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Effects of Polyembryonic Seed Types on Early Seedling Growth of Duku (*Lansium domesticum* Corr).

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Abstract. Duku (Lansium domesticum Corr.) is an important tropical fruit tree in Indonesia, but the supply of uniform and vigorous planting material for orchard establishment remains limited. Polyembryony in duku seeds offers a potential source of clonal seedlings, yet the effects of different polyembryonic seed types and handling methods on seedling vigor are not well documented. This study evaluated early seedling growth of duku derived from zygotic and polyembryonic seeds under farmer managed nursery conditions in Eris Village, North Sulawesi. A randomized complete block design was used with five seed treatments and four replications: zygotic seed with a single embryo (Z1), intact polyembryonic seeds with two embryos (PU2), intact polyembryonic seeds with three embryos (PU3), polyembryonic seeds cut into two sections (PB2), and polyembryonic seeds cut into three sections (PB3). At the end of the nursery phase, number of leaves, plant height, stem diameter, leaf area, and root weight were recorded and analyzed by analysis of variance followed by the least significant difference test at the five percent level. Seed type significantly affected all traits. Zygotic seeds produced seedlings with the highest leaf number, plant height, stem diameter, leaf area, and root weight. Seedlings from cut polyembryonic seeds (PB2 and PB3) showed intermediate performance, with stem diameter and root weight close to those of zygotic seedlings. In contrast, seedlings from intact polyembryonic seeds with three embryos (PU3) had the lowest leaf number, leaf area, and root weight. These findings indicate that zygotic seeds remain the best option for producing vigorous duku seedlings, while sections of polyembryonic seeds, especially PB2 and PB3, can provide acceptable alternatives when zygotic seeds are limited or when clonal propagation is desired.

Keywords: duku; Lansium domesticum; polyembryonic seeds; seedling vigor; rootstock; nursery management

INTRODUCTION

Duku (Lansium domesticum Corr.) is an important tropical fruit tree in Southeast Asia, particularly in Indonesia, where it is valued both for its sweet, aromatic fruit and for its cultural and economic roles in rural communities[1], [2]. Duku trees are traditionally planted in mixed home gardens and small orchards, and the fruit is sold fresh in local markets[3], [4]. Despite its popularity, the expansion of duku orchards is often slow because growers face difficulties in obtaining sufficient quantities of uniform, vigorous planting material. Many plantings are established from seeds collected from local trees, and the resulting seedlings can be highly variable in growth and fruit quality[5]. This variability complicates orchard management and may reduce yield stability over time[6].

Vegetative propagation techniques such as budding, grafting, and air layering are widely used in other fruit crops to produce uniform planting material, and these methods are also possible for duku[7]. However, vegetative propagation requires selected mother trees, nursery infrastructure, and skilled labor[8]. In many smallholder systems, farmers may not have consistent access to materials and training for vegetative propagation, so seed propagation remains the most common method for producing duku seedlings[9]. Seed propagation is simple and inexpensive, but it does not always guarantee uniformity[10]. In this context, any biological trait that could help bridge the gap between simplicity and uniformity becomes particularly important.

One such trait is polyembryony. In polyembryonic seeds, more than one embryo develops inside a single seed[11]. In many tropical fruit species, some of these embryos originate not from fertilization, but from maternal nucellar tissue, and they often produce seedlings that are genetically identical or very similar to the mother plant[12]. Polyembryony has been reported in several perennial fruit crops, including citrus, mango, and some Sapindaceae and Meliaceae species, and it has also been observed in duku[13]. The presence of polyembryonic seeds in duku suggests that, at least in theory, growers might obtain clonal or near clonal seedlings directly from seeds, reducing the need for vegetative propagation[14].

In practice, however, the use of polyembryonic seeds is not straightforward. A single polyembryonic duku seed may contain two or three embryos. When such a seed is planted intact, more than one seedling may emerge, and farmers must decide whether to keep all seedlings, to thin them to a single plant, or to handle them in some other way[15]. Some farmers or nursery workers may choose to cut polyembryonic seeds into two or three sections before sowing, with the expectation that each section will carry an embryo and give rise to a separate seedling[16]. These practices are based on practical experience and intuition, but the physiological consequences for seedling vigor and root development are not always well documented[17].

Polyembryony also raises questions about the balance between genetic uniformity and seedling vigor[18]. Zygotic seedlings, which arise from sexual reproduction, are genetically diverse and may vary widely in growth and performance, but they often develop from a single embryo with full access to seed reserves[19]. Nucellar seedlings from polyembryonic seeds may be genetically uniform and more similar to the mother tree, but competition among embryos or damage caused by cutting the seed could reduce their early growth. For nursery managers and farmers, the key question is not only whether polyembryonic seeds exist, but whether seedlings produced from different types of polyembryonic seeds can match or surpass the vigor of seedlings from zygotic seeds.

Information on polyembryony in duku is still limited compared with better studied crops such as citrus or mango[13]. Most available reports focus on the occurrence of polyembryonic seeds or on general descriptions of the phenomenon, rather than on quantitative comparisons of seedling growth from different seed types under controlled nursery conditions[20], [21]. There is therefore a need for empirical data that show how different ways of using polyembryonic duku seeds, such as sowing them intact or cutting them into sections, influence early seedling growth traits that are critical for successful transplanting. Number of leaves, plant height, stem diameter, leaf area, and root weight are commonly used indicators of seedling vigor and can be measured accurately in a nursery setting[22], [23].

The present study was designed to address this gap by evaluating the early growth of duku seedlings derived from different types of polyembryonic seeds as well as from zygotic seeds. Specifically, the experiment compared seedlings obtained from intact zygotic seeds, intact polyembryonic seeds with two or three embryos, and polyembryonic seeds cut into two or three sections. By quantifying shoot and root traits at the end of the nursery phase, this study aims to identify which seed type and handling method produces seedlings with the most desirable growth characteristics for use as planting material. The results are expected to provide practical guidance for smallholder farmers and nursery managers who wish to use duku seeds more efficiently, and to contribute to a better understanding of how polyembryony can be exploited in the propagation of this important fruit tree.

MATERIALS AND METHODS

Study site

The study was conducted in a farmer managed nursery in Eris Village, Eris Subdistrict, Minahasa Regency, North Sulawesi, Indonesia. The site represents typical smallholder conditions for duku seedling production, with nursery beds and polybags maintained under partial shade. All nursery operations followed local practices for raising fruit tree seedlings.

Plant material and seed classification

Ripe duku fruits were collected from productive trees in the surrounding area. After removal of the pulp, seeds were washed and carefully observed to identify the number of embryos. Seeds with only one visible embryo were classified as zygotic seeds. Seeds that showed more than one embryo were classified as polyembryonic seeds and were further grouped according to whether they contained two or three embryos. Germination of the seeds in the nursery bed is illustrated in Figure 1.

Figure 1. Germination of duku (Lansium domesticum Corr.) seeds in the nursery bed. Several seeds show more than one emerging seedling, indicating polyembryony.

For the experiment, five seed types (treatments) were defined based on the number of embryos and the way seeds were handled before sowing. These seed types represent practical options that a farmer or nursery manager might use when handling polyembryonic duku seeds.

Z1 was defined as a zygotic seed with a single embryo, sown intact in the nursery medium.

PU2 was defined as an intact polyembryonic seed containing two embryos, sown whole.

PU3 was defined as an intact polyembryonic seed containing three embryos, sown whole.

PB2 was defined as a polyembryonic seed with at least two embryos that was cut longitudinally into two sections before sowing. Each section was sown separately, with the expectation that each section contained at least one embryo.

PB3 was defined as a polyembryonic seed with three embryos that was cut longitudinally into three sections before sowing. Each section was also sown separately.

The visual difference between a normal zygotic seedling and polyembryonic seedlings is shown in Figure 2.

Figure 2. Normal zygotic duku seedling (Above) and polyembryonic duku seedlings (Below). The polyembryonic seed shows more than one seedling arising from a single seed.

Nursery management

Seeds from each seed type were sown in a seedbed containing a well drained nursery medium suitable for duku. After germination, uniform seedlings were transplanted into polybags filled with a mixture of topsoil, organic matter, and sand in proportions commonly used by local growers. Seedlings were maintained under partial shade and watered regularly to avoid drought stress. Weeds were removed by hand, and any pest or disease incidents were controlled using measures normally employed in the nursery. All management practices were applied uniformly across all treatments to ensure that differences in seedling performance could be attributed mainly to seed type.

The arrangement of the seedlings in polybags on raised nursery beds under partial shade is shown in Figure 3.

Experimental design

The experiment used a randomized complete block design with five seed treatments and four replications. Each replication contained one seedling per treatment. Thus, for each trait, the mean of a treatment was based on four individual seedlings. The experimental units (polybag seedlings) were arranged in blocks to reduce the influence of any small environmental gradients within the nursery.

Figure 3. Duku seedlings growing in polybags on raised nursery beds under partial shade in the experimental nursery.

Measured variables

At the end of the nursery phase, when seedlings had established and produced several leaves, the following variables were measured for each seedling.

Number of leaves per seedling was counted as the total number of fully expanded leaves on the main shoot.

Plant height was measured from the surface of the growing medium to the apical bud of the main shoot, in centimetres.

Stem diameter was measured at the base of the stem just above the medium surface, in centimetres, using a ruler or calliper.

Leaf area per seedling was estimated in square centimetres. For each seedling, the area of all leaves was calculated using a length–width method with an appropriate correction factor or another standard method appropriate for broad leaves. The sum of all leaf areas on the seedling was used as leaf area per plant.

Root weight per seedling was measured after gently removing the plant from the polybag, washing the root system free of soil, and weighing the fresh roots on a balance. Root weight was expressed in grams.

These traits were chosen as key indicators of seedling vigor and transplant quality.

Data processing and statistical analysis

For each trait, the mean of a treatment was obtained as the arithmetic mean of four seedlings. If Xi represents the value of an individual seedling and n is the number of seedlings observed for a given treatment, the mean was calculated as

$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n} X_i$$

A one way analysis of variance was planned for each trait using the model

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

Where $Y_{ij}$is the observation for treatment i in block j, $\mu$is the overall mean, $\tau_i$is the effect of the ith seed type, $\beta_j$is the effect of the jth block, and $\varepsilon_{ij}$is the random error term. In cases where the F test indicates a significant treatment effect, comparisons among treatment means can be carried out using the least significant difference test at the five percent probability level. The least significant difference can be calculated as

$$LSD = t_{\alpha/2,\, df_{error}} \times \sqrt{\frac{2 \times MSE}{r}}$$

Where $t_{\alpha/2,\, df_{error}}$ is the t statistic at a given significance level and the error degrees of freedom, MSE is the mean square error from the analysis of variance, and r is the number of replications. In the tables presented here, treatment means are shown as descriptive values; significance letters can be added after the analysis has been completed using the actual error mean square from the experiment. All raw and processed data used in this study, including treatment means for number of leaves, plant height, stem diameter, leaf area, and root weight, are summarized in an online interactive dashboard entitled "Duku Growth Dashboard". The dashboard presents the same field experiment dataset used in this article and is available at: https://sanriomisintaro.github.io/data-duku/.

RESULTS & DISCUSSION

Number of leaves, plant height, and stem diameter

Mean values for number of leaves, plant height, and stem diameter of duku seedlings derived from the different seed types are presented in Table 1.

Table 1. Mean number of leaves, plant height, and stem diameter of duku seedlings derived from different seed types

Treatment	Description	Leaves (number)	Height (cm)	Stem diameter (cm)
Z1	Zygotic seed, intact	4.17	12.66	0.443
PU2	Intact polyembryonic seed with two embryos	3.08	10.68	0.362
PU3	Intact polyembryonic seed with three embryos	2.25	9.61	0.365
PB2	Polyembryonic seed cut into two sections	3.17	9.62	0.427
PB3	Polyembryonic seed cut into three sections	3.17	9.41	0.415

Table 1 shows that zygotic seeds (Z1) produced the most vigorous seedlings for all three traits. Z1 seedlings had the highest mean number of leaves, the greatest plant height, and the thickest stems. Seedlings from intact polyembryonic seeds (PU2 and PU3) consistently had fewer leaves, shorter height, and thinner stems than Z1. Cutting polyembryonic seeds into sections (PB2 and PB3) improved performance, so that leaf number and stem diameter in PB2 and PB3 approached the values obtained from zygotic seeds, although plant height remained below Z1.

The pattern for number of leaves is illustrated in Figure 1. Z1 produced the highest mean number of leaves, 4.17 leaves per seedling. PU2 seedlings produced 3.08

leaves on average, while PU3 seedlings produced only 2.25 leaves and clearly formed the fewest leaves. PB2 and PB3 both produced 3.17 leaves per seedling, giving intermediate values between Z1 and PU3.

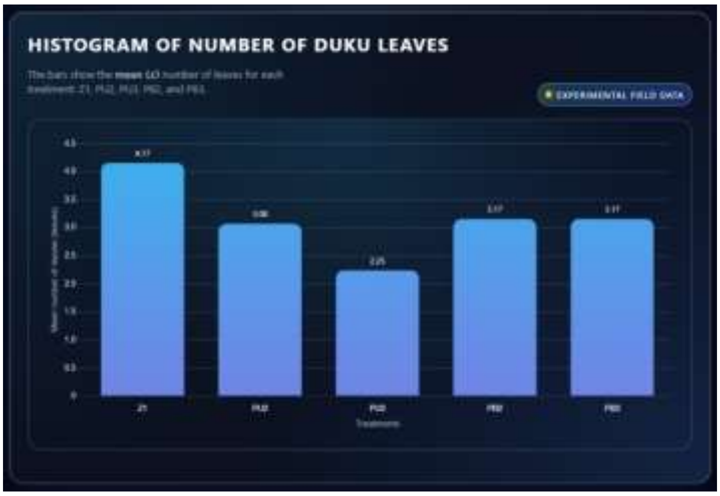


Figure 1. Histogram of the mean number of leaves per duku seedling for each seed type (Z1, PU2, PU3, PB2, and PB3). Bars represent treatment means derived from experimental field data.

The pattern in Figure 1 confirms the numerical differences in Table 1. Z1 seedlings formed the most leaves, while seedlings from intact polyembryonic seeds, especially PU3, showed reduced leaf production. Seedlings from PB2 and PB3 produced slightly more leaves than PU2 and markedly more than PU3, suggesting that cutting polyembryonic seeds into sections reduced the negative effect of competition among embryos on leaf formation.

Plant height followed a similar trend, as shown in Figure 2. Z1 seedlings reached a mean height of 12.66 cm, clearly taller than seedlings from any polyembryonic treatment. Seedlings from PU2 attained 10.68 cm on average, and those from PU3 reached 9.61 cm. PB2 and PB3 produced seedlings with mean heights of 9.62 cm and 9.41 cm respectively.

Figure 2. Histogram of the mean plant height of duku seedlings for each seed type. Heights are expressed in centimetres and represent treatment means from experimental field data.

Figure 2 indicates that all polyembryonic treatments produced shorter seedlings than Z1. Among the polyembryonic seeds, PU2 gave slightly taller seedlings than PU3, while seedlings from PB2 and PB3 were similar in height to those from PU3. Cutting the seeds therefore did not restore plant height to the level of Z1, but it prevented a further decline in height relative to intact polyembryonic seeds.

Differences in stem diameter among treatments are presented graphically in Figure 3. Z1 seedlings had the largest mean stem diameter at 0.443 cm. Seedlings from PB2 and PB3 also developed relatively thick stems, with mean diameters of 0.427 cm and 0.415 cm, whereas PU2 and PU3 seedlings had thinner stems, with mean diameters of 0.362 cm and 0.365 cm.

Figure 3, together with the data in Table 1, shows that stem thickening was greatest in Z1 and in seedlings from cut polyembryonic seeds. Cutting polyembryonic seeds into two or three sections allowed PB2 and PB3 seedlings to develop stems almost as thick as those of zygotic seedlings, while leaving polyembryonic seeds intact resulted in visibly thinner stems in PU2 and PU3. Thick stems in Z1, PB2, and PB3 are important indicators of mechanical strength and potential field performance

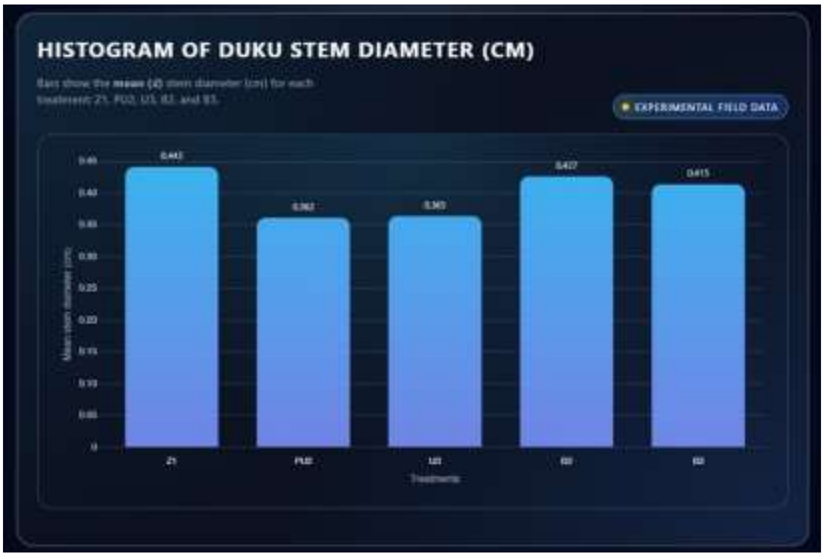


Figure 3. Histogram of the mean stem diameter of duku seedlings for each seed type. Stem diameter is expressed in centimetres and represents treatment means from experimental field data

Leaf area and root weight

Mean leaf area and root weight per seedling for each seed type are presented in Table 2.

Table 2 shows that leaf area per seedling was highest for zygotic seeds. Z1 seedlings reached a mean leaf area of 68.94 cm^2, which was substantially larger than the leaf area of any polyembryonic treatment. Cut polyembryonic seeds (PB2 and PB3) produced intermediate leaf areas of about 44 cm^2, higher than those of intact polyembryonic seeds but still below Z1. Among the intact polyembryonic seeds, PU2 seedlings had a mean leaf area of 38.77 cm^2, and PU3 seedlings had the smallest leaf area at 33.37 cm^2. Root weight followed a very similar pattern. Z1 seedlings developed the heaviest roots, with a mean root weight of 1.125 g. PB2 and PB3 seedlings also had relatively heavy roots, 0.975 g and 0.925 g respectively, whereas seedlings from PU2 and PU3 had much lighter roots of 0.575 g and 0.325 g.

Leaf area patterns are illustrated in Figure 4. Z1 seedlings again had the largest mean leaf area, 68.94 cm^2. Seedlings from PB2 and PB3 reached 44.22 and 44.42 cm^2, while PU2 and PU3 seedlings had smaller leaf areas of 38.77 and 33.37 cm^2.

Table 2. Mean leaf area and root weight of duku seedlings derived from different seed types

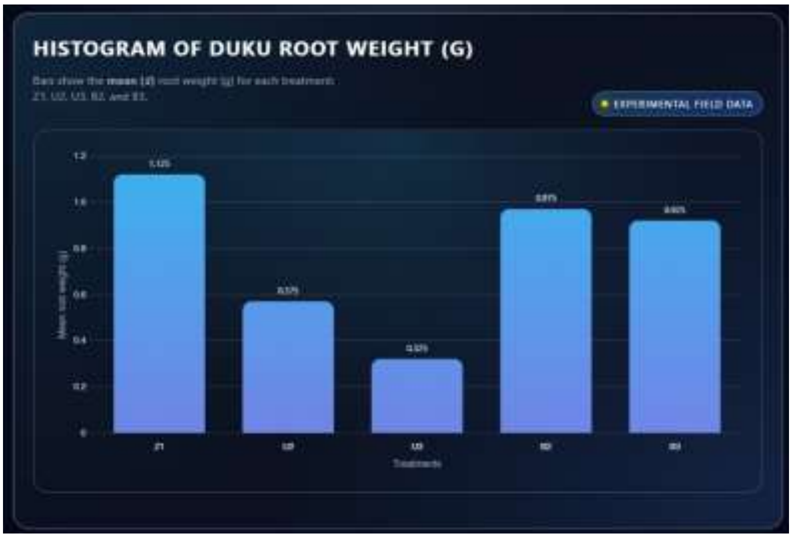
Treatment	Description	Leaf area (cm²)	Root weight (g)
Z1	Zygotic seed, intact	68.94	1.125
PU2	Intact polyembryonic seed with two embryos	38.77	0.575
PU3	Intact polyembryonic seed with three embryos	33.37	0.325
PB2	Polyembryonic seed cut into two sections	44.22	0.975
PB3	Polyembryonic seed cut into three sections	44.42	0.925

Figure 4. Histogram of the mean leaf area per duku seedling for each seed type. Leaf area is expressed in square centimetres and represents treatment means from experimental field data.

The distribution in Figure 4 emphasizes the strong advantage of zygotic seeds in producing a large photosynthetic surface. It also shows that cutting polyembryonic seeds into sections increases leaf area compared with sowing polyembryonic seeds intact. The smallest leaf area in PU3 reflects the combined effects of multiple embryos and unmodified seed structure on shoot development.

Root weight patterns are shown in Figure 5. Z1 seedlings had the heaviest roots at 1.125 g per seedling. Seedlings from PB2 and PB3 also developed strong root systems, with mean root weights of 0.975 g and 0.925 g. In contrast, seedling roots from PU2 and PU3 were considerably lighter, with mean values of 0.575 g and 0.325 g respectively.

Figure 5, together with Table 2, demonstrates that cut polyembryonic seeds can produce seedlings with root systems much stronger than those from intact polyembryonic seeds and not far below those of zygotic seedlings. Z1, PB2, and PB3 seedlings formed the largest root systems, whereas PU2 and especially PU3 seedlings developed weak roots. This pattern supports the conclusion that competition among embryos in intact polyembryonic seeds restricts root development, while separating embryos by cutting the seeds allows better root growth and results in more vigorous seedlings.



Discussion

The present study shows that the type of seed and the handling method used before sowing have a clear influence on early duku seedling growth. Across all measured variables, zygotic seeds produced the most vigorous seedlings. Seedlings from zygotic seeds developed more leaves, attained greater height, formed thicker stems, and built larger leaf areas and heavier root systems than seedlings from any of the polyembryonic treatments. These characteristics are typical of robust nursery plants and are desirable for field establishment.

The relatively poor performance of seedlings from intact polyembryonic seeds, especially those with three embryos (PU3), suggests that there is strong competition among embryos when they share the same seed reserves and root space. When a polyembryonic seed with multiple embryos is planted intact, more than one embryo germinates and begins to grow at the same time. As they grow, these seedlings must share not only the food reserves originally stored in the seed, but also water, nutrients, and physical space in the surrounding soil. This competition can limit the growth of each individual seedling, resulting in fewer leaves, smaller leaf areas, and weaker root systems, as observed for PU2 and particularly PU3 in this study.

Cutting the polyembryonic seeds into two or three sections before sowing changed this situation. In the PB2 and PB3 treatments, each embryo or seed section was given its own space in the nursery medium. Although cutting the seed can cause some mechanical damage and may reduce the initial reserve tissue attached to each embryo, the separation of sections appears to reduce competition during seedling establishment. In this experiment, seedlings produced from cut polyembryonic seeds had stem diameters and root weights that almost matched those of zygotic seedlings. Their leaf areas were lower than those of zygotic seedlings but still higher than those of seedlings from intact polyembryonic seeds. This indicates that separating embryos from a polyembryonic seed before sowing can partially restore seedling vigor that would otherwise be limited by within seed competition.

The balance between shoot growth and root development is particularly important for transplant success. Seedlings with larger root systems are better able to absorb water and nutrients after transplanting, and thicker stems are more resistant to mechanical damage and stress. In the present study, Z1, PB2, and PB3 seedlings consistently exhibited both relatively thick stems and heavy roots, while PU2 and PU3 seedlings showed thinner stems and lighter roots. From a practical standpoint, this pattern suggests that seedlings from intact polyembryonic seeds, especially those with three embryos, may be less desirable as planting material because their reduced root systems could compromise their ability to adapt to field conditions.

An additional aspect, not directly evaluated in this experiment, concerns the genetic background of the seedlings. Zygotic seedlings are genetically distinct from one another and from the mother tree, whereas some of the embryos in polyembryonic seeds may be nucellar in origin and therefore genetically similar to the mother tree. If polyembryonic seeds in duku behave in this way, cut polyembryonic seeds could in principle provide seedlings that combine acceptable vigor with greater genetic uniformity. At the same time, any propagation system based on polyembryonic seeds must ensure that seed handling does not strongly reduce vigor in the nursery. The present findings indicate that sowing polyembryonic seeds intact is not optimal from the perspective of seedling growth, while cutting the seeds offers a better compromise.

It is also important to recognize some limitations of the present work. The experiment focused on early seedling growth in a nursery environment and did not follow the seedlings into the field. It therefore remains to be tested whether the differences observed at the nursery stage persist after transplanting and how they influence long term growth and fruit production. Future studies could extend the evaluation to field performance and could also include genetic analyses to distinguish zygotic and nucellar seedlings. Such information would help clarify the extent to which polyembryony can be exploited to produce uniform and vigorous planting material in duku.

Nonetheless, the present results already provide useful guidance for nursery practice. They suggest that when zygotic seeds are available, they remain the most reliable source of vigorous duku seedlings. When growers wish to take advantage of polyembryony, they should avoid sowing polyembryonic seeds intact and instead consider cutting them into sections, particularly into two or three parts, to reduce competition among embryos and improve seedling vigor.

CONCLUSION

This study evaluated the effects of different seed types and handling methods on early seedling growth of duku. Zygotic seeds with a single embryo produced the most vigorous seedlings, characterized by the largest number of leaves, greatest plant height, thickest stems, largest leaf areas, and heaviest roots. Seedlings raised from intact polyembryonic seeds, especially those with three embryos, showed reduced vigor in all measured traits, indicating strong competition among embryos when the seed is planted whole. Cutting polyembryonic seeds into two or three sections before sowing improved seedling performance. Seedlings from cut polyembryonic seeds developed stem diameters and root weights close to those of zygotic seedlings and intermediate values for leaf area and leaf number.

From a practical perspective, these results suggest that zygotic seeds are still the best option for producing vigorous duku seedlings when they are available. However, when growers wish to use polyembryonic seeds, sowing cut seed sections rather than intact seeds can be recommended as a way to obtain seedlings with acceptable vigor. Further research that follows these seedlings into the field and examines their genetic background would add important information on the long term suitability of these different seedling types as planting material for duku orchards.

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