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Review Paper

Genetic resources of cocoa (*Theobroma cacao* L.) and their utilization- An Appraisal

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Abstract

Cocoa (*Theobroma cacao* L.) is an important plantation crop adapted as a mixed crop in palm based cropping systems of India which effectively utilised the land, air and root space. Four decades of cocoa research paved way for identification of potential clones and development of varieties suitable for different agro climatic conditions and tolerant to both biotic and abiotic stresses. The achievements are well recognised by International cocoa research communities especially in the Asia Pacific region which is mainly on coconut based cropping models. The challenges of management of genetic resources in the introduced environment coupled with long term breeding strategies resulted in positive achievements to take Indian cocoa to satisfy the requirement of chocolate industries. Supply of quality planting material of elite clones and hybrids encouraged cocoa cultivation in non traditional areas as well.

Key words: Cocoa, *Theobroma cacao*, beans, genetics, germplasm, breeding

Introduction

Cocoa (*Theobroma cacao* L.), represented as 'Food of Gods', is an important beverage crop next to tea and coffee. The economic produce of cocoa, the cocoa bean, when fermented and dried, becomes the source for chocolate, the world's favourite food. Cocoa is native to Amazon basin of South America and was domesticated and distributed to different regions by the natives Mayas, Aztecs and Pipil-Nicaraos. The natives roasted and ground the cocoa beans and made an energy drink called 'xocoatl', which the Spanish introduced into Europe added with sugar and milk and christened as 'chocolate' which subsequently spread throughout the continents (Wood 1991). Currently 40-50 million people depend on cocoa for their livelihood and the production is 3.97 mt in which Africa contributes 71%, Latin America 14% and Asia and Oceania 14% (Jean Marc Anga 2013). Ivory Coast is the major cocoa producer followed by Ghana, Cameroon, Nigeria and Brazil. In the Asian continent, Indonesia and Malaysia are the leading cocoa producers (Malhotra and Hubbali 2016).

Cocoa was introduced into India way back in 1798 at Courtallam in Tirunelveli district of the old Madras state (Ratnam 1961). Cocoa cultivation was then widely taken up in the Western Ghats region, mainly the rubber and coffee growing zones of Malabar and Mysore states, having more rainy days and short dry spells. From 1970 onwards, cocoa is commercially being cultivated as a plantation crop and the current area under cocoa is 71.34 thousand ha covering the states of Kerala, Karnataka, Tamil Nadu and Andhra Pradesh with production of 18.10 tonnes of cocoa beans. Cocoa is a crop of the humid tropics, shade loving and grown as an under storey crop in agro-forestry systems in Latin American and African countries, and adapted as a mixed crop in palm based cropping systems of Asian and Pacific nations. In South India, cocoa is mainly grown under arecanut, coconut and to some extent in oil palm gardens. Cocoa is a perennial tree with typical plant habit, specific

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fruit characteristics, highly responsive to climate change and growing environment, which makes it necessary to have long term and dynamic breeding programme (Malhotra and Hubbali 2016).

History of cocoa improvement

Cocoa breeding efforts are one of the oldest improvement programme started way back in 1930 by Cocoa Research Unit (CRU), Trinidad and in 1944 by West African Cocoa Research Institute (WACRI), Cocoa Research Institute of Ghana (CRIG) and Cocoa Research Institute of Nigeria (CRIN). In 1950's, Cameroon (IRAD) and France (CIRAD) initiated cocoa improvement, and in 1963 Brazil after the foundation of CEPLAC (Executive Commission for the Cocoa Development Plan) and Ecuador (INIAP) intensified the resistance breeding programme. In 1980's, in the Asia Pacific region, Malaysian Cocoa Board (MCB), Indonesian Coffee and Cocoa Research Institute (ICCRI) and Coconut and Cocoa Research Institute (CCRI) of Papua New Guinea, have taken up systematic breeding programs. In 1994, the USA formed International Group for Genetic Improvement of Cocoa (INGENIC) to co-ordinate cocoa breeders around the world.

In India, the oldest plantations with Criollo type cocoa were established in Kallar and Burliar Fruit Stations in the Nilgiris in 1930-35 and evaluated for their performance. In 1962, Indian Council of Agricultural Research adopted a policy of introducing and growing Criollo type of cocoa in South India and Forastero type of cocoa in North East India. In 1965, a research-cum-demonstration unit of Cadbury India Pvt. Ltd. was established in Chundale in Kerala (Peter et al. 2002). In 1969, systematic research was started in Central Plantation Crops Research Institute (ICAR-CPCRI) 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) has initiated cocoa research with funding from Mondelez International.

Diversity in cocoa populations

Theobromo cacao L. the cultivated species of cocoa is a diploid (2n = 20), originally classified under Sterculiaceae and reclassified under Malvaceae family, having a small genome of 380 Mbp (Lanaud et al. 1992; Figueira et al. 1992). There are 22 identified species in this genus, *Theobroma angustifolium, T. bicolor, T. grandiflorum, T. mammosum, T. microcarpum, T. simiarum, T. speciosum* and *T. Subincanum* are being utilised in breeding programs. *Theobroma grandiflorum* is consumed widely as 'cupuacu' in Brazil for its flavoured mucilage surrounding the beans.

Diversity among the genetic resources is important in achieving effective improvement of the crop. Bartley (2005) explained that the existence of diversity depend on the degree of human involvement in the establishment of cocoa populations. They are divided into three categories (i) natural or spontaneous establishment and development, (ii) sub-spontaneous population, which is established by human action but have no evidence in its exploitation and (iii) cultivated or domesticated population formed by human action and are under continuous utilisation. Larger diversity is documented from cocoa populations from both natural and adopted habitats.

The three basic types of cocoa are, Criollo, Forastero and Trinitario, which have specific pod and bean characteristics and form the base for many old plantations as well as the new varieties developed from research institutes (Wood and Lass 1955). *Theobromo cacao cacao* consists of Criollo populations of Central and South America and *Theobromo cacao sphaerocarpum* includes all the other populations like Forastero and Trinitario (Cuatrecasas 1964).

Criollos

Criollos are the traditional types, with red or purple colour fruits having anthocyanin pigmentation, elongated with a sharp end, rough surfaced and has a hard husk. The beans are usually large and thick, 20-30 in number, white or pinkish in colour when fresh and cinnamon colored after 3 days of fermentation. Trees are slow growing and susceptible to pests and diseases. Pure Criollos of Central and South American origin are found only in isolated groups, characterized with specific flavour and are called as 'fine' cocoa. Soria (1970) described Colombian Criollo, Mexican Criollo, Nicaraguan Criollo or Cacao Real and Pentagona or Lagarto based on their origin and distinct characteristics.

Forasteros

Forasteros are vigorous trees, with rounded and smooth fruits, generally green colour pod turning to yellow when ripe, have hard husk, flat, dark purple beans, more than 30 in number, turn dark chocolate brown after 6 days of fermentation. Forasteros are grouped according to their geographic origin like West African

cocoas, which have different flavour suitable for manufacture of mild chocolates. Forasteros are generally referred as basic cocoa or 'bulk' cocoa. Amelonado, Comum, West African Amelonado, Cacao Nacional, Matina or Ceylan, Guiana Wild Amelonado and Amazon populations are grouped under Forasteros (Toxopeus 1985).

Trinitarios

Trinitarios are the hybrid types obtained from natural crosses between Criollos and Forasteros from Trinidad. They are mostly cultivated types, have characteristics of both types (Cheesman 1944). They are vigorous and distributed across many countries and almost 90% of the plantations today are with Forasteros and Trinitarios. Trinitarios in South East Asia and Oceania have contributed largely to the cocoa economy in the region.

Attempts are underway in all cocoa producing countries of the world to exploit the available variability, which is the stock for breeders to produce varieties with good economic characteristics, by conserving and evaluating them in field gene banks.

Indicators of variability

Expression of crop diversity is estimated from different indicators of variability especially, the morphological traits which are important for the cataloguing and characterisation of germplasm (Table 1). The variation in the phenotypic expressions is the result of the action of different alleles of genes that occur and control the specific characteristics and the total of the alleles that make up the plant's genotype (Bartley 2005). Bioversity International has standardized the descriptor status for cocoa, which comprises of 60 characteristics including plant habit, leaf, floral, fruit, seed traits and special features like reaction to biotic and abiotic stresses. Characterisation and passport data documentation on diversity has been undertaken in thirty year old cocoa collections at CPCRI (Elain Apshara and Rajan 2009) and national identity numbers (IC/EC No.) are obtained from National Bureau of Plant Genetic Resources (NBPGR), New Delhi for further utilisation in the breeding programme.

Plant habit, reproduction and flowering pattern in cocoa

Cocoa has a typical plant habit. Germination is 'epigeal' with the cotyledons raised above the soil and this first phase of development is called 'soldier' stage. Vertical or orthotropic growth continues, at a certain height the growth of the terminal bud of the main stem ceases, forms five lateral plagiotropic branches or fans and this process is called 'jorquetting'. These arise in whorls and growth of the lateral branches continues through elongation and successive new growths. Further vertical growth (chupon) continues on the main stem by the activation of the orthotropic buds in the leaf axils below the whorl of lateral branches. This pattern continues until the tree reaches an average of 20 m height in wild. But in the intercropping systems,

Table 1. Indicators of morphological variability in cocoa

Tree architecture	Erect, Intermediate and Pendulous growth habits
Leaves	Shades of green and purple, with or without pulvinus in the petiole
Flowers	Colour, Petal diameter, Pedicel length
Fruit shape	Angoleta- deeply ridged, warty, square at the stalk endCundeamor- bottle necked angoletaAmelonado- smooth, shallow furrows, melon shaped, blunt end, slight bottle neckCalabacillo- small and nearly sphericalOblong/ Elliptic/ Obovate/ Orbicular
Basal constriction	Absent/ Slight/ Intermediate/ Strong
Apex form	Attenuate/ Acute/ Obtuse/ Rounded/ Mammilate
Surface rugosity	Absent/ Slight/ Intermediate/ Intense
Prominence of ridges	Slight/ Distinct
Primary furrow depth	Superficial/ Intermediate/ Deep
Husk hardness	Soft/ Intermediate/ Hard
Anthocyanin in ripe pods	Absent/ Slight/ Intermediate/ Intense
Pod size	Big/ Medium/ Small
Beans	Shape, Color, Size

(Wood and Lass 1985; Bartley 2005)

single tier canopy is maintained and managed with proper training and pruning measures. Genotypes with medium vigour, small stature and canopy are being selected for easy management and evaluation.

Cocoa is a cross pollinated crop, reproduces sexually and this has resulted in lot of variation in the progenies. Vegetative propagation is also practised through cutting, grafting and budding. Clones produced from orthotropic shoots will give seedling like architecture whereas, that from plagiotropic shoots will give bushy appearance which require formation pruning. Criollos are less suitable for clonal propagation than Forasteros (Eskes and Lanaud 2001). Cocoa flowers are small and hermaphrodite, appears in large numbers on floral branches called 'cushions' on the shoot on attaining a minimum physiological age of two to three years. The flowering is referred as 'cauliflorous' or truncate bearing and the flowers are about 15 mm in diameter, borne on long pedicels, have 5 free sepals, 5 free petals, 10 stamens and ovary of 5 united carpels. The petals have an interesting shape, they are very narrow at the base but expand into a cup shaped pouch and end in a broad tip or ligule. The ten stamens which form the androecium are in two whorls, the outer whorl consisting of five long non fertile staminodes, the inner whorl with five fertile stamens. The stamens bear two anthers which lie in the pouch of the corresponding petal. The ovary has five parts containing many ovules arranged around a central axis. The style has the appearance of a single style and about twice as long as the ovary. The ovary contains 30 to 60 ovules and it is a highly heritable characteristic of cocoa. Fruit setting and ovular fertility depend upon the pollination and nutrition conditions (Lachenaud 1995). The flowers are generally pink with darker tissue in the staminodes and the petals, but there is considerable variation between cultivars in the size and colour of the flowers. The inflorescence primordia arises from old leaf axils and it takes about thirty days from initiation for the bud to emerge through the bark and mature. Though cocoa trees produce large number of flowers, only 1-5 percent of the flowers are successfully pollinated to produce a pod. Higher proportion of pod set is generally reported in Amelonado type cocoa.

Floral biology and pollination

When a bud matures the sepals split during the afternoon and continue to open during the night, early in the following morning the flowers are fully open and the anthers release their pollen, i.e., the anthesis will be between 14.00-16.00 hrs, which will be completed 2.00-4.00 hrs the next day. Anther dehiscence will be at 4.00-6.00 hrs and completed between 8.00-10.00 hrs. The style matures a little later. This is the best day for pollination and failure of fertilisation on this day will cause the flower to abscise the following day (Zamora et al. 1960). The pollination process is effected by various small insects in cocoa, in particular midges of the genus *Forcipomyia*, which pollinates around 5 to 10% of all flowers produced. A self-incompatibility system prevails among cocoa populations and the allogamy ranges from 50 to 100%. Upto 97% pollen viability is recorded in cocoa and 66% *in vitro* germinability is observed (Rajamony and Mohankumaran 1995).

Incompatibility mechanism

Self-incompatibility in cocoa was first reported by Pound (1932) when he showed that certain trees in Trinidad could not set fruit with their own pollen nor with one another. Since then the existence of self-compatible and self- incompatible trees has been established in most cocoa growing countries. In many plants incompatibility occurs in the style or stigma, preventing the development of pollen tubes, but in cocoa, the pollen tubes develop normally in all cases, but when the mating is incompatible the male gamete does not fuse with the female gamete and considered as 'gametophytic' incompatibility (Bartley and Cope 1973). Cope (1962) in Trinidad proposed five different S-factors or S-alleles to explain the results of extensive selfing and crossing studies between many cultivars. The dominance relations of these alleles are expressed in the following formula: Sa=Sb=Sc>Sd>Sf. Previously Knight and Rogers (1955) studied the compatibility relations within few families of Amazon material in Ghana and explained the dominance relations as S1>S2=S3>S4>S5. In an incompatible pollination, the proportion of non- fusion between the gametes will be 25, 50 or 100 per cent. In a failed set, the flower falls off three or four days after pollination. The degree of incompatibility varies between different populations. Self compatible genotypes are found in Lower Amazon Forastero, Criollo and Trinitario, whereas the Upper Amazon Forasteros are generally self incompatible (Eskes and Lanaud 2001). Amazon cultivars are all self-incompatible but are generally cross- compatible. Trinitario cultivars have a high proportion of self-incompatible trees, which will not cross with other self-incompatible trees, requiring pollen from self- compatible trees for successful pollination. The Amelonado population is entirely self- compatible.

Enzymic markers have enabled verification of the origin of seed collected depending on gene combinations and about 0 to 100% may result from selfing (Lanaud 1987). It is always suggested to plant mixture of populations. Self-incompatibility is made use of in the design and operation of seed gardens to ensure that seed of a certain desired parentage is produced (Wood and Lass 1985).

Seed gardens/clonal orchards

Based on the compatibility reactions self-incompatible but cross-compatible high yielding parents are selected multiplied as clones through soft wood grafting and established as clonal orchards or seed gardens. Two self-incompatible parents grown together in a bi- clonal orchard will produce hybrid pods of specific identity or known parentage through natural crossing. In poly-clonal orchard more self-incompatible clones are assembled together and all the pods harvested are hybrids. These clonal orchards are established at ICAR-CPCRI, Research Centre, Kidu, Nettana, Karnataka (Elain Apshara, 2013) and at College of Horticulture, KAU, Vellanikkara, which meet the demand of planting material for the area expansion programs in India. In cocoa, seed pods, seedlings and clones (grafts/budded plants) are being used as planting materials.

Breeding strategies

Systematic and long term cocoa improvement programs are being taken up (Table 2) with the following strategies *viz*., 1. Introduction of germplasm, conservation, cataloguing, characterization and evaluation of collections, 2. Breeding through selection and hybridization, 3. Establishment of seed garden/ clonal orchards and vegetative multiplication, 4. Comparative yield trials (CYT), 5. Multi locations trials (MLT), 6. Screening for biotic and abiotic stress, 7. Biotechnology and bioinformatics approaches and 8. Demonstration gardens (Elain Apshara et al. 2005).

Introduction of germplasm

The basic step in any breeding program is introduction of germplasm from both the primary and secondary centres 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 programs and to ensure continuous breeding activities in cocoa research centres in different regions, Intermediate Cocoa Quarantine Centre (ICQ, C) is established in Europe, far away from the production zone. The University of Reading took over the responsibility for cocoa quarantine and created the facility with twin-clad polyethylene covered tunnels (green house) in 1987 (Hadley and Lee 1992). The centre is collecting important clones from international and national gene banks and wild collections, carrying out the virus indexing with a susceptible West African Amelonado type cocoa. It conducts strict quarantine for other major diseases and pests as per the guidelines for safe movement of germplasm formulated by FAO/ Bioversity International (formerly IPGRI) (Frison and Feliu 1989; Michelle End et al. 2014). Clones which have cleared the quarantine, after two years are supplied as bud sticks, with proper import permits and sanitary certificates to cocoa researchers in over 20 countries. CPCRI initiated systematic cocoa research in 1969 through introduction of collections from Malaysia and Nigeria and later through ICQ, C for which NBPGR is the nodal agency within country for all the imports. Around 400-500 collections are being maintained in the National Active Germplasm Site (NAGS) for cocoa at CPCRI, Regional Station, Vittal, Karnataka and also by Kerala Agricultural University in Kerala. The germplasm collections include clones of Amazon, Brazil, Ecuador, England, Ghana, Jamaica, Mexico, Nigeria, Peru and local collections from Kallar in Tamil Nadu, Wayanad in Kerala and Shiradi Ghats of Karnataka. All these are being conserved and evaluated for their precocity, compatibility, adaptability, stability of yield, productivity and quality of produce in the introduced environment.

Breeding through selection

Presence of genetic variability offers wide scope in selection of potential types. In cocoa, the variability is so high that in a seedling population, about 75% yields are obtained from 25% of the trees. 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 and with favourable compatibility reaction. Throughout the world the initial selections were made from native types and land races. Traditionally trunk girth has been used to measure yield efficiency in cocoa, which ultimately represents the vigour of genotypes (Thong and Ng 1978) to produce more

number of pods. 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 five to eight years in varietal trials. 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 programmes as well as in establishment of seed gardens (Elain Apshara et al. 2005). Seven clones CCRP 1 to 7 were identified and released through these selections for cultivation. Though individual tree selections are 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 (Soria 1975) but higher when based on yields from plots containing several trees (Lockwood and Pang 1995) and so clonal varieties are gaining importance. In the beginning of this century, clonal selection programmes were initiated with pod index, bean size and disease resistance as selection criteria. To assess the phenotypic value of genotype even in hybrid selection programmes, clonal trials are advised (Eskes and Lanaud 2001). From the clonal trials three varieties VTLCC-1, VTLCS- 1 and VTLCS-2 have been released by ICAR-CPCRI for commercial cultivation in different agro-climatic zones in the country (Table 3). Genetic analysis on 17 plant, pod and bean characters in 44 Nigerian cocoa clones resulted in selection of superior genotypes for higher performance with traits of high heritability with high genetic advance. Based on pod yield, VTLC-25, VTLC-15, VTLC-18, VTLC-36, VTLC-13, VTLC-37 and VTLC-17 have been identified as heavy bearers with optimal canopy (Table 4). These clones recorded single dry bean weight of more than 1 gram, 10-15 percent shell, high nib recovery and more than 50 percent fat making them suitable for industries as well (Elain Apshara et al. 2009 and Elain Apshara and Nair 2011).

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 single tier architecture. In the evaluation trials of Trinitario type of 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 (Elain Apshara 2015; 2014) with optimal canopy of 15-20 m^2. Trait specific improvements are being taken up in the current breeding programs. Bean index of 100 beans/ 100 g i.e., dry bean weight of 1 g is considered as standard for grade I beans (GOI 1997) and so selections are aimed at larger bean size of 1 g and above, which will have high butter content suitable for chocolate industry. Bean size is influenced by genotype, environment and also the processing methods. Variation in bean indices have 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 hybridisation programs (Minimol et al. 2011) in KAU. CPCRI also collected white beaned genotypes and evaluating for quality parameters. Cocoa is considered as functional food

Table 2. Breeding criteria and traits involved

Yield	**Quality**
• Vigour of young trees Relationship between yield and vigour of adult trees • Interaction with planting density • Ability to adapt in the field • Productivity of young trees (earliness) • Productivity of adult trees • No. of pods per tree • Bean wt. per pod (pod index: no. of pods needed for 1 kg of dry beans)	• Physical criteria: dry bean size (1 gram and above), shell percentage (10-15%), nib recovery (85-90%), butter content (>50%) hardness, colour of cotyledons • Organoleptic characters: flavour, viscosity, taste (bitter, astringent)
Resistance to diseases	**Resistance to insects**
• Intrinsic resistance: resistance to infection, colonisation or pathogen multiplication, grading of damage levels • Escape: period of fruiting or vegetative growth, duration of pod ripening, low productivity	• Attractiveness • Screening: grading with level of infection, husk characters, • Intrinsic resistance (antibiosis), Antixenosis,tolerance

Eskes et al. (2000); Eskes and Lanaud (2001); Eskes and Efron (2006)

Table 3. Characteristic features of CPCRI cocoa varieties developed through selection

VTLCC-1	VTLCS-1	VTLCS-2
Vittal Cocoa Clone 1 Clonal selection, heavy bearer, both self and cross compatible Colour-Green to Yellow No. of beans/pod-37, Single dry bean weight-1.05 g Dry bean yield/tree/year-1.33 kg	**Vittal Cocoa Selection 1** Selection, Stable, high yielder both under arecanut and coconut, withstands biotic and abiotic stresess Colour-Red to Orange No. of beans/pod-42 Single dry bean weight-1.13 g Dry bean yield/tree/year-2.5 kg	**Vittal Cocoa Selection 2** Selection, Stable, high yielder both under arecanut and coconut, bold and bigger beans, less incidence of pests and diseases Colour- Green to Yellow No. of beans/pod-42 Single dry bean weight-1.21 g Dry bean yield/tree/year-2.7 kg

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 (Counet et al. 2004). Cocoa beans of different clones evaluated at CPCRI 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 to 55% and antioxidant activity of 77 to 98% among cocoa clones studied. In general, cocoa beans with high polyphenol and procyanidin contents exhibited high antioxidant activities which are utilised for qualitative improvement. Cocoa butter with free fatty acid content (FFA) of 1% or less together with acceptable flavour is the best indication of good quality beans. Prolonged storage may cause fat degradation and rise in FFA concentration. Type of fatty acids in 18 hybrids and 10 elite clones has been assessed and from the profile it is clear that there are 11 fatty acids involved in the quality of cocoa beans. The fatty acids palmitic, stearic, oleic, linolic 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 percentage of expression (Senthil Amudhan and Elain Apshara 2012).

Breeding through Hybridization

Hybrid vigour between parents showing good combining ability is readily exploited in cocoa improvement programs. Posnette (1951) demonstrated inter-population heterosis in cocoa. The initial crosses involving Pound's seedling collections showed exceptional vigour, precocity and high yield in Ghana. These observations and similar ones in Trinidad are attributed to hybrid vigour (Bell and Rogers 1956). Most commonly adopted method is developing hybrids between two distant genotypes and selecting hybrid varieties, which is based on heterosis and hybrid vigour (Toxopeus 1972).

Method of hand pollination and fruit set

For the production of true hybrids with specific

Table 4. A list of best cocoa clones with desirable characteristics

Clone	Canopy area (m^2)	Pod no./tree/yr.	Bean no./pod	SBW(g)	DBY kg/tree/yr.	Shell(%)	Fat(%)
VTLC-25	11.81	61.9	42.7	1.03	2.45	10.9	51.0
VTLC-15	20.40	53.3	42.3	1.12	2.53	11.5	51.3
VTLC-18	12.26	49.4	43.0	1.17	2.48	13.1	53.2
VTLC-36	10.74	48.4	43.3	1.00	1.45	12.7	50.6
VTLC-13	11.34	45.1	43.0	1.00	1.93	11.2	54.5
VTLC-37	10.11	44.2	43.3	1.00	1.83	13.9	50.1
VTLC-17	13.39	43.1	40.3	1.24	2.16	13.1	53.2
VTLCS-1	15.25	55.0	41.0	1.13	2.52	11.0	52.0
VTLCS-2	14.60	54.5	41.0	1.21	2.70	15.0	53.0

SBW = Single dry bean weight; DBY = Dry bean yield; yr = Year

objectives and to confirm the compatibility reaction, hand pollination is being practiced. In artificial pollination, a flower bud which will open the following day, recognized by its whitish colour and swollen appearance, is selected. The bud is covered with hood of plastic tube/hose pipe piece 5 cm x 1.5-2 cm, 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. This ensures circulation of air and exclusion of insects. 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 style is surrounded by a ring of staminodes and if these are long, removal of two or three staminodes should be done for easy access to style. Emasculation is not necessary due to the presence of self- incompatibility. For selfing, hand pollination is done using stamens from the same flower. The pollinated flowers are labelled using tin foil pieces fixed in the cushion using ball pins. The hoods are removed 24 hrs after pollination and in three to five days, fertilization is confirmed by the visual swelling of the ovary. In order to prevent undue shedding and wilting of fruits from hand pollinations, it is usual to remove all the developing fruits on the tree produced by open pollination (Vikraman Nair et al. 2002; Chaudhary and Malhotra 2001).

In the natural pollination, too many fruits are normally set for the tree to carry through to maturity. The young developing pod of cocoa is called 'cherelle' and cocoa has its fruit thinning mechanism of 'cherelle wilt' which occurs by yellowing, blackening and shrivelling. The incidence of wilt increases from pollination to a peak at about 50 days, then falls off and rises to a second peak at 70 days. After 95-100 days no further wilting takes place, cherelles then become immature pods, reach full size, become mature and finally ripe. The ovule is filled with jelly like endosperm, which is utilised by the embryo about 140 days after pollination. During this period there is a rapid accumulation of fat. It takes 5-6 months from pollination to ripening. The mature fruit or pod is botanically an indehiscent drupe which remains on the tree unless harvested. Seeds are recalcitrant, without dormancy and so should be sown on the day of extraction for raising seedling nurseries. The mucilagenous pulp surrounding the beans is important for fermentation in the primary processing for chocolate making but for sowing it should be removed. Viviparous germination is observed in over ripe pods.

Table 5. Progeny Trials of CPCRI

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

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 centres with objectives of more number of pods, high dry bean yield, big bean size and drought tolerance (Table 5). Of these, 20 hybrids were identified as best hybrids and further evaluated as clones.

Table 6. Characteristic features of CPCRI varieties developed through hybridization

VTLCH-1
Vittal Cocoa Hybrid 1
Hybrid, vigorous, early, heavy bearer
Colour-Green to Yellow
No. of beans/pod-42 Single dry bean weight-1.00 g
Dry bean yield/tree/year-1.48 kg

VTLCH-2
Vittal Cocoa Hybrid 2
Hybrid, early, heavy bearer with medium canopy, tolerant to black pod rot Colour-Green to Yellow
No. of beans/pod-40 Single dry bean weight-1.15 g
Dry bean yield/tree/year-1.15 kg

VTLCH-3
Vittal Cocoa Hybrid 3
Hybrid, heavy bearer, suitable for water limited conditions
Colour-Green to YellowNo. of beans/pod-45
Single dry bean weight-1.07 g
Dry bean yield/tree/year-1.45 kg

VTLCH-4
Vittal Cocoa Hybrid 4
Hybrid, heavy bearer, suitable for water limited conditions
Colour-Red to OrangeNo. of beans/pod-43
Single dry bean weight-1.01 g
Dry bean yield/tree/year-1.25 kg

Among them 17 progenies exhibited high vigour and cropping efficiency even at early years of development (Elain Apshara et al. 2008). 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 (Table 6).

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 are done with HD^2 (H-Height, D-Diameter) value. A total of 3126 superior seedlings were field planted in series I,II,III,IV and progeny trials I,II,III,IV, 163 superior hybrids were selected, utilized in various breeding programme 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 Vascular Streak Die back (VSD) have been released as varieties for cultivation in the country (Minimol et al. 2011).

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 upto six to seven generations to attain homozygosity and thereafter to be utilized for crossing to exploit the hybrid vigour. 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 (Mallika et al. 2002; Vikraman Nair et al. 2002; Minimol et al. 2011).

Comparative Yield Trials (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 VTLCP-6, VTLCP-20, VTLCP-15, VTLCP-1 and VTLCP-19 have been identified as best performers even in their initial years of growth (Elain Apshara et al. 2008). Comparative study of parents and progenies as clones, resulted in identification of VTLCP-6, VTLCP-2 and VTLCP-20 and parents VTLC-1 and VTLC-56 as potential high yielders. In another trial, clones suitable for both arecanut and coconut canopies have been identified (Elain Apshara et al 2013) and released as varieties. Under coconut in double hedge system of planting, hybrids VTLCP-22, VTLCP-18 and VTLCP-1 showed the best performance with optimal canopy and high yield. Evaluation of clonal and seedling progenies of selected genotypes has resulted in identification of 4 hybrids and 2 clones for multiplication both as clones and seedlings for utilization in the area expansion programme (Elain Apshara 2014).

Multi Location Trials (MLT) and Demonstration plots

To assess the adaptability 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 non traditions states, namely Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, West Bengal and Assam, for studying genotype x location x environment interactions. Further, 94 front line demonstration plots are established under participatory research cum demonstration plots scheme funded by Directorate of Cashewnut and Cocoa Development (DCCD), Kochi in eight taluks in Karnataka and one taluk of northern Kerala and five AICRPP (All India Co-ordinated Research Project on Palms) centres for identification of location specific varieties and to tackle the climate change effects.

Resistance breeding

The perennial crop stand of cocoa in the native and productive zones is facing severe setback with debilitating diseases, which causes an estimated loss of 30% of world production. Three of the most important diseases of cocoa are black pod rot caused by several *Phytophthora* species, frosty pod rot caused by *Moniliophthora roreri* and witches' broom caused by *Moniliophthora perniciosa* (Ploetz 2007). *P. palmivora* is present in the entire cocoa growing area and *P. megakarya* is the most aggressive species with losses upto 50%, but is limited to some countries in West Africa. In Brazil, *M. perniciosa* is responsible for the drastic yield loss, which brought down its ranking in world cocoa production. Vascular Streak Die back (VSD) caused by *Oncobasidium theobromae* is found in South East Asia causing 20-30% loss in Papua New Guinea. In Africa, the cocoa economy is extensively affected by several viral diseases caused by Cacao swollen shoot virus (CSSV), Cacao yellow

mosaic virus (CYMV) and Cacao necrosis virus (CNV) which necessitated strict quarantine measures in germplasm exchange programs (Michelle End et al. 2014). Chemical control can be effective against fungal diseases but will pollute the environment and make the cultivation expensive. Integrated disease and pest management by use of resistant materials, cultural and biological methods is probably the best way to contain pathogens and pests in the longer run for sustainable resistance. And so, disease resistance became the primary trait targeted by cocoa breeders worldwide. Sources of resistance have been identified for black pod rot (Iwaro et al. 2006), witches' broom (Umaharan et al. 2005) and frosty pod rot (Philips-Mora and Wilkinson 2007). Since cocoa genome is sequenced, it is expected to provide models for plant pathogen interactions and also facilitate identification of resistance genes.

The insects causing severe damage in cocoa are mirids (*Sahlbergella* sp., *Distantiella* sp., and *Helopeltis* sp.) and thrips (*Selanothrips rubrocintus*). In Africa, mirids pose severe threat and in South East Asia, the cocoa pod borer (*Conopomorpha cramerella*) causes heavy loss (Azhar 1986). Many cocoa pests like mealy bugs, aphids, caterpillars and borers are also reported in India (Mariamma Daniel 2002) which are being managed with need based chemical control measures. Screening of available germplasm for prevailing diseases, existing and emerging pests are very important in light of the seasonal weather variations.

Black pod rot

India is free of most of the debilitating viral and fungal diseases of 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. In Costa Rica, through screening nine cultivars *viz*., EET 59, EET 376, UF 713, UF 715, SCA 6, SCA 12, Pound 7, Catongo and Diamantes 800 have been found to have promising degree of resistance (Lawrence 1978). In Java, SCA 6, SCA 12 and ICS 6 were found to exhibit resistance. At CPCRI based on field screening, clones have been categorized into <10%, 10-25% and >25% damage levels. *In-vitro* screening using isolates of *P. palmivora*, *P. capsici*, *P. citrophthora* indicated that collections of Nigerian origin exhibit certain degree of tolerance (Chandramohanan 1982). Few Wayanad collections have also expressed field tolerance to pod rot when tested over three seasons at CPCRI RS Vittal. Further, 21 exotic clones collected exclusively for *Phytophthora* pod rot resistance are identified for utilization in cocoa hybridization programme at ICAR-CPCRI. KAU has taken up screening and hybridization programme for combining desirable traits of CCRP released varieties and black pod resistance in cocoa.

Vascular streak die back

In India, Vascular streak die back (VSD) caused by *Oncobasidium theobromae*, was first reported from Kottayam, Kerala by Abraham (1981) 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 programme 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 utilised for establishment of clonal gardens for seedling supply (Mallika et al. 2000). Malaysia, Indonesia, Philippines, Vietnam and Papua New Guinea in the Asia- Pacific region have taken up VSD resistance programs and most of the Trinitario hybrids have been found to be resistant to this disease.

Tea mosquito bug

Tea mosquito bug (TMB) (*Helopeltis* sp.) incidence became severe during last three years in summer and post monsoon seasons. *Helopeltis antonii, H. theivora* and *H. bradyi* are reported in 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 ûeld damage (N' Guessan et al. 2004). Tolerance to *Sahlbergella singularis* and *Distantiella theobromae* has been measured by observing cumulative and recent damage in the field trials in Cameroon and Ivory Coast. This method permits ranking of genotypes according to their global reaction to mirid attacks (Anikwe et al. 2009).

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 at CPCRI in 100 cocoa genotypes and interpreted with reference to insect resistance (Elain Apshara 2013). Husk characteristics have been given special importance in the recent years in the Asia-Pacific region especially for bod borer resistance. 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 linked to the ability of a tree to contain damage and recover from it. Red coloured pods with smooth surface have been identified as tolerant to TMB damage among Wayanad collections at ICAR-CPCRI.

Low moisture stress

Cocoa plants are susceptible to environmental conditions especially temperature and drought and considerably influences the pod yield (Daymond et al. 2000). Cocoa is very sensitive to water scarcity and undergoes a period of low moisture stress for five to six months in its current growing condition in India. Breeding for drought tolerance is unique to our country and is taken up with systematic screening of available germplasm 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 (Balasimha 1999). 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 behaviour, 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 at CPCRI RS Vittal. Recently, photosynthesis, chlorophyll fluorescence and water potential under stress and non stress conditions were estimated in 11 genotypes from different geographical origins, Columbia, Brazil, Peru, Mexico and Ecuador (Elain Apshara 2013). Seasonal and varietal differences were found and transpirational water loss was found to be reduced with increased stomatal closure, which is considered as a favourable drought trait in any crop. Among the 52 new introductions, 5 Amazon and Pound collections have been found to be adaptable to water limited conditions (Balasimha et al. 2013) with high yields, which will be further utilized in the breeding programme.

To make the breeders task easier Bioversity International, The Common Fund for Commodities (CFC) and The International Cocoa Organization (ICCO) have also designed the working procedure for field screening and physiological evaluation of cocoa germplasm (Eskes et al. 2000). It is concluded that cocoa yield can be considered within a relatively simple physiological framework that helps to explain yield capacity and growth of cocoa genotypes under certain climatic conditions. Hadley (2000) detailed the visual estimates of physiological traits in cocoa, which are to be taken in clonal plants on plot basis with 6- 8 trees and in hybrid plants on individual tree basis. Morpho-physiological parameters include measurements on 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 optimum canopy shape and structure of cocoa, different spacing and canopy sizes have been studied at CPCRI 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 m x 2.7 m and 2.7 m x 5.4 m) and three canopy sizes (small, medium and large) and similar results were noticed with transpiration rate and stomatal conductance also (Balasimha 2006). 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.

Biotechnology and bioinformatics

One of the central applications of molecular biology is in the use of molecular markers in studying relationships between accessions using phylogenetic

analysis. For cocoa, this has been approached with isozymes, Randomly Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphisms (RFLP) and other types of genomic DNA markers. Most recently, microsatellite markers (SSR's) have gained acceptance as the most accurate and reliable method.

Quantitative traits refer to phenotypes or characteristics that vary in degree and can be attributed to polygenic effects, *i.e.,* product of two or more genes, and their environment. Quantitative trait loci (QTLs) are stretches of DNA containing or linked to the genes that underlie a quantitative trait. Mapping of regions of the genome that contain genes involved in specifying a trait can be done using molecular tags or markers. This is an early step in identifying and sequencing the actual genes underlying trait variation. QTL analysis of agronomic traits, quality parameters and disease resistance have been undertaken and results have been obtained on trunk diameter, canopy height, earliness in flowering, number of ovules per ovary, pod length, bean number, bean weight and resistance to *Phytophthora*. This will enable introgression of particular traits in varieties using marker assisted selection (N'goran et al. 1995; Crouzillat et al. 1996). TropgeneDB is maintained by CIRAD, France which comprises of genetic and physical maps, marker information, QTL's, sequence data and molecular data on genetic resources (www.tropgenedb.cirad.fr). Penn State University conducted an extensive comparative study on flower development in *Arabidopsis thaliana* and *Theobroma cacao* and examined the expression of several key floral regulatory genes (Swanson 2005) in continuation of the reclassification of cocoa from Sterculiaceae to Malvaceae (Alverson et al. 1999; Bayer et al. 1999; Whitlock et al. 2001). The INGENIC Study Group for Molecular Biology was chartered in 2003 to coordinate the activities of the members interested in molecular approaches (Guiltinan 2007).

DNA fingerprinting with RAPD markers has been done at CPCRI earlier on 76 collections and the clones VTLC-11, 67, 83 and VTLC-93 were identified as highly divergent. DNA extraction protocol of cocoa with fully expanded but soft leaves is standardized with modified SDS method. Recently 16 SSR primers specific to cocoa were used to assess diversity in 44 exotic clones of Nigerian origin. An attempt has been made to identify the markers for drought sensitivity by utilizing susceptible and tolerant parents and progenies of cocoa (M'bo Kacou et al. 2014). Cocoa genome has been successfully sequenced by CIRAD, France and Penn State University, USA along with a group of institutes and 75% of the genomic data is available in the public domain which has paved the way for analyzing genes related to specific needs (Xavier et al. 2011). CPCRI hosts one of the Agri Bioinformatics centre under Department of Information Technology and through bioinformatics tools, proteins involved in drought tolerance, *Phytophthora* resistance and carotenoid biosynthetic pathways have been analysed and databases, CocoaESTdb, CocoaSTRESSdb have been developed (Naganeeswaran et al. 2014; Naganeeswaran et al. 2015; Naganeeswaran and Elain Apshara 2011; Naganeeswaran et al. 2012; Elain Apshara et al. 2013a).

Future prospects

Cocoa improvement has attained a positive phase with the sequencing of its genome. Identifying genes responsible for incompatibility and disease resistance are 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 programme. The past few decades of research have been restricted with single cross hybrids and future development should focus on double and triple crosses, backcrosses and possible use of inbred lines. Development of early selection, detection and diagnostic methods for resistance will enable rapid screening of plant material and permit preselection activities. Because of the health benefits of dark chocolates, biochemical constituents and antioxidant properties of cocoa is to be given greater attention in the breeding programs. Farmers participatory plant breeding, *in-situ* conservation of land races, exploitation of flavour components from genotypes belonging to specific geographic region, varieties for changing climatic conditions and environment friendly management strategies are to be considered. Adaptability of cocoa genotypes in traditional and non-traditional zones should be verified and location specific varieties should be developed. At the National level, expansion of cocoa cultivation with quality planting material of elite clones, collaborative approach between research institutes, universities, state horticulture departments and developmental agencies are required. In the International level, participation of India in cocoa genetic resources networking and regional breeding groups of both developed and developing countries is important, to tackle the common and specific problems faced by the cocoa growers.

Declaration

The authors declare no conflict of interest.

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