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Chapter

Emerging Trends to Improve Tropical Plants: Biotechnological Interventions

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Abstract

Tropical plants are an integral part of the ecosystem and are of significance for the well-being of humanity. Since their domestication in 10,000 BC, conventional breeding has played a crucial role in their conservation and widespread adaptation worldwide. Advancements in multi-omics approaches, that is, genomics, metabolomics, transcriptomics, proteomics, whole genome sequencing, and annotation, have led to the identification of novel genes involved in crucial metabolic pathways, thus helping to develop tropical plant varieties with desirable traits. Information retrieved from the pan-genome, super-pan-genome, and pan-transcriptome has further uplifted marker-assisted selection and molecular breeding. Tissue culture techniques have not only helped to conserve endangered plant species but have also opened up new avenues in terms of mass-scale propagation of ornamental plants. Transgenic technology is increasingly contributing to the betterment of tropical plants, and different plant species have been engineered for valuable traits. Likewise, genome editing is appearing to be a promising tool to develop tropical plants having the potential to fulfill future needs. Hence, this chapter highlights the importance of conventional and modern scientific approaches for the conservation and improvement of tropical plant species.

Keywords: tropical plants, ornamental, medicinal, value addition, biotechnological interventions, genetic engineering, domestication, tissue culture, endangered species

1. Introduction

Tropical plants are those that grow in warm climates. They typically grow best in areas where the temperature ranges consistently between 75 and 85 degrees Fahrenheit (24 and 29 degrees Celsius). The term “tropical” is used for a variety of plants, from palms to orchids. Tropical plants often have thick leaves that are waxy or shiny to help reduce evaporation and protect them from too much light. These plants also have flowers with colorful petals or leaves that attract pollinators like butterflies [1].

Tropical plants are among the most colorful and beautiful of all plant species. They are very diverse, ranging from small herbaceous plants to tall trees. Many tropical plants have been introduced in areas outside their natural range because they can tolerate colder temperatures than other species of the same genus or family. Tropical plants grow in a wide range of habitats, from the equator to the tropics. Tropical plants thrive in warm weather and grow in rainforests and deserts, and even on mountainsides [2]. These plants have adapted to their environment over time by developing unique features that help them survive in hot climates without suffering from heat stress or lack of moisture. Some tropical plants have thick stems that provide support during strong winds, while others still use their roots as storage organs called tubers or rhizomes that store nutrients until they are needed by the plant itself or by animals or humans [3].

The tropics have been a source of many new food crops that have been introduced to other regions of the world: sorghum (which originated in Africa, Winchell, [4]), soybean (from China, Singh, [5]), mung bean (from China, Shahrajabian, [6]), peanut (from South America, Ozias-Akins, [7]), cassava (from Brazil, Bester, [8]), lima bean, and fava bean (from Peru, [9] Santos, 2008). Several plant families have their center of diversity in the tropics. These include *Arecaceae* (palms), *Lamiaceae* (mint family), *Lauraceae* (laurel family), and *Rubiaceae* (coffee family). The largest family by the number of species is the *Orchidaceae*, with about 25,000 species.

Because most living forms thrive here, the tropical zone is home to the majority of plant and animal species in the world. The domestication of food crops is one of the most important developments in human history that made it possible for people to settle down and live in communities rather than wandering around with their herds and flocks.

2. Tropical plants and ecosystem

The tropics are usually defined as the area between the Tropic of Cancer and Tropic of Capricorn bounded by lines of latitude running through the Arctic and Antarctic circles, which is 23.5 degrees north and south of the equator, respectively. This area spans among Central America and South America, Africa, India, and Southeast Asia. Some tropical plants can also be found in subtropical regions such as California. The region lies beneath the intertropical convergence zone (ITCZ), a belt where tropical cyclones often form during their lifetime before moving into more temperate latitudes where they die out. However, they can be found year-round in some areas as well as at higher altitudes inside the tropics—like in Indonesia or Ecuador [10].

Tropical climates generally do not experience marked changes in temperature throughout the year, with average temperatures ranging from 18 to 24°C (64 to 75°F). The average annual rainfall varies greatly from region to region within this climate zone, with some areas receiving more precipitation annually, while other areas may receive less than that. Humidity is high throughout these regions, and climatic conditions are relatively constant throughout the year. Within tropical regions, distinct wet and dry seasons are caused by changes in the direction of wind flow that result in periods of more intense (wet) or weaker (dry) rainfall. The length and onset dates for wet and dry seasons vary by the geographic location within tropical zones [11].

The climate in the tropics is characterized by high temperatures and a nearly constant rate of evaporation. The evaporation results in a higher concentration of

water vapor in the air, which produces high relative humidity. The high temperature reduces the capacity of the air to hold water vapor so that even though there is high humidity, the air remains very dry. High temperatures also cause a lot of water to evaporate from the ocean's surface, which make it rain more on land and make the air even more humid [12]. The tropics are characterized by wide diurnal temperature variations with large day–night differences (diurnal range) and small annual ranges. The diurnal range is due to a strong daily heating-and-cooling cycle as well as large daily fluctuations in solar radiation on land surfaces (high solar intensity). Also, there are no big changes in the way the atmosphere moves, like seasonal winds or changes in cloud cover, that would have a long-term effect on the surface temperature, like months [13].

Tropical plants have a significant impact on the ecosystem. The tropics contain more than half of the world's species, and the majority of plants and animals live in this region. Tropical rain forests are vital to the planet because they act as carbon sinks and produce oxygen, which helps to regulate the global climate [14]. Tropical rain forests are also home to endangered species such as the Sumatran tiger, Bornean orangutan, Sumatran rhinoceros, sun bears, and Asian elephants, which need preservation. These animals depend on tropical rain forests for food and shelter. Tropical rain forests are also a valuable economic resource because they provide many useful things that people use every day [15]. Tropical plants are essential to the survival and preservation of other living organisms. One of the most important traits of tropical plants is that they purify water through their roots. This filtering of water through soil acts as a safeguard against many different diseases, such as cholera, dysentery, and typhoid. The roots grow deep into the ground and hold onto small amounts of water that can sustain plant life. Tropical plants also prevent soil erosion by acting as a natural buffer against strong winds, storms, floods, and wildfires. Due to their deep root systems, this allows for proper drainage of water from floods and droughts alike. Tropical plants also provide food for animals and people by acting as a natural canopy for fruits and nuts, which are used for both food and trade [16].

3. Improvement of tropical plants using conventional approaches

The Agricultural Revolution began when people started cultivating crops on a large scale to feed larger populations. This allowed them to grow more food per unit of land than hunting and gathering. Enhanced agricultural productivity led to population growth and an increase in urbanization. Traditional methods for the improvement of tropical plants have been developed over thousands of years. Plants were selected and bred by farmers and gardeners, who then shared their knowledge with other growers. Because of these changes, there are now many beautiful plants that can be used as decorations or as food.

3.1 Domestication

Crop domestication is the process of selecting and breeding plants or animals to produce food and other desired products. It is a human-mediated artificial selection process that gives rise to domesticated organisms with certain desirable characteristics. Plants were first domesticated around 10,000 BC when early farmers selected and bred the best edible plants they found in their fields. They later moved on to domesticating animals, cultivating grain, and storing food [17].

3.2 Selective breeding

One of the most common ways to improve plants is through selective breeding. This is a process where desirable traits are selected and passed on to offspring generation after generation. The selection of naturally occurring varieties in the wild and, subsequently, in cultivated areas was the first type of plant breeding. Planting-harvesting cycles exert selection pressure on genetic diversity. Some plant phenotypes were profoundly altered as a consequence of this process, as shown by the derivation of maize from teosinte. The result is a plant that has better survival characteristics or greater productivity than its ancestors [18]. The earliest evidence for selective breeding dates back to 7000–7500 BC in Jiahu, China; here, rice was bred from wild rice, *Oryza rufipogon*, and domesticated through artificial selection with a combination of harvesting and replanting the plant's seeds. Other early examples of selective breeding are emmer wheat, barley, flax, and cotton [19].

Selective breeding requires careful selection of individuals with desirable traits, and they must be further propagated by vegetative means (such as cuttings) so that all their descendants have these same traits. It takes time and patience but can be very rewarding if done right! Another common method is simply growing out large numbers of seedlings until one appears that has the desired trait(s). This type of selection does not require vegetative propagation or any genetic engineering—just patience! [20].

3.3 Intuitive farmer selection

One way that farmers have traditionally developed new varieties is through intuitive farmer selection (IFS). This is a form of plant breeding where farmers select plants that exhibit desirable traits and save seeds from those plants to plant the following year. This process can be repeated for several generations until the desired characteristics are fixed in the population. The first step of IFS is to observe what happens to plants over time, including which ones express certain traits and which ones do not. Farmers then select the best examples of these traits and save their seeds for planting the next season. This process can be repeated for many years before any new varieties are created [21].

3.4 Pure line breeding

Pure line selection (PLS) is a method in which a new variety is created by selecting an individual with desirable characteristics from an existing population (often consisting of many different varieties). It involves repeated cycles of crossing or self-pollination between related plants or clones that are genetically identical to each other and then selecting for one or more traits. This process can be used to produce a wide range of new varieties, including dwarfing rootstocks, disease-resistant plants, fruits with improved flavor and color, etc. PLS has been used in agriculture since ancient times, but it became more important after the nineteenth century when it helped breeders create new varieties with desirable traits such as yield, drought tolerance, frost resistance, disease resistance, and so on. PLS is also known as single gene selection or monogenic selection. It involves selecting a plant that has one desired characteristic and eliminating all other plants that do not possess that trait. So, it is possible to make a variety with only one type of flower or fruit. This makes it easier for farmers to buy seed stock of the right variety [22].

3.5 Mass selection breeding

Mass selection is the process by which farmers select the best seeds from their crops and save them for the next year. This was the main way that new varieties were developed before modern times. To select seeds, farmers would have to grow them out in their fields and observe which ones were the most productive and hardy and had good taste. Once they had chosen a few plants to grow again, they would save their seeds for the following season's crops. Over many generations, this process gradually produced new varieties of plants that were well suited to local conditions and tastes. Mass selection is a form of artificial selection that allows only those plants that exhibit the desired trait to reproduce. This method does not require any knowledge about genetics or the mechanism by which genes are inherited. The breeder just picks a desirable trait and grows out plants with that trait over and over again to choose the best ones for breeding [23].

4. Improvement of tropical plants using innovative approaches

4.1 Cell and tissue culture

Tropical plant communities contribute an enormous proportion of the global plant species and represent more than 42% of the total carbon reserves throughout the world [24]. They play a critical role in the well-being of humanity. Our daily dependence on tropical plants and/or their products is outstanding. They are not only major contributors to food and feed but also a valuable source of spices, essential oils, fruits, sugar, and beautiful hardwoods. In addition, tropical regions also produce different types of fibers, resins, gums, plant essences, and dyes, which are extensively used in therapeutics and various industrial by-products. They are the dominant source of trade among the continents; for example, Africa and Latin America are the major suppliers of cacao and coffee, South America is the largest producer of sugar and bioethanol, whereas Asia produces most of the natural rubber.

With the increased demand for tropical plants, that is, rubber, oil palm, cocoa, banana, pepper, and pineapple, their large-scale production is direly needed. High-quality planting material can only be produced through tissue culture, thus the only potential strategy to fulfill the increased demand for ornamental tropical plants and plants of economic value. So, different types of explants, cultural media, and conditions have been worked out by different research groups. Micropropagation by somatic embryogenesis has been established through direct or indirect routes. The number of clonal plants is usually lower in the case of direct embryogenesis as compared with indirect somatic embryogenesis, so indirect embryogenesis is preferred for the commercial-scale production of ornamental plants, particularly in the case of endangered plant species, where only limited explant material is available. However, callus induction and somatic embryogenesis are dependent on exogenous auxin and culture conditions [25]. Exogenous auxin is involved in the downregulation of essential genes involved in embryogenesis perhaps by DNA methylation or other cellular processes, which is still to be explored. Removal of auxin from the culture media supports embryogenesis in most plant species [26]. Exploring the developmental trajectory of callus induction, indirect somatic embryogenesis, and direct somatic embryogenesis will be of great help for the commercial-scale production of indoor tropical plants and edible tropical plant species. Different types of explants including

immature zygotic embryos, mature zygotic embryos, immature female inflorescence, immature male inflorescence, immature leaves, mature leaves, young plantlets, and shoots were tested for the micropropagation of oil palm *via* indirect somatic embryogenesis [27].

Advancements in molecular biology have not only explored the critical pathways and genes involved in different phases of *in vitro* growth but also led to manipulation of certain genes to improve somatic embryogenesis and regeneration. Though most of the research has been reported on *Arabidopsis*, yet these findings may be extended for the betterment of other plant species including tropical plants. LEAFY COTYLEDON genes (LEC1 and LEC2) were found to be involved in the key pathways of somatic embryogenesis, and their overexpression triggered the upregulation of YUC genes, which are responsible for the increase of endogenous auxin levels. Other valuable genes involved in somatic embryogenesis are BBM, WOX, and SERK. Genetic manipulation of these genes can be of great help to further explore and understand fundamental cellular processes involved in somatic embryogenesis and clonal propagation [28]. In addition, proteomics studies have helped to elucidate the fundamental processes involved in these crucial cell growth phases, thus helping out to promote plant growth under *in vitro* conditions. Three valuable proteins linked with somatic embryogenesis were identified as osmotin-like proteins, chitinase, and β -1,3-glucanase. In *Picea glauca*, 48 differentially expressed proteins were observed to be of crucial importance during different stages of the development of somatic embryos. Efforts have been made to explore the proteome profile of oil palm embryogenic lines, embryogenic cell suspensions of coffee [29], auxin-induced embryogenic and non-embryogenic tissues of tamarillo, secondary somatic embryogenesis in cassava, and somatic embryos of avocado, which could further be exploited to improve clonal propagation of these valuable tropical plant species [30].

Though efforts have been made to unravel the fundamental cellular processes involved in key regulatory pathways of cell differentiation, dedifferentiation and commercial scale production of tissue culture plants have been possible. Still, certain impediments are there that need to be addressed for further improvement of existing clonal propagation systems. These include the genotype-dependent nature of the cultures, slow response of the cultured tissues, lower conversion rate of embryonically competent tissues, and heterogeneity of the cultured samples.

4.2 Genomics approaches

Breeding of the tropical tree plants is complicated owing to polyembryony, parthenocarpy, long juvenile phase, polyploidy, generation cycle, heterozygosity, and insufficient genomic resources. Advancements in multi-omics approaches, that is, genomics, metabolomics, transcriptomics, proteomics, whole genome sequencing, and annotation, have led to the identification of novel genes involved in crucial metabolic pathways responsible for sugar metabolism, fruit development, fruit ripening, stress tolerance, shelf life, etc. Interventions in genome-wide association (GWAS), genomic selection (GS), genetic transformation, and genome editing through CRISPR/Cas9 have helped to develop tropical plant varieties with desirable traits.

Developments in sequencing techniques have not only helped researchers devise molecular markers but also paved the way to explore genetic diversity in different plant species. The world's largest germplasm collection of bananas has been maintained at Biodiversity International Transit Centre (ITC), Belgium [31]. Diversity arrays technology (DArT) was employed for the selection of carotenoid-rich

bananas, thus helping out to promote nutrient-enriched bananas [32]. In papaya, 21,231 SSR markers were developed from genic regions, of which 73 SSR markers were validated for fruit ripening. The SCAR marker (CPFC1) was developed for the fruit flesh color in papaya, facilitating the identification of progenies based on pulp color. Likewise, AFLP markers were developed for the characterization of pink-fleshed and white-fleshed guava genotypes. Comparative mapping and germplasm characterization were performed by SSR markers in *Musa* species. These studies helped out to shortlist the *Musa* accessions with relatively higher content of minerals including calcium (111.1-fold higher), potassium, and magnesium (4.7-fold higher) [33]. Jackfruit draft genome assembly has helped to explore numerous gene families involved in starch synthesis and fruit development. SSR and ISSR markers have also successfully been employed for the identification of dragon fruits with white pulp and pink pulp [34].

Molecular breeding, that is, marker-assisted selection (MAS), marker-assisted backcrossing (MAB), and marker-assisted introgression (MAI), is quite helpful in the efficient development of new genotypes, mapping population, phenotyping, and genotyping. QTLs (quantitative trait loci) are of pivotal importance in this context, to track particular desired traits. In papaya, 21 QTLs were identified for the key quality traits of fruits, that is, fruit weight, fruit width, fruit length, flesh thickness, flesh sweetness, fruit firmness, and skin freckle. Similarly, 460 SNPs were predicted as potential molecular markers for the selection of particular fruit traits and diversity studies [35]. In mandarin, four QTLs were identified to be associated with fruit weight, three with peel puffing, and one with sugar content. The whole genome assembly of mango has helped to explore polyembryony, identification of non-coding RNAs, and QTLs for flavonoid biosynthesis and fruit weight [36].

The discovery of genome-wide SNPs has opened up new avenues in high-throughput genotyping and marker-assisted breeding, thus helping out to develop the novel genotypes. These SNPs have been used for the GWAS and identification of QTLs relevant to fruit traits in tropical plants, that is, mango, papaya, avocado, cassava, banana. These QTLs can effectively be used for the betterment of traditional breeding in terms of reduced time and cost. In addition, information retrieved from the pan-genome, super-pan-genome, and pan-transcriptome has been employed in the mining of genetic determinants of various phenotypes, thus helping out the betterment of tropical plant species.

4.3 Genetic engineering

Transgenic technology is increasingly contributing to the betterment of tropical and sub-tropical plants. Numerous fruit trees, crop plants, and ornamental plant species have been engineered for valuable traits, esthetic value, and the cleanup of the environment. Compared with the annual crops, tree plants are tough targets as far as their genetic manipulation is concerned. The complexity of the genome, low transformation efficiency, complex cultivation environment, long breeding cycle, and recalcitrant nature of the plant tissues are the major impediments in the genetic transformation of tree plants. Researchers have worked out to resolve these bottlenecks, and protocols have been established for the genetic transformation of numerous tropical and subtropical plants including citrus, mango, banana, pineapple, litchi, passion fruit, plantain, longan, and avocado. These plant species have been engineered not only for valuable agronomic traits but also for the improved quality and quantity of the fruits.

Populus alba is taken as a model plant for the establishment of genetic transformation in tree plants. It has been engineered for insect resistance, herbicide tolerance, and decreased lignin content. Insect-resistant transgenic poplar plant has been approved for commercial-scale cultivation in China, wherein stable expression of the transgene was observed in 8- to 10-year-old transgenic plants, hence providing broad-spectrum resistance against insect pests [37]. Stable transformation of Cavendish banana cv. Grand Nain was also reported using *uidA* and the potential virus-resistance gene (BBTV) along with the *nptII* gene as a selectable marker expressed.

Diverse tropical plants including papaya, oil palm, cassava, Picea, Ulmus, and Pinus have been engineered for disease resistance, herbicide tolerance, and resistance against insect pests [38]. The first draft of the papaya genome sequence from the commercial virus-resistant transgenic fruit tree opened up new avenues for the genetic transformation of tree plants [39]. Papaya has a relatively small genome of 372 Mb, with nine pairs of chromosomes and diploid inheritance. Other desirable features of the papaya are short generation time (9–15 months), primitive sex chromosome system, and continuous flowering throughout the year. These features make papaya a promising system for the exploration of fruit tree genomics and tropical tree genomes.

The first commercialized transgenic papaya carrying coat protein for papaya ring spot virus, PRSV CP gene, was introduced in Hawaii in 1998. CP-transgenic papaya plants appeared to have variable levels of resistance against ring spot viral isolates from different geographical regions. Isolates from Florida, Bahamas, and Mexico have delayed and made symptoms mild, whereas isolates from Thailand and Brazil have delayed symptoms; as a result, the virus can overcome resistance and thus may cause pathogenicity. Rainbow, a hemizygous line, also appeared to be susceptible to viral isolates from Taiwan [40]. Hence, the level of resistance appeared to be different against isolates from different regions. Broad-spectrum resistance has also been attempted to develop through RNAi by targeting the conserved domain of the PRSV CP gene [41]. The disease-resistant transgenic papaya was reported to be environmentally safe, with no harmful effects on human health. Oil palm is another valuable tropical plant that has extensively been used for the production of edible oil. Parveez and Christou [42] reported its genetic transformation through a biolistic transformation using multiple gene(s) constructs, that is, *gusA*, *bar*, *hpt* under ubiquitin, and CaMV35S promoters. The resultant transformants were selected on 50 mg/L Basta. Bahariah et al. [43] published biolistic transformation, and resultant transformants were selected through a mannose selection system containing mannose @ 30 g/L. Oil palm genome has also been targeted for the production of polyhydroxybutyrate (biodegradable plastics). Three genes (*phaB*, *bktB*, and *phaC*) responsible for the bacterial PHB biosynthesis were expressed under the Ubi promoter. The expression of biodegradable plastic was detected to be in the range of 0.33 to 0.58 mg/g of dry weight. The transgenic oil palm plants showed normal growth; thus, no deleterious effects of transgene expression were observed on plant growth.

Cassava (*Manihot esculenta*) is the third major contributor of staple food in sub-Saharan Africa, where it is grown as a starch-storing root crop. It has been engineered for valuable agronomic traits as well as to boost its nutritional value. Zeoline protein has been expressed in roots wherein the total soluble protein was detected to be increased up to 12.5% of the dry weight, thus showing a fourfold increase in protein content as compared with non-transgenic plants. The Nigerian cultivars, TMS 91/02324 and TMS 95/0505, were engineered for resistance against CBSD and CMD.

The transformed cultivars showed an increased level of resistance against the noxious viral pathogens [44]. AtFER1 and AtIRT1 were overexpressed in cassava for the increased accumulation of zinc and iron in roots (40 $\mu\text{g/g}$ and 145 $\mu\text{g/g}$ dry weight, respectively).

So, tropical plants have not only been engineered for improved agronomic performance and additive nutrients but also been engineered to clean up the indoor air and increase esthetic values. Indoor air often contains benzene, formaldehyde, chloroform, and other volatile organic compounds. Modern lifestyle has promoted the production of undesired molecules coming from furniture, smoking, and showering. A normal room needs at least 20 plants for the removal of these toxic molecules. The detoxifying ability of the plants can be increased through transgenic technology. Incorporation of mammalian cytochrome P450 2e1 (rabbit CYP2E1) in pothos ivy boosted its ability to detoxify the abovementioned hazardous molecules. The engineered plants were able to detoxify chloroform and benzene in the closed vials within 8 days of culture, thus showing great potential to detoxify the undesired molecules [45]. Likewise, sulfur metabolism can be engineered to improve resistance to SO_2 . Transgenic tobacco plants overexpressing serine acetyltransferase and cysteine synthase gene(s) were highly tolerant to sulfite and SO_2 [46]. Engineering tropical plants with the said gene can uplift their ability to tolerate sulfite and sulfur dioxide.

4.4 Role of biotechnology to secure endangered plant species

Tropical plants occupy approximately 1/20th of the earth's surface [47]. They comprise 2/3rd of the terrestrial plant species globally. Most tropical regions are categorized into biodiversity hotspots, but they possess different challenges due to the increasing rate of the human population. The tropical biodiversity hotspots have increased habitat loss, species richness, and an increasing number of endemic species [48]. An important cause of these changes is deforestation due to agricultural and industrial expansion during the past 30 years. As a result, the diversity in the tropical ecosystem is endangered, which has high environmental concerns regarding biodiversity degradation and extinction of species. One example of a biodiversity hotspot is Sumatra, where ~ 0.84 million hectares of forest land has declined [49]. The same is happening in other developing countries. Intensified agriculture and changes in the use of tropical land have a durable impact on the global biodiversity, and their future consequences are just estimated.

It has been studied by Rodriguez-Echeverry et al. [50] that fragmentation, changes in land use, and habitat loss are causing the decline of biodiversity and the composition of species is changing. The ecosystem processes and invasive species are altering. In the past decade, various studies conducted to find its impact on the genetic composition of tropical plant species and genetic alterations were observed [51]. Habitat loss, population differentiation, and genetic diversity losses have consequences that are caused by inbreeding, genetic drift, and increased distances by isolation [52]. The outcome of these changes is also caused by different life traits such as dispersal strategy, the density of plant species, mating, and gene flow in tropical lands. The information on genetic resources collected from one of the few species cannot reflect the plant community. However, genetics and molecular biology provide efficient approaches to precisely calculate the genetic variability in tropical plants.

Conservation management is an important issue in the tropical ecosystem. Various human and social factors have been identified that are responsible for biodiversity loss [51]. Agriculture expansion, corruption, human growth, and incompetency in the

development of genetic conservation strategies have increased the risk to the tropical ecosystem and sustainable management. The genetic information of the tropical species has increased the probability to maintain the genetic conservation of targeted plant species. The recommendations on the monitoring program and sustainable management in a particular fragment of tropical land are based on the genetic information of species, species richness, and processes of the ecosystem. Lack of sufficient genetic study along with population fragmentation data of different plant species could lead to the development of poor management practices for the conservation of plant species. The land-use change process is fast in the tropics, and there is a need for a robust method for identification of biodiversity hotspots and development of strategy in the identified area. Genetics alone does not provide a sufficient solution for the determination of the hotspots in the tropical plant community. Therefore, the emerging molecular and biotechnological techniques provide the required solutions to identify the genetic diversity in the high number of plants. These biotechnology-based tools provide the pre-requisite baseline information of the dominant species composition. It also helps to identify the high conservation value of different habitats that could be used in the conservative management practices of endangered species.

One important technique to investigate multiple plant species is amplified fragment length polymorphism (AFLP). After DNA extraction of the selected plant species from nearly 1 cm² leaf tissues of each plant, the AFLP protocol of Vos et al. [53] provides better results. The extra genetic material can be stored at -20°C for a longer period at optimized conditions. The samples were excised with the single enzyme to incubate overnight or to amplify with a single primer. In this regard, different PCR protocols are optimized for different plant species [54]. For the efficient reproducibility of the AFLP procedure, two to ten samples of each plant species provide reliable information. The fragments occurring from the restriction steps in the repetition of samples are considered important. The results obtained from the AFLP linked with PCR techniques can be visualized by transforming into the fragment presence-absence matrix. The analysis can be done by collecting samples of more than one hundred genotyped species. Different values of variation per species use the common genetic diversity indices [55].

In vitro conservation of germplasm included a large number of techniques involving the incubation of plant germplasm under controlled conditions. Although the nutrient requirement and conditions of growth vary for each tropical plant species, it provides a robust and reliable solution for the conservation of endangered species in the tropical region [56]. More commonly, the younger developing tissues of the explant can be used as the source. Genetic transformation has become an important tool in the additive one-point improvement in comparison with the mutation breeding that develops the subtractive one-point improvement. The genetically modified ornamental plants can be more acceptable to the consumers due to their vibrant colors as compared to food crops, where different ethical concerns are present regarding the use of recombinant technology [57]. Different GM technologies include zinc finger proteins, RNA interference, miRNAs, and CRISPR-Cas-9, which can be applied in the development of transgenic plants (**Figure 1**). The quality of the explant and type of tissue help to develop the biotechnology-based strategy for the regeneration of endangered species. The genome-editing technology has been vastly improved in the previous years. After the advent of genome-wide scan analysis and next-generation sequencing, varied information becomes available on tropical indoor and outdoor plants. The available information on the sequences of tropical plants could provide great help in breeding and basic research.

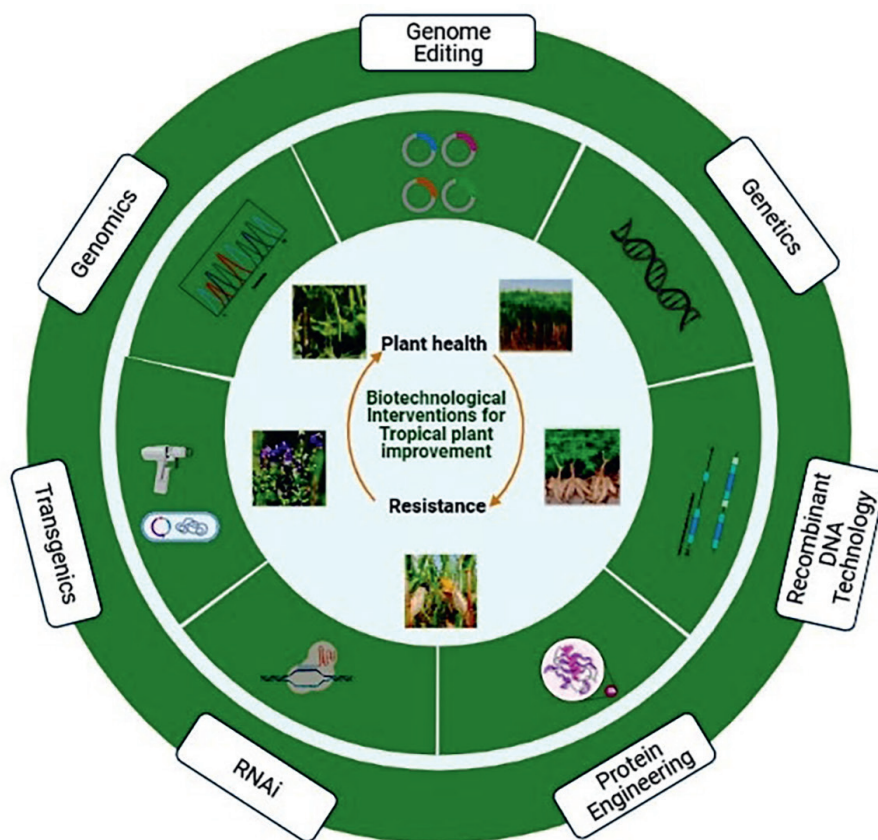


Figure 1. Contribution of biotechnology to the improvement of germplasm and agriculture in tropical areas. The DNA of tropical plants can be genetically engineered for the improvement of different traits, conservation of endangered species, and resistance against pathogens.

The heterogeneity of the different biodiversity plots and land-use systems can be studied by using the fragment distance matrix that is based on the principal component analysis. The bioinformatics and statistical tools can be used by applying the function `betadisper` and R-package `ggplot2`. Precise estimation of the genetic diversity in an area of dominant species depends on the spatial scales of alpha, beta, and gamma. The alpha scales respond to the diversity within the plot, the beta scale is for the land-use system, while the gamma scale is the highest level of spatial scales. In the fragment pool approach, the differentiation at the alpha level is determined by using at least 10 fragment pairs within each plot. However, the β diversity level can be calculated by taking at least 40 pairwise fragments in every land system. On the other side, the γ diversity is based on using the 160 genetic fragments from the plots where the concerned species are dominant. To apply this technique, the Shannon Index can be applied within each plot.

5. Biotechnological challenges to improving tropical plants

The flora of tropical areas suffers from biotic and abiotic stresses. In the past few years, the effects of climatic changes are significant on tropical plants. It has been reported by the Intergovernmental Panel on Climate Change (IPCC) that the climatic conditions are a precursor to various stresses on plants and are considered the most important influencing factor in the decline of agricultural production in developing countries. Global warming negatively influences tropical and sub-tropical plants due

to rapid alterations in the ecosystem, drought, rainfall patterns, floods, and biological outbreaks [58]. The balanced environment and lack of nutrients are the major constraints that enhance the losses of biological stresses. The losses due to abiotic stresses could be around 50 percent in tropical areas possibly due to a decline in plant metabolism [59].

The tropical plants of agricultural importance are suffering from viruses, bacteria, and insect attacks. Although there has been a substantial increase in food production in developing countries due to advances in breeding practices, the challenges of food security have never been met. The use of biotechnological approaches enables the genome alterations in plants to withstand abiotic and biotic stresses (**Figure 2**), which are usually difficult to achieve by conventional approaches. Advanced genome editing tools such as RNA-induced gene silencing, CRISPR-Cas, genome mapping, and next-generation sequencing have paved the way toward desirable genetic alterations in plants. However, the available biotechnological applications to agriculture in conventional crop species are the use of selective breeding approaches to bring improvement in genetic materials [66]. Some of the conventional crops of the tropical areas include maize, tomato, sweet potato, beans, pulses, nuts, cassava, sorghum, etc. These crops are present in the wet climate in tropical areas. On the other hand, the important crops of dry tropical climates are coffee, rubber, cotton, tobacco, tea, and sugarcane. The ornamental plants have their range such as hibiscus, plumeria, palms, ebony, teak, gardenia, fire brush.

Biotechnology has contributed to the solution of food security challenges by making robust genetic mutations. According to ISAAA infographics, more than 17 million farmers are benefiting from the cultivation of biotechnology crops. More

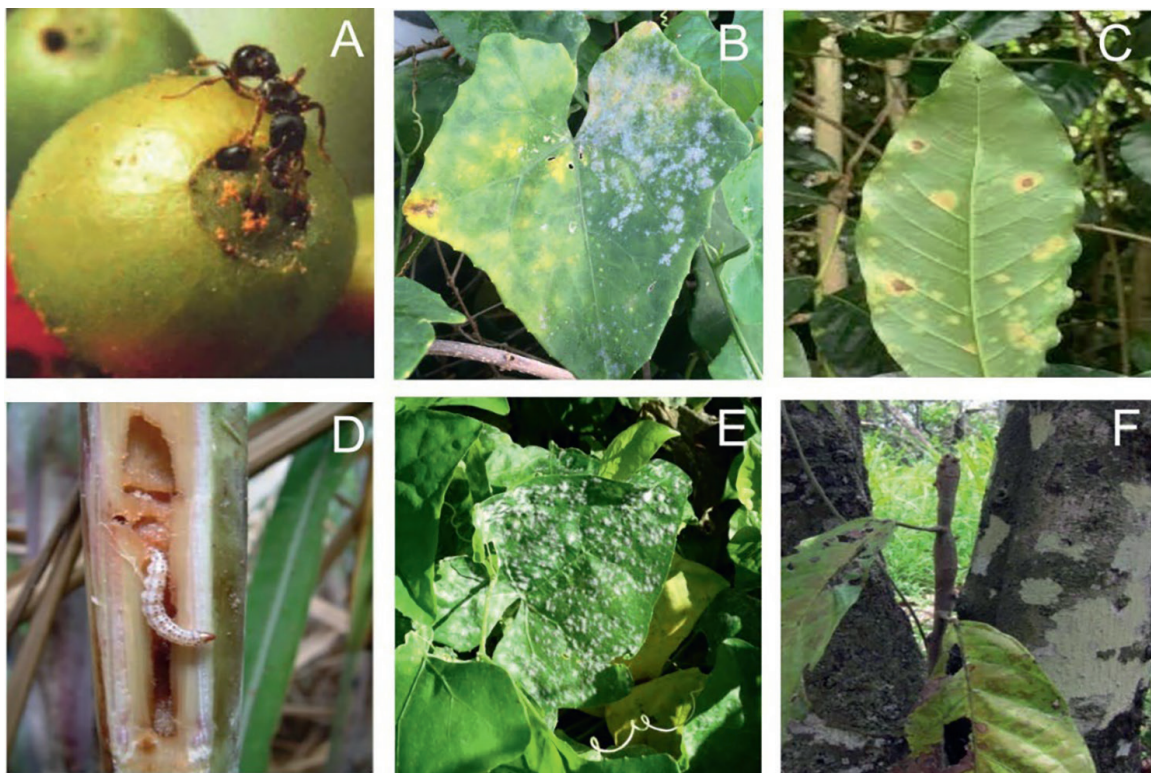


Figure 2. (A) Coffee berry borer (modified from Benavides et al. [60]), (B & E) *Coccinia grandis* (modified from; Goebel et al. [61]), (C) coffee leaf rust (modified from Nelson, [62]; Gichuru et al. [63]), (D) stem borer larval caterpillar on sugarcane stalk (modified from Cilas et al. [64]), and (F) cacao swollen shoot virus (modified from; Kouakou et al. [65]).

than 28 countries are growing 14 biotechnology crops precisely, and major contributors are tropical countries such as Brazil, India, Argentina, China, Indonesia, and Paraguay. The area under biotech crops is 2.7 billion hectares, and 17 million farmers' earnings are associated with it [67]. The genetic alteration strategies are designed to increase crop production, improve quality, and develop resistance against biological agents such as pests, herbs, and bacterial and viral diseases. With the advancement of technology, biotechnological approaches have become robust, accurate, and more reliable, but there are some ethical and environmental concerns about their agricultural applications.

The first contribution of biotechnology to crop improvement came with the development of crops resistance to a broad range of selective herbicides. The biotechnological techniques proved valuable on a socioeconomic as well as on an ecological scale because they reduced the consumption of questionable herbicides. However, due to unorganized applications of herbicides over growing seasons, the resistance to herbicides is emerging that could overcome the advantage of selective tolerance. The scientific challenge to address this issue is diversification of herbicide consumption along with crop rotation. There is the need to make continuous improvements in resistance technologies and the identification of new biochemical pathways in plants to find new targets. A similar challenge resides in another trait of resistance to insect pests, which is also commercially exploited. Many commercially important crops in tropical climate regions are threatened by different insect pests such as coffee rust, Lepidopteran stem borers, Helicoverpa, coffee berry borers [64].

The most important biological challenges are plant viruses because of their complexity in their life cycle, replication, movement, diversity, and unique capability of developing mutations against resistance. Identification of a causative virus and its pathogen vector is crucial for estimating the epidemiology and economic losses that are essential for the development of management strategy. Viruses can only be detected by using molecular diagnostic techniques. Some potential virus threats of tropical crops are cacao swollen shoot virus, banana bunchy top virus, cassava mosaic virus, plum pox virus, potato virus X, cucumber mosaic virus, African oil palm ring spot virus, rice yellow stunt virus, etc. [68].

Since after their inception, biotechnological approaches have established a reputation as a potential way to overcome food shortages. The choice of biotechnological approaches depends on the targeted pathogen or abiotic stress [69]. For example, the short RNA-based RNAi approaches are efficient against RNA viruses because the viral genome can be targeted before replication and formation of proteins, but to target the DNA genome, CRISPR-Cas is a superior technology. Violation of biosafety measures could cause unwanted gene flow to the other plant or pathogen species.

Although biotech crops are becoming an important hope in the tropical region, some challenges also emerged with the increase in production of biotech crops. For example, with the continuous application of broad-spectrum herbicide on the GM crop plants, resistance also emerges in the weeds, which lose the selective advantage in genetically modified plants. Another area that has been addressed more by biotechnological approaches is the use of bacterial toxins to develop transgenic plants for durable resistance to insect pests. So far, the toxins from *Bacillus thuringiensis* are mostly applied for pest tolerance.

Plant biology is facing an additional level of complexity as compared to mammals because plants are sessile and grow in varied environmental conditions that are far from optimal climate conditions. There is evidence that changes in the ecosystem alter the pathogenicity of viruses and plant pests. In the past few years, various

unpredictable changes have been reported in the tropical ecosystem, which need continuous investigation of the genome characterization of viruses, pests, and the response of host plants. Changes in humidity, temperature, and atmospheric pressure affect the growth of insect pathogens. The development of the post-transcriptional gene silencing strategy needs a very comprehensive study of the host, environment, and pathogen; otherwise, there are chances of horizontal gene transfer to other species [70]. Climate changes lead to ecosystem disturbances at different levels, which affect the efficiency of biotechnology techniques. The climatic changes affect the interaction of the ecological and biological communities including soil, natural habitats, plants, and biodiversity. For example, the plant susceptibility to disease increases due to high temperature and humidity [71]. However, these challenges can be overcome by combining conventional and biotechnological approaches to address biotic and abiotic stresses.

6. Future prospects

Tropical plants comprise two-thirds of the terrestrial plant species and have great importance in plant biodiversity. Due to increasing population, there is an urgency to double the food production globally. Thus, a series of biotechnological interventions can be made to conserve plant biodiversity (Van Montagu, 2020) and improve crop plants in tropical areas. Medicinal and fruit plants also need attention with regard to biotechnology-assisted breeding to combat biotic and abiotic stresses. Tropical forests can make an important contribution to the global demand for fruits, timber, and biomass. Biotechnological tools should be used to identify the potential of plants as new crops to mitigate malnutrition in tropical regions. Some important crops in tropical regions such as cassava, cowpea, sweet potatoes can be improved as cash crops by biotechnological efforts. Moreover, the nutritional contents of plants can be improved. For example, lathyrus is a leguminous protein-rich crop that is used after overnight soaking to remove the toxins on its split seeds. Engineering lathyrus genome could help in this regard. There are also gaps in the study of marker-assisted breeding on tropical plants, which can become an important tool in crop improvement. The tropical region has a range of medically important plants, and their properties can be broadened to increase diversity. Latest molecular biotechnology tools such as CRISPR/Cas 9 have promising applications in gene activation, repression, gene mutation, and epigenetics. It has been effectively applied to citrus, apple, petunia, and various other plants. Similar research models can be applied to economically important tropical plants.

7. Conclusion

The sustainability of the food supply chain depends on tropical plants, which are also vital sources of medicine, fiber, wood, and energy. Tropical plants are present in more than 60% of all terrestrial plant species worldwide. In addition to domestication, attempts have been made to enhance these plants using both conventional and cutting-edge scientific interventions. Parthenocarpy, polyembryony, polyploidy, protracted juvenile phase, heterozygosity, and generation cycle problems have been helped by genomic techniques. Innovations in cell and tissue culture have enabled micro-propagation and the commercial plantation of various plants, in addition to

aiding in the conservation of endangered tropical species. Additionally, transgenic technology has made it feasible to combine genes from multiple species, enabling plants to withstand biotic and abiotic challenges as well as other types of stress. Another useful intervention that has helped to create plants with the desired features and the capacity to mitigate potential climate change difficulties is genome editing. Therefore, scientific advancements are essential for both the preservation of tropical plant species and the cultivation of tropical plants that will meet future demands.

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