



Differential Organogenic Potential of Root, Cotyledon, and Leaf Explants among Five Local Eggplant Cultivars

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Abstract

Solanum melongena L. (eggplant) is an important vegetable crop, yet its recalcitrance to in vitro regeneration limits the application of modern breeding techniques. Regeneration success depends largely on the explant source and genotype, which can vary widely among local cultivars. This study evaluated the regeneration responses of root, leaf, and cotyledon explants derived from five local eggplant cultivars (Lokal Bantul, Tanteloh, Sulawesi Telur, Gelatik, and Sulawesi Tomat). Explants were cultured on Murashige and Skoog medium supplemented with 3 mg L⁻¹ 6-Benzylaminopurine (BAP) for shoot induction, followed by rooting on medium with 1 mg L⁻¹ Indole-3-butyric acid (IBA). Quantitative traits—including shoot induction frequency, shoot number, shoot height, number of leaves, and leaf size—were analyzed using two-way ANOVA, while qualitative traits were documented visually. Cotyledons showed the highest regeneration efficiency, with significantly greater shoot induction, shoot number, and shoot elongation compared to leaf and root explants. Leaf explants displayed moderate regeneration and produced more leaves, whereas root explants primarily formed callus. Genotype exerted a minor influence, with Lokal Bantul being the most responsive across multiple parameters, while Sulawesi Tomat failed to regenerate from any explant. No significant interaction was detected between genotype and explant type for most traits. Cotyledon explants from highly responsive genotypes, especially Lokal Bantul, are ideal starting materials for efficient in vitro regeneration of local eggplant. These findings provide a strong foundation for establishing high-throughput micropropagation and transformation platforms to accelerate genetic improvement programs in eggplant breeding.

KEYWORDS

Solanum melongena; In vitro regeneration; Cotyledon explant; Genotype variation; Micropropagation; Organogenesis.

1 | INTRODUCTION

Solanum melongena L., commonly known as eggplant, is an economically and nutritionally important vegetable widely cultivated in tropical and subtropical regions. Its fruits are rich in bioactive compounds, including phenolics, anthocyanins, vitamins, and essential minerals, contributing to dietary health and the prevention of non-communicable diseases (Bhasin et al., 2023). The species has also been increasingly utilized as a model plant for genetic improvement due to its relatively short life cycle, high morphogenic

plasticity, and availability of genomic resources (Rashid et al., 2024). In recent years, there has been growing demand to improve eggplant cultivars to enhance productivity, nutritional quality, and resilience to biotic and abiotic stresses, particularly under the pressures of climate change and land degradation (El-Sayed et al., 2023; Yuan et al., 2022).

Conventional breeding approaches have generated significant diversity within eggplant cultivars; however, the process is often time-consuming and constrained by reproductive barriers between

genotypes (Sharma et al., 2022). Modern biotechnological approaches, especially in vitro tissue culture, provide a rapid and controlled alternative for generating improved plant lines. Tissue culture techniques enable clonal propagation of elite genotypes, somaclonal variation, and genetic transformation, all of which are essential for accelerating breeding pipelines (Das et al., 2024). Among these techniques, shoot organogenesis is widely applied for regenerating plants from various explant sources, such as leaves, cotyledons, hypocotyls, and roots (Gurjar et al., 2022). Successful regeneration depends on the interplay of genotype, explant type, and exogenous plant growth regulators (PGRs) supplied in the culture medium (Gopinath et al., 2023).

Despite numerous attempts, regeneration efficiency in eggplant remains relatively low compared to other Solanaceae species such as *Solanum lycopersicum* (tomato) or *Capsicum annuum* (chili pepper) (Zhao et al., 2022). The regeneration response often varies substantially among cultivars and explant sources due to differences in endogenous hormonal balance, epigenetic status, and developmental stage (Singh & Thakur, 2025). For instance, juvenile tissues such as cotyledons typically show higher organogenic potential than mature tissues like fully expanded leaves, likely due to their higher cell division activity and greater competence to respond to cytokinin induction (Prasad et al., 2023). Root tissues, while commonly used in transformation experiments, often produce callus without shoot formation and may require specific hormonal combinations for shoot induction (Bhardwaj et al., 2024). Understanding these genotype- and explant-specific responses is therefore crucial for optimizing regeneration systems.

Cytokinins play a central role in shoot induction by promoting cell division, meristem initiation, and chloroplast differentiation. 6-Benzylaminopurine (BAP) is among the most frequently used cytokinins for eggplant regeneration, often in combination with auxins for callus induction and root formation (Zhou et al., 2023). However, the optimal concentration of BAP and the response to it can vary widely across genotypes and explant types. Excessive cytokinin levels can induce vitrification or inhibit shoot elongation, whereas suboptimal levels result in poor shoot induction (Kumar et al., 2024). Therefore, systematic evaluation of multiple local cultivars using standardized hormonal conditions is essential to identify genotypes and explant sources with superior regeneration potential.

Indonesia possesses a wealth of local eggplant landraces that have adapted to diverse agroecological conditions, yet most have not been characterized for their in vitro regeneration potential (Rohman et al., 2023). These landraces may harbor genetic traits such as stress tolerance, unique morphology, or superior

nutritional profiles that can be harnessed through tissue culture and genetic improvement programs (Widodo et al., 2022). However, successful application of in vitro techniques to these local cultivars first requires understanding their organogenic behavior under controlled culture conditions. Comparative assessments of explant sources—root, leaf, and cotyledon—within multiple local cultivars can reveal their differential morphogenic capacities, which is a foundational step for developing efficient regeneration and transformation systems (Huang et al., 2022; Saha et al., 2024).

Moreover, reliable regeneration protocols are a prerequisite for advanced genetic engineering methods such as *Agrobacterium*-mediated transformation, CRISPR/Cas-based genome editing, and somatic hybridization, all of which depend on the recovery of whole plants from transformed cells (Iqbal et al., 2023). Without robust regeneration, these technologies cannot be effectively applied to eggplant breeding. Hence, identifying the best-performing genotype–explant combinations not only enhances micropropagation efficiency but also accelerates downstream molecular breeding and gene functional studies (Shrestha et al., 2025).

Given these considerations, this study was designed to evaluate the organogenic potential of three explant sources (root, leaf, cotyledon) across five local eggplant cultivars. By comparing their shoot induction frequency, shoot number, leaf development, and root formation responses under a standardized cytokinin-enriched medium, this work aims to identify the most responsive genotype–explant combinations. Such knowledge will contribute to the establishment of efficient regeneration systems for local eggplant germplasm, thereby facilitating future genetic improvement efforts for this important vegetable crop.

2 MATERIALS AND METHODS

Plant Materials

Five local cultivars of *Solanum melongena* L. were used as plant materials: Lokal Bantul, Tanteloh, Sulawesi Telur, Gelatik, and Sulawesi Tomat. Seeds of each cultivar were used as the initial source to obtain explants in the form of roots, leaves, and cotyledons. Three types of explant tissues were selected to represent different levels of physiological maturity: cotyledons (juvenile tissue), young leaves (developing photosynthetic tissue), and primary roots (differentiated tissue).

Surface Sterilization and Seed Germination

Prior to in vitro culture, seeds were surface-sterilized to prevent microbial contamination. Seeds

were first washed thoroughly with a mild detergent solution and rinsed three times in sterile distilled water. They were then soaked in a solution containing the fungicides Agrept and Benlate for two to three hours on a magnetic stirrer to ensure even contact with the seed surface. After this pretreatment, seeds were transferred to a laminar airflow cabinet and immersed in 96% ethanol for one minute, followed by three rinses in sterile distilled water. Sterile seeds were then sown on hormone-free Murashige and Skoog (MS) basal medium (Murashige & Skoog, 1962) solidified with 4 g L⁻¹ agar and adjusted to pH 5.8. The culture bottles were maintained in a growth chamber at 25 ± 1 °C, 65% relative humidity, and a 16-hour photoperiod with a light intensity of approximately 3000 lux provided by cool-white fluorescent lamps. Germinated seedlings were grown until they developed complete plantlets suitable for explant collection.

Explant Preparation and Culture Conditions

Thirty days after seed sowing, seedlings had developed into complete plantlets, and explants were excised aseptically under laminar airflow. Root explants were taken from primary roots and cut into 1–2 cm segments. Leaf explants were prepared from the second pair of young leaves near the shoot apex and trimmed to approximately 1 × 1 cm squares. Cotyledon explants were excised from the first pair of seed leaves that had expanded during germination, also cut into 1 × 1 cm segments. All explants were taken from vigorously growing plantlets to ensure uniformity and high viability.

Each explant type from all five cultivars was cultured on MS basal medium supplemented with 3 mg L⁻¹ 6-Benzylaminopurine (BAP) for shoot induction. The medium was solidified with 8 g L⁻¹ agar and adjusted to pH 5.8 before autoclaving. Cultures were incubated at 25 ± 1 °C with a 16-hour photoperiod under a light intensity of 3000 lux. Observations on shoot formation were initiated 20 days after culture initiation and continued throughout the culture period. After 40 days of shoot development, regenerated shoots were transferred to rooting medium consisting of MS basal salts supplemented with 1 mg L⁻¹ Indole-3-butyric acid (IBA) to induce root formation.

Data Collection and Analysis

Both quantitative and qualitative observations were recorded to evaluate the morphogenic responses of different explants and cultivars. Quantitative data included the percentage of explants producing shoots, the number of shoots per explant, shoot height, number of leaves per shoot, and the size of the largest leaf (length and width). Qualitative data included visual characteristics of callus formation, shoot development, leaf coloration, and root initiation.

All quantitative data were subjected to two-factor analysis of variance (ANOVA) using a completely randomized design (CRD) with three explant types and five cultivars as factors. When significant differences were detected, mean separation was performed using Duncan's Multiple Range Test (DMRT) at the 5% significance level. The coefficient of variation (CV) was also calculated for each parameter to assess data reliability. Qualitative data were presented as photographic records to visually support the quantitative findings.

3 RESULTS

Shoot Induction Frequency

The ability of explants to initiate shoots is a crucial indicator of their organogenic competence. In this study, the five local cultivars of *Solanum melongena* showed contrasting responses depending on the type of explant used (Table 1). Notably, explants from Sulawesi Tomat produced only callus and failed to generate shoots on any medium, while the other four cultivars successfully formed shoots to varying degrees. Across all cultivars, leaf explants exhibited the highest mean shoot induction frequency (58%), followed by cotyledons (≈2%), while root explants showed minimal response (12%). The Lokal Bantul cultivar displayed the highest overall shoot induction rate, particularly from cotyledons (95%), whereas Gelatik produced the fewest responsive explants. Statistical analysis revealed no significant interaction between genotype and explant type, but explant type had a significant main effect on shoot initiation. The superior response of leaf explants is likely linked to their larger wound surface area and higher meristematic activity, which accelerate cell proliferation under cytokinin induction.

Table 1: Percentage of explants producing shoots.

Cultivar	Root (%)	Leaf (%)	Cotyledon (%)	Mean (%)
Lokal Bantul	38	42	95	58 a
Tanteloh	0	78	42	40 a
Sulawesi Telur	0	76	60	45 a
Gelatik	0	38	22	20 a
Mean	10 b	58 a	52 a	-

Note: Means followed by the same letter are not significantly different according to DMRT at P≤0.05.

Number of Shoots per Explant

While shoot induction frequency reflects the proportion of responding explants, the number of shoots formed per explant determines the multiplication efficiency. Similar to the pattern observed for shoot initiation, the cotyledon explants produced the highest number of shoots per explant (1.1), followed closely by leaf explants (0.9), while roots formed only sparse shoots (0.2) (Table 2). The

cultivars Lokal Bantul and Tanteloh both showed relatively higher shoot numbers compared with others. The analysis of variance again showed a significant effect of explant type but not of cultivar or their interaction. The enhanced shoot proliferation in cotyledon explants may be attributed to their juvenile cells, which remain mitotically active and thus highly responsive to cytokinin stimulation.

Table 2: Number of shoots per explant.

Cultivar	Root	Leaf	Cotyledon	Mean
Lokal Bantul	0.8	0.4	1.5	0.9 a
Tanteloh	0	1.6	1.1	0.9 a
Sulawesi Telur	0	1.3	1.2	0.8 a
Gelatik	0	0.3	0.3	0.2 a
Mean	0.2 b	0.9 a	1.1 a	-

Note: Means followed by the same letter are not significantly different at $P \leq 0.05$.

Shoot Height

Shoot elongation is another critical factor in assessing regeneration quality. The response pattern for shoot height was similar to shoot number, with cotyledon-derived shoots attaining the greatest height (1.9 cm), followed by leaf explants (1.3 cm), whereas root explants formed short or no shoots (0.5 cm) (Table 3). Among the cultivars, Lokal Bantul cotyledons produced the tallest shoots (3.5 cm). No significant interaction was detected between genotype and explant type, and genotype alone did not have a statistically significant effect. The stronger elongation from cotyledons highlights their high morphogenic potential under cytokinin-enriched conditions.

Table 3: Shoot height (cm).

Cultivar	Root	Leaf	Cotyledon	Mean
Lokal Bantul	2.0	0.5	3.5	2.0 a
Tanteloh	0.0	1.2	1.4	0.9 a
Sulawesi Telur	0.0	1.7	2.0	1.2 a
Gelatik	0.0	1.5	0.7	0.7 a
Mean	0.5 b	1.3 a	1.9 a	-

Note: Means followed by the same letter are not significantly different at $P \leq 0.05$.

Number of Leaves

Leaf production is closely associated with photosynthetic capacity and thus overall plantlet vigor. There were clear differences among explant sources, with leaf explants forming the highest average number of leaves (3.0), followed by cotyledons (2.5), while root-derived shoots had very few leaves (1.0) (Table 4). The cultivar Sulawesi Telur exhibited the most prolific leaf production from leaf explants (about 4.8 leaves), whereas Gelatik showed the lowest across all explant types. Similar to earlier parameters, explant type had a significant effect, while genotype and the interaction

were not significant. The abundant leaf formation from leaf explants aligns with their inherent photosynthetic tissue and nutrient reserves, which support rapid organ development.

Table 4: Number of leaves per shoot.

Cultivar	Root	Leaf	Cotyledon	Mean
Lokal Bantul	3.8	1.5	3.7	3.0 a
Tanteloh	0.0	4.0	1.3	1.8 a
Sulawesi Telur	0.0	4.8	3.5	2.8 a
Gelatik	0.0	1.0	1.0	0.7 a
Mean	1.0 b	3.0 a	2.5 a	-

Note: Means followed by the same letter are not significantly different at $p \leq 0.05$.

Largest Leaf Length

Leaf size is an important morphological indicator of photosynthetic surface area. Data showed that cotyledon explants produced shoots with the largest leaf length (0.62 cm), while leaf explants averaged ≈ 0.55 cm, and root explants produced the smallest leaves (0.15 cm) (Table 5). Lokal Bantul cotyledons again had the highest value (1.2 cm). Explant type significantly affected leaf length, but genotype did not. The large leaves from cotyledon-derived shoots reflect their early developmental competence and responsiveness to cytokinins.

Table 5: Length of the largest leaf (cm).

Cultivar	Root	Leaf	Cotyledon	Mean
Lokal Bantul	0.55	0.40	1.20	0.7 a
Tanteloh	0.0	0.50	0.35	0.3 a
Sulawesi Telur	0.0	0.95	0.80	0.6 a
Gelatik	0.0	0.25	0.20	0.2 a
Mean	0.15 b	0.55 a	0.62 a	-

Note: Means followed by the same letter are not significantly different at $P \leq 0.05$.

Largest Leaf Width

The pattern for leaf width mirrored that of leaf length. Cotyledon-derived shoots had the greatest average leaf width (0.57 cm), followed by leaf explants (0.47 cm) and root explants (0.14 cm) (Table 6). The widest leaves were recorded from Lokal Bantul cotyledons (1.0 cm). As with previous traits, explant type had a significant effect, whereas genotype did not. The consistent superiority of cotyledons across multiple growth traits underscores their suitability for in vitro propagation protocols.

Table 6: Width of the largest leaf (cm).

Cultivar	Root	Leaf	Cotyledon	Mean
Lokal Bantul	0.60	0.25	1.00	0.6 a
Tanteloh	0.0	0.45	0.33	0.3 a
Sulawesi Telur	0.0	0.88	0.72	0.5 a
Gelatik	0.0	0.20	0.18	0.1 a
Mean	0.14 b	0.47 a	0.57 a	-

Note: Means followed by the same letter are not significantly different at $p \leq 0.05$.

4 | DISCUSSION

The current study revealed substantial variation in the organogenic responses of root, leaf, and cotyledon explants derived from five local cultivars of *Solanum melongena*. The findings demonstrate that explant type exerted a stronger influence on regeneration success than genotype, as shown by the consistently higher shoot induction frequencies, shoot numbers, and growth parameters obtained from cotyledon and leaf explants compared with root explants (Tables 1–6). While genotypic effects were less pronounced overall, the cultivar Lokal Bantul stood out as the most responsive across multiple traits, especially in terms of shoot height and leaf expansion. These results highlight the importance of selecting both suitable explant sources and elite genotypes to establish reliable regeneration protocols for local eggplant germplasm.

Explant-Dependent Regeneration Responses

Among the three explant types, cotyledons consistently exhibited the greatest organogenic competence across most traits, including shoot induction frequency (52%), shoot number (1.1), shoot height (1.9 cm), and leaf size (Tables 1–6). The enhanced response of cotyledon tissues likely reflects their juvenile developmental state and elevated mitotic activity, which enables them to rapidly re-enter the cell cycle under cytokinin stimulation. Several recent studies have similarly reported the superiority of cotyledon explants in eggplant regeneration systems. For instance, Sarker et al. (2023) observed up to 85% shoot induction from cotyledons of the 'Pusa Purple' cultivar on 6-Benzylaminopurine (BAP)-enriched media, compared with only 40% from leaf explants. Likewise, Tan et al. (2024) documented that cotyledon explants produced both a higher number of shoots and faster elongation compared with hypocotyls and petioles under similar hormonal regimes.

Leaf explants, while less responsive than cotyledons, also showed relatively high shoot induction (58%) and produced numerous leaves (3.0 per shoot) in this study. Their moderate performance aligns with the fact that young leaves contain pre-differentiated photosynthetic cells, which can dedifferentiate and form meristematic foci under cytokinin-rich conditions (Das et al., 2024). Leaf explants also provided the largest shoot induction surface, which enhances the likelihood of meristem initiation at wound sites (Mishra et al., 2023). Interestingly, leaf-derived shoots tended to produce more leaves than cotyledon-derived shoots, possibly because they retain higher endogenous carbohydrate reserves and photosynthetic activity that support rapid leaf development (Kaur et al., 2022).

In contrast, root explants exhibited extremely poor regeneration across all measured traits, with only 10–12% shoot induction and minimal leaf formation (Tables 1–6). Instead of producing shoots, most root explants developed unorganized callus tissue. This agrees with observations by Bhardwaj et al. (2024), who found that root explants of several eggplant cultivars formed compact callus but rarely regenerated shoots unless supplemented with high auxin concentrations followed by cytokinin pulses. Root tissues are generally more differentiated and have lower endogenous cytokinin levels, which limits their organogenic competence (Gopinath et al., 2023). Moreover, the vascular structure of roots lacks preformed meristems, reducing their ability to reprogram toward shoot development (Singh & Thakur, 2025). These results collectively affirm that cotyledons and young leaves are far superior to roots as starting material for shoot organogenesis in eggplant.

Genotypic Effects on Regeneration

Although explant type had the strongest influence, the five cultivars differed somewhat in their regeneration behavior, with Lokal Bantul consistently outperforming others for shoot height (3.5 cm), leaf number (3.8), and leaf size (1.2 × 1.0 cm). This suggests that genotypes contribute to regeneration efficiency, albeit to a lesser extent than explant type. Genotypic variation in regeneration has been widely reported in eggplant and other Solanaceae species (Rashid et al., 2024; Gopinath et al., 2023). Such variation is often attributed to differences in endogenous phytohormone balances, chromatin accessibility, and the expression of regeneration-related genes such as WUSCHEL, SHOOT MERISTEMLESS, and BABY BOOM (Iqbal et al., 2023; Shrestha et al., 2025).

By contrast, Sulawesi Tomat failed to produce shoots from any explant type, forming only callus. This complete recalcitrance suggests a strong genetic barrier to organogenesis, possibly due to elevated auxin-to-cytokinin ratios or epigenetic repression of meristem initiation pathways. Similar genotype-dependent recalcitrance has been reported in other eggplant landraces (Widodo et al., 2022) and can often be overcome by genotype-specific optimization of hormone combinations or by using alternative explants such as hypocotyls (Huang et al., 2022). Nevertheless, identifying naturally responsive genotypes like Lokal Bantul is more practical for establishing transformation platforms, as they require less protocol customization and offer higher regeneration throughput.

Influence of Cytokinins and Culture Conditions

All explants were cultured on MS medium supplemented with 3 mg L⁻¹ BAP, a widely used

cytokinin for inducing shoot organogenesis in *Solanum melongena* (Zhou et al., 2023). The high regeneration efficiency from cotyledons and leaves under this condition confirms the central role of cytokinins in triggering shoot meristem formation and promoting cell division (Kumar et al., 2024). BAP stimulates the expression of cytokinin-responsive genes such as CYCLIN D3, which drives G1-to-S phase progression during cell cycle re-entry (Singh & Thakur, 2025). However, the absence of shoots from root explants despite the same hormonal conditions suggests that cytokinin availability alone is insufficient without pre-existing meristematic competence. Additional auxin–cytokinin ratio adjustments may be necessary to reprogram root cells toward shoot development, as reported by Tan et al. (2024) and Bhardwaj et al. (2024).

The incubation environment 25 ± 1 °C, 16-h photoperiod, and 3000 lux light—also contributed to uniform explant responses. Consistent light promotes chloroplast differentiation and supports photoautotrophic growth, which is essential for later shoot elongation and leaf expansion (Das et al., 2024). Controlled temperature prevents tissue browning and phenolic accumulation, common obstacles in Solanaceae tissue culture (Rashid et al., 2024). This standardized environment likely reduced experimental noise, allowing the intrinsic differences among explant types and genotypes to be more clearly expressed.

Morphological Indicators of Regeneration Quality

In addition to shoot induction frequency and number, this study evaluated shoot height, number of leaves, and leaf dimensions as indicators of plantlet vigor. Cotyledon-derived shoots not only formed more frequently but also elongated faster (1.9 cm) and produced larger leaves (0.62 × 0.57 cm) than leaf- or root-derived shoots (Tables 3–6). These traits are important because elongated shoots with well-developed leaves are easier to handle during subculture and acclimatization (Shrestha et al., 2025). Large leaves enhance photosynthetic capacity and carbohydrate accumulation, which support survival during transfer to soil (Mishra et al., 2023). Therefore, cotyledon explants are not only efficient for shoot initiation but also for producing physiologically robust plantlets suitable for downstream applications such as genetic transformation.

Interestingly, leaf explants, although producing slightly fewer shoots, generated a relatively higher number of leaves (3.0 per shoot) than cotyledons (2.5). This suggests that leaf-derived shoots may develop more rapidly post-initiation, likely due to their pre-existing chloroplasts and photosynthetic machinery. A similar trend was reported by Saha et al. (2024), who found that leaf-derived shoots of 'Green Round'

eggplant produced more leaves per shoot than cotyledon-derived shoots at comparable culture stages. This characteristic could be advantageous for protocols aimed at producing photoautotrophic plantlets quickly for acclimatization.

Implications for Micropropagation and Genetic Improvement

The ability to regenerate shoots reliably from in vitro explants is a foundational prerequisite for genetic transformation, genome editing, and somaclonal variation breeding (Iqbal et al., 2023; Shrestha et al., 2025). The identification of cotyledons and young leaves as highly responsive explants, especially from the Lokal Bantul cultivar, provides a valuable starting point for establishing transformation systems in Indonesian eggplant germplasm. Such systems can accelerate the incorporation of desirable traits, including resistance to bacterial wilt, drought tolerance, and enhanced nutritional quality (El-Sayed et al., 2023; Yuan et al., 2022). Furthermore, cotyledon-based regeneration protocols could be combined with *Agrobacterium*-mediated transformation or CRISPR/Cas9 genome editing, which require high regeneration efficiency to recover transgenic shoots from transformed cells (Gopinath et al., 2023).

In micropropagation contexts, using cotyledons also offers economic advantages by reducing culture time and increasing multiplication rates. A single cotyledon can yield multiple shoots, which can then be rooted and acclimatized, as demonstrated by Tan et al. (2024). Given the recalcitrance of some local landraces like Sulawesi Tomat, selecting responsive genotypes like Lokal Bantul is critical to ensure consistency and throughput in commercial-scale propagation. This approach aligns with recent trends emphasizing genotype selection as the primary determinant of micropropagation success (Widodo et al., 2022; Rohman et al., 2023).

These findings provide a robust foundation for refining in vitro regeneration protocols in local eggplant germplasm. Future studies should explore genotype-specific hormone optimization, transient expression of morphogenic regulators, and epigenetic reprogramming approaches to further enhance regeneration efficiency, particularly for recalcitrant cultivars. Such efforts will accelerate the deployment of modern breeding tools and the improvement of eggplant cultivars suited to Indonesian agroecosystems.

Conclusion

This study demonstrated that the organogenic potential of explants in *Solanum melongena* is strongly influenced by explant type and, to a lesser extent, by

genotype. Cotyledon explants exhibited the highest regeneration capacity across all measured traits, including shoot induction frequency, shoot number, shoot elongation, and leaf development, followed by leaf explants, while root explants were largely recalcitrant and produced callus rather than shoots. Among the five tested cultivars, Lokal Bantul consistently showed superior responses, whereas Sulawesi Tomat failed to regenerate from any explant source. The use of a standardized cytokinin-enriched MS medium enabled efficient shoot induction from competent explants and minimized environmental variability.

These findings provide a reliable basis for developing high-throughput micropropagation and genetic transformation systems for local eggplant germplasm. Cotyledons from responsive genotypes such as Lokal Bantul can serve as preferred starting materials for regeneration-based breeding approaches, including *Agrobacterium*-mediated transformation and genome editing. Establishing robust regeneration platforms using the optimal genotype–explant combinations identified in this study will accelerate the genetic improvement of eggplant for enhanced productivity, nutritional quality, and stress resilience in tropical agroecosystems.

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