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Abstract: Cocoa (*Theobroma cacao* L.) is the major input into the chocolate manufacturing industry, particularly the fine or flavor of the indigenous variety Trinitario, which is the hybrid of Crillo and Forastero. One key problem encountered by researchers is the inability to differentiate the various phenological stages of the cocoa reproductive cycle and to assess or predict the yield. This study sets out to map the various stages of the reproductive biology of cacao and to develop a set of photographic illustrations that appropriately describe the floral biology and development stages of Caribbean fine cocoa as a tool for agronomist and farmers. It used a modified version of the codification of the "extended BBCH" scale (Biologische Bundesantalt, Bundessortenamt and Chemische Industrie, Germany), which is a numerical system that differentiates between principal, secondary and tertiary growth stages. The phases and all floral stages to fruit development are described and illustrated in a set of photographs, which can now be used for training and referencing purposes. This is the first attempt to undertake and document this study.

Key words: Cacao, flavor, extended BBCH, fine cocoa, growth stages.

1. Introduction

Cacao, one of the world's most important perennial crops, is almost exclusively explored for chocolate manufacturing. Most cacao varieties belong to three groups that vary according to morphology, genetic and geographical origins [1]. The Caribbean cocoa industry is based on the breeding improvements of the indigenous variety Trinitario, which is the hybrid of Crillo and Forastero [2]. Both Crillo and Forastero are characterized as "fine or flavor" cocoa beans and are the premium flavor cocoa in Europe [3]. The fine flavors are heritable characteristics and are expressed based on a combination of agronomic and ecological conditions as evidenced by the yield and quality of the Trinidad selected hybrids (TSH) [2-5].

Previous studies were undertaken to examine the phenotypic relationships in a diverse cacao accessions [6]. It was found that there is a paucity of cacao phenological information when floral phenological studies in commercial cocoa plantations were conducted over a two-year period in three Caribbean islands. A major problem encountered was the inability by researchers to differentiate the various phenological stages of the cocoa reproductive cycle. This became more complex, as it was difficult to assess or predict the yield at any time due to the deficiency of this information. This was manifested in the assessment of yield potentials after agronomic interventions made or the likely effects of climatic stress of extreme dry spells or tropical storms, or disease or pest outbreak.

Growth and development of cacao are highly dependent on temperature, which mainly affects

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vegetative growth, flowering and fruit development. Cacao produces caulescent flowers, which begin dehiscing in late afternoon and are completely open at the beginning of the following morning, releasing pollen to a receptive stigma. Non-pollinated flowers abscise 24-36 h after anthesis. The percentage of flowers setting pods is in the range of 0.5%-5%. The most important parameters for determinants of yield are related to: (1) light interception, photosynthesis and capacity of photo-assimilate distribution, (2) maintenance respiration and (3) pod morphology and seed fermentation. These are events that can be modified by abiotic factor [1].

Studies in other parts of the world have found that the yield of cocoa is related to the floral phenology of the trees [7]. Clarke [8] confirmed that a good understanding of the floral phenology of tree crops provided significant insights into the functional attributes of growth and reproduction of the trees. Further, the synchronization between the phenology of flowering of cocoa trees and the pollinator population cycle will constitute a significant step towards the realization of increased yield of cocoa. Information on the floral phenology of the cocoa trees could be used to enhance the production potential of the trees for increased economic returns.

Early description of the phenological growth stages in some crops and weeds were compiled by Lancashire et al. [9] and Meier et al. [10]. Hack et al. [11] and Vogel [12] also have undertaken studies on cocoa phenological growth stages. Niemenak et al. [13] used the extended BBCH scale (Biologische Bundesantalt, Bundessortenamt and Chemische Industrie, Germany) to differentiate between the principal, secondary and tertiary growth stages of cocoa, using detailed line illustration. They found that a detailed description, aided by illustrations using a common code or scale, can enhance the transfer of information and technology between agronomist, extension workers and farmers. They further observed that this codification facilitated crops description

based on varietal, environmental and ecological differences. Swanson et al. [14] did a comparative flower development in *Theobroma cacao* based on temporal morphological indicators and morphological measurements using time-lapse photography. This was used to create mathematical models of flower development, and a comparison of the *T. cacao* floral developmental program to the rosid model system.

The BBCH scale provides a detailed description of the morphological and anatomical details of the plant development independent of variation in time [11]. It is based on a scale that provides an overall description of the phenological developmental stages, with a similar code for similar phenological stages of plant species. This is supported by a full code descriptor with illustration.

Niemenak et al. [13] used the BBCH extensively to describe *T. cacao* about germination of seeds and budding establishment, and vegetative and generative growth under the eight principal growth stages. The comprehensive description covered leaf, main stem elongation, formation of a jorquette, chupon initiation and chupon growth, fan branch elongation, inflorescence emergence, development of fruit, and ripening of fruit and seed. The codification follows the "extended BBCH scale". This is a numerical system that differentiates between principal, secondary and tertiary growth stages.

The purpose of this study was to map the various stages of the reproductive biology of cacao and to develop a set of photographic illustrations that appropriately describe the floral biology and development stages of Caribbean fine cocoa as a tool for agronomist and farmers.

2. Methodology

A floral phenological study was conducted during the period 2013 to 2015 in three islands of the Caribbean, *viz.*, Jamaica, Trinidad and Tobago over six commercial cocoa plantations by the University of Trinidad and Tobago. All observations were conducted during the hours of 7:30 to 11:00 am every 14 d, in all locations. The photographs were taken at regular intervals and selection was made to best represent all the macro and micro elements of influences, based on the BBCH descriptions [15].

The study was conducted on 5-year-old and even older mature trees that were of the Trinidad selected hybrid (TSH) variety and were all into the full reproductive phase. The observations were recorded and photographed throughout the two distinct flowering and fruiting stages and across the bimodal wet/dry season.

In this study, the BBCH scale was amended to include days at first bud visible (FBV) for each stage and was used to compute the length of each reproductive phase. The BBCH scale described by Bleiholder et al. [15] and the extended BBCH scale described by Hack et al. [11] covered the 10 principal

growth stages numbered from 0 to 9. Each phase further describes the 10 secondary growth stages in combination of a two-digit numeric code. The vegetative and generative growth is considered fewer than eight principal growth stages.

3. Results and Discussion

For the purpose of this study, only four of these stages were considered, namely, principal or "macro stages" numbered from 0 to 9, and each macro stage was further subdivided to consider the 10 secondary growth stages using the two-digit numeric code, *viz.*, inflorescence emergence (BBCH 5), flowering (BBCH 6), development of fruit (BBCH 7) and ripening of fruit and seed (BBCH 8).

The specific BBCH scale for cacao plant based on the two digits and the description of the individual developmental steps is presented in Table 1.

Growth stages	FBV (d)	Code	Description
Stage 5: inflorescence emergence	0-30	52	Flower buds expanded, emergence of sepal primordia (bud < 1 mm long)
		55	Flower buds expanded, sepals enclose bud (bud 1-2 mm long)
		56	Flower bud expanded, emergence of pedicle (bud 2-3 mm long)
		58	Flower bud expanded, bud turning from green to white (bud 2-4 mm long)
		59	Flower bud growth complete (buds 6 mm long and 3 mm large; pedicle 14 mm), buds closed
Stage 6: flowering	30-31	61	Beginning of flowering
		62	10% of flowers open
		65	50% of flowers open
		69	90% of flowers open
Stage 7: development of fruit	32-75	71	Beginning of fruit growth; endosperm cellularization; ovule and pericarp development; beginning of the cherelle wilt phase; fruits have reached 10% of final size (zygote dormant)
		72	Division of the zygote and preliminary development of the embryo; fruits swell; fruits have reached 20% of the final size
		75	End of the cherelle wilt phase; diameter/length 0.35; fruits have reached 50% of the final size
		76	Beginning of the non-wilting phase; ovule filled with jelly like endosperm; fruits have reached 60% of the final size
		77	Fat, storage proteins and anthocyanin accumulated in the cotyledons; endosperm is gradually resorbed by the embryo; fruits have reached 70% of final size
		79	Embryos are full-grown, only traces of endosperm remain around the fleshy cotyledons; increase in the external; embryos is full-grown, only traces of endosperm remain round the fleshy cotyledons; increase in the external
Stage 8: ripening of fruit and seed	75-225	81	Change of fruit color from green to yellow or orange
		85	Increase in fruit color intensity
		89	Fruit is fully ripe, attached to the main stem or branches and can be harvested with knife or cutlass

 Table 1
 The principal reproductive growth stages 5-8 of T. cacao var. TSH according to the modified BBCH scale.

3.1 Principal Growth Stage 5: Inflorescence Emergence (BBCH 5)

The flowers emerged and grew on the main trunk and branches (Figs. 1a-1f). The development of a



(a) BBCH 51: flower buds visible (1 FBV)

flower bud on a leaf scar (young plant) or on flower cushions (tree) is coded as BBCH stages 51-59, and described as the emergence of flower primordium (Fig. 1). The inflorescence emergence marked the first



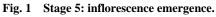
(b) BBCH 52: flower buds expanded



(c) BBCH 55: sepals enclose bud



(e) BBCH 58: bud turning from green to white





(d) BBCH 56: emergence of pedicle



(f) BBCH 59: bud growth complete (30 FBV)

visible sign of flowering and the buds primordia are $150 \ \mu m$ wide at 1 FBV. This continued over 30 FBV days and terminated at stage BBCH 59, when the flower bud growth is completed, but the bud is still closed. Usually, the individual flower cushion can produce many flowers at different stages of development over this growth stage.

There are two main flowering seasons observed occurring between January and June, and a single cocoa tree had the potential to produce over 100,000 flowers/year. Bayer and Hope [16] and Swanson [17] described the development of an individual flower, which encompassed 12 stages and took 30 d. They reported that stages 1-6 involved meristem development and the organogenesis of the floral organs. This was completed within 10 d, whilst the stages 7-12 implied that active elongation and differentiation of the individual organs were going on, but not visible to the naked eye until the flower was fully developed.

The BBCH scale does not describe the timing and micro events of inflorescence, but only the macro events are considered. At BBCH 55, sepals are relatively thick and displayed half of the total thickness of the developing flower [17]. Through elongation, the outside of the flower bud changed colour from green to white or reddish.

3.2 Principal Growth Stage 6: Flowering (BBCH 6)

Flowering began on day at 30 to 31 FBV, and occurred when the bud matured, and the sepals formed a series of five longitudinal abscission zones from the top to the base of the floral bud. Both the sepals and petals opened simultaneously and can occur over a 12 h period (Figs. 2a-2d). Swanson [17] reported that the sepals split opened during the afternoon, continued throughout the night, and by the next morning the flowers were fully opened causing the anthers released their pollen. Niemenak et al. [13] ascribed the flowering stages according to the BBCH code to cover the period BBCH 60 to 69. This period covered the

first flower opening at 30-flower day visibility (FDV) to the time when 90% of all flowers are open within the 12 h period.

Cheesman [18] observed that flower opening was synchronized between flushes of mature flowers, which open at approximately the same time and rate, regardless of their location on the same tree. He further noted that abscission of unfertilized flowers occurred 24-36 h after opening. When a bud matures, the sepal tissues form a series of five longitudinal abscission zones from the tip to the base of the floral bud. These longitudinal abscission zones separate, forming the five individual sepals, which then expand and open outwards. As the sepals open outwards, the petals open at the same time. The opening of flowers occurs over a 12 h period. Sepals usually split during the afternoon and continue to open during the night. At early the following morning, the flowers are fully open and the anthers release their pollen [17].

Flower opening is very well synchronized between the cohorts of mature flowers opening each night. The flowers open at almost exactly the same time and rate, irrespective of their position on the trunk. Unfertilized flowers abscise from the trunk approximately 1 d after flower opening [18]. This is completed just prior to opening of the next set of flowers, resulting in increased visitation of pollinators to the newly opened flowers by the reduction in competition of the day-old flowers.

When cacao plants are in the juvenile stage of development, their structure growth and characteristics are easier observe, to and measurements are made with greater accuracy than that is possible in the case of adult trees. The intrinsic characteristics of older trees are often indiscernible owing to the alterations to their architecture and canopy form that result from external factors [19]. In cultivated fields, particularly, pruning and harvesting practices and competition create significant changes in tree structure, and more emphasis is on the juvenile stages. Identification of differences between cacao



(a) BBCH 60: flowers open (30-31 FBV)



(b) BBCH 62: 10% of flower open



(c) BBCH 65: 50% of flower open

Fig. 2 Stage 6: flowering.

plants with regards to vegetative development in the early stages of juvenile growth is usually scanty.

3.3 Stage 7: Development of Fruit (BBCH 7)

The cacao fruit development stage spans a period of 35 d and are described by the BBCH 71 to 79 (Figs. 3a-3g). It occurred immediately after anthesis. The growth and maturation process of the cacao fruit, which is approximately 150 d, are observed into two phases [20].

The first is a developmental phase and lasted 75 d, during which the pericarp enlarged in conjunction with the ovules (BBCH 71). The endosperm cellularization is initiated and both dry and fresh



(d) BBCH 69: 90% of flower open

weight of the pulp increased exponentially. This phase also comprised two periods. The first of these covered an interval of about 50 d, during which the zygote is dormant [21].

At BBCH 71 to 72, the cocoa fruits on the main stem or branches are visible. This marked the beginning of fruit growth both internally and externally. Internally, the process involved the endosperm cellularization, ovule and pericarp development and the beginning of the cherelle wilt stage. The pods are approximately 10%-20% of final size, and had undergone cell division of the zygote and the preliminary development of the embryo. The fruits have attained 20% the final pod size at 51 FBV.



(a) BBCH 70: fruit visible

(b) BBCH 71: endosperm cellularization, ovule development; cherelle wilt stage; fruits reaching 10% of final size (50 FBV)



(c) BBCH 72: fruits have reached 20% of the final size (51 FBV)



(d) BBCH 75: end of cherelle wilt phase; fruits reaching 50% of the final size (75 FBV)





(e) BBCH 76: fruits reaching 60% of the final size (f) BBCH 77: fruits reaching 70% of the final size (85 FBV)



(g) BBCH 79: embryos fully grown; only traces of endosperm around the fleshy cotyledons; increase in the fruit external dimensions ceases; fruits reaching 90% of the final size (150 FBV)

Fig. 3 Stage 7: development of fruit.

At BBCH 75 to 77, which occurred during the period 75 to 85 FBV, the pods had entered the end of the cherelle wilt phase, and had reached 50% to 60% of the final size. Internally, the ovules are filled with jelly-like endosperm. Also, this phase is characterized by accumulation and storage of fat, proteins, anthocyanins and thocyanins in the cotyledons (BBCH 77). The endosperm has been resorbed by the embryo, and the pods have attained 70% of the final size (85 FBV). At BBCH 79, the embryos are fully grown with traces of endosperm remaining around the fleshy cotyledons, and the increase in the external dimensions of the fruit ceases. The fruits have reached 90% of the final size at 150 FBV [22].

The second phase (50-75 d) commenced with the division of the zygote and the preliminary development of the embryo. Fruits began to swell up and their length also increased. At the end of the first



(a) BBCH 81: change of fruit colour from green/red to yellow/orange (151 FBV)

phase, the ratio between the diameter and the length of an individual fruit was about 0.35. Fruits are called cherelles and displayed about 50% of their final size (BBCH 75). During this phase, fruits are susceptible to physiological deterioration named "cherelle wilt". Cherelle wilt is the shrivelling and blackening of young cacao fruit, which accounts for a considerable loss of fruits [23].

3.4 Stage 8: Ripening of the Fruit and Seed (BBCH 8)

The principal growth stage 8 of cocoa (BBCH 81 to 89) described the ripening process of the cacao fruit and seed maturity for harvest and is illustrated in Figs. 4a-4c. During this phase, there was an increase of the external dimensions of cocoa fruit which ceased at 150 d after fertilization. Internally, it was the period during which the embryos are almost fully grown (BBCH 81) and externally there is a change of fruit



(b) BBCH 85: increase of fruit colour intensity



(c) BBCH 89: fruit fully ripe, attached to the main stem or branches and can be harvested with knife or cutlass (181 FBV)

Fig. 4 Stage 8: ripening of the fruit and seed.

color from green/red to yellow/orange (151 FBV).

At BBCH 85, physiological maturity and ripening, which took about 20-30 d, had been completed with pronounced and distinct varietal color of the husk. Internally, at the seed level, the cells of the hypodermal layer of the outer integument and some of the adjacent layers beneath became prismatic in shape and highly mucilaginous, their walls ultimately disappearing, so that the ripe seeds are surrounded by a continuous sheath of mucilage [24].

At BBCH 89, the mature pods were fully ripe and still attached to the main stem or branches. This indicated the harvesting stage when the pods can be harvested with knife or gauntlet. It further marked the culmination of pollination, fertilization and fruit maturity phases of the reproduction of a single flower at 181 FBV.

The husk of the fruit is woody and is of varying thickness according to variety. According to the five loculi in the superior ovary, the number of ovules per ovary is clone dependent and varies between 37 and 65 ovules per ovary. The seeds are arranged in five rows in the fruit [24]. The number of seeds per fruit ranged between 20 and 50. The cotyledons of the seeds were more or less convoluted and their color varied from white to dark purple.

Depending on the variety and agro-ecological conditions, the maturation process of the fruit, from the pollination to fully mature fruit, takes 160-210 d [24]. The fruit of cacao is an indehiscent drupe and do not abort, but is attached to the tree by its peduncle until harvested. However, it is predisposed to animals, weather conditions, praedial larceny or will naturally deteriorate on the trunk, if not picked.

4. Conclusions

This study has confirmed that cocoa exhibited a bi-modal season related phenological pattern of flowering and fruit set with significant overlapping cycles. There are two distinct crops, but four floral stages can appear to occur simultaneously. Visually, it is observed that on any tree flower emergence can occur at some time of harvest, particularly when the wet season is earlier or delayed. Also, there is no marked "harvest season"; given that there is a minimum of 180 d for harvest, the overlapping cycles are more predominant. The photographic illustration, when tested by some workers, enabled them to better identify the stages, and thus were able to manage their cultural operation more easily. They were able to differentiate when particular TSH hybrids will be in different reproductive stage, and identify crop responses to micro-ecological variations within and between estates. All cocoa workers can now have access to a guide that defines phenological-phase of flowering, fruiting and harvesting more than once per year, under different intrinsic and extrinsic controls.

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