Phenology of Flowering and Pod Maturity on Some Cocoa (*Theobroma cacao* L.) Clones

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Abstract

Phenology of flowering is an important aspect in the growth cycle of cocoa (*Theobroma cacao* L.) because the performance of plant especially flowering is linked with anthesis time, time and duration of stigma receptivity, fruit formation, crossing, and seed development which determine the plant breeding programs. Research was conducted at the Kaliwining experimental station of Indonesian Coffee and Cocoa Research Institute (ICCRI). The cocoa plant was originated from top grafting seedling with the age of eight years. Design of experiment was randomized complete block design consisted of eight clones as treatment with three replications. Each plot consisted of 20 sample flowers. The parameters observed were phenology of flowering and pod maturity. Phenology of flowering observed were age of flowering, number of opened flower buds, number of flower buds that dropped before being opened, number of flower buds that dropped after being opened, and number of opening flowers that developed into tiny fruits. Observation on pod and been quality was done on pod size, pod weight, pod length, pod girth, and bean number. The result of experiment showed that difference in phenology of flowering and age of pod maturity existed within eight cocoa clones tested. KKM 22 showed the shortest flowering age and pod maturity, and lowest number of beans. The highest success of pollination was observed on Sca 6 and KW 617. ICCRI 03, Sulawesi 03, ICCRI 07, KKM 22 and KW 617 showed relative low success of pollination, that is was between 10 to 17.6%. Age of flower opening did not significantly influence age of pod maturity, on the other hand it did not negatively influence percentage of flower opening and percentage of pollinated flowers. The characteristic of flowering age had high genetic coefficient of variation as well as high estimated value of heritability. Percentage number of flower buds that dropped before open showed high genotypic and phenotypic coefficient of variation, however the estimated heritability was grouped in low category. The characteristic of pod maturity age, percentage of flower opening and bean number in each pod had low values of genotypic and phenotypic coefficient of variation, and low heritability.

Keywords: phenology, flower opening age, pod maturity, *Theobroma cacao* L.

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a cauliforous plant and the flowers of which are formed on flower cushion at the stem or branch (Young, 1994). From morphological and physiological point of view cocoa flower is hermaphrodite in which each flower has male and female reproduction organ (Young, 1994). The flower emerge when the plant has reached three to five years old. The top flowering occurs at night when the temperature is not more than 27^{O}C. The flower opens in the afternoon, and in the next morning it will fully open. Only 1-5% of total number of flowers will be pollinated and develop into fruits (Young, 1994).

The phenology of flowering is an important aspect in the plant growth cycle of cocoa because during that phase early process of plant propagation takes place (Yulia, 2007). Each plant has different characteristic of flowering and fruiting, however generally it is preceded by flower bud emergence and ended by pod maturity (Tabla & Vargas, 2004). Phenology of plant is the change of vegetative phase to generative phase and the length of generative phase of plant such as flowering, seed formation, and harvesting time (Sitompul & Guritno, 1995). Phenology of flowering and fruit maturity varied between species (Rauf *et al*., 2011; Kukade & Tidke, 2013).

Different age of flowering is presumed to be closely related with flower receptivity in cocoa plant. The difference in receptivity between flowers will cause failure of pollination. Determination of clone composition planted in an area influence the success of pollination. In general receptive time of cocoa flower is about 48 hours and if pollination is fail the flower will fall. Failure in pollination happen because there is no fusion between male and female gametes (Baker *et al*., 1997). Information on flowering is needed before determining composition of clones to be planted because flowering is related to receptivity of male and female flowers. Beside phenology of flowering, pod maturity is also important aspect in relation to harvesting management especially for the plant to be used as seed source. Nurtjahjaningsih *et al*. (2012) reported that parameter of flowering which tend to be not simultaneous might influence the quantity and quality of bean produced.

MATERIALS AND METHODS

Research was done at Kaliwining experimental station of Indonesian Coffee and Cocoa Research Institute (ICCRI) located 45 m asl., soil type of low humic gley, soil texture silty clay loam, and climate type D based on Schmidt-Ferguson classification. The cocoa plant was originated from top grafting of seedlings at the age of eight years. Plant management was conducted according to the standard of cocoa plantation in ICCRI. Design of experiment was randomized complete block design consisted of eight clones as treatment with three replications. Each plot consisted of 20 sample flowers.

The parameters observed were phenology of flowering and pod maturity. Phenology of flowering observed were flowering age, number of opened flower buds, number of flower buds that dropped before being opened, number of flower buds that dropped after being opened, and number of opened flowers developed into tiny fruits. Observation on pod and bean quality was done on pod size, pod weight, pod length, pod girth, and bean number. Age of flowering was counted from time between emergence of flower bud up to the flower opened. Age of pod maturity was observed since pollination up to the pod was physiologically mature. Observation on age of flowering as well as pod maturity was conducted every two weeks.

Table 1. Analysis of variance

Source of variation	Degree of freedom	Mean square	Expected mean square
Replication (r)	r-1	MS Block	ó^2_e + gó^2_r
Clone (r)	g-1	MS Genotype	ó^2_e + ró^2_g
Error	(r-1) (g-1)	MS Error	ó^2_e

The data was analyzed using SAS 9.1 programme.

Genotypic variance (σ^2_g) =

$$\frac{\text{MS genotype – MS error}}{\text{Replication (r)}}$$

Phenotypic variance (σ^2_p) = $\sigma^2_g + \sigma^2_e$

Estimated the broad heritability (h^2) = σ^2_g / σ^2_p

Genotypic variance, phenotypic variance, genotypic coefficient of variation, and phenotypic coefficient of variation were analyzed using Excell program based on methods of analysis developed by Singh & Chaudary (1979) as followed:

Phenotypic coefficient of variation:

$$CVP = \sqrt{(\sigma^2_p)X \text{ x } 100\%}$$

Genotypic coefficient of variation:

$$CVG = \sqrt{(\sigma^2_g)X \text{ x } 100\%}$$

Notes:
s^2_p = phenotypic variance
s^2_g = genotypic variance
X = general mean

According to Knight (1979) genotypic variance was classified as follows:

- High (σ^2_g > 14.5%)
- Medium (5% < σ^2_g < 14.5%)
- Low (σ^2_g < 5%)

According to Qosim *et al.* (2000) phenotypic variance was classified as follows:

- High (σ^2_p > 50%)
- Medium (25% < σ^2_p < 50%)
- Low (0 < σ^2_p < 5%)

Classification of heritability value was based on Mc.Whiter (1979):

- High H > 50%
- Medium 20% < H < 50%
- Low H < 20%

RESULTS AND DISCUSSION

Results of analysis showed that there was difference in phenology of flowering between eight cocoa clone tested. KKM 22 showed shortest flowering age as well pod maturity compared to the other clones (Table 2). Meanwhile Cheesman (1932) reported that flower opened at the same time and the same intensity. Flower opening of cocoa took place within the period of 12 hours, beginning at the afternoon with opening of sepala which lasted until night time. Flower will fully open in the morning with formation of pollen and mature pistil (Swanson, 2005). Phenology of flowering was an important aspect that influenced the success of pollination, pod and seed formation (Kukade & Tidke, 2013), time of anthesis, time and duration of stigma receptivity, success of fruiting, and seed development (Rout *et al.*, 2009), which support to the breeding programe (Nurtjahjaningsih *et al.*, 2012). The occurrence of variation of flower, pod and seed formation was probably due to plant genetic factor (Nelsonnavamaniraj, 2005) and the specific condition of environment (Dhillon *et al.*, 2009). According to Divakara *et al.* (2010), the hight genetic variation occurred on seed and pod maturity charactersistic, bean size, and pod colour. Results of research in other commodity showed that there was variation of time length between flower initiation until flower opening which was influenced by plant growth pattern and climate variation in the growing location of plant (Sedgley & Griffin, 1989 *cit*. Baskorowati *et al*., 2008). The same result of research was also presented by Srimathi *et al*. (2013) in *Pongamia pinnata*.

Sca 6 showed the highest percentage of flower bud death (35.6%) with lowest percentage of flower opening (64.4%). On the other hand, KW 516 showed the highest percentage of flower blooming up to 97.6%. The highest success of pollination was observed in Sca 6 (31.7%) and KW 617 (30.6%) and different significantly to ICCRI 03 the pollination success of which was only 10% from total of the flowers observed. ICCRI 03, Sulawesi 03, ICCRI 07, KKM 22, and KW 516 showed high failure of pollination with level of success of only 10–17.56% (Table 2). Only 1–5% from total number of flowers will be pollinated and developed into fruits (Young, 1994). In self incompatible genotypes, egg cell which could be fertilized was only 0–75%. KKM 22 had partially self compatibility with formation of young pod that further fall in the second week (Susilo, 2006). Fertilization in cocoa plant required 7–8 hours (Purseglove, 1968), 12–20 hours (Baker & Hasenstein, 2000) after pollination, therefore the fallen flower performed failure of fertilization.

Beside the specific character of cocoa as cross pollinated plant, the compatibility between flowers also influence the success of pollination and fertilization. In addition, pattern of pollen spreading seems to determine the effectiveness of population number and level of genetic variance in and between population (Nurtjahjaningsih *et al*., 2012). Phenology of flowering such as flowering ability, number of flower, compatibility of maturity between male and female flower seems to determine that pattern of spreading (Robledo-Amuncio, 2004). Phenology of flowering is controlled by gene for controlling synthesis of flowering hormone and phytohormone (Burczyk & Chalupka, 1997).

Age of flowering did not significantly influence age of pod maturity (Table 3). Age of flowering inflluenced pod and bean formation (Kukade & Tidke, 2013; Divakara *et al*., 2010). On the other hand, age of flowering age did not have significant negative influence on percentage number of flowers opened and percentage of flowers pollinated. Length of time the flowers opened seems to inhibit maturity of pistil and pollen so that the number of flowers pollinated was lower. Difference of anthesis time and receptivity of male flower and female flower much influence the low success of pollination.

Table 2. Characters of flowering and pod maturity on eight cocoa clones

Clones	Flower blooming time, day	Pod maturity, week	Percentage of flower buds mortality, %	Percentage of flowers blooming, %	Percentage of pollinated flowers, %
ICCRI 03	9.4 a	19.3 a	27.3 ab	72.7 ab	10.0 b
SCA 6	8.3 ab	17.4 ab	35.6 a	64.4 b	31.7 a
Sulawesi 3	9.3 a	17.3 ab	22.8 ab	77.2 ab	17.6 ab
ICCRI 7	10.7 a	17.4 ab	23.3 ab	76.7 ab	16.7 ab
KKM 22	6.0 b	16.9 b	11.7 ab	88.3 ab	16.7 ab
KW 516	6.5 b	17.1 ab	2.4 b	97.6 a	23.8 ab
KW 617	6.5 b	19.0 ab	14.4 ab	85.6 ab	30.6 a
TSH 858	6.7 b	18.0 ab	26.7 ab	73.3 ab	17.3 ab

Note: Number within the column by the same letter are not significantly different at 5% level according to Duncan multiple range test (DMRT).

Tabel 3. Correlation of some flowering characters and pod maturity on cocoa

Characters	Correlation, r	T-value	T-table	Note
Opened flower age vs mature pod age	0.03	0.14	2.06	NS
Opened flower age vs opened flower number percentage	-0.027	-0.14	2.06	NS
Opened flower age vs pollinated flower number percentage	-0.34	-1.8	2.06	NS
Mature pod age vs opened flower number percentage	-0.32	-1.6	2.06	NS
Mature pod age vs pollinated flower number percentage	-0.16	-0.77	2.06	NS
Opened flower mature percenntage vs pollinated flower number percentage	0.11	0.56	2.06	NS

Note: NS (Not significant).

Meanwhile, percentage of number of flowers opened did not have significant positive correlation to the percentage of number of flowers pollinated. The higher the number of flowers opened, the higher the chance of occurrence of pollination between flowers either in or between plants. The existence of relationship between that parameter provide more easy selection, because for conducting selection of a certain characteristic it could be done by using other characteristic which was more easily observed. Pomper *et al.* (2008) mentioned that variation of flowering character and pod maturity existed between *Asimina triloba* L. cultivar where flowering period and number of flower did not have relationship with increased number of pods.

Variation of pod size and number of beans per pod existed in all of the tested clone (Table 4). TSH 858 and KW 516 showed the greatest pod size and bean. On the contrary Sca 6 showed smallest pod size and bean. The lowest number of beans was observed in KKM 22 with an average 35 number of beans per pod. Number and quality of flower determined production or yield (Hanke *et al.*, 2007). KKM 22 which had shortest flowering age and pod maturity showed small size of pod and low number of beans or not optimum.

Results of analysis on genotypic and phenotypic variance coefficients (CVG and CVP) showed that flowering age had high coefficient i.e. 19.35 with high estimated heritability (H) i.e. 57.91%. High estimated heritability indicated that genetic factor was more important in determining variance of a character compared to environmental factor, this value determined progress of selection (Hadiati *et al*., 2003). Number of timer bud that dropped before being open showed high CVG and CVP, but estimated heritability was included in low category. The pod maturity age, percentage of opened flower, and number of bean per pod had low CVG and CVP as well as low H (Table 5) so that those characters were more influenced by environmental factor than by genetic factor. Breed heritability estimation showed that those characters were easily inherited, therefore the selection activity could be done in early generation (Hadiati *et al*., 2003). Heritability value could be used as a basic in simultaneous selection (Nasution, 2010).

Phenotypic expression was reflexion of a genotype in a certain environment (Bartley, 2005). Level of variation showed level of difference of each individual in a population. The higher of variation in a population means the higher heterogenity in the population and the easier to make improvement by means of plant breeding. The broad genetic variability had higher chance in selection for the best character compared to the characters having narrower genetical variability. Mangoendidjojo (2003) mentioned that the broad genetical variability had different genetic background, therefore if selection was done it could increase genetic advance

Table 4. Characteristics of pod quality and beans number per pod on eight cocoa clones

Clones	Pod length, cm	Pod girth, cm	Pod weight, g	Wet bean weight per pod, g	Number of beans
ICCRI 03	18.83 bc	24.83 b	267.2 cd	80.66 cd	40.67 ab
Sca 6	16.07 bc	20.77 c	218.7 d	59.14 d	41.66 ab
Sulawesi 03	16.00 c	25.00 b	332.5 cd	87.70 bcd	46.00 a
ICCRI 07	19.67 ab	26.83 ab	480.3 ab	108.59 abc	46.33 a
KKM 22	17.83 bc	25.33 b	376.7 bc	99.69 abc	35.00 b
KW 516	21.67 a	29.60 a	575.8 a	130.90 a	46.66 a
KW 617	20.33 ab	27.50 ab	490.5 ab	119.32 ab	44.66 ab
TSH 858	20.85 ab	30.10 a	601.9 a	120.37 a	41.67 ab

Note: Numbers within the same column followed by the same letter are not significantly different at 5% level according to Duncan test.

Table 5. Genotypic variation coefficient (GVC), phenotypic variation coefficient (PVC) and broad heritabilty estimation (H) for some flowering characters, pod maturity and bean number

Characters	CVG, %	CVP, %	Heritability, %
Opened flower age	19.35 (high)	25.43 (moderate)	57.91 (high)
Mature pod age	3.22 (low)	11.81 (low)	7.45 (low)
Bean number per pod	6.47 (low)	12.98 (low)	24.83 (moderate)
Dead flower bud percentage	22.52 (high)	82.47 (high)	7.46 (low)
Opened flower percentage	5.81 (low)	21.30 (low)	7.44 (low)
Pollinated flower percentage	13.41 (low)	42.59 (high)	9.92 (low)

and gave more chance to produce the genotype wanted. On the contrary, the selection would not be successful if the character had narrow genotypic variability although it had broad phenotypic variability, because the expression of phenotypical difference was due to environmental effect, so that the selection did not have the same effectiveness if being done in character with high genotypic and phenotypic variabilities.

CONCLUSION

1. KKM 22 showed the shortest age of flowering and pod maturity, small pod size and small number of beans.
2. The highest success of pollination was observed in Sca 6 and KW 617 clones. ICCRI 03, Sulawesi 03, ICCRI 07, KKM 22 and KW 516 showed relative low success of pollination.
3. Age of flowering did not have significant positive effect on age of pod maturity, on the contrary age of flowering did not have significant negative effect on percentage of number of opened flower and percentage of flower pollinated.

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REFFERENCES

Baker, R. Paul & K.H. Hasenstein (1997). Hormonal changes after compatible and incompatible pollination in *Theobroma cacao* L. *Horticultural Science*, 32, 1231-1234.

Burczyk, J. & W. Chalupka (1997). Flowering and cone production variability and its effect on parental balance in a Scots pine clonal seed orchard. *Annual Science Forest*, 54, 129-144.

Bartley, B.G.D. (2005). *The Genetic Diversity of Cacao and Its Utilization*. Wallingford, UK: CABI Publishing.

Baskorowati, L.; R. Umiyati; N. Kartikawati; A. Rimbawanto & M. Susanto (2008). Pembungaan dan pembuahan *Melaleuca cajuputi* subsp. cajuputi Powell di kebun benih semai Paliyan, Gunung Kidul, Yogyakarta. *Jurnal Pemuliaan Tanaman Hutan*, 2, 189–202.

Cheesman, E.E. (1932) The economic botany of cacao. *Tropical Agriculture*, 9, 1–16.

Cope, F.W. (1962). The mechanism of pollen incompatibility in *Theobroma cacao*. *Heredity*, 17, 157–182.

Dhillon, R.S.; M.S. Hooda; K.S. Ahlawat & S. Kumari (2009). Floral biology and breeding behaviour in karanj (*Pongamia pinnata* l. Pierre). *Indian Forester*, 135, 618–628.

Divakara, B.N.; A.S. Alur & S. Tripati (2010). Genetic variability and relationship of pod and seed traits in *Pongamia Pinnata* (L.) Pierre., a potential agroforestry tree. *International Journal Plant Production*, 4, 1735–8043.

Hadiati, S.; H.K. Murdaningsih; A. Baihaki & N. Rostini (2003). Parameter genetik karakter komponen buah pada beberapa aksesi nanas. *Zuriat*, 14, 47–52.

Hanke, M.V.; H. Flachowsky; A. Peil & C. Hattasch (2007). No flower no fruit-genetic potentials to trigger flowering in fruiting trees. *Genes, Genomes and Genomics*, 2, 16.

Knight, R. (1979). *Quantitative Statistics and Plant Breeding*. ***In:*** R. Knight (Ed.) Plant Breeding. Brisbane Australia.

Kukade S.A. & J. Tidke (2013). Studies on pollination and reproductive biology of *Pongamia pinnata* L. (Fabaceae). *Indian Journal of Fundamental Applied Life Science*, 3, 149–155.

Mangoendidjojo, W. (2003). *Dasar-dasar Pemuliaan Tanaman*. Kanisius. Yogyakarta.

Mc.Whiter, R.S. (1979). *Breeding of Cross Pollination Crop*. ***In:*** R. Knight (Ed.) Plant Breeding. Brisbane Australia.

Nasution, M.A. (2010). Analisis korelasi dan sidik lintas antara karakter morfologi dan komponen buah tanaman nenas (*Ananas comosus* L. merr). *Crop Agro*, 3, 1–8.

Nelsonnavamaniraj, K. (2005). *Studies on Phenology, Seed Collection and Post-Harvest Seed Management Techniques for Production of Quality Planting Stock in* Bixa orellana. *Thesi*s. Tamil Nadu Agricultural University, Coimbatore, India.

Nurtjahjaningsih, P.; A.Y.P. Sulistyawati; B.C. Widyatmoko & A. Rimbawanto (2012). Karakteristik pembungaan dan sistem perkawinan nyamplung (*Calophyllum inophyllum*) pada hutan tanaman di Watusipat, Gunung Kidul. *Jurnal Pemuliaan Tanaman Hutan*, 6, 65–80.

Purseglove, J.W. (1968). *Tropical Crops Dicotyledons*. Longman, London.

Qosim, W.A.; A. Karuniawan; B. Marwoto & D.S. Badriah (2000). Stabilitas parameter genetik mutan-mutan krisan generasi VM3. *Laporan Hasil Penelitian Lembaga Penelitian Universitas Padjajaran*. Jatinangor. 53p.

Rauf, S.S.; S.S. Narkhede; A.D. Rane & R.P. Gunaga (2011). Seed and fruit variability in *Pongamia pinnata* (L.) Pierre from Konkan Region of Maharashtra. *Journal Biodiversity*, 2, 27–30.

Robledo-Arnuncio; J.J. Alia & R. Gil (2004). Increased selfing and correlated paternity in a small population of apredominantly outcrossing conifer, *Pinus sylvestris*. *Molecular Ecology*, 13, 2567–2577.

Sitompul, S.M. & B. Guritno (1995). *Analisis Pertumbuhan Tanaman*. UGM Press: Yogyakarta.

Srimathi; N. Mariappan; L. Sundaramoorthy & K. Sudhakar (2013). Studies on floral phenology, fruit and seed maturation and harvest index based on fruit colour in *Pongamia pinnata* (L.) Pierre. *African Journal of Plant Science*, 7, 513–520.

Susilo, A.W. (2006). Kemampuan menyerbuk sendiri beberapa klon kakao (*Theobroma cacao* L.). *Pelita Perkebunan*, 22, 159–167.

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. Thesis*. The Pennsylvania State University, USA.

Tabla, P.V. & C.F. Vargas (2004). Phenology and phenotypic natural selection on the flowering time of a deceit pollinated tropical orchid, *Myrmecophila christinae. Annal of Botany,* 94, 243–250.

Young, A.M. (1994). *The Chocolate Tree: A Natural History of Cacao*. Smithsonian Institution Press, Washington and London.

Yulia, N.D. (2007). Kajian fenologi fase pembungaan dan pembuahan *Paphiopedilum glaucophyllum*, J.J. Sm. var. *Glaucophyllum. Biodiversitas*, 8, 58–62.

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