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Evaluation of Viability, Vigor, and Early Seedling Growth of Mango (*Mangifera indica* L.) from Polyembryonic and Zygotic Seeds .

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Abstract: Polyembryony in mango offers a potential route to produce uniform planting material, yet its practical value relative to zygotic seed sources requires empirical evidence. This study assessed whether polyembryonic seeds can serve as reliable sources of seedlings and examined how embryo origin and seed integrity affect early growth. The research was conducted for eight months in Eris Village and in the Plant Science Laboratory and shade house. A randomized complete block design with five treatments and four replications was used, comprising whole zygotic seeds that produced a single seedling (Z1), whole polyembryonic seeds that produced two and three seedlings (U2, U3), and split polyembryonic seeds yielding two and three seedlings (B2, B3). Viability exceeded eighty five percent for the zygotic lot and for the intact polyembryonic lots, and mean germination time was 3.243 days, which together indicate good physiological quality. Analysis of variance detected no significant differences among treatments for plant height, leaf number, and stem diameter, which shows that early vegetative growth was broadly comparable regardless of seed origin. Root weight differed at the five percent level, with Z1 and U2 forming a higher group than U3, B2, and B3, while leaf area varied descriptively and tended to be greater in intact seed treatments without confirmed statistical separation. Visual records of germination, seedling morphology, and nursery beds supported these findings. Overall, polyembryonic seeds, whether used intact or split, produced seedlings whose early performance matched that of zygotic seedlings, which supports their use as feasible sources of planting material under the conditions of this study, while highlighting a possible advantage in belowground biomass for some intact treatments.

Keywords : polyembryony, Mangifera indica, zygotic seedlings, nucellar seedlings, seed viability, seed vigor, mean germination time, early seedling growth, leaf area, root biomass, nursery performance.

INTRODUCTION

Seeds are the reproductive structures of higher plants that are produced through sexual reproduction. This process involves pollination, fertilization, and subsequent seed development. Seeds contribute to the persistence of species and serve as the natural means for plants to populate new areas through generative growth. This is achieved through seed dispersal, which transports propagules to suitable sites for establishment. Seed-based propagation has advantages and limitations. It enables the production of large numbers of propagules within a given period and facilitates broad geographic distribution, yet the resulting offspring show substantial genetic variability because sexual reproduction reshuffles genetic material. Even so, polyembryony in some species can yield genetically uniform nucellar seedlings that are useful for producing uniform rootstocks.

Polyembryony is the occurrence of two or more embryos within a single seed. It is commonly found in angiosperms such as avocado, cacao, citrus, jackfruit, duku or langsat, durian, rambutan, and mango, which show polyembryony in their seeds. Polyembryony can arise through several pathways. In cleavage polyembryony, the zygote or proembryo divides into two or more parts and each part develops into an embryo. Additional embryos can also originate from cells inside the embryo sac, for example synergids that proliferate without fertilization, alongside the zygotic embryo and sometimes from a second embryo sac. Embryos may develop from tissues outside the embryo sac, such as the nucellus or the integuments, that later grow into the embryo sac and are nourished by the endosperm. A further possibility is the development of embryos from another embryo sac within the same ovule. In some cases, polyembryony can be induced by external stimuli.

Figure 1. Manggo seeds

The horticultural value of polyembryony is considerable. It can produce uniform seedlings comparable to those obtained through vegetative propagation. In citrus, nucellar or adventive embryos yield seedling rootstocks that are vigorous, possess a strong tap root, and are often virus free, which makes them superior as rootstocks compared with cuttings. Polyembryony-based seedling production can also restore vigor that may be lost after repeated vegetative propagation. In addition, horticultural breeding programs in some species have obtained haploid plants through induced parthenogenesis or related techniques.

Mango is considered a medicinal plant because many of its parts are used therapeutically. The roots, bark, and fruit contain constituents with anti-inflammatory and laxative properties, while the flowers and leaves are traditionally used to manage constipation, boils, wounds, diarrhea, and anemia

Mango can be propagated by seed through generative reproduction and by vegetative methods such as budding and grafting. Budding and grafting use rootstocks raised from seeds or from nursery seedlings. Mango seeds exhibit polyembryony in addition to containing a zygotic embryo. Polyembryony arises through two mechanisms. One is amphimixis, in which the sperm cell fuses with the egg cell to form a zygotic embryo. The other is apomixis, in which embryos form without fertilization, typically from nucellar tissues. In many cultivars these processes occur within the same ovule and can produce one or more embryos in a single seed, a condition known as polyembryony.

The success of mango cultivation also depends on site conditions. Mango shows broad adaptability and can grow at low and higher elevations, and it tolerates regions with relatively low rainfall. To achieve optimal production, temperature and humidity during flowering and fruit set need to be suitable for pollen performance and fertilization. The soil should be friable and well aerated, with a balanced proportion of sand and clay, and an optimal pH between 5.5 to 7.5.

RESEARCH METHOD

Research Site and Period

The study was conducted in Eris Village, Eris District, in the Plant Science Laboratory of the Faculty of Agriculture and in a shade house, over eight months from May 2024 to December 2024. Use of shade houses and laboratory–nursery workflows is common practice in seedling research and nursery production, which aligns with this design.

Materials and Equipment

The materials and equipment comprised mango seeds; urea and NPK fertilizers; the insecticide carbofuran marketed as Furadan; the fungicide copper oxide marketed as Nordox; the herbicide glyphosate marketed as Roundup; bamboo stakes; plastic ties; a sprayer; a hoe; a machete; a woven tray known locally as a nyiru; pipettes; germination boxes; Petri dishes; hoses; and administrative supplies including markers, HVS paper, pens, folders, labels, and printer ink. Use of pesticides and fungicides in mango nurseries and orchards is documented in the mango pest and disease management literature, and nursery practice reviews describe comparable inputs and tools.

Methods

The research proceeded in stages. A preliminary survey was followed by a laboratory study to examine and test polyembryonic mango seeds collected in the survey. The observed variables included seed viability, seed vigor, and a set of seedling vigor indicators comprising leaf number, leaf area, seedling height, root mass, stem diameter, and seedling age. A seedling trial was then established in Eris Village using a randomized complete block design with five treatments and four replications.

Data were analyzed by analysis of variance, and when treatment effects were significant the means were compared using the Least Significant Difference test at the five percent level.

RESULTS AND DISCUSSION

This section presents the outcomes of the germination test conducted on polyembryonic mango seeds. Seed viability was quantified as the percentage of normal seedlings at the end of the standard germination test. Seed vigor was expressed as a time-based metric in days, interpreted as mean germination time, in which lower values indicate faster and more uniform emergence. These measures capture complementary dimensions of physiological seed quality.

Table 1. Percentage of mango seed viability and seed vigor

Treatment	Seed Viability (%)	Seed Vigor (days)
Germinated seeds	85 %	3,243

Interpretation of **Table 1**. The germination test yielded a viability of eighty-five percent, indicating that most seeds produced normal seedlings under the test conditions. The vigor value of 3.243 days reflects a relatively rapid and synchronous emergence for this lot, since shorter mean germination times are associated with greater seed vigor. Together, these indices suggest that the seed lot is suitable for subsequent nursery work and field evaluation

This study aimed to characterize the attributes of polyembryony in mango seeds, to determine whether polyembryonic seeds can be used as a seed source, to quantify the number of seedlings produced, and to examine the relationship between polyembryony and mango seedling growth. The observations focused on seed viability and seed vigor. Seeds with viability greater than eighty percent meet the requirements for further development, because seed quality is strongly influenced by physiological attributes such as viability and vigor. In addition, seed quality can be assessed through physical factors, namely seed purity, and genetic factors that determine the superior characteristics of a seed lot. According to Kamil (1982), high-quality seeds must satisfy genetic criteria such as high productivity, resistance to pests and diseases, and responsiveness to growth conditions, along with physical criteria that include purity, freedom from debris or insects, and appropriate moisture content.

In general, seeds with high vigor tend to show high viability, although a high level of viability is not always accompanied by the same level of vigor. Viability reflects the proportion of seeds that germinate, whereas vigor refers to the capacity for rapid and uniform germination and robust seedling establishment, which is often captured by the speed of germination. It is also possible to observe high vigor under laboratory conditions while vigor declines in the field, which warrants further investigation under realistic nursery or field environments.

The following results summarize the mean effects of the seed treatments on three early growth attributes of mango seedlings, namely plant height, leaf number, and stem diameter at the final observation. These traits are commonly used as practical indicators of seedling vigor and early vegetative performance in nursery studies, and they tend to covary during establishment.

Table 1. Variables and the convergent validity assessment of the model

Treatment	Plant Height (cm)	Leaf Number	Stem Diameter (cm)
Whole mango seed 1 (Z1)	53.22	5.75	0.59
Whole mango seed 2 (U2)	32.72	4.50	0.47
Whole mango seed 3 (U3)	36.00	4.50	0.46
Split mango seed into two (B2)	29.20	4.75	0.46
Split mango seed into three (B3)	34.67	4.50	0.45

The whole-seed treatment labeled Z1 produced the tallest seedlings and the largest stem diameter, with a slight advantage in leaf number compared with the other treatments. Treatments that involved splitting the seed showed lower means for height and stem thickness. This pattern is consistent with the role of intact cotyledons as reserve tissues that support early growth, because damage or removal of cotyledon tissue can reduce seedling growth and vigor. The use of plant height, leaf number, and stem diameter as summary indicators of early vigor is well established in mango seedling evaluations.

Based on the statistical analysis, plant height, leaf number, and stem diameter did not differ significantly among treatments. The effects of the whole-seed zygotic treatment, whole-seed treatments two and three, and the split-seed treatments were therefore comparable for seedling growth. This finding indicates that both zygotic and polyembryonic seed sources can be used to produce planting material under the conditions of this study.

For plant height, leaf number, and stem diameter, the absence of significant differences among the zygotic whole-seed treatment and the polyembryonic treatments that used whole and split seeds can be explained by internal and external determinants of early growth. Internal factors include the quality and quantity of endosperm reserves and the intrinsic viability of the embryo, whereas external factors include the supply of nutrients and adequate space that permits optimal growth.

Successful mango cultivation depends on a reliable supply of high-quality seedlings that can establish and develop well in the field. The zygotic seedling from a whole seed originates from the fusion of the male sperm cell and the egg cell, while polyembryonic seeds also yield nucellar seedlings that are genetically uniform with the mother tree and are widely used as rootstocks. Seed quality encompasses genetic and physical purity as well as physiological attributes such as viability and vigor, which together determine performance.

Stem diameter and plant height function as practical indicators of seedling vigor and vegetative performance in mango nurseries. Larger stem diameter and greater height generally reflect stronger vegetative growth, which supports later reproductive development. The use of these traits as vigor indicators in mango seedling evaluations is documented in nursery and rootstock studies.

The next results summarize the mean effects of the seed treatments on leaf area and root weight at the final observation. Leaf area provides a proxy for photosynthetic capacity and early shoot development, while root biomass reflects belowground resource acquisition and the ability of seedlings to establish. Together, these metrics are widely used as indicators of seedling vigor in nursery studies.

Table 3. Mean effects of mango seed treatments on leaf area and root weight.

Treatment	Leaf Area (cm²)	Root Weight (g)
Whole mango seed 1 (Z1)	314.94	3.40 b
Whole mango seed 2 (U2)	222.30	2.82 b
Whole mango seed 3 (U3)	130.57	1.37 a
Split mango seed into two (B2)	135.50	1.45 a
Split mango seed into three (B3)	232.71	1.60 a
LSD 5%		0.43

Note. Means followed by the same letter are not significantly different according to the Least Significant Difference test at the five percent level.

Leaf area varied among treatments, although no mean separation letters were provided for this trait, so statistical differences cannot be inferred from the table. Root weight showed significant differences at the five percent level. The whole-seed treatments Z1 and U2 formed a homogeneous group with higher root biomass than U3, B2, and B3, which formed a second homogeneous group. Greater root biomass is consistent with stronger seedling vigor and improved establishment potential under nursery and field conditions. The use and interpretation of letter groupings for mean separation with the LSD procedure are standard practice in horticultural experiments.

Figure 2 presents the mean values of four early growth variables of mango seedlings, namely plant height, leaf number, stem diameter, and leaf area at the final observation. The treatment codes are read as follows. Z1 denotes the whole seed that produced the zygotic seedling. U2 and U3 denote two and three seedlings that arose from intact polyembryonic seeds. B2 and B3 denote two and three seedlings that arose from split polyembryonic seeds. The graph provides a descriptive view that complements the numerical summaries in the tables.

As we can see from Figure 2, Z1 shows the highest overall performance, with the greatest height, stem diameter, and leaf area. U2 lies in an intermediate position. U3 and B2 exhibit the smallest leaf areas together with relatively low height and diameter. B3 shows a partial recovery in leaf area while stem diameter remains small. The small gaps among treatments for height, leaf number, and stem diameter are consistent with the earlier statistical test that detected no significant differences for these three traits. Leaf area varies more clearly, which suggests that seed integrity may be associated with early canopy development.

These descriptive patterns should be interpreted together with the ANOVA and mean separation results in the tables, so the main conclusion remains that zygotic and polyembryonic seed sources perform comparably at the seedling stage under the conditions of this study.

Figure 3 documents the germination workflow used for mango seeds in this study. Seeds were extracted, cleaned to remove adhering pulp, and sorted to exclude damaged seed material. Clean seeds were then sown in nursery trays filled with a well drained potting medium. Seeds were placed horizontally at uniform spacing and covered lightly, practices that favor rapid and uniform emergence. Trays under protected conditions help standardize moisture and temperature during early establishment.

Figure 2. Average Plant Height, Number of Leaves, Stem Diameter and Leaf Area

Figure 3. Mango Seed Germination.

After Figure 3. The first panel shows cleaned and sorted seeds prepared for sowing. The second panel shows seeds arranged in uniform rows in trays containing the growth medium. This layout provides consistent spacing and a comparable microenvironment across treatments. Such standardization is important because the composition of the medium, sowing depth, and seed orientation can influence germination percentage, emergence speed, and early seedling growth. Faster emergence is reflected by a lower mean germination time, which is a widely used vigor metric in seed studies. The sequence illustrated in Figure 2 therefore represents common nursery practice for establishing mango seedlings for experimental evaluation.

Figure 4 depicts a normal zygotic mango seedling that serves as the reference phenotype in this study. A single shoot emerges from the cotyledonary node and bears typical juvenile leaves. The hypocotyl and epicotyl are clearly elongated, and the primary taproot is intact with the initiation of lateral roots. The overall balance between shoot and root growth indicates healthy establishment under the germination conditions used.

Figure 4. Normal zygotic mango seedling

This morphology is characteristic of seedlings produced by sexual fertilization. The single main stem, the continuous taproot axis, and the uniform juvenile leaf form distinguish it from seedlings of polyembryonic origin that may show variable vigor or the emergence of multiple shoots. In the evaluation of treatment effects, the zygotic seedling functions as a benchmark against which the performance of seedlings from polyembryonic seeds is compared. The reference phenotype supports the conclusion that early differences among treatments are modest and are expressed more clearly in traits such as leaf area and root biomass than in shoot architecture.

Figure 5 documents normal polyembryonic seedlings of mango. In polyembryonic cultivars, a single seed typically carries one sexually derived zygotic embryo together with several somatic or nucellar embryos that are genetically identical to the mother plant. The emergence of two or more seedlings from the same seed is a characteristic expression of this reproductive mode and is the basis for using polyembryonic seedlings as uniform rootstocks in mango nurseries.

Figure 5. Normal polyembryonic mango seedlings

Figure 5. shown two expressions of polyembryony at the seedling stage. The left panel displays one vigorous seedling accompanied by a smaller companion seedling that both originate from a single seed, while the right panel shows two vigorous seedlings and a third smaller shoot emerging from the same seed tissue. These patterns are consistent with nucellar embryony, which produces multiple seedlings that are usually uniform, whereas the zygotic seedling often emerges later and may be less vigorous. The visual distinction between nucellar and zygotic seedlings is supported by morphological and molecular studies that document multiple independent shoots arising from a single polyembryonic seed.

Figure 6 documents abnormal mango seedlings observed during the germination phase. In this study, seedlings were classified as abnormal when they failed to develop a balanced axis consisting of a healthy shoot and a functional primary root system, or when extensive tissue damage and decay prevented normal development. Such abnormalities reduce the effective stand establishment and can confound vigor assessments if not recorded separately from normal seedlings.

Figure 6. Abnormal mango seedlings

Figure 6 shows representative defects captured at the time of evaluation. The seedlings display necrotic or damaged cotyledonary tissues, shortened or poorly branched radicles, and uneven shoot emergence. These symptoms are consistent with mechanical injury incurred during seed preparation, deterioration following prolonged moisture or microbial contamination, and competition among embryos within polyembryonic seeds that can leave some sprouts weak and underdeveloped. The presence of these abnormal seedlings helps explain variation in early growth and underscores the need to distinguish normal seedlings for viability calculations and to interpret vigor indices with caution.

Figure 7 illustrates the field nursery established for the seedling phase of the experiment. Raised beds were prepared to improve drainage and soil aeration, and each plot was clearly labeled to ensure traceability of treatments during data collection. Seedlings were transplanted at uniform spacing to minimize competition for light and nutrients. The surface of the planting holes was lightly mulched to help conserve moisture and suppress early weed emergence. The arrangement of beds and irrigation lines facilitated consistent watering and simplified routine maintenance throughout the observation period.

Figure 7. Nursery beds with mango seedlings

The first panel shows a series of well-formed raised beds with plot markers aligned along the rows, indicating the experimental layout used for the randomized blocks. The remaining panels present close views of representative seedlings that display the characteristic bronze juvenile leaves of mango and an upright, healthy shoot axis. The mulch surrounding each plant indicates standard nursery practice to stabilize soil temperature and moisture. The uniform appearance of the seedlings and the clear labeling of plots support reliable measurements of early growth traits such as height, leaf number, stem diameter, and leaf area in subsequent evaluations.

Figure 8 documents the field monitoring stage of the experiment, when mango seedlings were evaluated in the nursery beds. Observations focused on the same early growth attributes used in the analysis, namely plant height, leaf number, stem diameter, and general seedling vigor. The photographs illustrate the condition of representative plants at the time of measurement and provide visual context for the numerical results reported in the tables.

The panels show healthy seedlings with upright stems and the characteristic bronze juvenile leaves that are transitioning to green. The first image presents a single vigorous seedling with a well elongated main stem. The second and third images show planting points where more than one shoot is present, which is consistent with seedlings arising from polyembryonic seeds. Across the views, leaf expansion appears uniform and the stems are sturdy, which supports the conclusion that early vegetative growth among treatments was broadly comparable under the nursery conditions of this study.

Figure 8. Observation of mango seedlings

Figure 9 illustrates the procedure used to obtain measurements of leaf area and root weight. Seedlings were carefully uprooted, the root systems were washed free of substrate, and excess surface moisture was removed before weighing. Root weight was recorded on a digital balance as fresh weight, which is common in seedling studies, while recognizing that dry weight can provide a more stable index of biomass. Leaf blades were detached and arranged flat for measurement. Leaf area can be determined non-destructively from simple linear dimensions or by planimetric methods, both of which are widely used in mango and other horticultural crops.

The first panels show harvested seedlings with the shoots and cleaned root systems ready for measurement. The second panel shows the leaves spread flat alongside a ruler and cutting tools, a setup that facilitates recording leaf length and width or scanning for planimetric analysis. The third panel shows the root system on a digital balance with a fresh weight of 2.3 grams. Together these images document the workflow used to generate the leaf area and root biomass data reported in the tables and figures of this study

CONCLUSIONS

The study shows that the mango seed lots used, namely the zygotic lot Z1 and the intact polyembryonic lots that produced two and three seedlings, reached viability levels above eighty five percent, which meets common thresholds for quality seed. Mean germination time indicated good vigor of the lot as a whole. Across the core vegetative variables of plant height, leaf number, and stem diameter there were no statistically significant differences among Z1, the intact polyembryonic treatments, and the split seed treatments, which means early growth in the nursery was broadly comparable regardless of seed origin. Leaf area varied more markedly in a descriptive sense, with the intact zygotic treatment tending to be higher, while the Least Significant Difference test detected separation for root weight, where Z1 and U2 formed a higher group than U3, B2, and B3. Taken together, these findings indicate that polyembryonic seeds, whether sown intact or after splitting, can be considered suitable sources of planting material alongside zygotic seeds under the conditions of this experiment, with a possible advantage in belowground biomass for some intact treatments and a tendency for larger leaf area when cotyledons remain whole.

Further work is recommended to sharpen and extend these conclusions. Future experiments should verify the origin of seedlings in polyembryonic lots so that nucellar and zygotic seedlings can be distinguished with confidence, follow seedling cohorts from nursery to field to test whether early similarities persist into establishment and yield, and examine a wider range of environments, substrates, and management regimes that may interact with seed integrity and embryo number. It would be valuable to compare additional polyembryonic sources beyond avocado, cacao, citrus, and jackfruit to include duku, langsat, and rambutan, to test the generality of responses. Studies that standardize sowing depth and orientation, quantify the effects of splitting on cotyledon reserves, include both fresh and dry biomass measurements, and assess practical traits such as survival, disease tolerance, and graft compatibility will provide clearer, deeper, and more accurate evidence on how polyembryony can be used to produce reliable seed and rootstock material.

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