Strategies and Policies for Biotechnology Research in Tropical Root and Tuber Crops

Sujatha, T.P., Senthilkumar, K.M., Krishna Radhika, N. and Sangeetha, B.G.

Established in 1963, ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI), a pioneer research organization under the aegis of Indian Council of Agricultural Research (ICAR), undertakes research and development on tropical root and tuber crops. The mandate crops of the institute are cassava (*Manihot esculenta* Crantz), sweet potato (*Ipomoea batatas* (L.) Lam.), greater yam (*Dioscorea alata* L.), lesser yam (*Dioscorea esculenta* (Lour.) Burk.), white yam (*Dioscorea rotundata* Poir.), elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson), taro (*Colocasia esculenta* (L.) Schott.), tannia (*Xanthosoma sagittifolium* (L.) Schott.), giant taro (*Alocasia macrorrhiza* (L.) Schott.), swamp taro (*Cyrtosperma chamissonis* (Schott.) Merr.), Chinese potato (*Solenostemon rotundifolius* (Poir.) J.K. Morton), yam bean (*Pachyrhizus erosus* (L.) Urban) West Indian arrowroot (*Maranta arundinacea* (L.)), East Indian Arrowroot (*Curcuma angustifolia* Roxb.) and Queensland arrowroot (*Canna edulis* (Ker-Gawler)).

In the remarkable 60 years of its progress, ICAR-CTCRI has released 71 varieties and is recognised as a national repository for germplasm of major tuber crops, maintaining a wealth of germplasm collections of 5588 in total (4337 accessions in 50 tuber crop species in the headquarters, Thiruvananthapuram and 1251 accessions in the Regional Station, Bhubaneshwar). The institute is working on conventional and molecular breeding with international collaborations, various production systems including soil, water and integrated nutrient management (INM) as well as site-specific nutrient management (SSNM), integrated crop protection technologies and crop utilisation comprising post-harvest technologies for value addition, extension programmes for technology adoption and research programmes for students from various universities.

With the challenges such as climate change and declining land area for cultivation, meeting the food requirement of the rising global population with a limited number of mainstream domesticated crops, and having high vulnerability to biotic and abiotic stresses is challenging. Reckoned as 'hidden treasures of the soil, the tropical root and tuber crops such as cassava, sweet potato, yams etc., grown in the tropical and sub-tropical regions of the world are rich sources of carbohydrates, therefore forming the staple food and vital components of food security in resource-limited low-income countries. These crops are climate-resilient, with the ability to survive under marginal environmental conditions such as low fertility soils, drought etc., have high yield potential, lower incidence of insect pests and diseases and produce bioactive compounds. Tropical root and tuber crops are important for sustainable food production acting as supplementary diets and emergency foods and few are also indigenous Traditional Food Plants (TFPs).

Advances in biotechnology and molecular biology tools such as next-generation sequencing (NGS) technologies at a rapid pace, have led to the generation of vast genomic data and various omics technologies such as genomics, transcriptomics, proteomics, metabolomics etc., revealing the immense scope of application of these along with computational biology and bioinformatics tools, to decode major biological processes in crop plants. However, many tropical root and tuber crops are under-utilised when compared to other crops but have great potential for applications. The status of biotechnology research at ICAR-CTCRI and its future prospects are briefly discussed.

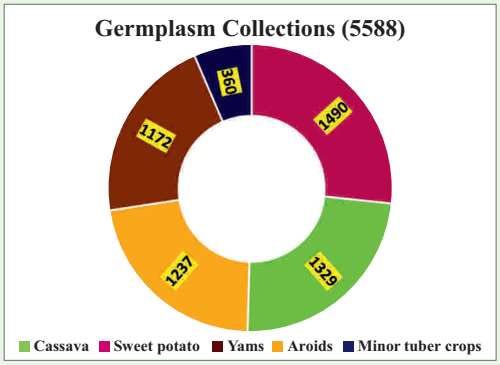
Status of biotechnology research

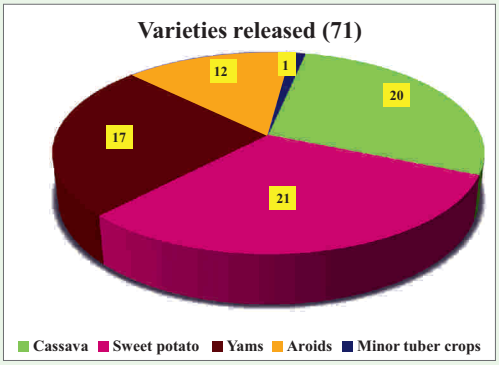
Methods and tools of molecular biology and biotechnology are employed and applied in almost all aspects of conservation, improvement, production, protection and utilisation of tropical root and tuber crops.

Biotechnological tools for crop improvement

Germplasm conservation

ICAR-CTCRI conserves 5588 germplasm accessions covering all tuber crops in the field gene bank (FGB). *In vitro* active germplasm (IVAG) using a core from the FGB is conserved and maintained under *in vitro* conditions in nutrient medium under controlled conditions of temperature, humidity and light. For medium-term conservation (up to 5 years) slow growth media has been standardised for tuber crops (Edison et al., 2006). Sorbitol was better than mannitol in imparting slow and healthy growth in cassava (Unnikrishnan and Sheela, 2000). Addenda like silver nitrate and activated charcoal were effective in maintaining the cultures for up to 18 months (Edison et al., 2006). *In vitro* micropropagation of yams has been standardized (Nair and Chandrababu, 1996). Taro cultures could be stored for up to ten months under light (3000 lux) on MS basal medium containing sucrose and mannitol (3 %) without vitamins or growth regulators. Half-strength basal medium also induced slow growth in taro (Unnikrishnan and Sheela, 2000). Long-term conservation of core collection from huge germplasm is possible in a small area and the cultures are not affected by climatic variations or natural calamities. Micropropagation of tuber crops such as cassava, sweet potato, taro, elephant foot yam, *Xanthosoma*, yams and Chinese potato have been standardised (Asha et al., 2013; Asha et al.,





2016; ICAR-CTCRI Annual Report, 2012-13; ICAR-CTCRI Annual Report, 2017-18). Production and multiplication of virus-indexed plants make the exchange of germplasm easier and also protect the crops from pests and diseases. ICAR-CTCRI has also successfully standardized protocols for the conservation of pollen grains from cassava and taro through cryopreservation technique at an ultra-low temperature of -196°C.

Molecular characterization of germplasm for distinguishing accessions

Morphologically similar plants may be genetically distinct. Molecular markers such as simple sequence repeats (SSRs) and inter simple sequence repeats (ISSRs) are employed for molecular diversity analysis of the germplasm and to characterise as well as distinguish germplasm accessions so as to maintain core germplasm and eliminate duplicates from the germplasm.

DNA fingerprinting

Each plant is genetically unique and the genetic identity of a plant is determined by DNA fingerprinting, which also helps a farmer or a scientist or any other stakeholder to register his plant variety under the PPV&FRA (Protection of Plant Varieties and Farmers Rights Act) and to settle disputes and claims of right over varieties developed. ICAR-CTCRI has generated DNA fingerprints of most of the varieties of cassava and yams developed at the institute.

Bioprospecting of tuber crops

ICAR-CTCRI has a rich genetic resource of tuber crops which opens avenues to discover and identify novel traits, which are otherwise unexplored from tuber crops. Coloured and aromatic tubers identified from the germplasm are being evaluated for anti-angiogenic, antimicrobial and anti-metastatic effects through a structured set of biochemical and molecular assays. Tuber crops with high antimicrobial effects were identified.

Development of genetic and genomic resources

Genetic and genomic resources are pre-requisite for the identification of trait-specific QTLs/genes/alleles/*cis*-regulatory sequences. Mapping populations are developed for mapping various traits viz., high starch, cassava mosaic disease (CMD) resistance, drought tolerance and post-harvest physiological deterioration (PPD) tolerance in cassava. High-quality draft genome assembly of two cassava genotypes, Sree Kaveri and 9S-127, were developed through whole-genome sequencing. Analysis of these draft genome sequences revealed the presence of a large number of SNPs and InDels which can be utilized to identify and develop molecular markers linked to important agronomic traits including high starch, profuse flowering and CMD resistance. The high-quality draft assembly can be used for mining SSRs and the development of molecular markers for marker-assisted backcross breeding in cassava.

Genome-wide identification resulted in the identification and characterisation of various abiotic stress-responsive gene families viz., *Mehsp20*, *Mehsp70*, *MeMADS*, *MeNAC* and *MeGRAS*. Transcriptome sequencing studies were initiated to identify and understand the roles of genes involved in various traits including drought tolerance, PPD tolerance and early bulking in cassava and tuber development in sweet potato.

Varietal improvement through transgenics and genome editing

There is always a demand for improved varieties with higher yield, disease resistance, better morphological and physiological attributes, climate resilience and improved quality attributes like high starch, modified starch, higher nutrient contents, low antinutrients and nutrient use efficiency. Biotechnology has been applied for varietal improvement over decades. Transformation strategies have been standardized using *Agrobacterium* in cassava and sweet potato. Friable embryogenic callus, the best-reported transformation system in cassava has been developed in Indian cultivars like H-226 and 9S-127 (ICAR-CTCRI Annual Report, 2015-16). Transgenic experiments as well as gene silencing using RNAi technology for developing improved cassava varieties with modified starch were attempted until the advent of gene editing technology. In 2020, a gene editing project was initiated to develop cassava with waxy starch.

Biotechnological tools for crop protection

1. Biotechnological approaches for the management of viruses infecting tuber crops

a. Molecular diagnostics tools

Primers specific to cassava mosaic viruses [*Indian cassava mosaic virus* (ICMV) and *Sri Lankan cassava mosaic virus* (SLCMV)] full-length coat protein (CP), replicase (AC1), movement protein (MP) and partial coat protein (CP1) were designed to detect/diagnose the cassava mosaic virus infection in cassava. The real-time PCR assay for the quantitative assessment of DNA A and DNA B genomic components of cassava mosaic virus in different cassava genotypes was optimised and analysed using absolute quantification experiments. DNA-B components accumulated in genotypes with severe disease symptoms. In elephant foot yam (*Amorphophallus paeoniifolius*), the coat protein gene of the *Dasheen mosaic virus* (DsMV) was characterised by RT-PCR using the coat protein (CP) gene and the 3′ untranslated region (UTR). A reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay based on the coat protein gene was developed for DsMV. About 20 viruses in traces along with DsMV were revealed by the whole transcriptome sequencing of virus-infected elephant foot yam (Kamala et al., 2015). Serological and nucleic acid-based techniques or protocols were standardized for the diagnosis of yam mild mosaic virus, yam mosaic virus, yam mottling virus and yam badna virus. Nanopore-based detection (MinION sequencing approach) and characterization of yam viruses were done (Filloux et al., 2018). Detection and identification of DsMV which infects *Colocasia esculenta* in India were done using Potyvirus group-specific primers (327 bp), designed to amplify the core region of the coat protein gene.

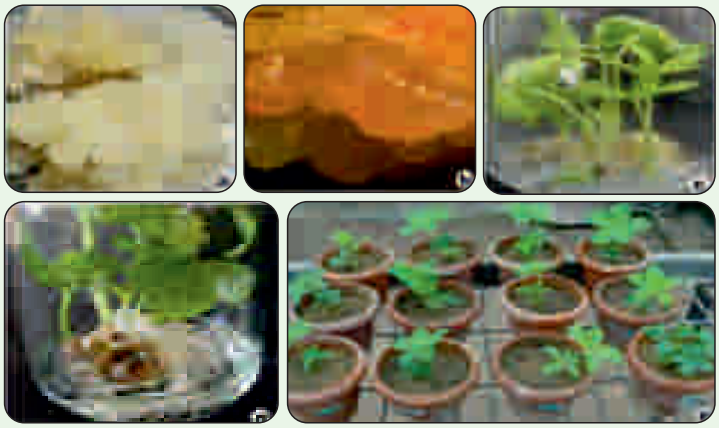
b. Gene regulation and other basic studies

Small RNA (sRNA) mediated gene regulation during SLCMV infection was studied from the Indian cassava cultivar H226. The mes-miR9386 was detected as the most prominent miRNA expressed in the control and infected leaf, while mes-miR156, mes-miR395 and mes-miR535a/b showed significant downregulation in the infected leaf (Asha et al., 2023). A hairpin construct of DsMV (DsMV-hp) was designed and its expression was assessed in a model host, *Nicotiana benthamiana*. The construct provided complete resistance towards the DsMV upon challenge inoculation of transgenic lines. An efficient *Agrobacterium*-mediated transformation protocol was established with *GUS* reporter gene for developing resistance in elephant foot yam. The transgenic elephant foot yam expressing the DsMV-hp construct is expected to be completely resistant to DsMV. The complete genome sequence of DsMV of elephant foot yam was assembled by transcriptome sequencing.

c. *In vitro* protocols and methods

Somatic embryogenesis and friable embryogenic callus (FECs) were produced in Indian cassava cultivars (H-226, H-165, Sree Vijaya, Sree Sahya) preferred by farmers (Dhanya et al., 2017). A high-frequency *in vitro* mass propagation protocol was developed for elephant foot yam, using corm bud, leaf and petiole explants. The corm bud explant was found more suitable for mass production due to its high callusing and less time required for plantlet establishment (Kamala and Makeshkumar, 2015).

Tissue-culture-based protocols were developed for generating virus-free plants for yam mild mosaic virus and sweet potato feathery mottle virus



Virus elimination in elephant foot yam

a. Callus phase, b. Somatic embryos (bar=2 mm), c. Shoot clusters regenerated from callus, d. *In vitro* rooting in liquid medium. e. Hardened virus-free plants maintained in net house.

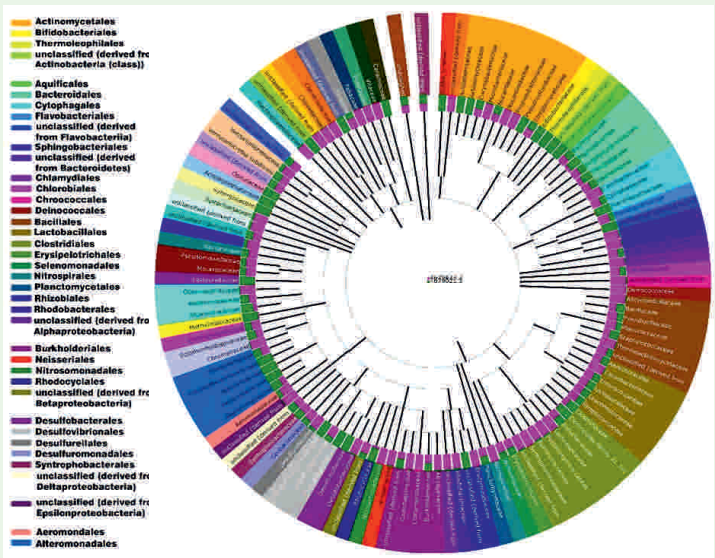
2. Biotechnological approaches for the management of fungal pathogens of tuber crops

Diagnosis of fungal pathogens causing cassava tuber rot such as *Phytophthora palmivora, Sclerotium rolfsii* and *Colletotrichum gloeosporioides* was standardised using molecular techniques like polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), nucleic acid spot hybridisation (NASH) and quantitative PCR (qPCR) using leaves, tubers and soil. Many potential fungal and bacterial biocontrol agents along with growth-promoting characters have been identified against major pathogens infecting tuber crops. Twenty-seven *Trichoderma* isolates (*Trichoderma asperellum*, *T. erinaceum* and *T. longibrachiatum*) which showed growth promotion and pathogen suppression, were characterized by sequencing internal transcribed spacer (ITS) and translation elongation factor EF-1 alpha (TEF1) regions. Based on field trials, *T. asperellum* was recommended to manage *Sclerotium rolfsii* and *Phytophthora colocasiae*. Sixteen isolates of *Bacillus* viz., *Bacillus subtilis* (2 isolates), *B. amyloliquefaciens* (6 isolates), *B. cereus* (2 isolates), *B. siamensis* (2 isolates), *B. pumilus*, *B. halotolerans*, *B. altitudinus* and *B. licheniformis* with pathogen suppression and growth promotion were isolated from tuber crops ecosystem and identified by 16S rRNA sequencing. Field studies were taken up with *Bacillus subtilis, B. amyloliquefaciens* and *B. licheniformis* against *Sclerotium rolfsii* and *Phytophthora colocasiae.*

3. Biotechnological tools for pests infesting tuber crops

A new biotype of whitefly, *Bemisia tabaci* as a pest of cassava was reported for the first time in the year 2018. Biotype-specific ISSR primers for *B. tabaci* were screened and molecular identification of different pests of tuber crops viz., *B. tabaci, Ferissia virgata, Aleurodicus disperses, Aleurodicus rugioperculatus, Paracoccus marginatus* and *Bedellia somnulentella* were carried out. Next-generation sequencing of the endosymbionts of *Bemisia tabaci* and *F. virgata* was carried out (Harish et al., 2019). The role of endosymbionts in the virus transmission ability of whiteflies was identified. Gene-specific protease inhibitor primers for *Ipomoea sp.* were screened and variation in the expression of protease inhibitor genes was studied, with respect to sweet potato weevil infestation.

Barcoding of *Bedellia somnulentella* infesting sweet potato



Phylogenetic tree of endosymbiotic bacteria of *Bemisia tabaci* from two cassava biotypes

Bioinformatics

The miRNAs in cassava genome with potential targets in the cassava mosaic virus genome were identified by *in silico* tools and 14 cassava miRNA families had putative targets in cassava mosaic virus with nearly perfect complementarity (Haridas et al., 2020). Forty genes involved in β-carotenoid biosynthesis or metabolism pathway were identified in cassava by *in silico* comparative genomic analysis of the genes encoding key enzymes of apocarotenoid biosynthesis using databases of plants such as *Arabidopsis*, tomato, potato and sweet potato. Three microRNAs, namely miR159a, miR171b and miR396a were also observed to be associated with the regulation of β-carotenoid biosynthesis in cassava (Sreekumar et al., 2022).

Way forward

Biotic stress due to viral diseases and pests, deleterious mutations due to clonal propagation, genetic or breeding barriers, limited genomic information or lack of knowledge of the genetic, molecular and physiological basis of key agronomic traits, limit the productivity and genetic improvement of the tropical root and tuber crops. Tropical root and tuber crops are also low in essential nutrients such as proteins, minerals and vitamins and contain antinutritional factors.

Germplasm characterisation, conservation and utilisation

Molecular characterisation, genetic diversity and phylogenetic analysis of vast germplasm collection will be carried out using molecular markers such as ISSRs, SSRs, SNPs etc. DNA barcoding will be employed for identifying and distinguishing germplasm, varieties or new species using nuclear internal transcribed spacers ITS1, ITS2 on either side of 5.8S ribosomal unit, or chloroplastic *rbc*L or *trn*H-*psb*A intergenic sequence. *In vitro* culture techniques such as cell, tissue and organ culture which includes meristem and callus culture, direct and indirect organogenesis and somatic embryogenesis will be employed for large-scale production of healthy and disease-free planting material and for the maintenance, multiplication and preservation of germplasm accessions of all mandate tuber crops *in vitro*. Somaclonal variations can be employed for creating variations in germplasm where it is lacking. Novel genes and pathways involved in the synthesis of bioactive or commercially important compounds can be isolated or prospected from the germplasm, especially for under-utilised minor tuber crops.

Generating genomics or functional genomics resources

Genome sequences are not available for the tropical root and tuber crops except cassava, sweet potato, greater yam, white yam, taro and draft genome assembly of yam bean (Prochnik et al., 2012; Bredeson et al., 2016; Yang et al., 2017; Sugihara et al., 2020; Tay Fernandez et al., 2021; Yin et al., 2021; Bredeson et al., 2022). Genomic or functional genomic studies are lacking in tropical root and tuber crops. The African Orphan Crop Consortium (AOCC), an international effort established in 2011 to reduce malnutrition had identified 101 African orphan crops including a few tuber crops such as cassava, sweet potato, yam, cocoyam, yam bean, potato and African potato for sequencing, assembling and annotating the genomes to generate genomic resources such as reference genome sequence, transcriptome sequence, and re-sequencing 100 accessions/species, using next-generation sequencing (NGS) technology through a network of international-regional-public-private partnerships and academic collaborations (Dawson et al., 2019). Such collaborations at the national and international level will be developed at ICAR-CTCRI to generate whole genomic sequence data of major and minor tuber crops of economic significance. T-DNA insertional mutagenesis, a functional genomics tool can be used to create T-DNA tagged lines in the tuber crops where genome sequences are available so that the functional genomic resources can be used to identify, isolate and clone the genes and promoters from tropical root and tuber crops. Most tropical root and tuber crops are polyploids and vegetatively propagated. Epigenetic mechanisms, comparative transcriptome and proteome studies, functions of small RNAs and miRNA regulation of major biological pathways affecting yield and quality attributes in tropical root and tuber crops can also be studied.

Comparative 'omics' and bioinformatics

Understanding the molecular mechanisms governing several biological processes such as storage root development, nutrient metabolism and uptake, response to biotic and abiotic stresses and mechanisms of virus or fungal resistance are not well-studied in root and tuber crops compared to that of cereals and potato, although comparative transcriptome and proteome studies were undertaken in cassava and sweet potato by international research groups. *In silico* analysis of the genome and transcriptome sequences for genes, promoters, molecular markers, regulatory molecules and structural variations in the genome using various bioinformatics tools available online will provide useful leads in delineating the regulatory components of a metabolic pathway. Thus, through a comparative approach and through the integration of omics tools such as genomics, transcriptomics, proteomics, metabolomics and epigenome, valuable information on genes controlling important traits and several basic biological processes can be discovered or identified, in addition to creating functional genomic resources.

Molecular breeding and marker-assisted selections

Allele mining, association mapping, map-based cloning of QTLs, marker-assisted selection and genomic selection are some of the methods used to identify alleles or genes linked to a trait. Molecular markers can be used to characterise germplasm and to introgress a desirable trait into elite lines or breeding stock or pre-breeding lines or germplasm for their genetic improvement. Simple sequence repeats (SSRs) and inter simple sequence repeats (ISSRs) are routinely used to characterise the genetic and molecular diversity of germplasm. Genes linked to a trait can be identified by genome-wide association studies (GWAS) by scanning the entire genome sequence for variations in nucleotide sequence or single nucleotide polymorphisms (SNPs) associated with a particular trait. Genomic selection (GS) or genomic prediction employing high-density SNPs/SSRs can be used for higher genetic gains, where the time required for progeny testing is shortened in breeding programs.

Genetic modification and genome editing

Genetic barriers such as high heterozygosity, allopolyploidy, erratic flowering, poor seed set and accumulation of deleterious mutations in clonally propagated progeny are major impediments to the development of varieties in tropical root and tuber crops through conventional breeding. Variability is also less in the germplasm of tropical root and tuber crops for traits such as mineral nutrients and therefore germplasm screening for potential gene sources for improvement through conventional breeding is futile (Elegba et al., 2021; Sugihara et al., 2020). Genetic engineering through the introduction or overexpression of exogenous genes or genome or gene editing through modification or knockout of endogenous gene sequences using CRISPR/Cas 9 (Clustered regularly interspaced short palindromic repeats / CRISPR-associated protein 9) is the only feasible alternative. Transformation and *in vitro* regeneration protocols are already standardised for major tropical root and tuber crops such as cassava, sweet potato etc. Direct or indirect, clean (preferably with minimal exogenous DNA and marker-free) and highly efficient transformation protocols can be developed for major tropical root and tuber crops and other minor tuber crops. Also, vector constructs for multiplex gene editing and trait-stacking for developing varieties with multiple traits and visual or easily identifiable markers for screening of transformants or marker-free transgenics can be developed .

Traits of tropical root and tuber crops for research focus

The molecular mechanism, regulation and the genes or proteins involved in the following traits can be identified, although efforts have already been initiated and are underway. Tropical root and tuber crops can be used as valuable sources of genes.

Tuberisation and yield

Tropical root and tuber crops are diverse in habitat and belong to different families. The mechanism of tuberisation, genes and promoters responsible for tuberisation, tuber size and the number of tubers can be identified and compared among various species of tuber crops.

Crop duration and plant type

Some of the tropical root and tuber crops such as cassava, yams and

aroids are perennials cultivated as annuals and have long duration such as 10-12 months. The duration of the crop can be decreased without compromising the yield. Sweet potato and yams are vines or creepers or climbers requiring an artificial support system for trailing. The production costs for erecting artificial support systems in climbers can be reduced using non-trailing and dwarf plant types.

Nutritional quality

Tropical root and tuber crops such as cassava though rich in carbohydrates, have very low protein, essential amino acids, vitamins and minerals. Most tropical root and tuber crops contain anti-nutritional factors such as cyanogenic glucosides, alkaloids, phytates, oxalates, phenols and tannins which need to be removed or reduced to a safe level. Biotechnological approaches will be employed to understand the mechanism of nutrient metabolism or nutrient homeostasis in the tubers and to identify the genes and the regulatory pathways involved, so that such genes can be introgressed or introduced or edited for the biofortification of tuber crops. Such biofortified nutritionally enriched tropical root and tuber crops with high protein, iron, zinc as well as vitamin A content and reduced levels of antinutrients, can address the issue of malnutrition (micronutrient and protein-energy) or "hidden hunger" arising due to the consumption of the tubers as a staple food. Efforts are already initiated at ICAR-CTCRI to modify the starch quality and create waxy cassava with low or no amylose through gene editing.

Modified starch and starch quality

Tropical root and tuber crops are starchy crops and tuber starch is an important nutrition and dietary component as well as industrial raw material. Modified starches especially from cassava have much demand from cassava-based industries. Starch from taro is used as a weaning food and arrowroot starch has medicinal properties as well. Resistant starch (resistant to digestion by amylases) from tuber crops is promising as the future food to prevent diabetes and high cholesterol. A range of starches with variable proportions of amylose and amylopectin can be developed through gene-editing approaches. Efforts are already initiated at ICAR-CTCRI to modify the starch quality and create waxy cassava with low or no amylose through gene editing. The amylose content of cassava tubers is nearly 20 %. Increasing the amylose content through gene editing will enable the development of resistant starch.

Nutrient use efficiency

Most tropical root and tuber crops can come up in marginal soils with low fertility, but for better yield, nutrients are supplied as fertilisers. Potassium is "the element of quality" and the micronutrient zinc also determines tuber yield and quality. Potassium and zinc nutrition also reduces cyanogen content in cassava tubers. Understanding the molecular regulatory pathway of major, secondary and micronutrients as well as the nutrient interactions and identifying the genes responsible for nutrient use efficiency in nutrient use efficient genotypes, will enable the incorporation of these genes into less nutrient use efficient ones. Thus, fertiliser input can be reduced, thereby reducing the expenditure and wastage of fertilisers, which otherwise will have serious impact on the soil and environment. Molecular characterisation of soil microbial population through 16S ribosomal or ITS sequencing or metagenomics can be used to identify and understand the role of soil microbes such as plant growth-promoting microbes (PGPMs) in growth, tuberization, tuber characteristics, nutrient uptake and nutrient metabolism. The identified microbes or microbial consortium can be used as biofertilisers for efficient uptake and use of soil nutrients.

Abiotic stress tolerance

Tropical root and tuber crops are considered climate-resilient having an innate ability to tolerate adverse environmental conditions such as drought. The habitats of tropical root and tuber crops are also diverse. Cassava can tolerate drought or water stress, while taro which grows well in marsh or swampy regions can tolerate excess water. Genes and molecular mechanisms responsible for tolerance to extreme conditions of water stress (flooding/ drought), temperature (heat/ cold), salinity and soil pH (acidic/ alkaline) can be identified from the genotypes with such traits.

Host-pathogen / pest interactions and plant-soil / microflora interactions

Tropical root and tuber crops are infected and infested with some serious bacterial, fungal and viral diseases, and pests that affect crop productivity, tuber quality and post-harvest storage or shelf life (eg. sweet potato weevil and post-harvest fungal rot in elephant foot yam and cassava). Molecular diagnostic kits for many viral diseases in major tuber crops are already developed. Through studies on molecular mechanisms of interactions between the pest/pathogens, novel genes and pathways of tolerance or resistance to major or emerging pests such as cassava mealy bug can be identified and better control strategies devised. Research is already in progress at ICAR-CTCRI for the identification of genes for the production of semiochemicals responsible for sweet potato weevil infestation from sweet potato, as well as the identification of the proteins and chemical compounds responsible for the mortality of sweet potato weevil from wild species *Ipomoea mauritiana.* The interaction of plants or host with the soil microflora can be identified to promote beneficial interactions and mitigate harmful ones.

Post-harvest storage and quality

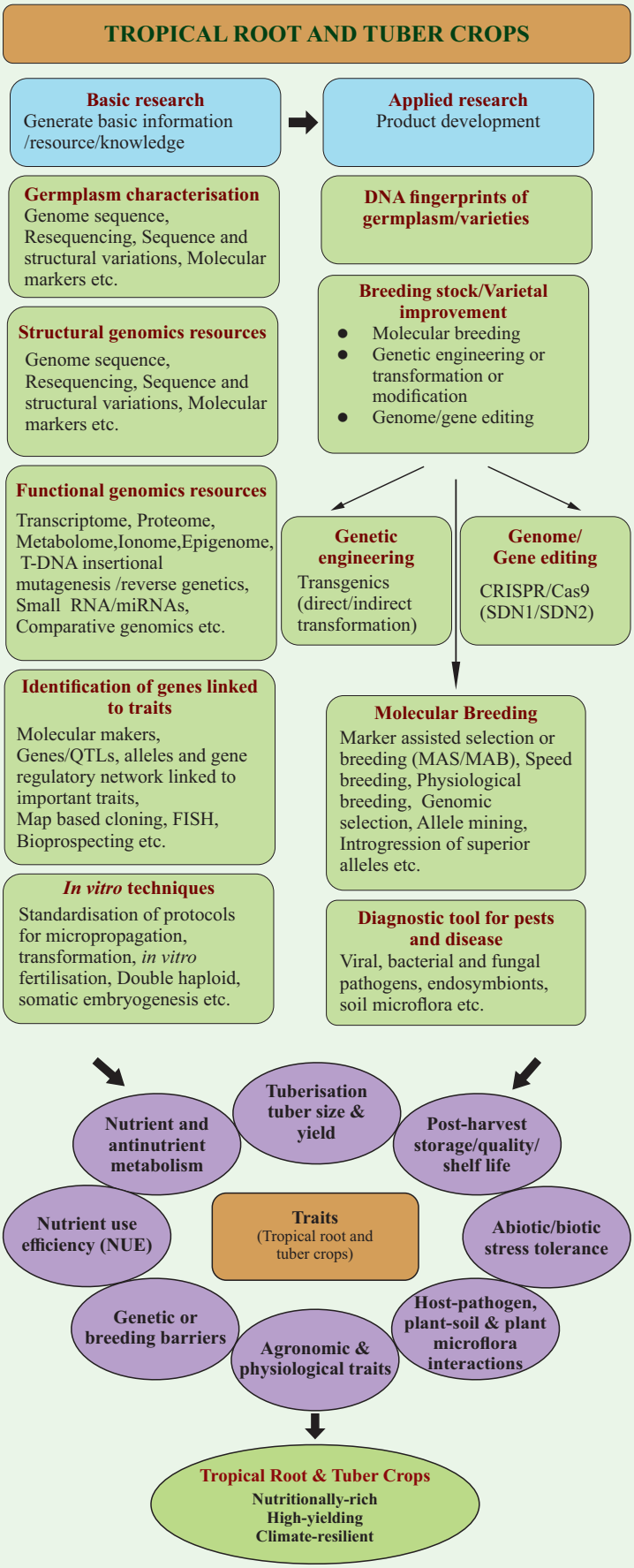
Understanding the molecular mechanism of post-harvest physiological deterioration (PPD) (most of the genes are known in cassava) in crops such as cassava and elephant foot yam and knocking out the upregulated genes responsible for PPD can enhance shelf life and storage quality. Similarly, post-harvest loss due to pests and diseases can be controlled. Research work is already initiated to develop sweet potato weevil-resistant genotypes through genome editing.

Breeding or reproductive barriers

Tropical root and tuber crops are mostly vegetatively propagated and deleterious mutations that accumulate due to clonal propagation are detrimental to crop improvement. Varying levels of polyploidy, high heterozygosity, irregular flowering, sterility, self-incompatibility, low seed set etc., are major genetic barriers in tropical root and tuber crops. Investigations into molecular aspects of the reasons for genetic barriers can help to devise strategies to overcome the breeding or genetic barriers.

Gene prospecting or bioprospecting

Tropical root and tuber crops such as yams, aroids, arrowroot, *Curcuma* and certain other tuber crops are rich sources of bioactive compounds which have nutritional, pharmaceutical and industrial applications. Many tropical root and tuber crops are used in traditional medicine as well due to their medicinal properties and are potential sources of novel metabolic pathways and the associated genes or biomolecules. Identification and characterisation of such novel genes and biomolecules involved in the synthesis of such bioactive compounds can be carried out for better utilisation of these crops.



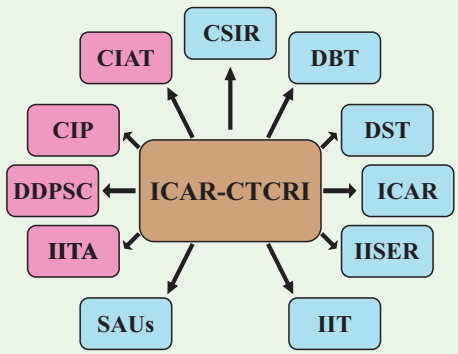
SDN: Site-directed nuclease
SDN1: Site-directed mutagenesis without using DNA template
SDN2: Site-directed mutagenesis using DNA template

National biotechnology policy and tuber crops research

The government of India has formulated a 'National Biotechnology Development Strategy' for knowledge and innovation-based bio-economy and these strategies and policies for the year 2021-2025 are focused on capacity building in terms of infrastructure and human resources, creating a strong basic research innovation system in both public and private research laboratories and institutes, promoting translation and commercialisation of products developed with an emphasis on the balance of basic and translational biotechnology research, to build start-ups, industrial and entrepreneurial base, and to make India a global manufacturing hub for innovative products in the global market. ICAR-CTCRI uses biotechnology tools in crop improvement and protection to develop improved varieties with desirable agronomic traits. The institute has an Institute Biosafety Committee (IBSC) for monitoring the biosafety aspects of research especially transgenic research or genetic modification of crops and also a techno-incubation centre for training entrepreneurs and farmers on the technologies developed by the institute. Also, training is provided as part of the research work to students from various universities. ICAR-CTCRI is a recognised research centre of many universities to undertake post-graduate and PhD research programmes. Basic research on the molecular mechanisms governing important traits such as biotic and abiotic stress tolerance and nutritional quality are undertaken along with an emphasis on translational research such as developing molecular diagnostic kits, genetic transformation and gene editing for pest and disease resistance as well as nutritional quality. The future thrust of biotechnology research in the institute aligns with the biotechnology policy of the country to achieve excellence in 'food and nutrition', one of the major missions of national biotechnology programmes.

Prospective collaborations

ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI) shall collaborate with various national and international research institutes in the field of molecular biology and biotechnology to achieve its goals in various aspects of improvement, protection, production and utilisation of its mandate tropical root and tuber crops. Collaborative research efforts will be undertaken with various institutes under ICAR, CSIR, DBT, DST, IISERs, IITs, SAUs and other universities as well as private biotechnology service firms at the national level. At the international level, ICAR-CTCRI will develop close associations with CGIAR institutes such as CIP, CIAT and IITA, Donald Danforth Plant Science Centre, USDA and other universities of repute working in the area of tuber crops.



CSIR: Council for Scientific and Industrial Research, DBT: Department of Biotechnology, DST: Department of Science and Technology, ICAR: Indian Council of Agricultural Research, IISER: Indian Institute of Science Education and Research, IIT: Indian Institute of Technology, SAUs: State Agricultural Universities, CIAT: International Centre for Tropical Agriculture, Colombia, CIP: International Potato Centre, Peru, DDPSC: Donald Danforth Plant Science Centre, Missouri, USA, IITA: International Institute of Tropical Agriculture, Nigeria

Target for the next 5 years

Year				
2023-2024	2024-2025	2025-2026	2026-2027	2027-2028
■ Identification and characterization of abiotic stress response genes in cassava. ■ Gene editing for waxy (low or no amylose) cassava. ■ Identification of resistant genes in *Ipomoea mauritiana* responsible for the antixenosis effect on sweet potato weevil. ■ Identification of the proteins and chemical compounds responsible for the mortality of sweet potato weevil from wild species *Ipomoea mauritiania.*	■ Identification of genes/ proteins/involved in protein and vitamin A homeostasis in cassava tubers. ■ Gene editing in cassava for waxy starch and high amylose starch. ■ Validation of the resistant genes in *Ipomoea mauritiana* against sweet potato weevil. ■ Validation of the insecticidal proteins from *Ipomoea mauritiana* against sweet potato weevil.	■ Identification and functional characterisation of genes associated with early bulking in cassava and sweet potato. ■ Understanding the molecular mechanisms of nutrient metabolism/ homeostasis in tubers. ■ Gene editing in cassava for high amylose starch & acyanogenic cassava. ■ Identification of genes in sweet potato responsible for the production of semiochemicals for sweet potato weevil infestation. ■ Validation of the genes in sweet potato responsible for the production of semiochemicals for sweet potato weevil infestation.	■ Identification and association of allelic variations (SNPs/Indels) associated with genes responsible for early bulking in cassava as well as abiotic stress response and tuberisation in sweet potato, to develop molecular markers. ■ Identification of genes involved in iron and zinc homeostasis in tubers. ■ Waxy cassava variety through gene editing ■ Elucidation of the genetic pathway responsible for the production of semiochemicals from *Ipomoea batatas* related to sweet potato weevil infestation. ■ Elucidation of the genetic pathway responsible for the production of insecticidal compounds from *Ipomoea mauritiana* related to antixenosis effect.	■ Gene editing for biofortification of cassava (protein and vitamin A). ■ High amylose cassava variety through gene editing ■ Gene editing for acyanogenic and post-harvest physiological deterioration -(PPD) resistant cassava ■ Gene editing for developing sweet potato - weevil resistant genotypes in sweet potato. ■ Publication in high-impact factor journals (NAAS rating: >7-12).

Conclusion

Tropical root and tuber crops are the 'future crops', vital to ensure food and nutritional security of resource-poor regions of the world.

Through conventional breeding, cassava varieties with resistance to cassava mosaic disease (Sree Reksha, Sree Sakthi, Sree Suvarna and Sree Padmanabha) and PPD tolerance (Sree Reksha) have been developed with tuber yield of 35-40 t/ha but having maximum potential yield of 70-80 t/ha. Similarly, the maximum average yield and maximum potential yield of the released varieties is 20-25 t/ha and 37 t/ha (H-41) respectively, for sweet potato and 25-35 t/ha and 55 t/ha (Sree Roopa) respectively, for greater yam. Early or short duration varieties of cassava (Sree Jaya, Sree Vijaya and Sree Prakash) have 6-7 months crop duration when compared to 9-10 months, while for sweet potato, early varieties (Sree Bhadra, Sree Rethna, Sree Nandhini and Sree Vardhini) had a 90-105 days duration when compared to 120 days and for yams early varieties with 6-7 months duration(Orissa Elite and Bu Swar) when compared to normal duration of 9-10 months, have already been released (Mukherjee et al., 2019).

For nutritional traits such as β carotene content, varieties were developed through conventional breeding in cassava (Sree Visakham with 466 IU/ 100g carotene) and in sweet potato such as Sree Rethna (3200-3500 IU /100g), Gouri (7500-9100 IU /100g), Bhu Ja (9160-10670 IU/100g), Bhu Kanti (10833 IU/100g), Sree Kanaka (14666 -16666 IU /100g) and Bhu Sona (19100-20800 IU/100g), the β carotene content being expressed in terms of fresh weight of tubers (Mukherjee et al., 2019). Through transgenic approaches, the highest levels of total carotenoids achieved in cassava roots were 60 µg/g dry weight (Jaramillo et al., 2022). The anthocyanin-rich sweet potato variety Bhu Krishna contains nearly 90 mg/100g anthocyanin. Also, the cassava varieties with high starch content such as Sree Harsha (38-41%), Sree Athulya (30.2%), Sree Apoorva (29.9%) and with medium starch content Sree Reksha (27-31%) and Sree Suvarna (25-27%) were developed and released by the institute. In yams, varieties with low starch, medium to high protein content and vitamins such as Sree Swathy (high protein content of 16.94% dry weight and ascorbic acid content of 6.9 mg/100g dry weight) were released through conventional breeding. Sree Prakash is a cassava variety with low cyanogen content of 30-50 ppm (Mukherjee et al., 2019).

The biotechnological approaches of crop improvement aims to achieve the potential yield of the tropical tuber crops or increase the average yield by a factor of two, reduce the crop duration to 4-5 months in cassava, 7-8 months in yams and 2-2.5 months in sweet potato, increase the nutritional content such as β-carotene and protein content in cassava by 20 %, develop resistant starch with high-amylose by increasing amylose content by 10 %, nullify the amylose content in starch (amylose-free or waxy cassava) and cyanogen content (to 0 ppm) in cassava tubers and develop tropical tuber crops resistant to major and emerging pests and diseases as well as tolerant to abiotic stress factors such as drought, flooding and salinity.

Basic research is essential to generate the basic knowledge or information on the molecular regulatory pathways of key traits of tuber crops which can be applied to develop superior or elite genotypes with desirable traits. Application of biotechnology and molecular biology tools to understand the basic molecular mechanisms governing major biological processes is imperative for crop improvement, to achieve the goal of high-yielding, nutritionally rich and climate-resilient genotypes of tropical root and tuber crops with multiple traits, but without compromising the yield.

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Policy Brief

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(भारतीय कृषि अनुसंधान परिषद्)
श्रीकार्यम, तिरूवनन्तपुरम 695 017, केरल, भारत

ICAR-Central Tuber Crops Research Institute
(Indian Council of Agricultural Research)
Sreekariyam, Thiruvananthapuram 695 017, Kerala, India
Tel. No. : 91 (471)-2598551 to 2598554; E-mail: director.ctcri@icar.gov.in, Website: https://www.ctcri.org