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Cassava breeding: Classical to recent breeding approaches for food, industry and climate resilience

M. Velmurugan[1], R. Janaharshini[2]*, C. Indu Rani[2], S.R. Venkatachalam[1] and P.A. Saravanan[1]

[1]*Tapioca and Castor Research Station, TNAU, Salem– 636 119, India*
[2]*Department of Vegetable Science, Horticultural College and Research Institute, Coimbatore– 641 009, India*

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***Corresponding Author Email :** janakeru@gmail.com ***ORCiD:** https://orcid.org/0009-0006-7973-610X

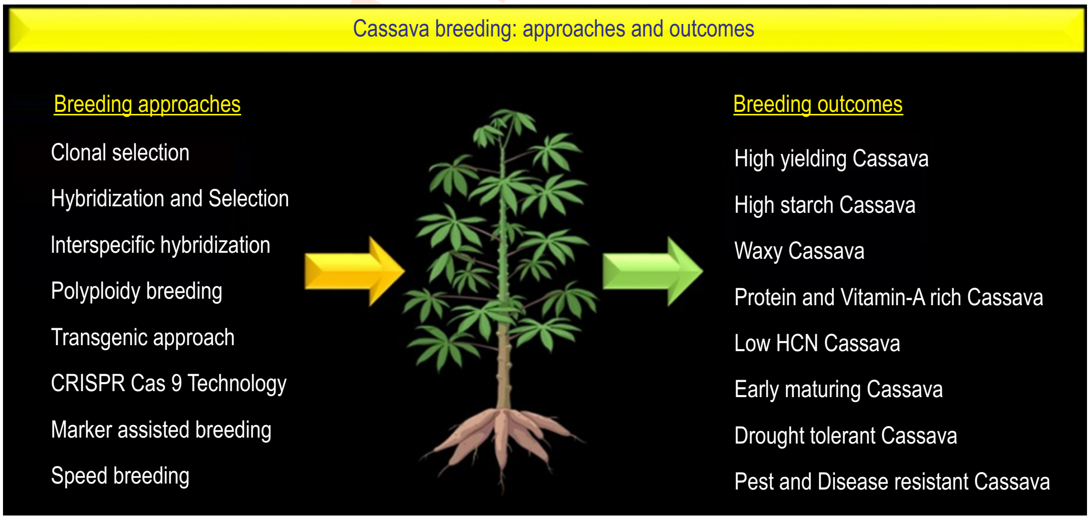
Abstract

Cassava ranks as the fourth-most significant starchy root crop in underdeveloped countries in terms of future food and acting as a key source of income for small and marginal farmers. To meet the growing demands for food security and economic development, it is imperative to develop improved cassava varieties that offer higher yields, enhanced nutritional content, safer for consumption, greater resistance to diseases and climate change.

The development of these improved varieties necessitates advancements in breeding techniques, leveraging both traditional methods and modern biotechnological tools. However, a major challenge in cassava breeding is heterozygous nature and the crop's sparse flowering, which limits the potential for sexual reproduction, thereby constraining breeding efforts for predominantly clonal selection. The continuous clonal propagation impedes genetic diversity and the introduction of novel traits, narrowing the overall progress of breeding programs.

Integrating genomic tools and accelerating the adoption of biotechnological advancements can overcome these limitations and expedite the development of superior cassava varieties. This review highlights the need of cassava breeding for addressing these challenges with conventional as well as with new breeding techniques with the aim to provide a comprehensive understanding of the current scenario and future directions of cassava breeding research.

Key words: Climate change, CRISPR/Cas 9, New breeding techniques, PPD, speed breeding, Waxy cassava



Cassava breeding: approaches and outcomes

Breeding approaches

- Clonal selection
- Hybridization and Selection
- Interspecific hybridization
- Polyploidy breeding
- Transgenic approach
- CRISPR Cas 9 Technology
- Marker assisted breeding
- Speed breeding

Breeding outcomes

- High yielding Cassava
- High starch Cassava
- Waxy Cassava
- Protein and Vitamin-A rich Cassava
- Low HCN Cassava
- Early maturing Cassava
- Drought tolerant Cassava
- Pest and Disease resistant Cassava

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Introduction

Cassava (*Manihot esculenta* Crantz.) a starchy root crop, occupies fourth position in terms of calories after rice, maize and wheat. Cassava is grown globally in tropical and subtropical nations due to its adaptability and variable development cycle, making it suitable for areas with high population pressure. About 186 thousand hectares of land and 6853 thousand MT of tapioca are produced in India (Press Information Bureau, 2022). Globally, processing of cassava has gained momentum of 311.50 MT during 2022 and 319.9 MT in 2023. Nigeria, Brazil, Indonesia, Thailand, Democratic Republic of the Congo, Angola, Ghana, Mozambique, Vietnam, India, Sierra Leone are the leading producers of cassava. The global cassava producing countries is depicted in the (Fig. 1). In India, Tamil Nadu has contributed 83.75% share in production followed by Kerala (8.74%) besides cultivated in Assam, Nagaland, and some parts Andhra Pradesh. The diverse use of cassava in food additives, cattle feed, and industrial processing into starch, sago, sweeteners, and ethanol makes it a significant export crop (Kumar *et al.,* 2021).

FAO (2013) highlights that the plant's distinct characteristics, including its richness in carbohydrates, ease of cultivation, low labor and nutrient requirements, resistance to pests and diseases, and ability to withstand dry conditions, has led to its widespread cultivation. This makes cassava a reliable source of food in regions with unpredictable weather patterns. Under these circumstances, breeding of cassava is essential to address various challenges and improve the crop for better productivity and climate resilience. This review explores the diverse and vivid dimensions of cassava improvement through multi-locational evaluation with the existing local landraces, in vitro and in vivo germplasm screening, hybridization, polyploidy breeding, genetic transformation, molecular markers and mutation breeding are the notable cassava breeding strategies.

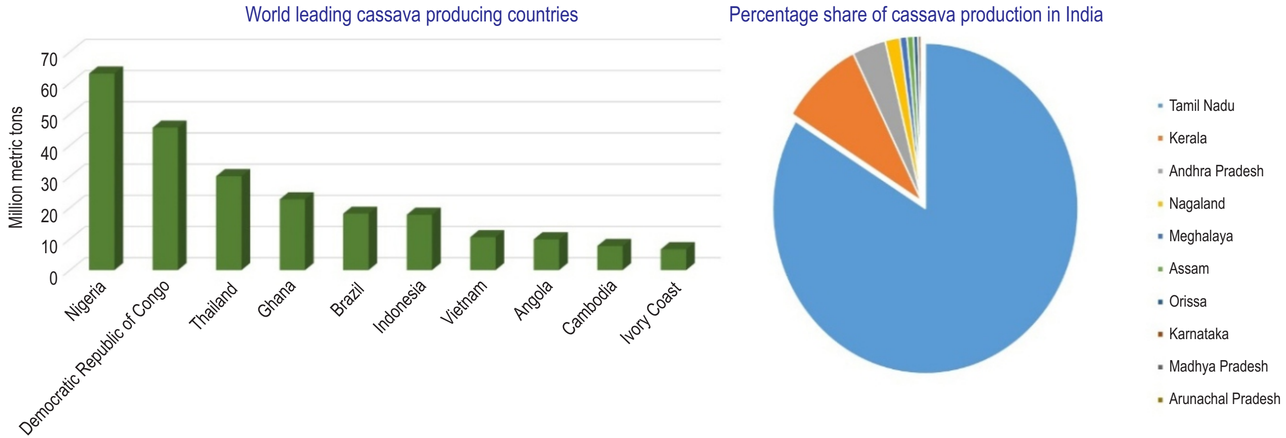
History, origin and domestication of cassava

In Euphorbiaceae, all the wild Manihot species have a chromosome number of 2n = 36, exhibiting diploid behavior during meiosis. Cassava might have originated from crossings between two closely related parental species, as it is segmental allo-tetraploid (x = 9). The chromosome behavior during meiosis varies significantly throughout varieties, which may be related to cassava's allopolyploid origin (Nassar, 2002). In 1766, Crantz coined first scientific name to cassava and later by subsequent classifications of Pohl (1827) and Pax (1910) as two diverse species viz., *M. utilissima* (bitter type) or *M. aipi* (sweet type). The origin is believed to have been in South America and closest wild relative of cassava was found to be *Manihot esculenta* ssp. *flabellifolia*.

Later, in the 16th century, Portuguese explorersfrom Brazil took cassava to Africa and subsequently, Asia (Jennings, 1979). Following a severe famine, the King of Travancore promoted Malayan varieties of cassava, which introduced the crop to Kerala.The ICAR-CTCRI, a global research institution since 1963, focuses advancing technologies and site-specific recommendations to address sustainability and resource-poor farmer's challenges (Malik *et al*., 2020).

Botany and Floral Biology

Cassava is a monoecious perennial shrub grown for its tubers with high starch and caloric content, it can be harvested between 6 to 24 months after planting, depending on the cultivar and climate conditions (Velmurugan *et al.,* 2023b). Cassava can be propagated by seeds or more commonly by stem cuttings. Genotypes with more branches produce more flowers, aiding breeding programs by shortening cycles and increasing seed production. The inflorescence bear flowers in clusters with five tepals that are either red or yellow or purplein colour. Each male



Source: *Statista (2024)*

Source: *Press Information Bureau (2022)*

Fig. 1: Global cassava producing countries and percentage share of cassava production in India.

flower contain 10 stamens that are borne above, while the female flowers are borne at the base of the inflorescence. Being protogynous, female flowers open roughly ten days before the anthesis of male flowers. The receptivity of stigma is between 6.30 a.m. and 2.30 p.m. Eight to nineteen hours after pollination, fertilization takes place (Andersson and de Vicente, 2010). Fruit is a trilocular, ovoid to globular capsule that dehisces and has noticeable ridges. For hybridization, a nectar drop at the pistil base indicates female flower opening. Female flowers are covered to prevent foreign pollen. Male flowers open one to two weeks later, making emasculation unnecessary. Fertilized flowers are covered to prevent foreign pollen hybridization and ensure seed formation.

Breeding objectives of cassava

Breeding objectives differ from nation to nation and are primarily influenced by how the food is ultimately consumed (Fig. 2). The general goals of cassava breeding are listed below:

To develop varieties with more crop yield: Cassava breeding programme aims to achieve maximum target on tuber yield. The global average production of cassava is 12–13 tons ha^{-1} as against 80 tons ha^{-1} under ideal conditions (FAO, 2013). The potential yield of cassava is limited by climate change, pests and diseases. High-yielding varieties can achieve their potential when resistant to biotic and abiotic stresses.

To develop varieties for tolerance to abiotic stresses conditions: Cassava, though drought-tolerant, suffers yield loss and reduced consumer acceptance due to fiber formation under climate change (Koundinya *et al.,* 2023a). Water deficit/ drought and elevated CO_2 concentration are serious problems due to climate change. The heterogeneous population method is suggested for drought tolerance as it is governed by polygenic nature, which makes it difficult to combine all genes into a single cultivar (Koundinya and More, 2021).

The breeding program should incorporate traits such as higher photosynthetic activities, leaf area, leaf retention, and harvest index to produce drought tolerant cassava varieties (More *et al.,* 2020). Developing cultivars resistant to drought along with using specialized agronomic techniques are essential for the successful cultivation of cassava in regions that are susceptible to drought (More *et al.,* 2023). Targeting the best crop in poor to marginal soil is another mandate to reach the subsistence farming (Kalarani *et al.,* 2018).

To develop early maturing varieties to reduce crop cycle: Early maturing and short duration varieties are becoming the prime objective of breeding to avoid the water deficit period (Koundinya and More, 2021). Reduced crop cycle favors better weed and nutrient management practices as well (Velmurugan *et al.,* 2017b; Velmurugan *et al.,* 2017c). Two varieties of cassava viz., Sree Jaya and Sree Vijaya developed by CTCRI matures in 6 months avoids the water deficit period (Koundinya and More, 2021).

Enhancement of quality traits of tubers

Improving starch content: Cassava's high starch content, averaging 84.5% of its dry weight, is crucial for its quality and applications in starch-based products, biofuels and food processing (Pugalendhi *et al.,* 2021b). Varieties with higher starch content are favored for their economic returns and versatility. Cassava starch contributes to its attractiveness with its high paste clarity, great texture, low protein complex, and reasonably strong stability against retrogradation and swelling capacity (Krishnakumar *et al.,* 2021).

Improving dry matter content: A higher dry matter content is preferred since it enhances the processing qualities, energy content, and shelf life of cassava products. The starch and dry matter content of tubers increase with their length of tuber bulking period. Okogbenin *et al.* (2013) demarcated the correlation between dry matter content, starch content and harvest index in cassava.

Developing waxy cassava: Recent advancements in cassava breeding have focused on the developing waxy varieties, aiming to produce cassava with either no or less amylose content. Waxy cassava starche possess more viscosity, limited or no retrogradation, high swelling power with poor to low solubility for diversified application in food processing industries. Lower amylose content cassava cultivars are frequently chosen for culinary applications because they cook out softer, mealy and stickier. In cassava, the average amylose level is approximately 20.7%. The cassava community is interested in amylose-free starch products (Karlstrom *et al.,* 2016).

Reducing anti-nutritional factor: Hydrogen cyanide in cassava is a significant concern and based on HCN generation, cassava varieties are categorized based on the glucoside concentration viz., >100 mg kg^{-1} fresh weight as "bitter" and <100 mg kg^{-1} fresh weight as "sweet" (Alves, 2002). Two cyanogenic glycosides, linamarin and lotaustralin, release HCN when plant tissues are disturbed. HCN release, heightened during droughts, comes from cassava roots and leaves. Breeding for low HCN cassava is crucial for safety, but achieving low HCN with high starch content is challenging (More *et al.,* 2023).

Increasing protein content and beta-carotene content: Cassava possess low protein and beta-carotene content in tubers, which can be increased through the application of biotechnological techniques and interspecific hybridization. Enrichment with β-carotene does not reduce the yield (Chavez *et al.,* 2005). Moreover, a high carotenoid concentration could help to prolong the shelf life of tubers (Morante *et al.,* 2010); however, because of altered carbon partitioning into starch, storage roots have a lower dry matter content (Beyene *et al.,* 2018).

Reducing post-harvest physiological deterioration (PPD): Cassava suffers huge economic and quality loss due to post-harvest physiological deterioration from increased oxidative

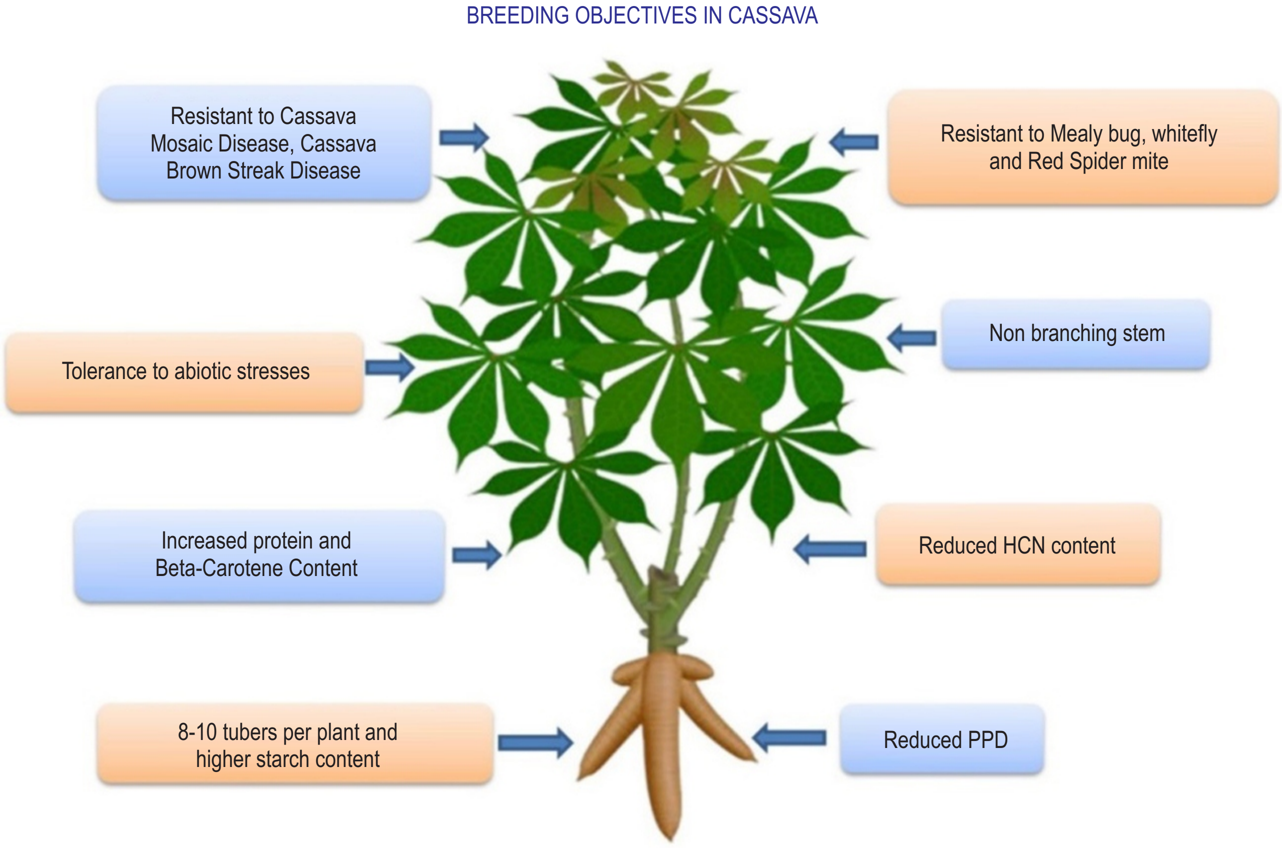


Fig. 2: Major breeding objectives of cassava (PPD – post-harvest physiological deterioration).

potential that restricts the storability and usage of tubers within 1-3 days post harvest (Sowmyapriya *et al.,* 2017). Tubers with neck portions are less prone to PPD and there is a positive correlation between root length, weight, dry matter and PPD. PPD mainly affects the proximal and distal ends of tubers (Visalakshi *et al.,* 2023). One of the potential methods to increase shelf life is to manage PPD by using targeted mutagenesis methods and RNA-dependent DNA methylation.

Developing varieties resistant to major pest and diseases: Two common pests that harm cassava plants are red spider mites (*Tetranychus* spp.) and cassava mealybug (*Phenacoccus manihoti*). Mealybugs cause yield loss up to 80% (Saravanan *et al.,* 2023; Velmurugan *et al.,* 2023c) and red spider mites reduce yield by 20–30% by causing yellowing and early leaf loss (Velmurugan *et al.,* 2023d). Cassava mosaic virus (CMV) cause yield reduction from 30% to 80% (Allemann *et al.,* 2004). Bacterial blight of cassava (*Xanthomonas axonopodi* spv. *manihotis*) and Brown streak disease of cassava (BSD) are two more threats that results with severe damage to cassava. Under these circumstances, it is imperative to develop cultivars exhibiting resistance to key diseases and pests at the earliest to cater the prevailing field problems.

Breeding strategies

Clonal selection: The technique of selecting desired clones from a heterogeneous diverse population of crops produced vegetatively is known as clonal selection. The clone's phenotypic value is determined by the interactions between the environment (E), genotype (G), and both (GxE). Heritable effects are limited to genotype, while selecting highly heritable traits like color and disease resistance plants and reliable rather than selecting from lesser number of plants. Recent days development of nutrient efficient lines (especially for nitrogen and potash) is need of the hour to save fertilizer cost (Velmurugan *et al.,* 2020).

In cassava, clonal selection helps to maintain the genetic homogeneity of the resultant crop. Clonal selection can be done from the existing gene banks either *in vitro* or *in vivo* gene banks. Tamil Nadu Agricultural University, Tapioca and Castor Research Station, Salem is maintaining 376 cassava germplasm accessions (Velmurugan *et al.,* 2017a; 2023a) to screen the best performing cassava germplasm accessions and promoted for the conduct of multi-locational trials across the state (Velmurugan *et al.,* 2023c). Some of the clonal selections made in Cassava in furnished in Table 1.

Table 1: Varieties developed through clonal selection

Variety	Special features	References
Sree Prakash	Short duration, non-branching plant, high yield and 29% starch, appropriate for lowlands	Nair *et al.* (1988)
Sree Jaya	Early maturing type, starch (27%),appropriate for lowlands and better cooking quality	
Nidhi and KMC -1	Short-duration variety contains 26% of starch	Abraham *et al.* (2001)
CO- 2	High yielding variety (35 t/ha), 25% starch with better cooking quality	
MVD- 1	Suitable for chips making and contains > 28% starch	
CO-1	Tuber contains 35% starch	
CO-3	Tuber yield (42.6 t/ha) and starch (35.6%)	Malik *et al.* (2020)
CO (Tp)- 4	Tuber with 40% extractable starch and 50.6 t/ha of yield	
CTCRI CO (Tp) – 5	Tuber with 28% starch and resistant to CMD	
Sree Vijaya	Suitable for cooking and contains 27–30% starch	Abraham *et al.* (2001)
Sree Swarna	Yellow-fleshed, CMD-tolerant variety with 25% of starch	
Sree Pavithra	High tuber yield and low cyanogenic content, starch (25 to 27%), suited for processing industry	Malik *et al.* (2020)
PDP-CMR 1	High yielding, CMD and drought-tolerant cultivar	
CR24-4	Better tolerance to PPD and resistance to cassava mosaic disease with 28% of starch.	
Sree Sakthi	High tuber yielding CMD resistant variety with high starch (29-32%)	
Sree Suvarna	Excellent yielder with CMD resistance, 25–27% of starch, amenable for processing	Malik *et al.* (2020)
YTP- 1	Erect, non-branching with starch content of 25 to 27%	
YTP- 2	Drought and CMD-tolerant selection with higher tuber yield (42.20 t ha-1) with starch content (28.40%)	Pugalendhi *et al.* (2021a)

Hybridization and selection: Hybridization is an important method of breeding for creation of variations in vegetatively propagated crops like cassava. The goal of hybridization is to create offspring that are superior to the parents by combining beneficial genes of interest from two or more different varieties. Once the F_1 populations are generated, subsequent generations can be clonally maintained without any variations. Here are some of the hybrids developed through hybridization Table 2.

Interspecific hybridization: Crossing of plants from two different species is referred to as interspecific hybridization. Interspecific hybridization is utilized to impart desired traits, especially resistance to pests and diseases, to lower the cyanide content and increase the protein content in cassava. Wild cassava species with specific traits (Table 3) can be utilized for interspecific hybridization. Gomes *et al.* (2013) reported that protein content in most cassava cultivars is limited to 2%. The interspecific hybrid exhibited a higher protein content up to 5.7% between *M. esculenta* and *M. oligantha.* In order to increase the protein content of cassava, interspecific hybridization was attempted that involved wild species viz., *M. saxicola* and *M. melanobasis.* Nassar and Sousa (2007) found *M. saxicola* crosses produced seedlings with up to 2% protein in the fresh root through amino acid profiling.

Manihot glaziovii (Ceara rubber tree) has been able to transfer low cyanide content and resistance to bacterial blight and mosaic virus to locally adapted African types (Hahn *et al.,* 1980). Cassava interspecific hybridization with *M. glaziovii* produced UnB 110, a progeny with increased root yield, drought tolerance, and resistant to cassava mealy bug (Nassar *et al.,* 1996). Nassar *et al.* (2004) restored the fertility of interspecific hybrids through polyploidization. Because there was no chromosomal matching during interspecific hybridization, the hybrid that resulted was sterile and prevented subsequent backcrossing.

Polyploidy breeding: Polyploidy is a state in an individual that have more than two basic or monoploid sets of chromosomes. In nature, chromosomal doubling can occur asexually (somatically) or sexually (gametically), giving rise to polyploids. The relative studies on polyploidy in tuber crops are countable and those which have been published are summarized here under. The most effective method for inducing tetraploidy in the cassava variety "Xinxuan 04" was discovered by Zhou *et al.* (2017) by giving colchicine treatment (0.05 g l^{-1}) for two days. The results evidenced that tetraploids have greater photosynthetic activity than diploids. Usually in breeding programs, triploids are created by crossing diploid with artificially induced tetraploids. Upon screening, the triploids had comparatively high starch and accumulated more dry matter content than their parents. In order to combat protein deficiencies in cassava, polyploidy breeding techniques were used. Sprouts of Malayan-4 stem cutting were treated with 0.5% colchicine solution to induce auto tetraploidy which produced 42.3% more crude protein in tetraploids than diploids (Magoon *et al.,* 1969). In order to explore the anatomical structure required for drought resistance breeding, Nassar *et al.* (2008) produced tetraploidy in UnB 530 cassava clone by spraying 0.02% aqueous form of colchicine solution to the buds

Table 2: Hybrids developed through hybridization and selection in cassava

Hybrids	Parentage	Special features	References
H- 97	Manjavella x Brazilian seedling progeny (Acc. No.300)	Drought tolerant, high yielding hybrid with 27- 31% starch content	
H- 165	Chadyamanagalam Vella x Kalikalan	Short duration hybrid, resistant to scale insects, spider mites with starch content 25%	Magoon *et al.* (1970)
H- 226	Ethakkakaruppan × M-4	Drought, scale insects and red spider mite tolerant hybrid with starch content of 29%	
Sree Visakham	Acc.No.1501 x Madagascar (S-2312)	Carotene rich hybrid with 28% starch	
Sree Sahaya	Multiple hybrid	High yielding drought tolerant hybrid with moderate leaf spot resistance with 31% starch	Abraham *et al.* (2001)
Sree Rekha	TMS.63198 (selfed) × Sree Visakham	High yielding top cross hybrid with starch content of 27-29%, amenable for upland and low land growing areas	
Sree Prabha	TMS63173-4 × Sree Visakham	High yielding top cross hybrid with starch content of 26-28%, amenable for upland and low land growing areas	

Table 3: Cassava species and their utilization in interspecific hybridization

Species	Important traits
Manihot anomala	Normal meiosis, well adapted to humid tropics and shade conditions
Manihot caerulescens	Adopted to low temperatures, tolerant to poor soil conditions and drought resistant
Manihot catinga,	
Manihot dichotoma,	Resistant to mosaic disease
Manihot glaziovii	
Manihot oligantha sub sp. nesteli	Roots contain 7% protein, cyanogenic glycoside content lesser, withstand drought
Manihot pseudo glaziovii	Drought tolerant
Manihot stipularis	Resistance to drought conditions, well acclimatized to low temperature
Manihot tripartite	Flesh is white in colour with high protein content. Roots are compact arranged
Manihot tristis sub sp. saxicola	Protein content is higher

on lateral sides for 12 hours. In comparison to diploids, the tetraploid exhibited larger pericycle fibers, and wider secondary xylem with thinner walls and less starch. CTCRI developed and released triploidvarieties, which are more suitable for starch extraction. The first triploid variety, 'Sree Harsha' had superior culinary qualities, better tuber yield and starch content than diploid (Sreekumari *et al.,* 1999). High yielding triploids, released from CTCRI, that are suitable for starch extraction by industries as well for culinary use are Sree Apoorva and Sree Athulya (CTCRI, 2017).

Mutation breeding

The use of physical and chemical mutagens to alter plants' genetic makeup in order to select desirable traits in the progeny is known as mutation breeding. This technique is helpful where hybridization barriers like sterility, incompatibility and heterogamy prevent the use of existing diversity. Summary of various applications of mutant breeding in cassava:

Reduced hydrogen cyanide content: Oyeyemi *et al.* (2010) exposed three different varieties of cassava with gamma rays and opined that 100 Gy was appropriate for reducing cyanide by 58%. Further, this experiment proves the existence of linear relationship between the cyanide content and radiation dosage of gamma rays.

Modified starch properties: True cassava seeds were subjected to gamma ray mutation and underwent selfing to generate M_2 generation. In M_2 generation, anomalies in the shape of starch granules, poor viscosity in the gels were notable concerns during evaluation phase (Ceballos *et al.,* 2008). A local genotype called 'Iding' was compared with Adira-4, the national variety after being exposed to gamma radiation at different doses. During evaluation phase, in fourth generation, higher tuber yields and higher amylose contents were observed in Adira-4, especially under 2 and 30 Krad dosage. All the genotypes and mutants exhibited greater levels of amylose than untreated control (Sudarmonowati *et al.,* 2021).

Increased beta- carotene content: The *in-vitro* shoots of cassava clone 'UbiKuning' was irradiated with 10 Gy gamma rays and the results radiation revealed that enhanced levels of beta carotene (0.252 µg ml^{-1}) was observed in mutant (UKRad3), while the unexposed control accumulates 0.219 μg ml^{-1} of beta carotene. All the mutants exhibited significant variations in color intensity of tubers and beta carotene levels (Rahman *et al.,* 2020). Currently, only two varieties of cassava viz., Tekbankye and Fuxuon 1, has been officially registered in the Mutant Variety Database of the International Atomic Energy Agency (Koundinya *et al.,* 2023b).

Biotechnological approaches: Numerous opportunities presented in plant biotechnology can be explored for developing cassava more suitable for adverse environmental conditions without compromising the tuber yield.

Transgenic Approach: Genetically modified (GM) crops, often known as transgenic crops thatgreatly increases the production potential of cultivars besides managing insects, diseases and other environmental stresses. The transgenic approaches in cassava are appended as follows:

Zhang *et al.* (2005) developed transgenic plants conferring resistance to African Cassava Mosaic Virus (ACMV) through antisense RNA technology. Through targeting particular viral mRNAs, such as AC1 (Rep), AC2 (TrAP) and AC3 (Ren), the transgenic plants showed decreased viral replication that are either asymptomatic or attenuated. Protease inhibitors, α-amylase inhibitors, Bt Cry proteins, and plant lectins are examples of insecticide proteins that may help in developing transgenic cassava to promote insect resistance. In comparison to wild-type plants, transgenic cassava plants expressing SAG12-IPT showed longer shelf life, pronounced role in climate resilience, especially for mitigating drought situations and altered cytokinin metabolism (Zhang *et al.,* 2010). Zhang *et al.* (2003) introduced the *ASP1* gene involving CaMV 35S promoter that led to higher amounts of amino acids required for the accumulation of enhanced levels of protein content in tubers of cassava. Waxy cassava plants developed through transgenic technology expediting through antisense GBSSI RNA with promoter as CaMV 35S (Raemakers *et al.,* 2005). Starches obtained from waxy transgenic plants showed altered physio-chemical and biological characteristics (Zhao *et al.,* 2011) than control. Under these circumstances, altered GBSS regulation activity was found to be a useful strategy for controlling amylose production.

CRISPR Cas 9 Approaches: In the cutting-edge and editing of genome technology, the researchers can alter the sequences of DNA, and target interference is generally achieved using one of the three primary classes of (Clustered Regularly Interspaced Short Palindromic Repeats) CRISPR–Cas systems. The appropriate utilization of CRISPR/Cas9 in promoting climate resilience, overcoming stresses, biofortification for nutritional importance, improvement of quality and conventional system of value addition, etc., can be easily achieved. The CRISP-Cas9 approaches attempts in Cassava are summarized as follows:

As cassava being a low protein diet, various approaches have been used to increase its content. Cyanide produced from linamarin reduce the nitrogen content in Cassava root protein synthesis. So disrupting gene encoding for cyanogenic glycoside synthesis is taken as strategy to improve protein synthesis as well as reducing HCN. The activity of two cytochromes CYP79D1/D2 and P450s genes were inhibited through Gene silencing technology. The modern biotechnological approaches such as artificial micro RNA (amiRNA), Trans-acting small interfering RNA (tasiRNA), CRISPR/Cas9 and Targeting Induced Local Lesions in Genomes (TILLING) are highly useful in developing cassava with resistance to virus, drought tolerance and reduced levels of HCN in tubers (Fondong *et al.,* 2018). Over expression of Hydroxynitrile lyase (HNL), an enzyme involved in detoxification of cyanohydrins to lesser toxic compound in the root accelerated cyanogenesis and cyanide volatilization during processing (Siritunga *et al.,* 2004). A practical and efficient strategy to delay the post-harvest physiological deterioration can be achieved through CRISPR/Cas 9 by targeted mutagenesis, i.e., Knockout of MeF6 genes such as (H1, H2 and H3), which actually took part in synthesis of scopoleptin, secondary metabolite which cause PPD during harvest of tubers especially upon wounding or any mechanical damage to cassava tubers (Mukami *et al.,* 2024).

By introducing specific amino acids substitutions, which reduces the binding affinity for glyphosate, and modifying the expression profile of EPSP gene in cassava plants that provides tolerance to glyphosate. The endonucleases targets lead to a 3.2 kb deletion and subsequent repair using HR/NHEJ and the targeted Integration with Point Mutation and Allele exchange (TIPA) resulted in tolerance to glyphosate (Hummel *et al.,* 2018). In Potyviridae family, the positive-sense RNA virus such as Brown Streak Virus and Ugandan cassava brown streak virus were the major threats in East and central part of Africa. The cultivar 60444 was utilized to create mutants viz., ncbp-1, 2 and ncbp1/ncbp2 involving CRISPR/Cas9 approach. Among these mutants, ncbp1/ncbp2 had diminished or weaker expression of brown streak disease symptom and reduced levels of severity of root necrosis during storage period (Gomez *et al.,* 2019).

The monoecious nature of cassava led to asynchronous state of maturation, which is a challenge to introgress the traits of interest at shorter time. Two genes related to biosynthesis of amylose such as PTST1 and GBSS helps to exclude amylose content in tubers of cassava through CRISPR-Cas9 approach (Bull *et al.,* 2018). TFL-1 (Terminal Flower 1 gene) acts as a repressor that inhibits transition of shoot apical meristem to flower, thereby promoting the production of leaves and stem. The interruption of Floral repressor through CRISPR/Cas9 technique helps to accelerate flowering in cassava (Odipio *et al.,* 2020).

Marker assisted selection breeding

For developing a new cassava variety, a time span of around ten years is required due to its lengthy growth cycle (12–18 months). With fewer cycles of phenotypic evaluation and faster genetic gain, Marker Assisted Section can significantly improve selection precision and shorten the time required for varietal development. In six years, varieties would be evaluated for release under the present cassava molecular breeding strategy. Furthermore, using MAS during seedling stage drastically lowers population size which increases the economics of breeding and emancipates breeders to deal with a greater number of populations (Ige *et al*., 2022). In addition to phenotypic screening, four KASP assays were employed to estimate the quantity of carotenoids in a cassava population, which was obtained through open pollination. This technique is more reliable to increase both the efficiency and accuracy of selection. Further from the studies of Codjia *et al.* (2023) it was evident that there was a strong association between the root tissue color score and total carotenoid content. The results of the investigations have recommended that marker for marker assisted selection for 'provitamin A' content in cassava germplasm in Africa was PSY572/S124155522.

Using a scale of 1–5, five numbers of cassava seedlings populations were assessed for CMD resistance during 1st, 3rd and 5th months after planting. Six molecular markers (NS 158, NS169, NS198, RME1, SSRY 106, SSRY 28) connected to the CMD2 gene were also used to screen the genotypes in five populations. The CMD Severity Score indicated that, on average, 70.5% of the progenies across all populations were resistant, ranging from 53 to 82%. Using the marker data, it was possible to identify approximately 70% of the progenies as disease-resistant throughout a range of 62–80% of populations. By combining marker data and CMDSS, 40–60% of progenies, or an average of 52%, was shown to be CMD resistant in each group (Olasanmi *et al.,* 2021). The Marker-assisted selection (MAS) has largely failed to enhance polygenic traits is one of the major disadvantages, and also, this method overlooks genes with minor effects when selecting for quantitative traits such as drought tolerance (Koundinya and More, 2021).

Speed breeding

Speed breeding is an innovative method that speeds up plant growth by adjusting environmental factors like temperature, light duration, intensity, humidity and photoperiodic regime. This technique aims to shorten generation time, enabling quicker crop improvements. Traditional breeding methods, which can take over ten years, are often too slow to address the increasing food demand in a rapidly changing world. In the case of cassava, speed breeding is particularly important due to specific challenges related to its floral biology and growth patterns. The lengthy generation time in cassava and complicated flowering hinder conventional breeding methods. By utilizing speed breeding, cassava breeding programs can overcome these obstacles and expedite the development of enhanced cassava varieties. Traits such as branching and flowering are directly correlated with each other. While, the erect or non-branching cassava varieties produces limited and delayed flowering, which interfere with breeding and genetic research. Ceballos *et al.* (2017) developed grafting technique for flower induction in cassava. Grafting non-flowering cassava genotypes onto early-flowering rootstocks led to faster branching and flowering. However, the effectiveness of this technique varied with different genotypes as the technique being genotype dependent, limiting its overall reliability. The extension of photoperiod had profound influence on the flowering time in 150 breeding cassava progenitors. The flowering time from six to seven months period was drastically reduced to three to four months in cassava progenitors which were basically the later flowering type. Additionally, pruning (removal of small branches <5 to 8 mm) and application of growth regulators (6-benzyladenine @ 0.5 mM) with extended time of photo period by illuminating with 50 W red LED (625– 635 nm) at night time, profusely set more fruits than the untreated ones (Rodrmguez *et al.,* 2023). From the above investigations, speed breeding has a greater scope in near future considering the time, money and precised results of the crossing programmes.

Cassava, the major starch yielding food crop of tropics especially in alleviating the hunger and poverty in developing nations besides having versatile application in food and feed industry. Being a vegetatively propagated crop, variable populations are very much limited and can be improved through hybridization and mutation breeding. Clonal selection is also one of the efficient methods of developing varieties for industrial expeditions. Many varieties developed through clonal selection has ruled for more than two decades. Precision breeding for desired features may benefit greatly through molecular breeding methods like CRISPR/Cas 9. Speed breeding is another recent approach which shortens the time crop cycle through the manipulation of growing environment. The major focus will be on improving tuber yield, disease and insect pest resistance and enhancing the nutritional value for ensuring the food security. Emphasis must be given for developing varieties exhibiting field tolerance to mosaic disease, red spider mites and cassava mealy bug for sustained tuber yield and starch content. Waxy cassava or amylose free starch is the need of the hour to meet the diversified application in food industries. Researchers, farmers, and policy makers working together is essential to the success of sustainable cassava breeding initiatives.

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