Self-Pollinated (Geinotogami) of *Uncaria gambir* (Hunter) Roxb. Type Udang

ABSTRACT

Aims: The research aimed to study the ability of natural and artificial self-pollinated (Geitonogamy) of gambir.

Study design: Experimental method consisted of determining the sample plants. Sampling determination was conducted by *non probability sampling, purposive sampling*.

Place and Duration of Study: This research was conducted in the UPT Experimental Garden of the Faculty of Agriculture, Andalas University, Padang from September 2019 to December 2019

Methodology: an experimental method consisted of determining the sample plants, conducting natural and artificial geitonogamy self-pollinated systems, observing sample plants, collecting data based on the experiment, and sample-testing in the laboratory. Sample determination selected by non-probabilistic sampling with purposive sampling method. Observation included percentage of pollination, percentage of fertilization, number of fruit formed, pollen fertility and sterility

Results: Data observations were analyzed with simple statistics. The results showed that (1) the percentage of pollination through the geitonogamy natural pollination system of udang gambier type was 89.30%, (2) the percentage of fertilization in the geitonogami's own natural pollination system was 10.68% and artificial was 27.5%, (3) the average percentage of pollen fertility was 56.56% and the pollen sterility was 43.44% tested with the bromothymol blue staining method.

Conclusion: Artificial geitonogami self-pollination was potential to develop for gambir cultivation

Keywords: Catechin, fertilization, gambier, geitonogami, self-pollinated

1. INTRODUCTION

Gambir (*Uncaria gambir* (Hunter) Roxb.) is high valuable Indonesian crops. This plant is classified to shrub plant, vine and wooden [1]. Twig and leaves extract contains catechins, tannin, flouresins, catechutants, fats, mucus, quercetin and waxes. From these chemical compounds, catechin and tannin are widely used [2]. The reason of gambir is widely cultivated due to this plant has many advantages such as fabric dye in *batik* industri, prevention of skin root and elasticity, material for cosmetic industry and lotion in India and France. Furthermore, in medical, gambir can be used for medicine material for cough, burn, dysentery, hemorrhoids, diarrhea and sore throat in Malaysia and also anti nicotine candy in Japan [3].

West Sumatera is one region in Indonesia which gambir is widely cultivated. According Statistic data of West Sumatera, the gambir area in this province increased 48.5% from 21,411.5 ha in 2012 to 31,791.25 in 2016. Unfortunately, the production just increased 19.8% from 14,220 ton to 17,036.03 ton [4]. However, 80% of Indonesian gambir export from West Sumatera with the main destination countries are India, Pakistan, Singapura, Thailand and Malaysia [5].

West Sumatera gambier is potential to develop because many benefits have been found from gambir and Indonesia is main exporter country of the world [6]. The other advantage of gambir than other crop plantation is the plant can adapt in marginal and sulphur land. Unfortunately, the productivity of gambir is still low. One of cause of the condition is the use of superior variety in plantation so that the yield quality is not suitable for international standard. One of effort to solve the problem is build up the pure strain through plant breeding [5]. This pure strain will be selected to obtain cross material to build up the superior variety.

The formation of pure strain is started from avoiding the genetic mixture that caused by crossed pollination of gambir. A research reported that plant reproductive organ of gambir is classified to crossed pollination system. Another

report support this result is according the pollen/ovule (p/o) ratio. Crossed pollination plants cause random pollination so that the population is heterozygote [7].

The possible way to obtain pure strain is geitonogamy self-pollinated process. Gambir is a plant that quite high potential to do self-pollinated naturally. Geitonogamy self-pollinated is sticked pollen of one reproductive organ to stigma of another in same plant. This type is commonly found in hermaphrodite plants. Autogamy self-pollinated can also occur even though the stigma position is higher than pollen. Lopita (2018) [8] reported that according the analysis percentage of fertilization in autogamy self-pollinated, no fertilization result can be obtained. Otherwise, in geitonogamy self-pollinated, 22% of fertilization succeeded. This result revealed gambir plant can do self-pollinated. The result aimed to study the ability of natural and artificial self-pollinated (geitonogamy) of gambir.

2. MATERIAL AND METHODS

2.1 Experimental site

This research was conducted in the UPT Experimental Garden of the Faculty of Agriculture, Andalas University, Padang from September 2019 to December 2019.

2.2 Method

An experimental method consisted of determining the sample plants, natural and artificial geitonogamy self-pollinated systems, observing sample plants, collecting data based on the experiment, and sample-testing in the laboratory. Sampling determination was conducted by *non probability sampling, purposive sampling*. The plant material was gambir type Udang. The age plant was 8 weeks and undergoing flowering process. Natural self pollinated geitonogami required five plants and artificial geitonogami self-pollinated required one plant.

2.3 Procedure

For each sampling plant, eleven clusters were chosen for natural geitonogami self-pollinated and eight for artificial geitonogami self-pollinated. Big bud stage (F2) was chosen as cluster that was started since emergence of a flower crown from ovary to opening of flower crown which is the beginning of opened flowering stage (F3). From natural self-pollinated stage, twenty five single flower were chosen from each cluster, meanwhile from artificial self-pollinated, five single flower were chosen from each cluster. One cluster was chosen from the plants that have conducted natural and artificial self-pollinated. In that cluster, four single flower were chosen in opened flowering stage (F3) to examine fertility and and sterility of pollen by using *bromothymol blue* coloring method. The observation was conducted under light microscope with 40 times magnification, fertile pollen looked blue for whole part and sterile pollen looked yellow, clear and pale blue. The observed parameter included pollination percentage, fertilization percentage, amount of formed fertilization, fertility and sterility of pollen.

3.RESULTS AND DISCUSSION

3.1. Percentage of pollination

According to the percentage of pollination, the potency of gambir pollination was 89.30% (Table 1). Event though this result showed the promise result, but it can be referred the gambir is self-pollinated plant. This condition caused by in pollination that was conducted in this research by covering whole plant using thin fabric so that stigma can be pollinated by pollen of another flower both of same and different cluster [7]. Single flower that was successfully pollinated by pollen marked by the pollen in stigma (Figure 1.b) while if the pollen failed to pollinate the stigma could be marked by the clear stigma from pollen (Figure 1.b). Fitri (2012) [9] reported that gambir could conduct pollination in different flower in same cluster.

Table 1. Percentage of pollination of natural geitonogami self-pollinated

Sampling	Number of pollination											Average of pollination (%)
	1	2	3	4	5	6	7	8	9	10	11	
A1	68	96	80	76	88	100	100	52	80	100	76	83.27
A2	100	100	96	92	100	80	76	100	100	100	100	94.90
A3	100	60	100	44	100	84	16	100	100	72	100	78.54
A4	100	100	100	88	88	84	60	84	84	100	100	90.18
A5	100	100	100	96	100	100	100	100	100	100	100	99.63
Average of pollination (100%)												**89.30**

Gambir reproductive organ is classified to *inflorencetial*/*cluster*. From reproductive organ structure, gambir is classified to complete flower. This type is hermaphrodite due to male and female structure in same place so this plant can conduct geitonogami self-pollinated [7].

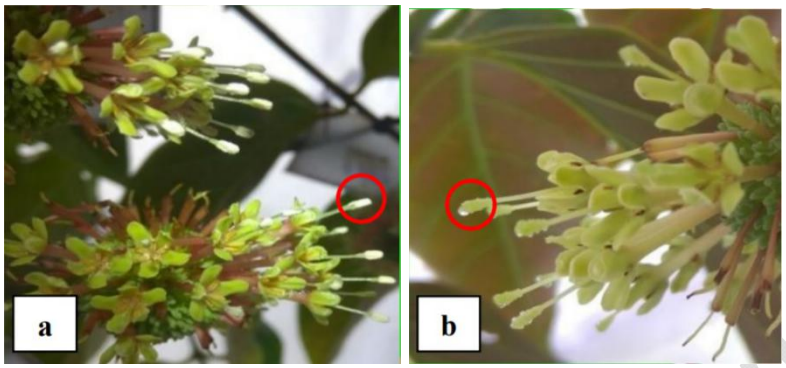

Figure 1. a) Pollinated flower that marked by pollen in stigma, b) unsuccessful pollinated flower by pollen

3.2 Percentage of fertilization

Isolation was conducted early by covering entire plant. After a week, flower structure such as petal, anther and stigma turned wilt and flower structure gradually fell and just ovary remained. If the fruit formed, ovary became bigger and long, meanwhile ovary that could not form fruit could be marked by brown color and finally fell down so that the fruit formation failed. The formed fruit then counted and analyzed including the fertilization percentage of natural and artificial geinotogami self-pollinated (Tabel 2 and Table 3). According the result, the percentage of pollination of natural geitonogami self-pollinated was 10.68%. This result caused by the occurrence of pollination of natural geitonogami self-pollinated was unknown due to pollen just relied wind flow as pollination vector [10]. This condition caused the possible of pollination could occur in anthesis and post-anthesis stage. If the process occurred in anthesis stage, the source of pollen that stick in stigma was still viable due to anther broke firstly and possibility of formed fruit bigger. Otherwise, if the pollination occurred in post-anthesis, the sticked pollen in stigma had low viability due to affected by environmental factor. Survival ability of pollen is different for each species [11].

Table 2. Percentage of fruit formation of natural geitonogami self-pollinated

Sampling	Number of flower that formed fruit per cluster											Average of fertilization (%)
	1	2	3	4	5	6	7	8	9	10	11	
A1	0	0	0	0	88	100	100	0	0	0	0	26.18
A2	100	16	0	0	0	0	20	52	0	4	60	25.45
A3	0	0	0	0	0	0	0	0	0	0	0	1.09
A4	0	0	0	0	0	0	0	0	0	0	0	0.72
A5	0	0	0	0	0	0	0	0	0	0	0	0
Average of pollination (100%)												10.68

For result of artificial geitonogami self-pollinated, the percentage of fertilization was 27.5%. This result explained the artificial geitonogami self-pollinated is better than natural geitonogami self-pollinated. Several factor affected the formation of fruit in gambir plant through artificial geitonogami self-pollinated such as genetic, treatment technique, climate, number and viability of pollen. For genetic factor, parental viability was main factor due to its relationship to male and female genes of parental. Treatment technique caused low fruit formation due to many treatments were conducted to flower for pollination process such as emasculation, castration, material covering and even pollination [7] [10]. Meanwhile, the size of gambir flower is small and soft so that it is very sensitive if many treatments were conducted. This affected the fertilization successful. Climate condition such as rainfall is the limited factor for artificial pollination process. This condition was obtained when artificial pollination process occur where the rainfall cause the pollen was difficult to stick in stigma. Wet pollen caused the pollen mucus decreased. Syukur et al. (2015) [12] stated that mature stigma released mucus that contained glucose and other chemical compounds that required for pollen germination. This mucus made the pollen stuck in stigma.

Table 3. Percentage of fruit formation of artificial geitonogami self-pollinated

Cluster	Number of formed fruit per cluster	Fertilization (%)

1	-	0
2	-	0
3	2	40
4	5	100
5	-	0
6	4	80
7	-	0
8	-	0
Average of fertilization		27.5

Note : (-) the fertilization did not occur

Another hand, number and viability of pollen were main factor to support fertilization in artificial pollination. This condition was caused by more number of pollen enlarged possibility of fertilization. Furthermore, pollen viability should be noticed due to even though number of pollen is widely available, but if pollen viability is low, the fertilization can not occur [3]. This condition was caused by in fertilization, the pollen should have good fertility. The value of pollen fertility is directly proportional to viability value because high viability value affects the fertilization. This was proven by the fertilization percentage of artificial geitonogami self-pollinated was higher than natural geitonogami self-pollinated [10].

In artificial geitonogami self-pollinated, number and viability of pollen were not obstacle because source of pollen was collected in first anthesis flower. Therefore, source of pollen that was lubricated in different stigma had good viability. Other than that, pollen stickiness process to stigma was helped by human so that the number of lubricated pollen to stigma was optimal and caused the level of fertilization was higher. Meanwhile, in natural geitonogami self-pollinated, pollination process was unknown because this process just relied wind flow [7]. This condition could occur in anthesis or post-anthesis. If pollination process occurred in anthesis, the fertilization occurred due to it became from viable pollen. Meanwhile, if the pollination occurred in post anthesis, the percentage of fertilization was lower due to the viable of stuck pollen was low. Low viable pollen caused low pollen fertility. The reduction of pollen viability was caused by the death of most pollen that underwent water evaporation, consequence of temperature and humidity in opened environment. Different pollen survival for different species [10].

3.3 Fertility and sterility of pollen

Pollen fertility is pollen ability to pollinate. The fertilization could occur if pollen had high fertility. Fertility value was correctly proportional to pollen viability. High viability affected cross pollination successful. According the data, pollen fertility was 56.56% and pollen sterility was was 43.44% (Figure 2). Udarno and Seytono (2013) [3] stated that the pollen fertility of gambir type Udang was 95.8%. According the data, the pollen fertility of the research was lower than previous research. The low fertility was caused by the pollen was collected in 7 am in the morning meanwhile the pollen was available in few hours after anthesis period, 6 pm to 5.00 am. The pollen storage was not conducted in the research. This condition caused the sampling pollen was susceptible to death. The reduction of pollen fertility caused by the death of most pollen that underwent water evaporation. Perveen (2007) [13] stated that humidity was the main factor affected pollen fertility.

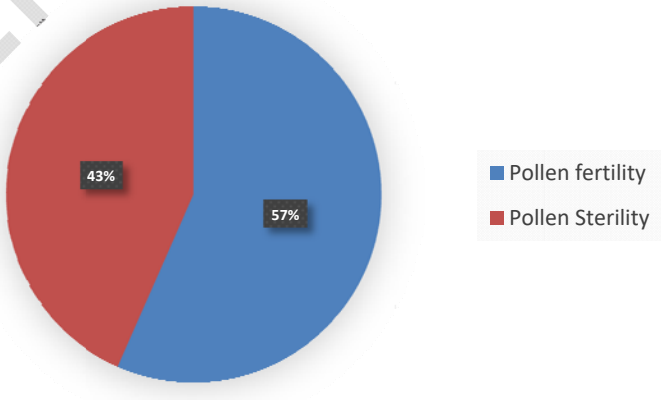

Figure 2. Average of pollen fertility and pollen sterility by using bromothymol blue method

Effect of environmental temperature caused the degradation of pollen fertility due to the change of macro molecule to simple molecules. Carbohydrate such as starch became sucrose cause the starch content decreased. Fertile pollen contained 5% sucrose, but if the environmental temperature increased, the sucrose content became 12% [14]. Fructose

played role in respiration process meanwhile formed glucose was used for energy availability. High respiration caused food storage lost in pollen and it caused the pollen became sterile. Coloring method was used for examining pollen fertility through nutrients content approach in pollen. If it was sufficient, it was assumed as fertile pollen (Figure 3). Nutrient content was detected by using *bromothymol blue.* Actually, the tested component was callose compound in wall tube of pollen.Lersten (2004) [15] stated that callose was carbohydrate that separated main cell micro spore from another and covered pollen after meiosis process. Pollen turned dark blue if contained callose. Callose content showed fertile pollen meanwhile yellow, clear or pale blue of pollen showed sterile pollen.

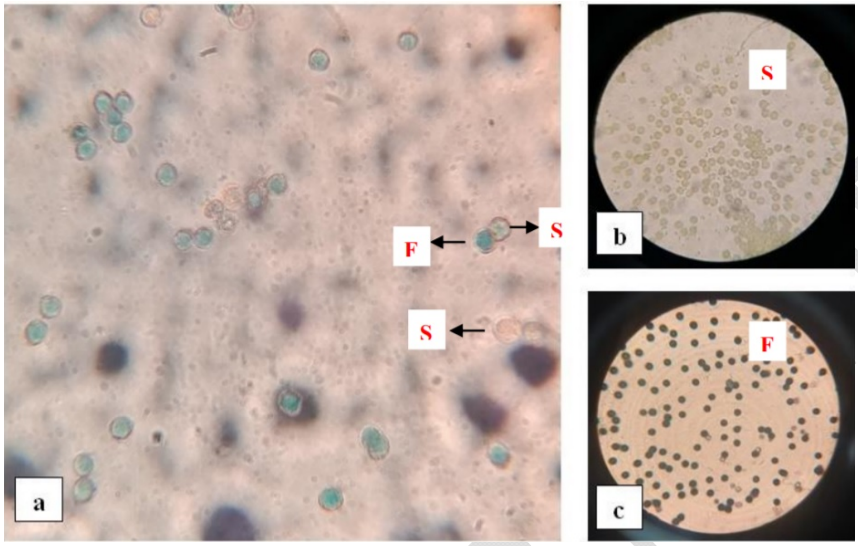

Figure 3. Pollen of gambir type Udang by using *bromothymol blue* coloring method. (a) Fertile and sterile pollen, (b) sterile pollen, © Fertile pollen. Note : F= Fertile, S = Sterile

4. CONCLUSION

Pollination Percentage of natural geitonogami self-pollinate was 89.30% and it indicated the pollination percentage was high. Natural geitonogami self-pollinated fertilization was 10.68 and for artificial geitonogami self-pollinated was 27.5%. Percentage of pollen fertility was 56.56% and pollen sterility was 43.44% by using *bromothymol blue* method.

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