Enhancing Salt Stress Tolerance in Solanum torvum L.: An In Vitro Approach Using NaCl and KCl Selection

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***Abstract*—Environmental stresses, particularly soil salinity and drought, significantly reduce agricultural productivity worldwide by inhibiting plant growth and lowering crop yields. Soil salinity, mainly caused by sodium chloride (NaCl) and potassium chloride (KCl), induces ionic toxicity, osmotic stress, and metabolic disturbances, affecting plant survival and development. Understanding the molecular and physiological mechanisms of salt stress tolerance is crucial for developing resilient plant varieties.**

This study aimed to develop NaCl- and KCl-tolerant cell lines in *Solanum torvum*, a medicinally valuable species. Seeds were surface-sterilised and germinated aseptically on Murashige and Skoog (MS) medium. Leaf explants from eight-week-old seedlings were cultured on a regeneration medium supplemented with NaCl (0.1–1.0%) and KCl (0.1–1.25%) to determine the tolerance thresholds. Concentrations causing 90% inhibition of regeneration were identified as 0.75% NaCl and 1.0% KCl. Selected stress-tolerant shoots were subcultured on fresh selective medium, and rooted plantlets were transferred to soil supplemented with NaCl/KCl for acclimatisation.

Results indicated a gradual decline in regeneration with increasing salt concentrations, with complete inhibition at 1.0% NaCl and 1.25% KCl. Salt-tolerant shoots showed enhanced proliferation compared to unselected controls, with KCl-tolerant shoots producing more roots (16.4 ± 0.24 roots/explant) than NaCl-tolerant ones. Upon soil transfer, survival rates were 46% and 64% for NaCl- and KCl-tolerant plantlets, respectively. Screening confirmed stable salt tolerance, as unselected shoots did not survive under stress.

These findings provide a foundation for further genetic and physiological studies on salt stress resilience in *S. torvum*, which can be leveraged for breeding programs and biotechnological approaches aimed at improving salt tolerance in solanaceous crops.

***Index Terms*—Solanum torvum, salt tolerance, NaCl, KCl, in vitro selection, plant stress physiology.**

I. INTRODUCTION

Soil salinity is recognized as one of the most critical abiotic stresses limiting global agricultural productivity (Munns & Tester, 2008). Excessive accumulation of salts such as sodium chloride (NaCl) and potassium chloride (KCl) leads to severe consequences for plants. The presence of high salt concentrations in the soil induces osmotic stress, which restricts water uptake and causes ion toxicity due to excess sodium and chloride. Furthermore, salinity disrupts the balance of essential nutrients, creating nutrient deficiencies that impair plant metabolism (Zhu, 2001). These combined effects significantly reduce plant growth, development, and crop yield. With the rapid expansion of salinity-prone areas worldwide, the development of salt-tolerant cultivars has become an urgent priority for ensuring food security and sustainable agriculture.

One promising approach to tackle this challenge is ***in vitro* selection**, a biotechnological method that allows researchers to generate and screen for salt-tolerant plant cell lines under controlled laboratory conditions. By exposing plant callus tissues, cell suspensions, or organ cultures to specific concentrations of NaCl or KCl, it is possible to identify tolerant variants. These selected lines can then be propagated and further studied for their physiological, biochemical, and genetic traits associated with salt tolerance (Rai *et al.,* 2011). Such methods have been successfully applied in several major crops. For instance, salt-tolerant lines have been developed in rice (*Oryza sativa*), wheat (*Triticum aestivum*), and tomato (*Solanum lycopersicum*), providing strong evidence of the utility of this strategy for crop improvement (Rao *et al.,* 2013; Singh & Prasad, 2014).

Solanum torvum Sw., commonly referred to as wild eggplant or turkey berry, belongs to the Solanaceae

family and is valued for its remarkable hardiness. It is widely used as a rootstock for cultivated eggplant (*Solanum melongena*) due to its natural resistance to soil-borne pathogens and tolerance to various abiotic stresses, including drought and poor soil fertility (Khan *et al.,* 2020). However, its potential as a salt-tolerant species has not been extensively studied. Investigating the response of *S. torvum* to NaCl and KCl stress and developing tolerant cell lines through in vitro selection could greatly expand its role in saline agriculture, particularly as a resilient rootstock for solanaceous crops.

Salt stress triggers a wide array of morphological, physiological, and biochemical changes in plants. To cope with high salinity, plants adopt mechanisms such as osmotic adjustment, which helps maintain water balance, and ion compartmentalization, which sequesters excess sodium and chloride ions into vacuoles to protect vital cellular processes. Additionally, plants accumulate osmoprotectants, such as proline and glycine betaine, which act as compatible solutes to stabilize proteins, membranes, and enzymes under stress (Dubey, 1997). Studying these adaptive responses in *S. torvum* not only enhances our understanding of its stress physiology but also provides valuable insights that can be applied in crop breeding and biotechnological programs.

Aim: The present review aims to highlight the potential of *Solanum torvum* Sw. for developing salt-tolerant cell lines using in vitro selection techniques, with a focus on NaCl and KCl stress responses.

II. OBJECTIVES

1. To discuss the role of in vitro selection as a strategy for generating salt-tolerant plant cell lines.
2. To explore the importance of *Solanum torvum* as a hardy solanaceous species with potential for salt stress resilience.
3. To summarise the key physiological and biochemical mechanisms involved in plant salt tolerance.
4. To emphasise the significance of developing NaCl- and KCl-tolerant *S. torvum* lines for saline agriculture and rootstock improvement.

III. MATERIALS AND METHODS

Plant Material and Surface Sterilisation

Seeds of *Solanum torvum* were used as the experimental plant material for in vitro studies. Before culture initiation, seeds were pre-soaked in sterile distilled water for 24 hours to soften the seed coat and facilitate germination.

Surface sterilisation was carried out under aseptic conditions in a laminar airflow cabinet following these steps:

1. Seeds were immersed in 70% ethanol for 2–3 minutes to remove surface contaminants.
2. The ethanol was decanted, and the seeds were rinsed once with sterile distilled water.
3. The seeds were then treated with 1% sodium hypochlorite (NaOCl) solution for 3–5 minutes for disinfection.
4. Finally, the seeds were rinsed thoroughly three times with sterile distilled water to remove all traces of sterilising agents. This sterilisation protocol ensured contamination-free seed material for subsequent in vitro experiments.

Seed Germination and Culture Conditions

Sterilised seeds of *Solanum torvum* were inoculated on Murashige and Skoog (MS) medium (Murashige & Skoog, 1962) supplemented with 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. The pH of the medium was adjusted to 5.8 before autoclaving. Cultures were incubated at 25 ± 2°C under a 16 h light / 8 h dark photoperiod, with a light intensity of 40–50 µmol m^{-2} s^{-1} provided by cool-white, fluorescent lamps.

Initiation of *In Vitro* Cultures

Leaf explants (approximately 1 cm^2) were excised from 8-week-old in vitro-grown seedlings and cultured on MS regeneration medium (RM). The medium was supplemented with 0.5 mg/L indole-3-acetic acid (IAA) and 2.5 mg/L 6-benzylaminopurine (BAP) to induce morphogenesis. To study the effect of salinity, cultures were exposed to varying concentrations of NaCl (0.0–1.0%) and KCl (0.0–1.25%) incorporated into the medium.

Screening for Salt Tolerance

For salt tolerance studies, leaf explants were cultured on regeneration medium (RM) supplemented with graded concentrations of NaCl (0.1–1.0%) and KCl (0.1–1.25%). The concentration at which approximately 90% of regeneration was inhibited was

considered the stress-inducing threshold, determined as 0.75% NaCl and 1.0% KCl.

To screen for tolerant regenerants, fifty explants were cultured under both stress (threshold concentrations of NaCl and KCl) and control (salt-free RM) conditions. Explants that successfully regenerated under stress conditions were considered putative salt-tolerant lines for further evaluation.

Shoot Bud Proliferation and Micros hoot Development

Emerging shoot buds obtained from selective media were sub cultured onto fresh regeneration medium to promote further proliferation. Developing micro shoots were then transferred to RM supplemented with 1.0 mg/L indole-3-acetic acid (IAA) and either 0.75% NaCl or 1.0% KCl to evaluate growth performance under salt stress conditions.

Establishment of Salt-Tolerant Plantlets

Rooted plantlets were carefully removed from the culture vessels and transferred to soil containing either 0.75% NaCl or 1.0% KCl for acclimatisation. Plantlets were maintained under controlled greenhouse conditions for three weeks. After successful acclimatisation, healthy plants were transplanted into the research field for further evaluation of growth and survival.

Data Analysis

Each treatment was represented by 15 replicates, and the experiments were performed twice to ensure reproducibility. Data were expressed as mean ± standard error (SE) and analysed using standard statistical procedures to assess the significance of observed differences between control and salt-treated groups.

IV. RESULTS

Selection of Salt-Tolerant Cell Lines

The response of *Solanum torvum* explants to NaCl and KCl stress was concentration dependent.

- NaCl Stress: Shoot regeneration declined progressively with increasing NaCl levels. At 0.75% NaCl, regeneration was reduced by approximately 90%, producing 2.4 ± 0.89 shoots per explant. Complete inhibition of shoot formation occurred at 1.0% NaCl (Fig. 1a; Table 1).
- KCl Stress: A gradual decline in regeneration was observed with increasing KCl concentrations. At 1.0% KCl, explants produced 3.8 ± 0.19 shoots per explant, whereas complete inhibition was recorded at 1.25% KCl (Fig-1: Table 2).

***In Vitro* Rooting**

Salt-tolerant shoots successfully rooted under selective conditions, although root numbers differed between treatments. KCl-tolerant shoots produced significantly more roots (16.4 ± 0.24 roots per explant) compared with NaCl-tolerant shoots, which formed fewer roots under stress conditions.

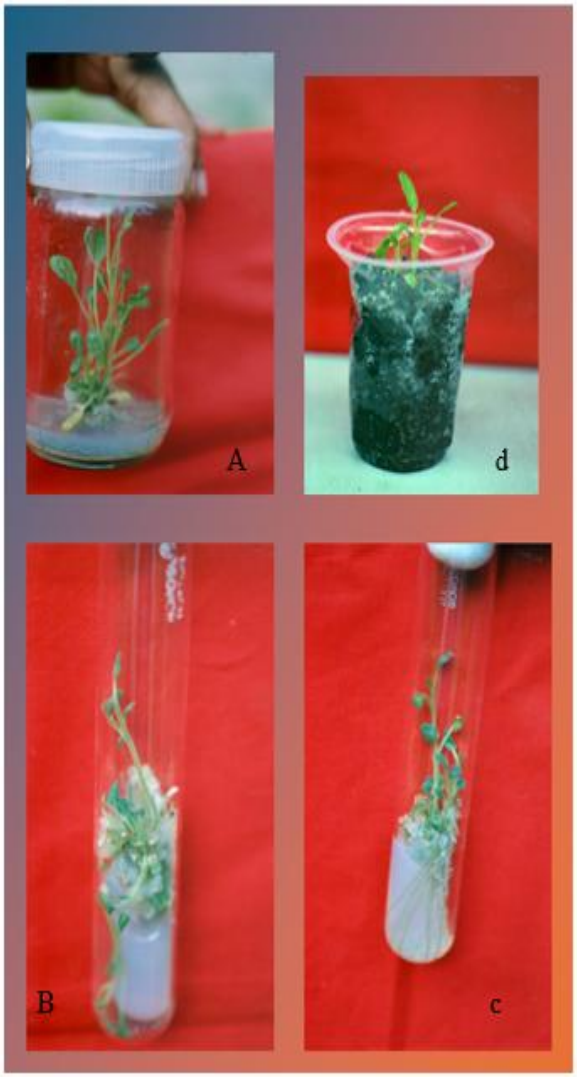

Figure 1. Effect of Different Salt and Hormonal Treatments on Regeneration and Hardening of Plantlets

(a) Shoot regeneration on medium containing 0.3% NaCl (average shoot length 9.2 ± 0.21 cm). (b) Enhanced shoot formation on medium supplemented with 0.1% KCl (average shoot length 11.0 ± 0.28 cm). (c) Root induction on regeneration medium containing

1.0% IAA. (d) Hardening of regenerated plantlets under acclimatisation.

Plantlet Establishment and Survival

Rooted plantlets were transferred to soil containing selective salt concentrations. Survival rates varied according to the type of stress:

- NaCl-tolerant plantlets showed 46% survival.
- KCl-tolerant plantlets showed higher survival at 64%.
- Unselected shoots failed to establish and did not survive under stress conditions.

Screening of Selected Lines

Further screening confirmed the regeneration potential of selected salt-tolerant lines:

- NaCl-selected lines: 70% of explants responded, producing 16.8 ± 1.04 shoots per explant.
- KCl-selected lines: 80% of explants responded, producing 10.0 ± 0.03 shoots per explant.
- Unselected controls: Failed to survive beyond two weeks under stress conditions.

Table 1. Effect of NaCl concentration on shoot regeneration of *S. torvum*

NaCl (%)	Explants Responding (%)	Average Shoots/Explant	Respond
0	100	12.3 ± 0.45	Control
0.1	92	10.8 ± 0.33	-
0.2	87	9.2 ± 0.21	-
0.3	82	7.5 ± 0.19	-
0.4	75	5.6 ± 0.23	-
0.5	62	4.3 ± 0.12	-
0.75	10	2.4 ± 0.89	90% inhibition
1	0	0	Complete inhibition

Table 2: Effect of KCl concentration on shoot regeneration of *S. torvum*

KCl (%)	Explants Responding (%)	Average Shoots/Explant	Respond
0	100	12.3 ± 0.45	Control
0.1	94	11.0 ± 0.28	-
0.25	88	9.5 ± 0.21	-
0.5	76	6.2 ± 0.14	-
0.75	60	4.1 ± 0.09	-
1	15	3.8 ± 0.19	Stress-induced
1.25	0	0	Complete inhibition

V. DISCUSSION

The present study demonstrated that in vitro selection is an effective strategy for developing salt-tolerant *Solanum torvum* lines, with stable regeneration observed under both NaCl and KCl stress. Salt tolerance is a complex trait influenced by multiple physiological and biochemical mechanisms, including osmotic adjustment, ion homeostasis, and the accumulation of compatible solutes such as proline and glycine betaine (Munns & Tester, 2008; Zhu, 2001). In this study, *S. torvum* exhibited differential responses to NaCl and KCl, with NaCl imposing stronger inhibitory effects on regeneration and survival than KCl. This finding aligns with earlier reports in *Brassica juncea*, *Nicotiana tabacum*, and tomato, where NaCl stress was more deleterious than KCl stress (Gangopadhyay *et al.,* 1997a, b; Cano *et al.,* 1998).

The inhibition of regeneration at higher salt concentrations in the present work reflects the osmotic and ionic stress that disrupts water uptake and nutrient balance, ultimately reducing morphogenic capacity. Similar observations have been reported in callus and shoot cultures of rice, wheat, and eggplant under in vitro conditions (Rai *et al.,* 2011; Singh & Prasad, 2014; Jain *et al.,* 1988). The comparatively higher tolerance of *S. torvum* to KCl suggests that chloride ions are less toxic than sodium ions at equivalent concentrations, which supports earlier studies showing that sodium toxicity is a primary determinant of salinity stress (Eberhardt & Wegmann, 1989; Rao *et al.,* 2013).

One of the key adaptive responses to salt stress is the accumulation of proline, which acts as an osmoprotectant, stabilises proteins and membranes, and scavenges reactive oxygen species (Delauney & Verma, 1990; Verbruggen *et al.,* 1993). Previous reports in stressed *Solanum* and *Brassica* species confirm that proline accumulation is positively correlated with stress tolerance (Leone *et al.,* 1994; Dubey, 1997). More recently, molecular studies have highlighted the role of proline biosynthesis genes such as *P5CS* and *P5CR* in enhancing stress resilience (Hu *et al.,* 1992; Shen *et al.,* 1997; Bartels & Sunkar, 2005). In addition to proline, glycine betaine also contributes to osmotic balance and photosynthetic protection under salinity stress (Weretilnyk & Hanson, 1990; Ashraf & Foolad, 2007). The performance of selected S. torvum lines under stress conditions suggests that similar osmoprotective mechanisms may be operative.

Importantly, the NaCl- and KCl-tolerant lines-maintained donor plant morphology and established successfully in soil, indicating the stability of tolerance traits beyond in vitro conditions. This is significant because many in vitro–derived variants fail during acclimatisation. The observed survival rates (46% in NaCl-selected and 64% in KCl-selected lines) are encouraging and provide a strong basis for their use as rootstocks in eggplant cultivation under saline soils, complementing the species' already known resistance to soil-borne pathogens (Khan *et al.,* 2020).

Overall, these findings suggest that *S. torvum* possesses untapped potential for salinity tolerance, making it a valuable genetic resource for breeding programs aimed at improving eggplant and related crops. Future research should focus on molecular characterization of selected lines, particularly gene expression studies on proline and glycine betaine biosynthetic pathways, ion transporters (e.g., *NHX* and *HKT* family genes), and antioxidant enzymes. Such insights will not only validate the physiological observations but also guide the development of transgenic or genome-edited crops with enhanced salinity tolerance (Flowers & Colmer, 2015; Isayenkov & Maathuis, 2019).

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