Floral Morphological Variation in Black Pepper (*Piper nigrum* L.) Varieties and Hybrid Lines

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ABSTRACT

The inter-variety crossing is a promising approach to increase black pepper production. However, successful hybridization hinges on floral characteristics. This study investigated the floral morphology of diverse pepper varieties and their hybrid offspring. Eighteen genotypes, including parent varieties and F1 hybrids, were cultivated in pots under greenhouse conditions at the Indonesian Spice and Medicinal Crops Research Institute, Bogor. Flower and fruit traits were observed. Results indicated variability in spike morphology. While most ripe spikes were yellowish-green, exceptions included LH 20-1 and LH 22-1. Ciinten and LH 4-5 displayed superior spike length and fruit set, contrasting with the shorter, less productive spikes of 20-1, 22-1, and 455-N2-97. Stigma receptivity and another dehiscence coincided in Ciinten, N2BK, LH 6-2, and LH 4-5, simplifying the hybridization process. Conversely, earlier stigma receptivity in Petaling 1, Petaling 2, Natar 1, Natar 2, LH 44-9, LH 20-1, and LH 22-1 facilitated castration procedures. These findings offer valuable insights for optimizing pepper breeding programs.

Keywords: Castration; Flower characteristics; Inter-varietal hybridization; Spices; Stigma receptivity

INTRODUCTION

Black pepper (*Piper nigrum* L.) is the world's most traded spice. Indonesia is one of the world's largest spice producers, with major commodities being black pepper, nutmeg, clove, cardamom, and cinnamon. Black pepper is native to the Western Ghats of India (Kumar et al., 2021), but nowadays, it has already been widely cultivated in several countries, including Indonesia. In Indonesia, black pepper has been cultivated in several provinces, such as Bangka, Lampung, South Sulawesi, East Kalimantan, South East Sulawesi, and West Java. Two product types of pepper are famous in the market, namely black and white pepper. White pepper is usually processed from varieties with big berries, such as Petaling and Ciinten. Meanwhile, black pepper is processed from varieties with smaller berries, such as Natar-1 and Natar-2. Currently, ten high-yielding varieties have been released, namely (1) Petaling 1, (2) Petaling 2, (3) Natar 1, (4) Natar 2, (5) Lampung Daun Kecil, (6) Chunuk, (7) Bengkayang, (8) Ciinten, (9) Malonan, and (10) Nyelungkup. Black pepper has several biological roles, including antioxidant, anti-inflammatory, anticancer, anti-obesity, antidepressant, antidiabetic, antimicrobial, gastroprotective, and insecticidal activities (Ashokkumar et al., 2021).

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The main constraints in black pepper cultivation are low productivity compared to the other producing countries, stem borer (Laba, 2018; Manohara, 2013), and foot rot disease (Manohara, 2013; Prasmatiwi & Evizal, 2020). Black pepper productivity in other pepper-producing countries could reach 3098 kg/ha in Brazil and 4650 kg/ha in Malaysia (FAOSTAT, 2022). Low productivity and the absence of pest, disease, and virus-resistant varieties may be due to the inadequate genetic variability within the cultivated varieties in the *Piper nigrum* species populations (Manohara, 2013).

Therefore, it is necessary to broaden genetic variability through intercrossing between varieties or species. However, successful hybridization may be obtained from understanding flower biology, especially the time of anthers dehiscence and stigma receptivity. Therefore, before inter-variety hybridization is carried out, studying biology and the morphological characteristics of pepper flowers is essential.

Apart from that, the choice of parents to be crossed is essential. The parents selected must have superior characteristics according to the breeding objectives to be achieved so that the hybrids produced would gain a good combination of characteristics from both parents.

Yield per vine correlated with the number of spikes per plant, the spike length, the number of fruits (berries) per spike, and the size. The more extended spike with many berries tends to produce a high yield. According to Bermawie et al. (2019), the ideotype of black pepper plants is those with good plant appearance with more branches, more prolonged spikes (≥ 12 cm) with a high number of berries (≥70) per spike, and big berry size. Moreover, berry size is related to the pepper products produced. There are two major pepper products in the international market, namely black and white pepper. White pepper is usually processed from varieties with big berries, while black pepper is processed from smaller ones.

The inflorescence characteristics are an important characteristic of the pepper variety. Pepper flower inflorescence is formed on fruit branches. Its position is the opposite of the petiole and is called a spike, but not every leaf produces a spike. The flower consists of female, male, and bisexual flowers (Wulandari et al., 2021). The stamen flanks the stigma on both the left/right sides. A larger size and whitish color characterize stigma receptivity. Mature stigma starts from the base of the spike. The ripe stamen looks white, protruding beside the stigma.

Flower characteristics in black pepper are often correlated with yield per vine, such as spike length, number of flowers per spike, and berry size. Several inter-variety crosses have been obtained. It is necessary to study the flower characteristics, that may be related to their potential yield and the most suitable recommended pepper products. This study aimed to observe the morphological characteristics of flowers for their potential success in hybridization and their further correlation with the potential yield per vine. This research can provide fundamental information to determine effective and efficient pepper breeding strategies, especially the crossing stage.

MATERIALS AND METHODS

The study was conducted in the greenhouse of the Indonesian Spice and Medicinal Crops Research Institute, Indonesian Agency for Agricultural Research and Development, Bogor, West Java, Indonesia, from January 2020 to December 2021.

Preparation of Planting Materials

Eighteen genotypes were used in this study, consisting of eight high-yielding varieties (Ciinten, Belantung, Lampung Daun Lebar, Bengkayang, Petaling 1, Petaling 2, Natar 1 dan Natar 2) and ten hybrid lines that have been screened foot rot diseases (LH 4-5, LH 4-5-5, LH 6-2, LH 20-1, LH 22-1, LH 37-16, LH 44-9, N2BK, LH N2BK x LDL-1, and LH 4-5-5x N2-97).

The plant material used was bushy pepper. The plants were prepared from fruit branches taken from each variety/genotype of mature trees. The branches were cut and then planted in a big pot 40 cm in height x 30 cm in diameter, containing soil and cow dung mature in a 1:1 ratio. Each genotype consisted of 5 pots. Plant care followed the procedures for bushy peppers. The plants began bearing flowers at 3 years old, and observation began.

Observation of Morphological Characteristics

Fifty mature spikes were randomly selected per each genotype/variety and used for observation.

Observation of Qualitative Morphological Characters

The selected spikes were observed for qualitative characteristics, such as spike color, the dense flower arrangement in the spike, time of stigma receptivity, and stamen dehiscent for fertilization.

Spike Color

The RHS color chart was used to observe the color by comparing the color of the spike with the color on the RHS color chart.

The Dense Flower Arrangement

The density of flower arrangements in the spike was observed visually with the help of a head magnifier. The arrangement of the stigmas in a spike was categorized as dense or moderate (relatively sparse).

Time of Stigma Receptive and Stamen Dehiscent

The timing of stigma receptivity and another dehiscence was observed visually using a head magnifier. The stigma elongating indicates stigma receptivity, fully open flowers, and a slightly white color. The theca flanking and the anthers dehiscing indicate stamen ripeness. The synchronization of stigma receptivity and anther dehiscence was observed in two scenarios: (1) on the same day or (2) with anther dehiscence occurring 1-2 days later.

Observation of Quantitative Morphological Characters

The quantitative morphological characteristics observed were peduncle length, spike length, spike diameter, number of flowers per spike, and number of berries per spike. The spike was observed when the stigma was in a mature stage.

Data Analysis

Qualitative morphological characteristics data were analyzed using descriptive statistics, and data of quantitative characteristics were analyzed using variance and correlation analysis.

RESULTS AND DISCUSSION

The spike characteristics of pepper varieties and hybrid lines are presented in Table 1. The spike characters of varieties and hybrid lines varied. Spike color mainly was yellowish green, but three accessions (LH 20-1, LH 22-1, LH N2BK) had a light-yellow color, and after pollination, the color became brownish yellow (YGG 153 D). The spike color of the black pepper of Malaysian cultivar was green, whitish green, or light yellow (Chen & Tawan, 2020a), with the color of inflorescence included in the Green group N144, Green group 144, and Green group 145 (Chen & Tawan, 2020b). Wulandari et al. (2021) reviewed that the color of the inflorescence of black pepper was light green-yellow. Pooja (2019) reported that the spike color of Panniyur 1 was light green and dark green. Variations in the color of the spike may be attributed to genetic differences.

Table 1. Qualitative characteristics of spike in several black pepper varieties and hybrid lines

No	Variety/lines	Young spike color	Mature spike color	Time of stigma receptivity vs anther dehiscence	Stigma size	Flower arrangement on the spike
1	Ciinten	YGG 145 A-B	YGG N144 D	Same time	medium	dense
2	Belantung	YGG 145 A-B	YGG N144 D	early	medium	moderate
3	Lampung Daun Lebar	YGG 145 B-C	YGG N144 D	Same time	medium	moderate
4	Bengkayang	YGG 145 A-B	YGG N144 D	early	medium	moderate
5	Petaling1	YGG 145 A-B	YGG N144 D	early	medium	moderate
6	Petaling 2	YGG 145 B-C	YGG N144 D	early	medium	moderate
7	Natar 2	YGG 145 A-B	YGG N144 D	early	medium	moderate
8	Natar 1	YGG 145 A-B	YGG N144 D	early	medium	moderate
9	LH 4-5	YGG 145 A-B	YGG N144 D	same	medium	dense
10	LH 4-5-5	YGG 145 A-B	YGG N144 D	same	medium	moderate
11	LH 6-2	YGG 145 A-B	YGG N144 D	same	medium	moderate
12	LH 20-1	YGG 145 A-B	YGG 153 D	early	big	dense
13	LH 22-1	YGG 145 B-C	YGG 153 D	early	big	dense
14	LH 37-16	YGG 145 A	YGG N144 D	early	small	dense
15	LH 44-9	YGG 145 A-B	YGG N144 D	early	Big and thick	moderate
16	LH N2BK	YGG 145 A-B	YGG 145 B-C	early	medium	moderate
17	LH N2BK x LDL-1	YGG 145 A-B	YGG N144 D	early	medium	moderate
18	LH 455-N2-97	YGG 145 A-B	YGG N144 D	early	big	dense

Note: YGG = yellow-green group

Anther dehiscence in the studied pepper varieties and F1 lines typically occurred at 10-11 AM on sunny days, with delays under cloudy conditions. However, this timing varies geographically. In Vietnam, Vinh Linh and Phu Quoc varieties dehisce earlier at 7-8 AM (Quyen et al., 2019). Indian Panniyur-1 exhibits a distinct pattern, with anthesis starting in the afternoon and anther dehiscence peaking between 2-3 PM (Pooja, 2019). Generally, black pepper anther dehiscence falls around 11-12 AM, with pollen viability lasting up to ten hours (Chen et al., 2018). Precise knowledge of anther dehiscence timing in both cultivated and wild peppers is crucial for successful interspecific crosses. It's important to note that both genetic factors and environmental conditions influence these timings.

The time of stigma receptivity versus anther dehiscence is one of the essential characteristics in determining the success of hybridization. Stigma receptivity and anther dehiscence are categorized as early, simultaneous, or late. In most varieties, stigma receptivity occurred one day earlier than anther

dehiscence (Table 1), indicating geitonogamy (Pooja, 2019). Varieties with stigma receptivity occurring earlier than anther dehiscence are beneficial for artificial crossing, as this timing can minimize mechanical damage to spikes and stigmas due to accidental contact during castration. The varieties with earlier stigma receptivity than anther dehiscence are better used as the female parent.

On the other hand, in hybridization involving varieties where anther dehiscence occurs earlier than stigma receptivity, it is preferable to use these varieties as the male parent. If the varieties are used as the female parent, the risk of failure is higher due to unavoidable castration. The stigma becomes damaged/dry from being accidentally touched during castration and is no longer receptive. Varieties and hybrids with anther dehiscence occurring at the same time as stigma receptivity include Ciinten, Lampung Daun Lebar, LH 4-5, LH 4-5-5, and LH 6-2. The Ciinten variety, which produces abundant pollen, is best used as a male parent. However, varieties with simultaneous stigma receptivity and anther dehiscence have a higher risk of self-pollination contamination and a reduced success rate for crosses due to accidental touch or injury during castration activities.

Stigma size also influences the success rate of hybridization. Varieties with large stigma sizes can increase the chances of sticking pollen during hybridization. Hybridization is more manageable in a hybrid line with an enormous stigma size, and the potential for success is high. However, in all crosses involving LH 44-9 with a big size stigma, as a female parent, it is easy to cross, but the success rate is low. Additionally, LH 44-9 has a moderate bloom density therefore, there are no issues with the castration procedure. The low success rate in crosses involving LH 44-9 may be attributed to other factors, such as the sterility of female flowers or other genetic factors that need further study. LH 44-9 is a hybrid of a cross between LDK and P2.

Table 2. Quantitative morphological characteristics of spike in several black pepper varieties and hybrid lines

No	Variety	Peduncle length (cm)	Spike length (cm)	Spike diameter (mm)	Numbers of flower	Numbers of berries
1	Ciinten	1.11abc	9.24a	3.24bc	107.25a	87.00a
2	Belantung	0.84def	6.75bcde	3.22bc	70.49def	34.67cdef
3	Lampung Daun Lebar	0.90cdef	6.20def	3.05cd	61.94ef	26.17efgh
4	Bengkayang	0.98bcde	5.98def	3.01cd	60.80ef	31.85defg
5	Petaling1	0.92cdef	6.73bcde	2.70d	75.02cd	35.94cde
6	Petaling 2	0.96bcde	6.3cdef	3.07cd	61.18ef	35.22cde
7	Natar 2	0.88cdef	6.82bcd	3.05cd	84.94bc	39.30cd
8	Natar 1	0.91cdef	5.32f	3.09cd	58.90f	30.26defg
9	LH 4-5	1.06abcd	9.64a	3.27bc	107.24a	63.84b
10	LH 4-5-5	1.06abcd	7.60b	3.23bc	92.08b	42.00c
11	LH 6-2	1.19ab	7.33bc	3.55ab	75.32cd	30.53defg
12	LH 20-1	0.80ef	3.26g	2.94cd	33.44g	16.79hij
13	LH 22-1	0.72f	3.40g	3.07cd	37.68g	15.18ij
14	LH 37-16	1.09abc	6.36cde	2.95cd	66.13def	25.11fgh
15	LH 44-9	1.23a	7.29bc	3.75a	72.10cde	7.78jk
16	LH N2BK	0.98bcde	5.73ef	2.74d	60.76ef	23.90ghi
17	LH N2BKxLDL-1	1.08abc	3.66g	2.71d	28.16g	-
18	LH 4-5-5 x N2-97	1.02abcde	3.26g	3.07cd	35.43g	3.88k

Note: Means followed by different letters are significantly different (Tukey HSD Test, α = 0.05)

LH 37-16 has a smaller stigma and high flower density in the spike. The smaller size of the stigma and the high density of the flower in spike are unfavorable, mainly if it is used as a female. A high density of flowers causes difficulty during castration. The risk of pistil damage is high from accidentally touching it during the castration process.

Natar 1, Natar 2, Petaling 1, and Petaling 2 have ideal flower characteristics for hybridization, with earlier stigma receptivity than anther dehiscence and moderate flower density, so a high success rate of crosses was obtained.

Spike length and number of flowers per spike vary among varieties and lines (Table 2). These characteristics are related to high yield per vine. The spike length from these studies ranges from 3.26 – 9.64 cm, with the numbers of flowers and berries of 28-107 and 4-87, respectively. Most varieties and lines have a moderate length of the spike. Varieties that have long spikes are Ciinten, followed by hybrid lines of LH 4-5 and LH 44-9. The hybrid lines with short spike lengths include LH 20-1, LH 22-1, and LH 455-N2-97. In the form of climbing pepper, the Ciinten variety has a spike length of 11 cm and a high number of berries, higher than the other superior varieties (Bermawie et al., 2019; Meilawati et al., 2020). There is no difference between Ciinten grown as a shrub and a climbing plant. Spike length and number of fruits (berries) per spike can be used to distinguish varieties (Chen & Tawan, 2020b). Spike character is directly related to attributes of various black pepper branch types (Bhasi et al., 2017).

Generally, a longer spike tends to have more flowers and berries. However, in LH 44-9, despite having a long spike and many flowers, the fruit set is low. The number of fruits per spike is less than 10. It may be caused by anther dehiscence of LH 44-9 is later, almost two days later than stigma receptivity. The first stigma appears to elongate in two days and continues to emerge wide 2-4 days later (Chen et al., 2018). The viability of pollens is high, which is up to ten hours after anther dehiscence. Stigma is the most receptive when it is in a fully emerged stadium. In LH 44-9, the duration for self-pollination is shorter because the time of stigma receptivity differs from anther dehiscence, with stigma receptivity occurring much earlier (by two days). The low fruit set may also be due to stigma damage from contact with nearby leaves or passing insects.

Spike length and the number of berries per spike are critical determinants of black pepper yield (Bermawie et al., 2019; Shango et al., 2021), in which spike length is categorized into short (<10 cm), medium (10-15 cm), and long (>15 cm) groups. A positive correlation has also been established between berry count per spike and pepper yield (Bermawie et al., 2019). The Ciinten variety exhibited a spike length of 9.24 cm with 87 berries, while other varieties and F1 hybrids in this study displayed shorter spikes. This indicates a narrow genetic base for spike characteristics among the parental lines. Incorporating parents with longer spikes into breeding programs to increase black pepper yield is essential for improving spike length.

Quality parameters such as essential oil, piperine, and oleoresin are important for pepper. Ciinten is a high-yielding variety with a berry yield of 4.30 kg per vine, piperine content of 4.73%, essential oil content of 2.79%, and oleoresin content of 15.98% (Bermawie et al., 2019). In Tanzania, the black pepper length of spikes is 5.6-12.4 cm, with the number of flowers varying from 55.4-93.5, and the percentage of fruit set of 46.7-86 %, depending on the varieties (Shango et al., 2021). In India, Pan-

niyur-1 has flowers ranging from 48 to 98, depending on the spike length, with a percentage of fruit set at 92.90% (Pooja, 2019). Meanwhile, the cultivar Kurimale in Karnataka has a spike length of 22.99 cm and produces many berries (103.7) (Pannaga et al., 2021). Studies on the breeding system in *Piper nigrum* revealed that high fruit sets resulted from geitonogamy, followed by autogamy, open pollination, and xenogamy (Pooja et al., 2022).

There are eight genotypes with long peduncles, namely LH 44-9, LH 6-2, Ciinten, LH 37-16, LH N2BKxLDL-1, LH 455-N2-97, LH 4-5, and LH 4-5-5. LH 44-9 has the longest peduncle and is significantly different from Belantung, Lampung Daun Lebar, Bengkayang, Petaling 1, Petaling 2, Natar 1, Natar 2, LH 20-1, LH 22-1, and N2BK based on Tukey test at 95% (Table 2). Ciinten is a superior pepper variety with the most extended spike length. LH 4-5 and Ciinten have the most extended spike and significant numbers of stigma. This study also shows that the Ciinten produces the highest number of berries. These varieties and lines are significantly different from each other. Although LH 4-5 has fewer fruits than Ciinten, it still has many fruits and differs significantly from other varieties and lines. LH 44-9 has the largest spike diameter, significantly differing from other varieties and lines. Meanwhile, LH 22-1 has the shortest peduncle, the shortest spike, and fewer flowers.

Table 3. Correlation between spike quantitative characteristics

Trait	Peduncle length (cm)	Spike length (cm)	Number of stigma	Spike diameter (mm)	Number of berries per spike
Peduncle length	1				
Spike length	0.19	1			
Number of stigma	0.15	0.93	1		
Spike diameter	0.17	0.17	0.08	1	
Number of berries per spike	0.08	0.60	0.64	-0.04	1

Based on this study, the quantitative characteristics are positively correlated except between fruit numbers per spike and spike diameter (Table 3). The correlation between the numbers of stigma and spike length is very strong, about 0.93. It means that the longer the spike, the more stigma. Spike length, numbers of stigma, and fruit numbers per spike are very strongly correlated. These characteristics are also the main yield component characteristics in pepper.

The spike length is positively correlated with the number of berries/spikes of black pepper. It follows previous research on black pepper in Ethiopia (Bekele et al., 2017). Spike length also has a significant and positive correlation with the starch content of leaves (Zu et al., 2018). A positive correlation between spike length and yield was also observed (Shango et al., 2021; Shivakumar & Saji, 2019). This characteristic has high heritability (Preethy et al., 2018). The number of berries per spike or percentage of fruit set for a yield of black pepper was also reported by Prayoga et al. (2020) and Oanh et al. (2021). However, the number of fruits per spike is negatively correlated with spike diameter, although the correlation is weak. This condition is a challenge for breeders in increasing pepper production because spike diameter is also one of the yield component characteristics in pepper.

CONCLUSION

The morphological characteristic of spikes varies among varieties and lines. The color of ripe panicles is generally yellowish green, except for LH 20-1 and LH 22-1. Ciinten and LH 4-5 varieties have the longest spike and the highest fruit sets. LH 20-1, LH 22-1, and L 4-5-5 x N2-97 have short spikes and low fruit sets. Stigma receptivity and anther dehiscence in spikes occur simultaneously in Ciinten, N2BK, LH 6-2, and LH 4-5. In contrast, in Petaling 1, Petaling 2, Natar 1, Natar 2, LH 44-9, LH 20-1, and LH 22-1, stigma receptivity precedes anther dehiscence. This earlier stigma receptivity facilitates easier castration. This finding will increase the effectiveness and success of pepper breeding programs.

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AUTHORS CONTRIBUTIONS

SW conducted research, observation, and data interpretation and wrote the original draft. NB conceptualized, supervised, observed, interpreted data, wrote, reviewed, and edited the original draft. NS conducted research and observation. MS conducted research, observation, data analysis, and data interpretation and wrote and edited the original draft.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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