Flowering characterization and its relation to the type of pollination on gambier (*Uncaria gambir* (Hunter) Roxb.)

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**Abstract.** This article reports on some of the flowering characteristics that are related to the type of pollination that occurs in the gambier plant. The observation has been carried out in the Gambier Research Station of Faculty of Agriculture, Andalas University, Padang. The research used descriptive method which observes flowering characteristics in ten plants using ten selected flowers within plants. Results from the observation showed that the bud initiation, small-bud, large-bud, anthesis, and fruit development phases occur in 19, 33, 6, 4, and 58 days. All phases complete in 120 days, ranging from 106-134 days. Anthesis occur at 18:30 – 05:00. The receptivity of the stigma and maturity of pollen occur before anthesis, while the highest receptivity occur at 00:00. Cleistogamy mechanism happens in the gambier plant. Self-pollination (both autogamy and geitonogamy) and cross-pollination (xenogamy) exist in gambier.

**Keywords:** phenology, self-pollination, cross-pollination, stigma receptive

1. **Introduction**

Gambier is a small-holder plantation commodity which has been cultivated for decades, especially in the island of Sumatra. Approximately 80% of the world's gambier production was from Indonesia, mainly from West Sumatera [1]. Gambier is one of the export commodities with high economic value. Nevertheless, the development of this commodity in the field has a complex technical and socio-economic problems [2,3,4].

The leaves and branches of gambier were extracted and utilized for various purposes. The extract of gambier contains a variety of flavonoids compounds from polyphenols group [5], especially Cathecin [6,7]. As a member of the genus Uncaria, the extract has activity as cytotoxicity, anti-inflammatory drugs, anti-viral, immuno-stimulant, anti-oxidant and anti-bacterial properties [8]. Gambier has been utilized as a complement of betel [3], dyeing in the textile industry [9], tanning agent in the leather industry [10] and a wood preservative[11]. Hence, due to the value of the gambier commodity as an industrial raw material, the commodity of gambier remains prospective for commercial development in the future.

The development of superior varieties plays a decisive role to increase crop productivity and low quality of gambier products. Some research related to aspects of biology needed to support the activities of the gambier breeding program [3,12]. This article reports the flowering characteristics that
are related to the type pollination of the gambier plant. This study is essential to the optimization of artificial cross techniques in gambier plants.

2. Materials and Methods
The observation has been carried out in the Gambier Research Station of Faculty of Agriculture, Andalas University, Padang. This research used descriptive method with direct observation of the flower objects in every stage of flowering, fruit formation and maturation in the field. Quantitative data obtained are analyzed using descriptive statistics.

Flowering phenology was preliminary of the research conducted by inspecting the flowering phases and the fruit formation of the gambier plant to determine the most appropriate stage time to obtain the desired flower samples in the succeeding studies. The observed phases were the initial and the final performance of bud-initiation, small-bud, large-bud of flower, flowering bloom (anthesis) and fruit maturity phases. The sequence of phenology observation referred to [13].

Sampling determination used the purposive sampling method. Samples used both in the observation of flowering phenology and determination the type of pollination are ten gambier plants in the flowering phase. The flower used from each plant as samples were ten in the determination of pollination type of pollination, while only two flowers used in the phenology characterization. We cut flowers off in the field which can contaminate pollination to ascertain the ability of gambier as a self-pollinated plant. The formulae to calculate the success of pollination occurred was:

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\text{Fruit set} = \frac{\text{number of fruit set}}{\text{number of flower}} \times 100\%
\]

3. Result and Discussion

3.1. Flowering Phenology
A flower of gambier is a single flower which set a compound floral structure (inflorescence). The type of inflorescence is capitulum which one axis (pedicel) support many single flowers. Based on its flower structure, the plant has complete flowers (flos completus), characterized by the completeness of the primary organs of the flower. The flower is hermaphrodite because it has a male organ (stamen) and a female organ (pistil). The fruit type is a capsule which contains hundreds of seeds. A seed is a reproduction material used for cultivation by all gambier plantation in Indonesia.

Based on the criteria of each phase of the flower development, the phases observed in the study can be seen in Figure 1.

![Figure 1. The development of the flowering phase in gambier. (a) bud initiation phase, (b) small-bud phase, (c) large-bud phase, (d) anthesis phase, and (e) fruit maturity phase](image)

The bud initiation was a phase when a macroscopic bud structure obviously detected. The small-bud was a phase when the inflorescence structure detected clearly. At the large-bud phase, the petals are starting noticeably out of the ovary and easily distinguished from the sepals. The anthesis is the phase of flower bloom. The opening of petals from a single flower in a set of composite flowers is an initial stage of the inflorescence intruded into the anthesis criteria. The dropping off the corolla tube
and other parts of flower indicate the end of anthesis stage and the start of the fruit development phase. The dried and brown fruit indicate the maturity of fruit. If we split off the capsule, the structure of seeds looks very small, light and easy to fly because it has a similar structure of wings.

The entire phase in Figure 1 occurred in 120 ± 8.74 days, ranging from 106 – 134 days (Table 1). The duration of the phase from the initiation until the maturity of fruit is not much different from those reported by [13] in the Udang-type which is 112 days, and [14] which is 116 days, both in the Udang- and Cubadak-type. Variation in the duration of flowering is may due to differences in the genotypic composition of gambier plant and the number of flower samples used as well as the influence of environmental conditions factors.

Table 1. The flowering development phases of gambier from bud initiation to the maturity of the fruit

| Phase       | Means (day) | Range (day) |
|-------------|-------------|-------------|
| Bud initiation | 19±2.16     | 15 - 22     |
| Small-bud    | 33±2.54     | 29 - 36     |
| Large-bud    | 6±1.33      | 4 - 8       |
| Anthesis     | 4±0.67      | 3 - 5       |
| Fruit maturity | 58±8.29    | 47 - 67     |
| Total        | 120±8.74    | 106 - 134   |

The anthesis phase is the crucial phase that determines how the type of pollination happens in the gambier plant. The phenomenon occurs before, during, and after anthesis become characteristic to determine whether the gambier perform within-flower self-pollination (autogamy), between-flower self-pollination (geitonogamy), or do cross-pollination (xenogamy). The gambier flower has a heterostyly type; the size of a stylus is longer than that of the anther. Theoretically, the lower position of stamen makes it not feasible to deposit pollen to a higher position of stigma.

Anthesis happened when the sun will be set from 18:30 until the sun will rise at about 05:00 WIB. The period of anthesis is in line with [13] presumptively that the blooms last from night to morning. Flowers blooming in one cluster were not simultaneous (Fig. 2). The more flowers bloomed in one cluster, the more intense the fragrance of the flowers. This blooming phase occurred for several days (3-5 days), varying among the flowers.

Figure 2. Variation in flowers bloom in one cluster at 00:00 hours

3.2. Pollination Type

To understand the process occurred within the flower before and during anthesis, we removed petal and observed the position of stigma and anther (Fig. 3). Before anthesis, anther and stigma are in the same high position. Anther swells, but pollen remains inside the anther. The stigma has an adhesive surface structure (Fig. 3a & 3b). Stylus grows elongated and bent due to restraining by the petal. At this time, pollens freed from the anther and deposit to the stigma surface (Fig. 3c). Finally, stylus and
stigma relief from the flower (Fig. 3d) and exhibit maximum length size when flowering blooms completely (Fig. 3e). These observations designate that pollination has occurred before the flower blooms (cleistogamy).

![Diagram of flower parts: Stigma, Anther, Petal, Corolla tube](image)

**Figure 3.** Development of flower at before and during anthesis. (a) & (b) large-bud flower, (c) flower begins to open, (d) flower blooms, (e) flower perfectly blooms

In the morning after anthesis, the pollen conditions scattered due to the fine pollen structure. The deposited pollen on the stigma surface of the same flower indicated that within-flower self-pollination (*autogamy*) existed in gambier. Cleistogamy is suspected to be a self-pollination mechanism. However, further study needed to examine whether entirely single flower in the cluster acts cleistogamy.

Artificial-crossing made in one day before anthesis was successful in setting fruit; however, the highest percentage of fruit set was the anthesis at 00:00 (data was not shown). The artificial-crossing was done with removing un-maturity anther, while the pollen derived from pollen which stick to stigma in another flower. This result indicated that receptivity of the stigma phase lasts before anthesis and leads a maximum at the time of anthesis.

Result of this research contradicted with [13]. They reported that the flower of gambier is protandry because the maturity of an anther (during anthesis) was before the receptivity of stigma (one day after anthesis). The result was also not in line with [14], who stated that the receptive stigma and the maturity anther phases coincide with anthesis. Based on the observation, the receptive stigma and pollen maturity occur earlier than the flower blooms. Hence, the investigation of pollen viability before anthesis is needed. Pollen viability notified by [14] was 95.8% in Udang-type, while 80.62% in the Cubadak-type at the anthesis, indicating the possibility in obtaining maximum pollen viability before anthesis.

How is the ability of gambier plant perform between-flower self-pollination (*geitonogamy*)?
To examine that gambier do *geitonogamy*, we set covering plant using a translucent plastic and a thin white textile. It turned out that the use of both sheets caused the temperature in the shade is too high; thus it is difficult to conclude whether the pollination failure is caused by plant genetic factors or due to environmental factors. Similar results were also found when we covered a flower with similar materials. It looks that flowering and fruit development of gambier is very sensitive to environmental factors.

To prove this, then all flowers in the field that could bloom on a period of pollination of plant samples emasculated. Thus, the implementation of the experiment was not carried out concurrently, even though it requires prolonged observation time. The results showed that the fruit set is quite high, i.e. 72%. The number of capsules per cluster produced, varied due to the different environment condition throughout the night (data was not shown). Availability of pollen in one plant also limited. This condition differed with that of cross-pollination when pollen produced by many flowers in the field. The fruit set signified that the gambier performs *geitonogamy* naturally. This result was in line with [15]. Reff [16] noted that *geitonogamy* acquires a fitness cost pollen exported and effects to reduce the viability of the seed.

Pollination vector is needed to transfer pollen from one anther to another stigma in different flower. This study has not been able to answer whether the wind can transmit pollen to other flowers within
the same plant as well as different plants. Scattered of pollen on leaves surface is not enough to prove that the wind assists the transmission of pollen.

Observations of insect frequenting flower showed that bees (Apis sp.) are a vector capable of transferring the pollen. An interesting phenomenon related to this insect is the pollen which covered the stigma since anthesis disappeared after the arrival of bee insects in the morning (Fig. 4). The pollen that is carried away by the bees is possible to adhere to stigma in the different flower within the same or different plants. The presence of pollinator insect indicated the possibilities of cross-pollination (xenogamy) in gambier plants. This is in line with [17] stated that geitonogamy occurs in the same mechanism as out-cross pollination hence obliged pollinator visitation.

The ability of gambier to perform xenogamy was in line with the success of artificial pollination set fruit (data was not shown). It is necessary to test further the degree of self- and cross-pollination occurs in the gambier plants. The degree of pollination type existed in gambier determines the direction of the gambier breeding program in the future.

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Based on the ratio of pollen to ovules, Silfia [18] reported that gambier is a cross-pollinated plant. The cross-pollinated plants resulted in a heterozygous and heterogeneous population. Consequently, the population will have broad genetic variability based on morphological and molecular characterisation of [19,20].

4. Conclusion

Based on the observation, the bud-initiation phase, small-bud, large-bud, anthesis and maturation of the fruit occur in 19, 33, 6, 4, and 58 days. Total time required for the entire phase of the bud initiation until fruit maturity is 120 days, ranging from 106-134 days. The anthesis or flower blooms occurred at 18:30-05:00. The receptivity of the stigma and maturity of pollen occurred before anthesis, while the highest receptivity occurred at 00:00. Cleistogamy mechanism occurs in the gambier plant. Flower of the gambier does self-pollination (both autogamy and geitonogamy) and cross-pollination (allogamy).

A study of the flowering characteristics in this study requires further observation regarding the duration of stigma receptivity and pollen viability. A more detailed study on the magnitude of self- and cross-pollination is vital to explain the variability in gambier plants.
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