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A review of the role of pollination on the yield of cocoa plant

Abstract. Yield of cocoa (*Theobroma cacao* L.) plant, in form of cocoa seeds, is produced through the process of sexual reproduction. Pollination plays an important role in the successful formation of cocoa fruit and seeds. In addition, number of ovules per ovary, degree of self-incompatibility, and xenia effect are also affected the final yield. Those characters are determined by genetic factors. Cocoa flowers that are not pollinated fall within 24 hours and fail to develop into fruits and seeds. Pollination intensity up to a certain level showed a significant effect on increasing the percentage of fruit formation and the number of seeds per fruit. The role of pollinating insects, especially from the Ceratopogonidae family, is very important in dispersing pollen grains, allowing the natural pollination process to be occurred.

Keywords: Fertilization · Pollinator · Xenia · Meta-xenia

Review peran penyerbukan pada hasil tanaman kakao

Sari. Hasil tanaman kakao (*Theobroma cacao* L.) adalah berupa biji yang dihasilkan melalui proses reproduksi seksual. Polinasi memegang peranan penting dalam keberhasilan pembentukan buah dan biji tanaman kakao. Selain itu, hasil akhir tanaman kakao juga dipengaruhi oleh karakter jumlah ovul per ovarium, tingkat inkompatibilitas sendiri, dan efek xenia. Karakter-karakter tersebut dikendalikan secara genetik. Bunga kakao yang tidak diserbuki akan gugur dalam waktu 24 jam dan gagal berkembang menjadi buah dan biji. Intensitas polinasi hingga level tertentu menunjukkan pengaruh yang nyata terhadap peningkatan persentase pembentukan buah dan jumlah biji per buah. Peran serangga penyerbuk, terutama dari famili Ceratopogonidae, sangat penting dalam menyebarkan polen sehingga proses polinasi secara alami dapat terjadi.

Kata kunci: Pembuahan · Polinator · Xenia · Meta-xenia

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Introduction

The main products of cocoa plants, as well as coffee and oil palm, are generative organs, namely fruits (pods) and seeds (beans). Cocoa productivity is described as dry bean weight (usually in tons) produced per acreage unit of land. Fruit production and fruit value/fruit index (pod value/pod index) are the two main parameters of cocoa yields. The pod value is determined by the number of beans per pods and dry beans weight (Tan, 1990) and it used to estimate the number of pods required to obtain 1 kg of dry cocoa beans (Elisabeth, 2009; García-Alamilla *et al.*, 2012).

From the description above, it can be concluded that in order to increase the productivity of cocoa plants, three main components must be improved, namely the number of ripe pods, the number of beans per pod, and the weight of the beans (Lopes *et al.*, 2011). According to Adewale *et al.* (2014), increasing the number of beans per pod and weight of beans has indeed become one of the main objectives of the current cocoa plant breeding program. Genetically, the two traits are known to be independently behave (Cilas *et al.*, 2010). However, genetic approach only involves potential improvement of those two traits, their actual performance in the field is often influenced by other genetic traits as well as environmental factors. For example, even though a genotype has a high potential for the number of seeds per fruit, if pollination does not occur during flowering, this potential will not be realized. This paper aimed to review genetic and non-genetic factors determining the successful pollination of cocoa plant.

Floral Biology of Cocoa Plant

History of the development of cocoa beans and pods begins with the appearance of flower buds on the bark or branches of cocoa plants. Flower buds grow and mature, marked by the start of rupture of the flower crown which continues overnight. In early hours of the next day cacao flowers were in full bloom and the anthers consisting of four stamens began to burst (Berbiye, 2014). Flowering in cocoa plants is influenced by external factors such as rainfall, temperature, and light. Among these three factors, rainfall is the most critical factor in cacao flowering phenology because it is responsible for variations in the number of mature buds and blooms by 78% and 75%, respectively (Adjaloo *et al.*, 2012).

Cocoa flowers are bisexsual, because it has both anther and pistil, aside the floral ornaments. Cocoa plants can produce up to thousands of flowers in one flowering period, but only a small percentage (1-5%) are successfully fertilized and develop into fruit sets. Flowers that are not fertilized will fall within 24 hours (Berbiye, 2014). Although it has been reported that the phenomenon of parthenocarpy in cocoa plant, developing pod is seedless, hence, it does not affect the yield (Menteiro *et al.*, 2009). In addition, there is no evidence that ovule in the ovary of cocoa plant can be developed apomictic seeds (Glendining, 1972). This fact indicates that pollination is an absolutely necessary factor in the process of fruit and seed formation in cocoa plants.

Genetic Effect of Pollen

Number of ovules per ovary in cocoa plants is highly variable as it is controlled by many genes (polygenic) (Bahia *et al.*, 2013). In Trinitario and upper Amazon Forastero groups the number is ranging between 30-40 and 50-65, respectively (Clement *et al.*, 2003). The Peruvian clone P 18, which belongs to the upper Amazon Forestero group, is even known to have up to 74 ovules per ovary and is thought to have a maximum of 80 ovules per ovary (Bahia *et al.*, 2013). Ovule number per ovary showed high inheritance ($h_b^2 = 0.810$), however, it is not be a good predictor of number of cocoa beans per pod (Cilas *et al.*, 2010). Number of ovules per ovary is a potential character, while its development into seeds is influenced by agroecological factors, including pollination intensity (Bahia *et al.*, 2013).

Based on the type of pollination, there are two groups of cacao plants, namely self-pollinating compatible group (SC) and self-incompatible (SI) group (Wahyudi *et al.*, 2008). In the SI group, although pollination has occurred in self-pollination mechanism, it will not be followed by the fusion of male and female gametes to form a zygote. Self-incompatibility mechanism in cocoa is unique because it occurs after sperm cell nucleus penetrates the embryo sac, to make late-acting self-incompatibility (LSI) (Ford and Wilkinson, 2012). According to de Nettancourt (2013), genetically, in cocoa plants, LSI is controlled by three different loci (S, A, and B) and in nature there are only three self-incompatible types, namely:

1) Individuals who have two S alleles that have different dominance status (eg S_1S_2, because $S_1 > S_2$), when selfing occurs, 25% of them are not experience gamete fusion.
2) Plants that have different and independent S alleles (eg S_2S_3, because $S_2 = S_3$), when selfing occurs, 50% of female gametes are not fuse with male gametes.
3) The group of plants that experienced 100% failure of fusion of male gametes and female gametes at the time of selfing, namely those with identical S alleles (eg S_1S_1).

The SI phenomenon, therefore, is one of the inhibiting factors for achieving optimum bean yields on cocoa plants. Therefore, combinatorial ability among commercial clones should be high to ensure the good yield. Many studies have been carried out extensively to evaluate combinatorial ability among cocoa clones (Susilo *et al.*, 2020; López *et al.*, 2021). Meanwhile, plant breeders also continue to strive to obtain superior cocoa clones that are self-compatible (SC). Royert *et al.* (2011) have succeeded in identifying a molecular marker that is known to be strongly associated with SC traits, namely mTcCIR222, that it can be used in marker-assisted selection (MAS) programs.

Another effect of male parental pollen on female parental tissue is known as xenia or metaxenia. Although the two terms are often used, the boundaries between the two seem confusing. The definition of metaxenia is the influence of parental pollen on the seed or fruit tissue of plants outside the embryo and endosperm associated with hormones produced by the embryo and endosperm after double fertilization (double fertilization). Denney and Martin (1990) defined that xenia is applied to the phenomenon exhibited by the fusion of the gametes in the ovule, namely the embryo and the endosperm, while metaxenia refers to the influence on other tissues of the plant outside of the two. According to Denney (1992) this limitation is only hypothetical and not supported by the availability of adequate data.

On the other hand, Olfati *et al.* (2010) made different definitions for both terms. Xenia is defined as the influence of genes from male parents on fruit and seed development, while metaxenia is the influence of pollen on fruit shape and other fruit characteristics. Piotto *et al.* (2013) also refers to another limitation, namely that metaxenia is a more specific part or case of xenia. Thus, the term xenia can be used in general terms, including the phenomenon of metaxenia. Apart from the polemic regarding the boundaries between the terms; xenia and metaxenia above, this phenomenon can be used to identify male elders as the best source of pollen that can increase the quantity and quality of yields on various types of plants.

Indications of the effect of xenia on cocoa plants of fine type (white seed color) and bulk (purple seed color) were shown by the character of seed color, fruit weight, length, fruit circumference, and seed weight (Anita-Sari and Susilo, 2011). The color of fine cacao beans changes from purplish white to dark purple when pollinated by bulk cacao (Figure 1). However, no indication of xenia effect on most of the important taste attributes, namely cocoa aroma, acidity, fruity aroma, and floral aroma (Sukha, 2008).

Xenia/metaxenia effect is also known in other plant species, such as in vanilla (*Vanilla planifolia*) to affec fruit pod size (Sasikumar, 2010). It also found to increase pilosity and reduce seed weight in two tomato cultivars cultivated with *Solanum lycopersicum* (Piotto *et al.*, 2013), increasing the nutrient and starch content of corn kernels (Sulewska *et al.*, 2014), reducing the percentage of empty seeds in hazelnuts (Fattahi *et al.*, 2014), increasing the quantity and quality of yield in grapes, (Sabir, 2015). The metaxenia effect has a potential to be used in regulating fruit ripening time, such as in date palms (*Phoenix dactylifera* L.) (Al-Khalifah, 2006), as well as potential could be used as a marker to check the combination of crosses (Tsuda *et al.*, 2011).

Figure 1. Example of xenia indication in noble type cocoa (DRC 16 clone). Beans were harvested from populations far from lindak-type cocoa (left) and from populations close to lindak-type cocoa (right).

Liu (2008) reviews various theories regarding the mechanism of xenia/metaxenia in plants. The first theory is the secretion of hormones, or other analogous dissolved substances, from the male parent tissue that diffuses into the female parent tissue. The role of transposons in the xenia phenomenon is the second theory. The third theory relates to endogenous mRNA which is believed to be able to move along the intercellular pathway to determine gene expression in distant tissues.

Abbas *et al.* (2012) supported the first theory by concluding that the effect of pollen on date fruit development could be related to the level of endogenous gibberellin content. In maize, although the effect of xenia on the chemical composition of seeds has been proven and explained, its effect on grain production and grain count is not clearly known (Bosinovic *et al.*, 2012). A microRNA (miRNA) could regulate the kernel size of almond (*Prunus dulcis* [Mill.] D. A. Webb). Distinct pollen donor could generate different hormonal signaling and metabolic pathways related to miRNAs (Jafari *et al.*, 2021).

Role of Pollinating Insects

Falque *et al.* (1996) translated the intensity of pollination (IP) as the number of pollen grains attached to the stigma after pollination process. Groeneveld *et al.* (2010) defined it as the percentage of flowers pollinated. Based on these two definitions, it can be seen that there are two variables that determine the success of pollination, namely the number of pollen grains and the percentage of flowers pollinated. These two variables determine the number of fruits and the number of seeds per fruit produced in each individual plant.

The experimental results of Falque *et al.* (1996) also showed an indication of a strong positive relationship between IP and the number of beans per pod. It takes 238 pollen grains to have the number of beans per pod close to the maximum. Ratio of pollen count to number of ovules per ovary is 3.8 (it takes on average 4 pollen grains to fertilize one ovule). However, these results were obtained based on the artificial pollination technique (hand pollination), thus they cannot describe natural pollination.

Cocoa flowers have a structure that makes it difficult for natural pollination to occur because the fertile stamens are blocked by sterile stamen structures (staminodia). In addition, the cacao flower does not emit a nectar aroma and the pollen grains are sticky. Natural pollination is only possible by insects that creep into the flower structure (Nair, 2010). Based on these facts, the cacao flower, as with its relatives in the *Theobroma* genus, can be classified into the entomophilous type (Santos *et al.*, 2012). It means that successful cocoa pollination as well as final harvested yield is only depended on insects. Insects belonging to the family of Ceratopogonidae (Diptera) are considered the most effective as pollinators, therefore they have a role in determining the productivity of cocoa plants (Adjaloo and Oduro, 2013). Despite their very small size (0.5–2 mm), these insects are known to be able to spread pollen within a radius of > 3 km (de Schawe *et al.*, 2013).

One species that resembles a mosquito, *Forcipomyia* spp., is known to play a role in spreading cocoa plant pollen, both over short and long distances (Silva *et al.*, 2011). The appearance of one species, namely *F. hardyi* as shown in Figure 2. The sticky pollen grains of the cocoa plant can stick to the side of the insect's thorax (O'Doherty and Zoll, 2012). Only the female insect has a role as cacao pollinator (Saripah, 2013). An average of 60 pollen grains per mm^3 were successfully pollinated by these insects on the stigma of cacao flower (Adjaloo and Oduro, 2013).

The intensity of natural pollination in cocoa plants is determined by the abundance of pollinating insect populations (Adjaloo *et al.*, 2013). The insect population is generally abundant in the rainy season and reduced in the dry season (Frimpong-Anin *et al.*, 2014). In addition, their visitation are more active in the morning compared to in the afternoon (Nugroho *et al.*, 2019). However, no positive correlation between *Forcipomyia* spp. population and microclimate condition (relative humidity, light intensity, and temperature) (Zakariyya *et al.*, 2016). The abundance of pollinating insects was also positively correlated with the proportion of intercropping with banana plants. Thus, the intercropping pattern of cocoa and banana is recommended to increase fruit set (Frimpong *et al.*, 2011). The rotting banana pseudo stem can provide a substrate and become a good habitat for ceratopogonid insects to breed (Adjaloo, 2012).

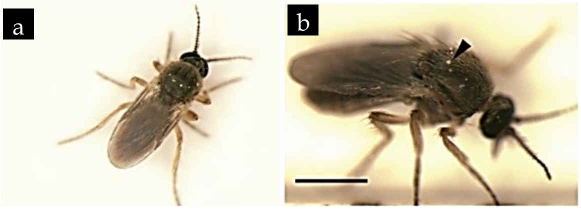

Figure 2. Appearance of the *Forcipomyia hardyi*: back view (a) and side view with attached pollen grains (arrows). The scale line on the image is 1 mm (O'Doherty and Zoll, 2012).

Conclusion

Cocoa yields are determined by the ability to produce a large number of fruits and seeds and high bean weight. Pollination is a step that must be passed for required for the formation of fruit and seeds. The success of pollination and fertilization in cocoa plants is influenced by genetic and non-genetic factors, especially the type and abundance of pollinating insects. Therefore, to optimize the yield of cocoa plants, it is necessary to select high yielding varieties with high yielding potential and suitable environmental conditions for the growth and development of pollinating insects.

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