Annals of Applied Biology ISSN 0003-4746

RESEARCH ARTICLE

Phenological growth stages of cacao plants (*Theobroma* sp.): codification and description according to the BBCH scale

N. Niemenak[1,2], C. Cilas[3], C. Rohsius[2], H. Bleiholder[4], U. Meier[5] & R. Lieberei[2]

1 Department of Biological Science, Higher Teachers' Training College, University of Yaounde I, P.O. Box 47 Yaounde, Cameroon
2 Department of Crop Science and Plant Ecology, University of Hamburg, 22609 Ohnhorststrasse 18, Hamburg, Germany
3 CIRAD Avenue Agropolis, TA A31/02, 34398 Montpellier, France
4 Dinkelackerring 4, 67435 Neustadt, Germany
5 Jülius Kühn-Institut, Institut für Kulturpflanzenforschung, Messeweg 11/12, D 38104 Braunschweig, Germany

Keywords
BBCH scale; growth stages; phenological development; *Theobroma* cacao.

Correspondence
Dr Nicolas Niemenak, Department of Biological Science, Higher Teachers' Training College, University of Yaounde I, P.O. Box 47 Yaounde, Cameroon.
Email: niemenak@yahoo.com

Received: 26 June 2008; revised version accepted: 29 June 2009.

doi:10.1111/j.1744-7348.2009.00356.x

Abstract

The detailed description of growth stages of useful plants followed by adequate codification facilitates communication between scientists and practicians if, for example, new findings of science have to be transferred to management procedures or if experiences made at one growing site have to be adapted to another. We describe the growth stages of the worldwide species of cacao trees (*Theobroma* sp.) to prepare the basis for production management, comparisons of epidemiological studies of disease, of growth patterns under different environmental factors and of genetically clone specific parameters. The codification follows the 'extended BBCH (BBCH, Biologische Bundesantalt, Bundessortenamt and CHemische Industrie, Germany) scale', a numerical system that differentiates between principal, secondary and tertiary growth stages. Each growth stage presented from seed germination to crown development and harvest is correlated with general management practices. This scale will be of great help to cacao growers and scientists around the world for better communication, more efficient planning of management practices and experiments.

Introduction

The cacao plant is a neotropical, small, evergreen tree native to South America (Motamayor *et al.*, 2002) growing between 20° latitude north and south of the Equator. Since its discovery by Europeans after the conquest of the New World, its cultivation has developed strongly since its introduction into Africa. The first attempt to plant cacao outside the American continent is attributed to the introduction of a plant from Mexico into the Philippines in 1670 (Blanco, 1837). In West Africa the first material was introduced in 1822 through São Tomé and Príncipe and was from 'lower' Amazonian origin (Bartley, 2005).

This plant, of the Malvaceae (formerly Sterculiaceae) family, is grown for its fruits, known as cacao pods. Botanically the fruits are berries. The pods contain seeds, which are fermented with the mucilage surrounding them and then dried to give fermented dried cacao, the raw material used to make chocolate. The cacao tree is a small tree whose fruits grow on both the trunk and the branches. The fruits arise from the pollination of flowers grouped in flower cushions. A distinction is currently made between three major groups of cultivated cacao trees: 'Criollo', 'Forastero' and 'Trinitario' (Cheesman, 1944; Bartley, 2005).

Several systems have been proposed to describe the phenological growth of cacao tree (Vogel, 1975), but no unified approach in describing phenological stages of growth that are compatible with other plant species has been defined for this species. Growth stage definitions for other plants have been developed by several groups. An interdisciplinary working group proposed a uniform decimal code to describe phenological growth stages of crops and weeds (Lancashire *et al*., 1991; Hack *et al*., 1992). The BBCH scale provides a uniform decimal code to describe the phenology of plants like cereals, maize, citrus, stone and pome fruits, coffee, roses and others. Reviews have been published by Meier (1997) and Meier *et al*. (2009).

In our work, we proposed to apply the BBCH system to describe the phenological growth stages of the cacao tree, from germination up to tree senescence (Hack *et al*., 1992). The BBCH scale describes the pattern of development independent of variation in timing. The basic principles of the scale integrated: (a) morphological characteristics which are used for the description of the phenological developmental stages; (b) the same code is applied for similar phenological stages of each plant species; (c) for each code, a description is given, and for some important stages, drawings are included; (d) development of the main stem is manly taken into consideration; (e) relative values relating to species and/or variety-specific ultimate sizes are used for the indication of sizes (Hack *et al*., 1992).

Material and methods

To describe the phenological growth stages of cacao plant, the extended BBCH scale will be used. The BBCH scale (Bleiholder *et al*., 1991) and the extended BBCH scale (Hack *et al*., 1992) consider 10 principal growth stages ('macro stages') numbered from 0 to 9 and each macro stage considers 10 secondary growth stages numbered from 0 to 9. The combination gives a two-digit numeric code.

For *T. cacao*, these begin with the germination of seeds or budding establishment (BBCH 0). The vegetative and generative growth is considered under eight principal growth stages corresponding to leaf development on the main shoot of the young plant and on the fan branches (BBCH 1); main stem elongation, formation of a jorquette with its fan branches and, subsequently, chupon initiation and chupon growth (BBCH 2); fan branch elongation (BBCH 3); inflorescence emergence (BBCH 5) and flowering (BBCH 6). Development of fruit (BBCH 7), ripening of fruit and seed (BBCH 8) and senescence (BBCH 9) complete the scale.

When cacao plants are in the juvenile stage of development, their structure and growth characteristics are easier to observe, and measurements are made with greater accuracy than 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 (Bartley, 2005). In cultivated fields, particularly, pruning and harvesting practices and competition create significant changes in tree structure. Therefore, during our description, we will put more emphasis on the juvenile stages. Identification of differences between cacao plants with regards to vegetative development in the early stages of juvenile growth is usually scanty.

The specific BBCH scale for cacao plant is based on a decimal code of at least two digits and a description of the individual developmental steps. In the present paper the scale for cacao plant is presented in two forms.

Two-digit code

The first digit describes the principal growth stage (0–9) and describes 10 clearly recognisable and distinguishable more long-lasting developmental phases. The second digit describes the secondary stage (0–9) or points of time or steps of plant development. In contrast to the principal growth stages, they are defined as short developmental steps characteristic of the cacao plant.

Three-digit code

The two-digit code is not precise enough in all developmental steps to describe a specific step. Therefore in this case a third digit is introduced between the principal growth stage and the secondary growth stage. The so-called mesostage gives the flexibility to provide a further subdivision of the secondary growth stages, if necessary. The scale is then built by three digits, where:

- the first digit indicates the principal growth stage (0–9),
- the second digit the mesostage (0–9),
- the third digit the secondary growth stage (0–9).

Results and discussion

Principal growth stage 0: germination–vegetative propagation

Cacao fruit reaches physiological maturity around 150 days after flowering and is harvested at 170 days. After harvest, seeds are removed from the fruit and are processed to obtain raw cacao by fermentation and drying. They die in the course of this treatment. Under permissive conditions (Fig. 1a) they germinate. The period from onset of imbibition to seedling emergence takes approximately 25 days. Imbibition is completed within 3 days and radicle protrusion appears around day 5 (BBCH 02). Radicle and hypocotyl elongation proceeds, hypocotyl is visible (BBCH 03) and the formation of root hairs follows (BBCH 05). Between days 7 and 18, depending on the conditions, seedlings have emerged from the soil (emergence) and the hypocotyl forms a hook

(3–5 cm long) lifting the cotyledons from the ground (BBCH 07, Fig. 1b and Fig. 1c); the cotyledons are upright but still closed (BBCH 09) (Hunter, 1959; Rohsius, 2000).

Different forms of vegetative propagation can be applied to cacao such as budding and somatic embryogenesis (Miller & Guiltinan, 2003). However, the planting materials found in the field come mainly from seeds and are improperly called 'clonal seeds' (seeds obtained from a number of trees of a single clone planted in isolation or from a mixture of several clones), most of which are derived from open-pollinated pods taken from the same tree clones. Budding is performed with softwood stem-cuttings either from plagiotropic (fans) or orthotropic material (chupons). Initiation of budding from orthotropic or fan shoots is characterised by the emergence of root through the cortical tissues just above the callus (Cheesman, 1944). Somatic embryos, when established display the same growth behaviour as seeds (Maximova *et al.*, 2002; Niemenak *et al.*, 2008).

Based on orthotropic materials, stage 00 encompassed stem cuttings with four or five leaves taken from the nursery trees, with their leaves trimmed to about half of their normal size. Stage BBCH 01 represented cuttings with their cut end dipped in a solution of naphthalene acetic acid (0.5%) and β-indole-butyric acid (0.5%) and then put in a rooting medium. Callus formation and root emergence are considered as stage BBCH 02.

Initiation of horizontal growth of tap roots constitutes the stage BBCH 03 whereas the switch from horizontal to vertical growth is the stage BBCH 05. The break and growth into a vertical leading shoot of terminal or lateral buds from orthotropic cuttings marked the stages BBCH 07 and BBCH 09, respectively (Charrier, 1969).

Principal growth stage 1: leaf development on the main shoot of the young plant and on the fan branches

Leaf development on upright shoots (main stem or chupon) as well as on the jorquette is considered. Instead of a regular sequence of successive leaves expanding in cacao, about 10 leaves start to grow at the same time, which is referred to as a leaf-flush (Greenwood & Posnette, 1950; Greathouse *et al.*, 1971). They expand almost simultaneously, and an interval of approximately 40 days intervenes before a new leaf-flush occurs again. The flush cycle shows a typical pattern: leaf unfolding (F-1), expansion (F-2), maximum leaf area and greening (I-1) and maturity (I-2) (Greathouse *et al.*, 1971; Sleigh *et al.*, 1984). The leaves, throughout expansion, are pale green or tinged with pink depending on the presence or absence of anthocyanins (Holden, 1957). They are soft and delicate, but gradually harden. Red pigmented leaves also become green during hardening. The new leaves attain their full size in the course of about 4 weeks after unfolding. The successive flushes can be easily distinguished by the degree of browning of the stem on which the leaves are attached. Young flushes display green stems whereas the oldest flushes appear on dark brown stem.

Leaves show dimorphic characters corresponding to the stem on which they arise. On the upright shoot, leaves have long petioles and are symmetrical; the petioles have a marked pulvinus or swelling at each end which allows the leaf to be oriented in relation to the light. The leaves on the fan-wood have shorter petioles and are slightly asymmetrical. Also the vigour and general size of the chupon leaves are usually greater than in leaves of the fan branches. The leaf blades are simple, lanceolate to lanceolate-ovate. The margin is entire and slightly wavy. The venation is pinnate, and the surface is glabrous on both sides (Brooks & Guard, 1952; Vogel, 1975).

The development of the first leaves (eophylls) from seeds begins after the cotyledons start to unfold (BBCH 10, Fig. 1d). The subsequent leaves occur as a succession of flushes. Each flush emerging from the main shoot or from a fan branch is counted up to the 9th flush and assigned BBCH codes 11 to 19, respectively.

The BBCH stage 110 is attributed to the emerging of unfolded leaves of the first flush, which are still pale green or red depending on the cultivar. When leaves expand and display 20% or 50% of their final size, BBCH stage 112 (Fig. 1e) or 115 is reached. The mature, flush–bearing, already hardened leaves with dark green colour and constant size are classified as BBCH stage 119 (Fig. 1f).

Principal growth stage 2: main stem elongation, formation of jorquette of fan branches and chupon

An individual shoot passes through alternate periods of growth and dormancy. The growth period is characterised by the expansion and the elongation of leaves of the shoot. During dormancy the length of the shoot remains constant, and no new leaves expand. Shoot-growth rhythm is correlated to the flush cycle and therefore displays the same patterns, that is bud swelling (F-1), stem elongation (F-2), maximum stem size (I-1) and maturity (I-2) (Greathouse *et al.*, 1971; Sleigh *et al.*, 1984). Maturation phase is achieved when the stem bearing the newly formed leaves begins to form phloem. In this phase, the stem is brown, the leaves are mature and the stipules covering the terminal bud are more or less closely appressed to it.

Shoot derived from seedling (BBCH 20) has a determinate orthotropic growth. Later this shoot constitutes the main trunk. After 1–2 years of growth, the shoot

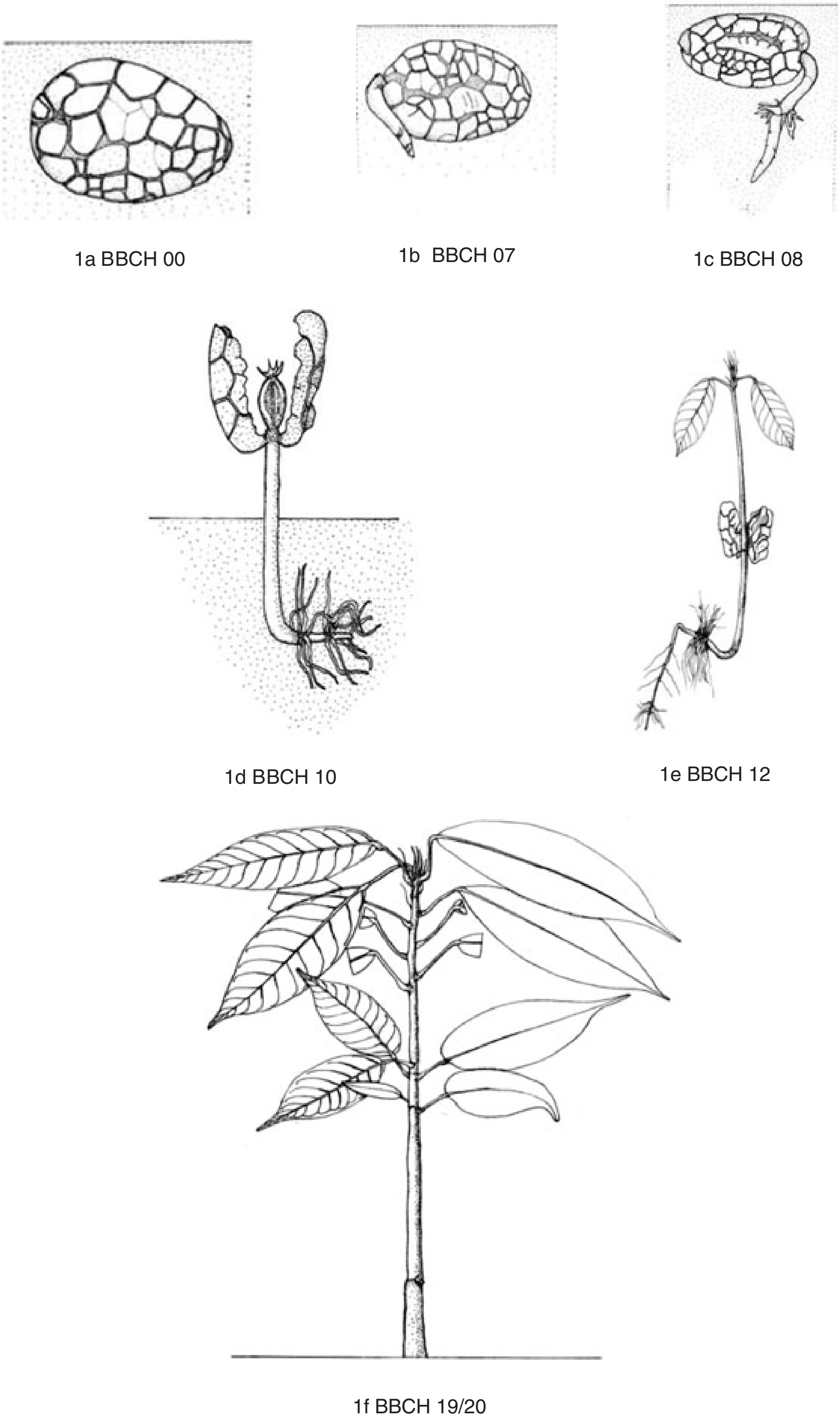

Figure 1 Principal growth stages of cacao plant 0 and 1.

reaches the physiological maturity and the apical meristem stops growth (BBCH 209). Branch dimorphism is then induced and a jorquette of plagiotropic branches is developed (BBCH 21, Fig. 2a and Fig. 2b). The height at which the first jorquette is produced depends on environmental conditions, such as light, soil fertility, the age of a plant at establishment in the field and on the genotype (Vogel, 1975).

A second vertical stem growth (first chupon) appears on the main stem by the activation of the orthotropic buds in the leaf axils below the whorl of lateral branches (BBCH 211). In the course of time a second whorl of fan branches is developed (BBCH 22, Fig. 2c). This process may be repeated several times (BBCH 29) when there is no competition from other vegetation and no pruning. This mode of development is a characteristic of growth architectural model found in tropical rain forest known as Nozeran model (Hallé *et al.*, 1978). As a consequence, the canopy becomes higher. In a cacao plantation, trees will grow to a height of 4–10 m depending on spacing, the degree of shade and the frequency of pruning. In the wild, under the heavy shade of the primary forest they reach a height of up to 20 m (Wood & Lass, 1985; Bartley, 2005).

Principal growth stage 3: fan branch elongation

A young cacao seedling produces a single vertical unbranched stem. When it attains physiological maturity, the terminal bud gives rise to jorquette of three, four or five fan branches which grow out obliquely to horizontally depending on the genotype and conditions. Branch elongation is devoted only to the jorquette which is considered as the primary fan branch. Fan branches branch, and their auxiliary buds produce shoots; that is fan branches of the second or higher orders (Greathouse *et al.*, 1971; Greenwood & Posnette, 1950).

According to the BBCH scale, when an individual fan branch produces 20 secondary fan branches stage 32 is applied (Fig. 2d). The 30th, 40th or more secondary fan branch is described accordingly with BBCH 33, 34, till 39.

Principal growth stage 5: inflorescence emergence

Cacao is cauliflorous, with flowers growing on the main trunk and branches. An individual flower cushion bears flowers at different stages of development (Fig. 3a). Cacao flowering is usually abundant with up to 120 000 flowers per tree each year. Flowering occurs mainly between January and June in West Africa and Brazil (Mossu *et al.*, 1981; Paulin *et al.*, 1983; Valle *et al.*, 1990). Lower-Amazon Amelonado genotypes stop flowering from July to November (Mossu *et al.*, 1981), whereas upper-Amazon genotypes usually produce flowers throughout the year. In all cases, flowering intensity decreases with increasing number of developing pods per tree because of the competition for assimilates (Valle *et al.*, 1990).

In *T. cacao*, flower development has been characterised using morphological and anatomical landmarks (Bayer & Hope, 1990; Swanson, 2005). Development of an individual flower encompasses 12 stages and takes 30 days. Stages 1–6 involve meristem development and the organogenesis of the floral organs, which is completed within 10 days. Stages 7–12 imply the process of elongation and differentiation of the individual organs up to the fully developed flower (Bayer & Hope, 1990; Swanson, 2005).

The BBCH scale does not describe the timing and micro events of inflorescence. Only the macro events are considered. The development of a flower bud on a leaf scar (young plant) or on flower cushions (tree) is coded by BBCH stages 51–59. BBCH stage 51 is defined by the emergence of flower primordium (Fig. 3a). Buds are approximately 150 μm long and visible. From BBCH stage 52 to 56, buds expand with subsequent sepals (BBCH 55) and pedicle growth (BBCH 56). At BBCH 55, sepals are relatively thick and display half of the total thickness of the developing flower (Swanson, 2005). Through elongation, the outside of the flower bud changes colour from green to white or reddish. At stage 59 flower buds are mature with an average length of 6 mm, an average width of 4 mm and a pedicle measuring about 14 mm. Buds are still closed.

Principal growth stage 6: flowering

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-hour period. Sepals split during the afternoon and continue to open during the night. Early the following morning, the flowers are fully open and the anthers release their pollen (Swanson, 2005).

Flower opening is very well synchronised 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. Unfertilised flowers abscise from the trunk approximately 1 day after flower opening (Cheesman, 1932). This is completed just prior to opening of the next set of flowers, and results in increased visitation of pollinators to the newly opened flowers by the reduction in competition of the day-old flowers.

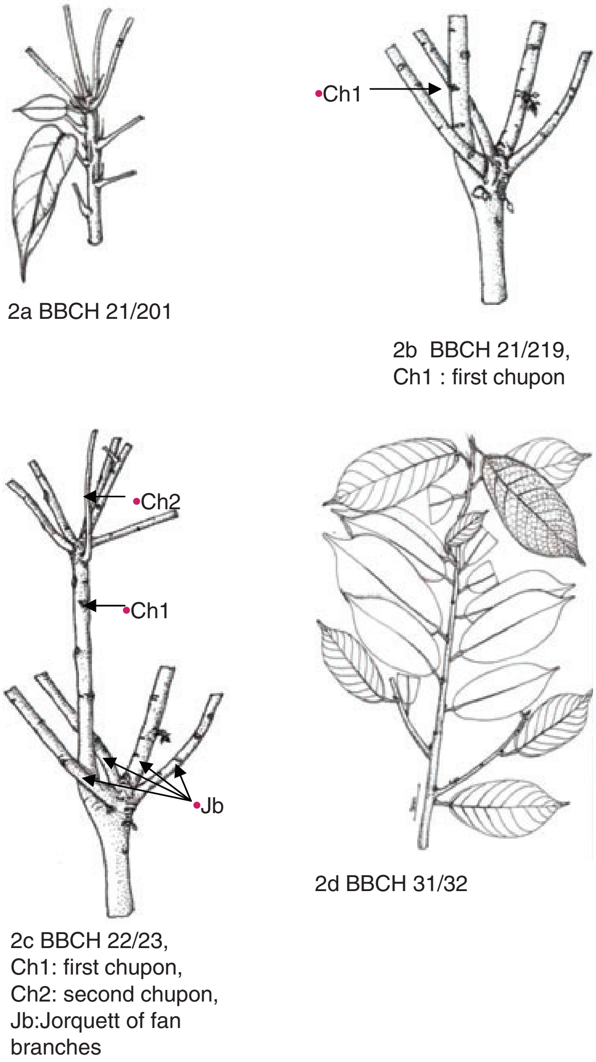

Figure 2 Principal growth stages of cacao plant 2 and 3.

When about 10% of the flowers on a plant are open, stage 61 is achieved. At stage 65, about 50% of the flowers have opened. At stage 69 about 90% of the flowers have opened (Fig. 3a and Fig. 3b).

Principal growth stage 7: development of fruit

After anthesis the growth and maturation process of the cacao fruit, which is approximately 150 days, falls into two phases (McKelvie, 1956).

The first phase is a developmental phase, occupying about 75 days during which the pericarp enlarges in conjunction with the ovules (BBCH 71, Fig. 3c and Fig. 3d). Endosperm cellularises and both dry and fresh weight of the pulp increase exponentially. This phase also comprises two periods. The first of these covers an interval of about 50 days during which the zygote is dormant (Cheesman, 1927). Growth in length is slow. The second period (50–75 days) of the first phase commences with the division of the zygote and the preliminary development of the embryo. Fruits begin to swell up and their length also increases. At the end of the first phase, the ratio between the diameter and the length of an individual fruit is about 0.35. Fruits are called cherelles and display about 50% of their final size (BBCH 75, Fig. 3e).

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 (Humphries, 1943a,b).

The second phase (BBCH 77, Fig. 3f and Fig. 1g), which starts about 85 days after pollination, is a period of active

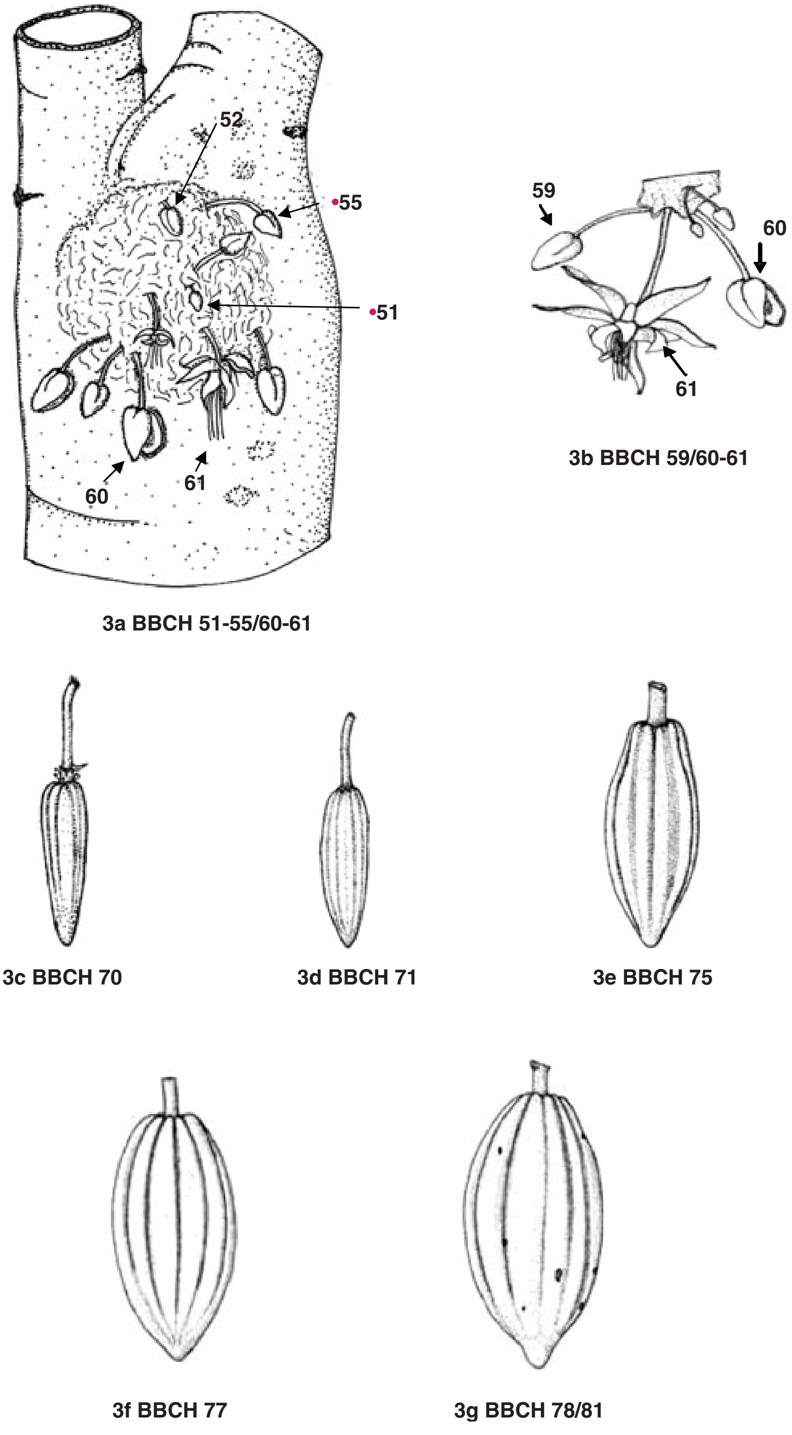

Figure 3 Principal growth stages of cacao plant 5–8.

metabolism, during which lipids, storage proteins and anthocyanins accumulate in the seed while the moisture content of the embryo decreases up to 30% (Lehrian & Keeney, 1980; Pence, 1991). Pericarp and ovule growth slows down at the expense of embryo growth. The embryo grows from 0.2 cm in length after 85 days to over 3 cm after 150 days. At this stage it completely fills the inner space, which is confined by the measures of the testa of the seed. Before embryo growth becomes rapid, the ovule becomes filled with a jelly like endosperm. When embryo growth ceases, there is no further resumption of fruit growth and ripening begins immediately (BBCH 79).

Principal growth 8: ripening of fruit and seed

The increase of the external dimensions of fruit ceases 150 days after fertilisation; at that time the embryos are almost fully grown. Fruit and seed have reached their physiological maturity and ripening follows immediately. It takes about 20–30 days to complete, and its characteristic is that the colour of the husk changes externally. Green pods become orange-yellow and red coloured pods turn into orange pods or stay red. Fruit colour increases its intensity with green/yellow or red/orange areas (BBCH 85). Internally, at the seed level, the cells of the hypodermal layer of the outer integument, and some of the adjacent layers beneath, become prismatic in shape and highly mucilaginous, their walls ultimately disappearing, so that the ripe seed is surrounded by a continuous sheath of mucilage.

Altogether, the maturation process of the fruit, from the pollination to fully mature fruit, takes 160–210 days (Berry & Cilas, 1994). The mature fruit of cacao is an indehiscent drupe that remains attached to the tree by its peduncle until harvested, distributed by animals or until deteriorated on the trunk. 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), the seeds are arranged in five rows in the fruit. The number of seeds per fruit range usually between 20 and 50. The cotyledons of the seeds are more or less convoluted and their colour varies from white to dark purple depending on the genotype combination after fertilisation.

Principal growth 9: senescence

The physiological life of a cacao tree can reach ages of more than 100 years because the tree can regenerate itself by the production of chupons. However, the economic life of a cacao field is influenced by the environmental conditions. The highest cacao yields are achieved between 25 and 40 years (Tafani, 1977).

Leaves on an individual flush have a determinate life time. The longevity of leaves of adult trees of cacao with closely packed crowns varies with their position within the canopy, and/or the irradiance received, and the time of emergence. In particular, longevity decreased greatly with the height of the leaf from ground level (Miyaji *et al*., 1997). Where conditions are accordingly, the leaves remain for three or four flushes. When the development of an individual flush is completed, the leaves are dark green and are of normal size (BBCH 90). After 4–5 months of maximum activity, senescence phase is initiated: the leaf changes its colour from green to yellowish (BBCH 92). This process is accompanied and finished by the formation of an abscission layer at the base of the basal pulvinus (BBCH 95). Post harvest treatments (fermentation and drying) or storage are included in BBCH 99.

Conclusion

The BBCH scale allows the description of cacao growth process and a comparison with other cultivated species. As the system exploits the presence or absence of distinct morphological criteria, it can be used to determine stage of growth in an objective, unequivocal manner so that communication of cacao research results and producer practices can be facilitated. This may also allow identifying the scarcity in the knowledge of the growth process in cacao. For example, the duration of different steps of cacao growth and development are not well characterised. In addition, the model can act as a stepping stone for constructing future mechanistic models, with the aim of better understanding cacao development in genetic, physiological, ecological, evolutionary terms as well as quality purpose. Application of mineral fertiliser is one of the most important measures in agricultural plant production whereby accuracy in distribution will influence success both in cultivation and economy. This study will help cacao culture by expressing the timing of agricultural operations on a standardised scale.

Phenological growth stages and extended BBCH-identification keys of cacao plant

Principal growth stage 0: seed germination/vegetative propagation

Code: Two-digit	Three-digit	Description
00	000	Fresh seeds /Orthotropic cuttings with four or five half-trimmed leaves
01	001	Seed imbibition/Cuttings planted in rooting media; no callus visible
02		Radicle protrusion through the seed coat/Callus formation begins on orthotropic cuttings; tap root emergence

Continued

03		Hypocotyl visible; elongation of radicle/Initiation of horizontal growth of tap roots
05		First side roots visible/Vertical orientation of tap root growth
07		Hypocotyl forms a hook; cotyledon emergence from the ground/Terminal or lateral buds from orthotropic cuttings break
09	009	Elongation of the hypocotyl completed; cotyledons are upright, but still unfolded/Growth of terminal or lateral buds into a vertical leading shoot

Principal growth stage 1: leaf development on the main shoot of the young plant and on the fan branches

Code		
Two-digit	Three-digit	Description
10	100	Cotyledon completely unfolded
		Stipules surrounding terminal bud on the main shoot or fan branches spread apart and bud swells
11	110	Leaf unfolding on the first flush. Leaves are pale green or red
	111	Leaf expansion is 10% of final size
	112	Leaf expansion is 20% of final size
	115	Leaf expansion is 50% of final size
	119	First flush growth complete. Leaves are dark green with maximum area
12	120	Leaf unfolding on the second flush. Leaves are pale green to red
	121	Leaf expansion is 10% of final size
	122	Leaf expansion is 20% of final size
	125	Leaf expansion is 50% of final size
	129	Second flush growth complete
19	190	Nine or more flushes completely mature

Principal growth stage 2: main stem elongation, formation of jorquette of fan branches and chupon

Code		
Two-digit	Three-digit	Description
20	200	Shoot derived from seedling displays 10% of growth
	202	Shoot with 20% growth
	203	Shoot with 30% growth
	205	Shoot with 50% growth
	209	Shoot reaches its physiological maturity and the growth of its apical meristem is arrested
21	210	Formation of the first jorquette and subsequently the first chupon
	211	First chupon appears under the first jorquette with 10% growth
	212	First chupon with 20% growth
	215	First chupon with 50% growth
	219	First chupon reaches its physiological maturity and the growth of its apical meristem is arrested
22	220	Formation of second jorquette and subsequently the second chupon
	221	Second chupon appears under the first jorquette with 10% growth
	222	Second chupon with 20% growth
	223	Second chupon with 50% growth
	229	Second chupon reaches its physiological maturity and the growth of its apical meristem is arrested
23	230	Formation of third jorquette and subsequently the third chupon
	231	Third chupon appears under the third jorquette with 10% growth
	232	Third chupon with 20% growth
	233	Third chupon with 50% growth
	239	Third chupon reaches its physiological maturity and the growth of its apical meristem is arrested
29	290	Formation of ninth or more jorquette and subsequently ninth or more chupon
	291	Ninth or more chupon appears under the ninth jorquette with 10% growth
	292	Ninth or more chupon with 20% growth
	293	Ninth or more chupon with 30% growth
	295	Ninth or more chupon with 50% growth
	299	Last chupon reaches its physiological maturity and the growth of its apical meristem is arrested

Principal growth stage 3: fan branch elongation

Code		
Two-digit	Three-digit	Description
31	301	Jorquette of primary fan branches visible
32	302	Primary fan branch with 20 secondary fan branches
35	305	Primary fan branch with 50 secondary fan branches
39	309	Primary fan branch with 90 or more secondary fan branches

Principal growth stage 5: inflorescence emergence

Code		
Two-digit	Three-digit	Description
51	501	Flower buds visible (buds primordium 150 μm wide)
52	502	Flower buds expanded, emergence of sepal primordia (bud <1 mm long)
55	505	Flower buds expanded, sepals enclose bud (bud 1–2 mm long)
56	506	Flower bud expanded, emergence of pedicle (bud 2–3 mm long)
58	508	Flower bud expanded, bud turning from green to white (bud 2–4 mm long)
59	509	Flower bud growth complete (buds 6 mm long and 3 mm large; pedicle 14 mm), buds still closed

Principal growth stage 6: flowering

Code		
Two-digit	Three-digit	Description
60	600	First flowers getting open
61	601	Beginning of flowering
62	602	10% of flowers open
65	605	50% of flowers open
69	609	90% of flowers open

Principal growth stage 7: development of fruit

Code		
Two-digit	Three-digit	Description
70	700	Fruits at the main stem or branches visible
71	701	Beginning of fruit growth. Endosperm cellularisation, ovule and pericarp development. Beginning of the cherelle wilt phase. Fruits have reached 10% of final size (zygote dormant)
72	702	Division of the zygote and preliminary development of the embryo. Fruits swell. Fruits have reached 20% of the final size
75	705	End of the cherelle wilt phase. D/L 0.35. Fruits have reached 50% of the final size
76	706	Beginning of the non-wilting phase. Ovule filled with jelly like endosperm. Fruits have reached 60% of the final size
77	706	Fat, storage proteins and anthocyanins accumulated in the cotyledons. Endosperm is gradually resorbed by the embryo. Fruits have reached 70% of final size
79	709	Embryos are full-grown, only traces of endosperm remain round the fleshy cotyledons. Increase in the external dimension of fruit ceases. Fruits have reached 90% of the final size

Principal growth stage 8: ripening of fruit and seed

Code		
Two-digit	Three-digit	Description
81	801	Change of fruit colour from green or red to yellow or orange
85	805	Increase in fruit colour intensity
89	809	Fruit is fully ripe, attached to the main stem or branches and can be harvested with knife or cutlass

Principal growth stage 9: senescence

Code		
Two-digit	Three-digit	Description
90	900	Flush completed its development, leaves appear dark green
92	902	Older leaves begin to discolour from dark green to yellow
95	905	Formation of abscission zone in the basal pulvinus of the old leaves
98	908	Abscission of old leaves
99	909	Post harvest or storage treatments

Acknowledgements

The study was supported by the Alexander von Humboldt Stiftung (www.humboldt-stiftung.de) via grant to Niemenak Nicolas (Grant No KAM/1115305). We thank Olivier SENE (Department of Plant Biology, Faculty of Science, University of Yaounde I) for drawings.

References

Bartley B.G.D. (2005) *The genetic diversity of cacao and its utilization*. Wallingford, UK: CABI Publishing. 341 pp.

Bayer C., Hope J.R. (1990) Floral development of *Theobroma cacao* L. *Beiträge zur Biologie der Pflanzen,* **65**, 301–312.

Berry D., Cilas C. (1994) Etude génétique de la réaction à la pourriture brune des cabosses de cacaoyers (*Theobroma cacao* L.) issus d'un plan de croisements diallèle. *Agronomie,* **14**, 599–609.

Blanco M. (1837) *Flora de Filipinas*. Manila: Imprenta de Santo Thome. 887 pp.

Bleiholder H., Kirfel H., Langelüddeke P., Stauss R. (1991) Codificação unificada dos estádios fenológicos de culturas e ervas daninhas. *Pesquisa Agropecuária Brasileira,* **26**, 1423–1429.

Brooks E.R., Guard A.T. (1952) Vegetative anatomy of *Theobroma cacao* L. *Botanical Gazette,* **113**, 444–454.

Charrier A. (1969) Contribution à l'étude de la morphogenèse et de la multiplication végétative du cacaoyer (*Theobroma cacao* L.). *Café Cacao Thé,* **13**, 97–115.

Cheesman E.E. (1927) Fertilization and embryogeny in *Theobroma cacao*, L. *Annals of Botany,* **11**, 107–126.

Cheesman E.E. (1932) The economic botany of cacao. A critical survey of the literature to the end of 1938. Supplement to Volume 9, No. 6. *Tropical Agriculture, Trinidad,* **1**, 16.

Cheesman E.E. (1944) Notes on the nomenclature, classification and possible relationships of cacao populations. *Tropical Agriculture,* **22**, 144–159.

Greathouse D.C., Laetsch W.M., Phinney B.O. (1971) The shoot-growth rhythm of a tropical tree, *Theobroma cacao*. *American Journal of Botany,* **58**, 281–286.

Greenwood D.C, Posnette A.F. (1950) The growth flushes of cacao. *Journal of Horticultural Science,* **25**, 164–174.

Hack H., Bleiholder H., Buhr L., Meier U., Schnock-Fricke E., Weber E., Witzenberger A. (1992) Einheitliche Codierung der phänologischen Entwicklungsstadien mono-und dikotyler Pflanzen Erweiterte BBCH-Skala, Allgemeine. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes,* **44**, 265–270.

Hallé F., Oleman R.A.A., Tomlinson P.B. (1978) *Tropical Trees and Forests. An Architectural Analysis*. Berlin: Springer-Verlag. pp. 441.

Holden M. (1957) An investigation on polyphenolic compounds of the cacao leaf in connexion with a chemical method for detecting virus infection. *Journal of the Science of Food and Agriculture,* **10**, 553.

Humphries E.C. (1943*a*) Wilt of cacao fruits (*Theobroma cacao*) I. An investigation into the causes. *Annals of Botany,* **7**, 31–44.

Humphries E.C. (1943*b*) Wilt of cacao fruits (*Theobroma cacao*) II. A preliminary survey of the carbohydrate metabolism with special reference to wilt susceptibility. *Annals of Botany,* **7**, 45–61.

Hunter J.R. (1959) Germination in *Theobroma cacao*. *Cacao (Turrialba),* **4**, 1–7.

Lancashire P.D., Bleiholder H., van der Boom T., Langelüddeke P., Stauss R., Weber E., Witzenberger A. (1991) A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology,* **119**, 561–601.

Lehrian D.W., Keeney P.G. (1980) Changes in the lipid components of seeds during growth and ripening of cacao fruit. *Journal of the American Oil Chemists' Society,* **57**, 61–65.

Maximova S.N., Alemanno L., Young A., Ferriere N., Traore A. (2002) Efficiency, genotypic variability, and cellular origin of primary and secondary somatic embryogenesis of *Theobroma cacao* L. *In Vitro Cellular & Development Biology–Plant,* **38**, 252–259.

McKelvie A.D. (1956) Cherelle wilt of cacao. I. Pod development and its relation to wilt. *Journal of Experimental Botany,* **7**, 252–263.

Meier U. (1997) BBCH-Monograph. *Growth stages of plants–Entwicklungsstadien von Pflanzen–Estadios de las plantas–Développement des plantes*. Berlin, Wien: Blackwell, Wissenschaftsverlag. 622 pp.

Meier U., Bleiholder H., Buhr L., Feller C., Hack H., Heß M., Lancashire P.D., Schnock U., Stauß R., Van den Boom T., Weber E., Zwerger P. (2009) The BBCH system to coding the phenological growth stages of plants–history and publications. *Journal of Cultivated Plants,* **61**, 41–52.

Miller C.R., Guiltinan M.J. (2003) Perspective on rapid vegetative multiplication for orthotropic scion and rootstock varieties of cacao. In International Workshop on Cacao. Cacao Breeding for Improved Production Systems (INGENIC), 189–194, 19–21 October 2003, Accra, Ghana.

Miyaji K-I., da Silva W.S., Alvim P.T. (1997) Longevity of leaves of a tropical tree, *Theobroma cacao*, grown under shading, in relation to position within the canopy and time of emergence. *New Phytologist,* **135**, 445–454.

Mossu G., Paulin D., De Reffye P. (1981) Influence de la floraison et de la pollinisation sur les rendements du cacaoyer. Liaisons mathématiques entre les données expérimentales. Equation du rendement. *Cafe Cacao Thé,* **25**, 155–168.

Motamayor J.C., Risterucci A.M., Lopez P.A., Ortiz C.F., Moreno A., Lanaud C. (2002) Cacao domestication I: the origin of the cacao cultivated by the Mayas. *Heredity,* **89**, 308–386.

Niemenak N., Saare-Surminski K., Rohsius C., Omokolo N.D., Lieberei R. (2008) Regeneration of somatic embryogenesis in *Theobroma cacao* L. in temporary immersion bioreactor and analyses of free amino acids in different tissues. *Plant Cell Reports,* **27**, 667–676.

Paulin D., Decazy B., Coulibaly N. (1983) Etude des variations saisonnière des conditions de pollinisation et de fructification dans une cacaoyère. *Cafe Cacao Thé,* **27**, 165–176.

Pence V.C. (1991) Abscisic acid in developing zygotic embryos of *Theobroma cacao*. *Plant Physiology,* **91**, 1291–1293.

Rohsius C. (2000) Proteolyse von Reserveproteinen bei Keimung und Fermentation der Samen von Theobroma-Arten. Diplomarbeit. Institut für Angewandte Botanik, Universität Hamburg, Deutschland. 122 pp.

Sleigh P.A., Collin H.A., Hardwick K. (1984) Distribution of assimilate during the flush cycle of growth in *Theobroma cacao* L. *Plant Growth Regulation,* **2**, 381–391.

Swanson J-D. (2005) Flower development in *Theobroma cacao* L. An assessment of morphological and molecular conservation of floral development between *Arabidopsis thaliana* and *Theobroma cacao* L. PhD Thesis, The Pennsylvania State University, USA. 191 pp.

Tafani R.R. (1977) Evaluaciones en teorias de inversión: Estimatura de vida económicade plantaciones de cacao en Bahia, Brazil. In Proceedings of the 5th International Cocoa Research Conference. Ibadan, Nigeria, pp. 624–636.

Valle R.R., De Almeida A-A. F., De O., Leite R.M. (1990) Energy costs of flowering, fruiting, and cherelle wilt in cacao. *Tree Physiology,* **6**, 329–336.

Vogel M. (1975) Recherche du déterminisme du rythme de croissance du cacaoyer. *Café Cacao Thé,* **19**, 265–290.

Wood G.A.R., Lass R.A. (1985) *Cocoa*, 4th edn. New York: Longman Scientific and Technical. 620 pp.