

# Genetic Compatibility Study With Various Pollination Methods On Three Types Of Lowland Passion Fruit (*Passiflora edulis*)

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## Abstract

This research aimed to study the genetic compatibility of three lowland passion fruit genotypes and to see the effect of various types of pollination on passion fruit characteristics. The research was conducted at the Research Station, Faculty of Agriculture, Andalas University, Padang, with an altitude of 168 m above sea level. The study was designed with a factorial complete randomised design (CRD) consisting of two factors. The first factor was the passion fruit type, which consisted of three levels, namely red and yellow passion fruit from *Passiflora edulis* f. *edulis* and yellow passion fruit from *Passiflora edulis* f. *flavicarpa*. The second factor is the type of pollination, which consists of four levels: artificial self-pollination, natural self-pollination, artificial cross-pollination, and natural cross-pollination. Each treatment combination was repeated three times, consisting of one pollination from three different trees. Results showed that the three types of lowland passion fruit have self-incompatibility, which resulted in the inhibition of the fruit formation process of natural and artificial self-pollination. At the same time, the results of natural and artificial cross-pollination showed high compatibility with fruit sets, each reaching 100%. Passion fruit type and pollination type showed no significant interaction on morphological characteristics and fruit flavour. However, passion fruit type had a significant effect on fruit characteristics and flavour. The yellow passion fruit type of *Passiflora edulis* f. *edulis* was superior to the red passion fruit type of *Passiflora edulis* f. *edulis* and the yellow passion fruit type of *Passiflora edulis* f. *flavicarpa*.

Keywords: cross-pollination, edulis, flavicarpa, self-incompability, self-pollination

## 1. Introduction

Passion fruit (*Passiflora edulis*) is one of the exotic fruits in Indonesia that has excellent potential to be developed commercially. The high nutritional content and diversity of health benefits make passion fruit one of the fruits that can be used as a functional food. Passion fruit extract is rich in carbohydrates, protein, fibre, potassium, magnesium, phosphorus, iron, sodium, calcium, zinc and various types of organic acids. (Ramaia et al., 2018; Ramaia, Bujang and Zakaria, 2018). In addition, more than 110 photochemicals have been identified from various parts of the passion fruit plant that have health benefits such as antioxidants, antihypertensive, antitumour, antidiabetic, hypolipidemic activity, analgesic activity, and anti-inflammatory (He et al., 2020; Dos Santos et al., 2021). The diversity of nutritional content and benefits of passion fruit opens up great opportunities for development in the food and pharmaceutical industries. However, various challenges, such as the limited growing environment of passion fruit, are the main obstacles in its development.

Generally, the types of passion fruit cultivated commercially are still limited to highland passion fruit. For example, purple sour passion fruit (*P. edulis* f. *edulis*) is widely cultivated to be processed into syrup as spread in the highlands of Karo, North Sumatra and Gowa, South Sulawesi (Zulfida & Rahmania, 2022), and konyal passion fruit (*P. ligularis*), which is found in the highlands of Alahan Panjang and Bukit Batabuah, West Sumatra, with an altitude of 1450 masl (Hayati, 2021). In comparison, passion fruit that is adaptive in the lowlands is from the forma *P. edulis* f. *flavicarpa* (Fischer & Miranda, 2021) which has a sour flavour. However, Sutoyo et al., (2023) reported that there is a natural cross of *P. edulis* f. *edulis* passion fruit that has

the ability to grow in lowlands and has a sweet flavour with TSS up to 19.6°Brix.

The existence of this lowland sweet passion fruit opens up opportunities for the development of high-yielding lowland passion fruit with a sweet flavour. However, special studies are needed regarding genetic compatibility as initial information in the variety assembly programme. Success in the formation of passion fruit is closely related to the right pollination process. In particular, the self-incompatibility of passion fruit flowers inhibits the process of self-fertilisation (Rêgo et al., 2013). So this plant generally has a type of cross-pollination that is assisted by wind or insects. Along with the decline in pollinating insect populations, this has become one of the most important threats to passion fruit production (Klein et al., 2007)

Studies related to the effectiveness of pollination in flavicarpa passion fruit have been conducted quite a lot. Barrera, Trinidad and Presas, (2021) reported that artificial pollination was proven to produce higher fruit set and better fruit quality compared to natural pollination assisted by *Xylocopa* spp. Meanwhile, Putra et al. (2023) reported that natural pollination assisted by insects produced higher fruit set, even though the quality of the fruit produced was the same as the results of artificial pollination. Thus, it is necessary to compare pollination methods and test genetic compatibility by selfing and crossing three types of lowland passion fruit so that the most effective and efficient pollination can be known to produce lowland passion fruit with superior quality.

This research aims to study the genetic compatibility of 3 types of lowland passion fruit and see the effect of pollination type on the quality of fruit produced by three types of lowland passion fruit.

## 2. Materials and Methods

The research was conducted from October 2023 to March 2024 at the Experimental Garden, Faculty of Agriculture, Andalas University, Padang (0°55'22.72" S, 100°27'17.60" E, 168 m above sea level). The materials used in this study were passion fruit plants propagated generatively from natural pollination of *P. edulis* f. *edulis* yellow owned by Kuswandi et al. (2021) in Solok, West Sumatra, and vegetatively propagated *P. edulis* f. *flavicarpa* plants, 0.1 N NaOH, distilled water, phenolphthalein indicator 1%, alcohol 96%, mask paper, pollination labels, and fruit nets. The equipment included a buret, hand refractometer, vernier calliper, digital scale, scissors, ruler, and writing utensils.

The research was designed using a factorial Completely Randomised Design (CRD) consisting of 2 factors, the first factor is the type of passion fruit, consisting of 3 levels, namely red passion fruit (M1) and yellow passion fruit (M2) of the *P. edulis* f. *edulis* type, and flavicarpa passion fruit (M3) of the *P. edulis* f.

*flavicarpa* type. The second factor was pollination type, which consisted of 4 levels, namely artificial self-pollination (P1), natural self-pollination (P2), artificial cross-pollination (P3), and natural cross-pollination (P4). Each treatment combination was repeated three times, each of which used one pollination from three different trees.

The experiment began with flower determination, emasculation, castration, pollination, and flower isolation. The flowers used were those in the large bud phase. Flowers in treatment P3 were emasculated using scissors on the day before the flowers bloomed at 5.00 pm. At the same time, the flowers in treatments P1 and P3 were castrated by removing the corolla and the additional corolla. Flowers that have been castrated and emasculated are then isolated by using paper masks, as well as in the P2 treatment. When the flowers had entered the blooming phase on the following day at noon, the flowers were pollinated by

hand (except P2 and P4). The P3 treatment was conducted by crossing one type with another, red type crossed with yellow type, yellow type crossed with flavicarpa, and flavicarpa crossed with yellow type. Treatment P1 was pollinated with pollen from its own flowers. Treatment P2 was left to pollinate itself because the stigma would touch the anther when the flower bloomed. Meanwhile, treatment P4 was left with open pollination. Afterwards, the pollinated flowers were isolated using a paper mask. Evaluation of the success of the cross was carried out at 5 days after pollination (DAP) by opening the paper mask. An enlarged ovary indicates successful pollination, while failed pollination is characterised by the drying and falling off ovary. Harvesting of pollinated fruits was carried out at 60-70 DAP with the criteria that the fruit skin had changed to yellow in *P.edulis f.edulis* yellow passion fruit and *P.edulis f. flavicarpa*, and red in *P.edulis f.edulis* red passion fruit.

Variables observed were fruit set (%), fruit weight (g), skin thickness (cm), number of seeds, edible portion (%), total soluble solid (°Brix), total titratable acid (%), and fruit sweetness. The total soluble solids

were observed using a hand refractometer by dripping passion fruit juice and looking at the value shown on the refractometer glass. While the observation of total titratable acid was done according to the procedure done Tadele et al. (2022), 10 grams of fruit juice was diluted with 250 mL of water. Then the solution was titrated with 0.1 N NaOH using 2-3 drops of 1% phenolphthalein as an indicator solution. Then the amount of NaOH used was recorded. The percentage of total acid titrated was then calculated using the following formula:

$$\text{TA} (\%) = \frac{\text{Volume titre} \times \text{Normality NaOH} \times \text{Meq of citric acid}}{\text{Volume of sample taken}} \times 100\%$$

Meanwhile, fruit sweetness was observed organoleptically using five panellists. The observed fruit flavour was divided into five groups: (1) very sour, (2) sour, (3) sweet-sour, (4) sweet, (5) very sweet. Observational data were analysed using the F test at a 5% level, followed by the DMRT test using Statistical Tools for Agricultural Research (STAR) software version 2.0.1.

### 3. Results and Discussion

#### Genetic Compatibility

The genetic compatibility of the three types of lowland passion fruit is determined based on the fruit set results of pollination types that have been carried out. The percentage of fruit set from each pollination treatment conducted on the three types of lowland

passion fruit showed consistently the same value (Figure 1). All types of passion fruit showed 100% fruit set value in artificial and natural cross-pollination treatments. Meanwhile, the artificial and natural self-pollination treatments showed a value of 0% because none of the selfing results succeeded in forming fruit.

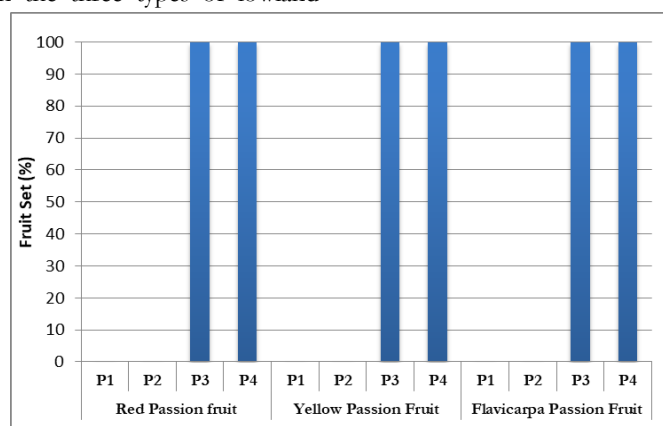


Figure 1. Percentage of fruit set resulting from pollination. P1: artificial self-pollination, P2: natural self-pollination, P3: artificial cross-pollination, P4: natural cross-pollination.

The results of artificial self-pollination and natural self-pollination treatments carried out on three types of lowland passion fruit consistently showed failure in fruit formation, characterised by decay in flower ovaries (Figure 2. P1 & P2). While in the cross-pollination treatment, both artificial and natural cross-pollination showed successful fruit formation, characterised by the enlargement of the flower ovary after pollination (Figure 2. P3 & P4). The failure in fruit formation indicates the existence of self-

incompatibility in yellow and red passion fruit, *P. edulis f. edulis*, and lowland passion fruit, *P. edulis f. flavicarpa*. Self-incompatibility also occurs in passion fruit *P. edulis f. flavicarpa* in the midlands of Central Java (Putra et al., 2023). In addition, self-incompatibility has also been reported in several passion fruit species, such as *P. cincinnata*, *P. maliformis*, *P. caerulea*, *P. mucronata*, *P. vitifolia* (Ocampo & Urrea, 2016), *P. alata* (Chavarri'a-Perez et al., 2020), and *P. incarnata* (Stafne, 2022).



Figure 2. The appearance of pollination results in each treatment at 5 days after pollination (DAP). P1: hand self-pollination, P2: natural self-pollination, P3: hand cross-pollination, P4: natural cross-pollination, a: ovary, b: stylus, c: stigma, the arrow lines indicate the difference in ovary development.

However, not all Passifloraceae species have self-incompatibility genes. Krosnick et al. (2015) reported that selfing *P. berbertiana* showed ovule development due to fertilisation. Passion fruit *P. ligularis* also showed successful fruit set from selfing, although the value was lower than the fruit set from crossing (Suarez et al., 2016). Meanwhile, selfing in *P. urnifolia* produced the same fruit set as the crossing results (García, 2023).

Self-incompatibility is a genetic mechanism in plants that prevents self-fertilisation and promotes cross-pollination. Rêgo and Rêgo, (2013) and Ribeiro et al. (2024) reported that the SI type in passion fruit is mostly sporophytic, meaning that pollen compatibility is determined by the genotype of the parent plant, not by the genotype of the pollen itself. This mechanism is regulated by glycoproteins encoded by the S-locus gene in the pistil. During incompatible pollination, the pollen tube grows slowly and becomes arrested at the stigma surface, followed by rapid callose deposition and membrane damage, ultimately preventing fertilisation (Madureira et al., 2014).

Differences in pollination compatibility between species are influenced by genetic variation and evolutionary adaptations. While SI is a barrier to selfing, it facilitates cross-pollination opportunities, including interspecific crosses. As reported by Soares et al. (2020), interspecific hybridisation in passion fruit can result in successful fruit set and generate novel genetic combinations, thereby increasing the diversity of SI responses among species.

In the present study, SI in three types of lowland passion fruit indicated that successful fruit set requires pollen from genetically distinct plants. Artificial and natural cross-pollination treatments in these passion fruit types achieved up to 100% fruit set, demonstrating comparable effectiveness between human- and insect-assisted pollination. However, considering efficiency, natural pollination by insects is more advantageous than artificial pollination. Common pollinator insects in passion fruit ecosystems include *Xylocopa latipes*, *X. eximia*, *Apis cerana*, *A. mellifera*, *Tetragonula laeviceps*, and *Bombus tucumanus* (Barrera et al., 2021; Putra et al., 2023; García, 2023).

### Morphological Characteristics

Self-incompatibility in the three types of lowland passion fruit causes fruit formation from artificial and natural self-pollination. Therefore, in the analysis of variance on morphological characteristics of passion fruit, the results of natural self-pollination and artificial self-pollination were not included in the fruit analysis.

The treatment of artificial cross-pollination and natural cross-pollination in these passion fruit types showed no significant interaction on the morphological characteristics of the fruit. However, each type showed significantly different effects on fruit morphological characters except for the number of seeds. Meanwhile, artificial cross-pollination and natural cross-pollination treatments gave the same effect on all morphological characteristics of the fruit. These results differ from those of Barrera et al. (2021) that natural pollination assisted by insects is significantly better in fruit weight, fruit length, number of seeds, and passion fruit juice yield.

In terms of fruit weight and fruit skin thickness, *flavicarpa* passion fruit types were significantly heavier and thicker than red and yellow passion fruit types. Meanwhile, fruit length and fruit width characters showed that the yellow and *flavicarpa* passion fruit types were significantly larger than the red passion fruit type. The differences between these types of passion fruit indicate that the three types of lowland passion fruit have different genetic makeup, thus giving different phenotypic expressions. The study by Charan et al. (2018) also showed that passion fruit *P. edulis* has high diversity, both in terms of quality characteristics and antioxidant activity in the fruit.

The high genetic diversity in passion fruit is one of the implications of self-incompatibility in passion fruit. Cross-pollination in passion fruit is either natural cross-pollination assisted by insects or artificial cross-pollination through plant breeding programmes. Cross-pollination between plants will produce new genetic variations due to crossing-over (CO) events during meiosis (Drouaud et al., 2006).

Table 1. Morphological fruit characteristics from artificial and natural cross-pollination in three lowland passion fruit types

| Passion Fruit Type       | Pollination Type             |                           |         | Pollination Type              |                           |       | Pollination Type             |                           |       | Pollination Type                |                           |       | Pollination Type             |                           |         |
|--------------------------|------------------------------|---------------------------|---------|-------------------------------|---------------------------|-------|------------------------------|---------------------------|-------|---------------------------------|---------------------------|-------|------------------------------|---------------------------|---------|
|                          | Artificial Cross-Pollination | Natural Cross-Pollination | Mean    | Artificial Cross-Pollination  | Natural Cross-Pollination | Mean  | Artificial Cross-Pollination | Natural Cross-Pollination | Mean  | Artificial Cross-Pollination    | Natural Cross-Pollination | Mean  | Artificial Cross-Pollination | Natural Cross-Pollination | Mean    |
|                          | ----- Fruit Weight (g) ----- |                           |         | ----- Fruit Length (cm) ----- |                           |       | ----- Fruit Width (cm) ----- |                           |       | ----- Peel Thickness (cm) ----- |                           |       | ----- Number of Seed -----   |                           |         |
| Red passion fruit        | 96.7                         | 98.7                      | 97.7 b  | 7.0                           | 6.7                       | 6.9 b | 6.1                          | 6.1                       | 6.1 b | 0.5                             | 0.5                       | 0.5 b | 211                          | 263                       | 237.0 a |
| Yellow passion fruit     | 117.3                        | 102.7                     | 110.0 b | 8.1                           | 7.6                       | 7.8 a | 7.1                          | 6.8                       | 7.0 a | 0.4                             | 0.5                       | 0.4 b | 273                          | 268                       | 270.5 a |
| Flavicarpa passion fruit | 126.7                        | 155.0                     | 140.8 a | 7.6                           | 8.2                       | 7.9 a | 7.0                          | 7.4                       | 7.2 a | 0.8                             | 1.2                       | 1.0 a | 226                          | 330                       | 278.0 a |
| <b>Mean</b>              | 113.6                        | 118.8                     |         | 7.6                           | 7.5                       |       | 6.7                          | 6.8                       |       | 0.6                             | 0.7                       |       | 237                          | 287                       |         |

Note: Similar letters in the mean column indicated no significant differences among passion fruit types based on DMRT 5%.

Table 2. Fruit flavor and edible portion characteristics from hand and natural-cross pollination in three lowland passion fruit types

| Passion Fruit Type       | Pollination Type                      |                           |        | Pollination Type              |                           |       | Pollination Type             |                           |       | Pollination Type               |                           |        |
|--------------------------|---------------------------------------|---------------------------|--------|-------------------------------|---------------------------|-------|------------------------------|---------------------------|-------|--------------------------------|---------------------------|--------|
|                          | Artificial Cross-Pollination          | Natural Cross-Pollination | Mean   | Artificial Cross-Pollination  | Natural Cross-Pollination | Mean  | Artificial Cross-Pollination | Natural Cross-Pollination | Mean  | Artificial Cross-Pollination   | Natural Cross-Pollination | Mean   |
|                          | ----- Total Soluble Solid (TSS) ----- |                           |        | ----- Total Acidity (%) ----- |                           |       | ----- Sweetness -----        |                           |       | ----- Edible Portion (%) ----- |                           |        |
| Red passion fruit        | 14.0                                  | 14.7                      | 14.3 b | 0.2                           | 0.2                       | 0.2 b | 2.6                          | 2.7                       | 2.6 b | 58.1                           | 58.5                      | 58.3 a |
| Yellow passion fruit     | 17.3                                  | 19.7                      | 18.5 a | 0.1                           | 0.1                       | 0.1 c | 3.4                          | 4.1                       | 3.8 a | 53.4                           | 54.7                      | 54.0 b |
| Flavicarpa passion fruit | 11.9                                  | 10.4                      | 11.1 c | 0.3                           | 0.4                       | 0.4 a | 1.3                          | 1.2                       | 1.2 c | 47.4                           | 48.0                      | 47.7 c |
| <b>Mean</b>              | 14.4                                  | 14.9                      |        | 0.2                           | 0.4                       |       | 2.4                          | 2.7                       |       | 53.1                           | 53.7                      |        |

Note: Similar letters in the mean column indicated no significant differences among passion fruit types based on DMRT 5%.

Unlike the weight, length, width, and thickness of the fruit skin, the number of seeds showed no significant differences between the three lowland passion fruit types or pollination types. The number of seeds formed on the fruit is related to the quality and quantity of pollen during pollination. The higher pollen amount used during pollination, the greater the fruit set and the number of seeds in the resulting fruit (Koffi et al, 2013)

### Fruit Flavour Characteristics

Based on the results of analysis of variance, passion fruit type and crossing type showed interactions that were not significantly different from the values of total soluble solid (TSS), total acidity (TA), sweetness, and edible portion of fruit (Table 2). However, all variables showed that passion fruit type had a significant affected TSS, TA, sweetness, and the edible portion of the fruit. Manual and natural crossing pollination types had the same effect on each fruit flavour variable.

The TSS value of fruit and sweetness showed that the yellow passion fruit type gave the highest value and significantly differed from the red passion fruit and flavicarpa passion fruit. Regarding total acidity, yellow passion fruit was significantly lower than red passion fruit and flavicarpa. Fruit flavour is an important characteristic of passion fruit quality. The higher the TSS value of the fruit, the higher the dissolved sugar content in the passion fruit, thus increasing the sweetness of the fruit. The increase is inversely proportional to the acidity value. Total acidity is the acid content of the fruit that can be titrated. Zhang et al. (2021) reported that passion fruit *Passiflora edulis* contains six organic acids, i.e. citric acid, malic acid, lactic acid, tartaric acid, ascorbic acid, and acetic acid. Based on the six organic acids in passion fruit, citric

## 4. Conclusions

Based on the results obtained in this study, three types of lowland passion fruit, namely *Passiflora edulis* f. *edulis* red and yellow and *Passiflora edulis* f. *flavicarpa* have self-incompatibility, which inhibits the fertilisation process in the results of natural self-pollination and artificial self-pollination. Meanwhile, cross-pollination done artificially or naturally gives the same effectiveness and does not significantly affect the morphological characteristics and taste of passion fruit. However, the three types of lowland passion fruit showed significant differences in each fruit characteristic. Flavicarpa passion fruit exhibits higher fruit weight and fruit skin thickness characteristics than other types. Meanwhile, in terms of fruit taste

acid is the most dominant organic acid among the other organic acids.

In the edible portion variable, red passion fruit showed the highest value and significantly differed from yellow and flavicarpa passion fruit. The edible portion is related to the thickness of the fruit skin because the edible portion indicates the proportion between the weight of the fruit skin and the fruit aril. The thicker the fruit skin, the smaller the edible portion will be. The flavicarpa passion fruit has a thicker skin than other types, so the edible portion in the flavicarpa passion fruit is also smaller than that of red and yellow passion fruit. Since the edible portion directly influences consumer preference and market value, evaluating flavor-related traits and morphological characteristics is equally important to determine the overall fruit quality and suitability for commercial production.

Based on the results obtained from morphological characteristics and fruit flavour, the three lowland passion fruit types exhibit substantial phenotypic variation, which likely reflects underlying genetic diversity among the genotypes. For instance, differences in edible portion, skin thickness, and flavour attributes suggest variation in loci controlling fruit morphology and biochemical composition. Such diversity provides valuable genetic resources for breeding programmes, enabling the combination of desirable traits to develop superior cultivars. However, the presence of self-incompatibility in these passion fruit types poses a challenge for achieving high levels of parental homozygosity. Therefore, further research is required to overcome self-incompatibility in these lowland passion fruit types, thereby facilitating the development of inbred lines and hybrid varieties

characteristics, yellow passion fruit exhibits higher TSS and sweetness values with lower total acidity than other types.

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