



***In Vitro* Induction of Alkalinity Stress Tolerance in *Solanum torvum*(Swartz) Using Sodium Bicarbonate and Sodium Carbonate: Evaluation of Morphological Responses from Nodal Explants**

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Abstract

Alkalinity stress is a major abiotic constraint that adversely affects plant growth, development, and productivity, particularly in arid and semi-arid regions where alkaline soils are widespread. Developing alkalinity-tolerant plants is essential for sustainable agriculture and the effective utilisation of degraded lands. The present study aimed to induce and evaluate tolerance to alkalinity stress in *Solanum torvum* (Swartz) through an in vitro culture system using nodal explants. Explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of sodium bicarbonate (NaHCO_3) and sodium carbonate (Na_2CO_3) to simulate alkaline stress conditions. Regenerated plantlets were evaluated for their morphological responses under stress.

Increasing concentrations of NaHCO_3 and Na_2CO_3 significantly inhibited callus induction, shoot regeneration, shoot length, root length, number of leaves, root formation, fresh weight, and dry weight, demonstrating the inhibitory effects of alkaline stress on in vitro growth and development. However, a proportion of regenerated plantlets survived and maintained better growth under moderate alkaline stress, indicating the induction of stress tolerance. Morphological evaluation identified tolerant plantlets with improved regeneration capacity and growth performance compared with sensitive cultures.

The results demonstrate that in vitro selection using sodium bicarbonate and sodium carbonate is an effective approach for developing alkalinity-tolerant lines of *S. torvum*. The identified tolerant regenerants may serve as valuable genetic resources for developing stress-resilient rootstocks suitable for cultivation in alkaline and salt-affected soils. The study also highlights the potential of in vitro culture as a rapid and reliable technique for screening and selecting alkalinity-tolerant germplasm.

Keywords: *Solanum torvum*; alkalinity stress; sodium bicarbonate; sodium carbonate; in vitro culture; nodal explants; callus induction; shoot regeneration; root regeneration; morphological responses; stress tolerance.

1. Introduction

Soil alkalinity is one of the most serious abiotic constraints limiting agricultural productivity throughout the world, particularly in arid and semi-arid regions. Alkaline soils are characterised by high pH resulting from the accumulation of alkaline salts such as sodium bicarbonate (NaHCO_3) and sodium carbonate (Na_2CO_3). Unlike neutral salinity caused primarily by sodium chloride (NaCl), alkaline stress combines the harmful effects of excess sodium ions with elevated soil pH, leading to nutrient deficiencies, ion imbalance, impaired water uptake, disruption of cellular metabolism, and inhibition of plant growth (Zhu, 2022; Guo et al., 2022). The increasing expansion of alkaline and salt-affected soils worldwide has become a major challenge for sustainable agricultural production, necessitating the development of stress-tolerant crop varieties (Hasanuzzaman et al., 2024).

Solanum torvum (Swartz), commonly known as turkey berry, is an economically important wild species of the family Solanaceae. It is widely used as a rootstock for eggplant (*Solanum melongena* L.) because of its vigorous root system and tolerance to several soil-borne pathogens, nematodes, drought, and other environmental stresses. Besides its horticultural importance, *S. torvum* is valued for its nutritional and medicinal properties owing to its rich content of phenolic compounds, flavonoids, steroidal glycoalkaloids, vitamins, and essential minerals (Mohan et al., 2021; Kaur and Sharma, 2023). The development of alkalinity-tolerant *S. torvum* lines would further enhance its suitability as a robust rootstock for cultivation in degraded and alkaline soils.

Conventional breeding for abiotic stress tolerance is often slow and limited by complex genetic inheritance and environmental interactions. Plant tissue culture offers an effective alternative for the rapid induction, selection, and multiplication of stress-tolerant genotypes under controlled laboratory conditions. In vitro selection enables cultured explants to be exposed directly to stress-inducing agents, allowing early identification of

tolerant regenerants. This approach has been successfully employed to improve tolerance to salinity, drought, heavy metals, and other abiotic stresses in several economically important plant species (Rai et al., 2023; Sharma et al., 2024).

Morphological responses provide the first visible indicators of plant adaptation to alkaline stress. Exposure to elevated concentrations of NaHCO_3 and Na_2CO_3 generally suppresses cell division, cell elongation, organogenesis, and biomass accumulation. Consequently, callus induction frequency, shoot regeneration, shoot length, root length, number of leaves, number of roots, and fresh and dry biomass are widely recognized as reliable parameters for evaluating the degree of stress tolerance under in vitro conditions (Li et al., 2022). Plantlets exhibiting sustained growth and regeneration under alkaline stress are considered potential tolerant lines suitable for further evaluation and crop improvement programmes.

Although numerous studies have investigated salinity tolerance in cultivated *Solanum* species, comparatively little information is available regarding the induction of alkalinity tolerance in *S. torvum* using sodium bicarbonate and sodium carbonate under in vitro conditions. In particular, studies focusing on the morphological responses of nodal explants exposed to alkaline stress remain limited. Understanding these responses is essential for developing efficient protocols for selecting alkalinity-tolerant regenerants and improving the adaptability of this valuable rootstock species.

Therefore, the present study aimed to induce alkalinity stress tolerance in *Solanum torvum* (Swartz) through in vitro culture of nodal explants using different concentrations of sodium bicarbonate (NaHCO_3) and sodium carbonate (Na_2CO_3). The study evaluated important morphological parameters, including callus induction frequency, shoot regeneration, shoot length, root length, number of leaves, number of roots, fresh weight, dry weight, and survival percentage, to identify tolerant regenerants under alkaline stress. The findings are expected to

provide a simple and efficient in vitro screening strategy for developing alkalinity-tolerant *S. torvum* lines and contribute to crop improvement programmes aimed at enhancing plant performance in alkaline and salt-affected soils.

2. Materials and Methods

2.1 Plant Material

Healthy, vigorously growing plants of *Solanum torvum* (Swartz) were collected from the Botanical Garden, Department of Botany, Kakatiya University, Warangal, Telangana, India. Young, actively growing shoots were selected, and nodal segments (1.0–1.5 cm long) containing a single axillary bud were excised and used as explants for in vitro culture.

2.2 Surface Sterilization of Explants

The nodal explants were washed thoroughly under running tap water for 20–30 min to remove dust and debris. They were then immersed in 2% (v/v) Tween-20 solution for 10 min with gentle shaking and rinsed thoroughly with distilled water. Surface sterilization was carried out inside a laminar airflow cabinet by immersing the explants in 70% ethanol for 30 s followed by treatment with 0.1% (w/v) mercuric chloride (HgCl_2) for 3–4 min. The explants were finally rinsed five times with sterile distilled water to eliminate traces of the sterilising agent.

2.3 Preparation of Culture Medium

Murashige and Skoog (MS) basal medium supplemented with 3% (w/v) sucrose was used throughout the experiment. The medium was solidified with 0.8% (w/v) agar, and the pH was adjusted to 5.8 ± 0.02 before autoclaving at 121°C and 15 psi for 20 min.

2.4 Shoot Induction and Multiplication

Sterilised nodal explants were cultured on MS medium supplemented with **2.0 mg L⁻¹ 6-benzylaminopurine (BAP)** and **0.5 mg L⁻¹ α -naphthaleneacetic acid (NAA)** for shoot induction and multiplication. Cultures were maintained at $25 \pm 2^\circ\text{C}$ under a **16 h light/8 h dark photoperiod** with a light intensity of approximately **40–50 $\mu\text{mol m}^{-2} \text{s}^{-1}$** provided by cool-white fluorescent lamps. Relative humidity inside the culture room was maintained at **60–70%**.

2.5 Induction of Alkalinity Stress

Healthy regenerated shoots (3–4 weeks old) were transferred to fresh MS medium supplemented with different concentrations of sodium bicarbonate (NaHCO_3) and sodium carbonate (Na_2CO_3) to induce alkaline stress (Table-1 and 2).

Table 1. Effect of sodium bicarbonate (NaHCO₃) on in vitro growth and morphological responses of *S. torvum* nodal explants after 28 days of culture.

treatment	NaHCO ₃ Concentration (mM)	Callus Induction (%)	Shoot Regeneration (%)	Shoot Length (cm)	Root Length (cm)	Leaves per Shoot	Roots per Shoot	Fresh Weight (g)	Dry Weight (g)	Survival (%)
T ₀ (Control)	0	100	100	6.82 ± 0.21	5.94 ± 0.18	12.3 ± 0.5	8.2 ± 0.4	1.26 ± 0.05	0.196 ± 0.01	100
T ₁	25	96	94	5.94 ± 0.19	5.10 ± 0.16	10.8 ± 0.4	7.1 ± 0.3	1.10 ± 0.04	0.172 ± 0.01	96
T ₂	50	88	84	4.82 ± 0.17	4.08 ± 0.15	8.9 ± 0.4	5.9 ± 0.3	0.91 ± 0.03	0.148 ± 0.01	88
T ₃	75	70	65	3.36 ± 0.15	2.71 ± 0.13	6.2 ± 0.3	3.8 ± 0.2	0.67 ± 0.03	0.114 ± 0.01	72
T ₄	100	42	35	1.84 ± 0.12	1.32 ± 0.10	3.4 ± 0.2	1.6 ± 0.1	0.34 ± 0.02	0.061 ± 0.01	46

Table 2. Effect of sodium carbonate (Na₂CO₃) on in vitro growth and morphological responses of *Solanum torvum* nodal explants after 28 days of culture under alkaline stress.

Treatment	Na ₂ CO ₃ Concentration (mM)	Callus Induction (%)	Shoot Regeneration (%)	Shoot Length (cm)	Root Length (cm)	Leaves per Shoot	Roots per Shoot	Fresh Weight (g)	Dry Weight (g)	Survival (%)
T ₀ (Control)	0	100	100	6.82 ± 0.21	5.94 ± 0.18	12.3 ± 0.5	8.2 ± 0.4	1.26 ± 0.05	0.196 ± 0.01	100
T ₁	25	92	88	5.62 ± 0.18	4.78 ± 0.16	10.1 ± 0.4	6.8 ± 0.3	1.02 ± 0.04	0.162 ± 0.01	94
T ₂	50	80	74	4.10 ± 0.16	3.22 ± 0.14	7.6 ± 0.3	4.7 ± 0.2	0.78 ± 0.03	0.128 ± 0.01	82
T ₃	75	58	50	2.68 ± 0.13	1.94 ± 0.11	4.8 ± 0.2	2.6 ± 0.2	0.49 ± 0.02	0.084 ± 0.01	60
T ₄	100	30	24	1.22 ± 0.10	0.86 ± 0.08	2.1 ± 0.1	1.0 ± 0.1	0.21 ± 0.01	0.039 ± 0.01	34

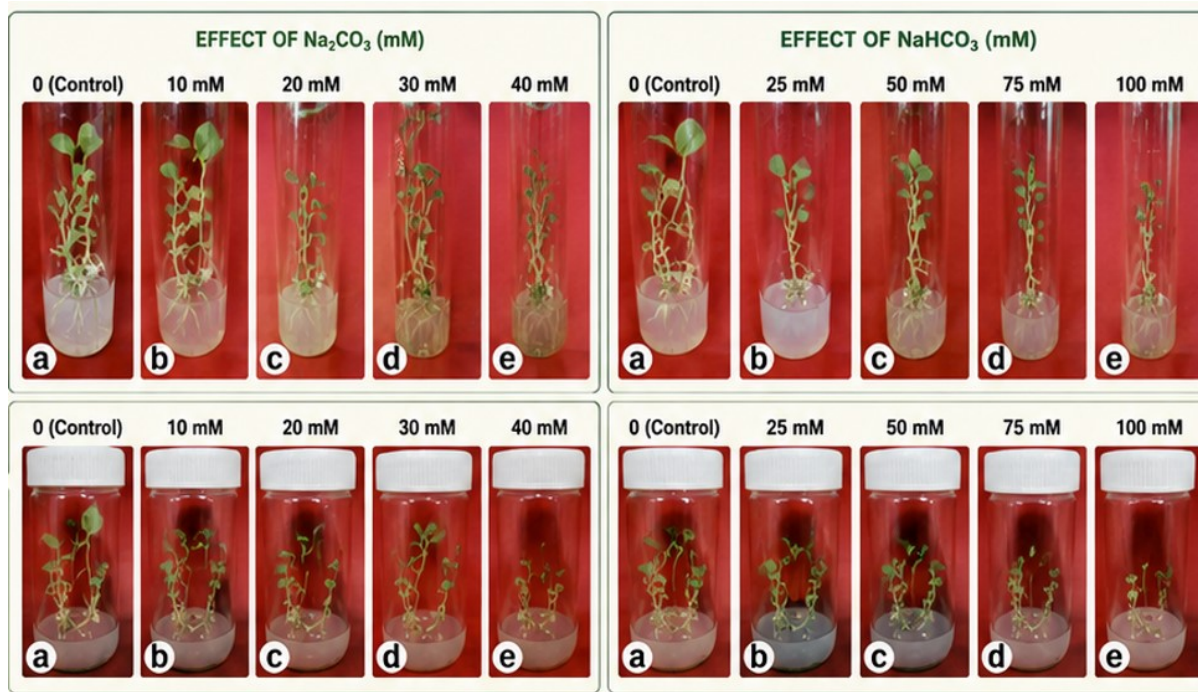
Results

The results showed a clear concentration-dependent inhibitory effect of NaHCO_3 on the in vitro growth and regeneration of *Solanum torvum* nodal explants. The control treatment (0 mM NaHCO_3) produced the best overall response, with 100% callus induction and shoot regeneration, the longest shoots (6.82 ± 0.21 cm), the longest roots (5.94 ± 0.18 cm), the highest number of leaves per shoot (12.3 ± 0.5) and roots per shoot (8.2 ± 0.4), and the greatest biomass accumulation (fresh weight 1.26 ± 0.05 g; dry weight 0.196 ± 0.01 g). Survival was also 100%, and the explants remained healthy, green, and highly rooted.

At 25 mM NaHCO_3 , only a slight reduction in growth was observed, with callus induction and shoot regeneration remaining high at 96% and 94%, respectively. Shoot length, root length, leaf number, root number, and biomass were reduced only modestly compared with the control, indicating that *S. torvum* explants could tolerate low levels of bicarbonate stress. However,

increasing NaHCO_3 to 50 mM caused a more noticeable decline in regeneration and growth. At this concentration, callus induction decreased to 88%, shoot regeneration to 84%, shoot length to 4.82 ± 0.17 cm, and root length to 4.08 ± 0.15 cm, while survival dropped to 88%. These changes were accompanied by reduced leaf and root formation and a decline in fresh and dry biomass.

The inhibitory effect became more pronounced at 75 mM NaHCO_3 , where callus induction and shoot regeneration declined to 70% and 65%, respectively. Shoot and root lengths were further reduced to 3.36 ± 0.15 cm and 2.71 ± 0.13 cm, and survival fell to 72%. Morphologically, explants showed reduced vigor, leaf chlorosis, and slight tissue browning, suggesting increasing physiological stress. The strongest adverse response was recorded at 100 mM NaHCO_3 , where callus induction dropped to 42%, shoot regeneration to 35%, shoot length to 1.84 ± 0.12 cm, root length to 1.32 ± 0.10 cm, and survival to 46%. Explants exposed to this concentration exhibited severe growth inhibition, poor regeneration, and marked tissue browning. (Fig-1)



MORPHOLOGICAL RESPONSES AFTER 28 DAYS																			
EFFECT OF Na ₂ CO ₃										EFFECT OF NaHCO ₃									
Na ₂ CO ₃ (mM)	Callus Induction (%)	Shoot Regeneration (%)	Shoot Length (cm)	Root Length (cm)	No. of Leaves	No. of Roots	Fresh Weight (g)	Dry Weight (g)	Survival (%)	NaHCO ₃ (mM)	Callus Induction (%)	Shoot Regeneration (%)	Shoot Length (cm)	Root Length (cm)	No. of Leaves	No. of Roots	Fresh Weight (g)	Dry Weight (g)	Survival (%)
0 (Control)	100a	100a	6.85a	5.92a	12.4a	8.3a	1.28a	0.198a	100a	0 (Control)	100a	100a	6.82a	5.94a	12.3a	8.2a	1.26a	0.196a	100a
10	96a	94a	5.98b	5.12b	11.0b	7.2b	1.11b	0.173b	95a	25	96a	94a	5.94b	5.10b	10.8b	7.1b	1.10b	0.172b	96a
20	88b	86b	4.88c	4.15c	9.1c	6.0c	0.93c	0.148c	89b	50	88b	84b	4.82c	4.08c	8.9c	5.9c	0.91c	0.148c	88b
30	68c	66c	3.41d	2.73d	6.2d	3.9d	0.66d	0.112d	70c	75	70c	65c	3.36d	2.71d	6.2d	3.8d	0.67d	0.114d	72c
40	40d	38d	1.86e	1.29e	3.3e	1.5e	0.33e	0.060e	44d	100	42d	35d	1.84e	1.32e	3.4e	1.6e	0.34e	0.061e	46d
Values are mean ± SE (n = 3). Means followed by the same letter in a column are not significantly different at P ≤ 0.05 (DMRT).										Values are mean ± SE (n = 3). Means followed by the same letter in a column are not significantly different at P ≤ 0.05 (DMRT).									

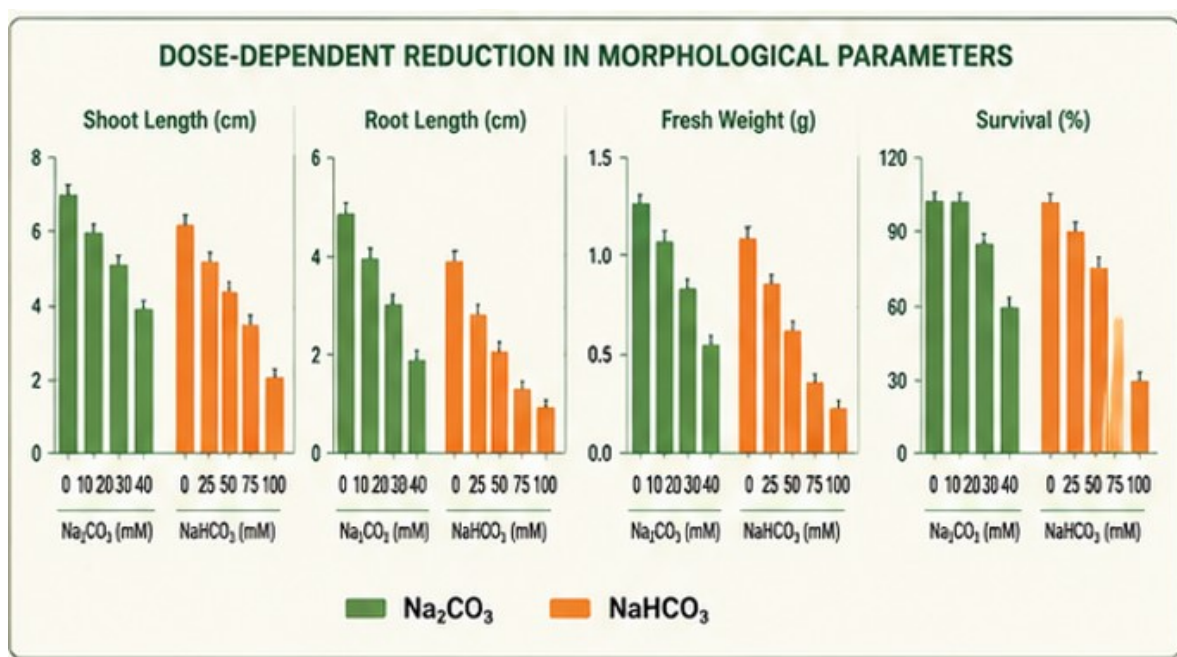


Fig. 1. Morphological response of *Solanum torvum* nodal explants after 29 days under different concentrations of Na₂CO₃ and NaHCO₃-induced alkaline stress.

Overall, the data indicate that NaHCO₃ exerts a strong dose-dependent negative effect on morphogenesis, biomass accumulation, and survival in *S. torvum* nodal cultures. This pattern is consistent with the known effects of salinity and alkaline stress, which disrupt ion homeostasis, reduce nutrient uptake, impair cell division and elongation, and ultimately suppress shoot and root development (Hasegawa et al., 2000; Munns & Tester, 2008; Zhu, 2001). In vitro cultures are especially sensitive to such stress because culture media have limited buffering capacity, and even moderate changes in ion

balance or pH can strongly affect regeneration and growth (George et al., 2008; Murashige & Skoog, 1962). The chlorosis and browning observed at higher NaHCO₃ concentrations further suggest oxidative and metabolic injury, which is commonly associated with reduced regeneration under saline conditions (Flowers & Colmer, 2008).

The results showed a clear concentration-dependent inhibitory effect of Na₂CO₃ on the in vitro growth and regeneration of *Solanum torvum* nodal explants. The control treatment (0 mM

Na₂CO₃) produced the best overall response, with 100% callus induction and shoot regeneration, the longest shoots (6.82 ± 0.21 cm), the longest roots (5.94 ± 0.18 cm), the highest number of leaves per shoot (12.3 ± 0.5) and roots per shoot (8.2 ± 0.4), and the greatest biomass accumulation (fresh weight 1.26 ± 0.05 g; dry weight 0.196 ± 0.01 g). Survival was also 100%, and the explants remained healthy, green, and highly rooted.

At 25 mM Na₂CO₃, only a slight reduction in growth was observed, with callus induction and shoot regeneration remaining relatively high at 92% and 88%, respectively. Shoot length, root length, leaf number, root number, and biomass were reduced modestly compared with the control, indicating that *S. torvum* explants could tolerate low levels of alkaline stress. However, increasing Na₂CO₃ to 50 mM caused a more noticeable decline in regeneration and growth. At this concentration, callus induction decreased to 80%, shoot regeneration to 74%, shoot length to 4.10 ± 0.16 cm, and root length to 3.22 ± 0.14 cm, while survival dropped to 82%. These changes were accompanied by reduced leaf and root formation and a decline in fresh and dry biomass.

The inhibitory effect became more pronounced at 75 mM Na₂CO₃, where callus induction and shoot regeneration declined to 58% and 50%, respectively. Shoot and root lengths were further reduced to 2.68 ± 0.13 cm and 1.94 ± 0.11 cm, and survival fell to 60%. Morphologically, explants showed reduced vigor, leaf yellowing, browning, and weak rooting, suggesting increasing physiological stress. The strongest adverse response was recorded at 100 mM Na₂CO₃, where callus induction dropped to 30%, shoot regeneration to 24%, shoot length to 1.22 ± 0.10 cm, root length to 0.86 ± 0.08 cm, and survival to 34%. Explants exposed to this concentration exhibited severe growth inhibition, necrosis, poor regeneration, and marked tissue browning.

Overall, the data indicate that Na₂CO₃ exerts a strong dose-dependent negative effect on morphogenesis, biomass accumulation, and survival in *S. torvum* nodal cultures. This pattern

is consistent with the known effects of saline-alkaline stress, which combine sodium toxicity with elevated pH, disrupt ion homeostasis, reduce nutrient availability, impair cell division and elongation, and ultimately suppress shoot and root development (Yang et al., 2008; Munns & Tester, 2008; Zhu, 2001). In vitro cultures are especially sensitive to such stress because culture media have limited buffering capacity, and even moderate changes in pH can strongly affect regeneration and growth (George et al., 2008; Murashige & Skoog, 1962). The chlorosis, browning, and necrosis observed at higher Na₂CO₃ concentrations further suggest oxidative and metabolic injury, which is commonly associated with reduced regeneration under alkaline conditions (Flowers & Colmer, 2008; Hasegawa et al., 2000).

Discussion

The present results demonstrate a clear concentration-dependent inhibitory effect of Na₂CO₃ on the in vitro growth, regeneration, and survival of *Solanum torvum* nodal explants. The control treatment (0 mM Na₂CO₃) produced the most vigorous response, with complete callus induction and shoot regeneration, the greatest shoot and root elongation, the highest number of leaves and roots per shoot, and the maximum fresh and dry biomass accumulation. The healthy green appearance and profuse rooting observed in the control indicate that the basal culture medium was suitable for normal morphogenesis and supported efficient regeneration under non-stress conditions (Murashige & Skoog, 1962; George et al., 2008).

At 25 mM Na₂CO₃, only a slight reduction in growth and regeneration was observed. Callus induction and shoot regeneration remained relatively high, and the explants retained good survival, suggesting that *S. torvum* can tolerate low levels of alkaline stress. However, even at this concentration, a decline in shoot length, root length, leaf number, and biomass was evident, indicating that early physiological disturbances had already begun. Such reductions are consistent with the initial effects of alkaline stress, which

include osmotic imbalance, sodium accumulation, and reduced nutrient availability due to elevated pH (Yang et al., 2008; Munns & Tester, 2008).

As Na_2CO_3 concentration increased to 50 mM, the inhibitory effects became more pronounced. Both callus induction and shoot regeneration declined substantially, and the explants showed reduced elongation, fewer leaves and roots, and lower biomass accumulation. The appearance of leaf yellowing and mild browning suggests that the stress was no longer limited to growth suppression but was also affecting tissue physiology and metabolic activity. Under alkaline conditions, high pH can reduce the solubility and uptake of essential nutrients such as iron, phosphorus, and manganese, thereby impairing chlorophyll synthesis, photosynthetic capacity, and overall growth performance (Hasegawa et al., 2000; Flowers & Colmer, 2008). In tissue culture systems, where nutrient buffering is limited, these effects are often amplified, leading to a rapid decline in regeneration potential (George et al., 2008).

At 75 mM Na_2CO_3 , the negative impact on morphogenesis was severe. Callus induction and shoot regeneration dropped to 58% and 50%, respectively, while shoot and root lengths were markedly reduced. The observed browning, weak rooting, and poor shoot elongation indicate substantial physiological injury. Browning in cultured explants is commonly associated with oxidative stress, phenolic oxidation, and cellular damage, all of which are frequently induced by salinity and alkalinity stress (Hasegawa et al., 2000; Zhu, 2001). The decline in biomass at this level further confirms that the explants were unable to maintain normal cell division and expansion under prolonged alkaline exposure.

The strongest adverse response was recorded at 100 mM Na_2CO_3 , where regeneration was severely suppressed and survival dropped to 34%. Explants exposed to this concentration exhibited necrosis, very poor shoot and root development, and minimal biomass accumulation. These symptoms indicate that the combined effects of sodium toxicity and high pH exceeded the physiological tolerance threshold of the explants.

Unlike neutral salt stress, alkaline stress imposes an additional burden through hydroxyl and bicarbonate accumulation, which can disrupt membrane integrity, enzyme activity, and ion homeostasis more severely than salinity alone (Yang et al., 2008; Munns & Tester, 2008). The drastic reduction in regeneration at 100 mM therefore reflects both direct ionic toxicity and indirect metabolic disruption.

Overall, the data show that Na_2CO_3 exerts a strong dose-dependent negative effect on in vitro morphogenesis, biomass production, and survival in *S. torvum* nodal cultures. The progressive decline in regeneration parameters from 25 to 100 mM indicates that the species has only limited tolerance to alkaline stress under in vitro conditions. This pattern is consistent with earlier reports showing that high pH and sodium-rich environments inhibit cell division, reduce nutrient uptake, impair chlorophyll formation, and suppress root and shoot development in plant tissues (Zhu, 2001; Hasegawa et al., 2000; Flowers & Colmer, 2008). The present findings also highlight the sensitivity of tissue culture systems to alkaline stress because the culture medium lacks the buffering and ion-regulating capacity of soil, making explants especially vulnerable to rapid physiological imbalance (George et al., 2008). From a practical perspective, the results suggest that *S. torvum* can withstand only low levels of Na_2CO_3 stress in vitro, while higher concentrations cause severe growth inhibition and loss of regenerative capacity.

Conclusion

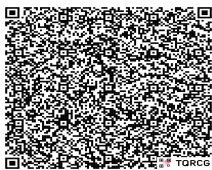
In conclusion, Na_2CO_3 caused a clear concentration-dependent inhibition of in vitro regeneration, growth, biomass accumulation, and survival in *Solanum torvum* nodal explants. The control treatment supported healthy morphogenesis, whereas increasing alkaline stress progressively reduced callus induction, shoot regeneration, shoot and root elongation, and overall biomass. Severe browning, necrosis, and poor survival at higher concentrations confirmed that *S. torvum* has only limited tolerance to

Na₂CO₃-induced alkalinity under in vitro conditions. These findings suggest that low levels of alkaline stress can be partially tolerated, but moderate to high concentrations severely impair regenerative capacity and tissue viability.

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