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ASSESSMENT OF GERMINATION AND SEEDLING DEVELOPMENT FACTORS OF SOYBEAN CULTIVARS IN DIFFERENT SALINITY LEVELS

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Abstract: Salinity poses a significant abiotic stress factor that exerts detrimental effects on plant growth during germination and early seedling stages. The global prevalence of high salt concentration has transformed salinity into a serious problem, impacting vast expanses of land worldwide. This experiment aims to examine the effects of various concentrations of sodium chloride (NaCl), including 200 mM, 150 mM, 100 mM, and 50 mM, on the seed development at early stage and germination of different cultivars of soybean to determine the variety with the highest value of tolerance, while exploring the underlying mechanisms responsible for salt tolerance in these plants. The parameters considered for measurement included relative injury rate, mean germination time, germination percentage, water uptake percentage, seedling height reduction, seedling biomass, and salt tolerance. Among these parameters, seedling height was highly affected with up to 72.58% reduction in 200 mM, followed by fresh weight and water uptake percentage. The parameters with minimum changes from 0 mM to 200 mM were mean germination time and relative injury rate. By assessing these parameters, a comprehensive understanding of the effects of salinity on soybean genotypes can be obtained. In conclusion, the study suggests that seedling traits are a reliable way to identify genotypes with increased tolerance to salinity stress by farmers according to the salinity situation in their soils.

Keywords: NaCl, Soybean, Germination, Salinity, Salt stress

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1. Introduction

Soybean (*Glycine max*) is one of the most economically significant crops worldwide, contributing extensively to food and oil production. It is widely cultivated for edible vegetable oil production and serves as a high-protein feed for livestock. Its chemical composition includes approximately 40-50% protein, 20-30% oil, and 26-30% carbohydrates (Linh et al., 2021). The majority of cultivated soybeans are used as animal feed, particularly in the USA. Soybean oil is used as raw material for biodiesel production and finds applications in industries such as bakery, food, textile, medicine, and chemicals (Hill et al., 2006).

Soybean cultivation faces numerous challenges, including abiotic stresses such as salinity, which can significantly impact germination, seedling establishment, and overall plant growth (Han and Lee 2005; El Sabagh et al., 2015). Salinity, resulting from high levels of soluble salts in soil or irrigation water, poses a major threat to the agricultural productivity of irrigated land worldwide (Zhang et al., 2017). Understanding the responses of soybean cultivars to varying salinity levels is imperative for devising strategies to mitigate its adverse effects and sustain crop yields (Kumar 2017). The ability of plants to tolerate salinity is a dynamic characteristic that undergoes constant changes and demonstrates variations within the same species. This variability is primarily influenced by the stage of growth that the plants are in (Hosseini et al., 2002). It has been observed that non-halophytes, such as white clover, wheat, and rice, tend to be more susceptible to salinity stress during the germination and the seedling stage (Hampson and Simpson, 1990; Rogers et al., 1995; Hosseini et al., 2002). However, it is essential to acknowledge that the sensitivity observed during the early seedling growth stage may not necessarily mirror the subsequent sensitivity of the mature plant to salinity. Remarkably, several species of plants demonstrate an enhanced ability to tolerate higher levels of salinity as they reach maturity, surpassing their sensitivity during the early growth of seedlings. This phenomenon highlights the dynamic nature of salt tolerance and underscores the importance of considering the growth stage when assessing a plant's ability to withstand salinity stress (Dehnavi et al., 2020).

The prevailing belief suggests that the detrimental effects of NaCl on plant tissues primarily arise from the absorption and accumulation of Na^+ ions, rather than Cl^- (Hosseini et al., 2002). Many studies were carried out on

common beans, soybeans, castor beans, corn, and wheat have provided evidence indicating the absence of a notable correlation between salt sensitivity and the concentration of Cl^- ions in plant tissues (Jeschke and Wolf, 1988a and 1988b; Kinraide, 1999; Hosseini et al., 2002). However, it is important to recognize that, in addition to Na^+, other cation concentrations present in plant tissues may also exert a noteworthy influence under saline conditions. Several authors have presented compelling evidence suggesting that the presence of Ca^{2+} and K^+ ions can provide a protective effect against the toxic impacts of Na^+ ions (Huang and Rozelle, 1995; Volkmar et al., 1998; Tester and Davenport 2003; Lindberg and Premkumar 2023). These findings underscore the complexity of ion interactions within plant tissues and emphasize the role of multiple cations in mitigating the harmful effects of Na^+ accumulation in saline environments.

The tolerance of plants to salinity is a trait that displays variability within the same species across different growth stages. Specifically, non-halophytic plants like white clover (Rogers et al., 1995), wheat (Hampson and Simpson, 1990), and rice (Pearson et al., 1966) exhibit higher sensitivity during the seedling stage compared to the germination stage. It is important to emphasize that the sensitivity observed during early seedling growth does not necessarily reflect the subsequent sensitivity of the mature plant to salinity. Interestingly, many plant species demonstrate a substantial increase in salt tolerance as they reach maturity, surpassing their sensitivity during the early seedling growth phase (Allen et al., 1986; Rogers and Noble, 1991).

The impact of salinity on plant growth can be attributed to two distinct mechanisms: osmotic effects and toxic effects, as highlighted in previous studies (Redmann, 1974; Hampson and Simpson, 1990). It is important to note that osmotic and toxic effects are not mutually exclusive, as evidenced by varying responses observed when comparing isosmotic solutions of NaCl and polyethylene glycol (PEG) during germination and growth. These differences indicate that the plant's response to salinity cannot be solely attributed to osmotic effects alone. For example, in PEG solutions, soybean germination is hindered at higher osmotic potentials but can recover when the seeds are transferred to deionized water. Conversely, in high concentrations of NaCl, germination inhibition seems to be a consequence of toxic effects, as recovery is not observed when the seeds are transferred to water (Hosseini et al, 2000). This highlights the complex interplay between osmotic and toxic effects in the context of salinity's impact on plant germination and growth.

2. Materials and Method

2.1. Plant Material and Seed Sterilization

In this experiment, Turbo, Lider, Agroyal, Arısoy, A3966 and Antsoy in total six soybean cultivars were used as plant material. The study was conducted at the Department of Field Crops, Faculty of Agriculture, Akdeniz University, Antalya, and Türkiye. Petri dishes, Whatman filter paper, and chemicals including NaOCl and NaCl used in this experiment were available in the molecular lab of the same department.

The seed sterilization procedure was carried out by Hassan's described method (Hassan et al., 2009). A total of 75 soybean seeds from each genotype, carefully chosen for their uniform size, were subjected to the sterilization process. Initially, they were thoroughly rinsed twice with a 2% solution of NaOCl to ensure proper disinfection. Subsequently, seeds were washed with deionized water for two minutes, effectively eliminating any residual chemicals from the seed surface.

2.2. Salt Solution Preparation and Design of the Experiment

To prepare the solution, the desired amount of distilled water was filled in four different flasks. The calculated weight of NaCl salt was measured with precise balance for each flask accordingly to prepare 50mM, 100mM, 150mM, and 200mM solution respectively (Table 1). Salt was added to distilled water contained in each flask and mixed on an electric stirrer for 15 minutes. The distilled water was used for control treatment.

Table 1. Preparation of salt solutions

Treatments	Grams of NaCl	Distilled Water
50 mM	0.584g	200 ml
100 mM	1.1688g	200 ml
150 mM	1.7532g	200 ml
200 mM	2.3376g	200 ml

The study was conducted using a completely randomized design (CRD) with five treatments, including the control treatment, and three replicates per treatment. Petri dishes with 9cm diameter were sterilized in the autoclave to prevent any possible fungal growth inside the treated petri dish. Two layers of Whatman filter paper were placed in each petri and 10 ml of the solution was added to filter paper layers in Petri dishes with three replications of each treatment and 5 ml same concentrate solution was 5 days later. The treatments, T0, T1, T2, T3 and T4 correspond to: 0; 50 mM, 100 mM, 150 mM and 200 mM NaCl respectively. Five seeds were placed in each petri dish with uniform size at a uniform distance. The seeds were then placed in a plant growth room with

a temperature range of 25 to 27.1ºC to trigger germination. To prevent any loss of water from the experiment with evaporation, petri dishes were wrapped with parafilm and placed in the dark.

2.3. Assessment of Germination and Seedling Growth

During each cycle of the experiment, daily counts were made of germinated seeds over 10 days. Germination was defined as root protrusion beyond the pericarp by at least 2 mm. At the end of the experiment, the soybean seedlings were preserved to record the data measurements including fresh weight, dry weight, and seedling length. These parameters allowed for an assessment of seedling growth and development, providing insights into their physiological, phenotypical, and biomass characteristics under experimental conditions.

2.3.1. Water uptake

After evaluating the seedling fresh weight and the seedling dry weight, the water uptake was determined using the given formula (equation 1).

Water Uptake %age = [(Fresh weight – dry weight)/Fresh weight] × 100 (1)

2.3.2. Germination time

The number of germinated seeds was counted daily from each replication and determined the mean value. The overall mean germination time was calculated by using the formula given below (equation 2).

Mean time of germination = $\sum Dn / \sum n$ (2)

Here, 'n' represents the count of seeds that have germinated on day 'D,' where 'D' denotes the number of days since the germination process started.

2.3.3. Germination percentage

Germination data was collected for 10 days daily and determined the germination time after 10 days. The given formula was used to determine the germination time (equation 3).

Germination Percentage = (Germinated seed count / total sown seed count) × 100 (3)

2.3.4. Relative injury

The calculation of the relative injury rate followed the formula established by Tsegay and Gebreslassie (2014). The calculation included subtracting the percentage of germination in salt-treated seeds from the percentage of germination in the control, followed by dividing the resulting value by the percentage of germination in the control (equation 4).

Rate of Relative Injury = [%age of germination in control – %age of germination in salt treatments] / %age of germination in control (4)

2.3.5. Reduction in seedling height

The reduction of the germinated plant height is obtained by subtracting the percentage of shoot length under salt stress conditions from the percentage of shoot length under normal conditions, relative to the percentage of shoot length under normal conditions. This reduction is calculated using the following equation 5.

Shoot height reduction = [(Controlled shoot height – salt-treated shoot height) / Controlled shoot height] x 100 (5)

2.3.6. Biomass of seedling

To ensure standardization of mass, the biomass of the seedlings was determined by weighing them using an analytical balance. Before weighing, the seedlings were placed in an oven at 55ºC temperature for 48 hours.

2.3.7. Salinity tolerance rate

The salt tolerance rate was carried out with the help of the prescribed standard formula provided hereafter (equation 6).

Salinity Tolerance Rate = (Salt treated seedling dry weight / Control seedling weight) x 100 (6)

3. Results and Discussion

The effect of different concentrations of NaCl on germination and early growth of soybean seedlings was analyzed in this study. The results revealed that there was no significant difference in germination percentage between seeds treated with 0 mM (control) and those treated with 50 mM NaCl. However, the germination percentage notably declined with increasing salinity levels. Under salt stress, the germination percentages decreased in the “Turbo” variety from 100% in the control to 86.67% at 200 mM NaCl. It decreased from 90% in the “Lider” variety at 0 mM, to 73.33%. In the “Agroyal” variety, from 86.67% (control) to 66.67% (similar to A3966). This percentage decreased from 93.33% in the controlled “AntSoy” to 70% and from 100% to 66.67% in “Arisoy”, with significant differences observed between all varieties (Table 2). The “Turbo” variety exhibited the highest cumulative germination percentage of 94.67%, while the A3966 genotype showed the lowest cumulative germination percentage of 77.33% (Table 3). Cumulative analysis across all varieties indicated a significant reduction in germination percentage from 92.78% (0 mM) to 71.67% (200 mM) for all treatments (Table 4).

In some previous studies, it has been stated that although salt stresses up to 50 ppm do not negatively affect seed germination, or plant growth, increasing levels of salinity stress have negative effects on these parameters (Shanko et al., 2017; Açıkbaş et al., 2023). In this respect, the results of this study are like those previously reported. These results suggest that the germination stage in the soybean is moderately salt-tolerant. However, high levels of salinity can still inhibit seed germination. This is likely due to the toxic effects of Na^+ and Cl^- ions, which can disrupt the water balance and metabolism of the seeds.

Table 2. Variance analysis of Soybean varieties under applied salinity levels

G	T	GP	SH (cm)	HR (%)	RIR (%)	MGD	FW (g)	B (g)	WUP (%)	ST (%)
Turbo	T0	100.000±0.000a	3.710±0.031a	100.000±0.000a	0.000±0.000b	2.550±0.040d	0.608±0.004a	0.210±0.002a	65.397±0.006a	100.000±0.000a
	T1	100.000±0.000a	3.510±0.059a	94.600±0.010a	0.000±0.000b	2.673±0.043d	0.568±0.001b	0.209±0.001a	63.285±0.002ab	99.212±0.004ab
	T2	93.333±0.033ab	2.500±0.012b	67.396±007b	6.667±0.033ab	3.030±0.035c	0.527±0.001c	0.205±0.002ab	61.261±0.003bc	97.632±0.012abc
	T3	93.333±0.033ab	1.533±0.133c	41.297±0.034c	6.667±0.033ab	3.273±0.014c	0.501±0.011d	0.200±0.001bc	59.842±0.009cd	95.110±0.013bc
	T4	86.667±0.033b	1.250±0.029c	33.695±0.008c	13.363±0.033a	3.463±0.019a	0.467±0.001e	0.197±0.001c	57.917±0.001d	93.520±0.011c
		0.0220*	<.0001*	<.0001*	0.0220*	<.0001*	<.0001*	0.0005*	<.0001*	0.0029*
Lider	T0	90.000±0.000a	3.800±0.023a	100.000±0.000a	0.000±0.000b	2.283±0.009d	0.733±0.002a	0.223±0.001a	69.637±0.001a	100.000±0.000a
	T1	90.000±0.000a	3.640±0.031a	95.806±0.014a	0.000±0.000b	2.410±0.010d	0.658±0.004b	0.218±0.001ab	66.714±0.003b	98.355±0.004ab
	T2	83.333±0.033ab	2.500±0.031b	65.787±0.006b	7.407±0.037ab	2.620±0.025c	0.548±0.002c	0.218±0.001ab	60.450±0.001c	97.461±0.005ab
	T3	76.667±0.033b	1.800±0.115c	47.335±0.028c	14.813±0.037a	2.783±0.060b	0.513±0.001d	0.210±0.004b	60.312±0.003cd	94.323±0.018bc
	T4	73.333±0.033b	1.233±0.120d	32.450±0.031d	18.517±0.037a	2.990±0.006a	0.484±0.001e	0.193±0.002c	58.799±0.006d	86.672±0.005
		0.0027*	<.0001*	<.0001*	0.0027*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
A	T0	86.667±0.067a	3.567±0.067a	100.000±0.000a	0.000±0.000b	2.730±0.091c	0.373±0.001a	0.112±0.007a	0.717±0.011a	100.000±0.000a
	T1	80.000±0.058a	3.000±0.115b	84.000±0.036b	7.500±0.038ab	3.027±0.054b	0.327±0.001b	0.095±0.003ab	0.700±0.006ab	85.843±0.062ab
	T2	80.000±0.058a	2.567±0.067c	72.046±0.028bc	7.500±0.038ab	3.130±0.015b	0.287±0.002c	0.087±0.002bc	0.688±0.011ab	78.132±0.039b
	T3	76.667±0.033a	2.300±0.115c	64.633±0.043c	10.833±0.058ab	3.150±0.029	0.255±0.001d	0.086±0.002bc	0.662±0.005bc	77.547±0.038b
	T4	66.667±0.033a	1.200±0.058d	33.719±0.022d	22.500±0.052a	3.400±0.021a	0.207±0.002e	0.077±0.002c	0.626±0.007c	69.533±0.041a
		0.1705	<.0001*	<.0001*	0.0418*	<.0001*	<.0001*	0.0007*	0.0002*	0.0040*
A3966	T0	86.667±0.033a	2.800±0.058a	100.000±0.000a	0.000±0.000b	3.167±0.089b	0.417±0.002a	0.107±0.001a	74.260±0.001a	100.000±0.000a
	T1	83.333±0.033a	2.100±0.100b	75.193±0.051b	3.703±0.037b	3.217±0.044b	0.310±0.001b	0.105±0.001ab	67.271±0.002b	97.834±0.013ab
	T2	76.667±0.033ab	1.367±0.120c	48.726±0.036c	11.573±0.005ab	3.553±0.024a	0.291±0.005c	0.101±0.002bc	66.708±0.002b	94.413±0.014bc
	T3	73.333±0.033ab	1.133±0.067cd	40.424±0.018cd	15.276±0.035ab	3.723±0.023a	0.282±0.001cd	0.099±0.000c	65.169±0.003c	91.615±0.000c
	T4	66.667±0.033ab	0.900±0.058d	32.129±0.018d	22.683±0.061a	3.770±0.025a	0.275±0.002d	0.090±0.001d	64.565±0.005c	83.850±0.003d
		0.0118*	<.0001*	<.0001*	0.0073*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
AntSoy	T0	93.333±0.067a	3.400±0.058a	100.000±0.000a	0.000±0.000b	2.623±0.039d	0.355±0.002a	0.103±0.001a	70.982±0.004a	100.000±0.000a
	T1	86.667±0.033ab	2.500±0.115b	73.631±0.042b	6.667±0.033ab	2.767±0.089d	0.301±0.001b	0.097±0.001b	68.488±0.002ab	94.202±0.014ab
	T2	76.667±0.033ab	1.700±0.100c	50.116±0.038c	17.500±0.025ab	3.150±0.029c	0.275±0.002c	0.090±0.002c	68.134±0.005b	87.084±0.022bc
	T3	73.333±0.033b	1.500±0.115cd	44.203±0.039cd	20.833±0.051a	3.400±0.021b	0.27±0.002c	0.085±0.001c	67.186±0.005b	82.219±0.013c
	T4	70.000±0.000b	1.133±0.033d	33.382±0.015d	24.167±0.058a	3.643±0.043a	0.178±0.001d	0.075±0.002d	57.859±0.010c	72.858±0.022d
		0.0096*	<.0001*	<.0001*	0.0069*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Arisoy	T0	100.000±0.000a	3.800±0.058a	100.000±0.000a	0.000±0.000b	2.730±0.091d	0.402±0.002a	0.133±0.002a	69.747±0.002a	100.000±0.000a
	T1	93.333±0.033ab	3.300±0.115b	86.975±0.044b	6.667±0.033cd	3.017±0.044cd	0.36±0.001b	0.122±0.002b	66.972±0.005b	91.741±0.011b
	T2	83.333±0.033bc	2.367±0.033c	62.332±0.018c	16.667±0.033bc	3.217±0.044bc	0.357±0.001b	0.108±0.002c	66.469±0.002b	81.226±0.011c
	T3	73.333±0.033cd	1.600±0.058d	42.102±0.013d	26.667±0.033ab	3.440±0.029ab	0.285±0.001c	0.096±0.001d	65.879±0.004b	72.194±0.009d
	T4	66.667±0.033d	0.900±0.058e	23.719±0.018e	33.333±0.033a	3.630±0.085a	0.215±0.003d	0.088±0.001e	59.106±0.010c	66.194±0.014e
		<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*

A= agroyal, G= genotype, T= treatment, GP= germination percentage, SH= seedling height, HR= height reduction, RIR= relative injury rate, MGD= mean germination days, FW= fresh weight, B= biomass, WUP= water uptake percentage, ST= salt tolerance.

Table 3. Pair-wise multiple comparisons of means of all of the 6 Soybean varieties for studied traits

	Turbo	Lider	Agroyal	A3966	AntSoy	Arisoy
GP (%)	94.667±0.020	82.667±0.020	78.000±0.050	77.333±0.033	80.000±0.033	83.000±0.027
SH (cm)	2.501±0.052	2.595±0.064	2.527±0.084	1.667±0.080	2.047±0.084	2.393±0.064
HR (%)	67.391±0.012	68.280±0.016	70.910±0.026	59.287±0.024	60.270±0.027	63.030±0.018
RIR (%)	5.333±0.019	8.15±0.022	9.667±0.037	10.646±0.027	13.828±0.033	16.667±0.027
MGD	3.00±0.030	2.620±0.022	3.086±0.042	3.488±0.041	3.120±0.044	3.213±0.058
FW (g)	0.534±0.004	0.587±0.002	0.290±0.001	0.315±0.002	0.276±0.002	0.324±0.002
B (g)	0.204±0.002	0.212±0.002	0.092±0.003	0.100±0.001	0.090±0.001	0.109±0.001
WUP	61.540±0.004	63.182±0.003	67.866±0.008	67.587±0.003	66.530±0.005	65.629±0.004
ST	97.091±0.008	95.364±0.006	82.21±0.036	93.535±0.006	87.266±0.014	82.297±0.009

GP= germination percentage, HR= height reduction, RIR= relative injury rate, MGD= mean germination days, FW= fresh weight, B= biomass, WUP= water uptake percentage, ST= salt tolerance.

Table 4. Pair-wise multiple comparison of means of all the 5 treatments for studied traits

T	GP	SH (cm)	HR (%)	RIR (%)	MGD	FW (g)	B (g)	WUP (%)	ST (%)
0mM	92.778±0 .019a	3.513±0. 086a	100.000±0 .000a	0.000±0.0 00a	2.681±0.0 68a	0.481±0.0 34a	0.148±0. 012a	70.294±0. 007a	100.000±0 .000a
50mM	88.889±0 .020ab	3.008±0. 137b	85.062±0. 024b	4.089±0.0 12b	2.852±0.0 67ab	0.421±0.0 034ab	0.141±0. 013a	67.124±0. 005b	94.531±0. 015ab
100mM	82.222±0 .019bc	2.167±0. 115c	61.067±0. 023	11.219±0. 015a	3.117±0,0 68bc	0.381±0.0 28ab	0.135±0. 013a	65.306±0. 008bc	89.325±0. 020bc
150mM	77.778±0 .021cd	1.644±0. 093d	46.667±0. 023	15.849±0. 021b	3.295±0.0 71cd	0.351±0.0 27b	0.129±0. 013a	64.0870.0 07c	85.501±0. 022cd
200mM	71.667±0 .020d	1.103±0. 042	31.516±0. 011	22.423±0. 022b	3.483±0.0 63d	0.304±0.0 30b	0.120±0. 013a	60.138±0. 007d	78.771±0. 025d
Sig.	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	0.0015*	0.5862	<.0001*	<.0001*

T= treatment, GP= germination percentage, SH= seedling height, HR= height reduction, RIR= relative injury rate, MGD= mean germination days, FW= fresh weight, B= biomass, WUP= water uptake percentage, ST= salt tolerance.

The seed germination process was meticulously monitored at regular intervals of 12 hours, providing insights into the germination initiation time, which was observed at the 36-hour mark after seed placement. Statistical analysis of observed data shows variation in mean germination time. A gradual increase in mean germination time was recorded with the increase in salinity level. We found that using a 200 mM solution made soybean seeds take longer to sprout. In the Turbo variety, it went from 61.2 hours to 83.1 hours. For Lider, it increased from 54.8 hours to 71.8 hours.

Agroyal showed the same effect, going from 65.5 hours to 81.6 hours. A3966 seeds took more time to germinate as well, moving from 76.0 hours to 89.4 hours. The AntSoy seeds went from 62.95 hours to 87.43 hours. Arisoy seeds also took longer, going from 65.52 hours to 87.12 hours when we used the 200 mM solution (Table 2). Variations among the varieties have been recorded in cumulative analysis, but there is no significance level between used genotypes. Lider showed the shortest mean germination time with 62.88 hours and A3966 showed the highest value of mean germination time with 83.71 hours (Table 3). In the treatment-wise cumulative statistical analysis, there was no significant difference between the control group and the group treated with 50 mM salt solution, which showed a moderate salt tolerance at the seed germination stage of soybean. However, there was a significant change with higher doses of salinity (Table 4). These findings reveal the varying responses of soybean genotypes to salinity stress during germination, offering valuable insights for future studies on stress resilience and crop improvement strategies. Previous soybean studies showed that salinity stress has been consistently documented to negatively impact various parameters, including seed germination, plant height, shoot dry weight (Essa, 2002), seedling fresh weight (Farhoudi and Tafti, 2011), germination percentage (Neves et al., 2005; Ahmadvand et al., 2012; Ndifon, 2013), as well as plant height and root length (Ahmed et al., 2023). Notably, higher salt concentrations have been demonstrated to reduce shoot dry weight significantly (Le et al., 2021).

The analysis demonstrates that the percentage of water uptake of soybean decreased inversely with increasing NaCl salt concentration. The water uptake percentage reduced significantly ($p<0.05$) at 200 mM NaCl, but there was no significant difference between the water uptake percentage of seeds treated with 50 mM NaCl solution and the control group. For all soybean varieties studied, statistical analysis shows a reduction in water absorption when treated with 200 mM saline compared to the control. The reduction ranging from 65.39 to 57.92% for the "Turbo" variety, from 69.64 to 58.80% for "Lider", from 71.71 to 62.67% for "Agroyal", from 74, 26 to 64.56% for “A3966”, from 70.98 to 57.86% for “AntSoy” and from 69.75 to 59.11% for “Arisoy” (Table 2).

Cumulative statistical analysis showed that there is slight difference without significance level between different used genotypes. The highest cumulative water absorption 67.87% recorded in “Agroyal”, and the lowest water absorption 61.54% has been recorded in the “Turbo” variety (Table 3). Cumulative treatment analysis showed a reduction in water uptake percentage from 70.29% at 0 mM to 60.14% at 200 mM salinity level (Table 4).

It's noteworthy to acknowledge that seed health and size play crucial roles in influencing the water absorption percentage during the germination and early seedling development stage. These findings emphasize the intricate dynamics of salt-induced water uptake reduction in soybeans, explain the potential impacts of seed characteristics on water absorption capacity, and offer valuable insights for future research in stress resilience and crop improvement strategies.

The decrease in water uptake percentage with increasing salinity is likely due to the dehydration of the cell cytoplasm caused by the high concentration of salt in the soil. Water absorption by the seed is also affected by the seed coat nature, which may become more impermeable to water as the salinity level increases. Higher salinity levels cause a reduction in water absorption during cell division and cell differentiation, which eventually leads to increased osmotic pressure. The high osmotic pressure of the soil solution inhibits water uptake in the seedlings, which in turn reduces the water uptake percentage of the seedlings.

Seedling height reduction is a common observation in crop plants grown in saline environments. This is due to the toxic effects of high salt concentrations, which can cause plant cells to dehydrate and shrink. In this study, we investigated the effects of different NaCl concentrations on seedling height reduction in different soybean varieties. We found that seedling height reduction increased significantly with increasing salinity, but the level of height reduction was different in every variety.

The statistical analysis showed a reduction of 66.31% in the height of the seedlings obtained at 200 mM saline solution compared to the control (0 mM) in the “Turbo” variety, a reduction of 67.55% in the "Lider", a reduction of 66.28% in "Agroyal", a reduction of 67.87% in the "A3966", a reduction of 66.62% of the mutant variety "Arisoy", and a maximum reduction of 76 .28% was recorded in the “Antsoy” variety (Table 2).

The cumulative height of all treatments recorded 67.39% in Turbo variety compared to the control group, 68.28% in Lider, 70.91% in Agroyal, 59.29% in A3966, 60.27% in AntSoy, and 63.03% has been recorded in the Arisoy (Table 3). Among these varieties, Agroyal showed the highest cumulative height and A3966 showed the lowest cumulative seedling height. Treatment wise cumulative analysis of all varieties showed that seedling height reduced from 100 percent at control to 31.52 percent at 200 mM salinity level (Table 4).

These findings shed light on the varied responses of soybean varieties to salinity stress, providing crucial data for advancing our understanding of salt-induced growth inhibition. The differential impact of salinity on seedling height among various genotypes warrants further investigation and offers valuable insights for devising strategies to enhance stress resilience in soybean cultivation. These results suggest that soybean is moderately salt-tolerant. However, high levels of salinity can still significantly reduce seedling height. This is likely due to the decrease in cell division and the cell elongation that occurs under salt stress. The reduction in seedling height can have several negative consequences for plant growth. For example, it can lead to slower leaf appearance and leaf size, which can reduce photosynthesis and overall plant productivity.

The greatest seedling biomass was achieved with seeds treated under 0 mM salinity in the control group, whereas the lowest values were observed with seeds subjected to 200 mM salinity. A reduction of 6.19% recorded in Turbo, 13.45% in Lider, 31.25% reduction in Agroyal, 15.89% reduction in A3966, 27.18% reduction in Antsoy, and 27.18% reduction in Arisoy variety from 0 mM to 200 mM salinity solution with a significant difference (Table 2). These results showed the highest reduction in the biomass occurred in the Agroyal variety while the lowest biomass reduction was recorded in the Turbo variety (Table 3). Treatment-wise collective results of all the varieties didn’t show a significant difference. Cumulative analysis showed that Lider variety had the highest biomass value (0.212g) and AntSoy had the lowest value (0.090g). No significant difference (p value 0.5862) was observed in seedling biomass among the different salt treatments while analyzing all the varieties cumulatively (Table 4).

Variations in the fresh weight of all varieties with changes in salinity level were more significant than the significance level in the biomass. The highest fresh weight value was recorded in the controls of all the varieties while the lowest fresh weight value was recorded in the seedlings treated with 200 mM salinity level. From the control group (0 mM) to the highest salinity level (200 mM) a reduction of 23.19% in “Turbo”; 33.97% in “Lider”; 44.50% in “Agroyal”; 34.05% in “A3966”; 49.86% in “AntSoy”; and 46.52% in “Arisoy” (Table 2).

Cumulatively, “Lider” showed the highest fresh weight value while the mutant “Arisoy” showed the lowest one (Table 3). Treatment wise the fresh weight of all genotypes cumulatively reduced from 100 percent at 0 mM treatment to 36.80%, at treatment applied with 200 mM solution (Table 4).

The biggest reason behind the relative injury is ion toxicity and osmotic stress caused by salinity. Relative injury rate increased with the increase in salinity level but with very low significance compared to other parameters. There was no relative injury in 0 mM and seedlings treated with 50 mM salinity solution in the

Turbo and Lider varieties. Any variety did not show relative injury in the seedlings of the control group (0 mM) seedlings.

The relative damage increased significantly, recording a maximum of 13.36% in the "Turbo" variety, subjected to 200 mM salinity; 18.52% in "Lider"; 22.50% in "Agroyal"; 22.68% in "A3966"; 24.17% in "AntSoy" and 33.33% in "Arisoy" (Table 2).

Cumulative treatment analysis of all varieties showed a 22.42% increase in relative damage rate under salinity conditions (200mM), compared to 0% in controls.

The maximum cumulative relative injury rate was recorded in "Arisoy" and the minimum was recorded in the "Turbo" variety (Table 3). Treatment-wise cumulative analysis of all varieties showed an increase of 22.42% relative injury rate at 200 mM solution from 0 percent at 0 mM saline solution (Table 4).

The precise mechanisms underlying the impact of salt on germination remain incompletely understood. Excessive salinity negatively impacts plant growth in several ways. The higher salinity creates an osmotic imbalance, making it harder for plants to take up water. It leads to osmotic stress, restricting water uptake and cell expansion. A severe imbalance of ions including K^+, Na^+, K^+/Na^+, Ca^{2+}, and Cl^- causes several physiological changes in the cell (Tunçtürk et al, 2011). Sodium and chloride ions accumulate in plant tissues, disrupting physiological processes and nutrient uptake. Nonetheless, it has been proposed that osmotic (Kingsbury and Epstein, 1986; Kumar and Sharma, 1990) and/or toxic effects may contribute to salt-induced injury (Essa, 2002). Furthermore, soluble salts can induce osmotic stress, leading to specific ion toxicity and ionic imbalances, potentially culminating in plant mortality (Munns, 2002).

Salt tolerance is often investigated by applying the NaCl solution to the medium of plant growth to create salinity. In this study, the levels of tolerance of soybean seedlings to salt stress were assessed using various salt solutions. The results demonstrated that soybean seeds exhibited tolerance across all salinity levels, with an inverse linear relationship between salt tolerance and increasing salt concentrations. Statistical analysis showed a significant difference between the treatments among all varieties. These results show that the minimum drop in tolerance level 6.5% was recorded in the "Turbo" variety, and the maximum drop in the tolerance level 33.81% was recorded in "Arisoy" (Table 2). Cumulatively Turbo showed the highest level of salt tolerance with 97.09%, and "Agroyal" showed the lowest value (82.21%) of relative tolerance (Table 3). Treatment-wise cumulative analysis showed a significant reduction from a 100% tolerance level in the control group to 78.77% in the 200 mM saline group (Table 4).

The current study reveals that soybean's salt tolerance decreases with higher salinity levels, but also confirms its capability to germinate under saline conditions. However, it is important to note that salt tolerance screening during the germination period may have limited implications for assessing crop salt tolerance since many germination studies are conducted in laboratory settings using Petri dishes with varying salinity solutions. It has been observed that in some plants, as the salinity rate increases, the plants' sensitivity to salt increases during plants germination stages.

4. Conclusion

The osmotic pressure resulting from salt stress adversely affects soybean's germination time and early growth of seedlings. Elevated levels of salinity result in a decrease in the percentage of water absorption, seed vitality, and an extension of the average germination time. Throughout this experiment, soybean seeds developed tolerance under lower salinity levels and experienced germination delays under high salinity. Germination percentage and index decreased with increasing salinity. Furthermore, variations in response to elevated salinity were evident in both seedling biomass and the relative injury rate. Increased NaCl concentrations had a noticeable impact on seedling height, contributing to a significant enhancement in salt tolerance for soybeans as salinity levels rose. Nevertheless, the early seedling growth phase revealed only modest salt tolerance. Interestingly, it was observed that increasing salinity levels did not yield significant changes in biomass.

It is crucial to raise awareness among people about the importance of cultivating tolerant varieties of soybean according to their soil salinity conditions. The selection of soybean varieties should be based on the specific environmental conditions of the area. It is essential to adopt recommended soybean varieties known for their tolerance levels and standard traits, while pairwise interaction results in a final yield.

This study provides valuable insights into the germination and seedling development factors of soybean varieties under different salinity levels. The observed variations in tolerance among varieties underscore the importance of genetic diversity in breeding programs aimed at developing salt-tolerant soybean varieties

Among the tested varieties, "Turbo" exhibited the strongest salt tolerance with a higher performance in all tested parameters comparing the others. However, although a high variation was observed in the tested parameters in terms of response to different salt concentrations, the variety "Arısoy" performed a higher susceptibility towards the highest salt concentration of 200 mM. When the average of all genotypes was evaluated, it was observed that while germination was 100% in the control groups, it decreased to 77% in the highest salt concentration solution.

Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	R.U.	M.A.
C	50	50
D	50	50
S	100	0
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	100	0
PM	100	0
FA	100	0

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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