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# Genotypic variation in flowering, fruit set, and cherelle wilt, and their relationship with leaf nutrient status in cocoa (*Theobroma cacao* L.) grown in humid tropics of India

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## Abstract

Cocoa is an important plantation crop grown for its beans, used in the chocolate and confectionary industry. It produces a large number of perfect flowers on the stem (cauliflorous), but the fruit set is low. There is a lack of comprehensive information regarding the genotypic variability in flowering, fruit set, and cherelle wilt, as well as their relationship with leaf nutrient status. Thus, a field study was undertaken at ICAR-Central Plantation Crops Research Institute, Regional Station, Vittal, to elucidate the influence of leaf nutrient status on flowering, fruit set, and cherelle wilt in cocoa. The study revealed significant variations among twenty cocoa genotypes for flower production, fruit set, and cherelle wilt. The highest number of flowers (106) produced on a one-meter marked area on the stem and fruit set (13.7) was recorded in VTLC 05. The lowest number of flowers was observed in genotype VTLCH 2 (16). The maximum fruit set percentage was found in VTLC 182 (13.8%), whereas the minimum fruit set percentage was observed in VTLC 30A (1.3%). Cherelle wilt was maximum in upper Amazon clone VTLC 155 (72.9%), whereas the lowest cherelle wilting percentage was recorded in VTLC 148 (22.9%). Pearson's correlation coefficients indicated that fruit setting percentage in different genotypes had no significant association with leaf nutrient status, except for calcium which had a significant association ($r = 0.596$). The result indicated that genotypic variability exists for fruit set and cherelle wilt, and an optimum level of calcium should be maintained in the leaf for better fruit setting in cocoa.

## Keywords

Cocoa, fruit set, cherelle, calcium

* Present address.

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# Introduction

Cocoa (*Theobroma cacao* L.) is the third most important beverage crop grown after tea and coffee. It is native to tropical South America (Cheesman 1944; Wood and Lass 2001) but cultivated throughout the globe for its beans, which are mainly used in the chocolate and confectionary industry. It is grown in agroforestry systems in major cocoa-growing countries; however, mixed-cropped in Asian countries. In India, it is grown as a mixed crop with coconut, arecanut, and oil palm.

Cocoa belongs to Malvaceae (Alverson et al. 1999), and it produces perfect flowers that attach to floral cushions by a long, thin pedicel (Wood and Lass 2001). They are cauliflorous. Although flowers are also formed on the secondary branches, most pods form on the trunk and main branches (Almeida and Valle 2007; Groeneveld et al. 2010). Several studies have shown that the flowers of cocoa are androgynous and essentially fertilized by midges, *Forcipomyia* spp. (Toxopeus 1985; Ollerton et al. 2011). The percentage of flowers that set pods in cocoa is usually very low, *i.e.*, 0.5–5% (Alvim 1984; Aneja et al. 1999). Abscission of flowers in cocoa occurs within 2–3 days after anthesis in the absence of pollination and fertilization, and even after manual pollination, only a small number of flowers develop and produce pods (Hasenstein and Zavada 2001). Various factors like genetics, environment, age of the tree, plant growth hormones, and the availability of soil moisture and nutrients determine flowering in cocoa. It also varies with different geographical locations. The seasonal differences in flowering and the intensity of flowering in cocoa have been reported (Delpinalrivero and Acunagale 1967; Rajamony and Mohankumaran 1995).

Pollinated flowers then develop into immature pods, commonly known as cherelles. Despite abundant flowers, few cherelles develop into mature pods. Up to 75% of cherelles are lost to a thinning condition known as cherelle wilt. During the first 50–100 days after the fruit set, the growth of a cherelle can stop, and the cherelle then becomes yellow, shrivels, and blackens (Valle et al. 1990). The cherelle wilt was a means for the cocoa tree to adjust the number of pods to a number that the tree could support.

Peaks of cherelle wilt are associated with a high fruit set which coincides with heavy leaf flushing, indicating internal competition for photosynthates or mineral nutrients between reproductive and vegetative tissues (Cunningham et al. 1961; Alvim and Nair 1986). This is further corroborated by the observation that the mineral nutrient content in cocoa leaves decreases during the minor and major cocoa harvest (Verlière 1981). Phosphorous stimulates flowering in cocoa (Asomaning et al. 1971; Jadin and Snoeck 1985) and the role of boron in auxin metabolism or transport is confirmed by increased wilted cherelles in the event of boron deficiency (Lachenaud 1995). However, not much information is available on the role of mineral nutrients in flowering, fruit set and cherelle wilt in cocoa. Hence, this study was conducted to know the genetic variability in fruit setting and cherelle wilt, and their relationship with leaf nutrient status.

# Material and methods

## Experimental location

The experiment was conducted at ICAR-CPCRI, Regional Station, Vittal, India. The experimental site is situated at 12°15'N, 75°25'E and elevation of 91 m above mean sea level in the coastal zone of Karnataka. The climate of Vittal is warm and humid, with an average annual rainfall of 3,670 mm in the last 30 years, distributed over 120 days from June to November, and the dry season prevails from December to May. The mean temperature ranged from 21 °C (minimum) to 36 °C (maximum), and the average relative humidity ranged between 61 and 94 percent.

## Plant materials

Four released hybrids and their parents and one clone planted in 2005, and eight upper Amazon genotypes planted in 2007 as a mixed crop with arecanut were used in this study.

## Flowering, fruit set and cherelle wilt

Number of flowers: A one-meter length of stem was marked just below the jorquette, and the number of flowers in that area was counted as and when they opened.

Number of flowers set fruit: The number of flowers set fruits were counted in a one-meter marked area on the stem in each genotype under study.

Fruit set percentage: Based on the number of flowers opened on one-meter stem length and the number of flowers set fruit, fruit setting percentage was calculated using the formula,

$$\text{Fruit set percentage (\%)} = \frac{\text{Number of flowers set fruit}}{\text{Number of opened flowers}} \times 100$$

Cherelle wilting percentage: Cherelles in each genotype were counted to get the total number of cherelles per tree. Healthy cherelles and wilted cherelles in the tree were counted to get the number of healthy cherelles and wilted cherelles, respectively. Wilting percentage (%) was calculated using the formula,

$$\text{Wilting percentage (\%)} = \frac{\text{Number of wilted flowers}}{\text{Total number of cherelles}} \times 100$$

## Nutrient estimation

Mature leaves (second leaf of the last maturing flush) were collected for nutrient analysis. The samples were cleaned with tap water followed by distilled water, air-dried, packed in brown paper bags, and oven-dried at 60 °C to a constant weight and ground. The ground samples were kept in labelled butter paper bags for further analysis.

The nitrogen content was analyzed following micro-Kjeldahl digestion method (Jackson 1973). The powdered plant samples were digested in a 3:1 nitric–perchloric acid mixture for total P, K, Ca, Mg and micronutrient estimations. Total P was estimated by the vanado-molybdate method using a spectrophotometer and total K by flame photometry method (Piper 1966). Total Ca and Mg, and micronutrients, *i.e.*, Fe, Mn, Cu, and Zn, were determined using an Atomic Absorption Spectrophotometer (AAS). Total B was estimated following Azomethine-H method (Gupta 1979).

### Data analysis

Data was collected from 20 cocoa genotypes in three replications. Statistical analysis of the data was done using standard analysis of variance (ANOVA) technique (Gomez and Gomez 1984). The data was subjected to RCBD analysis of variance with three replications using SPSS at a 5 percent level of significance.

## Results and discussion

### Flowering and fruit set in different cocoa genotypes

Flowering in cocoa is controlled by various factors such as environment, soil moisture, availability of nutrients, and genetic factors of the tree. Environmental factors like temperature, light intensity, and rainfall positively affect the production of flower cushions and the production of open flowers. However, rainfall had a greater influence on the phenological cycle of the cocoa plant (Adjaloo et al. 2012). In this study, the number of flowers, fruit set, fruit setting percentage, total number of cherelles, number of healthy cherelles, number of wilted cherelles, and cherelle wilting percentage showed significant variation among the genotypes. The flower count varied from 16 to 196. The highest number of flowers produced on a one-meter marked area on the main stem was recorded in VTLC 05 (192). The lowest number of flowers was observed in genotype VTLCH 2 (16). Such varietal differences in the number of flowers were also reported by other researchers (Sumitha et al. 2018; Vithya et al. 2018). Since flower production represents a considerable plant investment (Valle et al. 1990), the number of flowers can affect the auxin partition of each flower. This would explain the low concentration of endogenous auxin in clones with high flower productions (high-flower producers) (Hasenstein and Zavada 2001). Auxin concentration is significantly higher in clones with low production of flowers (low-flower producers).

**Table 1.** Flower production and fruit set in twenty cocoa genotypes.

| SI No. | Genotypes | No. of flowers | Fruit set | Fruit set percentage | |
|---|---|---|---|---|---|
| | | | | Original data | Transformed data |
| 1 | VTLCH 1 | 126 | 9.7 | 8.4 | 16.8 |
| 2 | VTLCH 2 | 16 | 1.7 | 10.1 | 18.3 |
| 3 | VTLCH 3 | 80 | 4.7 | 5.5 | 13.5 |
| 4 | VTLCH 4 | 137 | 9.0 | 7.2 | 15.5 |
| 5 | VTLCC 1 | 125 | 12.0 | 9.2 | 17.6 |
| 6 | VTLC 01 | 103 | 3.0 | 2.0 | 8.0 |
| 7 | VTLC 05 | 196 | 13.7 | 7.0 | 15.3 |
| 8 | VTLC 11 | 165 | 6.7 | 3.7 | 11.1 |
| 9 | VTLC 19A | 60 | 4.0 | 7.6 | 15.9 |
| 10 | VTLC 30A | 152 | 2.0 | 1.3 | 6.4 |
| 11 | VTLC 61 | 133 | 10.7 | 8.0 | 16.4 |
| 12 | VTLC 66 | 126 | 6.0 | 3.9 | 10.8 |
| 13 | VTLC 148 | 55 | 5.0 | 8.3 | 16.6 |
| 14 | VTLC 150 | 24 | 1.5 | 6.3 | 14.4 |
| 15 | VTLC 151 | 141 | 9.0 | 6.3 | 14.6 |
| 16 | VTLC 154 | 26 | 1.0 | 4.5 | 12.1 |
| 17 | VTLC 155 | 137 | 7.5 | 5.7 | 13.8 |
| 18 | VTLC 156 | 77 | 7.0 | 8.6 | 17.1 |
| 19 | VTLC 182 | 31 | 5.0 | 13.8 | 21.7 |
| 20 | VTLC 185 | 119 | 10.5 | 8.2 | 16.6 |
| | Mean | 101 | 6.5 | 6.8 | 14.6 |
| | S.Em.± | 22.31 | 1.80 | | 1.3 |
| | CD at 5% | 64.11 | 5.18 | | 3.74 |

The data on fruit set in the 1 m marked area on the stem showed significant variation among different cocoa genotypes (Table 1). Maximum fruit set was found in VTLC 05 (13.7), which was on a par with VTLCC 1 (12), VTLC 61 (10.7), VTLC 185 (10.5), VTLCH 1 (9.7), VTLCH 4 (9) and VTLC 151 (9), whereas, the least fruit set was observed in VTLC 154 (1). The data on fruit set percentage showed significant differences among different genotypes, in which the maximum fruit set percentage was found in VTLC 182 (13.8%), which was at par with VTLCH 2 (10.1%). In contrast, the lowest fruit set percentage was observed in VTLC 30A (1.3%). Genotypes VTLC 05, VTLC 61, and VTLC 30A produced 152–196 flowers but had a low fruit set percentage of 1.3–7%. The highest fruit set percentage was observed in VTLC 182, which produced only 31 flowers within the marked area on the stem. Pearson's correlation coefficients (Table 2) indicated that fruit set percentage in different genotypes had no significant association with leaf nutrient status, except for calcium which had a significant association (r = 0.596).

**Table 2.** Pearson's correlation coefficients for leaf nutrient status *vs* flowering and cherelle production parameters.

| - | N | P | K | Ca | Mg | Fe | Mn | Zn | Cu | B |
|---|---|---|---|---|---|---|---|---|---|---|
| Flower count | 0.092 | -0.027 | 0.088 | -0.270 | -0.178 | 0.360 | -0.343 | -0.260 | -0.368 | -0.368 |
| Fruit set | -0.091 | 0.019 | -0.180 | -0.075 | 0.141 | 0.196 | -0.193 | -0.330 | -0.283 | -0.283 |
| Fruit set % | -0.337 | -0.138 | -0.425 | 0.596* | 0.130 | -0.182 | 0.081 | -0.021 | 0.005 | 0.005 |
| Healthy cherelles | -0.032 | 0.089 | 0.096 | -0.194 | -0.111 | 0.351 | -0.353 | 0.02 | 0.019 | 0.019 |
| Wilted cherelles | -0.204 | -0.170 | -0.113 | 0.084 | 0.007 | -0.289 | -0.237 | -0.21 | 0.149 | 0.149 |
| Cherelle wilting % | -0.022 | -0.170 | -0.065 | 0.051 | -0.048 | -0.410 | 0.004 | -0.174 | 0.086 | 0.086 |

### Cherelles production in different cocoa genotypes

Pollinated flowers then develop into immature pods, commonly known as cherelles. The highest number of cherelles was found in the Nigerian clone VTLCC 1 (132), which was comparable to VTLC 155 (126), VTLC 156 (125), VTLC 151 (109), VTLCH 3 (102), VTLC 05 (91), VTLC 01 (87) and VTLC 154 (87). The lowest number of cherelles was recorded in upper Amazon genotype VTLC 148 (36). However, VTLCC 1 retained 66 healthy cherelles, whereas a lesser number of cherelles was recorded in VTLC 150 (16). This is because, despite abundant flowers and fruit set, only a few cherelles develop into mature pods. A large portion of developing cherelles is lost to a thinning condition known as cherelle wilt. The greater number of wilted cherelles was recorded in VTLC 155 (91). This wilting of cherelles varied from 22.9–72.9% in different cocoa genotypes, and it was maximum in upper Amazon clone VTLC 155 (72.9%), whereas the lowest cherelle wilting percentage was recorded in VTLC 148 (22.9%). The cherelle wilting percentage had a significant relationship ($r = 0.874$) with the total number of cherelles. Hand-pollinated trees had an increased number of pods but also an increased level of cherelle wilt when compared to naturally pollinated trees, causing hand and naturally pollinated pods to have the same number of mature pods (Valle et al. 1990). Pearson's correlation coefficients indicated that cherelle wilting percentage in different genotypes had no significant association with leaf nutrient status. Hence, cherelle wilt could be a means for the cocoa tree to adjust the number of pods to a number the tree could support.

**Table 3.** Cherelle production and their wilting in twenty cocoa genotypes.

| Sl No. | Genotypes | Total number of cherelles | No. of healthy cherelles | Wilted cherelles | Cherelle wilting percentage (%) |
|---|---|---|---|---|---|
| 1 | VTLCH 1 | 74 | 43 | 32 | 41 |
| 2 | VTLCH 2 | 59 | 36 | 23 | 37 |
| 3 | VTLCH 3 | 102 | 51 | 50 | 51 |
| 4 | VTLCH 4 | 61 | 42 | 18 | 32 |
| 5 | VTLCC 1 | 132 | 66 | 67 | 53 |
| 6 | VTLC 01 | 87 | 61 | 26 | 29 |
| 7 | VTLC 05 | 91 | 56 | 35 | 39 |
| 8 | VTLC 11 | 73 | 53 | 20 | 34 |
| 9 | VTLC 19A | 53 | 34 | 19 | 37 |
| 10 | VTLC 30A | 49 | 31 | 18 | 44 |
| 11 | VTLC 61 | 81 | 37 | 44 | 56 |
| 12 | VTLC 66 | 39 | 28 | 11 | 36 |
| 13 | VTLC 148 | 36 | 29 | 7 | 23 |
| 14 | VTLC 150 | 40 | 16 | 24 | 48 |
| 15 | VTLC 151 | 109 | 63 | 47 | 38 |
| 16 | VTLC 154 | 87 | 24 | 63 | 70 |
| 17 | VTLC 155 | 126 | 35 | 91 | 73 |
| 18 | VTLC 156 | 125 | 60 | 65 | 40 |
| 19 | VTLC 182 | 66 | 33 | 33 | 50 |
| 20 | VTLC 185 | 52 | 17 | 35 | 66 |
| | Mean | 77 | 41 | 36 | 45 |
| | S.Em.± | 17.1 | 7.5 | 13.7 | 9.33 |
| | CD at 5% | 49.2 | 21.7 | 39.5 | 26.83 |

## Conclusion

Cocoa produces flowers throughout the year, with one or two peak seasons. Flower production is enormous, but the fruit set is very low. The set fruits (cherelles) wilt at different stages of development. There exists variability for flowering, fruit set and cherelle production in different genotypes of cocoa. However, the studies on the relationship with leaf nutrient status are very limited. Our study revealed significant variations among twenty cocoa genotypes for flower production, fruit set, and cherelle wilt. Pearson's correlation coefficients indicated that fruit set percentage in different genotypes had no significant association with leaf nutrient status, except for calcium which had a significant association ($r = 0.596$). The result indicated that genotypic variability exists for fruit set and cherelle wilt, and an optimum level of calcium should be maintained in the leaf for better fruit setting in cocoa.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

**Table 4.** Pearson's correlation coefficients for the parameters related to flowering and cherelle production.

| | Flower count | Fruit set | Fruit set % | Total cherelles | Healthy cherelles | Wilted cherelles | Cherelle wilting % |
|---|---|---|---|---|---|---|---|
| Flower count | 1 | | | | | | |
| Fruit set | 0.704* | 1 | | | | | |
| Fruit setting % | -0.386 | 0.290 | 1 | | | | |
| Total cherelles | 0.260 | 0.397 | 0.052 | 1 | | | |
| Healthy cherelles | 0.433 | 0.421 | -0.048 | 0.718* | 1 | | |
| Wilted cherelles | 0.057 | 0.257 | 0.108 | 0.874* | 0.291 | 1 | |
| Cherelle wilting % | -0.068 | 0.075 | 0.067 | 0.355 | -0.336 | 0.724* | 1 |

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