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ARGININE AS A GROWTH STIMULANT: AN *IN VITRO* STUDY ON JACKFRUIT (*ARTOCARPUS HETEROPHYLLUS* LAM.)

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ABSTRACT

Nitrogen is universally recognized as a fundamental nutrient required for optimal plant growth and development. Among various nitrogen sources, amino acids serve as a primary reservoir of organic nitrogen in tissue culture media, thus effectively supporting plant growth. The current study was designed to determine the influence of the amino acid arginine on the in vitro growth of jackfruit (*Artocarpus heterophyllus*) plants. The experimental setup employed a completely randomized block design, incorporating four distinct treatments with varying concentrations of arginine (0, 1.5, 2.5, and 3.0ppm). Findings indicated that supplementation with 2.5 and 3.0ppm arginine resulted in marked callus induction rates (90% and 80%, respectively) and robust shoot proliferation from nodal explants. Notably, root development was not observed by the 180th day of culture. Additionally, arginine application at different concentrations exhibited varied effects on biochemical parameters. Elevated levels of arginine (2.5, 3.0ppm) significantly enhanced total soluble protein content (57.3 and 65.8mg/g, respectively) while simultaneously reducing total soluble sugar (7.43 and 4.97mg/mL) and proline content (9.39 and 8.30µg/mL) compared to the control group (36.93mg/g, 8.62mg/mL, 22.05µg/mL, respectively). Overall, the data suggest that arginine plays a pivotal role in early plant developmental stages and influences key biochemical processes. The present research highlights the potential utility of arginine as a growth-promoting agent in plant tissue culture, laying the groundwork for further exploration in this field.

Keywords: Arginine, *Artocarpus heterophyllus*, Biochemical parameters, Callogenesis, Jackfruit.

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1. INTRODUCTION

The growth and propagation of plants depend on several factors, whether achieved through traditional methods or modern technology (Pasternak and Steinmacher, 2024). Although growth regulators are required only in trace amounts throughout a plant's life cycle, they are indispensable for ensuring normal physiological and morphological development (Kraiser et al., 2011; Ghosh et al., 2022). These regulators coordinate cellular division, elongation, and differentiation, thereby governing the plant's overall architecture and productivity (Sabagh et al., 2021). It is well known that nitrogen is one of the primary limiting factors affecting a plant's productivity (Mahmud et al., 2020). Biostimulants, such as amino acids and polyamines, add an external form of nitrogen to the culture medium, thereby reducing the negative effects of nitrogen deficiency and enabling critical developmental pathways to occur (Greenwell and Ruter, 2018). Although plants possess the capability of biosynthesizing amino acids, the process is relatively metabolically burdensome, requiring high amounts of carbon and nitrogen (Zayed et al., 2023). For this reason, providing additional amino acids exogenously can significantly improve growth, enhance tolerance to stress and enable metabolic processes during vital stages of development (Rafiee et al., 2013).

Foliar and root absorption of amino acids occurs in plant tissues, which help to modulate growth regulators (Spanoghe et al., 2020). Carbohydrate biosynthesis, translocation, and nutrient uptake are all enhanced; these and other functions collectively elevate protein content, and together, they facilitate the escalation of developmental and physiological processes in higher plants (Liu et al., 2015). Moreover, the chelating function of amino acids has become a focus of attention, as amino acid-metal complexes can improve the nutrient supply and correct micronutrient deficiencies in many crops (Ram et al., 2024). Specific amino acids, acting as polyamines, assist plant tissue culture by promoting totipotency, cellular differentiation, mitosis and molecular signaling pathways (Rakesh et al., 2021).

Glutamine, glutamate, proline, and arginine are effective nitrogen donors for purines and pyrimidines, as well as macromolecules' bigger structures (Efzueni Rozali et al., 2014). The action of proline or arginine formation from glutamine is catalyzed by glutamine synthetase. L-arginine serves multiple functions, being a further source of

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biochemically important nitric oxide and polyamines (Liu et al., 2015). Furthermore, it is critical in protein metabolism for proline and polyamine synthesis, the cytoplasmic osmotic potential of the cell, stomatal conductance, growth of the plant's vascular tissues, and overall vegetative development (Nejadalimoradi et al., 2014). There is evidence that the incorporation of arginine has better outcomes in tissue culture systems. For example, Pant et al. (2025) showed that L-arginine supplementation to MS medium during in vitro strawberry propagation significantly enhanced shoot and root induction as compared to control media. Moreover, Kaur et al. (2024) illustrated with L-arginine treatment the increased somatic embryogenesis and shoot regeneration from the stems of Sugarcane (Saccharum officinarum), reinforcing the idea that arginine acts as a beneficial growth stimulant in tissue cultures.

Jackfruit, or *Artocarpus heterophyllus* Lam., is a species from the Moraceae family and is a module of the family Perennials and Evergreens Genus Artocarpus (Baliga et al., 2011). Members of this group are particularly noted for their distinctive character of latex bearing, in which all their tissues exude a characteristic milky latex upon injury. The fruits of *A. heterophyllus* species typically range in weight from 3.5 to 10kg, although some are reported to reach 25kg. In Pakistan, mature jackfruits are significantly smaller, with a weight range of 0.3 to 1.5kg, containing 100 to 500 seeds. Each seed is surrounded by a whitish, fleshy air sac which encloses the cotyledon known to be rich in starch and protein (Ruiz-Montañez et al., 2015). It is also mentioned that the leaves of the jackfruit tree produce coumarin, which is known to have anticoagulant, antifungal, and possible antitumor properties (Thapa et al., 2016).

Furthermore, the use of ethyl acetate, ethanol, and water in extracting seeds has shown potent anthelmintic activity, which can be attributed to the alkaloids, flavonoids, and triterpenoids present (Waisundara, 2020). Such a plethora of secondary metabolites highlights the remarkable potential of *A. heterophyllus* as a source of nutraceuticals (Siregar et al., 2018). Certain plant parts undergo simple processing to improve their commercial appeal. Jackfruit rind, for example, has been identified as a promising high-fiber ingredient for cookies when blended with wheat flour (Ramya et al., 2020; Palamthodi et al., 2021; Han et al., 2025). In addition, jackfruit peel biochar has served as a low-cost sorbent for the removal of heavy metals such as Cd, Pb, Cu, Fe, Mn from water (Ibrahim, 2020).

A. heterophyllus is not commonly accepted as a commercially viable crop despite its many advantages because its seeds are recalcitrant. The constraints arising from bisexual propagation necessitate the design of effective alternatives for augmentative reproduction and the production of genetically superior planting material. In this case, plant tissue culture has proven to be an efficient method for clonal propagation and rapid multiplication of jackfruit. Several reports have optimized the composition of growth regulators with the level of callus initiation and its subsequent regeneration in jackfruit. Ashrafuzzaman et al. (2012) prepared a protocol for in vitro shoot regeneration from shoot tip explants using benzyl aminopurine (BAP).

Das et al. (2025) noted that BAP and thidiazuron (TDZ) both stimulate in vitro shoot initiation and multiplication. Benzyl adenine (BA), along with naphthalene acetic acid (NAA) and kinetin, was reported by Abd El-Zaher (2008) to significantly enhance callus formation and differentiation when added to Murashige and Skoog (MS) medium. Other authors have also investigated the effect of different plant growth regulators on jackfruit tissue culture responses (Ali et al., 2017; Kader et al., 2022). There is a considerable gap in the literature, however, concerning the influence of amino acids, especially arginine, in the in vitro culture of jackfruit. This is the first study that, to our knowledge, attempts a comprehensive study on the effects of arginine on the growth and development of jackfruit in vitro. This study aims to investigate the potential of arginine supplementation in tissue culture media to improve growth performance and modulate biochemical characteristics in *A. heterophyllus*.

2. MATERIALS AND METHODS

2.1. Plant Material and Experimental Site

This research was undertaken at the Institute of Botany, University of the Punjab, Lahore, specifically in the Plant Developmental and Regenerative Biology Laboratory. The geographical coordinates of the facility are 31°49′ N and 74°29′ E, with an altitude of 236 meters above sea level. For this study, explants were procured from a 30-year-old jackfruit (*A. heterophyllus*) tree, which is maintained in the Botanical Garden of the University of the Punjab and has been clinically examined to be 30 years old.

2.2. Surface Sterilization of the Explants

For washing the plant material, both nodal and leaf explants were treated with a detergent solution (Lemon Max, Karachi, Pakistan) prepared at home for five minutes to scrub away any surface dirt and/or debris. After this step, the explants were washed thoroughly with distilled water to rinse away all residues of the detergent. In the case of nodal segments, surface sterilization was achieved by soaking the tissue in 0.1% (w/v) mercuric chloride for 5 minutes, followed by several washes with distilled water. This was followed by treatment in a 15% bleach solution, where 0.6% sodium hypochlorite was dissolved and 0.1% Tween 20 was added. The solution was then

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placed in a 500mL Erlenmeyer flask (Pyrex, Corning Inc., Corning, NY). On the contrary, leaf tissues were sterilized by soaking in a 10 percent bleach solution for 10 minutes, followed by multiple washes with sterile distilled water. The entire sterilization sequence was carried out in a Laminar Airflow Cabinet (ESCO, Singapore; model 1750) to guarantee the maintenance of aseptic conditions throughout the process.

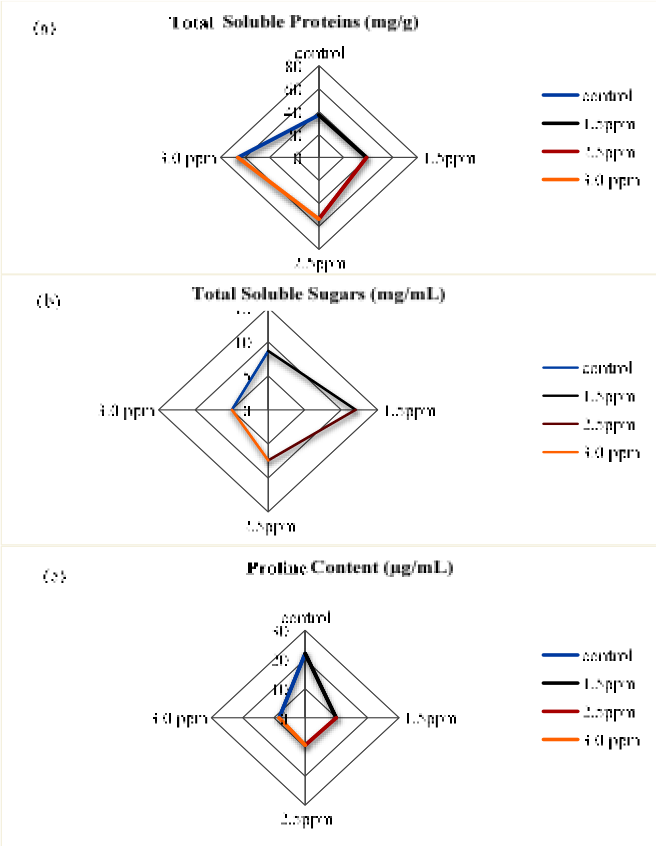

Fig. 1: Graphical Representation of Biochemical Alterations in Calli of *Artocarpus heterophyllus* grown on MS Medium Supplemented with Different Concentrations (1.5, 2.5, 3.0ppm) of arginine (a) Total Soluble Proteins (b) Total Soluble Sugars (c) Proline Content.

2.3. Selection and Preparation of Explants

In vitro studies explored the potential for callus induction in both nodal and leaf explants, while direct shoot induction was carried out on nodal segments alone. All steps of explant preparation and surface sterilization were performed using standard methods in order to avoid contamination. The explants could be inoculated on the culture medium following sterilization.

2.4. Media Formulation and Supplementation

The basal MS (Murashige and Skoog) medium served as the foundation for all tissue culture experiments. In the case of callus induction, an L-arginine gradient of 0, 1.5, 2.5, and 3.0ppm alongside varying levels of auxin and cytokine was used as a supplement to the MS media, as shown in Table 1. The inclusion of L-arginine was not required for IBA-induced roots, but it was maintained in the case of IBA-free additions. For the regeneration of shoots from the callus, the MS media was supplemented with BAP at a concentration of either 2 or 4mg/L, CK at 0.5mg/L and the remaining dosage of arginine. Micro-shoots that elongated from the nodal explants were separated from the culture containers and placed onto MS media with elevated doses of arginine and IBA to enhance rooting potential.

2.5. Incubation and Growth Conditions

All culture vessels were incubated in controlled growth chambers designed to maintain consistent environmental parameters. The temperature was controlled at 25±2°C to facilitate plant tissue culture development. The lighting was provided by cool-white fluorescent tubes (Philips, Pakistan) at a photosynthetic photon flux density of 45 µmol m^{-2} s^{-1}. Throughout the entire growth cycle, a photoperiod of 16 hours of light and 8h of darkness was maintained, which enhanced growth and supported morphogenetic and biochemical activities in the explants.

Table 1: The combinations of auxins and cytokinins with different levels of arginine used in the experiment

Sr. No.	Combinations of Growth Regulators	Concentration (mg/L)
1	KIN+IAA	2.5+2.5
		2.5+1.5
2	KIN+2,4-D	3.0+2.5
		2.5+2.5
3	BAP+NAA	4.0+2.0
4	BAP+NAA+Arginine	4.0+2.0+1.5
5	BAP+NAA+Arginine	4.0+2.0+2.5
6	BAP+NAA+Arginine	4.0+2.0+3.0

2.6. Measurement of Growth Parameters

The primary parameter measured and tracked for micro-propagated plants was shoot proliferation, which was further elaborated as part of plant growth. For callus cultures, data collection included the percentage of callus formation and the time taken (in days) for the callus to show, as well as a complete morphological description, which included color, type, size, texture, and overall biomass/build-up accumulated. The callus induction frequency was evaluated based on the number of explants exhibiting callus development relative to the total number of explants cultured. Callus fresh biomass was measured using an electronic balance (Shimadzu Corporation, Japan). In contrast, dry biomass was determined by drying the calli for 72h in a dry oven (DW-180G) and subsequently measuring their weight. Biochemical analyses, including total soluble sugars, total soluble proteins, and proline measurement, were performed on callus cultures and in vitro-derived plants.

2.7. Biochemical Assays

***2.7.1. Extraction and Quantification of Soluble Proteins*:** To quantify the total soluble protein content, a 1-gram sample of callus tissue from both control and arginine-treated groups was individually ground in an ice-chilled mortar and pestle. Each tissue sample was homogenized using 2mL of 1M phosphate buffer (pH 7.2), which also contained 0.1g of PVP to prevent proteolytic degradation of proteins. After homogenization, samples underwent centrifugation at 14,000 × g for 30min at 4°C to isolate the soluble proteins. The centrifuged clear supernatant was then subjected to the Biuret assay for protein quantification following the method of Racusen and Johnstone (1961). For each sample, Biuret reagent was added in a predetermined volume to provide a defined reaction volume, and absorbance was assessed at 545nm using a UV-visible spectrophotometer. Protein concentrations were determined from a standard curve prepared with Bovine Serum Albumin (BSA).

***2.7.2. Estimation of Total Soluble Sugars*:** The methods outlined by DuBois et al. (1956) were employed for the quantification of total soluble sugars. For this purpose, 0.1g of callus or plant tissue was individually collected and subsequently ground using a mortar and pestle. This process was aided by liquid nitrogen, which ensured complete cellular disruption and the liberation of soluble materials. The homogenized tissue was placed in a 25mL test tube containing 5mL of distilled water. The mixture was incubated in a water bath set to 100°C for 30 minutes. This treatment was rendered sufficient to extract the soluble sugars. The mixture was centrifuged at 1,900*g for 10min at 4°C. This results in the separation of the supernatant and any remaining solid. An aliquot of clear supernatant (extract) was taken, and together with 0.5mL of 5% (w/v) phenol solution, was measured for absorbance at 490nm using a spectrophotometer. Sugar concentrations were calculated by comparing the absorbance values to a standard curve generated using glucose.

2.8. Determination of Proline Content

The procedures of (Bates et al., 1973) were followed to determine the proline content. For free proline extraction, 500mg of callus or shoot fresh tissues were homogenized in 3% (w/v) sulfosalicylic acid. The mixture was centrifuged at 10,000×g for 10 minutes at 4°C, and the supernatant was collected for analysis. For quantification, 2mL of supernatant, 2mL of glacial acetic acid, and 2mL of acid ninhydrin were combined and heated at 100°C for 1 hour. After rapid cooling, the reaction mixture was extracted with 4mL toluene and mixed thoroughly. The toluene layer was separated, and its absorbance was read at 520 nm using toluene as a blank. Proline concentration was calculated from a standard curve and expressed in micrograms per gram (µg/g) fresh weight.

$$Amount\ of\ proline = \frac{x}{2} \times \frac{10}{25} \times 1000$$

2.9. Statistical Analysis

Data analysis was performed using one-way ANOVA in SPSS (version 25.0). Results are reported as mean±SE. Statistical differences between treatment means were assessed using Duncan's Multiple Range Test (DMRT), with significance determined at the 0.5% probability level.

3. RESULTS

3.1. Effect of Plant Growth Regulators on Callus Induction

This study focused on the impact of various combinations and concentrations of plant growth regulators on callus induction. Nodal explants displayed the highest level of callus formation, at 80%, when cultured on MS medium supplemented with 4mg/L BAP and 2mg/L NAA. In comparison, a medium containing 2.5mg/L KN and 1.5mg/L IAA yielded a modest response with only 32% callus formation from nodal explants. Other combinations of auxins and cytokinins tested did not elicit notable callus formation from nodal tissues. For leaf explants, culture on MS medium supplemented with 4mg/L BAP and 2mg/L NAA resulted in only slight swelling, with no callus formation. These results emphasize that the explant type, along with the specific hormonal composition of the culture medium, are crucial determinants in the successful induction of callus (Table 2).

Table 2: Effect of different combinations of plant growth regulators on percent response of callus induction

Plant Growth Regulators (mgL^{-1})		Number of Explants Cultured	Callus Induction Response (%)
KIN+IAA	2.5+2.5	25	0
	2.5+1.5	25	32
KIN+2,4-D	3.0+2.5	25	0
	2.5+2.5	50	0
BAP+NAA	4.0+2.0	50	80

The data presented here are the mean values of four replicates per treatment.

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3.2. Arginine Supplementation and Its Effect on Callus Formation and Shoot Proliferation

The inclusion of arginine in the culture medium markedly enhanced callus induction across multiple concentrations, as summarized in Table 3. When nodal explants were grown on MS medium containing 1.5ppm arginine under a 16/8-hour photoperiod, 90% developed green, friable calli within 30 days. Increasing arginine to 2.5ppm further accelerated and improved this response: 94% of nodal explants produced white to pale yellow callus in just 26 days, representing the peak induction efficiency observed. At the highest arginine level tested (3.0ppm), nodal explants still exhibited substantial callogenesis, with 80% forming healthy green callus in only 22 days and showing minimal tissue necrosis, far shorter than the 50-day initiation period recorded for the control medium without arginine. In contrast, leaf explants were far less responsive; only 10% formed callus when cultured on 1.5ppm arginine, and these calli were firm, browning within four to five days. These results indicate that arginine concentration and explant type have a critical influence on both the rate and quality of callus induction. Furthermore, all arginine concentrations tested (1.5, 2.5, and 3.0ppm) supported shoot proliferation from nodal explants, as illustrated in Fig. 2 to 4.

Table 3: Effect of Arginine on Callus Induction and Proliferation on Nodal and Leaf Explants of *Artocarpus heterophyllus*

Treatments	Conc. (ppm)	Explants	Callus Initiation (days)	Callus Induction (%)	Morphology
	0	Leaf	0	0	Brown to yellow callus, compact in texture
Control		Nodes	50.0 ± 0.32^{a}	80	
	1.5	Leaf	65.0 ± 0.04^{a}	10	Brown, compact
		Nodes	30.0 ± 0.04^{b}	90	Green, Soft and friable
	2.5	Leaf	0	0	White to pale yellow, friable
Arginine		Nodes	26.0 ± 0.01^{c}	94	
	3.0	Leaf	0	0	Green, soft and friable
		Nodes	22.0 ± 0.04^{d}	82	

The data presented here are the mean±SE of four replicates per treatment. Values with the same alphabetic letters within a column are not significantly different according to Duncan's Multiple Range Tests (DMRT).

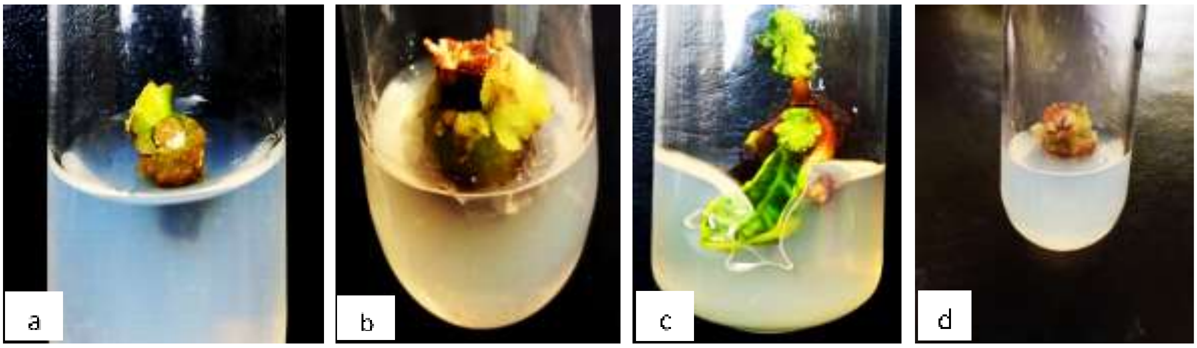

Fig. 2: Effect of 1.5 ppm arginine along with 4mgL^{-1} BAP and 2mgL^{-1} on callus induction and proliferation using nodal and leaf explant of Artocarpus: (a-b) Callus induction in nodal explant, (c) Proliferation of shoots, (d) Callus induction of leaf segment.

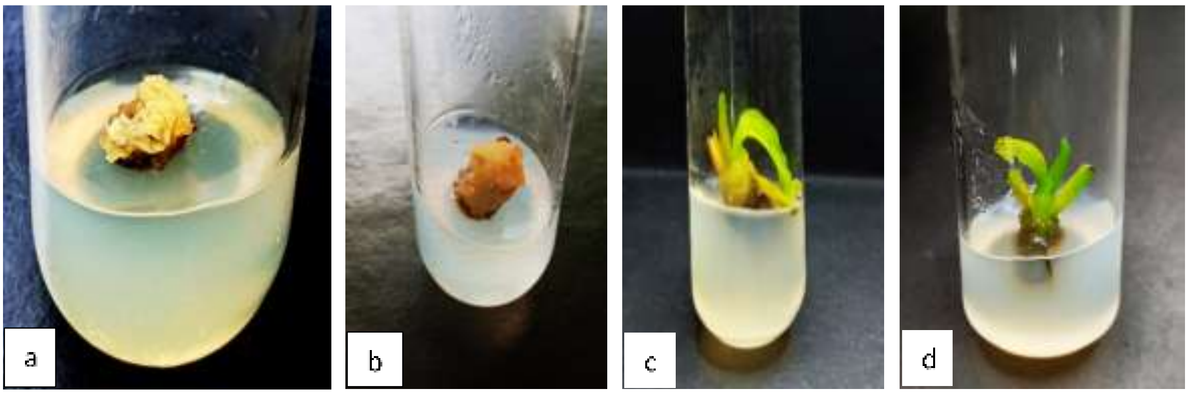

Fig. 3: Effect of 2.5ppm arginine along with 4mgL^{-1} BAP and 2mgL^{-1} on callus induction and proliferation using nodal and leaf explant of Artocarpus: (a-b) Callus induction in nodal explant, (c-d) Proliferation of shoots.

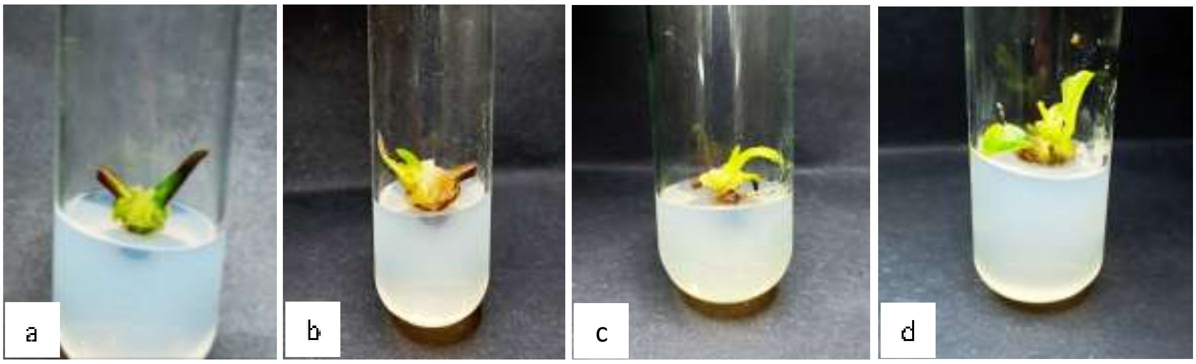

Fig. 4: Effect of 3.0 ppm arginine along with 4mgL-1 BAP and 2mgL-1 on callus induction and proliferation using nodal and leaf explant of Artocarpus: (a-b) Callus induction in nodal explant, (c-d) Proliferation of shoots.

3.3. Influence of Arginine on Shoot Regeneration

To assess the ability of callus tissues to regenerate shoots, samples were transferred to MS medium supplemented with 4mg/L BAP, 0.5mg/L kinetin (KN), and varying concentrations of arginine (1.5, 2.5, and 3.0ppm). However, across all arginine concentrations and combinations with BAP and KN, no evidence of shoot regeneration was observed during the study period. These results suggest that, under the tested conditions, arginine did not facilitate shoot organogenesis from the callus, either alone or in synergy with the chosen cytokinins, indicating that further optimization of hormonal regimes may be necessary for successful shoot induction.

3.4. Effect of Arginine on In Vitro Root Formation

Micro-shoots obtained from nodal explants cultured with 1.5 or 3.0ppm arginine, along with callus-derived tissues, were carefully transferred to Murashige and Skoog (MS) medium supplemented with 10µM indole-3-butyric acid (IBA) and a range of arginine concentrations (0, 1.5, 2.5 and 3.0ppm) in order to evaluate their potential for in vitro root development. Across all tested treatments, root development was absent throughout the 180-day observation period (data not shown).

3.5. Impact of Arginine Supplementation on Biomass Accumulation

Arginine supplementation had a marked effect on callus biomass production. Calli induced on MS medium containing both arginine and a combination of BAP and NAA accumulated significantly more biomass than those grown on BAP and NAA alone. Fresh weight analysis revealed that the highest biomass was achieved at 2.5ppm (5.66g) and 3.0ppm (4.67g) arginine, compared to just 2.0g in the control. Additionally, calli exposed to 1.5ppm arginine exhibited a 29% increase in fresh weight relative to the control. Dry weight measurements further indicated a 68% increase at 1.5ppm and a 99% increase at 2.5ppm arginine, demonstrating the substantial influence of arginine on biomass accumulation (Table 4).

Table 4: Effect of Arginine on Biomass Accumulation of *Artocarpus heterophyllus* Calli

Medium	Fresh Weight of Callus (g)	Dry Weight of Callus (g)
MS+2 mgL^{-1} NAA+4 mgL^{-1} BAP	2.0±0.58^{d}	0.024±0.00^{c}
MS+2 mgL^{-1} NAA+4 mgL^{-1} BAP +1.5ppm Arg	2.82±0.01^{c}	0.0400±0.01^{b}
MS+2 mgL^{-1} NAA+4 mgL^{-1} BAP +2.5ppm Arg	4.66±0.01^{b}	0.0473±0.00^{b}
MS+2 mgL^{-1} NAA+4 mgL^{-1} BAP +3.0ppm Arg	5.67±0.01^{a}	0.0800±0.01^{a}

The data presented here are mean±SE of four replicates per treatment. Values with the same alphabetic letters within a column are not significantly different according to Duncan's Multiple Range Tests (DMRT).

3.6. Influence of Arginine on Biochemical Properties

3.6.1. Total Soluble Protein Content: The impact of arginine supplementation on total soluble protein levels in callus tissues is depicted in Fig. 1a. Introducing arginine into the culture medium resulted in a notable increase in protein content compared to the untreated control. Calli cultivated on MS medium with 2.5 and 3.0ppm arginine reached protein concentrations of 57.3mg/g and 65.8mg/g, respectively. When compared to control values, arginine supplementation at 1.5, 2.5, and 3.0ppm led to respective increases of 5%, 45%, and 78% in total soluble protein content.

3.6.2. Total Soluble Sugars: Significant variation in total soluble sugar concentration was observed among the different treatments ($P \leq 0.05$; Fig. 1b). Control calli had a baseline sugar content of 8.62±0.004mg/mL. When exposed to 1.5ppm arginine, calli exhibited a 38% higher sugar content than the control. In contrast, calli treated with 2.5 and 3.0ppm arginine showed a reduction in soluble sugars, registering at 7.43mg/mL and 4.97mg/mL, respectively.

***3.6.3. Proline Content*:** A steady decrease in proline concentration was associated with increasing arginine levels (Table 5). Control calli, grown without arginine, displayed the highest proline content (22.05µg/mL). This amount declined to 9.92µg/mL with 1.5ppm arginine and was further reduced to 9.39µg/mL and 8.31µg/mL at 2.5 and 3.0ppm arginine (Fig. 1c), respectively.

Table 5: Effect of different concentrations of arginine on total soluble proteins, total soluble sugars and protein content of Calli of *Artocarpus heterophyllus*

Treatments	Conc. (ppm)	Total Soluble Proteins (mg/g)	Total soluble Sugars (mg/mL)	Proline Content (µg/mL)
Control	0	36.93±0.02^{d}	8.62±0.00^{b}	22.05±0.58^{a}
Arginine	1.5	38.60±0.01^{c}	11.96±0.01^{a}	9.92±0.01^{b}
	2.5	53.70±0.01^{b}	7.43±0.01^{c}	9.39±0.00^{b}
	3.0	65.80±0.01^{a}	4.97±0.01^{d}	8.31±0.01^{c}

The data presented here are mean±SE of four replicates per treatment. Values with same alphabetic letters within a column are not significantly different according to Duncan's Multiple Range Tests (DMRT).

4. DISCUSSION

The present study demonstrated that varying concentrations of L-arginine, in combination with BAP and NAA, significantly influenced both callogenesis and associated biochemical parameters. It is well-documented in the literature that the success of callus formation hinges on both the specific growth regulators employed and the source of explant tissue (Adil et al., 2018; Dar et al., 2021). Aligning with these prior studies, the highest rate of callus induction in this work was obtained when MS medium was supplemented with 4mg/L BAP and 2mg/L NAA. By contrast, combinations involving kinetin (KN) with either IAA (2.5+2.5mg/L or 2.5+1.5mg/L) or 2,4-D (3.0+2.5 or 2.5+2.5mg/L) yielded considerably lower induction rates.

These results align with the results of Rahman et al. (2019), where nodal explants and specific combinations of auxins and cytokinin promoted callus development in *Catharanthus roseus*. In contrast, leaf explants appeared to swell, exhibiting little to no callus formation.

Even though arginine supplementation in MS medium has visibly enhanced leaf explants, the rate of callus induction remained low, accompanied by necrosis within 4-5 days after inoculation. The reduced response noted in explants from leaves may be due to surface area. Leaves have a greater surface area, which makes them more prone to contamination (Campbell, 1985). Unlike leaf explants, meristematic tissues often facilitate a thorough purge of contaminants due to their size; however, leaf explants tend to trap microorganisms. The findings of Hemmati et al. (2020) demonstrated that nodal tissues significantly outperformed leaf tissues in callus induction, a result also reported by Kumlay and Ercisli (2015) for *Salvia tebesana* Bunge.

Moreover, the browning that occurs in leaf explants can also result from the oxidation of phenolic compounds, which are more plentiful in leaf tissues and can interact with inorganic and organic salts present in the culture medium (Bayraktar et al., 2020). The MS medium with BAP and NAA, without any other additions, functioned as a control for baseline comparison regarding the impact of arginine addition. Maximum callus induction from nodal explants occurred at an arginine concentration of 2.5ppm. The beneficial response of callus tissue to arginine indicates that amino acids may facilitate the action of auxins and cytokinins, thereby supporting growth and metabolism on a cellular level (Bajguz and Piotrowska-Niczyporuk, 2023). These results are consistent with those of Kumar and Kumari (2010) regarding the increase in indirect organogenesis reported in *Artemisia vulgaris* L. where a mixture of amino acids and certain growth regulators was employed.

Increase in fresh and dry weight of callus cultures with arginine supplements was greatest at 2.5 and 3.0ppm. This finding confirms the previously established hypothesis that amino acids positively impact biomass accumulation. The effects of arginine on callus growth and biomass are consistent with Bano et al. (2022), who noted that melatonin treatment resulted in increased callus growth and biomass. Also, El-Zohiri and Asfour (2009) documented the stimulatory effects of amino acids on the fresh and dry weight of soybeans and potatoes, respectively. The increase in biomass is best explained by amino acids being precursors in protein biosynthesis. Certain amino acids, including glutamic and aspartic acids, as well as arginine, not only aid in the sufficient metabolism of proteins but also support vigorous cell division and plant growth (Sadak et al., 2023; Asif et al., 2025).

Supplementation of arginine has remarkable effects not only on biomass yield but also on the biochemical composition of callus cultures. Increased levels of arginine were noted to markedly raise the total soluble protein content while simultaneously lowering total soluble sugars and proline levels. Calli grown on media with 3ppm arginine showed the highest total soluble protein content of 65.8mg/g compared to calli grown without any arginine supplementation. This increase in protein content may be attributed to higher amino acid levels available for protein synthesis due to the treatments reported by Geshnizjani and Khosh-Khui (2016) in *Gerbera jamesonii* with their amino acid supplementation.

Recent findings suggest that higher concentrations of arginine in the culture medium have a significant impact on the total soluble sugar content within callus tissues. Notably, calli maintained on medium containing 3ppm of arginine exhibited a decrease of 42% in their soluble sugar content when compared to the control group. This suggests that an increased supply of arginine may lead to certain metabolic changes that result in heightened lipolysis and reduced sugar accumulation. A possible explanation is that high concentrations of specific amino acids can stimulate the activity of sugar-utilizing enzymes, such as aldolase and hexokinase. These enzymes are central to glycolysis and the catabolism of carbohydrates, converting sugars into energy or metabolic precursors. With an increased supply of amino acids, the plant tissue can channel more carbon skeletons into amino acid and protein metabolism, thereby restricting the level of free soluble sugars within the cell.

Lowering the arginine concentration to 1.5ppm resulted in a significant 38% increase in soluble sugar content compared to the control group. This supports previous studies, including that of Abd El-Monem (2007), who noted that the application of amino acids can stimulate photosynthesis, thereby increasing the production and accumulation of sugars, polysaccharides, and carbohydrates in plant tissues. Furthermore, Hussein et al. (2022) reported the highest sugar levels in wheat with the least amount of arginine, suggesting similar tendencies in wheat crops. These observations reveal a finely tuned equilibrium between nitrogen and carbon metabolism: moderate amounts of arginine appear to enhance sugar accumulation, while excessive amounts seem to promote the conversion or utilization of sugars, indicating tightly regulated networks between amino acid and carbohydrate metabolism (Diniz et al., 2020).

Proline content was also found to decrease with increasing arginine concentrations in treated calli, with the highest proline levels recorded in the control group that did not receive amino acid supplementation. The observed decline in proline suggests that arginine may mitigate cellular stress by modulating proline metabolism (Hussein et al., 2022). In support of this, Sarropoulou et al. (2014) observed the decreased levels of proline in cultures of cherry rootstocks treated with arginine and IBA, proposing that either the degradation of proline to other metabolites or consumption during aerobic respiration could explain the observation.

5. CONCLUSION

This study demonstrated that arginine supplementation significantly improved growth parameters and the callogenesis process, encompassing callus induction, proliferation, and biomass accumulation in *Artocarpus heterophyllus*. Nonetheless, arginine showed varying effects on the total soluble sugars, total soluble proteins, and proline content. The optimal concentrations for inducing callogenesis were found to be 2.5 and 3.0ppm. These observations support the idea that arginine significantly aids in the growth and callus development, probably due to its function in nitrogen metabolism, signalling pathways at the cellular level, and other physiological functions. In addition, the results corroborate the prospective use of arginine as a plant tissue culture growth regulator, with possible applications for increasing crop yield and as a substitute for synthetic fertilizers. Based on these outcomes, further research is warranted to explore the utility of arginine as a biofertilizer in broader agricultural practices.

Abbreviation	Full Form
AA	Amino Acid
ANOVA	Analysis of Variance
Arg	Arginine
BAP	Benzylaminopurine
BA	Benzyl Adenine
BSA	Bovine Serum Albumin
CK	Cytokinin
DMRT	Duncan's Multiple Range Test
IBA	Indole-3-Butyric Acid
IAA	Indole-3-Acetic Acid
KN / KIN	Kinetin
MS	Murashige and Skoog Medium
NAA	Naphthalene Acetic Acid
NO	Nitric Oxide
PVP	Polyvinylpyrrolidone
TDZ	Thidiazuron
µg/mL	Micrograms per Millilitre
mg/g	Milligrams per Gram

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mg/L	Milligrams per Litre
ppm	Parts per Million

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