Identification of gambier plant [Uncaria gambir [Hunter] Roxb] pollination system

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Abstract. The gambier plant pollination system needs to be understood for a breeding program to be conducted. The purpose of this study was to determine the percentage of self-pollination [autogamy and geitonogamy] and cross-pollination, both natural and artificial allogamy, in these plants. This research was conducted in the Experimental Garden of the Faculty of Agriculture, Andalas University, Padang and used a survey method [identifying samples, conducting pollination, observing samples and collecting data obtained in the field] together with non-probabilistic purposive sampling. Observations made include pollen adhesion to the stigma and fruit formation. Data were analyzed using simple statistics. Fruit production was highest following artificial allogamous cross-pollination [86%], followed by natural allogamous cross-pollination [51%] and geitonogic self-pollination [22%]. Autogamic self-pollination failed to produce fruit. Gambier plants are cross-pollinating plants able to undergo geitonogamous but not autogamous self-pollination.

Keywords: determine, allogamy, autogamy, geitonogamy

1. Introduction

The gambier plant [Uncaria gambir [Hunter] Roxb] is a shrub with high economic value, obtained from the extracts [sap] of leaves and twigs containing catechins, catechutane tannins, fluorescein, waxy quercitin, fats and mucus [1]. Besides, catechins and tannins content are the most widely used chemical ingredients in the cosmetics, pharmaceutical, and agricultural industry, as a vegetable pesticide.

Gambier plant is a specific and superior commodity in West Sumatera, which exists in three varieties, including the shrimp, Cubadak, and Riau. About 80% of Indonesia’s gambier exports are sourced from this area. The main problem with this product is the shortfall in production and quality, this is not following the standards desired by the international market. One effort made is the assembly of superior cultivars through plant breeding. Fauza et al. [2] stated that the aspects of breeding had not been studied in-depth, based on the absence of sufficient publication.

The identification of plant pollination systems needs to be known related to the breeding method to be carried out. Silfia [3] researched pollination systems, which is based on the P/O ratio, obtained from pollen and ovule calculations. Furthermore, Fauza [4] observed the plant morphology of flower organs, including cross-pollinating plants.

Murdaningsih et al. [5] and Fitri [6] stated a different study outcome in contrast with the research by Silfia [3] and Fauza [4]. Murdaningsih et al. [5] stipulated the enhanced propensity to self-pollinate from different pollen and stigma of the same plant [geitonogamy]. This was in line with the investigation conducted by Fitri [6], which stated their possession of high natural potential.
There is a need to ascertain this phenomenon, by researching the system of pollination on stigma with pollen sourced from similar or different plants. Markers of this pollination system are indicated by pollen attached to the stigma and fruit formation.

The aim of this research, therefore, was to ascertain the percentage of autogamy and geitonogamy pollination systems and also to obtain the percentage of natural and artificial allogamous cross-pollination systems in Gambier plants.

2. Materials and Methods
This research was conducted from April to August 2019 in the Experimental Garden Technical Implementation Unit [UPT], Faculty of Agriculture, Andalas University, Padang. The material used gambier type of shrimp, cubadak, and riau is eight years old aquades and 70% alcohol. Identification was observed after 52 days from the beginning of the flowering initiation phase [7]. Other tools used encompass plastic pipette 1cm in diameter and 5cm long, oil paper, thin white cloth, scissors, tweezers, Petri dish, yarn, tissue, double tape, label paper, wooden support, stationery, loupe [magnifying glass], and camera.

The research was conducted via survey methods, involving, pollination system conduction, sample observation and data collection in the field. Selected sample plants are in flowering and a minimum of ten clusters in the plant are used as females. Experiments on the autogamy, geitonogamy, and natural cross-system using shrimp type. However, artificial cross-pollination used shrimp, cubadak, and riau types. Purposive sampling in a non-probabilistic way was used and the research was conducted in the form of isolation, using a thin white cloth on a gambier flower cluster for control. Isolation involved the use of a pipette and oil paper on a single flower for the autogamous self-pollination system, while emasculation and isolation on one plant, required using a thin white cloth for the geitonogamy, This process was conducted on the natural cross-pollination, gold circulation, and isolation, as well as artificial cross-pollination system, which was specifically carried out at 00.00 WIB. This was identified as the optimum time for gambier pollination, and the receptive period of stigma lasts for three days [6].

3. Results and Discussion

3.1. Self Pollination System

3.1.1. Self Pollination System Autogamy
The percentage of the autogamous pollination system isolated using a pipette is presented in Table 1.

| Sample number | The amount of flower interest pollinated per cluster | Pollination [%] |
|---------------|-----------------------------------------------|----------------|
|               | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    |         |
| 1             | 5     | 4     | 4     | 3     | 5     | 5     | 5     | 5     | 5     | 4     | 90±0.70 |
| 2             | 2     | 5     | 5     | 5     | 5     | 4     | 2     | 5     | 5     | 5     | 84±1.25 |
| 3             | 1     | 2     | 2     | 3     | 3     | 4     | 4     | 4     | 4     | 4     | 62±1.10 |
| 4             | 4     | 4     | 5     | 4     | 5     | 5     | 5     | 5     | 5     | 5     | 94±0.84 |
| 5             | 5     | 5     | 5     | 5     | 5     | 4     | 5     | 5     | 5     | 5     | 98±0.31 |

Average percentage of pollination 85.6±0.84
The initial isolation of a single flower was performed by using a pipette, and the percentage of pollination obtained was 85.6%. A week later, the structure of flowers, encompassing the petals, anther, and stigma had fallen with the expectation of being turned into fruits. This manifestation turns brown and eventually falls because 250 single flowers were covered using a pipette, and none eventually become fruit. Subsequently, isolation was continued using oil paper to ensure the genetic factor influence on the results obtained, and the percentage of pollination using oil paper was 94.4%, which is greater than using a pipette, based on the fact that it is lighter and easier to use, and the gambier flowers are not disturbed.

The gambier has type complete flower such as; petal, sepal, male [androecium] and female sexual [gynoecium]. The type of flower is hermaphrodites, male and female sexual at the one flower. Tjitosopeomo [8] reports that generally in Rubiaceae, the stamen is implanted in the petal. Gambier plants have flowers with a longer pistil stalk structure and shorter anther, stigma above the anther. Types of Rubiaceae, long stylus and short stamen [9]. This condition causes pollen cannot reach stigma by itself. Self-pollination of pistil by the pollen of the one plant is geitonogamy. Also, the observations obtained are presented in Table 2.

Table 2. Percentage flower in gambier plant cluster that occurs pollination on the autogamous pollination system using oil paper.

| Sample number | The amount of flower interest pollinated per cluster | Pollination [%] |
|---------------|-----------------------------------------------|------------------|
|               | 1 2 3 4 5 6 7 8 9 10                          |                  |
| 1             | 5 4 5 2 5 5 5 5 5 5                          | 92±0.96         |
| 2             | 5 4 5 5 5 5 5 5 5 5                          | 98±0.31         |
| 3             | 5 5 4 5 5 3 5 5 5 5                          | 92±0.69         |
| 4             | 5 5 3 5 5 5 5 5 5 5                          | 92±0.84         |
| 5             | 5 5 5 4 5 5 5 5 5 5                          | 98±0.31         |

The average percentage of pollination 94.4±0.62

It was also seen that the pollen grains attach to the pistil's head had a percentage that was high enough, with an unknown pollen grain nature. Based on the formation of fruit, it was established that none was formed, giving rise to the assumption that the alleged pollen did not germinate on attachment to the stigma. This also possibly occurs on instances where the pollen tube is shorter than the stalk of the pistil, and germination above the head allows for a downward elongated growth, which ensues into the pistil channel, which occurs not until it touches the embryo bladder [10] [11] [12]. Furthermore, conception does not occur, although fertilization takes place on occasions where the end of the pollen tube moves toward the ovule and also when it touches the nucellus through the micropile after entry into the tissue to the tip of the embryo bladder [13].

Gambier plants have flowers with a longer pistil stalk structure and shorter anther, which is the reason they are categorized into heteromorphic accuracy [heterocyclic]. Besides, the pollen that originates from a long anther tends to pollinate a long stigma.

If the pollen transfers from the same flower, fertilization tends not to occur based on the mechanism of self-incompatibility. Thus, pollen displacement requires pollinators and other factors for marital harmony, and the protrandry nature of maturity is a mechanism that prevents self-pollination. According to Syukur et al. [14] and Jamsari [15], the phenotype of pollen self-sufficiency is determined by the genetic constitution of the diploid plant. Moreover, it was established that inhibition
occurs in the stigma, few minutes after the initial contact between pollen and papillar cells [outer cells of the stigma that capture pollen quickly, which quickly communicates signals between cells and inhibits the development of pollen tubes]. The rejection of pollen was evidenced by the increase in β-1,3 glucan callose on the papillar cells at the site of contact with incompatible pollen, and none of the fruit remained fresh green, as all turned brown and eventually fell off, as shown in Figure 1.

3.1.2. Self Pollination System Geitonogamy

The results on the percentage of flowers that form geitonogamy fruit are presented in Table 3, and the diversity produced in the fraction that formed fruit is thought to be due to the flower location. These were located close to the pollinator, enhancing the formation of more fruits, as a result of the increased chance pollination, while the sample flowers located at some distance from a little fruit or none at all. This occurred because the pollen does not reach the sample flower, resulting in pollination difficulties. Besides, a variation is observed in the gambier flower bloom due to the occurrence on different days. Based on this examination, it is assumed that the sample flowers did not pollinate because blooming took place at the end of the receptive stigma. Environmental factors [wind] help in geitonogamy pollination systems and its speed affects the movement of pollens, as stronger winds facilitate the process.

| Sample number | Total flower formed fruit per cluster | Fertilization [%] |
|---------------|-------------------------------------|-----------------|
|               | 1 2 3 4 5 6 7 8 9 10                |                 |
| 1             | 3 - 5 - - - - - -                   | 16±1.75         |
| 2             | - 1 1 1 - 5 - - -                   | 18±1.52         |
| 3             | - - - 5 - - - 5 - - - 5             | 20±2.10         |
| 4             | - - - - 5 - 5 - - - - - 5           | 26±2.16         |
| 5             | - - - - - 5 5 - - - - - -           | 30±2.41         |

Average of percentage fertilization 22±1.99

The fruits formed are seen in Figure 2, and the average percentage of fertilization in geitonogamy gambier flowers is 22%, and it is assumed that the plants possess the capacity to self-pollinate.
Figure 2. The fruit formed self-pollination geitonogamy, [a] 1 fruit formed, [b] 5 fruits formed, [c] 5 fruits formed

3.2. Cross-Pollination System

3.2.1. Natural Cross-Pollination System

Data obtained from the observation of fruit formed in the natural cross-pollination system are presented in Table 4. and it is seen that the average percentage of flowers that form fruit is 51.2%. This was observed to be far greater than the geitonogamy fertilization in self-pollination system at 22%, due to numerous factors that assist in natural cross-pollination, as the flowers tend to be open and free.

Plant sample No. 4 displayed the least amount of fruit formed, due to the implementation at varying times, consequently different environmental conditions, and a smaller number of sources, resulting in less fruit formed. Generally, wind speeds blow off pollen, and the occurrence of rain influence the flying activity of some insects, and the abundant flowering attracts visitation to flowers and vice versa. Besides that, the availability of pollen resources is also an important factor in fruit production.

Table 4. Percentage fruit formed of natural pollination system

| Sample number | The mount flower formed fruit per cluster | Fertilization [%] |
|---------------|------------------------------------------|------------------|
|               | 1 2 3 4 5 6 7 8 9 10                     |                  |
| 1             | 5 5 5 5 5 5 - -                         | 70±2.41          |
| 2             | 5 5 5 5 5 2 1 1 2 -                     | 62±2.07          |
| 3             | 1 2 - 1 3 2 2 - 3 2                     | 32±1.07          |
| 4             | - 1 1 1 - - - -                         | 8±0.51           |
| 5             | 5 4 4 4 5 5 5 5 - 5                     | 84±1.54          |

Average of percentage fertilization 51.2±1.52

Therefore, the fruit formed from the natural cross-pollination system is seen in Figure 3.

Figure 3. Fruit formed of natural pollination system, [a] 1 fruit formed, [b] 2 fruits formed, [c] 3 fruits formed, [d] 4 fruits formed, [e] 5 fruits formed.
3.2.2. Artificial Cross-Pollination System

The average percentage of artificial pollination observed was 86%, which is higher than the geitonogamy self-pollination, at 22% and natural cross-pollination at 51.2%.

A major consideration is needed in artificial crosses, to adjust the flowering time of the elders used, and the process possibly ensues in the presence of matching flowering time. This is the reason pollen cooking and receptive stigma occur simultaneously. Before artificial crossing, it is important to note the source of pollen from male elders, which is influenced by the wind speed, as smaller sizes are moved more easily, and it is also important to note the stigma receptive period length.

On observation, the percentage of gambier plant flowers that form fruit after artificial cross-pollination is presented in Table 5.

**Table 5. Percentage fruit formed of artificial cross-pollination system**

| Sample number | The mount flower formed fruit per cluster | Fertilization [%] |
|---------------|------------------------------------------|-------------------|
| 1. [U×U]     | 5 - 3 5 5 5 5 5 5 5 5 5 86±1.63         |
| 2. [U×C]     | 5 5 - 5 5 5 5 5 5 5 90±1.58             |
| 3. [C×R]     | 4 5 5 1 2 5 4 5 5 5 82±1.44             |
| 4. [C×C]     | 5 5 5 - 5 5 5 2 - - 64±2.39             |
| 5. [U×R]     | 5 5 1 5 5 5 5 5 5 5 92±1.26             |
| [R×R]        | 5 5 5 5 5 5 5 5 5 5 100±0                |

The largest proportion was identified in sample number 6, which is a cross between two Riau type gambier and all the crossed flowers successfully form fruits. This was in contrast with the results of Silfia’s research [3], which stipulated that the Shrimp type possesses the highest average amount of pollen, and sample number 4 formed the smallest percentage of fruit. This occurred as a result of a crossing with two Cubadak gambier types at 64%, which is in line with the report by Silfia [3], stipulating that it contains the lowest amount of pollen. However, the success factor of artificial crosses is seen from the amount of pollen obtained from the male elders, and other environmental and genetic factors that influence the number of fruit formed.

Percentage fruit formed of natural pollination system is lower than Percentage fruit formed of artificial cross-pollination system. The causes are the availability of viable pollen, optimal pollination methods and anthesis, weather support [14] [16] [17] [18]. Pollination is assisted by humans so that the optimal amount of pollen and make more fruit formed. At the natural pollination system, It doesn’t know when pollination will occur. Wind-pollinated may occur during or past the anthesis. Fertilization occurs when pollen and pistil anthesis. Pollen viability is influenced by water evaporation, changes in temperature, humidity. Endurance of pollen in each species is different; several hours, several months, and several years [18] [19] [20] [21].

Other factors include the treatment techniques adopted, climate, as well as the amount and freshness of pollen. Besides, the genetic influences originate from the plant itself, alongside parental compatibility, which is related to the genes contained in male and female parents. Besides, the treatment technique that caused a lower percentage of fruit formed was presumably due to a large number of flower treatments for the process of artificial cross-pollination, encompassing gold circulation, castration, entrapping, and artificial pollination itself. Gambier plant flowers were soft and observed to exist in small sizes, hence highly sensitive to numerous treatments. Syukur et al., [14] reported the occurrence of pistil [stigma] maturity on instances where mucus secretion contains a
solution of sugar and other substances needed for the germination of pollen, which facilitates stickiness. However, before the crossing incidence, the exposure to rainwater and slime also reduced, and this affects the freshness of pollens used. and the success of artificial crosses is seen in Figure 4.

![Figure 4](image)

**Figure 4.** Fruits formed from the results of artificial cross-pollination systems, [a] 1 formed, [b] 2 formed, [c] 3 formed, [d] 4 formed, [e] 5 formed

From these observations, it is seen that gambier plants do cross-pollination, although they possess the ability to self-pollinate on the same plant flowers [geitonogamy]. This is in line with the information obtained from farmers of the Shrimp variety that tend to produce other varieties of seeds [Cubadak and Riau] and vice versa. Furthermore, the occurrence was because gambier cross-pollinates, enabling the seeds produced in one plant to exhibit numerous varieties.

The implementation of control is aimed at observing the plant's ability to fertilize without being pollinated by pollens. However, after opening the lid, none of the flowers forms a fruit, as the pistil which initially turns green became brown and falls, certifying its known-apical nature. Besides, the genes for sexual reproduction are not expressed in apomixis plants, fostering the requisition for pollination and fertilization in the seed formation process.

4. Conclusion

Based on the results, it is concluded that gambier plants undergo cross-pollination, as seen from the percentage of fertilization that occurred, being 86% for artificial, and 51.2% for natural. Furthermore, the percentage fertilization in the autogamous and geitonogamy self-pollination system was 0% and 22%, respectively.

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