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RESEARCH ARTICLE

Optimizing NaCl concentration for screening salt tolerance in cowpea (*Vigna unguiculata* L. Walp.) genotypes at germination stage using salt injury index

Shivam Kumar Rai[1,2], Rajesh Kumar[1], Abhinav Dayal[2], M. Linga Swami Sai Reddy[2] and Nakul Gupta[1*]

Abstract

Salinity is a major abiotic stress that impacts plant growth and crop yields, particularly in saline-prone regions. The main objective of this work is to determine the optimum NaCl concentration for screening salt tolerance using the Salt Injury Index (SII) by analyzing the effects of NaCl-induced salinity on germination and seedling growth in 30 cowpea (*Vigna unguiculata* L. Walp.) genotypes. Cowpea seeds were exposed to six NaCl concentrations (0, 75, 100, 150, 200, and 250 mM) in petri plates. Seedling growth parameters such as germination, shoot and root length, seedling length, dry weight, and vigour indices (I & II), were recorded to evaluate the effects of salinity stress. A consistent decline in all measured parameters was observed as NaCl concentration increased. Linear regression of the Salt Injury Index values revealed NaCl concentrations caused a 50% reduction in specific traits, including 215 mM affecting germination, 180 mM for shoot, 210 mM for root, 200 mM for seedling length and dry weight, and 130 and 160 mM for Vigour Index-I and -II, respectively, with an average threshold of 183.57 mM. Consequently, 200 mM NaCl was identified as the optimal concentration to evaluate salt tolerance levels, offering a standardized benchmark for assessing cowpea genotypes under saline conditions.

Keywords: Salinity stress, Cowpea, Salt tolerance, NaCl concentration, Salt Injury Index (SII), In-vitro screening.

[1]ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India.

[2]Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, India.

[*]**Corresponding author;** Email: nakulgupta1988@gmail.com

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Introduction

The escalating problem of soil salinity poses a significant threat to global agricultural productivity, affecting over one billion hectares of land in more than 100 countries (Abbas et al., 2013; FAO & ITPS, 2015; Hossain, 2019). Salinity, resulting from both natural processes and anthropogenic activities, has rendered approximately 76 million hectares of land unsuitable for cultivation. This issue continues to expand, with about 1.5 million hectares of arable land lost annually due to salinization (Hossain, 2019). The detrimental effects of salinity on seed germination and seedling development are of critical concern, as these stages are pivotal for successful crop propagation and establishment (Munns et al., 2020). Under saline conditions, seed germination is hindered by osmotic and ionic stress, which disrupt water absorption, metabolic activation, and enzymatic activity (Yadav et al., 2020; Wahid et al., 2014). The decreased activity of hydrolytic enzymes, such as α-amylase, further impedes the breakdown of starch into sugars, thereby restricting energy availability for embryonic development (Ucarli, 2020). These adverse effects are particularly pronounced in salt-sensitive genotypes compared to salt-tolerant ones. Beyond germination, salinity-induced oxidative stress exacerbates damage to cellular structures, further affecting seedling vigour and viability (Hasanuzzaman et al., 2021).

Consequently, understanding the mechanisms of salt tolerance and identifying genotypes with superior resilience at the germination stage are crucial for mitigating the impact of salinity on crop production.

Cowpea (*Vigna unguiculata* [L.] Walp) is an important legume crop cultivated in Africa, Asia, Europe, the U.S., and Latin America for multiple purposes including vegetable, pulse, fodder and cover crop (Wilson et al., 2006; Singh et al., 2003). Cowpea is nutritionally rich, offering high protein (24.5 g/100 g), essential minerals, and energy (Frota et al., 2008). Despite its genetic diversity, salt stress severely limits cowpea productivity, particularly in semi-arid regions (Zhang et al., 2012). Varietal differences in salt tolerance exist, with certain cultivars exhibiting resilience (Wilson et al., 2006; Praxedes et al., 2009). Therefore, early-stage screening is essential for developing salt-tolerant cowpea genotypes. Screening for salt tolerance in a laboratory or *in-vitro* is a cost-effective and reliable approach to evaluate genotype performance under controlled salinity stress. Unlike field-based evaluations, where variability in environmental factors such as moisture, temperature, and soil fertility can confound results, laboratory screening allows precise control over experimental conditions. This method also minimizes the resource requirements associated with large-scale field trials and accelerates the identification of salt-tolerant genotypes by focusing on critical growth stages, such as germination and seedling development. Additionally, laboratory screening facilitates the use of reproducible salt concentrations, ensuring consistency in evaluating genotypic responses to salinity. These advantages make laboratory screening an indispensable tool for advancing salinity research and developing resilient crop varieties.

Screening a large number of genotypes under varying concentrations of salt poses significant logistical challenges. Therefore, it becomes essential to optimize a single, standardized salt concentration that allows for effective screening of all genotypes. Establishing such a threshold concentration is critical to accurately assess the germination and seedling growth responses of cowpea genotypes under salinity stress. The current study aims to evaluate the responses of cowpea (*Vigna unguiculata* [L.] Walp) genotypes to salinity stress during germination. Additionally, it seeks to determine and optimize the threshold NaCl concentration that can reliably be used to screen genotypes for salinity tolerance.

Material and Methods

Experimental materials

The seeds of 30 cowpea genotypes with distinct genetic background were collected from the ICAR-Indian Institute of Vegetable Research (IIVR), Varanasi, India. The experiments were conducted during the 2023-2024 period in the Seed Technology Laboratory at ICAR-IIVR, Varanasi, following a completely randomized design (CRD). Prior to experiment, seeds were surface sterilized using a 0.5% sodium hypochlorite (NaOCl) solution. Seed viability was then assessed using the standard germination test protocol.

Salt stress treatment and experimental design

Five NaCl concentrations (75, 100, 150, 200, and 250 mM) along with distilled water serving as the control (0 mM NaCl) was used for applying salt stress (Table 1). For each genotype, 25 uniform and healthy seeds were placed in 15 cm petri-dish, each lined with two layers of germination paper, which were pre-moistened with 15 mL of the respective NaCl solution. Each treatment was replicated four times to ensure statistical robustness. The Petri dishes were incubated in a germination chamber set to 25 ± 3°C with 70% relative humidity. Germination was monitored by observing the emergence of radicles, defined as reaching or exceeding 2 mm in length. Germination percentage was calculated after 8 days, based on the number of normal seedlings observed, following the guidelines provided by ISTA (2024).

Seedling Growth Measurements

From each replicate, 10 seedlings were randomly selected for measurement of shoot and root lengths. Seedling dry weight was determined by drying the seedlings in a hot air oven at 70°C for 48 hours. Seedling vigour indices were calculated as follows (Gupta et al., (2022).

Vigour Index I = Vigour Index I = Germination Percentage × Seedling Length

Vigour Index II = Germination Percentage × Seedling Dry Weight

Determination of Optimal Salt Concentration

To identify the optimal salt concentration for evaluating salt tolerance, the Salt Injury Index (SII) using Salt Tolerance Index (STI) was calculated for each trait. STI is the ratio of the trait value under salt stress to the trait value under control conditions Whereas, SII reflects the extent of injury caused by salt stress, where a value approaching 1 indicates severe injury (Wu *et al*., 2019; Agrawal *et al*., 2024). The STI and SII for each trait was computed using the following formula.

$$Salt\,Tolerance\,Index\left(STI\right)=\frac{\text{Value of trait under salt Stress}}{Value\,of\,\,trait\,under\,control\,condition}$$

$$Salt\,Injury\,Index\left(SII\right)=1-\text{ value of STI of each traits}$$

The salt concentration at which the SII reached 50% of the control value was identified as the threshold for optimal salt stress, beyond which significant injury to the seedlings occurred.

Statistical analysis

Data were analyzed using SPSS software (version 16.0). Linear regression analysis was performed based on the SII values of different traits to optimize the salinity concentration

Table 1: Salinity levels and corresponding NaCl concentrations

S. No.	*Salinity level*	*Conc. (mM)*	*PPM*	*deciSiemens/meter*	*Grams/Litre (g/L)*
1.	S0	0 mM	0.00	0.00	0.00
2.	S1	100 mM	5844	9.13	5.84
3.	S2	200 mM	11688	18.26	11.69
4.	S3	225 mM	13149	20.54	13.15
5.	S4	250 mM	14610	22.82	14.61
6.	S5	275 mM	16071	25.11	16.07
7.	S6	300 mM	17532	27.39	17.53

for screening salt tolerance and to assess the relationship between salt stress and seedling performance.

Results

Effect of NaCl concentration on germination and seedling growth

To determine the ideal NaCl concentration for assessing salt tolerance during the germination and seedling stages, 30 cowpea genotypes were subjected to varying concentrations of NaCl (0, 75, 100, 150, 200, and 250 mM). After eight days, key germination parameters, including germination percentage (GP), shoot length (SHL), root length (RL), seedling length (SL), dry weight (DW), and vigour indices (VI-I and VI-II), were measured to evaluate the effects of salt on seedling growth (Fig. 1). The data represent the average of the 30 genotypes and provide insights into the detrimental effects of salinity on cowpea seed germination and seedling development (Fig. 2). Germination percentage was significantly reduced as salinity levels increased. Under non-saline conditions (0 mM NaCl), cowpea seeds exhibited optimal germination, but a slight decline of 1.2% at 75 mM NaCl. This reduction intensified to 17.3% at 100 mM, 27.4% at 150 mM, and 40.1% at 200 mM. The most critical inhibition occurred at 250 mM NaCl, where germination was reduced by 88.3%, effectively halting the germination process under extreme salinity stress.

The inhibitory effects of salinity extended beyond germination to vegetative growth, as evidenced by the progressive decline in shoot length (SHL). Shoot length (SHL) followed a similar trend, decreasing by 23.1% at 75 mM, 29.5% at 100 mM, 43.0% at 150 mM, 57.7% at 200 mM, and 91.7% at 250 mM, reflecting significant above-ground growth suppression.

Similarly, Root length (RL), declined by 21.2% at 75 mM, 29.5% at 100 mM, 39.7% at 150 mM, 44.9% at 200 mM, and 91.0% at 250 mM, indicating severe root impairment at higher concentrations. The combined negative impacts on shoot and root growth were evident in seedling length (SL), which progressively declined with increasing salinity. Compared to the control, SL was reduced by 21.8% at 75 mM, 29.5% at 100 mM, 41.3% at 150 mM, 51.3% at 200 mM,

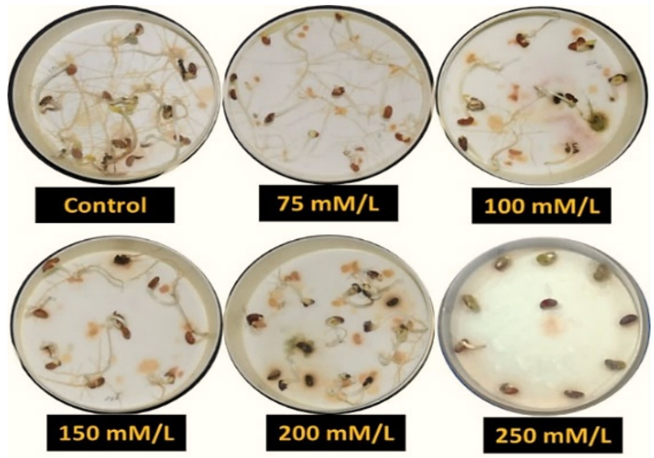

Fig. 1: Performance of representative cowpea genotypes under various NaCl-induced salinity concentrations, showing key growth parameters

and 91.3% at 250 mM. Similarly, Biomass accumulation, as indicated by dry weight (DW), was also severely impacted by salinity. . Dry weight (DW) exhibited a parallel decline, dropping by 10.5% at 75 mM, 19.3% at 100 mM, 29.8% at 150 mM, 56.1% at 200 mM, and 77.2% at 250 mM, underscoring reduced biomass accumulation. The detrimental effects of salinity were further validated by the marked reductions in vigour indices. Vigour Index-I (VI-I), integrating GP and SL, decreased by 22.9%, 41.3%, 56.6%, 69.9%, and 97.5% across the respective concentrations, while Vigour Index-II (VI-II), combining GP and DW, fell by 23.2%, 30.4%, 46.4%, 71.4%, and 94.6%, confirming the broad impact on seedling health. However, variability in the extent of inhibition across traits and genotypes ranging from 40.1% (GP) to 69.9% (VI-I), indicated that relying solely on these averages might oversimplify the optimal concentration, necessitating a more precise and standardized approach.

Determination of optimum NaCl concentration using salt injury index (SII)

To address the limitations of initial percentage reductions and establish a robust screening concentration, the Salt Injury Index (SII) was calculated for each parameter, quantifying salt-induced damage relative to the control (Table 2). The Salt Injury Index (SII) was calculated for each

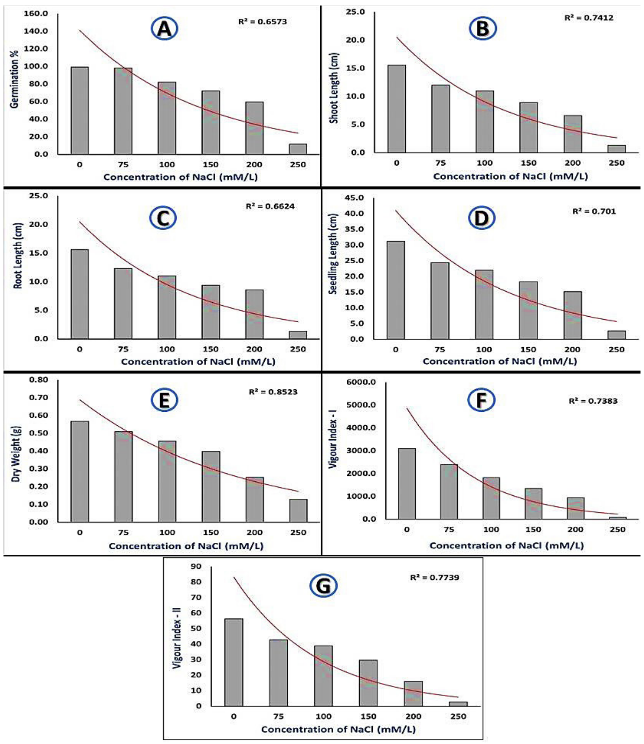

Fig. 2: Mean performance of 30 cowpea genotypes under varying NaCl concentrations: (A) Germination percentage, (B) Shoot length, (C) Root length, (D) Seedling length, (E) Seedling dry weight, (F) Vigour Index I, and (G) Vigour Index II

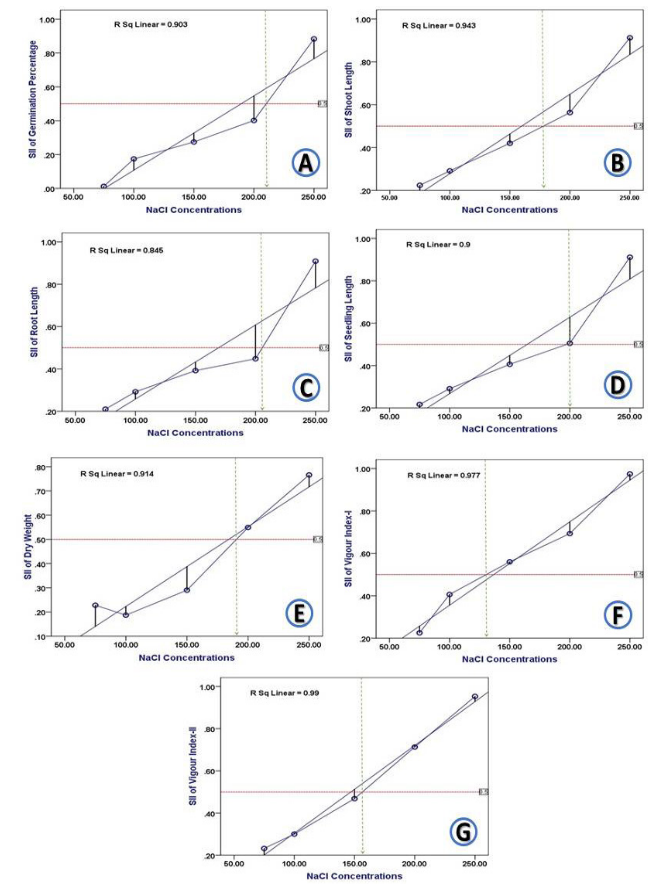

Fig. 3: Identification of the optimal NaCl concentration for screening salt tolerance in 30 cowpea genotypes, defined by a 50% reduction in the Salt Injury Index (SII) from control conditions. The figure shows linear regression relationships between SII values and NaCl concentrations for each parameter: (A) germination percentage, (B) shoot length, (C) root length, (D) seedling length, (E) seedling dry weight, (F) Vigour Index I, and (G) Vigour Index II

trait to estimate the degree of damage caused by salinity stress. The SII threshold of 0.5 represents the point at which half of the seedlings show salt-induced damage, providing a critical reference for determining the optimum concentration.

At 75 mM, SII values were low across all traits (0.012 for GP, 0.224 for SHL, 0.210 for RL, 0.217 for SL, 0.228 for DW, 0.225 for VI-I, and 0.232 for VI-II), reflecting minimal impact. At 100 mM, SII increased moderately (0.174 for GP, 0.291 for SHL, 0.292 for RL, 0.291 for SL, 0.187 for DW, 0.406 for VI-I, and 0.300 for VI-II), indicating a noticeable but variable effect. At 150 mM, SII values rose further (0.274 for GP, 0.420 for SHL, 0.392 for RL, 0.406 for SL, 0.290 for DW, 0.560 for VI-I, and 0.468 for VI-II), showing significant stress. At 200 mM, SII approached or exceeded 0.5 for most traits (0.401 for GP, 0.563 for SHL, 0.448 for RL, 0.505 for SL, 0.549 for DW, 0.693 for VI-I, and 0.713 for VI-II), while at 250 mM, SII values were consistently high (0.883 for GP, 0.912 for SHL, 0.909 for RL, 0.911 for SL, 0.766 for DW, 0.974 for VI-I, and 0.953 for VI-II), indicating near-complete inhibition. The linear regression analysis of SII values for all morphological traits revealed the following concentration points where SII reached 0.5: 215 mM NaCl for germination %, 180 mM NaCl for shoot length, 210 mM NaCl for root length, 200 mM NaCl for seedling length, 190 mM NaCl for seedling dry weight, 130 mM NaCl for Vigour Index-I, and 160 mM NaCl

Table 2: Salt injury index (SII) for various growth parameters of cowpea genotypes under different NaCl concentrations

NaCl Level	SII_{GP}	SII_{SDW}	SII_{RL}	SII_{SL}	SII_{DW}	SII_{VI-I}	SII_{VI-II}
75 mM	0.012	0.224	0.210	0.217	0.228	0.225	0.232
100 mM	0.174	0.291	0.292	0.291	0.187	0.406	0.300
150 mM	0.274	0.420	0.392	0.406	0.290	0.560	0.468
200 mM	0.401	0.563	0.448	0.505	0.549	0.693	0.713
250 mM	0.883	0.912	0.909	0.911	0.766	0.974	0.953

for Vigour Index-II (Fig. 3). The average SII across all traits was recorded at approximately 183.57 mM. Therefore, a NaCl concentration of 200 mM was identified as the optimum salt stress concentration for screening cowpea genotypes for salt tolerance. This concentration closely aligned with the 50% damage threshold for most traits (e.g., 200 mM for SL, 190 mM for DW) while providing a clear distinction between tolerant and sensitive genotypes, balancing the variability observed in raw percentage reductions.

Discussion

This study aimed to evaluate the salt tolerance of 30 cowpea genotypes under varying NaCl concentrations during the germination and seedling stages. The findings provide a comprehensive assessment of how increasing salinity affects key physiological parameters, including germination percentage; shoot length, root length, seedling length, dry weight, and vigour indices. Through this, the study highlights the detrimental effects of salinity on cowpea growth and identifies the optimal salt concentration for screening salt tolerance, as well as the critical threshold for salinity-induced damage using the Salt Injury Index (SII).

Impact of salt stress on cowpea seedling growth

Increasing NaCl concentrations significantly impaired the germination and seedling growth of cowpea. As NaCl concentration increased, a progressive decline was observed across all measured traits. The germination percentage, which serves as an indicator of seed viability, decreased markedly as salinity increased. This decline is consistent with previous studies on leguminous crops, where high salinity has been shown to hinder seed germination by disrupting physiological processes such as enzyme activity, water uptake, and membrane integrity (Munns et al., 2020; EL Sabagh et al., 2021; Hasanuzzaman et al., 2021).

Similarly, shoot length and root length exhibited a consistent decline with increasing NaCl concentration. Shoot length decreased progressively as salinity increased, while root length dropped significantly. This reduction in shoot and root growth is indicative of salt-induced inhibition of cell elongation and division, which are vital for proper seedling development (Wahid et al., 2014; El-Hendawy et al., 2019). The root system, being directly involved in water and nutrient uptake, is particularly sensitive to salt stress, which impairs its ability to absorb essential resources, exacerbating the overall negative impact on plant growth (Nahar et al., 2016; Gaikwad et al., 2022). Seedling length (SL), which combines both shoot and root growth, followed a similar trend, with severe stunting observed at higher NaCl concentrations. The dry weight (DW) of seedlings also progressively decreased as salinity levels increased, reflecting the negative impact of salt stress on metabolic processes involved in biomass accumulation. Salt stress often leads to reduced photosynthetic efficiency, disruption of nutrient uptake, and changes in hormone balance, all of which contribute to decrease biomass production (Munns et al., 2020; Zhao et al., 2021). Vigour indices, which provide a composite measure of seedling growth and health, also showed a significant decline with increasing NaCl concentrations. These declines highlight the overall negative impact of salinity on cowpea growth, indicating that high NaCl concentrations severely hinder seedling establishment and early development (Atta et al., 2023).

Salt Injury Index (SII) and its role in screening salt tolerance

The Salt Injury Index (SII) was used to determine the optimal NaCl concentration for salt tolerance screening. The SII provides a quantifiable measure of salt-induced damage across multiple growth parameters, offering a more holistic assessment of salinity stress (Wu et al., 2019; Agrawal et al., 2024). In this study, the SII was calculated for each trait, and employed regression analysis which revealed a range of concentrations at which SII reached 0.5 (130–215 mM), with an average of 183.57 mM. Selecting 200 mM as the optimum balanced this range, aligning closely with key traits like SL (200 mM) and DW (190 mM) while avoiding the extremes of minimal (75 mM) or excessive (250 mM) damage. This concentration induces moderate to severe damage across key growth parameters, providing an efficient means of screening for salt-tolerant genotypes. The SII is particularly valuable because it combines multiple physiological traits, providing a more comprehensive assessment of salt stress than any single trait alone. By identifying the concentration at which the SII reaches 0.5, researchers can pinpoint the level of salinity that leads to significant growth impairment, thereby optimizing salt tolerance screening. This approach is critical for the selection of genotypes that can perform well under saline conditions, particularly in regions where soil salinity poses a major constraint to crop production (Long et al., 2013; Wu et al., 2019; Agrawal et al., 2024). Earlier, a study was conducted on the effect of soil salinity in brinjal (Talwar, et al., 2023).

Conclusion

Salinity stress severely affects cowpea's physiological and morphological traits. This study showed that increasing NaCl concentrations severely impair cowpea germination and seedling growth, with salt-induced osmotic stress and physiological disruption reducing germination percentage, shoot and root lengths, seedling length, dry weight, and vigour indices. The study identified 183.57 mM NaCl as a critical threshold where notable growth inhibition begins, while 200 mM NaCl was determined as the optimal concentration for *in-vitro* screening salt tolerance using the salt injury index (SII). At this level, key growth parameters were reduced by 50%, providing a reliable benchmark for selecting salt-tolerant genotypes. These findings offer valuable insights into the screening of cowpea genotypes for salinity stress tolerance. They can be effectively utilized in breeding programs aimed at developing salt-resilient cowpea varieties for saline-prone regions.

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सारांश

लवणीय तनाव एक प्रमुख अजैविक दबाव है, जो पौधों की वृद्धि और फसल की उपज को वैश्विक स्तर पर प्रभावित करता है, विशेष रूप से उन क्षेत्रों में जहां मृदा में अधिक लवणीयता होती है। इस अध्ययन में 30 लोबिया (*Vigna unguiculata* L. Walp.) किस्मों पर सोडियम क्लोराइड प्रेरित लवणीय तनाव के प्रभावों का मूल्यांकन किया गया और लवणीयता सांद्रता स्तर की जांच के लिए लवणीय क्षति सूचकांक (SII) विधि का उपयोग किया गया। बीजों को छह विभिन्न सोडियम क्लोराइड सांद्रताओं (0, 75, 100, 150, 200, और 250 मिमी/ली) में पेट्री प्लेटों में रखा गया। अंकुरण प्रतिशत, प्ररोह लंबाई, जड़ लंबाई, कुल अंकुर लंबाई, सूखा वजन, और विगर सूचकांकों जैसे वृद्धि मापदंडों का मूल्यांकन किया गया। जैसे-जैसे लवणीयता बढ़ी, वैसे-वैसे सभी मापदंडों में गिरावट आई। लवणीय क्षति सूचकांक के रैखिक विश्लेषण से यह पाया गया कि सोडियम क्लोराइड की सांद्रता ने विभिन्न गुणों में 50% कमी की: अंकुरण के लिए 215 मिमी/ली, प्ररोह लंबाई के लिए 180 मिमी/ली, जड़ लंबाई के लिए 210 मिमी/ली, कुल अंकुर लंबाई और सूखा वजन के लिए 200 मिमी/ली, तथा विगर सूचकांकों के लिए क्रमशः 130 मिमी/ली और 160 मिमी/ली। औसतन, 183.57 मिमी/ली का सांद्रता स्तर पाया गया। इन परिणामों के आधार पर, 200 मिमी/ली सोडियम क्लोराइड को लोबिया जीनोटाइप्स में लवणीयता सहनशीलता की जांच के लिए आदर्श सांद्रता के रूप में निर्धारित किया गया। लवणीय क्षति सूचकांक विधि, जो वृद्धि मापदंडों में कमी पर आधारित है, लवणीयता सहनशीलता का प्रभावी मूल्यांकन करने में सक्षम है, जिससे इन विट्रो में लवणीयता सहनशील जीनोटाइप्स की स्क्रीनिंग अधिक लागत-कुशल बन सकी है।