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Cocoa Genetic Resources and Their Utilization in Palm-Based Cropping Systems of India

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Abstract

Cocoa (*Theobroma cacao* L.) became an integral part of palm-based cropping systems of India. It is being grown profitably as a mixed crop under arecanut (*Areca catechu* L.), coconut (*Cocos nucifera* L.), and oil palm (*Elaeis guineensis* Jacq.) gardens of the southern states Karnataka, Kerala, Tamil Nadu, and Andhra Pradesh. It is well adapted to the humid tropics with high rainfall and short dry spells as well as irrigated gardens of tropical belts, utilizing the shade provided by the palms. Research efforts of more than four decades at the ICAR-CPCRI (Indian Council of Agricultural Research-Central Plantation Crops Research Institute) and KAU (Kerala Agriculture University) have allowed efficient utilization of conserved cocoa genetic resources by farmers to provide additional income through multispecies cropping systems in the spices and plantation sector. National Horticulture Mission of Govt. of India identified cocoa as a potential crop for area expansion and development to meet both internal and export demands. Improved varieties were developed with high pod yield, bean quality, suitable to different agro-climatic zones and to tackle major biotic and abiotic stress. This chapter outlines the contributions of cocoa breeding efforts at the research institutes and State Agricultural Universities; developmental programs of Ministry of Agriculture and procurement and processing facilities to the growth of the cocoa sector in India.

Keywords: cacao, cocoa, dry beans, genetic resources, germplasm, breeding

1. Introduction

Cocoa was introduced into India way back in 1798 at Courtallam in Tirunelveli District of the Old Madras State (Tamil Nadu) by East India Company [1]. Cocoa was distributed then in the agro-climatic region covering Western Ghats Hills and Plains of Malabar (Kerala) and Mysore (Karnataka) states, having more rainy days and short dry periods [2]. Commercial cultivation of cocoa as a plantation crop under palms started in early 1970s, and the current area under cocoa is 82,940 ha with a production of 18,920 tonnes of cocoa beans [3]. From the traditional hilly regions, cocoa production has shifted and expanded to coconut gardens of non-traditional areas of Tamil Nadu and Andhra Pradesh states utilizing the 50% shade available in the gardens and irrigation. Safe conservation of genetic resources of this introduced crop and its utilization in breeding program is a top priority considering its perennial nature, adaptability, and compatibility with palms. Cocoa tree has a

typical growth habit and a distinct morphology highly responsive to climate change and growing environments, which necessitates long-term conservation of genetic resources and dynamic breeding programs [4] as systematically adopted in India.

2. History of cocoa improvement in India

Cocoa breeding is one of the oldest improvement programs in the world. In India, the oldest plantations with Criollo type cocoa were established and evaluated for their performance in Kallar and Burliar Fruit Stations in the Nilgiris between 1930 and 1935. Those found with high yield potentials were distributed wherever the agro-climatic conditions suited the crop. In 1962, Indian Council of Agricultural Research (ICAR) decreed to grow Criollo type in South India and Forastero type in North East India. In 1964, few Malaysian clones of Forastero and Trinitario types were imported, and research on arecanut + cocoa and coconut + cocoa mixed cropping systems were conducted at Central Plantation Crops Research Institute (CPCRI), Regional Station, Vittal, Karnataka and other centers, Peechi and Palode in Kerala and Kahikuchi in Assam and proved as profitable cropping models. In 1965, a research-cum-demonstration unit of Cadbury India Pvt. Ltd. was established in Chundale in Kerala [5]. In 1969, systematic research was started in CPCRI with additional introductions of germplasm, followed by Kerala Agricultural University (KAU) in 1979 and then continued at KAU in 1987 with Cadbury India Pvt. Ltd. funding. In 2008, Tamil Nadu Agricultural University (TNAU) initiated cocoa research with funding from Mondelez International.

3. Breeding strategies in India

Systematic and long-term cocoa improvement programs are being taken up with the following strategies: (1) germplasm collection, conservation, cataloging, characterization, and evaluation; (2) breeding through selection and hybridization; (3) standardization of vegetative multiplication, establishment of seed gardens/clonal orchards; (4) comparative yield trials (CYT); (5) multi-locational trials (MLT); (6) screening and resistance breeding for biotic and abiotic stress; (7) biotechnology and bioinformatics approaches; and (8) demonstration gardens [6, 7].

3.1 Introduction of germplasm

The basic step in any breeding program is the introduction of germplasm from both the primary and secondary centers of origin and distribution. Cocoa in its native zones of South and Central America and other major producing countries of Africa is suffering with many debilitating diseases caused by both viral and fungal pathogens and serious pests. To safe guard the germplasm exchange program, Intermediate Cocoa Quarantine Centre (ICQC, R) was established in Europe. The University of Reading took over the responsibility for cocoa quarantine in 1987 [8]. The center routinely collects important clones from international and national gene banks, wild collections and conducts virus indexing. It conducts strict quarantine for other major diseases and pests using the guidelines for safe movement of germplasm formulated by FAO/Bioversity International [9, 10]. Clones that have been cleared at the quarantine are supplied as bud sticks, with proper import permits and sanitary certificates to cocoa researchers over 20 countries. It also maintains the International Cocoa Germplasm Database to know about the gene banks, clones, traits, and even SSR/SNP profiles [11]. CPCRI also utilizes facilities offered by

ICQC, R and collected exotic clones with desirable traits for specific research purpose, for which ICAR-NBPGR (National Bureau of Plant Genetic Resources) is the nodal agency in India. Around 500 collections are being maintained in the National Active Germplasm Site (NAGS) for cocoa at CPCRI, Regional Station, Vittal, Karnataka and also at KAU, Kerala. The germplasm collections include clones from The Amazon, Brazil, Ecuador, Ghana, Kew, Jamaica, Mexico, Nigeria, Peru, and local collections from Kallar in Tamil Nadu, Wayanad, Idukki in Kerala, and Shiradi Ghats of Karnataka. All these are being conserved and evaluated for their adaptability, precocity, compatibility, stability of yield, productivity, and quality of produce in the introduced environment.

Diversity among the genetic resources is important for improvement program, and Bartley [12] explained the existence of diversity based on the degree of human involvement in establishment of cocoa groups. The three basic types of cocoa, Criollo, Forastero, and Trinitario, which have specific pod and bean characteristics [13] are also among the collections. Expression of diversity is estimated from different indicators of variability, especially, morphological traits that are important for cataloging and characterization of germplasm. Bioversity International has standardized the descriptor status for cocoa, which comprises of 60 characteristics. Turnbull and Eskes [14] developed visual aid to identify widely distributed cocoa accessions with a minimal descriptor of 20 characters. Morphological variability with regard to tree architecture, leaves, flowers, fruit shapes, apex form, pod rugosity, prominence of ridges and furrows, husk thickness, pod size, color, bean size, shape, and color are characterized, and passport data documentation has been undertaken in 30-year old cocoa collections [15]. National identity numbers (Indigenous/Exotic Collection, IC/EC No.) were obtained from NBPGR, New Delhi for further utilization in the breeding program. Assessment of distinctness, uniformity and stability (DUS) of traits is currently underway.

3.2 Breeding through selection

The presence of genetic variability offers wide scope in selection of potential types. Yield is the main selection criterion. An easy approach in yield improvement is to select superior plants and subsequently developing them into clones. The major selection criteria followed in cocoa are, trees yielding 100 pods/tree/year, pods weighing 350–400 g or more with 35–40 beans having a fermented single dry bean weight of 1 g. Dry bean production is in general considered as a combination of three yield components: bean weight per pod, number of pods per tree, and number of trees per hectare. It is expected to be 1 kg and above per tree in a year, and productivity is usually assessed over 6 years in varietal trials after stabilization. At CPCRI, seven high yielding clones VTLC-1, VTLC-5, VTLC-7, VTLC-8, VTLC-9, VTLC-11, and VTLC-30 were selected and utilized as parents in the breeding programs as well as in establishment of seed gardens [16]. KAU identified seven clones CCRP 1 to 7 and released these selections for cultivation. Though individual tree selections are being made from seedling progenies, they have to be further evaluated in clonal trials for confirmation. Heritability for yield is low or average when based on single tree harvests but higher when based on yields from plots containing several trees, and so clonal varieties are gaining importance [17]. To assess the phenotypic value of genotype even in hybrid selection programs, clonal trials are advised [18]. From the clonal trials, three varieties VTLCC-1, VTLCS-1, and VTLCS-2 have been released for commercial cultivation in different agro-climatic zones in the country (Table 1). Genetic analysis on 17 plant, pod, and bean characters in 44 exotic cocoa clones resulted in the selection of superior genotypes for higher performance with traits of high heritability and genetic advance. Based

Characters	VTLCC 1	VTLCS 1	VTLCS 2
Canopy area (m ²)	12	12	15
No. of pods/tree/year	55	55	55
No. of beans/pod	35	42	41
Single dry bean weight (g)	0.9–1.05	1.3	1.21
Dry bean yield kg/tree/year	1.3	2.5	2.7
Dry bean yield kg/ha	890	1700	1840
Shelling %	12	11	15
Nib recovery %	88	88	85
Fat content %	50	52	53
Features	Self compatible line suitable for North Eastern Zones	High yielder withstands both biotic and abiotic stress	High bean index, stable yielder withstands both biotic and abiotic stress

VTLCC—Vittal Cocoa Clone.

VTLCS—Vittal Cocoa Selection.

Table 1.
CPCRI cocoa varieties developed through selection.

on the pod yield, VTLC-25, VTLC-15, VTLC-18, VTLC-36, VTLC-13, VTLC-37, and VTLC-17 have been identified as heavy bearers with an optimal canopy. These clones recorded single dry bean weight of more than 1 g, 10–15% shell, high nib recovery 85–90%, and more than 50% butter fat content making them suitable for industries as well [19, 20].

In the palm-based inter cropping systems, the pod yield in general is expressed with respect to the canopy area which is mainly maintained as cone/ umbrella shaped single tier architecture. In the evaluation trials of Trinidad cocoa and Wayanad collections, 5 clones each are selected for high pod and dry bean yield ranging from 2.2 to 3.3 kg/tree/year [21, 22] with an optimal canopy of 15–20 m². Trait specific improvements are being taken up in the current breeding programs. A bean index of 100 beans/100 g, that is, dry bean weight of 1 g is considered as a standard for grade I beans [23], and so selections are aimed at a larger bean size of 1 g and above, which will have high butter content suitable for chocolate industry. The bean size is influenced by genotype, environment, and also the processing methods. Box, basket, and tray methods are being examined by research institutes as well as by farmers. Variation in bean indices has been observed across collections and the single dry bean weight ranged from 0.7 to 2.5 g. To improve the qualitative parameters, Criollo cocoa is used in hybridization programs [24], and white bean genotypes are being evaluated for quality parameters. Cocoa is considered as functional food, and so dark chocolates are consumed for their health benefits. Catechin, epicatechin, and procyanidines are the main polyphenols present in cocoa contributing to bitterness, astringency, and the organoleptic quality of cocoa. Cocoa beans of different clones evaluated for polyphenols and antioxidant activity exhibited distinct differences. Polyphenols ranged from 82 to 136 mg/g, procyanidin 49 to 64 mg/g, fat content of 24–55%, and antioxidant activity of 77–98% among cocoa clones studied. In general, cocoa beans with high polyphenol and procyanidin contents exhibited high antioxidant activities which are utilized for qualitative improvement [25].

Cocoa butter with free fatty acid (FFA) content of 1% or less together with acceptable flavor is the best indication of good quality beans. The type of fatty acids in 18 hybrids and 10 elite clones has been assessed, and from the profile, it is now known that 11 fatty acids are involved in the quality of cocoa beans. The fatty acids palmitic, stearic, oleic, linoleic, and arachidic acids present in all the genotypes invariably. The percentage of stearic acid is the highest in a range of 30.5% in VTLCP-7 to 44.2% in VTLCP-1. The other fatty acids differed among the genotypes in the percentage of expression.

3.3 Breeding through hybridization

Hybrid vigor between parents showing good combining ability is readily exploited in cocoa improvement programs along with inter-population heterosis. Most commonly adopted method is developing hybrids between two distant genotypes.

3.3.1 Method of hand pollination and fruit set

For production of true hybrids with specific objectives and to confirm the compatibility reaction, hand pollination is routinely practiced. In artificial pollination, a flower bud, which will open the following day, recognized by its whitish color and swollen appearance, is selected. The bud is covered with a hood of plastic tube of 5×1.5 – 2.0 cm size, which is sealed to the bark using materials like plasticine/glaze putty. The tube is covered with muslin cloth at the top, kept in place with a rubber band. Opened flowers are collected from the desired male parent, and stamens are carefully taken out by pushing the corresponding petal. One entire anther with a part of the filament is deposited on the stigma. The pollinated flowers are labeled using tin foil pieces fixed in the cushion using ball pins. The hoods are removed 24 hours after pollination, and in 3–5 days, fertilization is confirmed by the visual swelling of the ovary [26].

3.3.2 Progeny trials

Different cross combinations have been tried with specific objectives for development of hybrids. At CPCRI, five progeny trials have been evaluated with 76 cross combinations during 1983–1994 at Vittal, Kidu, and Kasaragod centers with objectives of more number of pods, high dry bean yield, big bean size, and drought tolerance. Of these, 20 hybrids were identified as best hybrids and further evaluated as clones. Among them, 17 progenies exhibited high vigor and cropping efficiency even at early years of development [27, 28]. From the progeny trials, four hybrids VTLCH-1, VTLCH-2, VTLCH-3, and VTLCH-4 have been released as improved varieties for cultivation in the country; in 2006, the Golden Jubilee year of CPCRI, RS, Vittal and VTLCH-5 is released as Nethra Centura for the centenary year of CPCRI, Kasaragod (**Tables 2 and 3**).

Hybridization program at KAU was initiated during 1983 and continued up to 1993. During this period, a total of 159 parents were included to produce 239 crosses, 187 pods, and 21,819 hybrid seedlings. Nursery evaluation of these hybrids is done with HD^2 (H—height and D—diameter) value. A total of 3126 superior seedlings were field planted in series I, II, III, and IV and progeny trials I, II, III, and IV, and 163 superior hybrids were selected, utilized in various breeding program for release of hybrids, and used as better combiners in clonal gardens. Three hybrids CCRP 8, CCRP 9, and CCRP 10 with high yield and good level of tolerance to

Trial	Progenies tested	Progenies selected	Dry bean yield (kg/tree/year)
Progeny I (1983)	5	VTLCP-1	1.01
Progeny II (1984)	25	VTLCP-7	1.48
		VTLCP-49	1.47
		VTLCP-50	1.42
		VTLCP-11	1.39
Progeny III (1987)	13	VTLCP-18	1.08
Progeny IV (1992)	15	VTLCP-29	1.25
		VTLCP-30	1.52
Progeny V (1994)	18	VTLCP-26	1.33
		VTLCP-27	1.62

VTLCP—Vittal Cocoa Progeny [27].

Table 2.
Progeny trials of CPCRI.

Characters	VTLCH 1	VTLCH 2	VTLCH 3	VTLCH 4	VTLCH 5
Canopy area (m ²)	16	15	18	18	16
No. of pods/tree/year	50	50	41	40	66
No. of beans/pod	40	40	41	40	43
Single dry bean weight (g)	1–1.11	1–1.5	1–1.05	1–1.07	1–1.11
Dry bean yield kg/tree/year	1.4	1.5	1.7	1.6	2.5–3.0
Dry bean yield kg/ha	959	1030	1150	1090	1500–1800
Shelling %	13	11	15	15	11
Nib recovery %	87	89	87	87	88
Fat content %	54	54	51	51	52
Features	Early stable heavy bearer	Heavy bearer, tolerant to black pod rot	Suitable for water limited conditions	Suitable for water limited conditions	Suitable for high density planting both under arecanut and coconut

VTLCH—Vittal Cocoa Hybrid [29].

Table 3.
CPCRI varieties developed through hybridization.

Vascular Streak Dieback (VSD) have been released as varieties for cultivation in the country (**Table 4**) [24].

3.3.3 Seed gardens/clonal orchards

Based on the compatibility reactions, self-incompatible but cross-compatible high yielding parents are selected and multiplied as clones through soft wood grafting and established as clonal orchards or seed gardens. Bi-clonal and poly-clonal gardens were

Variety	Breeding method	Important traits
CCRP-1	Single tree selection from local population	Heavy bearer, self incompatible Vascular Streak Dieback (VSD) tolerant No. of pods—56.2/tree/year No. of beans/pod—46.2 Single dry bean weight—0.8 g
CCRP-2	Selection	Heavy bearer, self incompatible, VSD tolerant No. of pods—53.9/tree/year No. of beans/pod—45.5 Single dry bean weight—1.0 g
CCRP-3	Selection	Heavy bearer, self incompatible, VSD tolerant No. of pods—68.5/tree/year No. of beans/pod—42.3 Single dry bean weight—1.0 g
CCRP-4	Selection	Heavy bearer, self incompatible, VSD tolerant No. of pods—66.2/tree/year No. of beans/pod—45.4 Single dry bean weight—1.1 g
CCRP-5	Selection	Heavy bearer, self incompatible, VSD tolerant No. of pods—37.9/tree/year No. of beans/pod—45.25 Single dry bean weight—0.8 g
CCRP-6	Selection	Heavy bearer, self incompatible, VSD tolerant No. of pods—50.1/tree/year No. of beans/pod—48 Single dry bean weight—1.9 g
CCRP-7	Selection	Heavy bearer, self incompatible, VSD tolerant No. of pods—78.1/tree/year No. of beans/pod—46.9 Single dry bean weight—0.9 g
CCRP-8	Hybridization	High yielder and VSD tolerant No. of pods—90.4/tree/year No. of beans/pod—48.8 Single dry bean weight—0.9 g
CCRP-9	Hybridization	High yielder and VSD tolerant No. of pods—106.7/tree/year No. of beans/pod—36.7 Single dry bean weight—0.8 g
CCRP-10	Hybridization	High yielder and VSD tolerant No. of pods—79.6/tree/year No. of beans/pod—41.6 Single dry bean weight—1.1 g

Ref. [26].

Table 4.
Cocoa varieties of KAU.

established for production of hybrid seeds with known parentage and performance. These clonal orchards are established and maintained at CPCRI, Research Centre, Kidu, Nettana, Karnataka [30] and at College of Horticulture, KAU, Vellanikkara, Thrissur. In cocoa seed pods, seedlings and clones (grafts/budded plants) are being used as planting materials. CPCRI nurseries are accredited by National Horticulture Board (NHB), with four star rating for quality planting material supply and acting as model nursery on cocoa. Sixteen regional nurseries were established in different states for the area expansion programs of Govt. of India.

Generation	Genotypes	Plants selfed	Flowers selfed	Pods obtained
S0	102	102	25,052	147
S1	51	178	6263	163
S2	9	41	693	24
S3	5	55	1720	48
S4	2	17	428	9
S5	1	1	132	0
Total	75	394	34,288	391

Table 5.
Inbred population field established (1989–2010) at KAU.

3.3.4 Inbreeding

Inbreeding forms a part of the breeding activities, not only to breed parents with some degree of homozygosity for the production of hybrids but also to breed materials homozygous for desirable traits like disease resistance. Existence of self-incompatibility makes inbreeding efforts in cocoa difficult. Since few self-compatible trees are also identified in the populations, selfing is possible but it should be continued up to six to seven generations to attain homozygosity and thereafter to be utilized for crossing to exploit the hybrid vigor. KAU has taken up this task of selfing self-compatible plants and with over 20 years of continuous effort, maintains two genotypes of S4 generation, 5 of S3, 9 of S2, and 51 genotypes of S1 (**Table 5**) [24, 26, 31, 32].

3.4 Comparative yield trial (CYT)

The clones and progenies developed through selection and hybridization programs are multiplied as clones and evaluated under comparative yield trials in different situations. Under high density planting in arecanut garden, five hybrids VTLC-6, VTLC-20, VTLC-15, VTLC-1, and VTLC-19 have been identified as best performers even in their initial years of growth [28]. Comparative study of parents and progenies as clones resulted in identification of VTLC-6, VTLC-2, and VTLC-20 and parents VTLC-1 and VTLC-56 as potential high yielders [33]. In another trial, clones suitable for both arecanut and coconut canopies have been identified [34] and released as varieties. Under coconut in double hedge system of planting, hybrids VTLC-22, VTLC-18, and VTLC-1 showed the best performance with an optimal canopy and high yield [35]. Evaluation of clonal and seedling progenies of selected genotypes has resulted in identification of four hybrids and two clones for multiplication both as clones and seedlings for utilization in the area expansion program [36].

3.5 Multi location trial (MLT) and demonstration plots

To assess the survival and stability of hybrids and clones in different agro-climatic conditions, multi-location trials are important. Elite clones of cocoa are under evaluation in both traditional and nontraditional states, namely Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Maharashtra, West Bengal, and Assam, for studying genotype \times location \times environment interactions. Further, 115 front line demonstration plots were established under participatory research cum

demonstration plot scheme in farmers plots as well as in five AICRPP (All India Co-ordinated Research Project on Palms) centers funded by Directorate of Cashewnut and Cocoa Development (DCCD), Kochi and Directorate of Arecanut and Spices Development (DASD), Calicut, for identification of location specific varieties, common varieties, and to tackle the climate change effects.

3.6 Resistance breeding

Chemical control can be effective against fungal diseases but will pollute the environment and make the cultivation expensive. Integrated disease and pest management by the use of resistant materials and cultural and biological methods is probably the best way to contain pathogens and pests in the long run for sustainable crop protection. Breeding for resistance, therefore, became the primary objective for cocoa breeders worldwide. Sources of resistance have been identified for major diseases, and since cocoa genome is sequenced, it is expected to provide models for plant pathogen interactions and also facilitate identification of resistance genes. Many cocoa pests such as mealy bugs, aphids, caterpillars, and borers are also reported in India [37] are currently being managed with chemical control measures. Screening of available germplasm for prevailing diseases, existing and emerging pests will therefore be very important in light of seasonal weather variations.

3.6.1 Black pod rot

India is free of most of the debilitating viral and fungal diseases known in cocoa. Since the current cocoa growing area comes under high rainfall zone and the main harvest season coincides with monsoon, incidence of black pod rot caused by *Phytophthora palmivora* is comparatively higher. Though the main harvest is safe guarded with systematic annual pruning, in the post monsoon period, the second season crop is still affected by pod rot. On field screening, clones have been categorized into having <10, 10–25, and >25% damage levels. *In-vitro* screening using isolates of *P. palmivora*, *P. capsici*, and *P. citrophthora* indicated that collections of Nigerian origin exhibit certain degree of tolerance [38]. Few Wayanad collections have also expressed field tolerance to pod rot when tested over three seasons. Further, 21 exotic clones collected exclusively for *Phytophthora* pod rot resistance was identified for utilization in cocoa hybridization program. The variety VTLCH-2, a combination of ICS 6 × SCA 6 is found to be tolerant to black pod rot in India as well. KAU has taken up screening and hybridization program for combining desirable traits of CCRP released varieties and black pod resistance in cocoa.

3.6.2 Vascular streak die back

In India, vascular streak die back (VSD) caused by *Oncobasidium theobromae* was first reported from Kottayam, Kerala, and it began to spread to adjoining cocoa growing areas of the state. As this disease cannot be controlled effectively by the use of fungicides, KAU breeding program concentrated mainly on production of VSD resistant varieties. Hybrid seedlings were screened in the nursery by subjecting them to high inoculum load by keeping them in the midst of infected seedlings. The tolerant and vigorous seedlings were selected and established in field for evaluation. CCRP varieties have been especially released for VSD resistance and also have been utilized for establishment of clonal gardens for seedling supply [31].

3.6.3 Tea mosquito bug

Tea mosquito bug (TMB) (*Helopeltis* sp.) incidence became severe in the recent years in summer and post monsoon seasons. *Helopeltis antonii*, *H. theivora*, and *H. bradyi* are reported on cocoa in South India. Insect population is influenced by many factors like temperature, humidity, water stress, condition of cocoa tree, etc. The development and use of mirid resistant cocoa varieties is one of the alternatives to chemical control and resistance studies in cocoa have mostly concentrated on assessment of field damage [39]. Damage on flushes, cherelles, and pods of individual trees and different grade levels of infection on cherelles and pods are assessed to work out the TMB tolerance among genotypes. Penetrometer readings for determining the hardness of sclerotic layer, thickness at primary and secondary furrows of pod husk have been recorded in 100 cocoa genotypes and interpreted with reference to insect resistance [40]. Mechanism of plant resistance to insects is a complex phenomenon. Plant attractiveness to some extent affects the level of infestation, antixenosis prevents feeding, while antibiosis disturbs the pest development, and finally cocoa tolerance is assessed from the ability of a tree to contain damage and recover from it. Red colored pods with the smooth surface have been identified as tolerant to TMB damage among Wayanad collections.

3.6.4 Low moisture stress

Cocoa plants are susceptible to environmental conditions especially temperature and drought and considerably influences the pod yield [41]. Cocoa is very sensitive to water scarcity and undergoes a period of low moisture stress for 5–6 months in its current growing condition in India. Detailed study on climate change and weather variability over 43 years (1970–2012) at Vittal, which is located between 12°15'N latitude and 75°25'E longitude, showed 38% yield variability in cocoa [42]. The trends of temperature increase are +0.4°C for mean maximum ($P < 0.001$) and +0.4°C for mean minimum during the last decade. Breeding for drought tolerance is unique to our country and is taken up with systematic screening of available germ-plasm as well as hybridization programs. Screening of accessions is conducted for physiological parameters like stomatal resistance, chlorophyll fluorescence, proline accumulation under stress and by studies on seed germination under low osmotic potential, etc. A total of 216 cocoa genotypes have so far been screened for physiological and biochemical parameters under different trials [43]. In all these studies, field measurements were taken during unstressed (October) and stressed (March) conditions. Few Nigerian collections have been identified as drought tolerant and used for hybridization with high yielding Malaysian collections under two progeny trials. Two hybrids VTLCH-3 and VTLCH-4 have been released as varieties suitable for cultivation under water limited conditions in the country. Studies on leaf morphology, stomatal behavior, water relation components, and biochemical factors indicated that thick leaf, higher wax content, efficient stomatal closure, and high tissue elasticity are responsible for better adaptation of cocoa plants to drought conditions. The application of chlorophyll fluorescence as a tool to screen cocoa for drought tolerance has been confirmed with a series of genotypes. Recently, photosynthesis, chlorophyll fluorescence, and water potential under stress and nonstress conditions were estimated in 11 genotypes from different geographical origins, Columbia, Brazil, Peru, Mexico and Ecuador [44]. Seasonal and varietal differences were found, and transpirational water loss was found to be reduced with increased stomatal closure, which is considered as a favorable drought trait in any crop. Among the 52 new introductions, five Amazon and Pound collections have been found to be adaptable to water limited conditions [45] with high yields, which will

be further utilized in the breeding program. Genotypic differences for morpho-physiological criteria, potential antioxidant enzymes, and biochemical components depicting drought tolerance in young seedlings were determined with cocoa clones and hybrids under controlled low moisture stress conditions [46–48]. From these trials, standards and thresholds for several physiological parameters related to cocoa were established.

Hadley [49] detailed the visual estimates of physiological traits in cocoa, and the morpho-physiological parameters include measurements of flowering, fruiting, cherelle wilting, leaf flushing, branching and pruning intensity, canopy shape, density, and light transmission on different point scales. In order to understand and elucidate the optimum canopy shape and structure of cocoa, different spacing and canopy sizes have been studied at CPCRI [50], which showed significant differences in crop yield. In an experiment with grafts, the photosynthetically active radiation (PAR) and light interception varied significantly over the years with two spacings (2.7×2.7 m and 2.7×5.4 m) and three canopy sizes (small, medium, and large), and similar results were noticed with transpiration rate and stomatal conductance [51]. It is important to note that the maximum leaf area should be maintained, self shading of leaves should be avoided, and pruning should be done to the extent of retaining 20–30 leaves/developing pod to ensure the yielding potential of the genotype. With an annual pruning of single tier canopy, fertilizer dose of 100:40:140 g NPK in two splits with 20 L of water as drip is being practiced in maintenance of field gene bank under arecanut and coconut-based cropping systems.

3.7 Biotechnology and bioinformatics

DNA fingerprinting with RAPD markers has been done earlier on 76 collections, and the clones VTLC-11, 67, and 83 and VTLC-93 were identified as highly divergent. DNA extraction protocol of cocoa with fully expanded but soft leaves is standardized with the modified SDS method. Recently, 16 SSR primers specific to cocoa were used to assess diversity in 44 exotic clones, and both morphological and molecular diversity were assessed in detail [52]. An attempt has been made to identify the markers for drought sensitivity by utilizing susceptible and tolerant parents and progenies of cocoa [53]. About 75% of the genomic data of cocoa is available in the public domain which has paved the way for analyzing genes related to specific needs. CPCRI hosts one of the Agri Bioinformatics center under the Department of Information Technology and through bioinformatics tools, proteins involved in drought tolerance, *Phytophthora* resistance, and carotenoid biosynthetic pathways have been analyzed, and databases, CocoaESTdb, CocoaSTRESSdb, and a Standalone EST microsatellite mining and analysis tool (SEMAT) have been developed [54–60].

4. Future prospects

Cocoa improvement has attained a positive phase with the sequencing of its genome. Identifying genes responsible for incompatibility and disease resistance is the main concern of geneticists and molecular biologists. Expression of genes for resistance and quality parameters and their validation with trait specific germplasm is very important for future cocoa improvement program. Possible use of inbred lines will be taken up. Development of early selection, detection, and diagnostic methods for resistance will enable rapid screening of plant material and permit pre-selection activities. Because of the health benefits of dark chocolates, biochemical

constituents and antioxidant properties of cocoa are to be given greater attention in the breeding programs. Farmers participatory plant breeding, *in-situ* conservation of land races, exploitation of flavor components from genotypes belonging to specific geographic region, varieties for changing climatic conditions, and environment-friendly management strategies will be considered. Adaptability of cocoa genotypes in traditional and nontraditional zones should be verified, and location specific varieties should be developed [61]. At the national level, expansion of cocoa cultivation with the quality planting material of elite clones, collaborative approach between research institutes, universities, state horticulture departments, and developmental agencies are required. At the international level, participation of India in cocoa genetic resources networking and regional breeding groups of both developed and developing countries is important. Collaboration of India with Asia Pacific regional countries, Malaysia, Indonesia, Philippines, Vietnam, and Papua New Guinea is essential with their common coconut-based cropping systems with known pest and disease problems. This will enhance region specific sustainability of cocoa cultivation.

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