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Interspecific and intraspecific cross-compatibility of *Durio kutejensis* and hybrid *Durio zibethinus x kutejensis*

RUDARMONO[1,♥], NOER RAHMI ARDIARINI[2], BUDI WALUYO[2], SUMERU ASHARI[2]

[1]Department of Agroecotechnology, Faculty of Agriculture, Universitas Mulawarman. Jl. Pasir Balengkong, Kampus Gunung Kelua, Samarinda 75123, East Kalimantan, Indonesia. Tel./fax.: +62-54-749159,♥email: rudarmono1@gmail.com

[2]Department of Agronomy, Faculty of Agriculture, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia

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Abstract. *Rudarmono, Ardiarini NR, Waluyo B, Ashari S. 2022. Interspecific and Intraspecific cross-compatibility of* Durio kutejensis *and* hybrid Durio zibethinus x kutejensis. *Biodiversitas 23: 1837-1843.* Lai (*Durio kutejensis*) and Mandong (*Durio zibethinus x kutejensis*) fruits that have advantages and high consumer preference values. Fruit productivity and development of Lai and Mandong plant varieties are still not optimal. The aim of this study was to analyze the level of incompatibility of interspecific and intraspecific crosses between Lai (*D. kutejensis*) and Mandong (*D. zibethinus x kutejensis hybrid*) plants. Artificial crosses were made utilizing flower samples from Mahakam, Kutai, and Batuah types of Lai plants, as well as Mandong varieties of Mandong, employing a scheme of 20 crossings that included a combination of these varieties (12 treatments), as well as each variety from the same flower and tree (8 treatments). The intensity level and incompatibility classification were determined according to Wang's (1963) method, which was based on the number of fruits formed from artificial crosses, and the pollen incompatibility intensity level (IIL) was determined according to the ratio of flowers that produced fruit from the cross to the total number of flowers crossed. The results showed that overall crosses of the same flower (selfing) from three varieties of Lai and one variety of Mandong were incompatible. Lai Mahakam x Lai Batuah crosses yielded 14.28 % harvested fruit, Lai Mahakam x Lai Mahakam crosses yielded 18.75 % harvested fruit, Lai Kutai x Lai Kutai crossings yielded 14.81 % harvested fruit, while a cross of Durian Mandong x Lai Batuah yielded 16.67 % harvested fruit. It shows partially compatible. The complete compatibility value showed the number of harvested fruit ranged from 23.44 to 46.77%, with the most significant being obtained from a Lai Mahakam x Durian Mandong cross. The Mahakam, Kutai, Batuah, and Mandong varieties are compatible with all pollen sources.

Keywords: Incompatibility, interspecific crosses, intraspecific crosses, Lai, Mandong, selfing

INTRODUCTION

East Kalimantan, one of the areas with a tropical rainforest climate, has a very diverse source of biological diversity in the form of germplasm and genetic resources (SDG), including plants from the genus Durio, which are native to Indonesia. The potential and biological richness of the Durio genus found in this area need to be preserved and appropriately managed to benefit the community.

The genus Durio has a high level of diversity, and according to Brown (1997), the Durio genus is estimated to be as many as 28 species in the world. Of these, there are 19 species scattered in the Kalimantan region. There are seven edible species among the 19 existing species (Gadug and Voon 2000). Several Durio species that have been identified and well-characterized are *D. zibethinus, D. kutejensis, D. graveolens, D. excelsus, D. dulcis, D. oxleyanus, D. acutifolius, D. affinis, D. beccarianus, D. crassipes* (Kostermans and Reksodihardjo 1958). Meanwhile, some edible Durio species are classified as rare, namely *kerantungan (D. oxleyanus), Lahong (D. dulcis), Lai* (*D. kutejensis*), and *Mandong (D. zibethinus x kutejensis)* (Saichol et al. 2020).

Lai fruit (*var. Mahakam, var. Kutai*, and *var. Batuah*) and Mandong fruit are widely known only by consumers of local people of East Kalimantan. Lai and Mandong fruits have a relatively high consumer preference and selling value (equivalent to other large local durians). It is because these fruits have advantages: sweet flesh taste, not fibrous, not pungent aroma, thick flesh, yellow-orange (strong orange-yellow) to slightly reddish-orange (moderate reddish-orange). The texture is relatively soft, and the content of alkaloid compounds is low. Mandong (*D. zibethinus x kutejensis*) is a natural cross between *D. Zibethinus* (*Durian*) and *D. Kutejensis* (Lai) (Sunaryo et al. 2015a), so it has fruit performance which is a combination of durian and Lai plants. Unlike *Durian*, *Mandong* has no unpleasant odor and long shelf life (10-15 days); therefore, it is popular with consumers and usually wins *Durian* contests. People enjoy *Mandong* because of its peculiar flavor, sweetness, soft texture, and dark yellow color (Sunaryo et al. 2015a; Sunaryo 2015b). The main characteristics of *Lai* and *Mandong* fruit are very easy to distinguish, i.e., yellow skin of the ripe fruit, rounded to elongated shape of the fruit with slightly angular (like star fruit) with slightly sharp angles to obtuse angles (Rudarmono and Rusdiansyah 2004; Rudarmono 2012).

Research and development efforts for *D. kutejensis* and *D. zibethinus x D. kutejensis* species are still relatively few and not optimal, due to the limited collection of genetic resources for superior varieties, limited duplicates of labeled parent trees, and relatively low fruit productivity.

Fruit productivity of the *Durio* spp group is a combination of the balance of external and internal plant factors to start flowering and fruiting involving environmental factors, and the hormones auxin, giberrellin, and cytokinins (Ashari dan Wahyuni 2012; Jawal et al. 2013; Fenn and Giovannoni 2021), sufficient to meet the nutritional and water needs of the plant in the soil (Didik 2018), the availability of efficient pollinators (Baqi et al. 2022), and the suitability of the environment/microclimate for plant growth and fruiting. Low fruit productivity is also caused by the relatively high intensity of flower and fruit shedding and abnormal fruit formation. Seasonal, fluctuating, and unstable fruit productivity is influenced by climatic factors and the ineffectiveness of natural pollinators. In addition, morphological factors and plant genetic factors are also the cause of the low productivity of durian fruit. Most of the Durio genera have different incompatibility traits (Indriyani et al. 2012). Incompatible means the incongruity of the pollen with the stigma inhibiting the pollination process in hermaphrodite plants, such as in the genus Durio.

Knowledge about the degree and mechanism of incompatibility in some *durian* species, especially *D. kutejensis* and hybrids *D. zibethinus x D. kutejensis*, including the factors that influence it, is still relatively limited. For the pollination process to produce flowers and fruit, insects or artificial pollination assistance is needed. This study aimed to analyze the interspecific or intraspecific cross-compatibility level between *Lai (D. kutejensis)* and *Mandong (D. zibethinus x kutejensis)* plants in increasing fruit productivity.

MATERIALS AND METHODS

Research place

The study was conducted during the flowering season of Mahakam (M), Kutai (K), and Batuah (B) *Lai* plants, as well as *Mandong* varieties of Mandong (Md), in the *Lai* and *Mandong* planting areas located in Samarinda City's administrative area, followed by research at the Laboratory Biotechnology, Faculty of Agriculture, Mulawarman University, Samarinda, Indonesia.

Crossing procedures

This research begins with exploration and identification activities to determine the location and diversity of Lai and Mandong trees used as research samples, then continues with the leading research activities, which include the following stages:

(i) Selection of female flowers used as parents based on the level of maturity of the flowers, by selecting normal and healthy flower buds which a few hours later are expected to fully bloom (anthesis);

(ii) The castration process is carried out in the morning or afternoon, 10-12 hours before the artificial pollination treatment, followed by labeling and isolation of the castrated flowers (wrapped in oil paper) so they are not pollinated by foreign pollen;

(iii) Preparation and selection of male elder flowers are carried out in the afternoon by selecting flowers that have just bloomed or flower buds that are expected to bloom at night. The flowers of the appointed male elders are cut placed in a container with a wet tissue at the bottom of the container to accelerate the rupture of the anther and the ripening of the pollen;

(iv) Conducting artificial pollination (crossing), carried out after the discharge of exudate or mucus on the stigma (observation with pocket loupe 25x magnification). Crossing activities in the field are carried out by (a) slowly unwrapping the castrated female flower so the pistil (style) is not broken or damaged, then attaching the anther to the stigma using a small soft brush. (b) repackaging female flower panicles that have been crossed with new oil paper.

Cross schema

Interspecies and intraspecies artificial cross-pollination treatment schemes are presented in Table 1.

Table 1. Treatment scheme of artificial cross-pollination

V_1 (M) (Female parent)	**V_2 (K) (Female parent)**
M x K	K x M
M x B	K x B
M x Md	K x Md
M x M (Same flower)	K x K (Same flower)
M x M (Same tree)	K x K (Same tree)
V_3 (B) (Female Parent)	**V_4 (Md) (Female Parent)**
B x M	Md x M
B x K	Md x K
B x Md	Md x B
B x B (Same flower /*Selfing*)	Md x Md (Same flower/*Selfing*)
B x B (Same tree)	Md x Md (Same tree)

Note: The tree used as research material comes from grafting seedlings whose source of entry comes from a single parent tree (SPT). V1…V4: Variety 1…4, M: Lai Mahakam, K: Lai Kutai, B: Lai Batuah, Md: Mandong

Cross compatibility measurement

The Incompatibility Intensity Level (IIL) was measured using Wang's (1963) approach, which was based on the number of fruits formed from crosses, then classified as follows: (i) Compatible if the artificial cross produces more than 20 % fruit; (ii) Partially Compatible if the cross produces fruit between 10-20 %; (iii) Fully Incompatible if the cross produces less than 10 % fruit.

The Incompatibility Intensity Level (IIL) of pollen or fruit formation of the sampled plant varieties (expressed in %) is determined based on the formula:

$$IIL = \frac{\text{the number of flowers produces fruit from the cross}}{\text{number of crossed flowers}}$$

RESULTS AND DISCUSSION

Samarinda has a climate type of A (very wet) and B (wet), with an average annual rainfall of 1500 - 2500 mm, an average daily air temperature of 27 ºC with a difference between day and night temperatures of 5-7ºC, and an average humidity reaches 83% (Bappeda East Kalimantan 2020). The climatic conditions of Samarinda have a significant influence on the flowering and fruiting processes of plants from the Durio spp. High rainfall intensity will inhibit the flowering process, causing flowers to fall and fruit to fall out (Shakidin et al. 2019). If the rainfall exceeds 150 mm per month for several consecutive months at the flowering time, it will affect the flower initiation process. The process of flowering and fruiting of plants of the Durio genus requires a dry season of 2-3 months or low rainfall intensity. Seng et al. (2020) added that the flowering process of the genus Durio could be stimulated by modifying the microclimate, namely a dry period of 18-20 days or a maximum rainfall of 1 mm per day, the temperature at night ranges from 20-23ºC and relative humidity 50-60%. In addition to climatic factors that affect the failure of the natural flowering and fruiting process, the incompatibility of Durio's generative organs causes pollination and fertilization to not take place.

The shape of the crown of the Lai plant is different from that of the Mandong plant. The Lai plant has a canopy shape like an umbrella, while the Mandong plant has a slightly tapered crown shape (Figure 1). *Durian* (*D. zibethinus*) is an annual plant with a roux-type growth model characterized by continuous monopodial orthotropic stem growth (Ken et al. 2019). The crown of the observed Lai and Mandong plants consisted of a rounded crown and a pyramid shape. The stem of Lai and Mandong plants were in three color categories, namely: brownish-gray, brown, and dark brown. Stem diameter will increase in size directly proportional to the availability of nutrients needed by plants (Gardner et al. 1991; Arumingtyas 2015).

Figure 1. The shape of the crown. A. Upper branching, B. Stem, C. Lai Mahakam variety. 1. Kutai variety, 2. Batuah variety, 3. Mandong variety. 4. Sample tree research. Samples of research trees are estimated to be more than 10 years old

The process of crossing is presented in Figure 2. Meanwhile, the results of crosses interspecifically (different species) and intraspecifically (same species) and the fruits from crosses formed before harvest until harvest time are presented in Table 1 and 2. The results of the cross were indicated by the value of compatible, partially compatible, and fully incompatible based on the percentage of fruit. Interspecies and intraspecies crosses are essential approaches to determine the level of incompatibility or compatibility of a variety (Zabicha et al. 2020). In addition, interspecific and intraspecific-species crosses are also intended to increase genetic diversity and assemble new genotypes in breeding programs.

Crosses of the same flower (selfing) from each of 3 (three) Lai varieties and 1 (one) Mandong variety showed 100% flower fall and were completely incompatible (Tables 1 and 2). All flowers from crosses fall before forming fruit. It is thought to be caused by several things, both pre-pollination and post-pollination, namely: (1) the inability of pollen to germinate on the stigma, (2) pollen germinates and forms a pollen tube but is unable to penetrate the stigma or tube. Pollen and 3) pollen can penetrate the stigma but cannot reach the ovule. The incompatibility process starts when the pollen attaches to the surface of the stigma. Still, the pollen is unable to germinate, even though the pollen is physiologically in a fertile condition (Lo et al. 2007). This condition is called cross incompatibility, caused by the pollen tube being unable to penetrate the stigma (Susanto 2012).

Furthermore, the inability of the pollen tube to penetrate the stigma causes fertilization to occur and the formation of ovules (Qosim 2013). In addition to the reasons above, based on observations, it was found that the flower pollen of the tested genus *Durio* was ripe before the stigma, which was characterized by the ease with which pollen was released from the pollen box. In contrast, the stigma was still closed (there is no liquid on the surface of the stigma). The liquid on the surface of the stigma (stigmatic fluid) captures and attaches pollen to the surface of the stigma and helps maintain pollen moisture as a medium to stimulate the pollen germination process (Jutamanee et al. 2017).

Partially compatible indicated by a cross of Lai Mahakam with Lai Batuah (V1 x V3) yielded 14.28 % of harvested fruit, a cross of Lai Mahakam with Lai Mahakam (V1 x V1) from the same tree yielded 18.75 % harvested fruit, a cross of Lai Kutai with Lai Kutai (V2 x V2) from the same tree yielded 14.81% of harvested fruit and treatment of Mandong with Lai Batuah (V4 x V3) yielded 16.67% of harvested fruit. Crosses showing Partially compatible or pseudo compatible, previously produced a relatively high percentage of fruit 1-5 cm in diameter (fruit formed before harvest) above >20% (range 22.45-75.00%), as shown in Figure 3, subsequently fell off before harvest due to abnormal fruit growth. The low percentage of natural durian fruit formation is due to incompatibility factors (Seng et al. 2020). In addition, it is also caused by differences in flower morphology (flower sex organs) of each species. The Durio plants observed had different flower morphology (phenotypic), particularly the length of the stalk, which differed *D. kutejensis* from *D. zibethinus x kutejensis*. Plants of the Durio genus have a hermaphrodite but protandry capitula, which causes self-incompatibility, namely the inability of the plant to form seeds and fruit because the anther matures earlier than the stigma (Baqi et al. 2022). Furthermore, the Durio genus, especially the *Durio zibethinus* species, has incompatibility properties in each of its varieties, so that the ability of pollen to be compatible with the pistil of each durian species is also different (Indriyani et al. 2012). Incompatibility factors in *durian* can be minimized by performing artificial cross-pollination (Qosim 2013).

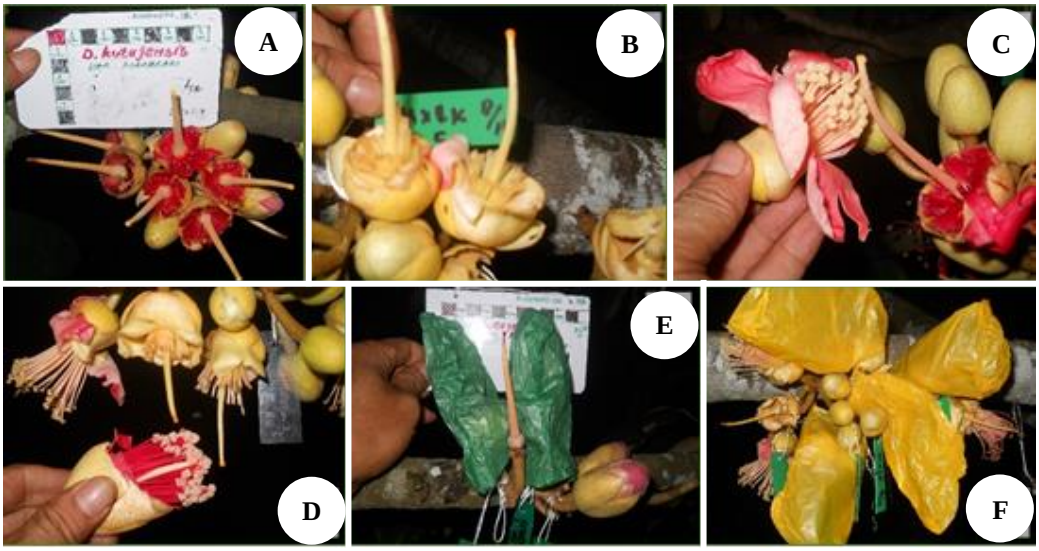

Figure 2. Crossing process. A. Lai flower castration, B. Mandong flower castration, C. Lai anther with Mandong pollen, D. Mandong anther with Lai pollen, E-F. Flower isolation after crossing

Table 1. Fruits from crosses formed before harvest

Cross combination		Set of cross	Falling flowers		Fruit formed before harvest	
			Total	Percentage	Total	Percentage
V1 (M)	M x Md	62	18	29.03	44	70.97
Lai Mahakam	M x K	56	25	44.64	31	55.36
	M x B	49	38	77.55	11	22.45
	M x M (Same plat)	48	12	25.00	36	75.00
	M x M (Selfing)	35	35	100	0	0
V2 (K)	K x Md	67	25	37.31	42	62.69
Lai Kutai	K x M	52	27	51.22	25	48.08
	K x B	32	17	53.12	15	46.88
	K x K (Same plant)	54	26	48.15	28	51.85
	K x K (Selfing)	48	48	100.00	0	0
V3 (B)	B x Md	38	20	52.63	18	47.37
Lai Batuah	B x M	36	11	30.56	25	69.44
	B x B	31	14	45.16	17	54.84
	B x B (Same plant)	24	9	37.50	15	62.50
	B x B (Selfing)	20	20	100	0	0
V4 (Md)	Md x M	71	29	40.85	42	59.15
Mandong	Md x K	74	36	48.65	38	51.35
	Md x B	24	18	75.00	6	25.00
	Md x Md (Same plant)	64	25	39.06	39	60.94
	Md x Md (Selfing)	34	34	100.00	0	0

Table 2. Fruits formed until harvested from crosses

Cross combination		Set of cross	Falling flowers and fruits		Fruit that is developed until harvest		Description
			Total	Percentage	Total	Percentage	
V1 (M)	M x Md	62	33	53.23	29	46.77	Compatible
Lai Mahakam	M x K	56	32	57.14	24	42.86	Compatible
	M x B	49	42	85.72	7	14.28	Partially compatible
	M x M (Same plant)	48	39	81.25	9	18.75	Partially compatible
	M x M (Selfing)	35	35	100	0	0	Incompatible
V2 (K)	K x Md	67	41	61.81	26	38.81	Compatible
Lai Kutai	K x M	52	39	75.00	13	25.00	Compatible
	K x B	32	24	75.00	8	25.00	Compatible
	K x K (Same plant)	54	46	85.19	8	14.81	Partially compatible
	K x K (Selfing)	48	48	100	0	0	Incompatible
V3 (B)	B x Md	38	26	71.05	11	28.95	Compatible
Lai Batuah	B x M	36	18	52.78	18	50.22	Compatible
	B x B	31	19	61.29	12	38.71	Compatible
	B x B (Same plant)	24	16	66.67	8	33.33	Compatible
	B x B (Selfing)	20	20	100	0	0	Incompatible
V4 (Md)	Md x M	71	45	63.38	26	36.62	Compatible
Mandong	Md x K	74	45	60.81	29	39.19	Compatible
	Md x B	24	20	83.33	4	16.67	Partially compatible
	Md x Md (Same plant)	64	49	76.56	15	23.44	Compatible
	Md x Md (Selfing)	34	34	100	0	0	Incompatible

Figure 3. Fruits with a diameter of 1-5 cm from a Lai and Mandong cross. A. Cross on the same tree, B. Lai x Mandong cross, C. Mandong x Lai cross

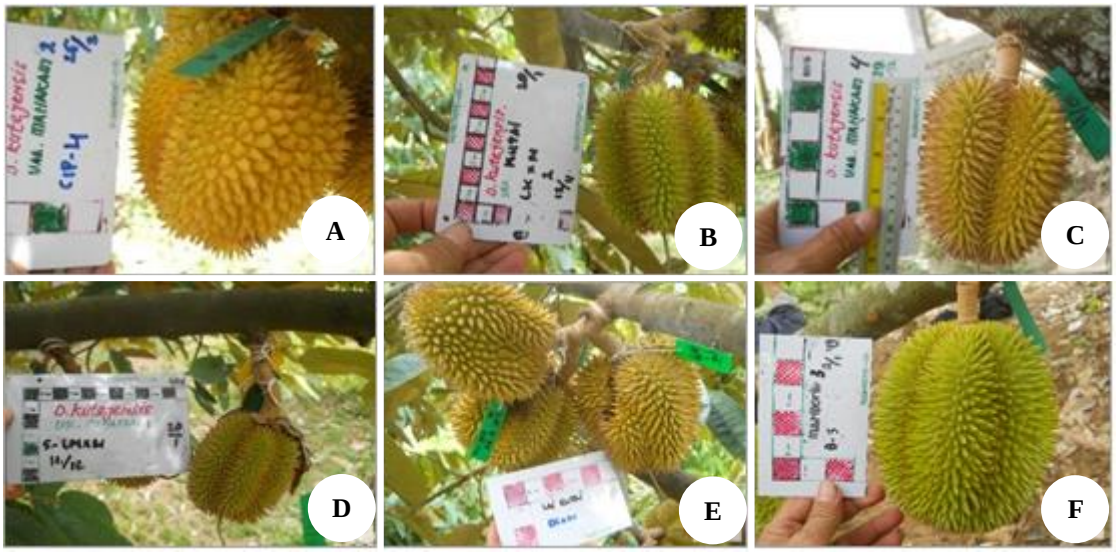

Figure 4.A. The fruit of a cross between flowers on the same tree, B. Lai Kutai x Mandong cross, C. Lai Mahakam x Lai Kutai cross, D. Lai Mahakam x Lai Batuah cross, E. Lai Kutai x Lai cross Batuah, F. The Mandong x Lai Batuah cross

Fruits with a diameter of 1-5 cm formed from the combination of interspecies and intraspecies reciprocal crosses have experienced high fruit loss, between 22.45-75.00%. The highest data on the rate of fruit loss comes from the pollination process of the same species but from pollen sources from different flowers. Plants that carry out open pollination tend to cause increased incompatibility traits that can result in abnormal fruit growth and subsequently result in fruit loss. Abnormal fruit growth and then fruit loss is thought to be caused by the inhibition of the cell development phase (mitosis) or endosperm, thereby reducing the quality of the fruit formed (Pancoro 2016: Lattier and Contreras 2017). The percentage of Durio fruit produced from self-pollination in nature (selfing) is only about 10% (Sakhidin et al. 2019; Mariani 2018). Artificial pollination or cross-pollination is necessary to improve the compatibility of plants of the genus Durio, and the results can be obtained by 50-75% of fruit (Husin et al. 2018).

The low percentage of flowers that can produce fruit on Durio plants is thought to be due to several factors, including low pollen viability, failure of the pollination process, when the pollen box (androecium) ripens, and the pistil (stigma) ripens at the same time. The length of the stamen (stylus) and the pistil (stamen). Abnormal pollen-tube growth causes fertilization failure and a mismatch between pollen and stigma (Seng et al. 2020).

The crosses carried out in this study consisted of interspecies (different species) and intraspecies (same species). The results of 6 combinations of reciprocal crosses between *D. kutejensis* and *D. zibethinus x kutejensis* were five cross combinations showing the classification of fully compatible and one cross combination with the category of Partially compatible, namely the Mandong cross (*D. zibethinus x kutejensis*) with Lai Batuah variety (*D. kutejensis*) as the pollen source. The full compatible value was indicated by the number of fruits ranging from 23.44-50.22% (Figure 4). The results of the cross showed that Lai Mahakam, Lai Kutai, Lai Batuah, and Mandong were compatible with all pollen sources. The highest fully compatible value (50.22%) was found in the cross treatment of Lai Batuah and Lai Mahakam (B x M).

Interspecies and intraspecies crosses of *D. kutejensis* and *Durio* sp. resulted in the compatible category of about 83.33% and in the partially incompatible category of 16.67% (Table 2). The results of this study are in accordance with the opinion that interspecies crosses

produce fully compatible, partially incompatible, and fully incompatible categories, depending on the pair of interspecies or intraspecies crosses (Warda and Waluyo 2020). The number of fruits formed until harvest from interspecies and intraspecies crosses of *D. kutejensis* (Lai) and *D. zibethinus x kutejensis* (Mandong) using a combination of reciprocal crosses indicates that the pollen source from the Batuah variety of the Lai plant is partially incompatible with the pistil of the Lai, Mahakam, and Mandong varieties.

In conclusion, the results showed that pollen viability in both Lai and Mandong plants was fertile. Meanwhile, the anthers on *D. kutejensis* and *D. zibethinus x kutejensis* flowers have matured earlier than the stigma. Flowers and fruit that fell from crosses between species *D. kutejensis* was 67.82% and interspecies *D. kutejensis* with *D. zibethinus x kutejensis* was 65.60%. Interspecific and intraspecific crosses of Lai with Mandong showed 8 (eight) reciprocal crosses in the compatible category and 2 (crosses) in the partially incompatible category, namely *Mandong* and *Lai Mahakam* against *Lai Batuah* varieties.

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