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Review

Excellence in Cassava Breeding: Perspectives for the Future

Hernan Ceballos [1,*], Chareinsuk Rojanaridpiched [2], Chalermpol Phumichai [2], Luis A. Becerra [1], Piya Kittipadakul [2], Carlos Iglesias [3], Vernon E. Gracen [4]

[1] Centro Internacional de Agricultura Tropical (CIAT), Recta Cali-Palmira km 17, Cai 763537, Colombia

[2] Department of Agronomy, Kasetsart University, Bangkok 10900, Thailand

[3] Department of Horticultural Science North Carolina State Univeristy, Raleigh, NC 27607, USA

[4] International Agriculture and Rural Development, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY 14853, USA

* Correspondence: Hernan Ceballos, Email: h.ceballos@cgiar.org.

ABSTRACT

Cassava (*Manihot esculenta* Crantz) is a major tropical crop. Regarded as an orphan crop a few decades ago, it now receives considerable attention from governments, industry and agencies investing in agricultural research. As a result, the cassava community generates vast amounts of information and develops useful technologies and products. This positions cassava as a key industrial commodity and a reliable food security staple. Significant genetic gains have been achieved through the early 2000s. Gains are particularly noticeable under better agronomic conditions, as was the case for the green revolution of cereals. However, further gains obtained in the past two decades have not been as impressive. Cassava breeding cycle is long, and its multiplication rate slow. Therefore, it takes no less than 8 years to develop a new variety. Cassava breeding is based on the use of heterozygous progenitors which has important drawbacks. One of them is the impossibility to implement conventional back-crossing. Cassava researchers have recently introgressed a single recessive trait (amylose-free starch) into elite varieties. This was unprecedented in cassava and revealed important problems incorporating single genes into elite varieties. In the absence of backcross, the process required essentially the development of new varieties, which exposed strong effects of undesirable genetic linkages. Breeding approaches to overcome the problems of introgressing single-gene traits have been developed and will be implemented to introduce resistance to cassava mosaic disease into SE Asia breeding populations. These methods will also be useful to exploit recently identified immunity to cassava brown streak disease, a serious problem currently restricted to Africa. Trait introgression is an important process in crop breeding and will be more important in the future as gene discovery and editing identify useful genes. This article consolidates and integrates information from cassava and other crops and proposes

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guidelines to maximize the returns on investments on this important commodity.

KEYWORDS: back-cross; genetic load; genetic linkages; inbreeding; reverse genetics; trait introgression

INTRODUCTION

Cassava (*Manihot escultenta* Crantz) is an economically important crop. It is among the most important sources of energy in the diet of most tropical countries. Historically, cassava was grown for human consumption (gari, fufu, sago, table consumption, etc.). In addition, a strong demand from worldwide markets such as starch, animal feed, and bioethanol, have emerged over the years. For example, it is now the second most important source of starch worldwide [1]. Cassava is a key food security crop for millions of people (particularly in Sub-Saharan Africa) and a key commodity for agro-industrial processes. Each of these markets have specific requirements with clearly identified breeding goals.

The first formal cassava breeding programs began around the 1930s in Eastern Africa and in Brazil. In the late 1960, two international centers that work on cassava breeding (IITA and CIAT) were created. At that time, several cassava research programs at the national level were also initiated in Africa, Asia and Latin America and the Caribbean [2,3]. Currently important breeding efforts are made in Brazil, Colombia, China, Ghana, India, Nigeria, Kenya, Mozambique, Tanzania, Thailand, Uganda and Vietnam, among tropical countries of the world. Financial resources to support research in cassava have increased considerably in recent years compared with a few decades ago [4]. Cassava breeding programs rely on conventional breeding approaches based on phenotypic recurrent selection. As new technologies have been developed (genetic transformation, marker assisted selection, gene editing, genomic selection, etc.), they have been adapted and applied to cassava. These new technologies have a much greater chance of success when used within an effective breeding program encompassing conventional evaluation stages, clear objectives, excellence in phenotyping, relevant site selection, proper handling of flowering and reliable crossing procedures.

The progress achieved in cassava research has been somehow uneven. There is a dynamic progress in biotechnology tools on one hand, but a significant vacuum of basic knowledge on the other. Protocols for the genetic transformation of cassava have been available since the 1990s [5]. The first molecular map of cassava was published 20 years ago [6] and its genome has been sequenced [7]. However, it is only recently [8] that Ramos Abril and co-workers reported that stigmas remain receptive for three days after anthesis, not just one day as previously reported [9]. Similarly, the first steps towards understanding the induction of flowering

in erect-plant genotypes, which are preferred by farmers, is very recent [10].

This article presents a thorough description of conventional breeding and other approaches used for the genetic enhancement of cassava. Relevant unpublished information on genetic gains and results from the introgression of a single recessive trait are also provided for a complete description of the state of the art in cassava breeding. New threats to cassava production such as cassava mosaic disease (CMD) in SE Asia will require a fast-track breeding methodology. The main objective of this article is to describe current bottlenecks and opportunities to maximize the efficiency of the genetic enhancement of cassava.

STATE OF THE ART IN CASSAVA BREEDING

Conventional Breeding

Many breeding programs have used the same conventional cassava breeding method, with only minor variations, for decades [3,11–13]. This involves the production of full- or half-sib seed in crossing blocks. Flowering in cassava results in branching of the stem which produces a plant type that is not preferred by farmers who usually prefer erect plant architecture, which facilitates husbandry, mechanization and extends the storability of planting material [14]. Adequate recombination and production of botanical seed is more difficult in erect varieties.

Cassava progenitors are heterozygous and crosses between them produce progeny that are genetically very diverse. Each F_1 seedling is genetically distinct and several years are required to produce enough stem cuttings for multi-location testing (the multiplication rate in cassava is 1:10). Figure 1 illustrates the traditional evaluation scheme used in most cassava breeding programs worldwide [11]. If botanical seeds can be produced within one year, the resulting seedlings can be grown in the F_1 seedling nursery in Year 2 (top of Figure 1). However, botanical seed from crosses of erect progenitors take more than a year to be obtained. The evaluation of these progenies (which are the most valuable) would be delayed for an additional year in the diagram presented in Figure 1. It will be assumed, however, that it takes only one year to produce the botanical seed and all progenies can be grown in seedling nurseries in Year 2.

Selection in the seedling nurseries is only for high-heritability traits. In Africa, drastic selection takes place to eliminate genotypes susceptible to CMD. Individual plants selected in the seedling nursery are cloned for single row trials (SRT) grown in Year 3. Each F_1 seedling plant produces a limited number of stem cuttings. Therefore, SRTs typically have 6–8 plants for each genotype. Although the number of genotypes evaluated in seedling nurseries varies widely, the number of genotypes tested in this first clonal evaluation usually ranges from 500 to 2500. The next stage in the selection process (preliminary yield trials—PYT) is planted in Year 4 and usually evaluates about 100–300 genotypes. Advanced yield trials

(AYT) in Year 5 have 40–80 genotypes and the final stage (uniform yield trials—UYT) in Year 6, has 10–30 genotypes. The number of plants per clone in the different stages of the evaluation process increases gradually.

The main purpose of growing F_1 seedling nurseries is to produce planting material for the SRT. A major problem in the breeding scheme is that extensive selection is done on SRTs, which are unreplicated and grown in a single location. The bottom of Figure 1 presents an innovation for the traditional evaluation system. The F_1 seedling plants are grown in the off-season and for only six months. At six months of age, selection for key high-heritability traits such as resistance to CMD, bacterial blight, thrips, late branching, pigmented root parenchyma (for high-carotenoids), amylose-free and/or small-granule starch, vigor, and potential harvest index can still be done [15]. There is no need to grow plants for a year to select for these traits. However, only three stem cuttings can be collected from these six-month old seedling plants. The three cuttings from each seedling are planted (at the normal planting time) and grown for a year. This is a new stage in the process called F1C1. The main difference between the traditional and the new system is that Year 2 begins with one plant per genotype in the original system and three plants in the modified scheme (Figure 1). To do this, breeders germinate the botanical seed six month earlier, when fieldwork is not intensive.

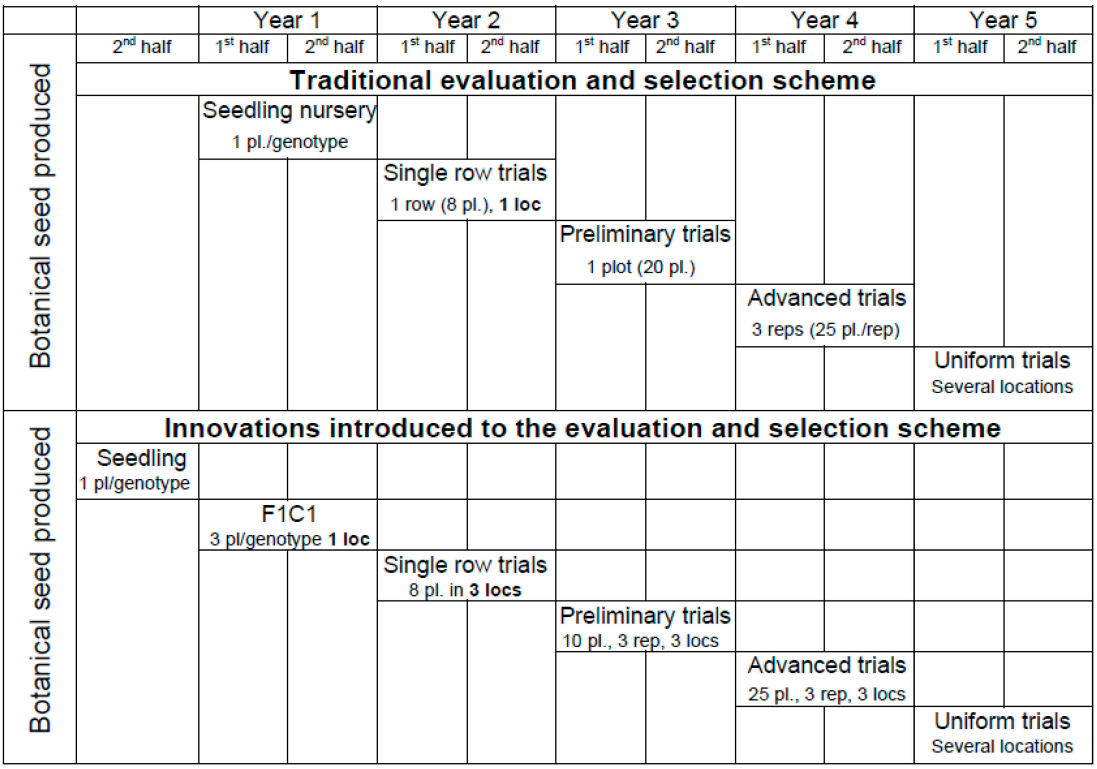

Figure 1. Illustration of the traditional and modified phenotypic recurrent selection schemes used in cassava breeding. The introduction of the F1C1 stage requires only six additional months in an off season, but allows growing SRTs in three locations rather than just one. Because multi-location trials begin at the SRT stage, advanced yield trials may be eliminated (pl: plant; loc: location; rep: replication).

The new system was originally developed to overcome logistical problems in breeding for high-carotenoids [16], but was quickly implemented for every breeding pipeline at CIAT because of its advantages. The growth of three plants per genotype at the F1C1 allows growing identical SRTs in three locations, overcoming a major weakness of the original system. Genotype-by-environment (G × E) interactions limit the reliability of SRTs. Preliminary results of this innovation indicate that only 25–35% of clones are simultaneously selected in two of the three locations, highlighting the influence of GxE in these non-replicated trials. A second important innovation is that PYTs are evaluated with three repetitions and 10-plant plots following a special field design [15]. These innovations were implemented to overcome problems in early stages of selection, which were based on single replications. Ceballos and co-workers described in 2004 [17] another innovation: selection of progenitors based on their breeding value. In spite of promising early results, this approach proved inefficient because of the relatively large within-family genetic variation in comparison with differences in breeding values [18].

Breeding clonally propagated crops such as root, tuber and bananas (RTB) requires consideration of the impact of the overall quality of the planting material. Epigenetic factors, nutritional and physiological status, pathogens and beneficial endophytes affect the general performance of the same genotype through the sequential stages of evaluation depicted in Figure 1 [19,20]. These features explain the poor correlations observed in the phenotypic evaluation of the same genotypes through these different stages of selection [21] and, to some extent, justify the lengthy evaluation process.

Genetic Gains in Cassava from the Conventional Breeding System

The conventional breeding approach described above has been successful in developing outstanding cassava varieties [22]. Thailand offers excellent conditions to assess genetic gains because the markets in SE Asia (dry root chips and starch) require simple yield parameters that are easy to quantify and monitor. The breeding goals in Thailand have not changed in the past 50 years. KU50, an outstanding variety released in 1992, is still widely grown throughout SE Asia [23]. Two major breeding programs are operational in Thailand: Kasetsart University (which has released KU50, HB60, HB80, and HB90) and Rayong Field Crops Research Center which released excellent varieties such as Rayong 5, 7, 9, 72, and 90.

A study, conducted by Kasetsart University involving 67 evaluation trials with replications from 2012 to 2019 in Thailand, compared varieties released through the years (Table 1). This unpublished study included an experimental clone (#21) that may be released soon. Eberhart and Russell stability analysis [24] was also done. These data, combined with the information presented in Figures 2 and 3, provide insight into genetic

gains achieved during the past 45 years. This is, to our knowledge, the first systematic attempt to study genetic gains in cassava.

The regression coefficients of the Eberhart-Russell analysis are listed in Table 1. Environmental conditions varied considerably as reflected by a wide variation in the environmental index in this analysis (Figure 3). Significant gains have been achieved in key traits such as fresh root and dry root yield per hectare and starch content in the root. The genetic progress and high adoption rate of improved cassava varieties in SE Asia demonstrate the power of sound plant breeding programs closely integrated with strong and clearly defined markets.

Table 1. Root yield and starch content of cassava varieties tested across 67 evaluation trials in Thailand during 2012–2019. Varieties are ordered from their time of release, with older varieties at the bottom [α].

Clone	Year of Release	Fresh Root Yield			Dry Root Yield			Root Starch Content (%)
		(t/ha)	% of R1	β [c]	(t/ha)	% of R1	β [γ]	
#21 [β]		34.5 [a]	156	1.24	12.7 [a]	197	1.38	24.5 [b]
HB90	2018	33.1 [ab]	150	1.09	12.3 [ab]	190	1.18	26.0 [a]
HB80	2008	31.4 [bcd]	142	0.97	11.8 [bc]	183	1.00	26.1 [a]
R9	2005	30.9 [cd]	140	1.14	11.9 [bc]	183	1.18	26.1 [a]
HB60	2003	29.6 [d]	134	0.98	10.4 [d]	161	0.87	23.7 [c]
KU50	1992	31.3 [bcd]	142	0.99	11.2 [c]	172	0.91	24.0 [bc]
R1	1975	22.1 [e]	100	0.65	6.5 [e]	100	0.57	17.9 [d]
Mean		30.6			11.1			24.1
F-test		**			**			**
CV (%)		17.75			20.17			8.24

[α] Values in a given column followed by the same letter are not statistically different; R1 and R9 stand for Rayong 1 and 9, KU50 is Kasetsart University 50. HB60, HB80 and HB90 are Huey Bong 60, 80 and 90, respectively; [β] #21 experimental clones from Kasetsart University not yet released; [γ] Regression coefficient in the Eberhardt and Russel stability analysis; ** Significant at the 0.01 probability level.

Figure 2 presents the information from Table 1 as linear regressions on years. Other regression lines could have been presented that offer slightly better R^2 values, but the straight line offers the advantage of simplicity and the R^2 values are acceptable. It is clear that major gains were achieved with the release of clones such as KU50 and R9 but further gains obtained after their release are not as impressive. Using data from 1975 (when the first official variety was released) as the base line, genetic gains per year can be estimated around 1.8%, 0.7% and 1.0%, respectively, for dry root yield, starch content in the roots and fresh root yield. These gains are quite acceptable for a crop whose breeding has often been considered complicated and inefficient and they align with earlier reports [12,25].

The results from this selected group of clones were consolidated in Figure 3 for dry root yield (combining fresh root yield and dry matter content—DMC). The old variety, Rayong 1, had a regression coefficient significantly lower than 1.0 suggesting that it was particularly adapted to

low-yielding environments. The data demonstrate that, after 45 years, Kasetsart University has developed varieties with higher capacity to take advantage of improved environmental conditions since the regression coefficients tend to increase with time of release of each variety. This follows the essential principles of the green revolution in cereals. All modern varieties out yielded R1 in low yielding environments and responded better to improved management. Although the study focuses on the clones released by Kasetsart University, the general performance of R9 suggests that varieties released by Rayong Field Crops Research Center would show a similar trend.

Optimizing the genotype by environment interaction is key to sustaining crop production gains [26]. Cassava is a resilient crop but, in spite of some misconceptions, it is very responsive to good agronomic practices [27]. The evolution of clones from different eras clearly supports this statement (Figure 3). However, further improvement over current elite varieties is becoming increasingly difficult. This is particularly the case for starch content in SE Asia where breaking the 26% threshold is proving to be very difficult. Gains in fresh root yield have been attained but usually at the expense of a slight reduction in starch content. Similar trends have been observed in Africa [28] and the Americas but data similar to that from Thailand is not available. Breeding efforts in cassava may be reaching a yield plateau like that seen for maize open pollinated varieties in the USA [29].

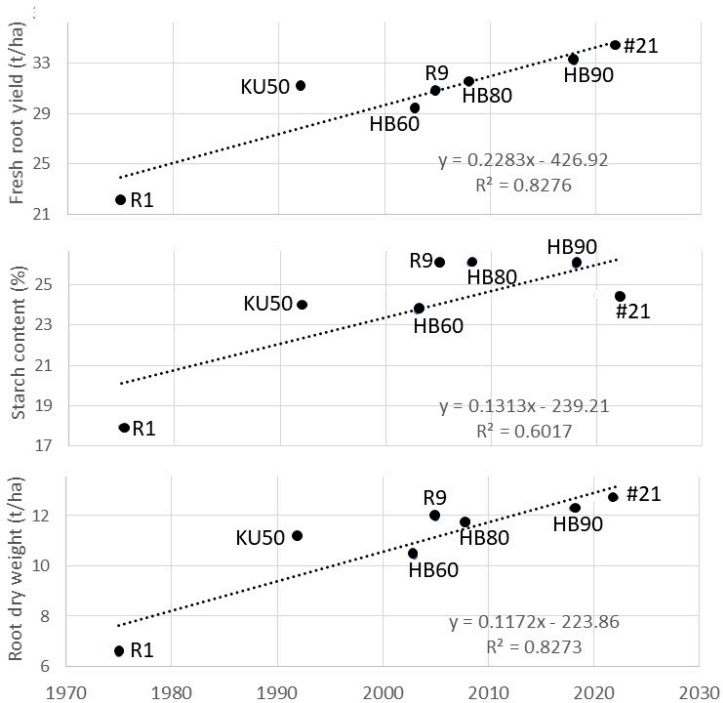

Figure 2. On average, genetic progress per year in Thailand has been 0.23 t/ha for fresh root yield, 0.13% for starch content and to 0.12 t/ha of root dry yield.

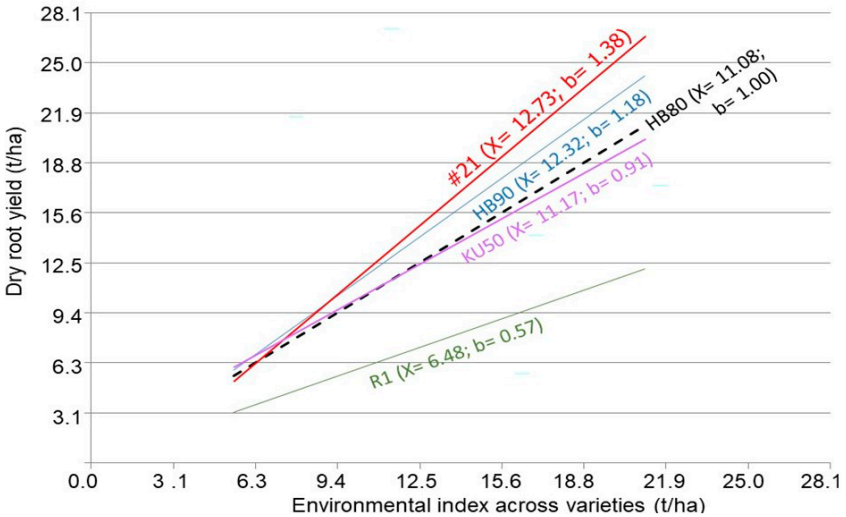

Figure 3. Eberhart and Russell stability analysis [24] of selected varieties released over decades by Kasetsart University in Thailand (#21 is not released yet). The performance of HB80 is very close to the average of the overall experiment.

The introduction of the F1C1 stage in breeding may help overcome some of the current problems but further increases in productivity are likely to require different breeding approaches including the potential use of fast track breeding methods, novel molecular technologies, development of inbred parents and complementary gene-pool breeding. As increases in productivity became harder to attain, breeders have used the same conventional recurrent selection method (or modified versions) to improve high-value traits with high heritability such as increased carotenoids content or special starch quality traits rather than increased yield and starch. High heritability in cassava is often linked to repeatability (e.g., selections made at the seedling stage are confirmed in later stages of selection). However, in the case of starch quality traits or carotenoids content, it is also linked to a simple inheritance that depends on one or few major genes [30].

Genetic Transformation and Gene Editing

Genetic transformation in cassava was initially restricted to a single model genotype but the array of germplasm that can be genetically transformed has been expanded. Many traits have been considered for genetic transformation: resistance to cassava brown streak disease (CBSD) and CMD [31–37]; enhanced nutritional quality of the roots including high carotenoids, Fe, Zn, proteins, and the reduction in cyanogenic glucosides [38–42]; quantity and quality of starch [43–45]; reduction of Postharvest Physiological Deterioration [46–48]; resistance to arthropods [49]; improving physiological traits such as leaf retention [50]; herbicide tolerance [51]; induction of flowering [52]; cold tolerance [53]; and enhanced yield through physiological components [54]. Overexpression of the *Arabidopsis thaliana* vacuolar iron transporter VIT1 in cassava accumulated 3–7 fold higher levels of iron in transgenic storage roots than

in the non-transgenic controls. Plants engineered to co-express a mutated *A. thaliana* iron transporter (IRT1) and ferritin (FER1) accumulated iron levels 7–18 times higher and zinc levels 3–10 times higher than those in non-transgenic controls in the field. Growth parameters and storage-root yields were unaffected by transgenic fortification [55].

No transgenic cassava has been grown commercially, partly due to the regulatory problems for this technology but also because of the difficulty in developing a transgenic cassava varieties that offer real advantages for producers and processors. Enhanced expression of phytoene synthase resulted in increased levels of carotenoids in the roots, but was reached at the expense of a drastic reduction in starch content [56]. Genetic transformation has been used to create resistance to CBSD. The genotypes transformed previously carried resistance to CMD and the ultimate objective was to develop varieties that were resistant to both major diseases. However, for unknown reasons, genetic transformation for the resistance to CBSD resulted in a loss of the resistance against CMD [57]. In spite of these regrettable drawbacks, transgenic research has provided valuable information on gene expression in cassava.

The CRISPR/Cas9 approach has been adapted to the crop and used to modify phytoene desaturase [58] to increase carotenoids content, the GBSS gene to produce waxy starch and PTST1 to induce earlier flowering [59]. The effects of altered gene sequences in the phenotype are strategic for guiding the application of this new technology, which may not have the regulatory limitations that genetically modified organisms have. Any gene modification, being made through transgenics or editing, must fit into the overall balance of gene expression and physiological functions to add to the performance of the target genotype. In that regard, editing seems to offer better prospects than conventional genetic modification. However, one of the limitations of gene editing, with the tools available today, is that it works quite well disrupting gene functionality, but not so well improving/optimizing gene functions [60].

Marker-Assisted Selection (MAS) and Genomic Selection (GS)

The first molecular map of cassava was published two decades ago [6] updates have been reported [61]. The initial molecular mapping was based on biparental populations targeting traits such as resistance to diseases induced by bacteria (CBB), fungi (anthracnose) and viruses (CMD and CBSD); tolerance or resistance to pests such as mites and whiteflies; tolerance to abiotic stresses and to post-harvest physiological deterioration; nutritional quality such as carotenoids, total protein content or cyanogenic potential in the roots; starch quality traits; fresh root and foliage yield; DMC; plant architecture and early bulking [61–64].

The biparental crosses, which usually aimed at maximizing phenotypic variation in the segregating progenies, were often unrealistic from the breeding point of view. For example, the cross between HB60 and Hanatee was used for developing QTLs for fresh root yield and DMC [65]. HB60 is

an improved clone with outstanding fresh root yield and high DMC (Table 1) developed for industrial processing (e.g., starch and animal feed production) while Hanatee is a landrace with excellent cooking quality but limited productivity. Breeders would seldom make crosses between clones that serve such different end-uses. In many of these studies, markers could only explain a limited amount of the total phenotypic variation and results were often not reproducible.

Classical MAS has had limited impact in cassava [61,63]. However, new technologies based on next-generation SNP markers may overcome many of the problems with conventional MAS. Large numbers of SNPs can be identified with high density at the genome level at relatively low cost. This has led to new applications such as GS and genome-wide association studies (GWAS), which have been used successfully to improve yield in homozygous crops like wheat [66]. The importance of non-additive, epistatic effects in the determination of attributes like yield limit the potential impact of GWAS or GS in heterozygous crops like cassava [62].

GWAS has been used in cassava for CMD and CBSD resistances and dry matter and carotenoids content in the roots [67–72]. GS simultaneously estimates the additive value of many marker effects across the entire genome (genomic estimated breeding value or GEBV). Factors affecting the prediction accuracy of GEBV include the degree of heterozygosity of the population, the training population size, heritability of the trait, phenotyping accuracy and data quality, marker density and genetic relationship between the training population and candidates for selection. GS is based on the assumption of negligible epistasis or over dominance, and must operate with "*closed*" population (e.g., trait introgression would drastically disrupt allelic frequencies).

GS does not require prior knowledge of QTL positions in linkage maps. A key advantage is that several different additive traits can be improved simultaneously through a selection index, similar to those used by breeders with phenotypic attributes. GS efficiently analyzes all markers in a population including loci with small effects (provided that there is a dense, genome-wide marker coverage). GS could maximize genetic gains by unit of time [73] by allowing faster and more efficient selection of progeny to advance. Unfortunately, GS cannot speed the actual advancement of the selected progeny from planting to flowering to harvest, so selections can be made rapidly but then selected progeny still must be grown to maturity to be crossed to other selected progeny to accumulate desirable alleles and then tested in seedling and clonal evaluations requiring several years to develop improved varieties. To speed sexual cycles in combination with GS, there are efforts underway to shorten the time to flowering in cassava. The need to reduce time to flowering in order to speed up recombination cycles should be balanced with the risk of selecting for poor plant architecture (e.g., short branching types). Using progenitors that allow seed-to-seed cycles within a year

promotes a reduction of height of first branch [69], which is highly undesirable in cassava.

The analysis of cassava's genome suggests a number of putative (hypothesized) deleterious loci in cultivated cassava [74], which is consistent with the phenotypic reports on the inbreeding depression observed in this crop [75,76]. Introducing some degree of inbreeding in cassava would facilitate reducing the high levels of genetic load currently affecting this crop. Once developed, the use of doubled haploids (DH) would increase the efficiency of GS by providing homozygous material. DH offer positive synergies with population genetics and breeding, including GS [77,78].

New Phenotyping Approaches

The efficiency of genotyping has increased exponentially in the past two decades. Cost per marker have been reduced and speed and reliability of analyses are outstanding. On the other hand, progress in phenotyping has been limited and continues to be a major bottleneck. Important progress in developing new phenotyping tools for cassava, however, has been made recently. A bottleneck was encountered by projects working to increase levels of pro-vitamin A carotenoids. The routine protocol to quantify these pigments was based on HPLC which was expensive and allowed assessment of only a few samples per day [6–8). Over the years, an efficient and reliable high-throughput system based on near-infrared spectroscopy (NIRs) was developed and deployed [79,80]. At IITA, a different approach has been used based on the enzymatic iCheck approach [81]. These innovations allowed the implementation of a rapid-cycling recurrent selection, which resulted in a 4-fold increase in β-carotene levels within a decade [82].

Ground penetrating radar [83] allows a non-destructive monitoring root growth during the season. Significant progress has been made improving the precision of the predictions made by the radar. Changes in the design of the device will help adapt it for other RTB crops. This technology will also enable breeders to effectively select for early bulking genotypes, which can extend the production season of cassava and allow early harvest/income for farmers [84].

Early Experiences Introgressing Traits in Cassava: the Case of Waxy Starch

Cassava is the second most important source of starch worldwide [1]. In 2007, a spontaneous mutation for the GBSS locus resulting in the production of amylose-free (waxy) starch was reported [85]. The cassava waxy starch phenotype offers important advantages for functional properties and potential uses. It depends on a single recessive gene [30,44]. The starch industry invested in the development of commercial varieties producing this type of starch. Description of the process to generate these varieties has been published [30].

The source of amylose-free starch was a landrace poorly adapted to the commercial production of cassava. It carried many undesirable genes in addition to the starch mutation. The amylose-free landrace was crossed onto elite cassava varieties to try to incorporate the waxy gene into desirable varieties. Thereafter, the traditional clonal evaluation and selection process was followed (top of Figure 1). About 550 genotypes were evaluated in SRT, 50 in PYT, 9 in AYT and finally 3 in UYT. Only one recombination cycle that could break undesirable linkages in the process of developing the first generation of waxy cassava varieties was used. In addition to waxy starch, harvest index and general vigor were the main selection criteria.

Table 2 presents the average performance of three selected waxy clones from the cross of the waxy starch source and an elite cassava variety as well as commercial checks from the 1970s (Rayong 1) and 1990s (KU50). The population from which these three clones were selected was relatively small compared with the ordinary number of genotypes used in elite variety development. The performance of the first generation of waxy clones was equivalent to that of Rayong 1, a variety released over 45 years ago and that is not commercially competitive today. The waxy varieties have a clear disadvantage in starch content. Introgression of the amylose-free starch mutation in Thailand was later replicated in a similar project in Colombia. Waxy materials selected in Colombia also have low starch content (unpublished data). Commercial production of waxy cassava clones began in Thailand and Colombia despite their lower productivity. This highlights the advantages related to the functional properties of amylose-free starch.

Table 2. Summary of the results leading to the release of the first and second generation of waxy starch varieties in Thailand and two commercial checks (Rayong 1, KU50 and HB80).

Genotype	Fresh root yield (t/ha)	Starch content (%)	Starch yield (t/ha)
Data leading to the release of the first generation of waxy varieties (2014)			
Average 1st generation	**28.7**	**17.0**	**4.9**
Rayong 1	28.6	19.4	5.5
KU50	39.5	27.1	10.7
Data leading to the release of the second generation of waxy varieties (2019)			
Average 1st generation	**21.24**	**19.27**	**4.10**
Average 2nd generation	**26.89**	**21.50**	**5.76**
Rayong 1	21.96	19.60	4.30
HB80	24.23	27.40	6.64

Molecular markers were used to identify heterozygous genotypes that carried the waxy starch allele and had an outstanding phenotype [30]. These and selected waxy-starch individuals, were crossed onto elite germplasm or among themselves to further break the undesirable linkages present in the first generation of waxy clones. Although different

strategies were followed, the critical objective was to allow for additional meiotic events to promote further genetic recombination. A new group of three clones has been selected representing the second generation of waxy clones. Significant improvement has been made improving plant architecture, but still productivity is lagging, particularly in relation to starch content (Table 2).

Fast Track Breeding

Branching is closely linked with flower development; hence, erect plants which are preferred by farmers, do not flower early or often. A critical vacuum in the ability to manipulate sexual reproduction in cassava limited the impact of the ongoing GS by the Next-Gen Cassava Project. Earlier flowering needs to be obtained in genotypes that otherwise have an erect plant architecture. These varieties are late or non-flowering types but have superior yields and plant types for farmers.

Earlier flowering in some erect genotypes has been obtained using the grafting technique [10,86]. However, this technique is time consuming and cumbersome. Other technologies have been successfully used to induce earlier flowering and fruit/seed set through extended photoperiod using red light treatments (RLT) at night, the application of plant growth regulators (PGR) and/or pruning young branches soon after flowering [87–90]. The RLT induces flowering within a few months after planting in most late-flowering genotypes tested. The application of PGR can induce earlier flowering in certain genotypes. Pruning the young branches on the first flowering event results in fruit and seed set in otherwise abortive first inflorescences. In addition, pruning increases the number of female flowers and thus the number of seeds produced per raceme. The combination of RLT with pruning and PGR, therefore, allows abundant production of seeds several months earlier compared to the conventional method. Hermaphrodite flowers are also produced abundantly. This unprecedented situation in cassava facilitates self-pollinations but would require the equally unprecedented emasculation of flowers to produce hybrid seeds.

RECENT CHALLENGES FROM PESTS AND PATHOGENS

The control of pest outbreaks in cassava have often relied on biological control solutions. Cassava is a perennial species grown as an annual crop. A key feature of the normal cropping system in cassava is the length of its growing cycle, which is typically close to a year. The tropical and subtropical environments in which cassava is grown also have important effects on the dynamics of pests and diseases because there is no winter breaking their cycles. In fact, it is common to see cassava that was planted at different times during the year growing in close proximity. These are ideal conditions for the development of pests and disease problems. However, these conditions are also propitious for the establishment of

biological control agents, which eventually could lead to a sustainable equilibrium.

Cassava breeders must be aware of the different alternatives available for overcoming biotic stresses. The introduction into Thailand of the pink mealybug (*Phenacoccus manihotis*) in 2008 resulted in drastic reduction of cassava productivity. The parasitic wasp *Anagyrus lopezi* was then brought in, mass-reared and released with excellent results [91,92]. Similar results had been obtained in Africa few decades earlier [93].

The control of cassava green mites (CGM) (*Mononychellus tanajoa*) is an interesting example of the interaction between genetic effects and biological control [94,95]. The most effective agents controlling the CGM are different phytoseiid mites. The presence of these predators in the Neotropics may explain why *M. tanajoa* has never been a major problem for cassava in the region [96]. As part of the collaborative efforts between CIAT and IITA, several phytoseiid species were introduced from South America into Africa, where CGM can be a serious pest. Only *Typhlodromalus aripo,* among the introduced species, succeeded in establishing and surviving the African conditions. *T. aripo* significantly reduced the populations of the CGM, which resulted in increased fresh root yield by at least 30% [93]. The interesting feature of the biological control of CGM is that the survival of the predator depends on the morphology of the plant's apex, while the efficiency of the biological control depends on the volatiles emitted by the plant host. The genotype determines both characteristics.

Emergence of CMD in SE Asia

Cassava Mosaic Disease (CMD) is a major disease problem endemic to Africa, southern India and Sri Lanka. It is not present in the Americas and was not present in SE Asia until recently. Unfortunately, CMD emerged a few years ago in Cambodia [97]. This is a catastrophic situation as Asian cassava germplasm lacks resistance to this devastating disease. Fortunately for the cassava community, there is an excellent monogenic source of resistance (CMD_2) that has remained effective in Africa for many years [98,99]. CMD_2 was successfully introduced into India [100], and pre-emptively introduced into Thailand in 2011. Molecular markers for this source of resistance have been available for almost 20 years [101]. They have been validated [102] and further improved [70,71,99]. In fact, the use of markers to track the resistance to CMD was the first (perhaps the only) example of applied MAS where selection for the resistance was done in Colombia in the absence of the pathogen. Wolfe and co-workers [99] have mapped CMD_2 to a specific region in chromosome 8 and reported thirteen additional regions (e.g., in chromosome 14) with small effects.

Cassava breeders are currently ill-prepared to efficiently introgress simply inherited traits like CMD_2. Waxy starch introduction demonstrated the drastic negative effects of undesirable linkages. Introgression of resistance to CMD should be easier because we are now better prepared,

it is a dominant trait and reliable markers are available. Still, massive amounts of undesirable DNA will be introduced along with CMD_2. Additional meiotic events can brake undesirable linkages and result in better performing progeny. Markers would be useful for selecting individuals that are homozygous for CMD_2.

Westward Spread of CBSD in Africa

The first reports of Cassava Brown Streak Disease (CBSD) came from northern Tanzania in the 1930s. Since then, the disease has been reported in coastal areas of East Africa [98,103]. CBSD has been gradually spreading westward. It is now found in the Democratic Republic of Congo, Western Kenya, Burundi and Lake Victoria region of Tanzania [104]. Without concerted efforts to control CBSD, its spreading is likely to continue to all major cassava-growing regions of Africa and possibly to SE Asia as well.

There is a prevalence of additive [68] but also non-additive [105] genetic effects controlling the resistance/tolerance to CBSD. The Namikonga landrace has been often used as source of resistance to CBSD [106]. Genotype-by-environment interactions are important and different virus species have been linked to the occurrence of CBSD symptoms [68,98,107,108]. The degree of resistance to CBSD is not as clear or stable as that observed for CMD_2 against CMD.

Several accessions from CIAT's germplasm collection were found to be immune to CBSD [108]. This is the first time such strong type of resistance has been reported for CBSD. Validation of these findings in the field is ongoing and preliminary results are promising. No information is available on the inheritance of this type of resistance. Incorporation of genes for resistance to CMD and CBSD into elite varieties of cassava in SE Asia and Africa (respectively) will be the most effective method to limit damage from the viruses. The discovery of resistance to CBSD in germplasm that has evolved in complete absence of the pathogen is intriguing. Understanding the physiological/immunological plant response responsible for such resistance could open up avenues to develop broader and more durable resistance against both, different biotypes of the same virus, and potentially different viruses [109].

A SCHEME FOR ACCELERATED INTROGRESSION OF RESISTANCE TO CMD

A scheme for the aggressive introgression of CMD_2 into SE Asian cassava breeding populations has been developed. This scheme may be used as example for introgressing other traits. Figure 4 illustrates the main features of this scheme. It assume that the main planting season is around March–April (a common situation in SE Asia, Africa and the Americas). The initial step would be to cross as many different sources of resistance to CMD as possible with elite cassava varieties adapted to SE Asia environments and prevailing markets. About 50% of the resulting progenies are expected to carry and express the resistance to CMD. The

seed from the this first batch of crosses would be germinated in the off-season in August. Seedling plants would be grown for only six months. During this period, marker-assisted selection would be carried out to identify genotypes carrying CMD_2. In March-April, the seedling nursery would be harvested and selection for other high-heritability traits would be made (e.g., late branching, adequate vigor, resistance to thrips, pubescence in the apical meristem, etc.). Visual selection of acceptable root yield potential could also be done. Because of variation in flowering habit in cassava, there will be large variation in the number of families in this first batch of segregating progenies. Selection would balance, to some extent, the number of genotypes representing each family.

<table>
<tr><th></th><th>Year 1</th><th>Year 2</th><th>Year 3</th><th>Year 4</th><th>Year 5</th><th>Year 6</th></tr>
<tr><td rowspan="2">Seedling nursery-I (6 months)</td><td rowspan="2">F1C1-I (3 locs)</td><td>SRT-I (3 locs)</td><td>PYT-I (6 locs)</td><td>AYT-I (6 locs)</td><td colspan="2">Regional trials for eventual release</td></tr>
<tr><td colspan="5">Promising materials identified as early as in F1C1 can be multiplied rapidly using approaches suchs as SAH</td></tr>
<tr><td colspan="2" rowspan="2"></td><td colspan="2" rowspan="2">Best plants from best families in F1C1-I back-crossed to elite clones & crossed among them</td><td rowspan="2">Seedling nursery-II (6 months)</td><td rowspan="2">F1C1-II (3 locs)</td><td>SRT-II (3 locs)</td><td>PYT-II (6 locs)</td></tr>
<tr><td colspan="2">Rapid multiplication of promising clones from F1C1-II</td></tr>
<tr><td colspan="5"></td><td>Best plants from best families in F1C1-II back-crossed to elite clones & crossed among them</td><td>Seedling nursery-III</td></tr>
</table>

Figure 4. Proposed scheme for fast track introgression of CMD_2 into SE Asian cassava breeding populations and rapid breaking of undesirable linkages.

F1C1-I: The young, selected seedling plants can produce only three stem cuttings. In the following stage (F1C1), each genotype would be represented by three plants. A key distinction with the schemes presented in Figures 1 and 4, is that for CMD the F1C1 will be planted in three different locations. Moreover, at each location, genotypes belonging to a given family would be split into three groups of about equal size, which would then be planted in different areas of the field. Individual genotypes would be planted once in each location. Families, on the other hand, would be replicated within and across the three locations. The main purpose of this complex approach is to have an early assessment of the performance of families and individual genotypes across locations.

The best genotypes from the best families across the three locations would be selected and planted as single row plots in three different locations. Harvest of the three locations will be coordinated so selection can be done within a day or two from each location. Plants will be pulled out of the ground and kept intact for a day or two until selection at all locations has been done. Then stem cuttings will be used to plant the SRT. Young branches will also be used as micro-cuttings for additional evaluations or used to initiate a rapid multiplication scheme to produce quickly a large number of planting material of promising genotypes. The limited information used to advance the selection process makes it quite risky. Selection would be based solely on the phenotypic performance of

three individual plants grown in three different environments. Perhaps 20–30 genotypes can be advanced this way initially and, as the rapid multiplication progresses (which at the beginning is painfully slow), additional phenotypic information on the performance at the SRT and PYT will allow the multiplication in 1-3 genotypes that might provide a first line of defense against CMD.

F1C1-II: The micro-cuttings from F1C1-I will also provide planting material for a crossing block to be initiated during the second year. This is a critical advantage of this scheme. Very quickly, a second meiotic event will occur to allow further recombinations to break undesirable linkages. The best genotypes from the best families identified in the F1C1-I will be "back-crossed" to elite clones (the quotation marks are used because true back-crossing in cassava is not possible) and crossed among themselves. This crossing block will be maintained for a year and a half and subjected to all the new technologies for induction of flowering and early fruit and seed set described above. During the second semester of the third year, a second seedling nursery will be planted. This seedling nursery will be grown and selections will be made as in the first batch. At this stage, breeders could start selecting genotypes homozygous for CMD_2. Selected genotypes from the second seedling nursery would be used for planting an F1C1-II in Year 4. The best genotypes from the best families would be selected to continue the standard selection process of planting SRT the following year in three locations.

F1C1-III: Genotypes selected in the F1C1-II would be quickly incorporated into a crossing nursery to go through a third meiotic event to further break undesirable genetic linkages. The focus now will be in generating genotypes homozygous for CMD_2, which offer the advantage of fixing the resistance. Homozygous genotypes would have twice the breeding value compared to their progenitors since all their progenies should be resistant. Once CMD_2 has been fixed markers will no longer be necessary to track resistance.

This is a novel breeding scheme that allows for a rapid breeding and selection process. However, the cassava sector in SE Asia is facing a crisis that merits extreme measures. A critical feature of this scheme is the selection of the best families and genotypes within the best families across locations. This early selection can identify promising materials for early recombination. Phenotypic selection for varietal release will have to follow the standard pipeline (although with the benefits of a more reliable information from the F1C1 stage).

THE NEED FOR NEW AND INTEGRATED BREEDING APPROACHES IN CASSAVA

During the past few decades, researchers, farmers and industry have managed to gradually position cassava in a prevalent food-security and industrial role that it plays today. Maintaining this prevalent position is a challenging endeavor that requires taking advantage of the lessons

learned in cassava and other crops. Two things can be taken for granted, things eventually need to change and the change requires a sensible integration of available technologies.

Rapid Multiplication of Planting Material in Cassava

The new breeding approach for cassava must shorten the time required to develop new varieties. The multiplication rate in cassava is low (one plant produces on average only 5–10 cuttings). It takes 6 years to have enough planting material for multi-location trials. There is technology available to shorten the time required to evaluate the performance of new varieties across locations. The leaf bud and tunnel methods have been developed at CIAT to allow rapid increase of planting material from an individual plant. The SAH (Semi-Autotrophic Hydroponic) method used at IITA [110] also allows for doubling the amount of planting material every few weeks, with the ability to increase from 100 boxes with 25 plantlets per box to 1600 boxes within 2 months.

Considering the large number of genotypes involved in the initial stages of selection, these rapid multiplication approaches are not practical unless there is a major threat like CMD in SE Asia requiring speed. Using SAH, for example, rapidly increases planting material of clones derived from crosses between a source of resistance and elite lines. This would allow phenotypic selection of plants over locations that have promising traits and carry markers for the CMD_2 gene.

Inbreeding

Inbreeding to develop (near-)homozygous parents in cassava would offer several advantages [62]. As stated by Rabbi in 2017, MAS has had negligible impact in cassava. Accelerated backcrossing is not feasible because of the lack of inbred recurrent progenitors to backcross to. The significant implications of this limitation have been made clear to the cassava community after the introgression of the single recessive waxy starch gene and is more critical now when the entire SE Asia region faces the serious threat of CMD. Trait introgression in cassava faces problems that could be easily solved if we had inbred progenitors in cassava.

Ramu and co-workers published in 2016 an interesting assessment of genetic load in cassava. They demonstrated that the frequency of deleterious alleles increased through the domestication and early selection processes. Current breeding approaches, however, are ineffective in purging the deleterious allele burden. These authors concluded that reducing the genetic load should be a key target for future cassava breeding. The advantages of partial inbreeding have already been reported in cassava [111]. Inbred lines in cassava would also allow identification of additional, desirable recessive alleles such as those mutations affecting starch functional properties [85,112].

If inbred parents were available, two parents could be crossed to develop 50 to 100 F_1 seedlings that were genetically identical, thus

providing several hundred genetically identical progeny for replicated evaluation trials in one clonal generation. This would allow reaching the multi-location evaluation phase in a shorter period of time, particularly when progenitors expected to express high levels of heterosis have been identified. The recently developed technologies to manipulate flowering in cassava [10,86–90] make it possible to produce fully- or partially-inbred cassava without favoring early branching plant phenotypes. Cassava inbreds would likely require self-pollinations to the S_2 generation since this level of inbreeding would be >75% homozygous (considering that some degree of homozygosity is present in S_0 genotypes). S_2 lines could be maintained by cloning, an approach not feasible in maize. Crosses between two lines of 85 to 90% homozygosity would likely allow identification of superior specific combinability (e.g., heterosis).

Heterosis and Reverse Genetics

Development of F_1 hybrids from inbreds may help break the productivity ceiling seen over the past decades in cassava. In maize, there was essentially no yield increase in open pollinated varieties from the 1860s to 1930s. However, since the introduction of hybrids from inbred progenitors in the 1930s, maize productivity has been improved consistently over the years [29]. Heterosis or hybrid vigor is conditioned by epistatic and over-dominance gene action. Pairs of inbred lines that express a high level of specific combining ability (SCA) exhibit the highest levels of heterosis. Improved maize inbreds have been bred for a divergence in alleles by recombining the most elite genetic material available. The recycling of elite inbred lines within separate heterotic families has resulted in the accumulation of genes within separate heterotic families that maximize hybrid yield by enhancing SCA [113,114].

Finding two inbreds that express very high levels of SCA is a big challenge since it is conditioned by non-additive gene action which is hard to predict. Non-additive genetic effects have been found to be significant in self-pollinated crops as well. Yield increases in hybrid rice ranging from 15% to 20% over pure line varieties have been reported [115–117]. The use of diploid inbred progenitors offers many advantages in potato breeding [118–120]. The development of diploid inbred lines with acceptable tuber shape and size is now a reality with at least three commercial companies producing them [121,122], Hans van Doorn-HZPC, personal communication]. The experiences in maize, rice and potato are very relevant for cassava. Cassava productivity relies on both additive and non-additive genetic effects. The evidence is supported by conventional field data using different quantitative genetic designs as well as from molecular studies [62,72,123].

Cassava competes with maize, not only as a source of starch, but for animal feeding and ethanol production. Cassava needs to keep pace with increasing productivity of maize in order to remain competitive. A major feature of maize breeding is the way heterosis has been exploited over the

years. The outstanding cross between Mo17 and B73 was the basis for identification of superior SCA to produce superior hybrids. It is feasible to create such a superior combination in cassava. The hybrid exists, it was released in 1992 and was named KU50 (one of the varieties in Table 1). The progenitors of KU50 are known and available. It is therefore possible to use a reverse genetic approach to identify inbred lines that might produce a F_1 hybrid variety that competes with KU50 [124].

Producing partially (or fully) inbred lines with outstanding specific combining ability would produce hybrids comparable to KU50 and fully exploit additive, dominance and epistatic effects that made this hybrid the success story described by Gracen and co-workers in 2018 [23]. This would be the first and only time in which all sources of genetic variation would be completely under control by the cassava breeder. The fact that commercial potato companies have shifted towards the use of inbred progenitors is very significant.

Thinking out of the Box for the Application of Novel Technologies

Most modern technologies come from developed countries and are applied on crops relevant for them (maize, rice, wheat, soybean, Arabidopsis, etc.). It is disappointing that the application of modern technologies in cassava tends to imitate those for which they were originally developed, ignoring the large differences between the crops. New technologies have been developed in crops whose progenitors are inbred and genetic resources fully screened. The number of major commercial traits in maize or rice that are still waiting to be discovered is probably limited. In the case of cassava, however, screening the germplasm in search of useful traits has just begun. Systematic evaluation of the large collection at CIAT has yielded only three important traits: resistance to white flies (which can be observed in the accessions themselves), waxy starch (which required the painful process of self-pollinations because of its recessive nature) and resistance to CBSD (which required grafting protocols because of the absence of the disease in Colombia and the complex nature of the disease).

On the other hand, there is a huge knowledge of Arabidopsis genome and comparisons with the sequenced *M. esculenta* genome could yield valuable information about gene functions in cassava. An array of useful traits ranging from herbicide tolerance, starch functional properties, haploid inducers, etc., could be easily identified using "*molecular sieving tools*" such as Eco-TILLING [125]. In spite of the early evidence demonstrating its feasibility [126], there has been no interest by the biotechnology community to seize the opportunity. The germplasm collection offers huge opportunities for identifying useful traits, yet no molecular technology has been applied to find them. Instead, there are plenty of articles on QTL, which as pointed out, are not used in MAS.

Demand-Led Breeding: Know What the Farmers Need and the Markets Want

The integrated approach of the different strategies described above requires that the breeder has an intimate interaction with farmers, industry, end users, sellers, intermediaries and other customers. Breeders need to have a clear understanding of the different end uses of the crop, the relative importance of these markets and, the requirements that the varieties must have to properly satisfy the expectations and demands for each product profile. Integration with food technologist and biochemists are essential for developing high throughput phenotyping tools and proper sampling procedures. The integrated approach should also seek for optimization of the GxE interaction for genotypes selected from advanced cycles [127].

SUMMARY AND CONCLUSIONS

Conventional cassava breeding is slow because of the year-long growing season and the slow rate of multiplication. It takes 1 or 2 years to make a cross and then the F_1 seed produced are genetically distinct. Each new variety must be developed by cloning an individual F_1 seedling. Since a plant can produce only few cuttings per year, planting material must be increased for about 5 years to get enough cloned plants for replicated, multi-location tests. Adopting the F1C1 stage, adds an extra 6-month long phase but allows testing the first clonal generation plants at three locations instead of one. It eventually leads to the elimination of the one-year long AYT stage.

Traditional breeding has been very successful but changes in market competitivity and pest pressures require changes in the traditional breeding methods. Previous experience with introgression of the single, recessive waxy gene demonstrate that new methods for introgression of single genes is needed. The arrival of CMD in SE Asia requires rapid introgression of a single resistance gene. We have incorporated the use of new methodologies and modified breeding methods to speed the introgression of the CMD_2 allele into elite varieties. The immunity to CBSD found in accessions from the germplasm collection must now be introgressed into African breeding populations.

Clearly, the use of inbred progenitors would greatly facilitate trait introgression in cassava.

The use of inbred progenitors would also facilitate the exploitation of non-additive genetic effects that are significant for fresh root yield. Reverse genetics would be an innovative approach to create heterotic groups in cassava. New protocols to induce early and abundant flowering in non-branching varieties are now available. They overcome earlier barriers that prevented inbreeding through successive self-pollinations.

Cassava holds a great potential to become an engine for agriculture development and food security in the tropics. However, excellence in

cassava breeding is needed to sustain genetic gains for the most relevant production and processing attributes while enabling the rapid introgression of traits that could provide stress tolerance and/or lead to novel products. Advances in molecular markers technology offer great potential identifying and tracking alleles in vegetatively propagated species like cassava. However, from the applied breeding perspective, *controlling* the way alleles segregate is more important than *tracking* them. Inbred progenitors are the only practical way to "*direct*" allelic segregation in breeding populations.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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