ACHIEVEMENTS IN COCOA BREEDING PROGRAMMES AT CPCRI

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Introduction

The first cocoa brought into India is said to be in 1798 after Malaysian and Sri Lankan introductions, when 8 plants were first shipped from Amazon islands and planted at Courtallam in Tirunelveli district of Madras state (Ratnam, 1961). Later in 1873 few plants were planted in Burliar fruit station by Mr. F. B. Thomas the then collector of Coimbatore. No attention was paid to these plants until 1955 and later an attempt on research was started at Kallar and Burliar under the auspices of ICAR. Wood (1964) found the states of Kerala, Madras and Mysore to be the most suitable areas for cocoa cultivation, with rains from both monsoons and short dry period, especially in extreme south and parts along the eastern side of Western Ghats. Many introductions were effected in the early 20th century, but cultivation was limited to few Government farms with both Criollo and Forastero types of cocoa. In 1969 cocoa improvement programmes were started at Central Plantation Crops Research Institute (CPCRI) with few introductions from Malaysian estates. From then on intensive procurement of cocoa germplasm from primary and secondary centres of origin and distribution, conservation of accessions in field gene banks, characterization for diversity, evaluation for desirable characteristics, selection and hybridization works are being done with significant success.

I. Genetic Resources Management:

1. Collection, conservation and cataloguing of cocoa germplasm

Exploiting the available variability in the cocoa populations is very important for breeding program and so germplasm collection was done with clones of native types, genetic groups (Criollo, Forastero, Trinitario, Upper and Lower Amazon Forastero, Amelonado and Refractario) and with desirable traits like high yielders with quality beans and resistant to biotic and abiotic stresses. The present collections at CPCRI is 291 which comprised of accessions from Amazon, Brazil, Ecuador, England, Ghana, Jamaica, Malaysia, Mexico, Nigeria, Trinidad and local collections from farmer's fields of Wynad in Kerala, Shiradi ghats of Karnataka and Kanyakumari of Tamil Nadu. Some clones are obtained from KAU, Lalbaugh gardens, Bangalore and Kallar in Tamil Nadu. All these are being conserved in field gene banks of CPCRI, Regional Station, Vittal, Karnataka under arecanut and coconut. Apart from germplasm blocks, compact block on varieties, scion bank and hybrid blocks are being maintained.

Documentation of passport data on 56 Kannara clones completed (Elain Apshara and Rajan, 2009). EC and IC numbers for 76 clones were obtained from NBPGR. Detailed descriptor with 60 characteristics comprised of general characters, plant habit, leaf, flower, fruit, seed and special characters along with photographs was done for 50 clones. Cocoa Germplasm Database was prepared and provided for online retrieval through cpcri website. Diversity in the available cocoa populations was assessed. Based on the pod shapes, 12 Amelonado, 13 Cundeamor and 31 Angoleta types were catalogued in the collections including hybrids.

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II. Breeding:

1. Selection

An easy approach to yield improvement in cocoa is to select plants superior in yield and their subsequent development into clones. Under our environment the following criteria are being followed.

Criteria for selection

- Trees with medium canopy under intercropping system.
- Vigor x yielding efficiency.
- Compatibility reaction.
- Trees bearing 50 or more pods/tree/year.
- Medium to large pods of not less than 350 g weight, smooth pods. Husk thickness of pods to be more than 1 cm.
- Pod value (number of pods required to produce 1 kg beans) to be not more than 12.
- Number of beans per pod should be more than 35.
- Bean dry weight should be more than 1 gram.
- Dry bean yield should be more than 1 kg/ tree/year.
- Shelling percentage-10-15%.
- Fat content- >50%.

Seven high yielding clones, I-56,1-14, II-67, III-35, III-105, IV-20 and NC-42/94 were selected and utilized as parents in the initial breeding programmes (Bhat, 1999).

2. Hybridization

Hybridization programme was started at Vittal in 1980 with selected self-incompatible but cross-compatible parents with specific objectives of more number of pods, high dry bean yields, quality beans and drought tolerance. Four sets of hybrids were produced at Vittal, planted and evaluated under progeny trials from 1983 to 1992. A comparison of parents and hybrids in progeny trials indicated that more vigour is exhibited by the progenies than parents and showed positive and significant heterosis over their mid- parental value (Bhat and Ananda, 1997).

Progeny Trial I (1983)

The parents in the first progeny trial included Upper Amazon collections/Imperial College Selections, Scavina and Nanay series and their hybrids. The progenies NA-33 x ICS-89, NA-31 x ICS-89, SCA-6 x IMC-67 and SCA-6 x ICS-6 were identified as best yielders.

ProgenyTrial II (1984)

It had a total of 17 hybrids, their parents and a check line I-56 seedling. All showed significant differences with regard to their bean yield. The progenies, I-56 x II-67, 1-14 x II-67, 1-14 x 111-105 and 1-14 x NC-42/94 were identified as promising hybrids.

Progeny Trial III (1987)

It involved 9 hybrids, 4 Malaysian hybrids and bulk Forasteros. From consistency point of view three progenies ICS-6 x SCA-6, ICS-6 x SCA-12 and IMC-67 x ICS-6 and a Malaysian hybrid Amelonado x NA-33 were identified with medium canopy and high yield.

Progeny Trial IV (1992)

Under the fourth set nine hybrids with their seven parents were evaluated for high yield and drought tolerance. Bio chemical components showed significant differences among hybrids and parents (Balasimha and Ananda, 1999). The hybrids II-67 x NC-42/94 and II-67 x NC-29/66 were found as suitable for water limited conditions.

Clonal Trial (1985)

Eight high yielding trees of Nigerian origin (NC-102, NC-119, NC-73, NC-63, NC-13, NC-116, NC-53 and NC-8) were selected and multiplied clonally and further evaluated. Among them the clone NC-45/53 had the highest yield range of

0.930-1.726 kg dry bean yield/tree/year and this clone is both self and cross compatible. Another clone NC-38/119 also selected as it showed the best stability indices.

Varieties

These hybridization works resulted in development of varieties which are vigorous, early, heavy bearing, stable yielders, VTLCH-1, 2, 3,4 and a clone VTLCC-1, with standard bean characters and industrial value (Elain Apshara *et a/.*, 2008). These are suitable for cultivation in Kerala, Karnataka, Tamil Nadu, Andhra Pradesh, Goa, Maharashtra and North Eastern states.

Table 1. High yielding varieties of CPCRI

Variety	Single bean wt(g)	Shelling %	Dry bean yield kg/tree/year	Dry bean yield kg/ha	Special feature
VTLCC-1	1.05	12.0	1.15	904	Early, heavy bearer, self & cross compatible
VTLCH-1	1.00	13.0	1.48	1007	Early, high yielder
VTLCH-2	1.10	10.9	1.15	800	High yielder with medium canopy
VTLCH-3	1.06	13.7	1.48	1005	High yielder & drought tolerant
VTLCH-4	1.01	12.1	1.25	847	High yielder & drought tolerant

3. Multiplication and establishment of clonal orchards

Along with progeny trials, for supply of quality planting materials clonal orchards or seed gardens were established in 1987. Based on the compatibility reactions self-incompatible but cross-compatible high yielding parents were multiplied as clones and assembled as clonal orchards. With known parentage and performance, two clones each were planted schematically in bi-clonal orchards and multiple clones in poly-clonal orchard for production of F1 hybrids through natural pollination. These orchards were established at CPCRI, Research Centre, Kidu, Nettana, Karnataka. On an average these will meet out the demand of around 20 lakhs seedlings.

Table 2. Details of clonal orchards

Bi-clonal	Poly-clonal
I-56 & NC-42/94	1-14
ICS-6 & SCA-6	I-56
I-56 & 111-105	111-105
1-14 & NC-42/94	NC-42/94
1-14 & I-56	
1-14 & IV-20	Total: 1480 trees

Vegetative propagation through soft wood grafting method was standardized for multiplication of selected accessions and high yielding hybrids for production of true to types, as well as for early evaluation. Grafts are being supplied to cocoa growers, demonstration farmers and developmental agencies regularly along with F1 seedlings and seed pods under National Seed Project of ICAR.

Table 3. Distribution of cocoa grafts/ seedlings

Year	No.	Year	No.
2000	69,287	2006	44,876
2001	26,992	2007	73,034
2002	54,697	2008	1,02,091
2003	36,718	2009	75,000
2004	48,572	2010	70,436
2005	51,244	**Total**	**6,52,947**

With funding from Directorate of Cashewnut and Cocoa Development (DCCD), Cochin, 17 regional nurseries were established in the year 2005 in five states of Karnataka, Andhra, Tamil Nadu, Maharashtra and Goa for unhindered supply of quality planting materials in cocoa growing regions. They were supplied with grafts of superior mother trees from CPCRI.

Table 4. Regional nurseries

State	No. of regional nurseries	No. of grafts supplied
Karnataka	9	11,250
Andhra Pradesh	2	2,500
Tamil Nadu	3	3,750
Maharashtra	2	2,500
Goa	1	1,500
Total	**17**	**21,500**

4. Breeding for stress Drought tolerance

Breeding for drought tolerance was taken up, since cocoa is sensitive to water scarcity and it has to undergo a period of five to six months in its growth period without rains. The germplasm was screened for physiological parameters like stomatal resistance, chlorophyll fluorescence, proline accumulation under stress and seed germination under low osmotic potential. The Nigerian collections were found to be good source for drought tolerance. Balasimha *et al.* (1985) observed considerable genotypic difference in specific leaf weight and epicuticular wax content and those with high values were found to perform better under stress conditions. Five accessions selected for drought tolerance were, NC-23, NC-29, NC-31, NC-39 and NC-42. Hybridization programmes identified the hybrids 1-21 x NC-42/94, 1-29 x NC-23/43, II-67 x NC-42/94 and II-67 x NC-29/66 as drought tolerant. All new collections are continuously being evaluated for drought tolerance.

Screening for Black Pod Disease

Cocoa growing in heavy rainfall zone made them susceptible to black pod rot. *In-vitro* screening of majority of the available germplasm against the black pod disease using isolates of prevailing three *Phytophthora spp.*, viz., *P. palmivora, P. capsici, P. citrophthora* has indicated that Nigerian collections exhibits certain degree of tolerance (Chandramohanan, 1982).

Evaluation for Tea Mosquito Bug resistance

Tea mosquito bug {*Helopeltis* sp.) incidence was severe during last three years in summer seasons. Individual treewise data on flushes, cherelles and pods were taken and the percentage damage ranged from 19.68 to 63.53%. The lowest damage was observed in the clone NC-51 whereas, it was the highest in NC-34. The damage was lesser in clones NC-39, NC-25 and NC-57. Among the 21 progenies the damage percentage varied from 23.21 to 100%. The lowest damage of 23.21% was observed in the progeny VTLCP-7 followed by VTLCP-5 with 24.68% damage. 100% damage was observed in four progenies. Among the 10 clones the percentage of incidence varied from 65.91 to 100% under dense plantings. Further systematic evaluation on antixenosis, antibiosis and tolerance is going on.

Asia-Pacific breeding program for pod borer resistance

Pod borer caused by *Conopomorpha cramerella* is a threat to cocoa cultivation in Asian countries. Under Asia- Pacific regional breeding initiatives husk characteristics of clones were given emphasis with penetrometer readings for further

interpretation with the possible level of insect resistance. 100 accessions including clones and hybrids were tested. At the top, middle and bottom portions of pods the readings ranged from an average of 4.42 at primary furrows to 4.82 at secondary furrows. Maximum values were recorded in the clones II-52, NC-9, K-179, W-12, NA-242 and P-4 which ranged from 5.17 to 7.82 and 6.28 to 8.00 at primary and secondary furrows respectively. The ridge thickness was the maximum in Nigerian collections which ranged from 1.08 to 1.86 cm and furrow thickness was the maximum in Malaysian collections which ranged from 0.78 to 1.14 cm. These will be utilized in the hybridization program and exchanged with other countries. Hybrids PBC-123 x LAFI-7, QH-22 x NA-33 and MCB.C.3 x KKM.22 from Malaysian cocoa board and BR-25 x K-2 and BR-25 x S-5 from Philippines, resistant to pod borer also included in the collections under this program. Biochemical characterization of Malaysian clones with regard to total phenol content to assess the antioxidant properties and level of resistance was done in shoot, leaf and bean samples, which ranged from 5 to 10 μ mol.

5. Evaluation trials

- Evaluation for compatibility was done with artificial pollination through selfing and crossing to find out the self-compatible, self-incompatible and cross-compatible clones. Among 23 Malaysian trees studied, 12 trees were self-incompatible and 11 self-compatible. Out of the 34 from the Nigerian collections, 22 were self-incompatible and 12 self-compatible. Both Malaysian and Nigerian collection had similar pattern of distribution for self-compatible and self-incompatible trees. The studies further revealed that different trees belong to same accession shouldn't be identical with regard to their compatibility reaction (Nair and Rekha, 1996). Among other germplasm collections 22 self-incompatible and cross-compatible, 26 self-incompatible and 7 self and cross-compatible clones were identified.
- Malaysian clones 1-21,1-14, II-67 and 11-51 were identified as yielding more than 2 kg dry beans per tree per year. Eleven Malaysian clones were tested for their adaptability under Kerala and Karnataka conditions and assessed for their pod yield performance with respect to no. of pods and pod weights over different growth periods. The clones Amelonado x NA- 32, Jerangau Red Axil, Landas- 357 and Landas- 364 have recorded high yields under both environments.
- Fifteen Lalbaugh clones were evaluated for their nature and degree of genotype x environment interaction over different seasons of growth. Based on magnitudes of stability parameters and high yield 7 clones were identified (Elain Apshara *et al.*, 2002). The clones V-1 and V-2 yielded 2.2 and 2.0 kg dry beans at Vittal, Karnataka whereas, the clones SCA-6, SCA-12, NA-242 and SIAL- 93 performed better under Kannara, Kerala conditions with more than 2.0 kg dry bean yields/ tree/ year.
- Computation of phenotypic and genotypic coefficients of variation, heritability and genetic advance studies on 17 plant, pod and bean characters was done in 44 Nigerian cocoa clones and the possibility of selection of superior genotypes for higher performance with traits of high heritability with high genetic advance was assessed. Based on the mean performance the clones, NC-37, NC-23, NC-26, NC-50, NC-20, NC-51, NC-27 and NC-25 were identified as the best performers (Elain Apshara *et al.*, 2009).
- Amelonado, Imperial College Selection, Nanay, Parinari and Scavina group of clones continued to yield more than 50 pods per tree per year. Landas and Ecuador clones recorded 1.5 kg dry bean yield per tree with good quality beans.
- Among the local collections Wynad clones yielded bold beans with more than 1 gram dry weight and high recovery percentages.

Table 5. Best Nigerian clones with desirable characteristics

Clone	Pod no.	Bean no.	SBW(g)	DBY(kg)	Shell %	Fat%
NC-37	61.9	42.7	1.03	2.45	10.9	51.0
NC-23	53.3	42.3	1.12	2.53	11.5	51.3
NC-26	49.4	43.0	1.17	2.48	13.1	53.2
NC-50	48.4	43.3	1.00	1.45	12.7	50.6
NC-20	45.1	43.0	1.00	1.93	11.2	54.5
NC-51	44.2	43.3	1.00	1.83	13.9	50.1
NC-27	43.9	43.7	1.07	2.06	12.5	51.6
NC-25	43.1	40.3	1.24	2.16	13.1	53.2
SBW-single dry bean weight				DBY-dry bean yield		

Quality assessment

Fat content was estimated in 152 samples comprised of hybrids and clones and expressed in percentage. Among the 20 progenies the fat content ranged from 26.66 to 61.58%. Among the clones the fat content ranged from 18.18 to 47.05%. The fat content in beans dried in open ranged from 21.32 to 37.83% whereas, the beans dried in oven recorded a high percentage of fat ranged from 37.59 to 58.53%. Under coconut canopy the fat content in cocoa beans ranged from 40 to 52.43%. With samples from Andhra Pradesh, beans of coconut garden recorded a highest of 44.53% fat compared to beans of oil palm shade which has 40.06% fat. From the fatty acid profile it was clear that there are 11 fatty acids *viz.*, lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, arachidic, eicosapentaenoic, behemic and lignoceric acids involved in quality of cocoa beans. The fatty acids palmitic, stearic, oleic, linoleic and arachidic acids were present in all the accessions invariably whereas, myristic acid was present in only one accession. The percentage of stearic acid was the highest among all in a range of 30.50% in VTLCP-7 to 44.20% in VTLCP-1. All fatty acids differed among the accessions in percentage of expression.

6. Comparative Yield Trial

20 progenies and 10 clones obtained from earlier progeny trials were subjected to comparative yield trial. Comparative studies of cocoa varieties under arecanut and coconut canopies, different spacings, parents and progenies, performance of clones and seedlings are going on.

Under arecanut canopy

20 progenies of different cross combinations showed considerable difference in their growth and yielding behaviour in their initial years of growth. Vigorous trees with sturdy stems were observed in VTLCP-11, 9, 15 and 5 with 43, 42.67, 39, 38.33 cms respectively. The progenies VTLCP-5, 6, 15 and 20 had comparatively compact canopies. The hybrids VTLCP-6, 5 and 1 were identified with high harvest efficiencies 0.88, 0.66 and 0.56 and had more pods 68.73, 61.73 and 50.87 respectively. Dry bean yield/tree/year was the maximum (2.64 kg) in the hybrid VTLCP-6. The hybrids VTLCP-1, 5, 15, 19 and VTLCP-20 recorded >1.5 kg dry bean/tree/year. They also had more big sized beans suitable for processing

industry with more fat content (Elain Apshara *et al.*, 2008). Among the 10 selected clones planted in the same plot, CII-148 and CI-161 recorded >1.5 kg dry beans and CI-75 and CIII-350 yielded >1 kg dry bean/tree/year with good bean quality.

Under coconut canopy

Annual plant growth and pod yields in elite clones planted under coconut shade in double row system at Vittal and Kidu were recorded. More pods were observed in trees of hybrids VTLCH-2, VTLCH-3 at Vittal and in VTLCP-8 at Kidu. The hybrids VTLCP-1, 9, VTLCH-1, 3 and 4 recorded >1.00 kg dry bean yield per tree per year. Nine clones grown under coconut at Kidu were tested for their genotype x seasonal effect over 5 years. The highest dry bean yield was recorded in VTLCH-2 (1.95 kg/tree/year).

7. Multi Location Trial and Demonstration plots

To assess the adaptability and stability of hybrids and clones evolved out of the breeding programmes, a multi location trial was laid out with hybrids and their parents. Elite clones were planted at Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, West Bengal and Assam under different agro climatic conditions. Apart from arecanut and coconut shades elite clones are being evaluated under oil palm and old rubber plantations. The performance of varieties under oil palm was poor compared to coconut, which might be due to the varying light intensities under oil palm (19.35% PAR) and coconut (37.32% PAR). Drought tolerant hybrids showed good adaptation under Tamil Nadu and Andhra Pradesh conditions.

94 front line demonstration plots were established under participatory research cum demonstration plots scheme funded by DCCD, Kochi in 8 taluks in Karnataka and 1 taluk of northern Kerala. They were supplied each with 500 grafts and other technologies on cocoa cultivation. Performance of CPCRI clones assessed in 12 gardens planted during 2004, showed 1 kg dry beans in Bantwal, Sampaje and Kasaragod taluks. Other plots are under evaluation.

8. Biotechnology and Bioinformatics

Molecular characterization

The genetic diversity in the cultivated species should be studied using morphological, isoenzyme and molecular markers. If the phenotypic characters are considered along with the fingerprinting data the identification process is foolproof and will serve as a valuable data to be documented in the germplasm holdings. With this view, RAPD (Randomly Amplified Polymorphic DNA) work was carried out. DNA fingerprinting was done in 76 accessions. Jaccard's coefficient assembled 6 groups based on genetic distance and the clones BE-10, EQX-78, I-56 and SCA-12 were proved as highly divergent (Anuradha Sane, 2002). Microsatellite markers were identified from cocoa EST (Expressed Sequence Tags) sequences with Bl tools, primers designed and verified in wet lab (Riju *et al.*, 2009). Screening of 12 cocoa accessions for level of polymorphism produced 27 polymorphic alleles which ranged from 2 to 6 alleles per locus with an average of 3.85 alleles per locus. The average polymorphism information content (PIC) value was 0.57. The similarity index, based on Dice coefficient, obtained after pair wise comparison among 12 cocoa accessions showed the highest index of 0.80 in the accessions Jerangau Red Axil (JRA) and VTLC-1. 2-D and 3-D principal coordinate analysis showed VTLCC-1 as a distinct accession. The accessions, in general, were scattered across the co-ordinates. The results of this study were made available online (http://www.riju.mybioscience.com/cocoa/). DNA extraction protocol was standardised with young

recently matured leaves using modified SDS method. PCR was performed with 18 Nigerian accessions, 50 alleles were detected using 11 microsatellite primers with an average of 4.5 alleles per primer. Maximum similarity is seen between NC-9 and NC-30 (0.91) and minimum similarity (0.14) is seen between NC-37, NC-13, NC-43 and NC-48 and NC-13 (Sahiti, 2011).

Identification of genes for biotic/ abiotic stress and quality

Totally 1,09,633 ESTs belong to 33 cDNA library of *Theobroma cacao* was retrieved from ESTtik database. They were analyzed using bioinformatics tools and identified 11 important enzymes involved in ethylene, hypusine, acetaldehyde and polyamine biosynthesis pathways. Totally 392 enzymes corresponding to 102 metabolic pathways were identified. Functional annotation showed most of the EST sequences were responsible for stress response, signal transduction and transcription factors. Five phenylpropanoid biosynthetic pathway enzymes involved in drought stress response were identified (Naganeeswaran and Elain Apshara, 2011). A database on genes responsible for drought stress was created and is made available in the following URL: http://220.227.88.254/cacao/index.html.

Analyzed 4 libraries of ESTs derived from *Phytophthora megakarya* infected cocoa leaf and pod tissues. Totally 6379 redundant sequences were retrieved from ESTtik database and performed EST processing using seqclean tool. Clustering and assembling was done using Cap3 and generated 3333 non-redundant (907 contigs and 2426 singletons) sequences. Blast, Interproscan, Gene ontology and KEGG search were executed and 1230 orthologous genes were annotated. Totally 272 enzymes corresponding to 114 metabolic pathways were identified. Functional annotation revealed that most of the sequences are related to molecular function, stress response and biological process.

Annotation of carotenoid biosynthesis pathway genes in cocoa genome was done. Total of 25,912 cocoa genome short gun sequence were retrieved from Genbank database. Important genes involved in the carotenoid biosynthesis pathways were retrieved from the plant model organisms *{Arabidopsis thaliana, Vitis vinifera* and *Populus trichocarpa).*

Conclusion

Wider genetic base will enable identification of more desirable characteristics to meet the production and processing needs. Flavour improvement should be given importance in the future breeding programmes. Screening and hybridization to impart satisfactory levels of resistance in varieties, to major diseases can be taken up. Screening of germplasm for established and emerging pests is important. Molecular strategies have to be formulated to confirm the breeding results. In the National level public private partnerships, collaborative approach between research institutes, universities, state horticulture departments and developmental agencies will contribute to improvement of cocoa. In the International level participation of India in cocoa genetic resources networking and regional breeding groups of both developed and developing countries is important.

References

1. Anuradha Sane. 2002. Final report of the Ad-hoc scheme on DNA fingerprinting of Cocoa accessions using PCR based markers. ICAR, New Delhi.
2. Balasimha, D. and Ananda, K.S. 1999. Final report of the Ad- hoc scheme (from AP Cess Fund, ICAR) on drought tolerance in Cocoa submitted to ICAR, New Delhi, p. 7-8 and 31-33.

3. Balasimha, D., Subramanian, N. and Chenchu Subbiah, C. 1985. Leaf characteristics in cocoa *(Theobroma cacao L)* accessions. *Cafe Cacao The. 29:*95- 98.

4. Bhat, V.R. 1999. Cocoa germplasm, characterization, conservation and utilization. *In:* Improvement of plantation crops, (eds. Ratnambal, M.J., Kumaran, P.M., Muralidharan, K., Niral, V. and Arunachalam, V.), CPCRI, Kasaragod.pp 77-85.

5. Bhat, V.R. and Ananda, K.S. 1997. Evolving high yielding varieties by selection and hybridization in cocoa. *Ann.Rep. of CPCRI:* 71-72.

6. Chandramohanan, R. 1982. Studies on the reaction of pods of different cocoa accessions to Phytophthora palmivora. *Planter,* 58:99-103.

7. Elain Apshara, S. and Rajan, P. 2009. Profile of cocoa collections at CPCRI, Research Centre, Kannara. CPCRI Publication, p.60.

8. Elain Apshara, S., Bhat, V. R. and Rajan, P. 2002. Stability of pod yield in cocoa *(Theobroma cacao, L)* under Kannara conditions. *In:* Proceedings of National seminar on technologies for enhancing productivity in cocoa, CPCRI, Regional Station, Vittal, November 29- 30, 2003, pp. 40-43.

9. Elain Apshara, S., Bhat, V. R., Ananda, K. S. and Nair, R. V. 2008. High yielding cocoa varieties of the Central Plantation Crops Research Institute, India. *INGENIC Newsletter,* 11, December 2007, p. 12-15.

10. Elain Apshara, S., Bhat, V. R. and Nair, R. V. 2008a. Comparative studies on elite cocoa progenies in their initial years of growth. *Journal of Plantation Crops, 36 (1):* 38- 44.

11. Elain Apshara, S., Bhat, V. R., Ananda, K. S., Nair, R. V. and Suma, D. 2009. Evaluation and identification of high yielding trees in Nigerian cocoa germplasm. *Journal of Plantation Crops, 37 (2):* 111-116.

12. Naganeeswaran, S. and Elain Apshara, S. 2011. Analysis of drought induced expressed sequence tags (EST's) library and identification of metabolic pathways in cocoa. *In:* Proceedings of seminar on strategies for enhancing productivity of cocoa, CPCRI, RS, Vittal, Jan 28-29, 2011. (Eds. Elain Apshara.S., Jaganathan.D. and Balasimha.D.) p.69-73.

13. Nair, R. V. and Rekha, A. 1996. Incompatibility in cocoa germplasm. *Indian Journal of Genetics. 56(2):* 17- 20.

14. Ratnam, R. 1961. Introduction of Criollo Cacao in the Madras State. *South Indian Hort.* 9:24-29.

15. Riju, A., Rajesh, M.K., Fasila Sherin, P.T.P., Chandrasekar, A., Elain Apshara, S. and Arunachalam, V. 2009. Mining of expressed sequence tag libraries of cacao for microsatellite markers using five computational tools. *Journal of Genetics* 88 (2): 217-225.

16. Sahiti, P. 2011. Molecular characterization of Nigerian cocoa collections using microsatellite markers. *M.Sc.Thesis.* CPCRI, Regional Station, Vittal, Karnataka. p. 50.

17. Wood, G.A.R. 1964. Cocoa Growing in India. Cadbury Brothers Limited Publication Department, Bournville, London, p.27.