MORPHOMETRIC ANALYSIS REVEALS A SEED SIZE-NUMBER TRADE-OFF IN *Reutealis trisperma*

ANALISIS MORFOMETRIK MENUNJUKKAN HUBUNGAN BERKEBALIKAN ANTARA JUMLAH DAN UKURAN BIJI PADA *Reutealis trisperma*

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ABSTRACT

The fitness of many angiosperm plants, including *Reutealis trisperma*, is affected by the size and number of fruit and seed. However, studies on the fruit and seed morpho-physiology of *R. trisperma* are still highly limited. This study aimed to identify the variation of locule and seed number besides the fruit and seed morphometric traits of *R. trisperma*. The number of locules and seeds per fruit was observed in immature, developing *R. trisperma* fruits. These observations were made by cross-sectioning *R. trisperma* that was obtained from field collections. Morphometric data collection was subsequently carried out on sampled mature fruits. The results showed that the locule and seed number of the single fruit of *R. trisperma* ranged from 2 to 4 and 1 to 4, respectively. Trilocular fruits were the most commonly found type. However, some of trilocular fruits were consisted of two seeds (two-seeded fruits) instead of three seeds (three seeded fruits). The proportion of two-seeded fruits was comparable to the three seeded fruits. No significant differences were found in fruit size or weight between two-seeded and three-seeded fruits. However, the seed weight, as well as the kernel weight, were heavier for two-seeded fruits compared to three-seeded fruits. Therefore, it revealed a seed size-number trade-off. These results can enrich the valuable informations related to the growth and development as well as the fitness of *R. trisperma*.

Key words: Biodiesel, Empty locule, Morphometric, Philipine tung, Resource-allocation

ABSTRAK

Daya reproduksi beberapa tanaman angiosperma, termasuk *Reutealis trisperma*, dipengaruhi oleh ukuran serta jumlah buah dan biji. Namun demikian, masih sangat sedikit penelitian yang telah dilakukan terkait morfo-fisiologi buah dan biji pada spesies tanaman tersebut. Penelitian ini bertujuan untuk mengidentifikasi variasi jumlah lokulus dan biji serta sifat morfometrik buah dan biji dari *R. trisperma*. Pengamatan jumlah lokulus dan biji per buah dilakukan pada buah muda *R. trisperma* yang sedang berkembang. Pengamatan dilakukan dengan cara memotong secara melintang *R. trisperma* yang didapatkan dari koleksi lapangan. Pengumpulan data morfometrik kemudian dilakukan pada buah matang yang diambil sebagai

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Sampel. Hasil penelitian menunjukkan bahwa jumlah lokulus dan biji dari satu buah *R. trisperma* berkisar antara 2 hingga 4 dan 1 hingga 4, berturut-turut. Buah trilokular adalah jenis buah yang paling umum dari spesies ini. Namun, beberapa buah trilokular terdiri dari dua biji (buah berbiji dua) bukan berisi tiga biji (buah berbiji tiga). Proporsi buah berbiji dua sebanding dengan buah bebiji tiga. Sementara itu, tidak ada perbedaan ukuran buah maupun bobot buah antara buah berbiji dua dan buah berbiji tiga. Di sisi lain, bobot per biji serta bobot per kernel lebih berat pada buah berbiji dua dibandingkan buah berbiji tiga. Hasil tersebut membukitkan adanya hubungan berkebalikan antara jumlah dan ukuran biji. Hasil penelitian dapat memperkaya informasi mengenai pertumbuhan dan perkembangan tanaman serta *fitness* pada spesies *R. trisperma*.

Kata kunci: Alokasi sumber, Biodiesel, Kemiri sunan, Lokus hampa, Morfometrik

INTRODUCTION

Reutealis trisperma, commonly known as Philippine tung or bagilumbang, is a tropical tree species native to the Philippines and introduced to West Java, Indonesia (Stuppy et al., 1999). It has garnered interest for its potential as a biofuel source and various medicinal and industrial applications, such as the production of varnishes, paints, insecticides and other industrial products (Lu et al., 2020; Riadi et al., 2019; Waseem et al., 2024). The seeds of *R. trisperma* are rich in oil (50-52 wt%), which can be extracted and processed into biodiesel (Holilah et al., 2015). The properties of this oil are similar to those of conventional diesel, thereby rendering it a viable alternative fuel source. (Riayatsyah et al., 2017). The cultivation of *R. trisperma* for biofuel production could contribute to energy security and reduce reliance on fossil fuels (Sari et al., 2020). Moreover, the tree species has good adaptability to unfavorable environmental conditions, even to heavy metal-contaminated soils, making it have the potential to become a solution for degraded soils (Prasetya et al., 2022).

Fruit and seed are the main yield components of *R. trisperma*. The fruit of *R. trisperma* is a woody capsule containing three large seeds, each is enclosed in a hard seed shell or seed coat. Mature seeds of *R. trisperma* contain a starchy-white color structure of endosperm, which is commonly named “kernel”. This tissue serves a vital role as a nourishment for developing embryos in the seeds (Li & Berger, 2012). The seeds fulfill an additional function, namely the multiplication of the plant. For this purpose, a high number and high quality of seeds are required. Many studies have reported a high positive correlation between fruit and seed size as well as between seed size and quality (Ambika et al., 2014; Kadapi et al., 2018; Pramono et al., 2019). However, there are many reports of seed size and number trade-offs, even not in a strict manner (Qiu et al., 2022).

Beyond the significant economic, pharmaceutical, and ecological benefits of *R. trisperma*, there is a lack of studies related to morpho-anatomy, especially for fruit and seed of this tree species. Ajijah et al. (2009) postulated that *R. trsiperma’s* ovary consisted of 2, 3, or 4 locules, a wider range of numbers compared to the earlier report by Stuppy et al. (1999). Meanwhile, Pranowo et al. (2015) stated that there was a variation in a number of seeds per fruit of *R. trisperma*, ranging from 3 to 5 seeds. However, no more detailed information related to the frequencies of each number of developed locule(s) or seed(s), as well as an indication of seed size-number trade-off. This study aimed to reveal the fruit and seed morphometric variation also their inter-relations to the seed number of *R. trisperma*. The importance of this study is for elucidating the biological nature of fruit and seed formation of *R. trisperma*.

MATERIALS AND METHOD

The research was conducted from June to October of 2021, at the Pakuwon Experimental Garden of Indonesian Industrial and Beverage Crops Research Insitute. This site had a collection of five *R. trisperma* ecotypes, including two released cultivars, Kemiri Sunan 1 and Kemiri Sunan 2. These two cultivars were consisted of 20 and 23 trees, respectively, whereas the other three ecotypes were consisted of only less than 5 individuals. The arrangement of the ecotypes was conducted in a manner that constituted a single row. It was located at the elevation of ~ 490 m above sea level (6°50'06.4"S 106°44'36.2"E), which had daily temperatures of 19 °C to 32 °C and total year-round precipitations of 2760 mm with the single dry month (< 60 mm) according to category cited by Nepal et al. (2021). Those *R. trisperma* populations in this location had reached the age of ~10 years.

Locule and seed number per fruit

One hundred immature fruits, which had an equatorial diameter of ~2 cm, were randomly collected from each of ten individuals of respective two *R. trisperma* cultivars, Kemiri Sunan 1 and Kemiri Sunan 2. To identify the locule and seed number of a single fruit, cross sectional cuts were made using the fine folding razor blades on those sample fruits. The number of bilocular (2 locules), trilocular (3 locules), and tetralocular (4 locules) as well as the number of single, double, triple, and quadruple seeded fruits were subsequently counted and recorded.

Fruit and seed morphometric

In order to elucidate the effect of seed number per fruit on fruit and seed morphometrics, a sample of 100 mature fruits from the Kemiri Sunan 1 cultivar was examined. Each of those sample fruits was subsequently weighed and measured to gain the fruit weight (g), fruit polar diameter (mm), and fruit equatorial (mm) data. Subsequent to this, the fruits were peeled off to separate the seeds from the fruit mesocarp, and both were immediately weighed and measured to generate seed weight per fruit (g), seed weight (calculated as seed weight per fruit divided by the number of seeds of a single fruit, g), mesocarp weight (g), and mesocarp thickness (mm), seed polar diameter (mm), and seed equatorial diameter (mm) data. The seeds of *R. trisperma* consist of a hard seed coat and a soft white color kernel inside. The seed coat was removed using specialized instruments designed to break down the hard seed shell. The seeds were then thoroughly cleaned and weighed using a digital balance. To obtain precise measurements, a digital caliper was used. Key parameters recorded included the total kernel weight per fruit (g), the average kernel weight (calculated by dividing the total kernel weight per fruit by the number of seeds per fruit) (g), the polar diameter (mm), and the equatorial diameter of each kernel (mm).

Statistical analysis

Locule and seed number per fruit data were subjected to descriptive statistical analysis and visualized by clustered column charts. Meanwhile, the fruit and seed morphometric data were subjected to Student's t-test analysis to compare the mean values of two and three seeded fruits. Data were also visualized by a boxplot diagram. All statistical analyses were conducted using Smartstat XL V. 3.6.5.3.

RESULTS AND DISCUSSION

Locule and seed number variations

Trilocular fruits were the common type (>90%) for both *R. trisperma* cultivars, i.e. Kemiri Sunan 1 and Kemiri Sunan 2. Only 7% and 8% of fruits were bilocular for cultivars Kemiri Sunan 1 and Kemiri Sunan 2, respectively. Meanwhile, the tetralocular fruit was only 1% for both cultivars (Fig. 1A). However, no unilocular and pentalocular fruits were detected in the samples.

Each locule contained only a single seed. It means, in the case of seeds developing normally, the locule number was identical to the seed number for *R. trisperma*. However, a few abnormal bilocular and some abnormal trilocular fruits, both of which had one empty locule, had also been identified. In consequence, there was a variation in the seed number of a single fruit, ranging from 1 to 4 seed(s) per fruit. The abnormal bilocular fruits were only found in a low frequency (< 5%) for both *R. trisperma* cultivars. The most common was normal trilocular fruits containing three seeds or three-seeded fruits, which had a proportion of 51 and 58% for Kemiri Sunan 1 and Kemiri Sunan 2, respectively. Meanwhile, abnormal trilocular fruits with one empty locule or two-seeded fruits had a proportion of 45 and 40% for Kemiri Sunan 1 and Kemiri Sunan 2, respectively (Fig. 1B). However, that deviation from trilocular fruit as well as the empty locule formation in *R. trisperma* was still remain unknown.

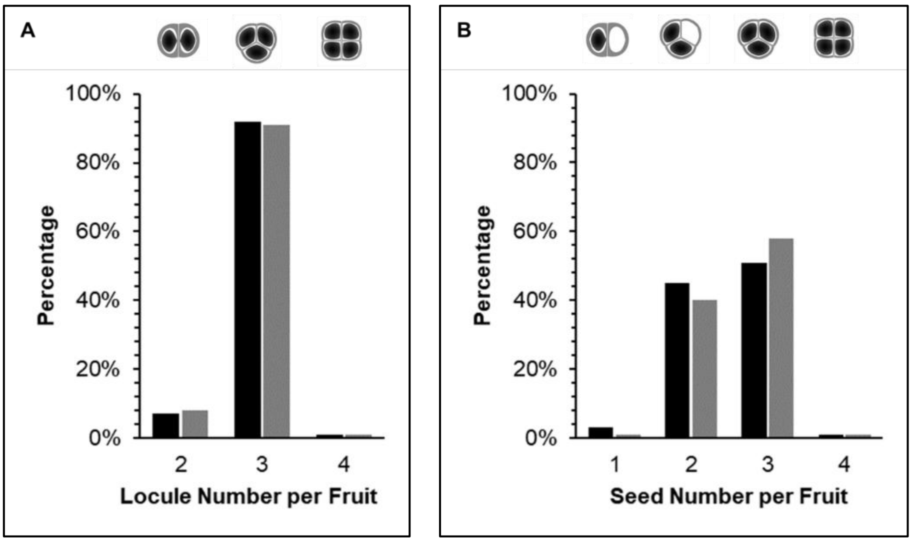

Figure 1. Locule and seed number variations of two *R. trisperma* cultivars, i.e.: Kemiri Sunan 1 (black-color bars) and Kemiri Sunan 2 (grey-color bars).

The fruit and seed of angiosperm develop form the ovary and ovule, respectively (Souza, 2022). The ovary could have a single or multiple locule(s) containing one or many ovule(s). The number of ovules is identical to the carpel and pistil number for the apocarpous gynoecium (Simpson, 2019). Ajijah et al. (2009) reported the number of pistils per single ovary of *R. trisperma* was varied, ranging from 2, 3 to 4 pistils. This is equal to the locule number variation revealed in this research work, i.e., bilocular, trilocular, and tertralocular.

In tomatoes, the locule number is depend on and identical to the number of carpels generated during flower development (Wu et al., 2024; Zhang et al., 2023). The Locule number of tomato fruits is controlled by genetic factors, such as lc and fas genes (Chu et al., 2019). These two genes are located in chromosome 4 and chromosome 11, respectively (Basaroh et al., 2024; Xiang et al., 2023). However, genetic regulation for locule number in *R. trisperma* is still unknown.

Interestingly, there was the appearance of empty locules in some bilocular and trilocular fruits, thus extending the variation in seed number into 1 to 4 seed(s) per fruit. However, research related to the process of empty locules formation in *R. trisperma* has never been carried out. This case might be similar to the formation of empty coffee beans formation. According to Nugroho et al. (2016), the percentage of empty beans was affected by genetic × environment interaction. The empty locule formation was related to imbalanced gametes of post-fertilization (Ram et al., 1990). Dani et al. (2024) confirmed that a high proportion of empty locules was observed in developed fruits of interspecific hybridization between *Coffea canephora* × *C. arabica*. Therefore, a study on imbalance gamete formation in *R. trisperma* is very important to generate a scientific explanation for empty locule formation.

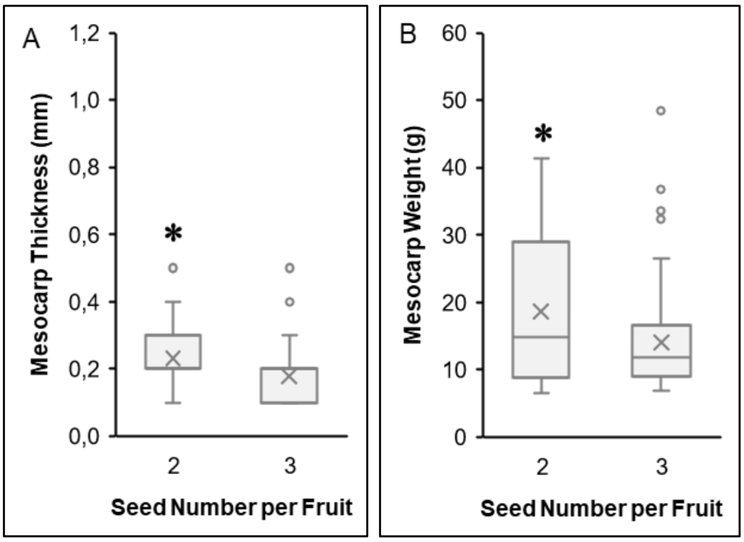

Figure 2. Fruit mesocarp thickness (A) and weight (B) of abnormal two-seeded and normal three-seeded trilocular fruits of *R. trisperma*. Asterics sign means there was a significant difference according to the *t*-test at *p*-value < 0.05.

Fruit and seed morphometric related to seed number

The study's findings show that only 4 and 1 fruits out of 100 mature-sampled fruits were bilocular and tetralocular type, respectively. Those bilocular fruits contained only a single seed, leaving another locule empty. Meanwhile, the solely tetralocular fruit contains of 4 seeds. Due to a limited number of bilocular and tetralocular fruits, finally it's decided to focus merely on trilocular fruits for morphometric traits analysis. Some of 28 the trilocular fruits were abnormal and filled with only 2 seeds inside a single fruit (two-seeded fruits). Another 67 trilocular fruits were normal, which contained 3 seeds per fruit (three-seeded fruits) instead.

Three of the five fruit morphometric characters, i.e., fruit polar diameter, fruit equatorial diameter, and fruit weight, are similar between two-seeded fruits and three-seeded fruits of *R. trisperma*. Mean values of those three characters were slightly higher for the two-seeded fruits, but not statistically significant according to the t-test analysis. It could be inferred from these results that the sizes of two-seeded fruits and three-seeded fruits are the same. Meanwhile, the mean values of the other two morphometric traits, i.e., mesocarp thickness and mesocarp weight, were significantly higher ($p < 0.05$) for two-seeded fruits compared to three-seeded fruits. This means that the mesocarp of two-seeded fruits was comparatively thicker and heavier than that of three-seeded fruits (Fig. 2).

Among eight seed morphometric traits, only seed weight per fruit, average seed weight, and average kernel weight were significantly different between two seeded fruits and three seeded fruits according to t-test analysis. Seed weight per fruit of three-seeded fruits was significantly higher ($p < 0.05$) compared to two-seeded fruits. In contrast, the average seed weight and average kernel weight of two seeded fruits were significantly higher ($p < 0.01$) compared to those of three-seeded fruits (Fig.3B, 3C).

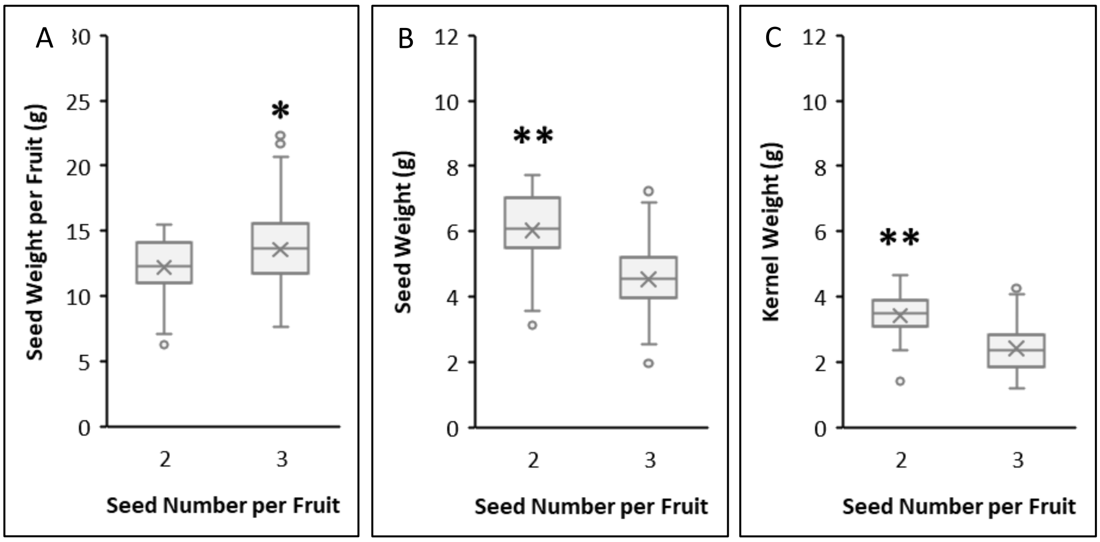

Figure 3. Seed trait comparisons between two different seed number per fruit of *R. trisperma*. Single and double asterics signs mean a significant differences according to the *t*-test at *p*-value < 0.05 and < 0.01, respectively.

It means that the average weight of seed per fruit, as well as average weight of kernel per fruit of two-seeded fruits was heavier compared to of three-seeded fruits albeit of no difference in their size.

Seed size is determined primarily by seed coat and endosperm (Xu & Zhang, 2023). Variations in seed size and number are affected by genetics and environment as well as genotype × environment interaction, even though they have different genetic regulations (Gnan et al., 2014; Larios & Mazer, 2022). Seed size could be affected by plant growing site elevation or shading. Some species tend to produce larger seeds at higher elevations or shade levels (Bogdziewicz et al., 2023; Olejniczak et al., 2018). However, those theories are unable to explain the seed size and number variation within individual plants or even within a single infructescence. Changes in resource allocation in plants could also have an impact on developed seed size and number. Lower competition for reserves could result in lower seed numbers but larger seed sizes as shown in Arabidopsis (Bennett et al., 2012).

CONCLUSIONS

1. *R. trisperma* fruits tested consisted of not only trilocular and tetralocular, but also bilocular. Moreover, the seed number per fruit was also more varied, ranging from 1 to 4 seed(s).
2. The two-seeded fruits had similar size and weight, but consisted of a thicker and heavier mesocarp as compared to three-seeded fruits. Meanwhile, the seed weight per fruit of two-seeded fruits was heavier, compared to of three-seeded fruits. This was an indicator of seed size-number trade-off in *R. trisperma*.

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