Bangladesh J. Bot. **54**(1): 137-144, 2025 (March) DOI: https://doi.org/10.3329/bjb.v54i1.80592

EVALUATION OF COCOA CLONES FOR YIELD AND QUALITY AS INTERCROP IN COCONUT GARDENS OF WESTERN GHATS, INDIA

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Keywords: Clones, Cocoa, Dry bean, Quality parameters

Abstract

Cocoa is an important cash crop grown in southern parts of India. Though many cocoa varieties have been released for cultivation, there is no variety recommended for cultivation exclusively for Tamil Nadu. Therefore, the present investigation was undertaken to study the performance of nine identified cocoa clones for its growth, pod yield and yield attributing traits for subsequent research programmes. Mean average pod yield among the clones varied from 9.40 to 15.3/tree^{-}/year. Clone TNAU CC 5 significantly higher pod yield (15.31), number of fresh beans/pod (37.55), pod weight (382.93 g). Pod set percentage in the juvenile stage varied from 2.1 (TNAU CC 9) to 3.3(TNAU CC 5). Dried beans are the prime economic product of cocoa, and single dry bean weight ranged from 0.80 to 0.92 g. In the present study, the mean value for theobromine and caffeine, catechin and epicatechin content was 7.35 and 1.76 mg/g, 0.91 and 2.19 mg/g, respectively. Considering the most economic traits of cocoa, TNAU CC 5 appears to be the most suitable clone for commercial cultivation in the region.

Introduction

Cocoa (*Theobroma cacao* L.) is a shade -loving crop and demonstrated as imminent scope to share the inter spaces of palm family (Arecaceae) in southern region of India and adapted to the microclimatic conditions available in such perennial gardens. The economic value of cocoa is determined by the quality of the beans. Cocoa has been produced in India for about half a century, Kerala and Karnataka are major leading producing states. 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. Adaptability of cocoa genotypes in traditional and non traditional zones should be verified, and location specific varieties should be developed (Malhotra and Elain Apshara 2017). Under ICAR-AICRP on Palms, 18 Cocoa entries screened for yield and quality were planted across different agro- climatic zones to evaluate their performance under coconut /oil palm based-cropping system (Maheswarappa *et al*. 2019).

Though various varieties and hybrids of cocoa are developed by ICAR- CPCRI and KAU, there are no reports of the performance of these varieties under Tamil Nadu conditions. Variation in pod yield performance with same cocoa varieties (CCRP 1 to CCRP 10) was also reported from KAU (Sujith and Minimol 2016) and TNAU (Sumitha *et al.* 2018). In contrast, planting materials were introduced as seedlings from polyclonal seed gardens of Kerala and Karnataka to Tamil Nadu. These trees have adapted and acclimatized to the local climatic conditions. This warrants breeding and development of cocoa varieties which are suitable for tropical hot climatic conditions. A good number cocoa clones/hybrid have been evaluated over years in different locations *viz.*, VTLCH 2 and VTLCH 4 in Gujarat (Bhalerao *et al.* 2018), VTLC 19 (under Arecanut) and VTLC 20 (under coconut) in Assam (Phukon *et al.* 2021, Singh *et al.* 2021).

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Lockwood *et al*. (2007) reported that some farmers try to identify their best trees for yield and either harvest seed from them or graft. Similarly, Karthikumar (2014) identified nine high yielding clones (TNAU CC 1 to TNAU CC 9) from farmer's gardens of Coimbatore district. As development of new variety is a long term process, the immediate step is evaluating best performing clones (soft wood grafting) to assess their suitability. Nowadays, cocoa is gaining importance as an intercrop in the irrigated coconut gardens of Tamil Nadu; hence, a field experiment was carried out to identify suitable cocoa clones for intercropping.

Material and Methods

Field experiment was conducted for three consecutive years from 2016 to 2018 at Coconut Research Station, TNAU, Aliyarnagar. The research station is situated at 10.49° N latitude and 77° E longitude with an altitude of 20 m above the mean sea level. The soil was sandy loam, non-calcarious, non-saline and neutral in pH. The station received an average annual rainfall of 818.52 mm during 2013-2018 and 50 percent of rainfall recorded in North East Monsoon period. The mean maximum air temperature was higher in the month of April (32.88°C) and mean minimum temperature (21.48°C) was in January. Nine high yielding clones (TNAU CC 1 to TNAU CC 9) selected based on their yield performance (ten year yield data). In generating experimental materials, scions from fan branches of the nine selected genotypes were soft wood grafting onto six-month old rootstocks. The clones were transplanted to field in July 2013, eight-months after grafting following a randomised complete block design with three replications consisting of six trees per clone. One row of cocoa was planted at a spacing of 2.7 m in between two rows of coconut spaced at 7.5 × 7.5 m. The cocoa plants were given cultural practices as per the package of practices recommended by Tamil Agricultural University, Coimbatore. Pruning was regularly done in the identified trees wherein excess chupons arising from the main stem and fan shoots were removed before and after each monsoon. All the experimental materials (cocoa tree) were flood irrigated during the study period.

Reproductive and yield data collection was carried out between July 2016 and June 2018. Morphological traits *viz*., number of flowers per tree, number of pods per tree, pod weight, pod set percentage, number of beans per pod, dry bean weight per pod, single dry bean weight and estimated dry bean yield per tree were recorded and analyzed. At harvest, the number of matured pods for each clone was combined and counted. A sample of 30 pods per clone was used to estimate the mean bean weight and number of seeds per pod after fermentation and drying to a moisture content of about 7%. Pod value (number of pods needed to produce 1 kg of dry beans) was estimated from the mean seed weight and number of seeds per pod.

Total phenolic content of the bean extracts was determined by a colorimetric Folin-Ciocalteu reagent assay. Total carbohydrates were estimated by anthrone method. The moisture and crude fat were determined following the procedures in AOAC (2005) methods. Analyses were carried out in triplicates.

Standard compounds such as (+) – catechin hydrate, (-) – epicatechin, caffeine and theobromine were purchased from Sigma-Aldrich. All solvents and water used for the analysis were of HPLC grade. Stock solution of all the standards were prepared to get working solution of different concentrations ranging from 10 to 200 ppm. The working standards were injected to establish different standard calibration curves. Preparation of fermented bean sample for HPLC analysis was followed as reported by Payne *et al.,* (2010).

The determination of phenolic compounds (catechin, epicatechin, caffeine and theobromine) was performed according to the method described by Elwers *et al*. (2009). Twenty microlitres of each sample solution was analyzed by HPLC system (Star pro Variant Model) equipped with C18

column (100 mm × 4.6 mm O.D.S.-2, 3 µm) and using the eluent comprising of 87% water: 8% methanol: 5% acetic acid, flowing at 1.0 ml/min. The compounds were monitored by UV detection at 280 nm wavelength. The total run time was 30 min and the temperature was 26°C. Quantitative analysis was carried out by using the individual standard curves for each type of compounds. Standards were dissolved in methonal (HPLC grade) and were injected separately to find out the retention time of each standard. Retention times identified for each standard in the study are given in Fig. 1 and Table 1.

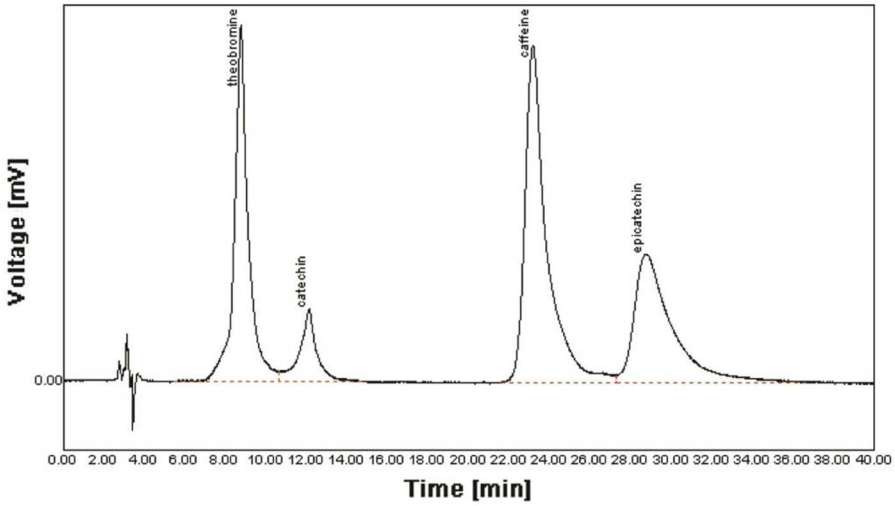

Fig 1. Representative chromatogram of a sample of defatted cocoa at λ = 280 nm.

Table 1. Retention time of different compounds of cocoa.

Compound	Retention times (Minutes)
Theobromine	8.61
Catechin	11.31
Epicatechin	20.08
Caffeine	26.15

Statistical analysis of recorded data was analysed by using the techniques described by Panse and Sukhatme (1985). Critical difference (CD) values at 5% level of probability were computed for comparing the treatment means.

Results and Discussion

The morphological characters of flowers, pods and beans pertaining to yield and quality traits were recorded for two consecutive years (pooled data 2017 to 2018) in Table 2. Results from the present study indicated the significant variation among clones for mean flower number per cushion (5.57) and number of cushions per tree (59.71) and number of flower per tree (344.36). Flowering in cocoa varies with different geographical locations, seasonal differences in flowering and intensity of flowering in cocoa has been reported by Efron *et al.* (2003) (Fig. 1). In cocoa flowering is also influenced by genetic characters and each genotype is a heterogenous population hence exhibiting differences in their performance (Sumitha *et al.* 2018). Pruning is an important

practice to ensure proper ventilation within the crown and penetration of sunlight to stimulate cocoa flowering and fruit setting (USDA, 2007). Further, the cocoa trees are normally pruned from January to February every year in Tamil Nadu. Influence of pruning on flowering in cocoa intercropped in coconut plantations has been reported by Anok Uchoi *et al.* (2018).

Table 2. Reproductive characters of the identified clone.

Clones	No. of flowers /cushions	No. of flowers cushions/tree	No. of pods harvested per tree	Pod set percentage	Pod weight (g)	No. of beans per pod	Single dry bean weight (g)
TNAU CC 1	5.31^{cd}	57.56^{c}	12.70^{c}	3.1^{b}	351.93^{b}	31.62^{def}	0.86^{bc}
TNAU CC 2	5.31^{cd}	62.40^{b}	12.91^{c}	3.4^{a}	345.81^{bc}	34.31^{b}	0.83^{cd}
TNAU CC 3	5.52^{c}	62.40^{b}	13.83^{b}	2.3^{d}	332.30^{cd}	34.05^{bc}	0.90^{ab}
TNAU CC 4	5.80^{b}	64.01^{b}	14.61^{a}	2.2^{de}	348.37^{bc}	32.41^{cde}	0.80^{d}
TNAU CC 5	6.56^{a}	68.73^{a}	15.31^{a}	3.3^{a}	382.93^{a}	37.55^{a}	0.92^{a}
TNAU CC 6	5.12^{de}	64.41^{b}	13.35^{bc}	2.5^{c}	356.26^{b}	35.15^{b}	0.82^{cd}
TNAU CC 7	5.20^{de}	53.12^{d}	12.62^{c}	2.3^{d}	350.40^{bc}	33.41^{bcd}	0.86^{bc}
TNAU CC 8	5.2^{de}	53.82^{d}	11.72^{d}	2.5^{c}	312.47^{e}	30.42^{f}	0.80^{d}
TNAU CC 9	5.01^{e}	51.19^{d}	9.40^{e}	2.1^{e}	318.81^{de}	31.34^{ef}	0.83^{cd}
Mean	5.57	59.71	12.92	2.63	344.36	33.34	0.76
CD (0.5 %)	0.29	3.19	1.54	0.139	18.35	1.83	0.048

Values followed by same alphabet in a column represent non-significant differences at 5% level.

Number of pods harvested/tree/year in the juvenile stage, showed significant variation among the cocoa clones (Table 2) and TNAU CC 5 registered the highest number of pods tree/yea (15.31) on par with TNAU CC 4 (14.61) whereas, the clone TNAU CC 9 recorded the lowest number of pods tree/year (9.40). The number of pods per tree harvested in a year is the prime factor in determining the yield in cocoa (Thondaiman *et al.* 2013). In present study the pod production was low during the initial years, later on, it increased every year as the tree gets older. The relationship between juvenile tree growth and early yields has been used for selecting high yielding cocoa genotypes in later years (Ofori *et al.* 2015, Phukon *et al.* 2021). Pods are produced throughout the year depending on management practices, but the main harvest usually begins at the June-July and subsequent harvest during Nov-Jan at Tamil Nadu condition

Pod set percentage in the juvenile stage varied from 2.1 (TNAU CC 9) to 3.3 (TNAU CC 5). Cocoa flowers profusely with low per cent of fruit set (0.5 to 7 per cent). A low count of pod set per cent in cocoa was reported by Vithya *et al.* (2018). The quantitative characters of the pod like pod weight, pod length, pod girth and husk thickness also registered significant variations among the clones. Among all the clones, the highest pod weight was recorded in TNAU CC 5 (382.93 g) followed by TNAU CC 6 (356.26 g) and it was lowest in TNAU CC 8 (312.47). Number of beans per pod ranged from 31.34 in TNAU CC 9 to 37.55 in TNAU CC 5 clone. Number of seeds per pod is largely influenced by the number of ovules per ovary, the fertility of the ovules and the tree reproductive nature. These attributes may vary among genotypes and may have accounted for the large differences between clones for number of beans per pod, which agrees with previous reports that number of bean per pod is very variable (Cilas *et al.* 2010). Pod weight of more than 350 g to ensure pod filling with > 35 beans is the ideal selection criteria for yield improvement (Vikraman Nair *et al.* 2000). Hence, improved yield attributing parameters in TNAU CC 5 observed during the present study could be justified.

Beans are an important parameter in the present study as most of them are traded as dry beans. Highest yield of single dry bean weight was recorded in TNAU CC 5 (0.92 g). Cocoa genotype with bean weight more than 1g is characterized as superior (Monteiro *et al.*2009). Bean weight on the other hand is highly heritable. Variation in dried bean weight is considered to be the direct and significant method for the selection of cocoa genotypes (Oyedokun *et al.*2011). In general, dry bean yield per pod trend was found to be in the order of TNAU CC 5(34.21g), TNAU CC 6 (31.82 g) followed by TNAU CC7 (29.69 g) (Fig. 2). The recovery was significantly influenced by number of beans per pod, fresh bean weight, dry bean weight, single wet and dry bean weight and fermentation method. Similar phenomenon was observed by Sajeevkumar *et al.* (2017). In Pod value, the lowest pod value was found in TNAU CC 5 (29.4) which was statistically at par with TNAU CC 6 (31.4). The highest pod value was found in TNAU CC 8 (42. 3). Pod value or index is defined as the number of pods required to produce one kg of dry cocoa beans. It is an indirect measurement of yield components. The higher the pod index, the smaller the pod and consequently the bean size and vice versa.

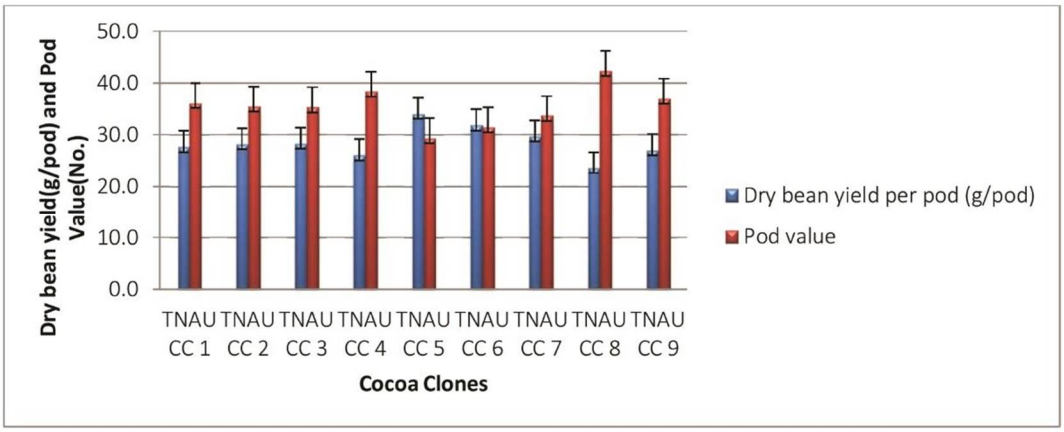

Fig. 2. Dry bean yield per pod (g) and Pod value of different TNAU cocoa clones.

Total phenolic and total carbohydrate content was determined in dry beans obtained after fermentation from each clone, which revealed significant differences (Fig. 3). In general, total phenol content ranged from 69.31 to 77.75 mg equivalent for pyrocatechol per gram. Highest total carbohydrate content was recorded by TNAU CC5 (21.80 %) which were followed by TNAU CC 4 (21.32 %). But it is known that TPC and CHO varies depending on the cocoa bean variety, geographical origin, ripeness degree (harvest season) and post-harvest conditions, such as fermentation, drying, roasting, processing and storage (Wollgast and Anklam 2000). Data pertaining to fat content of dried beans are shown in Fig. 2 indicating that TNAU CC 5 produced the highest (53 %) fat content, followed by TNAU CC 4 and TNAU CC 3 (50 %). Cocoa beans containing high fat content have higher marketability as they play a major role in chocolate industry (Fapohunda 2012). Similar results of variability in performance for fat content by cocoa clones, farm accessions, hybrids has been reported by Elain Apshara *et al.* (2009).

Each sample of defatted cocoa seed poly phenols exhibits 4 significant peaks at $\lambda = 280$ nm (Fig. 1). In the present study, the mean value for theobromine and caffeine content was 7.35 mg/g and 1.76 mg/g. Two of these peaks, correspond to the two purine alkaloids; caffeine and theobromine, which are commonly found in cocoa seeds. The mean value for the catechin was

0.91 mg/g and epicatechin content ranged from 2.04 mg/g in to 2.42 mg/g (Fig. 4). Niemenak *et al.* (2006), noted that, the predominant polyphenols identified in dried defatted cocoa bean was epicatechin followed by catechin. These values were a little lower than those obtained by Elwers *et al.* (2009) in cocoa seeds and this can be due to variation in the methods used by the authors for the extraction. Flavour quality of cocoa beans depends on the genotype and origin of the cocoa tree that has produced the beans and also influenced by many factors such as bean composition, soil type, age of cocoa tree, post harvest treatments such as pulp pre – conditioning, fermentation and drying, industrial processes such as roasting as well as storage and transportation (Crafack *et al.* 2014).

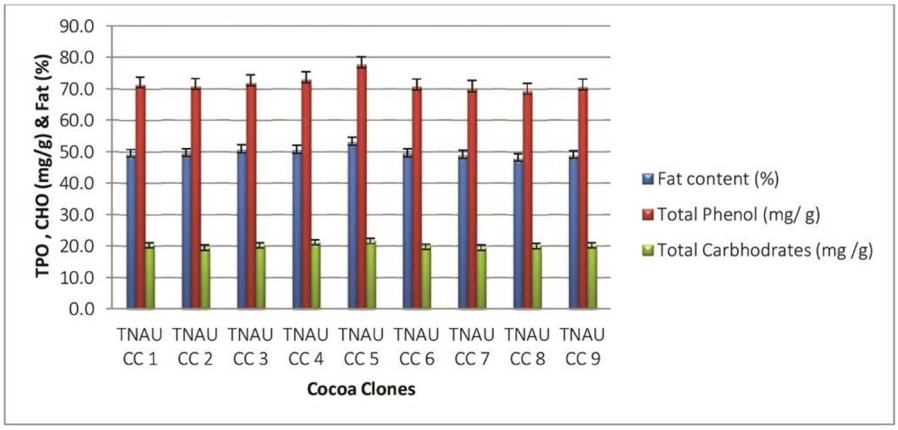

Fig. 3. Total phenolic content (mg/g), total carbohydrate content (mg/g) and fat content (%) of different TNAU cocoa clones.

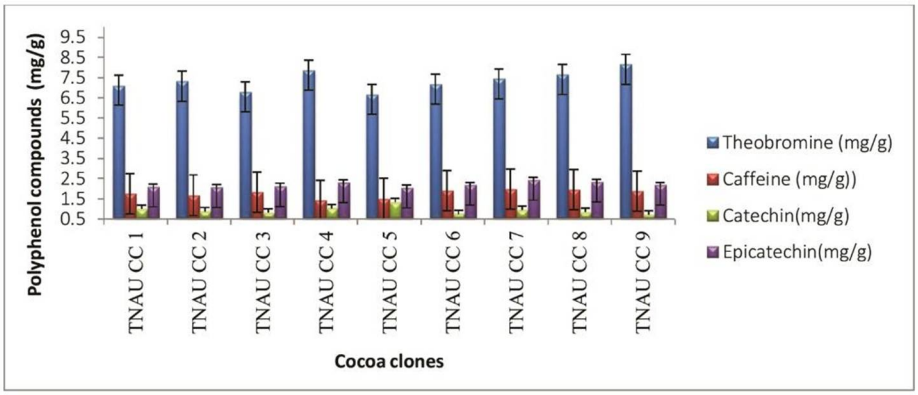

Fig 4. Polyphenol compounds of different TNAU cocoa clones.

Moreover, evaluation of the nine identified cocoa clones (TNAU CC 1 to 9) propagated through soft wood grafting in this present study revealed that all the growth traits such as tree characters, flower characters, pod and bean traits showed significant variation among the clones during three years after planting. Among the clones TNAU CC 5 performed better in qualitative and quantitative parameters. Selection of these genotypes for further evaluation over years and locations as sexual or clonal planting material may eventually lead to their release to farmers in a

short term breeding programme. In conclusion, the use of the above identified genotypes as parents in future breeding programmes will enhance the productivity of cocoa.

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(Manuscript received on 30 November, 2024; revised on 15 March, 2025)