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African Cassava: Biotechnology and Molecular Breeding to the Rescue

Michael A. Gbadegesin[1*], Charles O. Olaiya[1] and John R. Beeching[2]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan 200005, Nigeria.
[2]Department of Biology & Biochemistry, University of Bath, Bath BA2 7AY, United Kingdom.

Authors' contributions

This work was carried out in collaboration between all the authors. Author MAG suggested the topic and wrote the outline of the review, sourced necessary data and wrote the first draft of the manuscript. Authors COO and JRB contributed to the comprehensiveness of the manuscript through input of literature information on the subject. All authors read and approved the final manuscript.

Review Article

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SUMMARY

Cassava is an important African food crop, where it is a staple to about 250 Million people. It is a household name in Nigeria, the world largest producer of the root crop. It is propagated from stem cuttings and well known for its adaptation to wide range of adapho-climatic conditions and including those unfavourable for other crops. However cassava production, exploitation, utilization and acceptance are limited by diseases and pests, cyanogenesis, low protein content and quality, and post-harvest physiological deterioration. The breeding research activities of IITA (International Institute of Tropical Agriculture) Ibadan, Nigeria, CIAT (International Centre of Tropical Agriculture) located in Cali, Colombia and National Root Crop Research Institute (NRCRI), Umudike, Nigeria have transformed cassava to double as a food security crop as well as a cash and industrial crop. Of recent, Bio Cassava Plus, an initiative sponsored by Bill and Melinda Gates, has been using experimental biotechnology approaches to address several of the main constraints to African cassava. This review presents the many advantages of cassava to the small-scale farmer and its potentials for industrial applications. It also describes the roles of biotic and abiotic factors hampering the production yield, root quality, nutritional adequacy, marketability and acceptance, and commercial processes.

**Corresponding author: Email: magbadegesin@yahoo.com;*

The use of conventional breeding and biotechnology in unravelling the milieu of these constraints is discussed as well.

Keywords: Cassava; biotechnology; breeding; protein energy malnutrition; cyanide poisoning; post-harvest physiological deterioration; cassava mosaic virus.

1. CASSAVA

Cassava (*Manihot esculenta* Crantz) is a perennial shrub belonging to the Euphorbiaciae, a group including other agronomically important plants such as *Ricinus communis* (castor bean) and *Hevea braziliensis* (rubber). One characteristic of euphorbiaceae is the presence of lactifers and the production of latex. In the genus *Manihot* only *M. glaziovii* is used as a minor source of rubber while *M. esculenta* is the widest cultivated member of the 98 described species [1].

Cassava is a diploid (2n=36) angiosperm. The plant has a woody stem and grows to between 1 and 5 metres depending on the cultivar type with each plant producing 5-10 tuberous roots (Fig. 1). Cassava storage roots are not true tubers but developed by secondary root thickening storing starch within the proliferated xylem parenchyma. It is cultivated primarily for its starchy roots though in some areas the lobed palmate leaves are also eaten as a vegetable. In Africa, cassava is mainly cultivated by small-scale farmers who observe, select and name their cassava varieties based on morphology, food, social and economic interest. In general, there is a wide range of African cassava cultivars distinguished on the basis of a range of morphological characteristics including stem and leaf colour, branching pattern, leaf shape and lobing and root form and colour. Peter Illuebey of Yam-barn Unit, International Institute of Tropical Agriculture (IITA), Ibadan estimated that the institute has more than 1000 landraces of cassava in its collections (personal communication).

Cassava originated in South America possibly Brazil/Paraguay or Mexico/ Guatemala, and along the Southern border of the Amazon basin [2-3]. It was introduced to Africa in the 16th century and is now cultivated in tropical and subtropical Africa regions with mean rainfall ranging from 500 mm – 8,000 mm [4]. It is well known for its adaptation to wide range of adapho-climatic conditions and including those unfavourable for other crops making it easily adopted in non-intensive farming practices.

Fig. 1. (A) Cassava tuberous root produced by secondary root thickening. Parts of the woody stem to which the starchy root tubers are still attached are shown. (B) Potted cassava plants growing in green house

2. CASSAVA: AN IMPORTANT FOOD CROP WITH HUGE POTENTIAL INDUSTRIAL APPLICATIONS

In Africa, cassava is traditionally grown primarily as a staple food crop particularly by small-scale subsistence farmers in marginal areas. It is a crop with many advantages to the small-scale farmer and has potentials for industrial applications. Some of the advantages include; its high efficient carbohydrate production, tolerance to even prolonged drought and other adverse environmental conditions following an initial establishment period after planting. In addition, it thrives well and produces acceptable yields with poor soil fertility where other crops such as maize, sorghum, beans and soybeans die soon after germination. Furthermore, it is a famine reserve crop as there is no fixed period of maturity and tubers can be left on in the ground and harvested from 6 months to 3 years after planting. Moreover, it is propagated via the lignified stem cuttings so that none of the harvest need be set aside as subsequent planting material as with yam for example.

Cassava is the staple food crop for about 750 million people worldwide [5]. It is ranked 4th as a human calorie source and 6th in term of its production after wheat, maize, rice, potato and sugar beets. In tropical Africa, cassava has been the single most important source of calories in the diet, with the largest production in Nigeria, Democratic Republic of Congo and Uganda. Cassava is processed into different food types in Nigeria including gari, fufu, cassava chips, cassava flour puree (lafun), farina, tapioca, cassava bread and pudding.

Secondly, animal feeds processors are finding cassava as an effective low cost alternative to maize. Cassava products constitute important components for livestock feeds and have been used in compounding feeds for pigs and chickens (broilers, pullets and layers) in Nigeria.

Thirdly, cassava starch, cassava flour, cassava juice and fermented cassava have found applications especially in food industries. For instance, cassava flour is used in making products such as biscuits, bread and derivatives such as sagos and sauce [6]. Cassava starch has also been industrially modified to yield products with physical and chemical properties for specific applications such as preparation of jelly, as thickening agents, in gravies, custard powders, baby food, spaghetti, macaroni and beer [6]. However, cassava starch accounts for only a small percentage of internationally traded starch [7-8].

Fourthly, the rising energy cost, especially of liquid fuel has resulted in shift of attention to bio fuels such as bio-ethanol. Cassava, a renewable sugar containing biomass, is the preferred feedstock for ethanol production especially in situations where water availability is limited for the cultivation of sugar cane. Fig. 2 depicts the flowchart of bio-ethanol production from cassava starch [9]. Moreover, the possibility of using the cassava root peels as substrate for yeast monoculture and co-culture in the production of bio-ethanol provides a huge economic and environmental advantage. Fig. 3 is a schematic diagram of bio-ethanol production by fermentation process of sugar, starch and lignocelluloses feedstock [10].

Furthermore, the capacity to modify cassava large root organ into a novel sink for nitrogen with a huge potential to produce and accumulate a range of proteins with nutritional, industrial or pharmaceutical value has been experimentally demonstrated [11]. The uses and potential applications of this long neglected crop are therefore huge. The importance of cassava and the enormous potential for improvement also makes it a target crop for research.

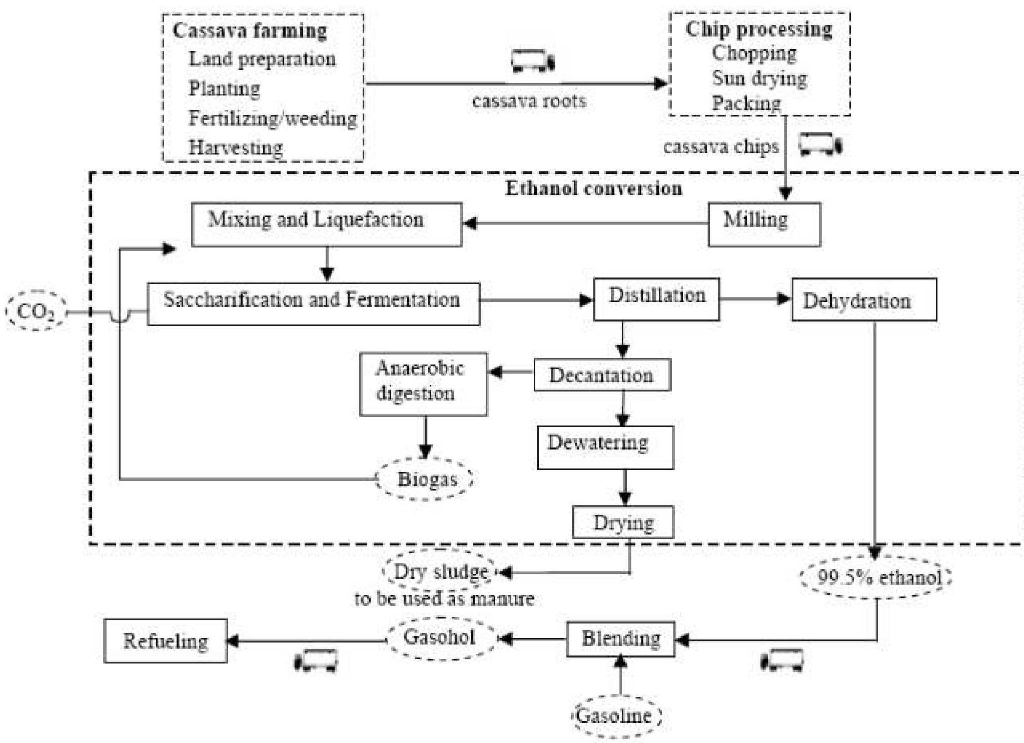

Fig. 2. Flowchart of cassava ethanol production [9]

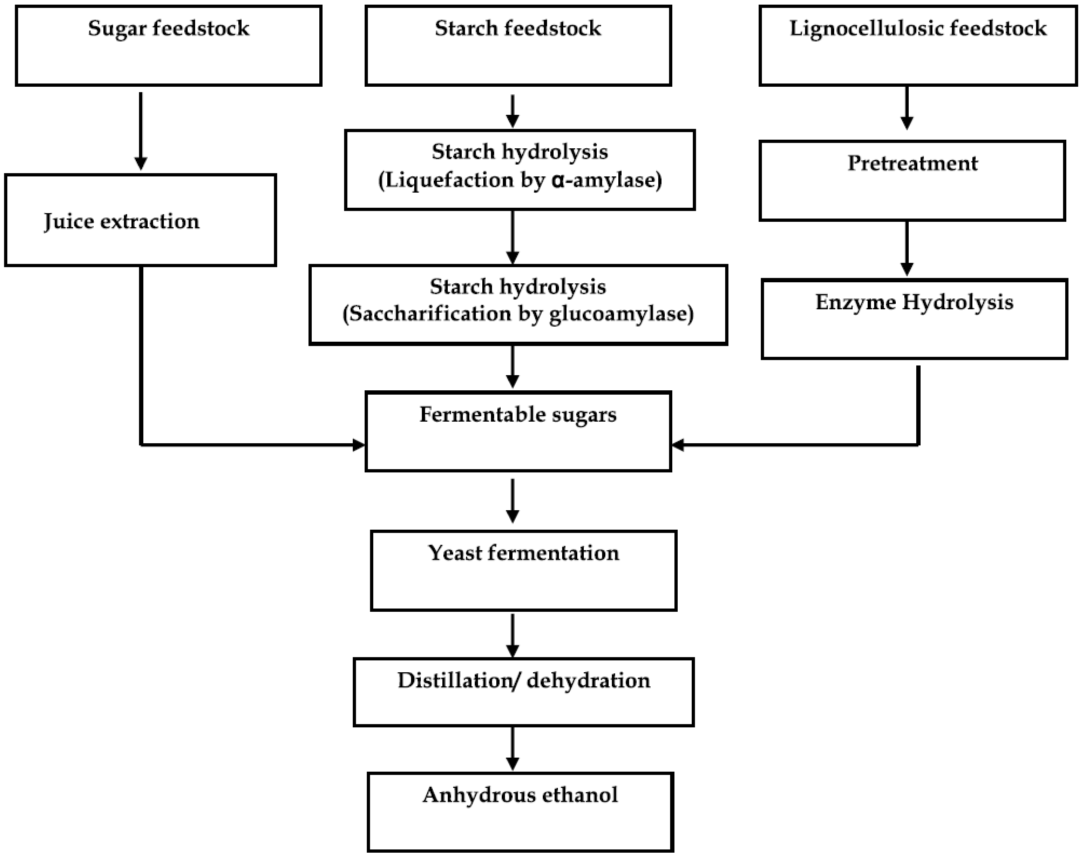

Fig. 3. Schematic diagram of bio-ethanol production by fermentation process of sugar, starch and lignocelluloses feedstock [10]

3. PROBLEMS AND LIMITATIONS TO CASSAVA PRODUCTION AND UTILISATION IN AFRICA

Despite the many advantageous traits of cassava, production yield in Africa is generally far below those obtained under optimal conditions [12-14]. Most of the observed increased production in African countries actually stems from increased cultivated land areas (Figs. 4a, b and c). In addition, the root quality, nutritional adequacy, marketability and acceptance, and commercial processes are hampered by both biotic and abiotic factors. These include propagation on infertile soils, planting of unimproved traditional varieties and general inadequate farming practices.

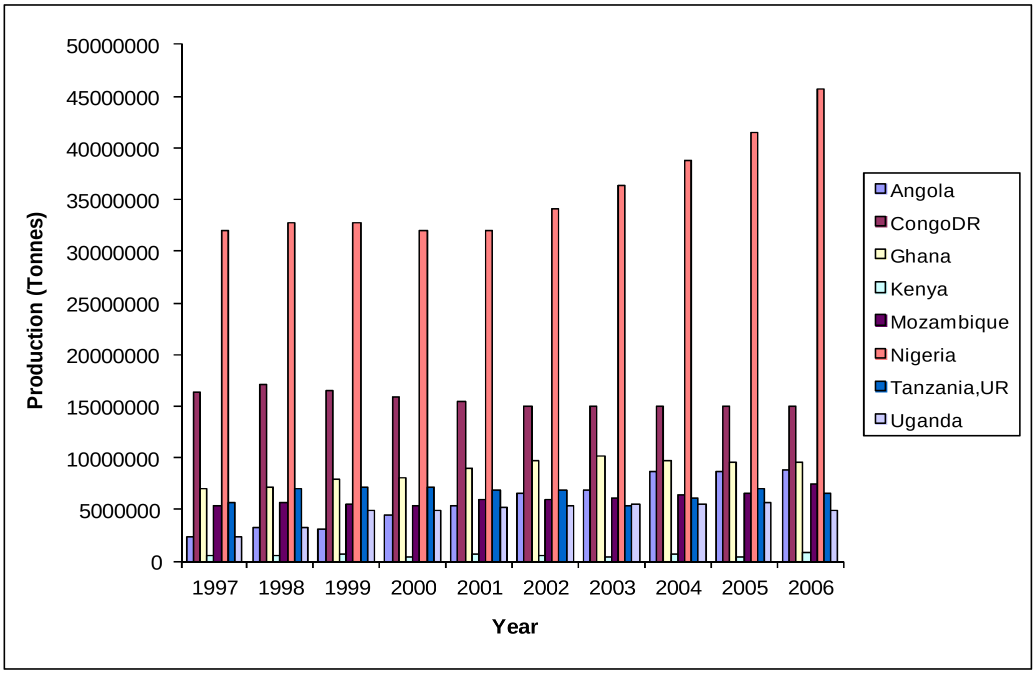

Fig. 4a. African cassava production from 1997 to 2006 [15]

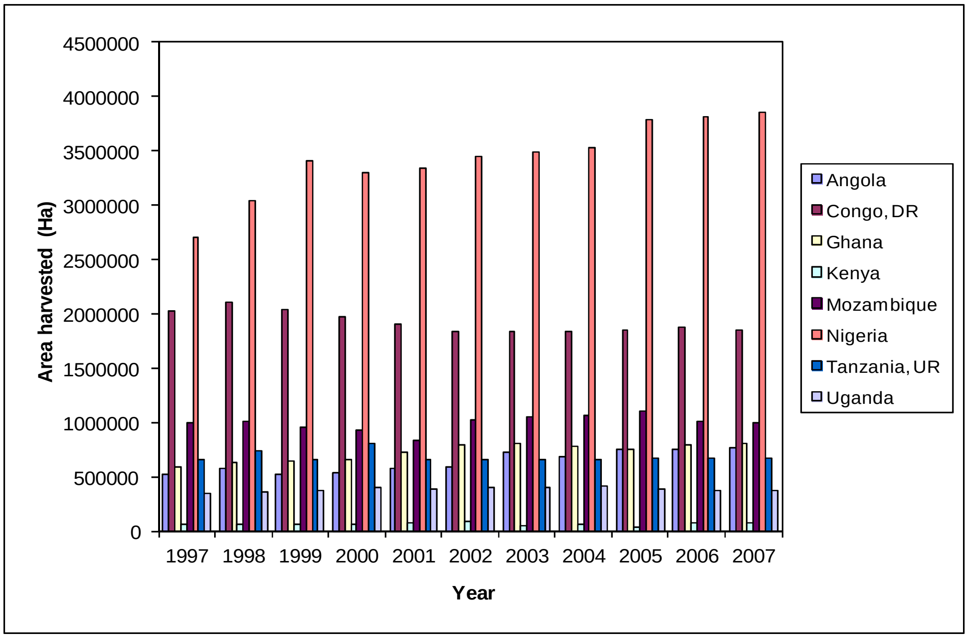

Fig. 4b. African annual land cultivated for cassava from 1997 to 2007 [15]

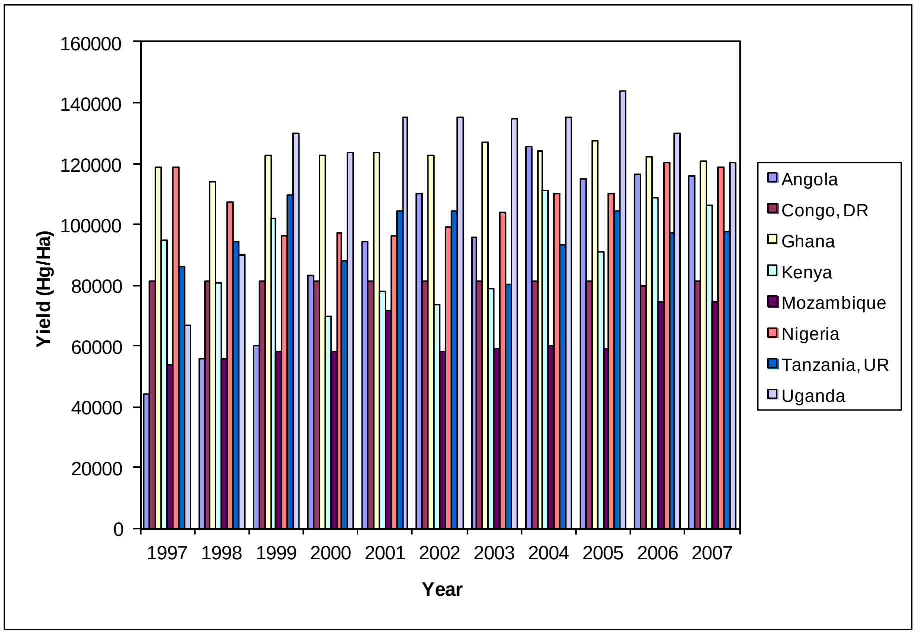

Fig. 4c. Yearly cassava production yield in African countries from 1997 to 2007 [15]

Other limitations are diseases and pests, cyanogenesis, low protein quantity and quality, and post-harvest physiological deterioration. The major diseases of cassava are African cassava mosaic disease (ACMD) cause by the African cassava mosaic virus (ACMV), Cassava brown streak disease (CBSD), cause by Cassava brown streak Uganda virus (CBSUV) and Cassava brown streak virus (CBSV) and the cassava bacterial blight (CBB) cause by *Xanthomonas axonopodis pv* [16-18]. The spread of these diseases is worsened by cassava vegetative propagation and the use of diseased cuttings [19]. The use of sanitation techniques and meristem tip culture had been found to alleviate the spread of these diseases [19-20]. Another pest of cassava is *Mononychellus tanajoa*, cassava green mite (CGM), which originated in South America, the genetic ancestral home of cassava. It causes up to 80 % yield loss of cassava in tropical Africa [21]. It has been effectively controlled biologically using *Typhlodromalus aripo*, a natural enemy of CGM from Brazil that has been introduced into Africa. On the other hand, both the leaves and tubers of cassava are cyanogenic, producing HCN by reaction sequence (Fig. 5), triggered by tissue damage.

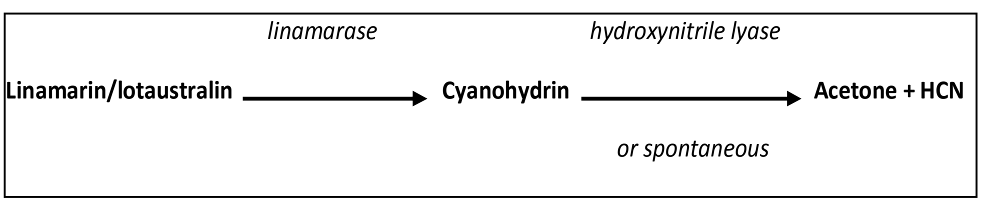

Fig. 5. Reaction sequence producing hydrogen cyanide from cyanogenic glycosides present in cassava leaves and tubers. Enzymes or mode of the reactions are shown in italics

Unprocessed cassava roots may contain 15–1,500 mg/kg cyanide equivalents [4], which must be removed by processing such as peeling, soaking, boiling and draining prior to use. Short-cut processing techniques, especially during time of crisis such as civil war, would lead to production and ingestion of toxic cassava food products. Long-term exposure of humans can trigger neurological disorders such as tropical neuropathy or goitre, and, in acute cases, the disease Konzo and/or death [22-23].

The low protein content and quality of cassava storage root due to low level of the amino acids methionine, lysine, tryptophan, phenylalanine, tyrosine and cysteine demand that cassava based food should be supplemented with other foods to supply an adequate protein diet [24]. Populations that rely on cassava as their major source of calories are at high risk of protein energy malnutrition [25], Kwashiorkor [26] and related pathological disorders [27]. It has been hypothesized that selecting for higher protein content in breeding may eventually lead to a lower yield because protein synthesis requires about twice the primary products of photosynthesis as the synthesis of a similar weight of starch [28]. However, a proof of concept research data on transgenic cassava, demonstrated the development of molecular strategies to meet minimum daily allowances for protein and iron in cassava based diets with strategies employed to increase root protein levels resulting in reduced cyanogen levels in the roots [11]. Cassava root also contain very little amount of essential micronutrients including beta-carotene, Fe and Zn so that individuals consuming cassava as a staple food are at risk for inadequate zinc, iron, and vitamin A intake [29].

Moreover, cassava storage roots must be processed soon after harvest because they do not store well but suffer from post-harvest physiological deterioration. This is symptomatically seen as an undesired discolouration [30]. It is due to the oxidation of phenolic compounds, in particular scopoletin (a hydroxycoumarin involved in plant defense [31], by reactive oxygen species [32-34]. PPD can occur within 48 h after harvest depending on the cultivar and climate, and renders the root unpalatable and unmarketable [8]. This therefore limits cassava exploitation and is a constraint to the producers, processors and consumers [8].

4. REMOVING THE BOTTLENECKS TO CASSAVA UTILISATION

Various approaches are being implemented to tackle the constraints to cassava roots utilisation, notably breeding and biotechnology. In fact, removing the constraints so as to boost food production in the tropics were priority research areas identified by the FAO (7). Two centres within the Consultative Groups on International Agricultural Research (CGIAR) concerned with cassava genetic improvement were set up in 1968. These centres, CIAT (Centro Internacional de Agricultura Tropical) located in Cali, Colombia and IITA (International Institute of Tropical Agriculture) located in Ibadan, Nigeria, have been instrumental in the breeding and introduction of high yielding cassava varieties in Africa. Consequently, Nigeria has become the World largest producer of cassava since 1990, with an estimated output of 31.4 million tonnes in 1995 and 38.2 million tonnes in 2005 [35]. In general, the research efforts of the international centres and national agricultural research systems (NARS) over the last few decades have seen improved knowledge of cassava as a crop, enhanced productivity and modernisation of cultural practises [36-38]. In particular, the conventional breeding research activities of IITA, CIAT and National Root Crop Research Institute (NRCRI), Umudike, Nigeria have transformed cassava in Nigeria from a mere food security crop to a cash crop.

Traditional breeding has resulted in the introgression of important traits into the cassava germplasm with major improvements recorded for bacterial blight resistance, virus resistance [39-40], protein content [41] and starch quality [42]. However, traditional breeding techniques face several limitations, notably the heterozygous nature of the crop renders it difficult to identify the true breeding value of parental lines, and also there is only limited knowledge of inheritance traits that have agronomic importance [36,38]. Thus, production of improved plant lines by conventional breeding can take about 10 years from the first parental crossing to distribution of the improved plants [43]. Moreover, introgression of the selected trait(s) into locally adapted and farmer-preferred cultivars without affecting their favoured characteristics remains difficult. However, advances in molecular mapping [40,44], sequencing of cDNA clones and expressed sequence tags [45-47], marker-assisted breeding [43] and in particular the recent elucidation of the cassava genome sequence offer exciting new tools for both conventional breeding and biotechnology research.

The Cassava Biotechnology Network (CBN) was first set up in 1988 to facilitate communication and collaboration among cassava researchers. The first set of objectives of the CBN were to enlist advanced laboratories for cassava biotechnology research around a common strategic agenda, in order to use existing research investment and facilities cost–effectively and to stimulate relevant research in cassava growing countries in an attempt to remove or reduce the constraints to cassava production [48]. Recently, Bio Cassava Plus (BC+), an initiative sponsored by Bill and Melinda Gates, is using experimental biotechnology approaches to address several of the main constraints to African cassava production and utilisation.

Transgenic approaches have been explored to produce disease, especially ACMD, resistant cassava [49-50] as well as increased cassava root protein and essential micronutrients content [11,51-52]. Also, increased expression of endogenous hydroxynitrile lyase gene has been shown to eliminate the precursor compound from harvested roots as volatile HCN [53]. Furthermore, Sirituinga and Sayre [54] have reported the generation of transgenic cassava plants with up to a 94% and 99% reduction respectively in leaf and root linamarin content compared with the wild plant by inhibition of CYP79D1 and CYP79D2 cytochrome P450 expression. This was achieved by using a leaf-specific promoter to drive the antisense expression of the CYP79D1/ CYP79D2 genes in the transgenic cassava plant. Beeching's research group in Bath is using transgenic strategy to extend cassava shelf life by enhancing the anti-oxidant status of the storage root through over-expressing in the root five genes (superoxide dismutase, catalase, ascorbate peroxidase, D-galacturonic acid reductase and γ-glutamylcysteine synthase) that either code directly for anti-oxidant enzymes or for enzymes involved in the biosynthesis of anti-oxidant compounds (JR Beeching, personal communication).

Production of transgenic 60444 served well the proof-of-concept phase of BC+, generating sufficient plants to screen transgene expression cassettes and test hypotheses for all the traits addressed in the project. However, to deliver products to farmers, the transgenically imparted nutritional traits must be integrated into genetic backgrounds of cassava favoured by farmers in the target regions of Nigeria and Kenya. As for all the major crop species, morphogenic potential and transformability of cassava varies significantly between varieties. In the case of cassava, however, the option of integrating transgenes into amenable backgrounds followed by backcrossing with favoured breeding stock to produce agronomically suitable cultivars is problematic. Therefore, the capacity to genetically transform a given cultivar for delivery to end-users needed to be developed empirically in each case. Special efforts have also been made to develop a cassava transformation protocol that can be adapted by African laboratories thereby easing the transfer of the biotechnology to African laboratories [55-56].

The above transgenic research on cassava makes use of either constitutive promoters of viral origin or a potato-derived promoter (patatin). The former are not the ideal candidates to drive most of the constructs designed to modulate the improvement of the traits listed above due to their constitutive nature and to concerns of their public acceptability. On the other hand patatin, whilst it shows general root-specificity, may not be used to target expression to specific tissue types within the root, neither is it advisable to attempt to stack multiple traits driven by the same promoter as this can lead to interference and even suppression of expression. Therefore, there is a critical need for a tool-kit of plant, ideally cassava, root-specific promoters with a range developmental and tissue specificities within the cassava storage root. Without access to such a tool-kit the transition from research to the release of cassava varieties improved for multiple traits will be delayed. Significant progress has been made in the isolation and characterisation of cassava root-specific promoters that could form the basis for such a tool-kit [57].

5. CONCLUSION

Cassava is unrivalled as the main dietary calorie for the majority of populace in sub-Saharan Africa. It has become an important cash/commercial crop providing raw materials for industries. Cassava starch, either as native or modified form, are finding uses for a broad range of food and non-food applications including paper, textile, pharmaceutical, building materials and adhesives. In addition, cassava starch is extensively been utilized for the production of sweeteners and derivatives including glucose syrup, fructose syrup, sugar alcohols (e.g. sorbitol, mannitol), and organic acids such as lactic acid and citric acid [10]. Moreover, the application of cassava as renewable feedstock has now expanded to biorefinery, i.e. a facility that integrates processes and equipment to produce fuels, power, chemicals and materials from biomass [58]. Therefore, although the destination to making cassava roots an ideal food is yet a long way ahead, the ‘beast’ is an industrially very viable and important source of materials for processing into higher value products.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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