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Comparative Morphological Effect of Plant Growth Regulators on Callus Induced from *in vitro*-grown Leaf Explants of *Solanecio biafrae*

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ABSTRACT

Callus culture is a technique used in plant tissue culture systems to obtain secondary metabolites utilized in agrochemicals, biopesticides, colorants, flavors, food additives, fragrances, and pharmaceuticals. The callus can vary in form and texture, with its induction influenced by factors such as explant type, growth medium composition, and the concentration or combination of growth regulators. In this study, leaf explants excised from in vitro-grown plantlets of *Solanecio biafrae* were placed on Murashige and Skoog (MS) media containing various concentrations of plant growth regulators to stimulate callus induction. At low concentrations of 2,4-D (1.0–5.0 mg L^{-1}), friable callus was predominantly produced. These calli varied in color, with cream and brown hues; specifically, 1.0 and 2.0 mg L^{-1} of 2,4-D resulted in embryogenic calli. Green calli were observed in media containing 4.0 mg L^{-1} 2,4-D combined with 1.0 mg L^{-1} BAP (compact), as well as in 1.0 mg L^{-1} 2,4-D combined with 1.0 mg L^{-1} kinetin, and 2.0 mg L^{-1} 2,4-D combined with 1.0 mg L^{-1} kinetin (friable). At varying concentrations of BAP (0.2–1.0 mg L^{-1}), the leaf explants enlarged and predominantly produced cream and friable callus. When BAP was combined with 2,4-D, the highest frequencies of callus induction were achieved with 0.8 mg L^{-1} BAP + 0.05 mg L^{-1} 2,4-D and 1.0 mg L^{-1} BAP + 0.05 mg L^{-1} 2,4-D. However, combinations such as 0.4 mg L^{-1} BAP + 0.10 mg L^{-1} NAA, 0.2 mg L^{-1} BAP + 0.05 mg L^{-1} NAA, and 0.4 mg L^{-1} BAP + 0.05 mg L^{-1} NAA did not induce any callus formation but resulted in root development. Additionally, 0.2 mg L^{-1} BAP + 0.05 mg L^{-1} IBA (the lowest concentration tested) also failed to induce callus response but was observed to promote rooting. The various types of callus formed can be utilized for different applications.

Introduction

Solanecio biafrae is a nutritious and medicinal African indigenous vegetable belonging to the Asteraceae family, recognized for its potential to treat various ailments and its cultural significance (Bello et al., 2018). It has been identified as a potential agent for addressing certain clinical infections caused by bacteria (Oyeniyi et al., 2019). The bioactive compounds found on the

foliar surface of this vegetable possess significant nutraceutical properties, affirming its potential as a source of plant-based drugs (Omotehinwa et al., 2023). Recommendations have been made to utilize biotechnological methods to enhance the production of *S. biafrae* (Bello et al., 2020).

In vitro callus induction plays a crucial role in plant biotechnology, serving as a pivotal technique for manipulating and propagating various plant species (Kruglova et al., 2023). This method allows for the regeneration of undifferentiated cell masses from explants, providing a versatile platform for studying plant physiology, genetic transformation, and the production of secondary metabolites (Efferth, 2019). It is particularly suitable for propagating diverse bioactive compounds and wholesome plant materials (Gonçalves and Romano, 2018).

Callus culture is a technique employed in plant tissue culture systems to obtain secondary metabolites for use in agrochemicals, biopesticides, colorants, flavors, food additives, fragrances, and pharmaceuticals (Murthy et al., 2014). Callus exhibits considerable diversity in shape and texture, ranging from firm, nodular clusters to delicate, easily crumbled structures. Soft, crumbly callus, composed of loosely attached undifferentiated cells, forms under conditions conducive to rapid cell division (George and Sherrington, 1984). In contrast, compact calli, which are densely packed, appear when rapid cell division is not favored and may be white or cream-colored, with sections exhibiting green due to chloroplast development or purple from anthocyanin accumulation in vacuoles. The individual cells within the callus mass vary in shape from nearly spherical to significantly elongated (Bhatia et al., 2015).

Various plant tissues, including both meristematic and non-meristematic regions, can generate callus (Efferth, 2019). Factors such as explant type, growth medium composition, and the addition of growth regulators, particularly auxins, significantly influence callus induction (Moshtaghi, 2020). Compact calli have been reported to produce shoot-like structures when induced on a medium containing 1 ppm 2,4-D and BA (Zamora et al., 1987). Conversely, friable callus is particularly valuable as an initial material for cell suspension cultures due to its ability to be easily fragmented in a liquid medium (Ashrafadeh et al., 2015). Friable callus has a higher potential for forming somatic embryos compared to compact callus (George and Sherrington, 1984). Compact callus has also been shown to induce somatic embryos with a reduced concentration of 2,4-D (Kong et al., 2023). Additionally, a preferred approach for selecting somaclonal variants with improved resistance to cadmium (Cd) has originated from non-friable potato callus (Ashrafadeh et al., 2015).

Different types of callus, such as rooty, shooty, or embryonic, exhibit varying degrees of organ regeneration (Ikeuchi et al., 2013). Plant growth regulators are critical components of culture media that govern cell growth, development, morphogenesis, and the formation of metabolites (Strydhorst et al., 2018). Zhai and Xu (2021) noted that callus develops from detached explants when exposed to a callus-inducing medium with a high ratio of auxin to cytokinin. Despite the potential of *S. biafrae*, studies on callus induction protocols from its leaves are limited, and there is a lack of information on the different callus morphologies or characteristics induced by various concentrations and combinations of plant growth regulators.

It is important to compare the morphology of the callus induced from in vitro-grown leaf explants of *S. biafrae* in response to varied auxins and cytokinins, both singly and in combination. This study aims to develop optimized callus culture protocols tailored for efficient callus induction in *S. biafrae*, enabling diverse applications in plant research and industrial/agricultural biotechnology.

Materials and Methods

Source of starting material

In vitro starting materials were sourced from stem cuttings excised from *Solanecio biafrae* plants cultivated in plastic pots.

Surface sterilization

The collected stem cuttings were rinsed under tap water to remove dust, debris, and surface contaminants. A few drops of liquid detergent were added to the water, agitated, and rinsed several times. The stem cuttings were then transferred to a laminar airflow cabinet and placed in a sterile conical flask. For final surface sterilization, the cuttings were treated with 70% ethanol for 5 min, followed by immersion in 10% sodium hypochlorite (NaOCl) for 20 min. This was followed by soaking in 5% sodium hypochlorite with periodic shaking for an additional 5 min. After disinfection, the stem cuttings were washed repeatedly with sterile distilled water. The bleached ends were trimmed, and the cuttings were subsequently cut into single nodal sections.

Medium preparation and explant culture

All culture media were prepared using Murashige and Skoog's (MS) medium (Murashige and Skoog,

1962), enhanced with 3% sucrose and solidified with 0.8% agar, adjusted to a pH of 5.7 ± 0.1. Test tubes containing 15 mL media were used for culture. MS-only media were utilized for establishing the nodal cultures. Additionally, MS media were enriched with varying concentrations of plant growth regulators (PGRs), including 1.0-5.0 mg L^{-1} of 2,4-D (auxin) alone or in combination with 1.0 mg L^{-1} of cytokinins (BAP or kinetin), as well as 0.2–1.0 mg L^{-1} of BAP either alone or paired with 1.0 mg L^{-1} of 2,4-D or 0.1 mg L^{-1} of GA_3. Concentrations of 0.05 or 0.1 mg L^{-1} of 2,4-D, NAA, or IBA were also used for callus induction. Control media were prepared without any hormones. Baby jars and test tubes containing 50 mL and 15 mL of media, respectively, were sterilized in an autoclave at 121 °C for 20 min. After autoclaving, all media were monitored for three days for contamination before use.

Establishment of cultures

The disinfected nodal cuttings were cultured on contaminant-free semi-solid media within test tubes. The experiments were conducted in a controlled culture room maintained at a temperature of 25 ± 2 °C. Uniform illumination was provided by fluorescent tubes, delivering approximately 1000 lux of light intensity with a photoperiod of 16/8 h. The cultures were observed for 14 days, during which in vitro stock plantlets were grown for further experiments. For callus induction, leaf explants excised from the in vitro-grown plantlets were placed on MS media supplemented with various concentrations of plant growth regulators. Each treatment was replicated ten times.

Data collection and statistical analysis

The cultures were monitored daily to record the days to callus induction. Data on callus induction, texture (friable or compact), and color (green, cream, brown, or yellow) were collected through visual observation. Each treatment combination was assigned ten explants. The data collected in this study comprised descriptive and qualitative information, which was directly elucidated.

Results

Callus induction response using varying concentrations of auxins singly and in combination with cytokinins or gibberellic acid

The study investigated callus induction in *Solanecio biafrae* using varying concentrations of auxins (2,4-D, NAA, and IBA), cytokinins (BAP and kinetin), and gibberellic acid (GA_3). The effects of different concentrations of 2,4-D (ranging from 1.0 to 20 mg L^{-1}) on callus induction from leaf explants were evaluated. It was found that at low concentrations (1.0-5.0 mg L^{-1}), the frequency of callus production was optimal, ranging from 51% to 100%. Most of the explants that fully callused exhibited a friable callus type (Table 1). The callus varied in color, predominantly cream and brown, with concentrations of 1.0 mg L^{-1} and 2.0 mg L^{-1} of 2,4-D yielding embryogenic calli, as illustrated in Figure 1. In contrast, at higher concentrations (10-20 mg L^{-1}) of 2,4-D, partial callusing (26-50%) was observed, primarily resulting in compact calli with non-defined colors.

Leaf explants were subsequently transferred onto MS media enriched with a combination of 2,4-D and BAP to initiate the caulogenesis response of *S. biafrae*. All the treatments led to the initiation of calli that are mostly friable, cream in color, and fully formed except for the treatments containing 2.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} BAP. Meanwhile, green callus was obtained on the media containing 4.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} BAP, which is compact in texture. Also, 1.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} BAP induced roots (Table 1 and Fig. 2). However, the combination of 2,4-D at concentrations of 1.0 and 2.0 mg L^{-1} with kinetin at a concentration of 1.0 mg L^{-1} showed an increased callus induction response. The highest frequency induced was obtained on MS medium supplemented with 1.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} kinetin and 2.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} kinetin. The callus induced in these treatment groups were mainly friable in texture and cream in color, but treatments 1.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} kinetin and 2.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} kinetin also induced green callus (Table 1).

The influence of varying concentrations of 6-benzylaminopurine (BAP) independently and combined with other plant growth regulators (PGRs) on callus induction

Table 2 presents the results of the experiment conducted using MS medium enriched with BAP, both alone and in combination with 2,4-D or GA_3, to investigate the caulogenesis response of *S. biafrae*. The leaf explants treated with varying concentrations of BAP (ranging from 0.2 to 1.0 mg L^{-1}) showed enlargement, resulting in predominantly cream and friable callus, with callus induction frequencies between 76% and 100%. However, the combination of 1.0 mg L^{-1} 2,4-D with varying concentrations of BAP led to poor results, as the leaf explants burnt out, with the exception of the medium enriched with 0.6 mg L^{-1} BAP and 1.0 mg L^{-1} 2,4-D, which produced

cream compact callus at a frequency of 26% to 50%. Similarly, the combination of GA_3 (1.0 mg L^{-1}) and BAP was ineffective, as the leaf explants again experienced burning, except in the case of the medium with 0.4 mg L^{-1} BAP and 1.0 mg L^{-1} GA_3, where frequencies ranged from 26% to 50%. Even then, the resulting cream-colored compact callus appeared necrotic

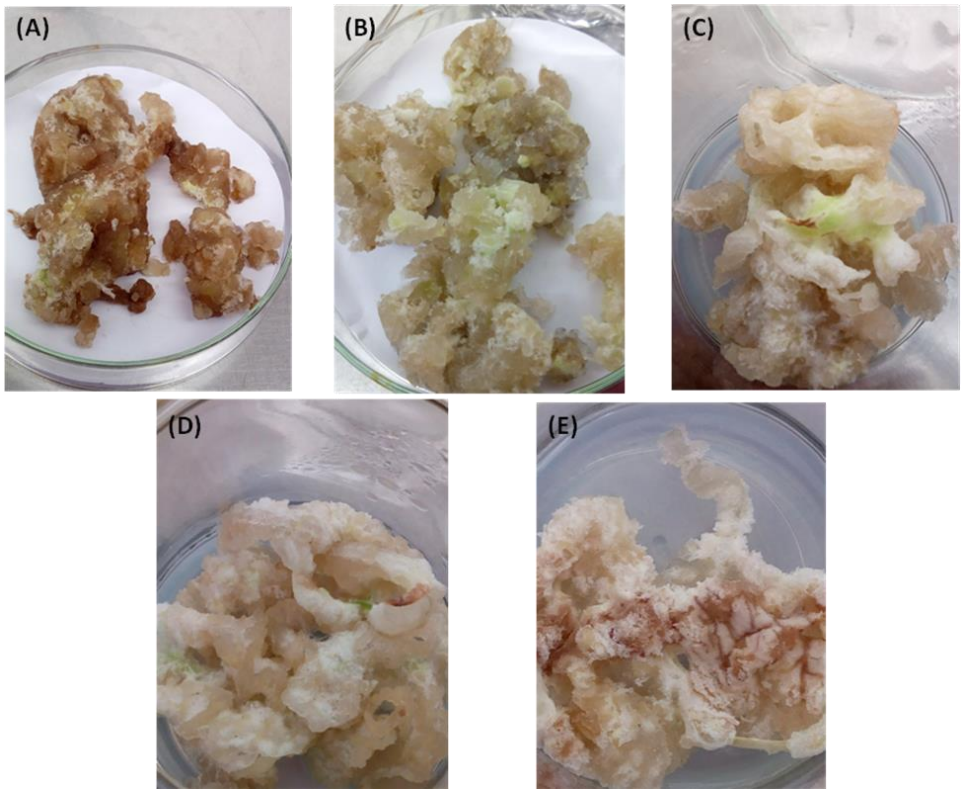

Fig. 1. Culture at 5 weeks showing callus induction in *S. biafrae* from leaf explant. (A) Callus cultured on MS medium containing 1.0 mg L^{-1} 2,4-D, (B) Callus cultured on MS medium containing 2.0 mg L^{-1} 2,4-D, (C) Callus cultured on MS medium containing 3.0 mg L^{-1} 2,4-D, (D) Callus cultured on MS medium containing 4.0 mg L^{-1} 2,4-D, and (E) Callus cultured on MS medium containing 5.0 mg L^{-1} 2,4-D.

Table 1. Effect of concentrations of 2,4-D (1- 20 mg L^{-1}) singly and in combination with BAP or kinetin on callus induction from leaf explants of *Solanecio biafrae*.

Treatment	Plant Growth Regulators (mg L^{-1})			Response	Callus type	Callus colour	Remarks
	2,4-D	BAP	Kinetin				
MSD0	-	-	-	-	-	-	No response
MSD1	1.0	-	-	++++	Friable	Cream/brown	Enlarged and fully callusing
MSD2	2.0	-	-	++++	Friable	Cream/brown	Fully callusing
MSD3	3.0	-	-	++++	Friable	Cream	Fully callusing
MSD4	4.0	-	-	++++	Friable	Cream/brown	Fully callusing
MSD5	5.0	-	-	++++	Friable	Cream/brown	Fully callusing
MBD1	1.0	1	-	++++	Friable	Cream/brown	Fully callusing and rooting
MBD2	2.0	1	-	++++	Friable	White/cream	Callus not fully formed
MBD3	3.0	1	-	++++	Friable	Cream/brown	Fully callusing
MBD4	4.0	1	-	++++	Compact	Cream/green	Fully callusing
MBD5	5.0	1	-	++++	Compact	Cream/brown	Fully callusing
MKD1	1.0	-	1	++++	Friable	Cream/brown	Fully callusing
MKD2	2.0	-	1	++++	Friable	Cream/green	Fully callusing
MKD3	3.0	-	1	++++	Friable	Cream/green	Fully callusing
MKD4	4.0	-	1	++++	Friable	Cream	Fully callusing
MKD5	5.0	-	1	++++	Friable	Cream	Fully callusing
MSD10	10	-	-	++	Compact	ND	Partially callusing
MSD15	15	-	-	++	Compact	ND	Partially callusing
MSD20	20	-	-	++	Compact	ND	Partially callusing

- = no response; + = 0-25%, ++ = 26-50%, +++ = 51-75%, ++++ = 76-100%; ND = Not defined.

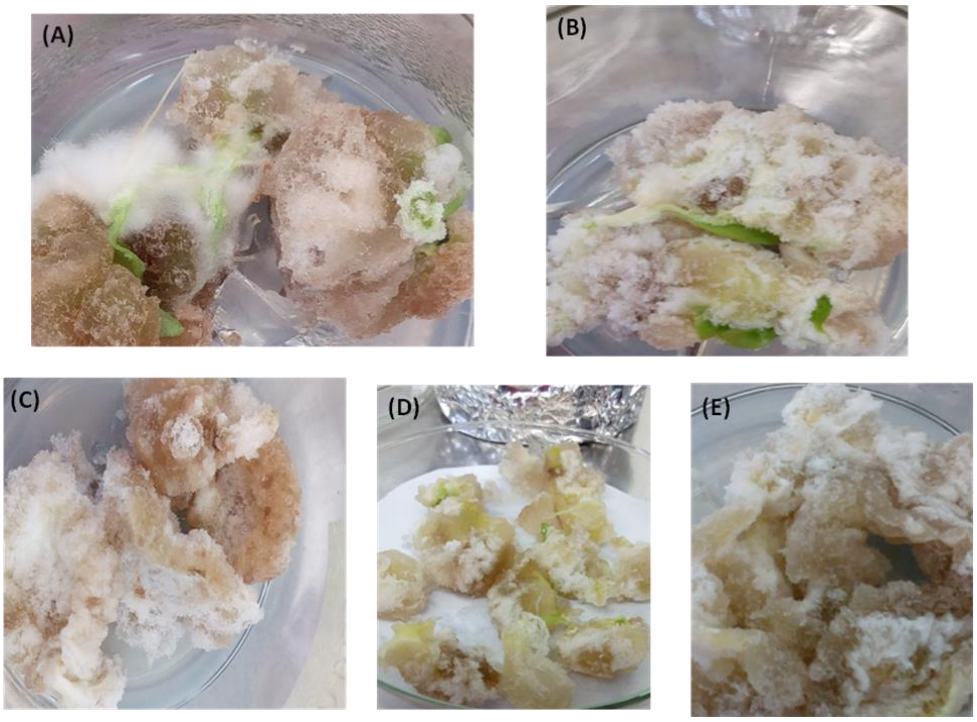

Fig. 2. Culture at 5 weeks showing callus induction in *S. biafrae* from leaf explant. (A) Callus on MS medium containing 1.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} BAP, (B) Callus on MS medium containing 2.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} BAP, (C) Callus on MS medium containing 3.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} BAP, (D) Callus on MS medium containing 4.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} BAP, and (E) Callus on MS medium containing 5.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} BAP.

Table 2. Factorial combinations of varied concentrations of BAP singly or paired with either 1 mg L^{-1} 2,4-D or GA_3 on callus initiation from leaf explants of *Solanecio biafrae* at four weeks after culture.

Treatments	Plant Growth Regulators (mg L^{-1})			Response	Callus texture	Callus color	Remarks
	BAP	2,4-D	GA_3				
MB0	-	-	-	-	-	-	No response
MB1	0.2	-	-	++++	Friable	Cream	Explants enlarged and callus fully formed
MB2	0.4	-	-	++++	Friable	Cream	Explants enlarged and callus not fully formed
MB3	0.6	-	-	++++	Friable	Cream/brown	Explants enlarged and callus not fully formed
MB4	0.8	-	-	++++	Friable	Cream	Explants enlarged and callus not fully formed
MB5	1.0	-	-	++++	Friable	Cream/green	Explants enlarged and callus not fully formed
MDB0	-	1	-	-	-	-	Burnt
MDB1	0.2	1	-	-	-	-	Burnt
MDB2	0.4	1	-	-	-	-	Burnt
MDB3	0.6	1	-	++	Compact	Cream	Few responded and others got burnt
MDB4	0.8	1	-	-	-	-	Burnt
MDB5	1.0	1	-	-	-	-	Burnt
MGB0	-	-	0.10	-	-	-	No response
MGB1	0.2	-	0.10	-	-	-	Burnt
MGB2	0.4	-	0.10	++	Compact	Cream	Callus not fully formed but necrotic
MGB3	0.6	-	0.10	-	-	-	Burnt
MGB4	0.8	-	0.10	-	-	-	Rooting observed but necrotic
MGB5	1.0	-	0.10	-	-	-	No response

- = no response; + = 0-25%, ++ = 26-50%, +++ = 51-75%, ++++ = 76-100%.

The MS medium was supplemented with varying concentrations of BAP (ranging from 0.2 to 1.0 mg L^{-1}) combined with lower concentrations of 2,4-D (0.10 and 0.05 mg L^{-1}) to investigate the caulogenic response of *S. biafrae*. The combination of 0.10 mg L^{-1} 2,4-D with different BAP concentrations resulted in low-frequency callus formation (21-50%) as BAP concentrations increased, with observed necrotic callus. In contrast, the addition of 0.05 mg L^{-1} 2,4-D increased callus frequency alongside increasing BAP concentrations. Notably, media enriched with 0.8 mg L^{-1} BAP + 0.05 mg L^{-1} 2,4-D and 1.0 mg L^{-1} BAP + 0.05 mg L^{-1} 2,4-D induced the highest callus frequency. At these hormone combinations, calli were fully formed on the leaf explants and remained non-necrotic after 4 ± 1 weeks of culture. However, the control treatment did not induce any callus, and no rooting was observed in any of the treatment groups (Table 3).

Table 3. Effects of BAP and 2,4-D on the induction and growth of callus.

Treatments	BAP	2,4-D	Response	Callus texture	Callus color	Remarks
LB0D0	0	0	-	-	-	No response
LB1D1	0.20	0.10	++	Compact	Brown	Necrotic
LB2D1	0.40	0.10	++	Compact	Brown	Necrotic
LB3D1	0.60	0.10	++	Compact	Brown	Necrotic
LB4D1	0.80	0.10	++	Compact	Brown	Necrotic
LB5D1	1.00	0.10	++	Compact	Brown	Necrotic
LB1D2	0.20	0.05	++	Compact	Brown	Necrotic
LB2D2	0.40	0.05	++	Compact	Brown	Partially callusing
LB3D2	0.60	0.05	+++	Compact	Brown	Few partially callusing
LB4D2	0.80	0.05	++++	Compact	Brown	Fully callusing
LB5D2	1.00	0.05	++++	Compact	Brown	Fully callusing

- = no response; + = 0-25%, ++ = 26-50%, +++ = 51-75%, ++++ = 76-100%.

NAA (0.10 and 0.05 mg L^{-1}) in combination with various concentrations of BAP (0.2–1.0 mg L^{-1}) showed that the treatments proved ineffective for caulogenesis, except in cases where the medium was supplemented with 0.2 mg L^{-1} BAP + 0.10 mg L^{-1} NAA, as well as 0.8 mg L^{-1} BAP + 0.10 mg L^{-1} NAA, both showing frequencies ranging from 26-50%. However, no callus was formed in the treatments supplemented with 0.4 mg L^{-1} BAP + 0.10 mg L^{-1} NAA, 0.2 mg L^{-1} BAP + 0.05 mg L^{-1} NAA, and 0.4 mg L^{-1} BAP + 0.05 mg L^{-1} NAA, but roots were visible (Table 4).

Table 4. Effects of BAP and NAA on the induction and growth of callus.

Treatments	Plant Growth Regulators (mg L^{-1})		Response	Rooting (%)	Remarks
	BAP	NAA			
LB0N0	0	0	-	0	No response
LB1N1	0.20	0.10	++	0	Callus not fully formed and necrotic, some are rotten
LB2N1	0.40	0.10	-	100	No callus response but rooting
LB3N1	0.60	0.10	-	0	No response and burnt
LB4N1	0.80	0.10	++	0	Callus not fully formed and necrotic, some are rotten
LB5N1	1.00	0.10	-	0	Lost to contamination
LB1N2	0.20	0.05	-	75	No callus response but rooting
LB2N2	0.40	0.05	-	60	No callus response but rooting and burnt
LB3N2	0.60	0.05	-	0	No response
LB4N2	0.80	0.05	-	0	No response
LB5N2	1.00	0.05	-	0	No response

- = no response; + = 0-25%, ++ = 26-50%, +++ = 51-75%, ++++ = 76-100%.

The results of treatments using MS medium enriched with varying concentrations of BAP in combination with IBA (0.10 and 0.05 mg L^{-1}) elucidated the results of studying the caulogenesis response of *S. biafrae* (Table 5). The combination of IBA (0.10 mg L^{-1}) with BAP resulted in callus formation, but the frequency of callus decreased as the BAP concentration increased, with no rooting observed. In contrast, the combination of 0.05 mg L^{-1} IBA yielded higher callus frequencies of 51-100% at elevated concentrations of BAP. Notably, the media enriched with 0.2 mg L^{-1} BAP + 0.05 mg L^{-1} IBA (the lowest concentration) did not elicit a callus response; instead, it was observed to induce rooting.

Table 5. Effects of BAP and IBA on caulogenesis.

Treatments	Plant Growth Regulator (mg L^{-1})		Response	Rooting (%)	Remarks
	BAP	IBA			
LB0I0	0	0	-	-	No response
LB1I1	0.20	0.10	+++	-	Partially callusing and necrotic
LB2I1	0.40	0.10	+++	-	Partially callusing and necrotic
LB3I1	0.60	0.10	++	-	Partially callusing and necrotic
LB4I1	0.80	0.10	+	-	Partially callusing
LB5I1	1.00	0.10	-	-	Necrotic
LB1I2	0.20	0.05	-	++	No callus response but some are rooting
LB2I2	0.40	0.05	++++	-	Fully callusing but necrotic
LB3I2	0.60	0.05	+++	-	Fully callusing with some partially callusing
LB4I2	0.80	0.05	++++	-	Fully callusing
LB5I2	1.00	0.05	++++	-	Fully callusing

- = no response; + = 0-25%, ++ = 26-50%, +++ = 51-75%, ++++ = 76-100%.

Discussion

Various types of auxins produce distinct morphological variations in calli. Identifying the appropriate auxin concentration in conjunction with cytokinins is crucial for initiating callus formation from leaf explants (Sivaraja and Packiam, 2022). Leaf explants have been reported as the most effective source for callus induction (Rao et al., 2015) and micropropagation (Aswathi and Thomas, 2023). To establish an efficient callus induction protocol, factors such as explant type, the combination of plant growth regulators, and culture conditions are essential (Kulus and Tymoszuk, 2020).

In this study, leaf explants were used to induce callus across various treatments, with observed enlargements and callus formation at different frequencies. This finding contrasts with Yaacob et al. (2022), who reported poor callus formation using *Capsicum frutescens* leaves as the explant source. The induction of callus from explants is influenced by hormonal action, with the auxin 2,4-dichlorophenoxyacetic acid (2,4-D), used either alone or in combination with cytokinins, being extensively employed to promote callus induction. In this study, callus was induced at varied frequencies across all media treatments containing 2,4-D. The effectiveness of 2,4-D in callus induction has also been reported in *Centratherum punctatum* (Aswathi and Thomas, 2023), supporting the results of this study.

Callus induced on 2,4-D was friable in texture and cream in color, which may serve as an indicator of its quality (Rahayu et al., 2023). This finding aligns with Chong et al. (2020), who reported higher calli in the 0.5 to 2.0 mg L^{-1} range of 2,4-D in *Taraxacum officinale*, and with Rao et al. (2015), who noted that 1.0 mg L^{-1} and 2.0 mg L^{-1} 2,4-D induced friable callus, though whitish-green in *Centella asiatica*. In contrast, higher concentrations of 2,4-D (10-20 mg L^{-1}) in this study failed to induce callus from leaf explants, likely due to the herbicidal properties of 2,4-D at elevated concentrations. A lower concentration of 2,4-D induced somatic embryos from friable callus in a study by Kong et al. (2023).

The combination of auxin and cytokinin regulates cell division and morphogenesis (Fehér, 2019). BAP enhances both the number and quality of callus (Rahayu et al., 2023). Combinations of 2,4-D (1-5 mg L^{-1}) and BAP (1 mg L^{-1}) across all treatments resulted in cream and friable callus induction. Green, compact callus formed on media containing 4.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} BAP. Rao et al. (2015) reported light green calli on media enriched with 2.0 mg L^{-1} 2,4-D + 1.0 mg

L^{-1} BAP. Roots emerged in the combination of 1.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} BAP, while green and friable callus was observed on 2.0 mg L^{-1} 2,4-D + 1.0 mg L^{-1} kinetin, consistent with the response of *Evolvulus alsinoides* to these treatments (Sivaraja and Packiam, 2022).

Ganie et al. (2022) noted that green callus typically represents embryonic tissue, which tends to regenerate rapidly to develop shoots, whereas yellow callus, which is non-embryonic, undergoes browning and ceases growth. Rao et al. (2015) reported that a greenish-yellow callus was stimulated on a medium enriched with BAP and NAA derived from *Centella asiatica*, attributed to the concentrations used in the study. Cytokinins are recognized for promoting cell division and encouraging lateral bud growth by inhibiting apical dominance (Slimani et al., 2021). The combination of low concentrations of cytokinins with high auxin concentrations reportedly induced callus in various species, with 2.5 mg L^{-1} 2,4-D + 1.5 mg L^{-1} BAP yielding optimal results in *Salacia macrosperma* (Mahendra et al., 2020). Lower concentrations of BAP produced friable, cream-colored callus. This friable callus, characterized by loosely connected cells, is believed to arise from rapid cellular proliferation facilitated by favorable environmental conditions (George and Sherrington, 1984).

The results of this study contrast with the findings of Chong et al. (2020), who reported no friable callus or root formation on media supplemented with BAP. Instead, they observed direct and indirect shoot regeneration, as well as various stages of somatic embryogenesis. Rahayu et al. (2023) noted that increasing the concentration of BAP in callus induced from leaves enhanced the number of calli exhibiting a compact texture. While a compact callus texture is beneficial for regenerating plantlets, a friable texture is preferred for the production of secondary metabolites through cell suspensions (Rahayu et al., 2023).

The color of the induced callus serves as an indicator of their activity, with white or cream colors suggesting active division and brown indicating aging (George and Sherrington, 1984). Moreover, the combination of IBA with varying concentrations of BAP led to higher frequencies of callus formation, reaching 51-100% at elevated BAP concentrations. This finding is consistent with Sulistyorini (2020), who observed similar callus induction from leaf explants of *Piper betle* at much higher concentrations (2 mg L^{-1} BAP and 2 mg L^{-1} IBA). Additionally, media enriched with 0.2 mg L^{-1} BAP and 0.05 mg L^{-1} IBA (the lowest concentration) showed root development. Previous research by Ikeuchi et al. (2013) indicated that callus types, either rooty, shooty, or embryonic, exhibit varying degrees of organ regeneration (root, shoot, or embryo).

Conclusions

In conclusion, varying concentrations of plant growth regulators, both singly and in combination, were effective in inducing callus from *Solanecio biafrae* leaf explants. However, it is recommended to use reduced concentrations of auxins and BAP. Higher concentrations, particularly 2,4-dichlorophenoxyacetic acid, can be herbicidal to the explants. The callus induced from the leaf explants of *Solanecio biafrae* holds potential for various research applications, as well as for agricultural and industrial uses. Regardless of the type of callus formed *in vitro*, it possesses value and significance. The choice of plant growth regulator should be guided by the intended application of the callus.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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