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FLORAL PHENOLOGY AND MORPHOMETRIC ANALYSIS OF THREE COMMERCIALLY GROWN *COFFEA* SPECIES

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SUMMARY

Coffea arabica, *C. canephora*, and *C. liberica's* coexistence in one location or proximity areas could promote interspecific pollination. However, information related to floral phenology and flower structure of these three coffee species is insufficient. This research aimed to identify the divergence of flowering phenology and flower morphometrics among *C. arabica*, *C. canephora*, and *C. liberica*. Flowering phenology observations continued daily from July to October 2020, extending to two locations of parapatric populations of three coffee species. Flower morphometric measurements ensued at the peak period of flowering. The results showed some co-anthesis periods among the three coffee species, with *C. liberica* starting to flower early. Moreover, *C. liberica* var *liberica* had the broadest flower diameter and the highest petal number, whereas the tetraploid *C. arabica* had the lowest. Consequences of flowering phenology and flower structure on a pre-zygotic barrier of spontaneous, as well as, controlled interspecific hybridization among the three coffee species were the discussion focus.

Key words: *Coffea,* interspecific crossing, flowering phenology, flower structure, pre-zygotic barrier

Key findings: Co-anthesis events occurred during the peak flowering periods in sympatric or parapatric populations of three coffee species (*C. arabica*, *C. canephora*, and *C. liberica)*. Therefore, interspecific hybridization using freshly collected pollen is feasible. These findings could enrich information on a pre-zygotic barrier among *C. arabica*, *C. canephora*, and *C. liberica* species, especially in Indonesia. Breeders, in turn, could utilize the results to manage their future work on interspecific hybridization among these three coffee species.

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INTRODUCTION

Coffee is the most widely consumed crop beverage worldwide because hundreds of billions of cups of coffee are consumed yearly (Gosalvitr *et al*., 2023). The global economic value of coffee claims to rank second after petroleum (Cure *et al*., 2020). On the other hand, the sustainability of the world's coffee supply faces various challenges related to global climate change, requiring a comprehensive mitigation strategy (Pappo *et al*., 2021).

One of the offered solutions is using new superior varieties that are more adaptive, have higher yields, and are highly resistant to major pests and diseases. Hybrid varieties are the prevailing right solution for the future. Efforts to develop hybrid varieties within and between coffee species have progressed in several coffee-producing countries, especially Indonesia. The aim is to exploit the heterosis effect and introduce genes controlling resistance traits and other essential traits from wild relatives and other species (Gimase *et al*., 2015).

Until now, records of 124 species exist in the genus *Coffea*, but only *C. arabica* (arabica coffee) and *C. canephora* (robusta coffee) have vast cultivation in all coffee-producing countries. The *C. liberica* (liberica coffee) species' development only has a much smaller scale; hence, it has no accounting in the world coffee trade (Davis *et al*., 2020). The third species' relatively wide cultivation in Southeast Asian countries included Indonesia. In some areas of coffee development in Indonesia, mixed populations of the three species can persist. Thus, there is a potential for natural intercrossing among these three coffee species.

At the center of the origin of the three coffee species, there are barriers in both spatial and temporal for natural crossing between species to happen. Spatial isolation related to the incompatibility of flowering times between species can become pre-zygotic barriers that are strong enough to hinder gene flow to continue between species. On the other hand, differences in climatic conditions, especially rainfall, and human interference in the distribution areas of the three coffee species can weaken this barrier. For example, on decades of neglected land in New Caledonia, one can find putative hybrids in mixed or adjacent populations of three coffee species (Gomez *et al*., 2016).

Mixed or adjacent populations of *C. arabica*, *C. canephora*, and *C. liberica* species are also prevalent in numerous coffee-growing areas in Indonesia. Pollen transfer from nearby species is possible in such conditions by a vector of wind and insects (Gomez *et al.*, 2023). Consequently, several spontaneous interspecific hybrids between the two interplanted *Coffea* species existed in Indonesia in the early 20th century (Cramer, 1957). However, increasing information regarding flowering phenology and morphological structure among the three coffee species requires attention. Thus, it is crucial to identify the potential for forming natural hybrids and a reference for developing strategies for artificial crosses between the three coffee species. This study aimed to analyze differences in flowering phenology and flower morphometric characters between coffee species *C. arabica*, *C. canephora*, and *C. liberica*.

MATERIALS AND METHODS

The study used genetic material in the form of populations of *Coffea arabica*, *C. canephora*, and *C. liberica* (var. *liberica* and var. *dewevrei*) growing close to each other. Populations of the three adjacent coffee species exist in two separate locations 1.6 km apart. Both are in the Pakuwon Experimental Station, Research Institute for Industrial and Beverage Crops, Sukabumi, West Java, with coordinates 6°50′00.4″S 106°44′31.6″E and 6°50′38.7″S 106°45′05.6″E (Figure 1). The study site has a rainfall pattern classification of monsoon, with one rainy and one dry season yearly (Azgha and Mukminan, 2019). During the observation period, daily rainfall ranged from zero to more than 30 mm, with an average daily temperature of 22 °C to 26 °C (Figure 2). The coffee plants' age in the two populations is

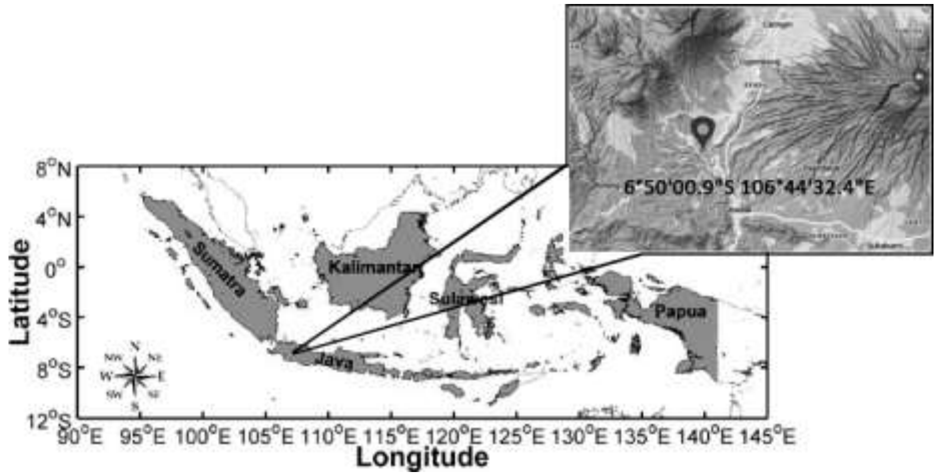

Figure 1. Location of sympatric and parapatric populations of three commercially grown *Coffea* species in the valley of two adjacent mountainous areas, Mount Salak and Gede-Pangrango, near Sukabumi District of West Java.

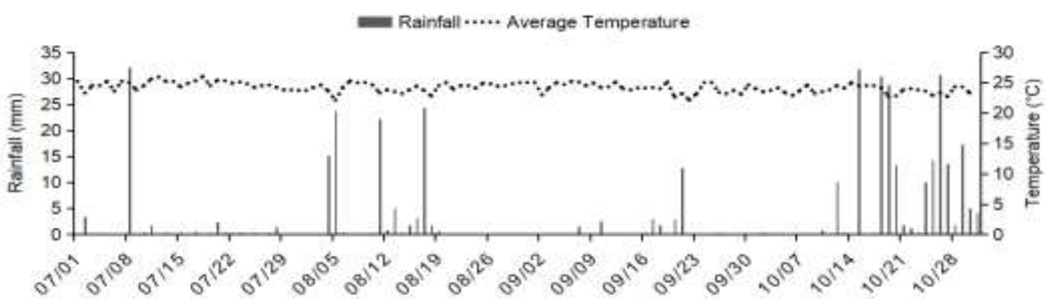

Figure 2. Graph of the rainfall pattern and the average of daily temperatures during flowering phenology observation in 2020 at Pakuwon Experimental Station, Parungkuda, Sukabumi, West Java.

relatively uniform because their planting was almost simultaneous from 2012 to 2013. Thus, in 2020, all individuals in the population were of productive age. Pruning continued to the main stem at a height of 1.60 m. The *C. liberica* is under the beneficial coconut tree (*Cocos nucifera*) shading, while *C. canephora* and *C. arabica* are under the shade of the Gamal tree (*Gliricidia sepium*).

Flowering phenology

Observing flowering phenology transpired in the months when maximum flowering took place, from July to October in 2020, for 30 sample plants per species (*C. arabica* and *C. canephora*) and sub-species (*C. liberica*) from each population. Thus, in total, 240 sample plants underwent scrutiny at the two sites.

Probing the sample plants included the frequency, amplitude, and duration of flowering in the anthesis phase. Flowering frequency is the number of anthesis flowering events during the observation period. Flowering amplitude is the average value of the number of flowers in the anthesis phase each day for the evaluation time. The flowering duration illustrates the days needed to complete the flowering of the anthesis phase in each flowering event. The obtained data incur processing with the *Microsoft Excel®* software to display a graph illustrating the flowering patterns of the three coffee species during the observation period.

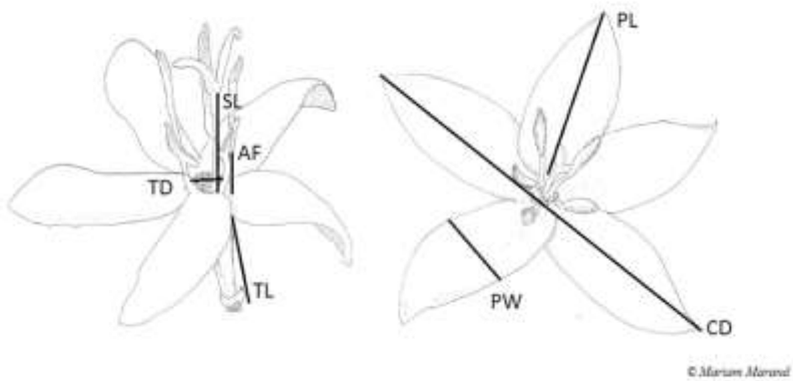

Figure 3. Scheme for measuring the quantitative characters of coffee flowers: TD = tube diameter, SL = stylus length, AF = stalk length, TL = tube length, PW = petal width, PL = petal length, and CD = crown diameter. Source: (Prado *et al*., 2019).

Flower morphology

Assessing the flower morphometric characteristics of the three coffee species went on for 30 flowers of the anthesis phase per sample plant. The observed flower morphometric variables included the petal's diameter, number, length, and width, the number, length, and width of the anthers, the diameter of the flower tube, the extent of the pistil, and the stretch of the anther. Technical examples of measurements follow, as listed in Figure 3. The observed data processing used the R Studio version 2023.06.1+524 "Mountain Hydrangea" with R version 4.3.1 packages to display a boxplot diagram and principal component analysis (PCA) that illustrates the diversity between the three coffee species.

RESULTS

Flowering phenology

The flowering frequency of the three *Coffea* species was more than two times per year in 2020. However, major flowering that displayed a massive bloom on entire individuals was only distinct once a year. A different flowering peak was noticeable between *C. liberica* and two other *Coffea* species. *C. liberica* var *liberica* and *C. liberica* var *dewevrei* bloomed at the beginning of October, whereas *C. canephora* and *C. arabica* were at the end of July and mid-August (Figure 4). On the other hand, several minor flowerings in each almost prevailed every month of the year. Slight flowering of *C. liberica* var. *liberica* and *C. liberica* var. *dewevrei* was more frequent (data not shown).

Each flowering period showed *C. liberica* blooming first, followed by *C. canephora*, with the last, *C. arabica*. This trend was similar to a previous report in New Caledonia (Gomez *et al*., 2016). However, the recording of anthesis intersection among those three coffee species was mainly during high flowering periods. The flowering intersection between *C. canephora* and *C. arabica* was the most frequent with the highest intensity compared with the other possible combinations, i.e., *C. canephora, C. liberica,* and *C. arabica* and *C. liberica*.

The arrangement of coffee flowers appeared in axillary clusters along physiologically mature plagiotropic branches, rarely borne on the main trunk. Anthesis completion of an individual flower took only a single day. However, anthesis in a single coffee plant may last more than two days, even up to five days, during high flowering periods. This phenomenon was according to asynchronous flower development among individual flowers of a single inflorescence (cluster) and among flower clusters of the same plant (Figure 5).

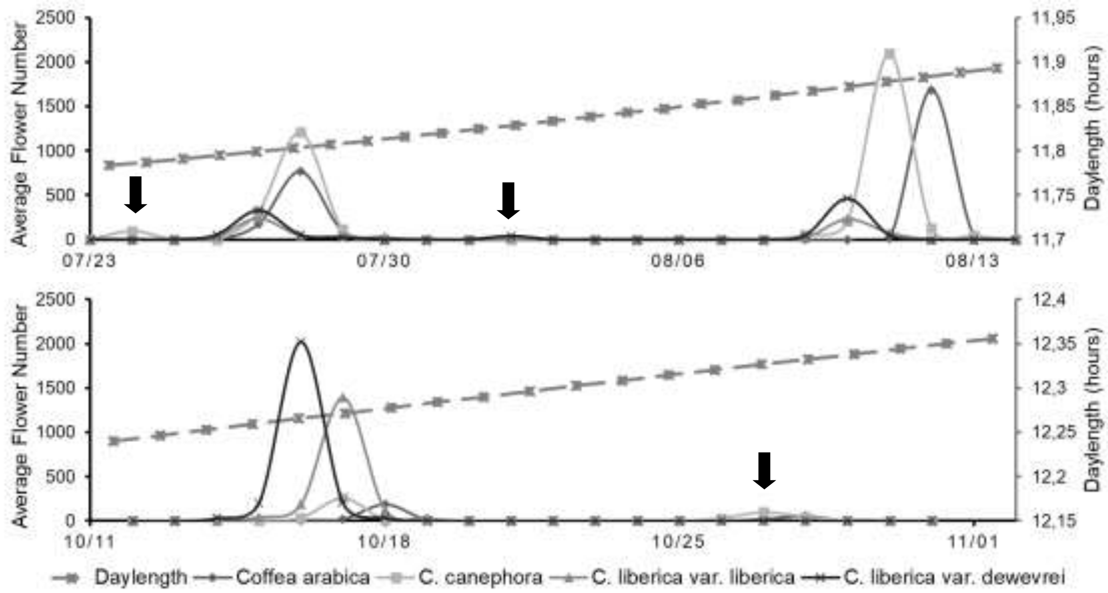

Figure 4. Schematic diagram of frequency, amplitude, and duration of flower anthesis of three cultivated coffee species during a major flowering period of 2020. Arrow pointed out a minor flowering sporadically happens in successive months of the year.

Figure 5. Asynchronous flower development observed at a single inflorescence (left), as well as, among clusters (right), which could consist of initiation, green bud, white candle, and anthesis stages.

Flower morphology

Morphological features of flowers were variable among the three *Coffea* species. *C. liberica* var. *liberica* has the largest flower diameter, followed by *C. liberica* var. *dewevrei*, *C. canephora*, and *C. arabica* has the smallest one. However, *C. liberica* var. *dewevrei* has the most variable sizes of flower diameters, ranging from 30 to 45 mm. Inversely, the *C. arabica* flower was the most uniform in diameter (Figure 6a).

Interestingly, the individual flower's mean number of petals was a distinctive feature for *C. liberica* var. *liberica*. It distinctly differed from the two *Coffea* species, even with

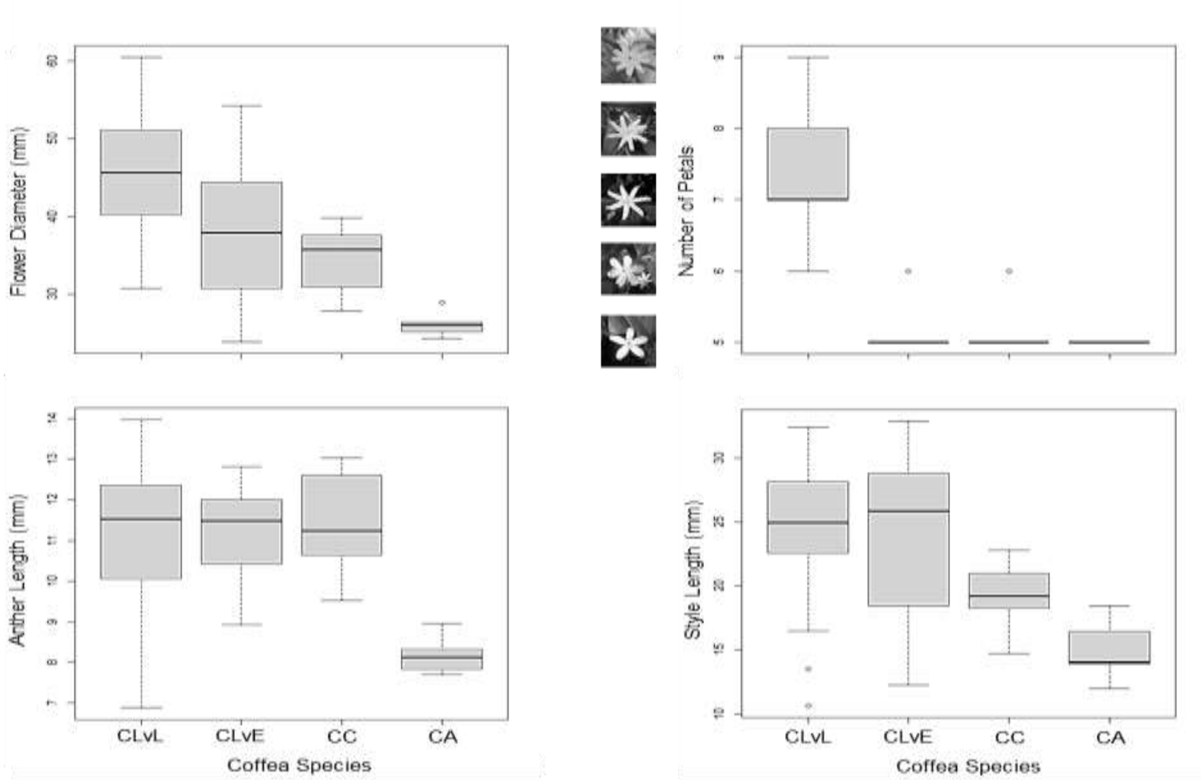

Figure 6. Morphometric variability among three coffee species: a. flower diameter; b. number of petals; c. anther length; and d. style length. CLvL = *Coffea liberica* var *liberica*, ClvE = *C. liberica* var. *dewevrei*, CC = *C. canephora*, and CA = *C. arabica*.

another botanical variety of the same species, i.e., *C. liberica* var. *dewevrei*. *C. liberica* var. *liberica* flower has six to nine petals, even up to 10 petals in the slightest frequency, while the rest of the genotypes only had five petals, on average (Figure 6b). It means the number of anthers per individual flower was in the same pattern and quantity. Indeed, the single flower of *C. liberica* var. *liberica* had the highest number of anthers. Anthers attached to filament dorsifixedly and uniseriate epipetalously. The number of anthers is identical to the number of petals (Melese and Kolech, 2021). Despite having the broadest flower diameter and the highest number of petals and anthers, *C. liberica* var. *liberica* had the lowest number of individual flowers per single inflorescence (cluster). This trade-off is common in several other plant families (Sargent *et al*., 2007).

In addition to the variability of flower perianths, as described earlier, researchers also analyzed the reproductive part of the Coffea flower, anther, and style. The average length of anthers of *C. liberica* was slightly longer than that of *C. canephora*. On the other hand, *C. arabica* flower has comparatively the shortest size of anthers (Figure 6c). Several reports stated a high positive correlation between anther length and the number of pollen grains (Astiz and Hernández, 2014). In other words, the anther length is a better estimate for pollen quantity. Therefore, an inference could be that the individual flower of *C. liberica* could produce the highest amount of pollen. Inversely, *C. arabica* generates the lowest number of pollen grains.

The average of the style length was also variable among the three coffee species. *C. liberica* had the longest style, as shown

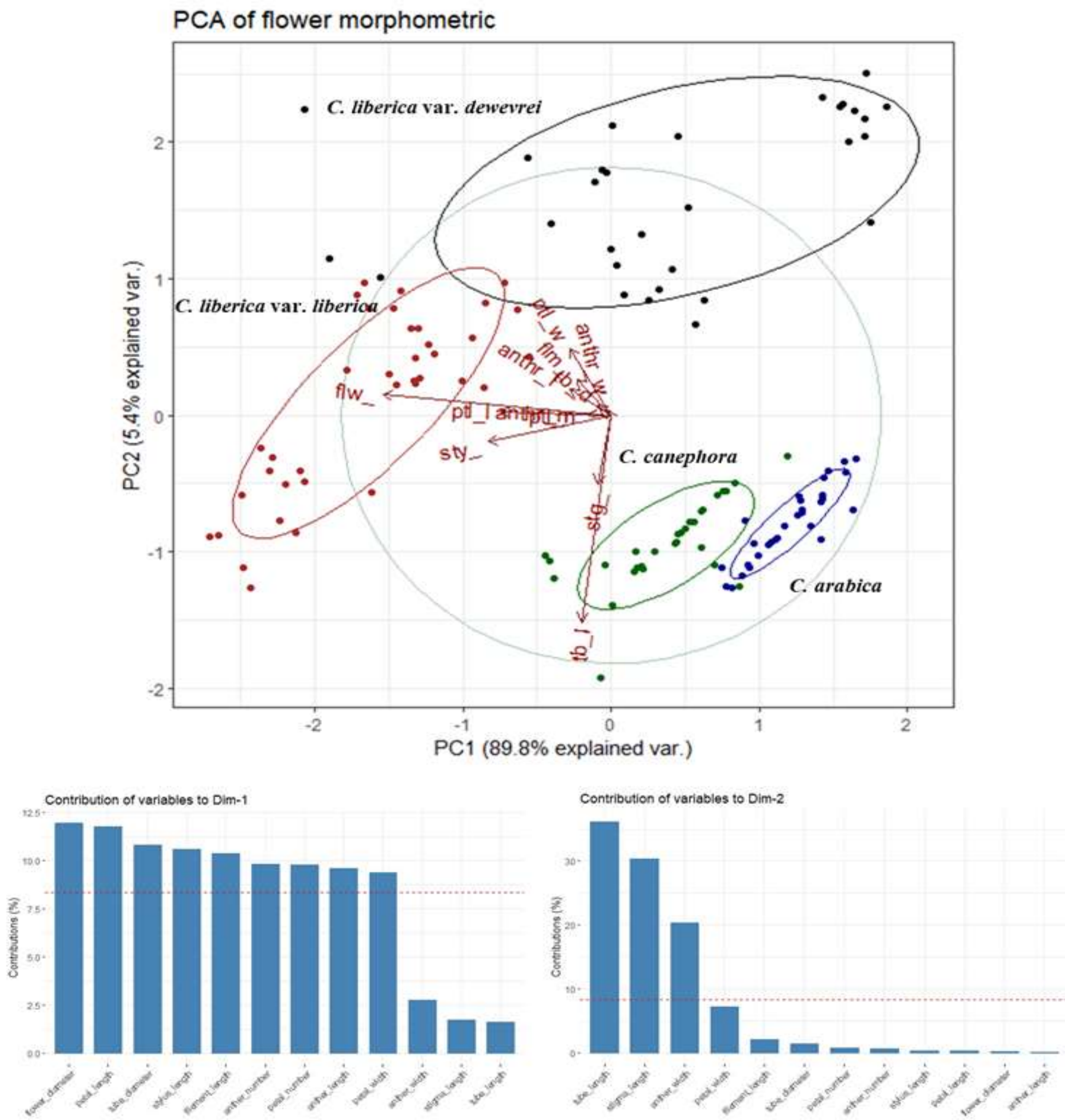

Figure 7. Principal component analysis (PCA) based on the flower's morphometric traits of three coffee species. PC1 and PC2 are responsible for >95% of cumulative variance. The most contributing characteristics to PC1 were flower diameter, tube diameter, petal number, petal length, petal width, anther number, anther length, filament length, and stylus length. The rest of the three traits, i.e. tube length, anther width, and stigma length, were most contributing to PC2.

by its flower, and that of *C. arabica* had the shortest style. As with the flower diameter, the style length of *C. liberica* var *dewevrei* was the longest polymorphic, having a range similar to that of *C. canephora* up to the longest in *C. liberica* var *liberica* (Figure 6d).

Based on the principal component analysis (PCA) generated from datasets of flower morphometric characteristics, the three coffee species could be distinguished into four distinctive groups (Figure 7). However, the *C. arabica* group is closer to the *C. canephora* group. Interestingly, dividing two botanical varieties of *C. liberica*, i.e., var. *liberica* and var. *dewevrei*, could still have two groups with only a slight intersection.

DISCUSSION

Floral phenology divergence among three coffee species

The introduction of three coffee species, namely *C. arabica*, *C. canephora*, and *C. liberica*, to Indonesia, gave rise to sympatric zones, especially in Sumatra and West Java, with a climate type that tends to be wet. The formation of these sympatric zones is inseparable from the role of coffee farmers, who generally are small-scale. Such conditions open up opportunities for physical contact between species with no geographical barriers, different from the conditions in their centers of origin.

Apart from the loss of spatial barriers, temporal blocks in the form of differences in flowering phenology still have the potential to hinder gene flow between species. However, based on observations of the flowering frequency and duration in the anthesis phase, there was a wedge between the three species, especially during maximum flowering. The flowering peak on coffee plants signified a close relatedness to day length (Quiñone *et al*., 2011), i.e., when the day length is <12 hours from July to October. It indicates a weakening of the temporal barrier between coffee species at the observation site, providing opportunities for crossing between species. Changes in the flowering phenology of the three coffee species have strong influences from differences in climatic conditions between the genetic center of origin and the development areas (Gomez *et al*., 2016; Azka *et al*., 2021).

Morphometric variation among three coffee species

Coffea species have a perfect or hermaphroditic flower structure where a single flower carries the male and female organs. This structure has considerably less pollen limitation than pistillate flowers (Mamut *et al*., 2014). The female organ consisted of a bi-ovulated carpel (Noirot *et al*., 2016), rarely mono- or tri-ovulated carpel. *C. arabica* was considered a self-compatible species, while *C. canephora* and *C. liberica* had a self-incompatible nature.

Pollination of coffee plants receives assistance from the wind (anemophile) and insects (entomophile) (Melese and Kolech, 2021). Flowers of *C. arabica*, *C. canephora*, and *C. liberica* produce sufficient nectar to attract pollinating insects. However, a nectar volume derived from the single flower of *C. arabica* is the lowest among the three *Coffea* species. *C. arabica* flowers produce a nectar of 1 µL to 6 µL, depending on the time of collection, with an average of 3.3 ± 0.2 µL. Meanwhile, nectar content of *C. canephora* was 1.3 times of *C. arabica*. Nectar volume of *C. liberica* was of 9.27 ± 3.90 µL. The highest nectar volume was visible in the morning when maximum air humidity was apparent (Bareke *et al*., 2021; Manila-Fajardo and Cervancia, 2020; Prado *et al*., 2019; Sari *et al.*, 2015).

Nectar volume positively correlated with flower size (Tavares *et al*., 2016). The size of *C. liberica* flower is the broadest compared with *C. canephora* and *C. arabica*. Flower size differentiation among the three coffee species has considerable consequences on preference for effective pollinating insects. A larger size of the flower could attract large-bodied insect species, which have better capability to transport pollen on longer distances. By implication, the pollination success of large flower species is comparatively higher (Kettle *et al*., 2011). However, human intervention might change the effectiveness of this reproductive cost. *C. liberica* could grow up to 9 m in a natural population, comparatively taller than *C. canephora* and *C. arabica* (Emanauli and Prihantoro, 2019). Meanwhile, in well-managed coffee plantations, the height of the three coffee species could only be up to 2 m because their main trunk would be topped immediately. Therefore, the large body size of pollinating insects might be inappropriate. On the other hand, interspecific pollen transfer should be higher in such conditions in case co-anthesis occurs. The pre-zygotic barrier between the two different coffee species could have immediate repression.

A challenge for successful interspecific-homoploid and interspecific-heteroploid hybridization is a post-zygotic barrier. Distant-hybrid seed failure, impacting unusual endosperm development, is typical in many plant species and is considerably an early-acting post-zygotic barrier. Interestingly, the effective balance number (EBN) and crossing direction could affect such failure aside from the divergence of genome number (Städler *et al.*, 2021). Therefore, further study on a post-zygotic barrier of interspecific hybridization between two distinct coffee species should be very challenging.

CONCLUSIONS

There was a co-anthesis period among three coffee species grown in the sympatric or parapatric populations, which might be a reliable indicator of an interspecific pollen transfer event. However, the co-anthesis between *C. arabica* and *C. canephora* is comparatively more frequent than between *C. arabica* and *C. liberica*. Based on the morphometric characteristics of the flowers, the three coffee species differ from each other. *C. liberica* had the largest flower size, while *C. arabica's* flower was the smallest. Therefore, the degree and mechanism of pollen transfer among those three coffee species in well-managed coffee plantations require further examination.

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