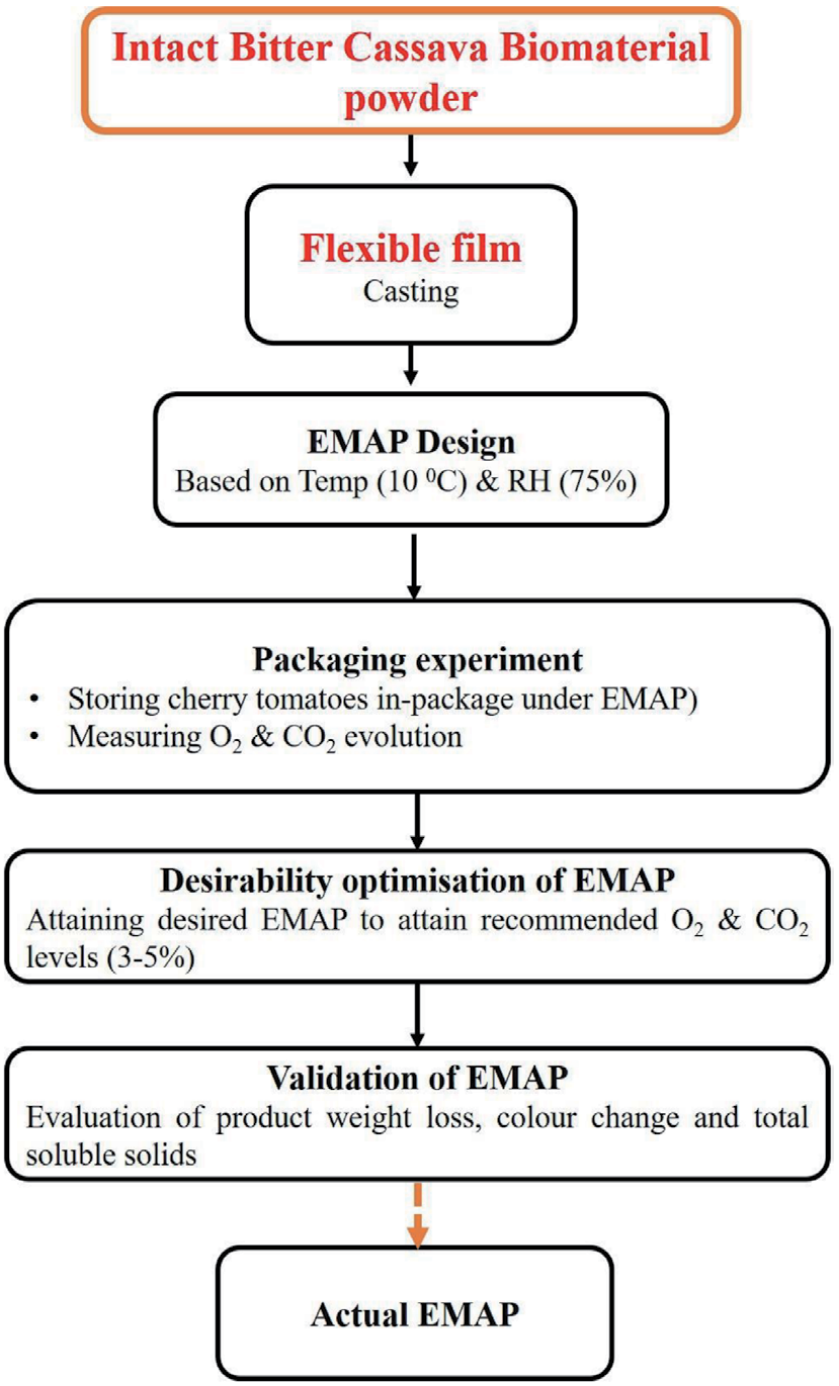


Figure 10.
Practical study of an EMAP design.

4.2.3 Properties of packed and stored product

Since the shelf-life of the products is associated mainly with microbiological quality, modifying the in-package atmosphere through EMAP is often intended to limit microbial contamination.

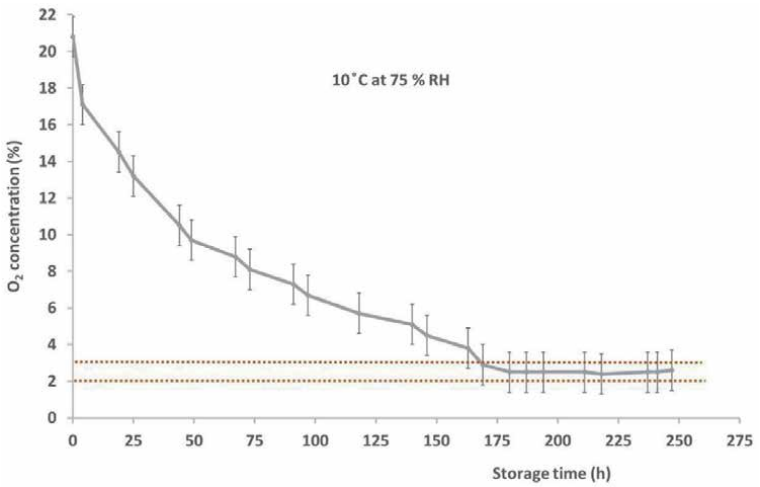


Figure 11.
Progress of headspace oxygen (%) of stored cherry tomatoes in EMAP.

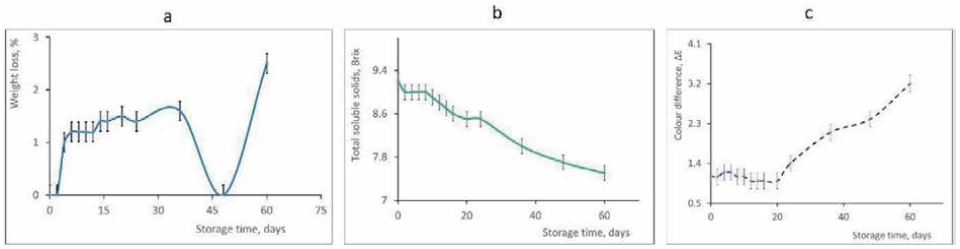


Figure 12.
Status of cherry tomato quality at different storage times: (a) weight loss; (b) TSS; (c) colour difference.

Cherry tomatoes stored in the EMAP show visible mould around 360 h at 10°C and 75% RH and demonstrated reduced weight loss. The loss in normal EMAP is attributed to the combined effect transpiration rate of cherry tomatoes and permeability of bitter cassava films [15].

Colour is a key indicator of market value of the product. An EMAP did not have significant effect on the colour of cherry tomatoes during the 21 days of storage; however insignificant changes are apparent due to the nature of biological materials. A colour index of 1.1 was observed with cherry tomatoes storage with EMAP.

Reduced total soluble solids (TSS) is often encountered when tomatoes are stored for a long time. Like any other storage medium, EMAP decreases cherry tomato TSS (**Figure 12**). In this case, there is a possibility of packaging contributing to the reduced hydrolysis of insoluble polysaccharides into simple sugar [31].

5. Other applications of cassava

According to [32], the global cassava processing market reached a volume of 298.8 Million Tons in 2020, implying that industrial application has grown correspondingly in food, ethanol, paper and cardboard, textiles, pharmaceutical, glues and adhesives. It is reported that food industry accounts for around a half of the total global cassava consumption followed by feed industry [32]. The use of cassava

in most industrial applications such as food, pharmaceuticals, beverages, civil works and textile industries is mainly done with sweet cassava starch and flour.

Food application of cassava. By improving properties of cassava flour using enzymatic and thermal modification, has been found to be acceptable in using modified flour as a key ingredient in the production of gluten-free baked products such as pasta and bread [33, 34].

Textile application of cassava. Because of starch qualities such as flexibility, resistance to abrasion and the ability to form a bond with the fibre, it is used in sizing, finishing, and printing in textile industry [35]. Of the total cassava starch used in textile, an estimated 80 percent go into sizing unit operation, which involves shaping and forming yarn fibres into warp. In this case, starch is used to coat the surfaces of the twisted warp that is then subjected to thermal treatment into a beam of warp ready for weaving. When the yarn is moisturised with cassava starch, the threads become smooth, greasy, slippery and hairless. In this case, starch behaves as a lubricant.

Pharmaceutical application of cassava. Tapioca starch, obtained from the roots of cassava by physical and chemical modifications (oxidisation, esterification, etherification, and treatment with enzyme) is applied in medicine and pharmaceuticals. Native and modified tapioca starch are used as diluents, binders and disintegrants in tablet and capsule formulations [36]. The excellent flowability and swelling power of native tapioca starch renders it useful as diluent for capsule and tablet formulations. Native tapioca starch produces tablets with higher tensile strength, less friability, least tendency to brittle fracture, longer disintegration time and slower drug dissolution rate, thus is preferred in paracetamol tablets when compared with cereal starches. Similarly, modified tapioca starch such carboxymethyl starch is generally used in medicine, pharmaceuticals, cosmetics and food due to their improved hydrophilicity, increased water absorption, reduced tendency of retrogradation, lowered gelatinization temperature, increased solubility in cold water with clear gel and higher storage stability [36, 37]. Acid-modified tapioca starch is an important filler or binder in direct compression with higher tensile strength, lower friability, faster dissolution than the native tapioca starch [38].

Application of cassava in civil works. Research has demonstrated that waste cassava is an ingredient in building materials [39]. It is demonstrated that when waste cassava, cement, charcoal and sand mixed in 2.5 kg composite and made into bricks, a denser texture of the bricks is obtained with perfect binding and compaction [39]. The relative strength is reported to be 711.5 kg/cm2 in addition to the brick being more environmentally responsive. Elsewhere, experimental trials of cassava starch modified concrete confirmed improved compressive, split tensile, flexural and elastic modulus of concrete at an optimum of 0.8% as well increased setting time and durability, with potential application in retarding admixtures [40, 41].

Application of cassava in beverages. Research trials confirm application of cassava into spirits and beers [42]. Using enzymes, cassava is liquefied and saccharified serially into, fermentable broth (circa 184 g/l of fermentable sugars), alcohol (circa10% ethanol) and spirits (40% ethanol by volume) with consumer acceptance [42].

Of recent, cassava coating is used in active packaging using both sweet and bitter varieties. Cassava-based edible coatings is used universally in preservation of foods. Trials have shown that edible cassava starch coating extended the shelf-life of Andean blackberries by 100% after 10 days in storage [43] and prevent decay and extend shelf life of black mulberries under refrigerated conditions [44].

6. Conclusion

Green environment, sustainability, resource renewability and efficiency, industry biomaterial supply, and circular produce-consume-dispose model could be spurred by exploiting innovative research solutions into cassava waste biomass. This chapter demonstrates that cassava varietal-specific waste can be transformed fully into sustainable and efficient feedstocks for bioplastics, packaging, and food supplement industries. Using innovative SRRC improved downstream processes and integrated sustainable process, up to 30% waste from bitter cassava can provide stand-alone feedstock requirements for food, medical, packaging industries. Valorisation of wastes reveals application in Iron-Zinc supplements and extending shelf life of tomatoes, which has advantage of improving nutrition status of vulnerable communities but also avoiding use of pesticides in fruit marketing. Either way, health is improved for the communities. Innovative SRRC improved processing methodology can be an alternative solution that eliminates the burden of drudgery and rudimentary process of small and medium enterprises (SMEs) to increase their market participation. As a supplementary bonus, valorisation of bitter cassava wastes into bioplastics would likely avert consequences of littering and burning of plastics (mainly carrier bags) that impact negatively on the environment and public health. Ultimately, committed used of SRRC in bitter cassava processing would help SMEs to have a sustainable non-food feedstock resource, contribute national environment programmes and improve community incomes.

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Conflict of interest

Author declare no conflict of interest for this work.

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Chapter 8

Primary Quality Control Parameters of Cassava Raw Materials

Shadrack Mubanga Chisenga

Abstract

Fresh cassava roots are transformed into shelf stable raw materials (flours and extracted starches). Chemical composition (moisture, protein, lipid, fibre and amylose content, cyanide contents), dry matter, starch extraction yields, particle size distribution and whiteness index are some of the quality characteristic requirements for selection of varieties in breeding programs, and raw materials for industrial processes. Starch yields ranges 20–35%, and vary with genotype. The crude protein (1–2%) and crude fat (~1%) are considered minor components of cassava and are indicative of the poor nutritional quality. The cumulative of particles passing finer than sieve (D90) is commonly selected for industrial applications because it yields a large proportion of flour in the range 90–96% finer particle than sieve size. The amylose is the main genetic trait for categorising starches into waxy, semi-waxy, normal/regular and high amylose types when amylose content is 0–2, 3–15, 16–35, and > 35% of the total starch, respectively. Additionally, amylose is basic criteria for blending flours of different botanical sources. Cassava varieties are classified as sweet and bitter varieties when cyanide values are in the range 15–50 and 50–400 ppm, respectively. The a* (redness-greenness) and b* (yellowish) are considered as impurities in white fleshed cassava.

Keywords: Cassava, *Manihot esculenta* Crantz, Flour, Particle Size, Chemical composition, Starch yields, Amylose Cyanides

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a staple food for over 800 million people in the tropics, and second highest dietary source of calories in sub-Saharan Africa where the crop is mainly produced for human consumption [1]. The cassava leaves are consumed as vegetable. In countries such as Democratic Republic of the Congo, Zambia, Tanzania, Sierra Leone, Liberia and Guinea, younger and tender cassava leaves are a major component of the diet. Nigeria is the largest producer of cassava worldwide. Thailand and Indonesia account for the largest share (75%) of cassava production in Asia. Thailand is the largest cassava exporter with 60% of global markets [2]. In the Americas, 40% of produced cassava is used as human food, and 30% for livestock feed. Cassava provides income, employment, and play a role as major food security crop. Cassava root is typically a carbohydrate material predominantly comprising of starch. Among the starches, including main cereal crops,

cassava is the highest producer of carbohydrates per hectare and is very tolerant to drought, heat stress and can thrives well on marginal soils. Cassava has gained tremendous use in food and beverages. It is added to wheat flour to form composite flour for making bread and other bakery products. In Nigeria, where the policy of cassava flour inclusion is mandatory, flour mills are required to partially substitute wheat flour with minimum of 10% cassava flour for bread making and other bakery products [3, 4].

Commercially, cassava flour and starch find application in bakery, brewing and pharmaceuticals industries. These industries have a number of specific quality requirements in raw materials. Proximate contents (moisture, crude protein, crude lipids, crude fibre and ash), colour, dry matter, starch yield, amylose contents, flour particle size and cyanide contents are some of the primary quality indicators for selection of cassava root raw materials in the industry. This chapter focused on production of cassava raw materials and criteria parameters for primary selection.

2. Cassava varieties

The cassava bulking roots are source of dietary calories. The cassava leaves serve as vegetable and contains significant levels of leucine, lysine, phenylalanine, valine and threonine, and crude protein content of ~26% per 100 g [5]. There are a number of cultivated cassava varieties. The breeding objective of most cassava program is to produce high yielding, early bulking, pests and disease resistant varieties [6]. Other breeding objectives include reduced cyanide content in cassava to produce sweet varieties. The bio-fortified cassava is yellow or orange fleshed and is the most recent genotype which has been bred for improved nutrition to supply pro-vitamin A carotenoids. The micronutrients, mainly of provitamin A, and dry matter content traits are among the primary selection objectives in cassava breeding. The yellow-fleshed cassava has other advantages such as delayed postharvest deterioration due to anti-oxidant carotenoids contained in the root [7].

3. Cassava primary products

The rapid physiological postharvest deterioration (PPD) and high amounts of cyanide content are the main factors limiting utilisation of fresh cassava [8]. Fresh cassava roots undergo PPD after harvest resulting in poor shelf life. The PPD decreases starch content resulting in poor functional properties. Preventing PPD requires that fresh cassava roots are processed into shelf-stable dried products immediately after harvest. Processing of cassava roots leads to decreased cyanide content and improved shelf life stability. The cassava primary products include flours and starches and serve as raw materials to the growing industrial utilisation of cassava. The fresh cassava roots can be processed into chips, and the dried chips can be milled into flour or starch at the later stage in the value chain.

3.1 Processing of cassava chips

Cassava chipping is the first primary step of processing peeled cassava into raw dried chips. The production of cassava chips is mainly a two steps process involving chipping and drying. Manually or motorised chipping machines are used. The peeled and cleaned fresh cassava roots are fed into the hopper of a chipping machine, and the chipped materials are collected at the bottom using cleaned receptacles such as trays, buckets or woven sacks. The collected fresh chips are

spread on the drying mats or trays. For large scale production, the chipper is mounted on the wheels to facilitate the spreading of chips on the drying mats. The chipping machine has an inbuilt rotating circular steel plate with chipping blades. The peeled cassava is chipped or sliced into finger-like strips of ~3 mm diameter. Thinly chipped materials are recommended for increased surface area for enhanced drying and release of volatile cyanides. The methods of drying include sun-drying, and hot air circulation using commercial ovens. The drying temperature ranges of 45–60°C are commonly applied. It is recommended to dry to a moisture content of 8 to l0%, after which the chips are cooled and packed.

3.2 Processing of cassava flour

Cassava roots are processed into unfermented flour as described by Chisenga et al. [9]. The harvested fresh cassava is sorted to select healthy and marketable roots which are cleaned to remove soil and debris. The cleaned roots are peeled and chopped into small pieces by hand with a knife, and washed 2x in potable water. Also, peeling can be conducted using motorised or engine driven peeler machine. The chopped cassava is grated using a motorised or engine driven grating machine with an inbuilt spiked stainless-steel sheet mounted on the wooden roller. The grating roller rotates against the clearance space of the adjustable wooden board at the bottom of the hopper, and can be adjusted according to the desired fineness of cassava pulp. The fine grated cassava pulp is then put into clean polypropylene woven sacks and dewatered to remove excess water by pressing using a manually operated vertical screw press. Alternatively, industrial electric motor or engine driven dewatering machines are used. The dewatered pulp is then granulated by crumbling by hand or using pulverising machine into small particles (grits) and spread on mats (polyethylene plastic sheet) placed on raised platforms. The grits are then sun-dried, and can also be dried using a hot air circulation oven drier at 35°C for 12 h. The dried grits are stored until milling into flour.

3.3 Production of starch

Wet milling of cassava is the primary step toward extraction of starch from fresh cassava. Subsequent steps include dilution, filtration, sedimentation, and decantation. Centrifugation of the filtrate can replace a batch step of sedimentation. The sedimented starch can carry impurities. Therefore, after decantation, it is essential to wash the surface of sedimented starch with potable water. The extracted wet starch can be dried through sun drying or oven drying at 35–40°C. Thorough peeling of fresh cassava roots before wet milling is a critical step. Cassava starch extracted from unproperly peeled roots can form a grey colour during wet storage and purple colour during drying (personal observations). Also, starch extracted from bitter cassava varieties are subject to purple colourations during drying (personal observations). The retained colour decreases the quality, and thus affecting the market value. The extraction methods are not standardised, to the extent that workers applied different amount of water for extraction. The ratio of water to cassava slurry of 2:1 was used by Abera and Rakshit [10], while Nand et al. [11] used ratio 10:1 of water to cassava slurry, respectively. In some procedures, grating was performed with sulphur-containing water for detoxification of hydrocyanic acids (HCN). The fresh starch can be stored in sodium meta-bisulphite solution to prevent the microbial growth.

Extracted starch is expressed in terms of percentage starch yield. The fresh cassava roots are washed, peeled, chopped into small pieces and then pulverising in a blender. The pulp is suspended in potable water in the ration 1:10 (the volume of

water 10x the volume of pulp), and the well-stirred mixture is filtered using double cheesecloth. The collected filtrate is allowed to sediment, and after decanting of the supernatant, the sediment is washed six times. The resultant starch is washed using distilled water, and after decanting, the starch is oven-dried at less than 35°C for 24 h. The starch yield is determined based on original weight of peeled and blended cassava.

$$Starch\ yield, \% = \frac{S_f - S_d}{W_o} \times 100 \quad (1)$$

Where, S_f = weight of fresh starch, S_d = weight of dried starch, W_o = original weight of peeled and blended cassava.

Starch is the main component of cassava. The values of starch extraction yield are expressed as fresh weight of peeled cassava and usually reported based on wet weight. Literature showed cassava starch yields in range of 20–35% [12]. On dry weight, the starch content from fresh cassava root is estimated at 80% [13]. Cassava genotype is the major factor influencing starch yields. Mtunguja et al. [14] reported that genotype had huge influence on variability of starch contents and yields, while environmental factors yielded insignificant variations. Similarly, Mejía-Agüero et al. [13] screened and compared starch content among cassava cultivars planted and harvested simultaneously in a single plantation. The authors observed that significant differences in starch contents due to differences in cassava varieties. Therefore, diversity of cassava genotypes accounts for differences in starch extraction rates (yields) and contents. The cassava industry is focused on growing cassava cultivars with high starch yields.

4. Cassava flour particle size distribution

The particle size can affect the pasting and functional characteristics of flours and starches. Ahmed et al. [15] reported that the onset gelatinization temperature decreased from 70 to 60°C with decreasing particle size, which suggested that the smallest particle fraction had a lower initiation temperature of gelatinization because of high water absorption for smaller particle size. Oladunmoye et al. [16] reported that the particle size of flours affects the rate of water absorption during processing as fine particles resulted in faster absorption of water. Lazaridou et al. [17] reported that coarse flour doughs exhibited increased stiffness and resistance to deformation and flow. A study on rice reported that coarse particles had lower solubility compared with fine and medium particles, and large particle size retarded digestion [18]. The reduced digestibility in large particle could suggest application in the formulation of resistant starch products. When wheat flour was fractioned by sieving into finer fractions (<75 and 75–118 μm) and coarser fractions (118–150 and > 150 μm), the finer fractions were reported to produce high-quality bread [19]. Reducing the particle size can strengthen gluten network of dough resulting in shorter development time and longer mixing stability of dough because of fast and high-water absorption [20]. The reduced particle size of cassava flours from 15 to 5 μm were reported to result in a decreased peak, trough and final viscosities [21].

The fractionation of cassava flour from the Zambian varieties was shown in the work of Chisenga et al. [22]. Flour particle size distribution was determined by sieving ~500 g of sample for 5 min using seven sieves with opening dimensions of 425, 300, 180, 150, 106, 90 and 38 μm. The sieves were serially stacked in descending order with the receiver pan at the base. The sample was loaded on the largest sieve on top and covered. The column was placed on the vibratory

mechanical shaker (DuraTap, Model DT168, Advantech Mfg. Co., New Berlin). After shaking was completed the sample weight on each sieve was measured. The weight of the materials on each sieve was then divided by sample weight to obtain the percentage retained on each sieve. The next step was then to find the cumulative percent of the retained in each sieve. The cumulative percent passing was calculated by subtracting the percent cumulative retained from 100%.

$$Retained(\%) = \frac{W_{sieve}}{W_{sample}} \tag{2}$$

$$Cumulative(\%)Finer\ particles = 100 - \%Cumulative\ Retained \tag{3}$$

where: W_{sieve} = weight of fraction retained on the sieve, W_{sample} = weight of the sample.

The weight of sieve and total weight (sieve and flour sample) retained on sieve after sieving is shown in **Table 1**. To obtain weight of sample retained on sieve (**Table 2**), the weight of sieve is subtracted from the total weight. For example, amount retained on sieve 425 μm for Bangweulu is obtained by subtracting 473.99 g from 484.14 g. The difference (10.15 g) is expressed as percentage of total weight of retained samples (**Table 3**). Percentage cumulative for Bangweulu of sieve 425 μm is 4.97% (**Table 4**). The %cumulative on sieve 300 μm is obtained by adding % weight retained on sieve 425 μm (4.97%) and %weight retained on sieve 300 μm (5.57%). Therefore %cumulative on sieve 300 is 10.53% which is then added to % weight retained on sieve 180 μm to obtain 25.10% cumulative on sieve 180 μm, and so forth. The percentage cumulative of flour particles passing finer than sieve size (**Table 5**) is obtained using Eq. (3).

The percent passing (finer than size) was plotted as the function of sieve sizes (**Figure 1**). The limits of D10, D30, D50, D60, and D90 were selected as they are commonly used in the classification of powder materials. These parameters refer to the percentages cumulative size distribution of passing particles finer than the particular sieve size. D10, D30, D50, D60 and D90 is defined as the size value corresponding to cumulative size distribution at 10%, 30%, 50%, 60% of 90% by weight, which represents the size of particles below which 10%, 30%, 50%, 60% of 90% of the sample lies. The D10, D30, D50, D60 and D90 were obtained from the plot by performing particle size trend analysis using Excel. For example, particle

Sieve Size (μm)	Weight of Sieve	Total weight of sieve and retained flour sample (g)					
		Ban	Kat	Mwe	Kar	Kam	Chi
425	473.99	484.14	482.4	480.64	485.6	485.94	483
300	325.05	336.43	333.9	332.68	337.94	339.45	334
180	303.96	333.72	323.14	319.7	328.01	334.09	325
150	310.25	325.16	318.6	323.87	318.8	324.92	318
106	310.63	355.5	342.64	371.99	392.07	417.4	432.46
90	280.79	302.98	305.15	313.42	293.55	284.94	292.3
38	442.66	495.13	518.4	493.03	489.13	465.48	462.09
Receiver pan	414.23	432.03	437.34	433.63	427.64	426.72	425.46

Cassava varieties: Ban = Bangweulu, Kat = Katobamputa, Mwe = Mweru, Kar = Kariba, Kam = Kampolombo, Chi = Chila.

Table 1.
Weight of sieve, total weight of sieve and flour samples retained on sieves after sieving.

Sieve Size (μm)	Amount retained on sieve (g)					
	Ban	Kat	Mwe	Kar	Kam	Chi
425	10.15	8.41	6.65	11.61	11.95	9.01
300	11.38	8.85	7.63	12.89	14.4	8.95
180	29.76	19.18	15.74	24.05	30.13	21.04
150	14.91	8.35	13.62	8.55	14.67	7.75
106	44.87	32.01	61.36	81.44	106.77	121.83
90	22.19	24.36	32.63	12.76	4.15	11.51
38	52.47	75.74	50.37	46.47	22.82	19.43
Receiver pan	17.8	23.11	19.4	13.41	12.49	11.23
Total weight retained	203.53	200.01	207.4	211.18	217.38	210.75

Cassava varieties: Ban = Bangweulu, Kat = Katobamputa, Mwe = Mweru, Kar = Kariba, Kam = Kampolombo, Chi = Chila.

Table 2.
Weight of cassava flour samples retained on sieve after sieving.

Sieve Size (μm)	%Weight retained					
	Ban	Kat	Mwe	Kar	Kam	Chi
425	4.97	4.19	3.20	5.49	5.34	4.15
300	5.57	4.41	3.67	6.10	6.43	4.12
180	14.56	9.57	7.57	11.37	13.46	9.68
150	7.30	4.16	6.55	4.04	6.55	3.57
106	21.96	15.97	29.53	38.52	47.70	56.06
90	10.86	12.15	15.70	6.03	1.85	5.30
38	25.67	37.78	24.24	21.98	10.19	8.94
Fines <38	8.71	11.53	9.34	6.34	5.58	5.17

Cassava varieties: Ban = Bangweulu, Kat = Katobamputa, Mwe = Mweru, Kar = Kariba, Kam = Kampolombo, Chi = Chila.

Table 3.
Percentage of weight of cassava flour samples retained on sieve after sieving.

size of Bangweulu flour (312 μm) at D90 was obtained by performing trend formula using Excel (Please see **Figure 2**) on %cumulative finer particles: TREND (B3:B4, C3:C4,90), where B3 = 425 μm mesh, B3 = 300 μm mesh, C3 = 95.03% finer particles passing through 425 μm mesh, C4 = 89.46% finer particles passing through 300 μm mesh.

The percentage of flour particles passing through 38, 90, 106, 150, 180, 300, and 425 μm standard sieves were in the ranges 6.47–11.77, 17.13–49.53, 20.51–61.69, 68.21–78.96, 77.03–82.05, 88.22–93.15, and 94.64–95.85%, respectively, and varied with variety. The flour particle size distribution between 90 and 10% cumulative of particles passing finer than sieve were estimated from the particle distribution curve (**Figure 1**). The average particle sizes of flours at D90, D60, D50, D30, and D10 were in the ranges 250.44–334.34, 103.76–142.42, 90.59–133.19, 63.09–114.75 and 35.56–48.52 μm, respectively (**Table 6**). The particle size distribution curve on each variety suggested that flours were a mixture of various particle sizes. The variety Kampolombo recorded the largest particle size across the distribution levels

Sieve Size (μm)	% Cumulative retained					
	Ban	**Kat**	**Mwe**	**Kar**	**Kam**	**Chi**
425	4.97	4.19	3.20	5.49	5.34	4.15
300	10.53	8.61	6.87	11.59	11.77	8.26
180	25.10	18.17	14.45	22.96	25.23	17.95
150	32.39	22.34	21.00	27.00	31.79	21.51
106	54.35	38.30	50.53	65.52	79.49	77.57
90	65.21	50.45	66.24	71.55	81.34	82.87
38	90.88	88.23	90.48	93.53	91.53	91.81

Cassava varieties: Ban = Bangweulu, Kat = Katobamputa, Mwe = Mweru, Kar = Kariba, Kam = Kampolombo, Chi = Chila.

Table 4.
Percentage cumulative of cassava flour samples retained after sieving.

Sieve Size (μm)	%Finer particles					
	Ban	**Kat**	**Mwe**	**Kar**	**Kam**	**Chi**
425	95.03	95.81	96.80	94.51	94.66	95.85
300	89.47	91.39	93.13	88.41	88.23	91.74
180	74.90	81.83	85.55	77.04	74.77	82.05
150	67.61	77.66	79.00	73.00	68.21	78.49
106	45.65	61.70	49.47	34.48	20.51	22.43
90	34.79	49.55	33.76	28.45	18.66	17.13
38	9.12	11.77	9.52	6.47	8.47	8.19

Cassava varieties: Ban = Bangweulu, Kat = Katobamputa, Mwe = Mweru, Kar = Kariba, Kam = Kampolombo, Chi = Chila.

Table 5.
Percentage cassava flour sample particles passing through finer than sieve.

except at D10 and exhibited a smaller amount of flour passing at all sieve sizes except at lowest aperture sieve (38 μm). Particle size can be a differentiating genetic trait among cassava varieties. In this study, cassava genotypes were cultivated simultaneously on a single plantation and harvested at the same time, with the same milling conditions. Thus, the variation in flour particle size was attributed to genetic differences among the cassava varieties [23]. The selection of sieve is guided by product nature because decreasing cumulative of particles passing finer than sieve results in reduced proportion amount of flour. In this study, D90 was selected for bread baking since it yielded a large proportion amount of flour in the range 90–96% finer particle at sieve size 300–425 μm, respectively.

4.1 Bulk density

The bulk density (g/cm^3) of flour is the density measured without the influence of any compression. The bulk densities of cassava flours are reported in the range 0.40–0.50 g/cm^3 [22] and 0.60–0.70 g/cm^3 [24]. These values are lower than 0.80 g/cm^3 reported in wheat flour. The bulk density is reported to associate positively with moisture, protein and lipid content, and negatively with fibre.

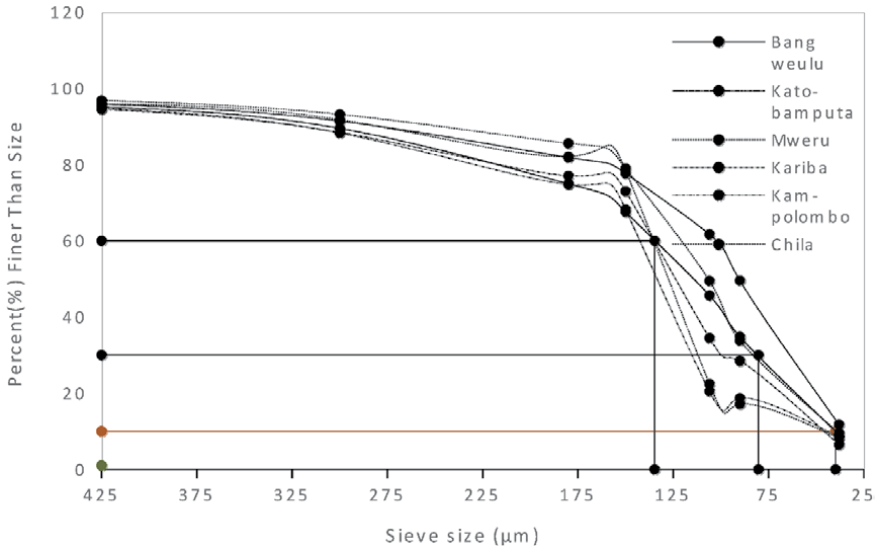

Figure 1.
Particle size distribution curves for cassava flours from six different varieties at selected percentage cumulative of particles passing finer than standard sieve size at 5% significance level LSD test (Variety = 0.055, Sieve size = 0.059). Classification and uniformity criteria of D10, D30, and D60 for the particle size distribution of flours derived from Bangweulu variety using TREND in Excel. Adapted from the work on Zambian cassava varieties [22].

	A	B	C	D	E	F	G	H
1	Sieve		%Finer particles than sieve size					
2	Sieve size (mm)	Sieve Size (µm)	Ban	Kat	Mwe	Kar	Kam	Chi
3	0.425	425	95.03	95.81	96.80	94.51	94.66	95.85
4	0.3	300	89.47	91.39	93.13	88.41	88.23	91.74
5	0.18	180	74.90	81.83	85.55	77.04	74.77	82.05
6	0.15	150	67.61	77.66	79.00	73.00	68.21	78.49
7	0.106	106	45.65	61.70	49.47	34.48	20.51	22.43
8	0.09	90	34.79	49.55	33.76	28.45	18.66	17.13
9	0.038	38	9.12	11.77	9.52	6.47	8.47	8.19

Figure 2.
Excel sheet used for performing TREND Analysis. Cassava varieties: Ban = Bangweulu, Kat = Katobamputa, Mwe = Mweru, Kar = Kariba, Kam = Kampolombo, Chi = Chila.

	Percentage cumulative				
Variety	**D90**	**D60**	**D50**	**D30**	**D10**
Bangweulu	312.00 (0.00)^{A}	134.80 (0.01)^{o}	114.71 (0.01)^{n}	80.29 (0.01)^{h}	39.78 (0.01)^{c}
Katobamputa	282.53 (0.03)^{z}	103.76 (0.01)^{l}	90.59 (0.01)^{j}	63.09 (0.01)^{g}	35.56 (0.01)^{a}
Mweru	250.43 (0.03)^{x}	121.69 (0.01)^{p}	123.71 (0.01)^{q}	81.92 (0.02)^{i}	39.02 (0.00)^{b}
Kariba	332.52 (0.02)^{B}	135.17 (0.03)^{u}	123.72 (0.03)^{q}	94.12 (0.01)^{k}	46.35 (0.00)^{e}
Kampolombo	334.43 (0.01)^{C}	142.42 (0.01)^{w}	133.19 (0.01)^{s}	114.75 (0.01)^{o}	45.82 (0.00)^{d}
Chila	278.49 (0.00)^{y}	135.48 (0.00)^{v}	127.64 (0.00)^{r}	111.94 (0.03)^{m}	48.52 (0.00)^{f}

All values are means of three replications. Within the same column, the values with different letters are significantly different at $p < 0.05$ by LSD test.

Table 6.
Flour particle size at selected percentage cumulative of particles passing finer than sieve size.

This suggests that flour with high bulk density contains high protein and lipid contents. Oladunmoye et al. [25] ascribed low bulk density in cassava flour to low protein and fat content of cassava flour. Flours with high bulk density exhibit low fibre content, implying that decreased fibre content results in finer flour particle size. Chandra et al. [26] revealed that bulk density depends on the particle size and initial moisture content of flours. The study on roasted Bengal gram flour showed that an increase in moisture content resulted in an increase in bulk density [27]. This means that bulk density increases with increase in moisture content. The strong negative correlation between bulk density and particle size [22] suggests that flours with smaller particle size at specified mesh size can yield higher bulk density. The bulk density values can find application in packaging, handling, and processing requirements.

4.2 Packed density

The packing of powder is the indication of the maximum packing density of flours attained under the influence of defined externally applied forces. The packed densities are higher than bulk densities. This variation could be due to factors such as geometry, size, solid density and surface properties of the flour materials and could be improved when the particles are small, compactable, properly tapped/vibrated and with suitable packaging material. Bulk density influences flowability of flours, package design and can be used in determining the requirements of packaging material [28]. The denser packaging materials are required for packing flours with higher bulk densities. The increase in packed density is desirable as it offers greater packaging advantage as a greater quantity may be packed within a constant unit volume.

The methods to determine bulk density and packed density have been reported [22, 24]. The bulk density is determined by adding 50 g of flour sample to a graduated cylinder, and the volume recorded.

$$Bulk\ density,\ g/cm^3 = \frac{V_b}{Weight\ of\ sample} \tag{4}$$

where: V_b = volume of flour.

The tapped density is determined by mechanically tapping (100x) a graduated cylinder containing flour sample (V_b) until no further volume change was observed. The final volume [29] recorded.

$$Packed\ density\ g/cm^3 = \frac{V_b - V_f}{Weight\ of\ sample} \tag{5}$$

where: V_f = final volume.

4.3 Root dry matter content

The dry matter content is used as a basis for accepting raw materials in the food industry. In addition, dry matter content is often used by breeders as a proxy for starch content when selecting cassava varieties. Dry matter content levels of above 30% are considered to be high. The differences in dry matter content among the varieties can be associated with months after planting. Teye et al. [30] reported dry matter content in the range of 30–40% for cassava root that were harvested at 13 months after planting, which are lower than the values (40 and 50%) for cassava roots harvested 18 months after planting [22]. The factors influencing dry matter

content include harvest age, seasons and growing locations. Beyene et al. [31] reported that bio-fortification of nutrients in cassava reduced dry matter contents.

The dry matter content can be determined by taking 200 ± 05 g fresh peeled cassava roots from undamaged roots selected randomly from 3 plants after medial sections are chipped into strips, mixed thoroughly and dried at 65°C, 72 h until constant weight. The dry matter content is estimated as the difference between the mass before drying and the mass loss on drying.

$$Drymatter\ content, \% = \frac{W_f - W_d}{W_f} \times 100 \quad (6)$$

where: W_f = weight of fresh cassava strips, W_d = weight of dried cassava strips.

4.4 Cyanide glucoside content

Cyanide glucosides content in cassava is a limiting quality trait for both human and animal consumption. The level of cyanides in roots and flours is an important trait for selecting cassava varieties in breeding programs. Consumption of high dietary cyanides causes konzo, a permanent neurological condition, causing spasticity. The groups at risk include children and women of child-bearing age. Furthermore, cassava dietary toxicity has been reported to cause tropical ataxic neuropathy in elderly people, a progressive myeloneuropathy that was first described in Nigeria and is characterised by a progressive onset of ataxia [32]. Cassava roots have been reported with high levels of cyanides (1090–1550 ppm) [33]. Other studies reported low levels of cyanides (23–350 ppm) [22]. The cyanides can vary with genotype and environment. The variation due to environmental was demonstrated in Tanzania by Mtunguja et al. [14], and the authors observed that the variety Kiroba recorded 800, 200, and 40 ppm of extracted cyanides from three separate regional sites (Chambezi, Amani and Magadu), respectively, at 15 months after planting. The variations due to genotype was shown in Chisenga et al. [22] the study in which the varieties were cultivated at the same site and were rain fed. The differences in cyanide levels among varieties were assumed to relate with genotype and water stress. Water stress experienced during the rainy season may cause a variation in cyanide levels [34]. The root cyanide levels were associated with genetic traits, protein and fibre content. The xylem and phloem are fibrous nature [35], and can retain higher cyanides after harvest. Cassava roots contain cyanides in different forms. The glycosides linamarin and lotaustratin are considered bound [36]. The non-glycosides which are hydrogen cyanide (HCN) and cyanohydride are considered free [37] and would be leached during processing. The cyanogens can lead to human toxicity problems and would require that cassava for food is processed to reduce cyanide-containing substances to safe levels. There are many cultivars of cassava and are classified as bitter and sweet varieties depending on the cyanogenic glucoside levels. The bitter and sweet varieties have high (≥ 100/mg/kg) and low (≤ 50 mg/kg) HCN content respectively. Cassava is consumed in various forms which may vary across countries. Generally, one target of cassava processing is to reduce its cyanogenic glucoside content to the lowest level possible.

Primary techniques of processing cassava are developed with a common goal of reducing cyanogens to safe levels in shelf-stable products such as cassava flour, chips, and starch. The processed cassava flours showed cyanide reduction levels of 60–90% compared to the levels in fresh cassava roots [22]. The method of processing is vital for reduction of cyanogens. Cyanides are largely removed by the traditional processing methods such as grating, dewatering (pressing), fermenting,

and drying. The highest level of cyanides retention in some cassava derived products could suggest that the cyanogens are chemically bound. The improved varieties referred to as sweet varieties have lower cyanides retention levels, which could indicate the higher levels of free cyanides such hydrogen cyanide and cyanohydride are readily extracted, or have higher levels of linamarase, which degrades cyanogenic glycosides. Hydrogen cyanide and cyanohydride are soluble in water and volatile (25°C boiling point). The total cyanide content can be reduced by soaking and air drying at low temperatures (28–40°C), which combines the enzymic action of linamarase with water extraction and volatilisation. Cassava varieties are classified as sweet varieties when their extracted cyanide values are in the range 15–50 ppm, and as bitter variety when their values are from 50 to 400 ppm of fresh cassava [34]. The recommended safe level of cyanides in a final food product is 10 ppm (FAO/WHO). Since cassava flour is a raw material, the total cyanide level is expected to reduce further down the processing stream. The temperatures for proofing (30–32°C) and baking (178–193°C) for bread making can significantly reduce cyanide levels in the final product.

4.5 Fungal contamination

Cassava raw materials (chips and flours) are subject to attacks by fungi such as Aspergillus, Fusarium and Penicillium during storage and distribution. These fungal contaminations can lead to mouldy taste, discolouration, and production off odours. In addition, the contamination can lead to production of mycotoxins of serious safety and health concerns. Some of the mycotoxins of public health importance include aflatoxins, fumonisins, ochratoxin A, zearalenone and deoxynivanelol [38] and harmful to animals and humans. The mycotoxins can act as nephrotoxins, hepatotoxins, immunotoxins, neurotoxins, teratogens, or carcinogens, however, the kidney is the primary target for toxicity. The quantity of mycotoxins in food and feed is strictly monitored and regulated by the bureau of standards in most of member states of World Trade Organisation. It is the common practice for food companies to inspect raw materials for presence of fungal and mould growth on receipt and during storage of raw materials. However, this method is limited to detecting fungal at visible growth level. Nevertheless, the recent advances in detection techniques accounts for the use of electronic nose system capable of early detection and identification of fungal [39]. The electronic nose technique involves analysing air in the vicinity of fungal and records of which are analysed using Principal Component Analysis.

4.6 Proximate composition

The chemical composition is cultivar dependent, and varies according to geographical location, maturity stage of the plant, and environmental conditions. On the wet basis, fresh cassava root is composed of 56–60% moisture, 0.3–0.6% protein, 30–35% carbohydrate, and 0.1–0.3% fat (**Table 7**). Moisture content is one of the most common tests in foods because the water content in food products influences the interaction between preservation and chemical-, physical- and microbiological- changes during storage. On the dry basis, 8–12% moisture levels are recommended for shelf stability. It is difficult to compare chemical constituents based on data derived from literature because analyses were based on different cassava varieties, variation in harvest time, and lack of complete description of sample materials in terms of genotypic traits, and insufficient information about the colour of the flesh (yellow or white fleshed cassava). Cassava is rich in carbohydrates and deficient in proteins and fats. On a dry matter basis, cassava root has

Cassava source, Country	Number of variety	White/yellow fleshed	Harvest time	Moisture (%)	Ash (%)	Protein (%)	Lipids (%)	Fiber (%)	Reference
Gannoruwa, SriLanka	1	-	-	62.92	-	0.72	0.41	0.92	[40]
Mansa, Zambia	6	White	18	10-11	1.16-1.80	1.21-1.87	0.15-0.63	0.03-0.60	[22]
Umudike, Nigeria	3	-	10	61.05-69.95	-	-	-	-	[41]
Umudike, Nigeria	3	-	13	62.85-70.21	-	-	-	-	[41]
Umudike, Nigeria	3	-	16	49.96-62.02	-	-	-	-	[41]
Pokuase, Ghana	6	-	12	33.14-45.86	-	1.76-3.48	0.74-1.49	1.38-3.20	[42]
Nassau, Bahamas	6	-	9	56.50-68.80	2.27-3.24	1.20-2.10	0.20-041	-	-
Chapare, Bolivia	6	-	-	-	1.46-2.71	1.46-2.49	0.58-1.4	7.40-8.50	[43]
IITA Ibadan, Nigeria	2	-	-	55.44-58.79	1.90-2.84	0.90-1.43	-	3.62-5.45	[44]
Nokornratchasrima, Thailand	1	-	10	-	2.41	1.83	0.14	1.79	[45]
Nokornratchasrima, Thailand	1	white	12	-	2.52	1.41	0.08	2.59	[45]
Umudike, Nigeria	1	white	-	12.28	1.92	-	0.95	1.78	[24]
Umudike, Nigeria	5	yellow	-	8.40-9.85	1.44-2.35	-	0.80-2.75	1.65-2.32	[24]

MAP = months after planting, hyphen (-) implies value or information not found. Dry matter basis, analysis based on dried cassava samples. Wet basis, analysis based on fresh cassava samples.

Table 7.
Proximate composition of different cassava varieties.

carbohydrate content of 70–82%, which is made up of starch containing amylopectin and amylose. Nevertheless, carbohydrate contents are season depenedent, and converts into sugars during hot and rainy season. These changes results in decreased starch yields, and this necessitate the need for optimal harvest time depending on genotype and geographical location.

The differences in proteins among the varieties can be accounted for in terms of environmental conditions such as soil fertility levels and environmental conditions. Nitrogen rich fertiliser contributed to increased protein contents in cassava varieties from the range of 4.3% unfertilized to 9.6% in fertilised varieties [46]. However, these results reported levels of protein in cassava flour that are far higher than other studies. The high levels, however, could have been due to the measurement of additional nitrogen from cyanogens during alkaline distillation of acid-digested samples. While it is not certainly clearly understood, the nitrogen in cyanide compounds can contribute to the crude content of nitrogen levels, which may be attributed to proteins. Moreover, the study Chisenga et al. [22] showed that proteins correlated positively with cyanogen contents in the roots. Nevertheless, protein in cassava flours can influence pasting properties. The entanglement of protein and starch is responsible for viscosity changes during gelation, and the resulting matrix can restrict swelling of starch granules. Proteins can bind water and limit starch swelling at low heating temperature. The negative association between protein and carbohydrates follows the 'dilution hypothesis', which explains the reduction of molecular interactions between protein molecules (aggregation) by increased saccharide contents [47]. During drying, saccharide replaces water molecules bonded to proteins. The elimination of water may alter the binding sites of proteins, which affects their activities, and presumably decreases the protein content.

Lipid such as monoglycerides and phospholipids can form a liquid-crystalline phase with water through hydrophilic (polar heads) or hydrophobic (methyl) groups. The polar lipid, due to their surface-active nature, accumulates at interfaces [48], and have a tendency to absorb water, which justifies their positive association with moisture in flours. The formation of amylose-lipid complexes is reported to increase viscosity levels achieved during starch pasting.

Ash content is an indicator of mineral content, and is used as measurement of the quality of flours in the food industry. The ash content of cassava varies 1–2%. Fibre is a major contributor to ash contents in varieties. Related studies showed that wheat flour varieties with higher fibre content had a higher ash content [49]. The objective of milling and fractionation is to separate the fibrous residue from the flour. The determination of ash content involves incinerating a known weight of flour under controlled conditions, weighing the residue, and calculating the percentage of ash based upon the original sample weight [50].

Studies showed that fibre contents associated negatively with smaller particle size, and positively with larger particle size and dry matter content of cassava flour. This suggests that high fibrous cassava flour would be characteristically coarse, while less fibrous flour would be finer. In addition, higher dry matter contents are likely to be associated with high levels of fibre and larger flour particle size. The negative interaction between fibre and ash content is indicative of the loss of mineral content in high fibrous cassava roots during dewatering (pressing). There could be a high level of nutrients release (loss) from highly permeable fibre during processing [51]. Furthermore, the negative correlation between fibre content and moisture content confirms that the high fibre cassava have lower moisture content. Edible fibres are mainly composed of polysaccharides such as cellulose, hemicellulose and pectin. The matrix combining cross-linking hemicelluloses and cellulose microfibrils with an inter-penetrating pectin network gives strength and rigidity to the cell wall. In cellulose, a system of micro fibrils composed of close packing of

unbranched β-1,4-glucan chains through intra- and inter-molecular hydrogen bonds, makes this polymer impermeable and water-insoluble [51]. Fibre is quick to take up water like a wick, however this water is loosely bound in the fibre structure, and can be easily lost during drying, resulting in decreased moisture contents. When fibre is present along with starch, it competes for the limited amount of water available in food system. The partial solubilisation of fibre present in mixtures can affect the initial viscosity. Pectin functions as a plasticiser and controls porosity [52], and depending on porosity, there could be differential moisture responses among the varietal genetics. In addition the fibre content in cassava flours have been observed to increase while protein and lipids decreased with increases in the age of the plant [52].

The total carbohydrate contents on dry matter basis associates negatively with other proximate contents (protein, lipid, fibre, ash and moisture). Carbohydrates bind proteins through hydrogen bonding via hydroxyl group on saccharides and amine group on proteins, which may result in highly carbonyl-substituted carbohydrates, and subsequently loss of protein activity and availability. Carbohydrates also interact with lipids to form glycolipids through glycosidic bonds, and this reduces the levels of free lipids. Carbohydrates binds water molecules through hydrogen bonding [53], hence limiting water mobility, which explains the inverse relationship between moisture and carbohydrates.

4.7 Amylose content

The extracted starch is a biopolymer of two major polysaccharides, namely amylose and amylopectin. The amylose is the principal molecule for classifying starches into waxy, semi-waxy, normal/regular and high amylose types when amylose content is 0–2, 3–15, 16–35, and > 35% of the total starch, respectively [54]. Waxy cassava varieties containing zero amylose content by weight was reported [55]. The common cassava varieties are mostly normal/regular starches. High amylose starches are reported in maize varieties [56], which implies that these corn varieties contained high content of amylose by weight, while wheat and potato are commonly regular starches. Amylose content can be suggested as a basis for selecting flours/starches from different botanical sources for blending application. Starches with similar amylose contents can exhibit similar functionalities. Amylose content is the basis of ascertaining *in vitro* enzyme susceptibility of cassava starch to α-amylases. Amylose content was reported to relate negatively with cassava starch digestibility [57]. This relationship suggests that amylose resist α-amylases digestibility. The resistance of a starch material to digestion is related with the extent of starch availability to enzymatic hydrolysis in the human digestive system [58]. The resistant starch (RS) and inclusion in human diets have elicited interest because it restricts calorie load for individuals such as diabetic patients [59]. RS is a dietary fibre that does not get digested in the small intestine and has the potential for human health benefits [59]. The RS concept could be utilised as the basis of describing nutrition quality and potentially as a criterion parameter for classification of cassava varieties in slowly and fast digestible starches.

4.8 Colour

The colour of cassava flour and extracted starch is commonly described using whiteness index and chroma. The whiteness of flours can be analysed using a HunterLab ColorFlex instrument (Hunter Associate Laboratories Inc., Reston, CA, USA). The colour parameters of flours regarding 'L' (degree of lightness), 'a' (redness to greenness) and 'b' (yellowness to blueness) are measured after being

standardised using Hunter Lab Colour Standards of Hunter L^*, a^*, and b^*. The whiteness index can be calculated using the equation:

$$Whiteness\ index = 100 - \left[(100 - L)^2 + a^2 + b^2\right]^{\frac{1}{2}} \quad (7)$$

$$Chroma = \left(a^2 + b^2\right]^{\frac{1}{2}} \quad (8)$$

where: L* = lightness, a* = redness to greenness, b* = yellowness to blueness.

The whiteness index values of the cassava flours were reported, 90–92 [22] and 80–90 [60]. The whiteness of flour is influenced by drying temperature and time. Higher temperatures and longer times can impact scorching effect on flours, and may show increased a* (redness) and b* (yellowish) values, which contributes to decreased whiteness. The whiteness of flour exhibits positive association with L*, and negative with a* and b*. The high whiteness index values are indicative of low a* and b* values, and high L* values. Elevated oven drying temperatures can cause scorching and discolorations leading to reduced lightness. Moreover, high temperatures combined with high moisture content of flours, can gelatinize the flours leading to loss of birefringence properties which can affect the pasting quality of cassava flours. The redness-greenness (a^*) and yellowness (b*) are considered impurities in white flours. The source of impurities redness/greenness in cassava flours are possibly due to the residue of cassava peels. The yellowness (b^*) of the cassava flours can be due to inadequate dewatering of grated cassava. The a* and b* are reported to associate positively with ash content in wheat flours [61], which implies that high ash contents can influence the whiteness of flours and subsequent products such as bread. Additionally, high mineral content can accelerate metal chelating activities to form metal ion-pigment complexes, which can confer greenness/redness or yellowness (b*) colour on the final flour product. The negative relationship of whiteness index with ash content along with a* and b* as shown in Chisenga et al. [22] is consistent with the above theories because starchy vascular ground tissue of white fleshed cassava do not contain pigments, whereas the formation metal ion-pigment complexes are prominent in the cassava peels. The dewatering stage is the critical quality control point. The water in the fresh cassava is the medium of reactive oxygen species (oxidants) [62], and can taint the flours yellowish during drying. In addition, the yellowness may be due to residual procarotenoids compounds or minor Maillard and/or caramelization reaction products formed on drying. During processing toward flours, it requires that the available water is expressed out from the grated cassava followed by granulating of pulp before drying. Granulation with use of pulverizer or hands is critical to crumble the mass into smaller particles for an increased surface area during drying.

5. Influence of chemical composition on physicochemical properties

The presence of non-starch compounds (lipids and proteins) are reported to have negative influence on swelling power of starches. The protein compounds can restrict swelling of starch granules in plasticizing water [63] due to increased hydrophobicity which limits uptake of water [64]. However, lipids and proteins are minor components of cassava starch, and may not limit swelling of starches. This could explain the highest swelling power and solubility values for cassava starches when compared to those reported for corn, wheat and potato starches [65, 66]. It is worth noting that swelling power and solubility of starches are in function of amylose contents. The negative correlation between swelling power and amylose

content was reported [57]. In addition, Sanchez et al. [67] reported that waxy cassava starches (containing less amylose) showed highest swelling powers. Swelling of starches in water results in release of soluble matters including amylose. However, presence of lipid can influence formation of lipid-amylose complex, and can restrict exudation of amylose [66] which could yield lower swelling power. The presence of amylose-lipid complex lowers gelatinization of starches. Lipids may impact diffusion of water into the starch granules, and their presence on starch granules was reported to reduce gelatinization. Li et al. [68] showed that defatted starch yielded lower gelatinization temperatures. The protein and starch granules compete for water molecules [69] which probably leads to inhibited swelling resulting in delayed gelatinization, and hence higher gelatinization temperatures.

Amylose contents influences pasting behaviour of starches in a food system. The lower amylose cassava starches (waxy) exhibited a narrow range of viscosities [70]. This implies that waxy starches are likely to limit exudation of amylose which could probably lead to decreased solubility, and hence reduced viscosity. The higher contents of proteins and lipids, and subsequent formation of lipid-amylose complex could be the reason for higher pasting temperatures in cereal and potato starches. At higher gelatinization temperatures, cassava starches form a clear paste with high starch paste viscosity. The chemical components such as amylose may affect light transmittance of starch gels. Paste clarity tend to relate negatively with amylose content. The waxy cassava starches are associated with high paste clarity and high swelling power [70].

6. Conclusion

Dry matter content, starch yields, particle size, and composition (protein, lipid, ash, fibre, moisture) including cyanide contents are some of the primary quality traits for selecting cassava raw materials. Flour particle size distribution can influence uniformity and efficiency of processing systems. Grating and dewatering are two operation steps considered as critical quality control points in the primary processing of transforming cassava roots into safe shelf-stable product. Reduction of cyanide contents in fresh cassava roots can be achieved through grating and dewatering. To achieve good shelf stability of raw materials, moisture levels <12% are recommended during storage. The high whiteness index and low ash content are some of the primary desirable quality traits for application of cassava flours in the food and non-food industry. Amylose content is basis of classifying flours into regular, waxy and high-amylose, and can be used as primary criteria for blending flours of same or different botanical sources. Amylose content influences starch digestibility and resistant starch content. The concept of resistant starch can be criterion parameter for describing nutrition quality and classification of cassava varieties in slowly and fast digestible starches.

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Cassava is a staple crop in a large number of countries due to its adaptability to a variety of climatic conditions. It has spread extensively throughout Latin America, tropical Asia, and Sub-Saharan Africa. Cassava, which is well known for its high carbohydrate content, is the third most carbohydrate-rich food after rice and maize. This book discusses the diversity of cassava and its microbiome, cassava cultivation and postharvest practices, as well as crop yield-reducing diseases. Due to its widespread use and market importance, cassava has been subjected to biological and technological intervention to ensure food safety. This book will help readers to gain knowledge about cassava, its biological properties, and some of the strategies and procedures necessary to increase cassava crop output.

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