



# **Effect of NaCl Stress on Seedling Parameters of Various Chilli (*Capsicum annuum* L.) Genotypes and Its Mitigation through Hormonal Priming**

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## **Authors' contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## **ABSTRACT**

Salinity is a major environmental challenge that leads to agricultural land degradation and reduced crop yields worldwide. In chilli (*Capsicum annuum* L.), high salinity conditions can significantly inhibit seed germination and seedling growth. This study aimed to achieve two main objectives: first,

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to evaluate the effects of NaCl stress on seedling parameters across various chilli genotypes, and second, to explore the potential of hormonal priming in mitigating salt stress. The experiment was conducted in the Seed Testing Laboratory of the Department of Genetics and Plant Breeding at SHUATS, Prayagraj, using 14 chilli genotypes exposed to varying salt concentrations (0 mM, 75 mM, 100 mM, and 150 mM). The results revealed that higher salt concentrations negatively impacted seedling parameters across all genotypes, with EC-492576 displaying superior salt tolerance.

The second part of the study focused on alleviating salt stress through hormonal priming, an approach that enhances seedling quality by improving germination and growth under adverse conditions. Under 150 mM salt stress, 12 treatments were applied, including GA<sub>3</sub>, salicylic acid, ascorbic acid, and IAA at varying concentrations, with unprimed seeds serving as the control. Among these treatments, salicylic acid at 100 ppm yielded the best results improving key parameters such as germination rate, seedling length, and overall vigour. These findings highlight the potential of salicylic acid priming to improve salt tolerance in chilli. These findings highlight the potential of salicylic acid priming to improve salt tolerance in chilli, paving the way for future research in developing salt-tolerant varieties and informing breeding programs aimed at enhancing agricultural resilience in salt-affected regions.

**Keywords:** Chilli, genotypes; salinity; NaCl; SA; GA<sub>3</sub>; IAA; ascorbic acid; hormonal priming.

## 1. INTRODUCTION

Chilli (*Capsicum annum* L.) is categorized as a glycophyte plant, which faces challenges in surviving under high salinity stress, often resulting in reduced yields [1]. It is noteworthy that chilli ranks as the world's second most widely cultivated solanaceous vegetable, trailing only behind tomatoes. In India, chili is a prominent crop, cultivated over 1,983,000 hectares, yielding 170.03 million metric tons with a productivity rate of 1900 kg per hectare [2]. The primary region for premium dried chili production in India is located in the southern states, covering approximately 287 thousand hectares. This region contributes significantly to India's chili output, producing around 3,406 thousand metric tons annually with a productivity of 2.1 kg per hectare. It is noteworthy that India contributes about 50% of global chili production, with 90% of the yield consumed domestically and only 6% exported to countries like the United States, Bangladesh, Nepal, and Mexico [3].

Salinity stress poses a significant challenge, aggravated by the increasing salinization of arable land worldwide, as indicated by the latest FAO report. Soil salinity is emerging as a major threat to sustainable agriculture, affecting approximately 20% of the world's cultivated land and 33% of irrigated agricultural land, covering about 1,128 million hectares [4]. In India, salinity impacts about 6.727 million hectares, representing 2.1% of the country's geographical area, with 2.956 million hectares being saline and the remaining 3.771 million hectares being sodic [5]. The Indo-Gangetic plains account for a

significant portion of salt-affected soils in India, with 2.347 million hectares, including 0.56 million hectares of saline soil and 1.787 million hectares of sodic soil. The majority of these salt-affected soils are concentrated in states like Gujarat, Uttar Pradesh, Maharashtra, West Bengal, and Rajasthan [6].

Salinity stress adversely impacts the seed germination process through various mechanisms, including osmotic stress, ion-specific effects, and oxidative stress, leading to reduced germination rates and prolonged germination times. Salinity increases external osmotic potential, which hinders water uptake during seed imbibition. The toxic effects of excess sodium and chloride ions on embryo viability can also hinder seed germination [7]. Additionally, salinity can negatively influence seed germination by altering hormone levels, particularly by reducing germination stimulants like GA<sub>3</sub> and increasing ABA levels. This can also affect membrane permeability and water behavior in the seed. [8].

Seed priming is a valuable physiological approach to enhance the adaptability of glycophyte species to saline conditions during germination and early seedling establishment. It involves exposing seeds to controlled stress levels, creating a stress memory that enables the plant to respond more effectively to subsequent stress events. Seed priming is cost-effective and can promote seed germination and seedling establishment in salt-affected agricultural lands [9]. Seed germination undergoes through different phases: Imbibitions phase (phase I) a

physical process that involves water uptake; lag phase (Phase II) involves little net water uptake where seed moves from dormant to germinating state and growth phase (phase III) with a marked increase in water uptake associated with radicle protrusion and growth. The lag phase is the most prominent phase in priming that initiates early activation event through the stress response restoration of metabolic activities, achieved through stress memory and enables a head start. The priming process encompasses distinct phases of seed germination, including the imbibition phase (Phase I), lag phase (Phase II), and growth phase (Phase III). Of these, the lag phase is particularly significant during priming, as it initiates early activation events through stress response, restoration of metabolic activities, and augmentation of DNA repair, enzyme activation, and metabolite accumulation essential for germination [10]. In many crops, germination and seedling emergence are more sensitive to adverse growing conditions and if unaffected can significantly contribute towards uniform crop stand, resulting in higher yield. Similarly, salt stress negatively affects the germination by disturbing physio-biochemical processes including ionic imbalance, oxidative stress, and osmotic stress. Various studies show that salt stress slows down the germination of seeds by reducing water imbibition, disruption of proteins structure, and affecting stored food mobilization. Conversely, priming of seeds helps in early seed germination by accelerating the pre-germination metabolic process [11]. In hormonal priming, seed imbibition occurs in the presence of plant hormones such as GA<sub>3</sub>, ethylene, auxins, and salicylic acid, which gave an effect on seed metabolism. Chemical priming is a promising seed priming technique to enhance germination under high salinity stress. Seeds were pre-treated with different chemical solutions used as priming agents. Chemical agents include a wide range of both natural and synthetic compounds such as antioxidants (ascorbic acid, glutathione, tocopherol, and melatonin [12].

Hence, the present study was planned with the objective of studying the response of various chili genotypes to different salinity levels during germination and seedling growth under salt stress and evaluating the effect of hormonal priming for overcoming salt stress.

## 2. MATERIALS AND METHODS

The study was conducted in the Seed Testing Laboratory (Notified by the Government of Uttar

Pradesh) of the Department of Genetics and Plant Breeding at Sam Higginbottom University of Agriculture, Technology, and Sciences, Prayagraj (Uttar Pradesh), during the Rabi season of 2023. The research comprised two experiments: the first aimed to evaluate the effect of NaCl stress on seedling parameters of various chilli (*Capsicum annuum* L.) genotypes, while the second focused on mitigating salt stress on the selected genotype Ec-492596.

The seed materials, consisting of 14 chilli genotypes, were procured from the Indian Institute of Vegetable Research, Varanasi. The genotypes included Kashi Sindhure, Ec-492596, BS DN – 5, CMB -15, Azeet -13, KA-2 SL, Good Fruiting, Roshni, Ec – 790570, LCA-357, IIVRC - 316, BS – 35, Pickle Type-9, and Akola – 13.

**Experiment–1:** In this experiment the fourteen chilli genotypes were put under salt stress with four NaCl concentrations. The experiment was carried out in a factorial experiment based on Completely Randomized Design with four replications. Factors were factor A (14 chilli genotypes) and factor B (Concentration of 0, 75, 100 and 150 mM of sodium chloride (NaCl).

**Method of Inducing Salinity Stress:** Germination paper used for conducting germination tests was moistened in the below four mentioned NaCl concentrations and then placed in Petri dishes. Later 25 seeds were allowed to germinate on this moistened germination paper in the Petri dishes. Before placing, seeds were disinfected by immersion of 1% sodium hypochlorite (NaClO) for 2 minutes. Later washed with distilled water for 5 min and dried at room temperature to bring back original moisture content before placing in Petri dishes.

- S<sub>0</sub> – Control (distilled water).
- S<sub>1</sub> – Salt stress is induced by the addition of 75mM NaCl (Dissolved 4.35gram NaCl in 1 litre distilled water).
- S<sub>2</sub> - Salt stress is induced by addition of 100mM NaCl (Dissolved 5.85gram NaCl in 1 litre distilled water.)
- S<sub>3</sub> - Salt stress is induced by the addition of 150mM NaCl. (Dissolved 8.77-gram NaCl in 1 litre distilled water.)

**Experiment–2:** In the second experiment, seeds of chilli genotype (EC-492596) were surface

sterilized by dipping them in a 1% sodium hypochlorite solution for two minutes and then dried on filter paper. These surface-sterilized seeds were primed using 12 hormonal treatments, with an unprimed as the control. The hormonal treatments included GA3 at concentrations of 50 ppm, 75 ppm, and 100 ppm; Salicylic acid at 50 ppm, 100 ppm, and 150 ppm; Ascorbic acid at 50 ppm and 100 ppm; and IAA at 10 ppm, 20 ppm, and 30 ppm, all under 150 mM sodium chloride (NaCl).

After preparing the priming solution for GA3, Salicylic acid, Ascorbic acid, and IAA as above-mentioned concentrations the seeds were soaked in their respective solutions for 12 hours at a temperature of 20-25°C. After the 12-hour soaking period, the solution was drained from the beaker, and the pre-soaked seeds were shade dried at room temperature to restore them to their original moisture content. Subsequently, the seeds were placed for germination using the top-of-paper method in petri dishes under controlled laboratory conditions. The germination paper used for conducting the germination test was moistened with a 150 mM NaCl solution.

**The observations recorded and methodology from present study are given as follow:**

### 1. Germination per cent [3]

Germination test was conducted by adopting top paper method as described by ISTA [3] procedures

*Germination per – cent =*

$$\frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

### 2. Root length (cm) [3]

Ten seedlings was selected randomly from each treatment on 14th day from germination test. The root length was measured from the tip of the primary root to base of hypocotyls with the help of a scale and mean root length was expressed in centimeters.

### 3. Shoot length (cm) [3]

Same randomly selected ten seedlings were used to measure the shoot. The length was measured with the help of measuring scale for all genotypes in every replication. The length was measured from the base of the primary leaf to

the base of hypocotyls and the mean was expressed in cm.

### 4. Seedling length (cm) [3]

The total length of ten seedlings was calculated by adding root and shoot length as recorded earlier for each replication of every genotype.

Seedling length = Shoot length + Root length

### 5. Seedling fresh weight (mg) [3]

For recording seedling fresh weight 10 seedlings from each sample will be taken randomly. The fresh weight of seedling weighed with the help of electronic balance in milligram.

### 6. Seedling dry weight (mg) [3]

10 normal seedlings after measurement of root and shoot length was kept in butter paper and dried in a hot-air oven maintained at 60 °C temperature for 20 min. The mean dry weight of the seedlings will be considered as seedling dry weight.

### 7. Vigour indices

Vigor index was calculated by adopting the method suggested by **Baki and Anderson [13]**.

Vigor index-I = germination (%) x total seedling length (cm).

Vigor index-II = germination (%) x seedling dry weight.

**8. Germination rate (GR) =**  $N1 \times D1 + N2 \times D2 + N3 \times D3 + \dots / \text{Total germinated seeds}$

Where: N = Number of germinated seeds per day, D = Number of days from the start of the count, according to **Scott et al. [14]**.

**9. Germination index (GI) =**  $N1/D1 + N2/D2 + N3/D3 + \dots$

Where, N = Number of emerged seedlings per day, D= Number of days from the start of the count, according to Association of Official Seed Analysis [15].

**10. Salt tolerance index (STI)** Salt tolerance index was calculated as **Goudarzi and Pakniyat [16]** by the following formula Salt tolerance index = Variable measured under stress condition / Variable measured under normal condition.

### Statistical analysis and experimental design:

For the first experiment all the data were analysed by the factorial CRD with factor A as fourteen chilli genotypes and factor B as four salinity levels. The mean was subjected to the critical difference at 5% level of significance.

For the second experiment to determine the mean significant differences among the GA3, salicylic acid, ascorbic acid and IAA treatment and the control samples for the seedling parameters, 4 replicates of 25 seeds each was kept under the Completely Randomized Design so that the data were analysed by one way analysis of variance (ANOVA) using the ICAR software WASP (Web agri stat package) version 1.0. Least Significant Difference (LSD) was calculated for each treatment at 5% significant level.

## 3. RESULTS

### Response of various chilli genotypes to different salinity levels during germination and seedling growth under salt stress:

**Germination percent:** The data presented in Table 1 illustrates the germination (%) of various chilli genotypes tested under different NaCl concentrations: 0 mM (control), 75 mM, 100 mM, and 150 mM, in a Petri dishes. Among the genotypes, at the final count, the highest germination percentage was observed in the control (S0) for genotype EC-492596 (86%), followed by Kashi Sindhure (83.00%) and CMB 15 (43.00%). However, as salt stress increased, the germination percentages showed a significant decline across S1, S2, and S3, with mean germination percentages of 61.7%, 49.9%, and 33% respectively. Notably, distinct variations in germination responses under salt stress were observed among the different chilli genotypes. For instance, genotype EC-492596 exhibited the highest germination (48%) at the highest NaCl concentration (S3, 150 mM), along with Kashi Sindhure (38%) while CMB-15 (14%) displayed the lowest germination percentage.

**Shoot length(cm):** The data presented in Table 1 illustrates the shoot length(cm) of various chilli genotypes tested under different NaCl concentrations: 0 mM (control), 75 mM, 100 mM, and 150 mM in a Petri dishes. Among the genotypes, at the final count, the highest Root length was observed in the control (S0) for genotype EC-492596 (5.10cm), followed by CMB-15 (4.96cm) and Kashi Sindhure (4.69cm).

However, as the salt stress increased, the Root length showed a significant decline across S1, S2, and S3, with mean Root length of 3.3cm, 2.8cm, and 2.0cm, respectively. Notably, distinct variations in Root length responses under salt stress were observed among the different chilli genotypes. For instance, genotype EC-492596 exhibited the highest Root length (3.31cm) at the highest NaCl concentration (S3, 150 mM), along with Kashi Sindhure (2.41cm), while CMB-15(1.6cm) displayed the lowest Root length.

**Root length(cm):** The data presented in Table 1 illustrates the root length(cm) of various chilli genotypes tested under different NaCl concentrations: 0 mM (control), 75 mM, 100 mM, and 150 mM in a Petri dishes. Among the genotypes, at the final count, the highest shoot length was observed in the control (S0) for genotype EC-492596 (4.64cm), followed by CMB-15 (4.49cm) and Kashi Sindhure (4.19cm). However, as the salt stress increased, the shoot length showed a significant decline across S1, S2, and S3, with mean shoot length of 2.9cm, 2.3cm, and 1.6cm, respectively. Notably, distinct variations in shoot length responses under salt stress were observed among the different chilli genotypes. For instance, genotype EC-492596 exhibited the highest shoot length (2.87cm) at the highest NaCl concentration (S3, 150 mM), along with Kashi Sindhure (2.04cm), while CMB-15 (1.24cm) displayed the lowest shoot length.

**Seedling length(cm):** The data presented in Table 2 illustrates the Seedling length(cm) of various chilli genotypes tested under different NaCl concentrations: 0 mM (control), 75 mM, 100 mM, and 150 mM, in a Petri dishes. The results reveal the Seedling length(cm) of these genotypes. Among the genotypes, at the final count, the highest Seedling length(cm) was observed in the control (S0) for genotype EC-492596 (9.74cm), followed by CMB-15 (9.40cm) and Kashi Sindhure (8.88cm). However, as the salt stress increased, the Seedling length(cm) showed a significant decline across S1, S2, and S3, with mean Seedling length(cm) of 6.3cm, 5.1cm, and 3.6cm, respectively. Notably, distinct variations in Seedling length(cm) responses under salt stress were observed among the different chilli genotypes. For instance, genotype EC-492596 exhibited the Seedling length (6.17cm) at the highest NaCl concentration (S3, 150 mM), along with Kashi Sindhure (4.50cm) while CMB-15 (2.80cm) displayed the lowest Seedling length(cm).

Table 1. Impact of NaCl stress on Seedlings parameters of different chilli genotypes

Genotype	Germination %				Shoot Length (cm)				Root Length (cm)			
	S0	S1	S2	S3	S0	S1	S2	S3	S0	S1	S2	S3
AZEET-15	74	61	50	38	3.29	2.87	2.26	1.30	3.75	3.31	2.71	1.75
BS DN-1	69	60	48	32	3.08	2.57	1.92	1.49	3.44	3.03	2.47	2.01
KA-2 SL	70	56	49	31	3.60	3.20	2.56	1.61	3.89	3.60	3.05	2.13
GOOD FRUTING	69	62	45	34	3.41	2.88	2.34	1.28	3.83	3.37	2.90	1.76
KASHI SINDHURE	83	74	60	35	4.19	3.52	2.99	2.04	4.69	4.00	3.39	2.41
ROSHNI	71	65	49	34	3.77	3.21	2.71	1.92	4.19	3.67	3.18	2.37
EC-790570	67	63	50	31	3.66	3.15	2.80	1.75	4.15	3.53	3.21	2.16
EC-492596	86	75	64	48	4.64	4.22	3.49	2.87	5.10	4.62	3.88	3.31
LCA-357	68	62.5	50	32	2.84	2.40	1.89	1.05	3.26	2.85	2.36	1.39
IIVRC 316	68	61	53	38	2.99	2.43	1.79	1.37	3.42	2.89	2.22	1.84
BS-35	66	60.5	51	32	3.07	2.64	2.01	1.31	3.51	3.06	2.39	1.84
CMB-15	81	39	30	14	4.49	2.34	1.63	1.24	4.96	2.78	2.06	1.75
AKOLA -13	68	62	49	36	3.17	2.64	1.89	1.16	3.56	3.04	2.39	1.63
Pickle type-9	69	64	51	32	3.86	2.78	2.47	1.46	4.31	3.31	2.78	1.74
Mean	72.07	61.78	49.92	33.35	3.57	2.92	2.34	1.56	4.00	3.36	2.78	2.00
Factor	G	S	G×S	G	S	G×S	G	S	G×S	G	S	G×S
Sed	0.039	0.019	0.0764	0.04	0.0204	0.864	0.039	0.0195	0.076			
CD (0.05)	0.76	0.41	0.153	0.81	0.043	0.163	0.76	0.41	0.153			

**Table 2. Impact of NaCl stress on Seedlings parameters of different chilli genotypes**

Genotype	Seedling length (cm)				Seedling fresh weight (mg)				Seedling dry weight (mg)			
	S0	S1	S2	S3	S0	S1	S2	S3	S0	S1	S2	S3
AZEET-15	7.03	6.18	4.96	3.05	52.8	48.3	42.3	34.5	27.5	21.5	18.0	13.0
BS DN-1	6.52	5.60	4.38	3.50	53.0	47.5	42.0	37.0	26.0	20.5	16.8	13.3
KA-2 SL	7.49	6.80	5.61	3.74	48.3	42.0	39.5	33.3	24.3	20.5	14.8	10.8
GOOD FRUITING	7.24	6.25	5.24	3.04	45.0	40.3	37.3	32.0	23.5	19.0	16.8	14.0
KASHI SINDHURE	8.88	7.51	6.38	4.44	58.3	51.0	48.0	42.0	28.3	22.3	18.0	13.8
ROSHNI	7.96	6.88	5.89	4.29	46.5	41.3	38.0	31.8	22.0	18.8	16.0	12.3
EC-790570	7.81	6.67	6.01	3.91	50.5	45.3	42.5	38.8	23.0	19.3	16.8	13.0
EC-492596	9.74	8.83	7.36	6.18	70.5	60.5	57.5	53.3	36.8	33.5	28.8	26.3
LCA-357	6.10	5.25	4.25	2.43	44.0	39.8	33.0	33.8	23.8	18.5	16.3	11.3
IIVRC 316	6.41	5.32	4.01	3.21	44.5	40.8	41.0	36.5	25.5	20.8	18.5	16.0
BS-35	6.58	5.70	4.39	3.14	47.8	43.3	40.5	34.8	23.8	20.0	17.5	14.8
CMB-15	9.45	5.12	3.69	2.99	62.3	41.0	51.5	29.3	32.8	18.0	11.8	9.5
AKOLA -13	6.73	5.68	4.27	2.79	44.5	42.8	38.8	33.3	22.8	18.5	16.8	11.8
Pickle type-9	8.17	6.09	5.25	3.20	46.8	41.8	39.5	34.8	25.8	20.8	16.5	13.0
Mean	7.58	6.28	5.12	3.56	51.0	44.7	42.2	36.1	26.1	20.8	17.4	13.8
Factor	<b>G</b>	<b>S</b>	<b>G×S</b>	<b>G</b>	<b>S</b>	<b>G×S</b>	<b>G</b>	<b>S</b>	<b>G×S</b>	<b>G</b>	<b>S</b>	<b>G×S</b>
Sed	<b>0.071</b>	<b>0.038</b>	<b>0.142</b>	<b>0.759</b>	<b>0.40</b>	<b>1.519</b>	<b>0.671</b>	<b>0.249</b>	<b>1.29</b>	<b>0.671</b>	<b>0.249</b>	<b>1.29</b>
CD (0.05)	<b>0.144</b>	<b>0.077</b>	<b>0.287</b>	<b>1.529</b>	<b>0.818</b>	<b>3.059</b>	<b>1.345</b>	<b>0.719</b>	<b>2.69</b>	<b>1.345</b>	<b>0.719</b>	<b>2.69</b>

**Table 3. Impact of NaCl stress on Seedlings parameters of different chilli genotypes**

Genotype	Vigour Index I				Vigour Index II				Salt tolerance Index		
	S0	S1	S2	S3	S0	S1	S2	S3	S1	S2	S3
AZEET-15	520	376	249	116	2027	1311	899	495	0.783	0.658	0.474
BS DN-1	450	336	210	111	1792	1228	805	421	0.790	0.649	0.511
KA-2 SL	524	381	275	116	1696	1145	733	331	0.847	0.605	0.439
GOOD FRUTING	500	387	236	103	1623	1181	757	473	0.809	0.713	0.598
KASHI SINDHURE	737	556	383	155	2348	1647	1081	481	0.791	0.641	0.489
ROSHNI	565	447	288	146	1563	1217	786	420	0.853	0.729	0.557
EC-790570	523	421	300	121	1541	1208	837	404	0.842	0.732	0.568
EC-492596	837	662	471	296	3162	2512	1838	1258	0.913	0.785	0.717
LCA-357	415	328	212	78	1620	1157	815	362	0.781	0.689	0.475
IIVRC 316	436	325	213	122	1733	1267	975	612	0.813	0.724	0.626
BS-35	434	344	225	101	1565	1210	891	479	0.850	0.755	0.640
CMB-15	765	200	111	42	2652	703	350	133	0.550	0.356	0.292
AKOLA -13	457	352	209	100	1548	1153	816	421	0.813	0.736	0.520
Pickle type-9	563	390	268	103	1782	1330	843	421	0.817	0.650	0.516
Mean	552	393	261	122	1904	1305	888	479	0.804	0.673	0.530
Factor	G	S	GxS	G	S	GxS	G	S	GxS	G	S
Sed	8.73	4.69	17.49	47.31	25.11	96.6	0.38	0.017	0.066		
CD (0.05)	17.69	9.44	35.3	95	51	195	0.77	0.36	0.133		



**Seedling Fresh weight (mg):** The data presented in Table 2 illustrates the Seedling Fresh weight (%) of various chilli genotypes tested under different NaCl concentrations: 0 mM (control), 75 mM, 100 mM, and 150 mM in a Petri dishes. The results reveal the Seedling Fresh weight percentages of these genotypes. Among the genotypes, at the final count, the highest Seedling Fresh weight percentage was observed in the control (S0) for genotype EC-492596 (70.5mg), followed by CMB-15 (58.3mg) and Kashi Sindhure (62.3). However, as the salt stress increased, the Seedling Fresh weight percentages showed a significant decline across S1, S2, and S3, with mean Seedling Fresh weight percentages of 44.7mg, 42.2mg, and 36.1mg respectively. Notably, distinct variations in Seedling Fresh weight responses under salt stress were observed among the different chilli genotypes. For instance, genotype EC-492596 exhibited the highest Seedling Fresh weight (60.4mg) at the highest NaCl concentration (S3, 150 mM), along with Kashi Sindhure (49mg), while CMB-15(29.3) displayed the lowest Seedling Fresh weight percentage.

**Seedling Dry weight (mg):** The data presented in Table 2 illustrates the Seedling Dry weight of various chilli genotypes tested under different NaCl concentrations: 0 mM (control), 75 mM, 100 mM, and 150 Mm in a Petri dishes. The results reveal the Seedling Dry weight of these genotypes. Among the genotypes, at the final count, the highest Seedling Dry weight percentage was observed in the control (S0) for genotype EC-492596 (36.8), followed by CMB-15 (32.8) and Kashi Sindhure (28.3). However, as the salt stress increased, the Seedling Dry weight percentages showed a significant decline across S1, S2, and S3, with mean Seedling Dry weight percentages of 20.8mg, 17.4mg, and 13.8mg respectively. Notably, distinct variations in Seedling Dry weight responses under salt stress were observed among the different chilli genotypes. For instance, genotype EC-492596 exhibited the highest Seedling Dry weight (95.00) at the highest NaCl concentration (S3, 150 mM), along with Kashi Sindhure, while CMB-15 displayed the lowest Seedling Dry weight.

**Vigour index I:** The data presented in Table 3 illustrates the Vigour index I of various chilli genotypes tested under different NaCl concentrations: 0 mM (control), 75 mM, 100 mM, and 150 mM in a Petri dishes. The results reveal the Vigour index I of these genotypes. Among

the genotypes, at the final count, the highest Vigour index I was observed in the control (S0) for genotype EC-492596 (837.18), followed by CMB-15 (765) and Kashi Sindhure (736.59). However, as the salt stress increased, the Vigour index I showed a significant decline across S1, S2, and S3, with mean Vigour index I of 393.2, 260.7, and 122.1, respectively. Notably, distinct variations in Vigour index I responses under salt stress were observed among the different chilli genotypes. For instance, genotype EC-492596 exhibited the highest Vigour index I (296.25) at the highest NaCl concentration (S3, 150 mM), along with Kashi Sindhure (155), while CMB-15(42.08) displayed the lowest Vigour index I.

**Vigour index II:** The data presented in Table 3 illustrates the Vigour index II (%) of various chilli genotypes tested under different NaCl concentrations: 0 mM (control), 75 mM, 100 mM, and 150 Mm in a Petri dishes. The results reveal the Vigour index II of these genotypes. Among the genotypes, at the final count, the highest Vigour index II was observed in the control (S0) for genotype EC-492596 (3162), followed by CMB-15 (2652) and Kashi Sindhure (2348). However, as the salt stress increased, the Vigour index II showed a significant decline across S1, S2, and S3, with mean Vigour index II of 1304, 887.5, and 479.3 respectively. Notably, distinct variations in Vigour index II responses under salt stress were observed among the different chilli genotypes. For instance, genotype EC-492596 exhibited the highest Vigour index II (1258) at the highest NaCl concentration (S3, 150 mM), along with IIVRC 316 (612) while CMB-15(133) displayed the lowest Vigour index II.

**Salt tolerance index:** The data presented in Table 3 illustrates the salt tolerance index of various chilli genotypes tested under different NaCl concentrations: 0 mM (control), 75 mM, 100 mM, and 150 mM, in a using Petri dishes. The results reveal the salt tolerance index of these genotypes. Among the genotypes, at the final count, the highest salt tolerance index value at 150mM was observed in the genotype EC-492596 (7.1) and least value in CMB-15 (0.29).

**Effect of hormonal priming on seedling parameters of the genotype EC under 150 mM salt stress:**

**Germination per cent:** In Table 4 the data for germination percentage after priming under salt stress is presented with the mean value of 48.83%. The highest germination percentage

(58%) was significantly reported in T<sub>5</sub>, where seeds were primed with salicylic acid at 100 ppm for 12 hours. Following closely, T<sub>1</sub> showed a germination percentage of 53.00%, with priming involving GA<sub>3</sub> at 50 ppm for 12 hours. Notably, the lowest germination percentage of 44.00% was observed in the control (T<sub>0</sub>) unprimed.

**Shoot length(cm):** In Table 4, the data for shoot length after priming under salt stress is presented with the mean value of 3.74cm. The highest shoot length (4.15cm) was significantly reported in T<sub>5</sub>, where seeds were primed with salicylic acid at 100 ppm for 12 hours. Following closely, T<sub>1</sub> showed a shoot length of 4.02cm, with priming involving GA<sub>3</sub> at 50 ppm for 12 hours. Notably, the lowest shoot length of 2.86cm was observed in the control (T<sub>0</sub>) unprimed.

**Root length(cm):** In Table 4, the data for root length after priming under salt stress is presented with the mean value of 4.02cm. The highest root length (4.73cm) was significantly reported in T<sub>5</sub>, where seeds were primed with salicylic acid at 100 ppm for 12 hours. Following closely, T<sub>1</sub> showed a root length of 4.54, with priming involving GA<sub>3</sub> at 50 ppm for 12 hours. Notably, the lowest root length of 3.16 was observed in the control (T<sub>0</sub>) unprimed.

**Seedling length(cm):** In Table 4, the data for seedling length after priming under salt stress is presented with the mean value of 7.77cm. The highest seedling length (8.89cm) was significantly reported in T<sub>5</sub>, where seeds were primed with salicylic acid at 100 ppm for 12 hours. Following closely, T<sub>1</sub> showed a seedling length of 8.56cm, with priming involving GA<sub>3</sub> at 50 ppm for 12 hours. Notably, the lowest seedling length of 6.05cm was observed in the control (T<sub>0</sub>) unprimed.

**Seedling Fresh weight (mg):** In Table 4, the data for seedling fresh weight after priming under salt stress is presented with the mean value of 58.77mg. The highest seedling fresh weight (62.50) was significantly reported in T<sub>5</sub>, where seeds were primed with salicylic acid at 100 ppm for 12 hours. Following closely, T<sub>1</sub> showed a seedling fresh weight of 61.50, with priming involving GA<sub>3</sub> at 50 ppm for 12 hours. Notably, the lowest seedling fresh weight of 50.75mg was observed in the control (T<sub>0</sub>) unprimed.

**Seedling dry weight (mg):** In Table 4, the data for seedling dry weight after priming under salt

stress is presented with the mean value of 27.18mg. The highest seedling dry weight (30.75mg) was significantly reported in T<sub>5</sub>, where seeds were primed with salicylic acid at 100 ppm for 12 hours. Following closely, T<sub>1</sub> showed a seedling dry weight of 29.00mg, with priming involving GA<sub>3</sub> at 50 ppm for 12 hours. Notably, the lowest seedling dry weight of 24.50mg was observed in the control (T<sub>0</sub>) unprimed.

**Seedling Vigour Index I:** In Table 4, the data for seedling Vigour Index I after priming under salt stress is presented with the mean value of 381. The highest seedling Vigour Index I (515) was significantly reported in T<sub>5</sub>, where seeds were primed with salicylic acid at 100 ppm for 12 hours. Following closely, T<sub>1</sub> showed a seedling Vigour Index I of 453, with priming involving GA<sub>3</sub> at 50 ppm for 12 hours. Notably, the lowest seedling Vigour Index I of 266 was observed in the control (T<sub>0</sub>) unprimed.

**Seedling Vigour Index II:** In Table 4, the data for seedling Vigour Index II after priming under salt stress is presented with the mean value of 1333.8. The highest seedling Vigour Index II (1783) was significantly reported in T<sub>5</sub>, where seeds were primed with salicylic acid at 100 ppm for 12 hours. Following closely, T<sub>1</sub> showed a seedling Vigour Index II of 1538, with priming involving GA<sub>3</sub> at 50 ppm for 12 hours. Notably, the lowest seedling Vigour Index II of 1079 was observed in the control (T<sub>0</sub>) unprimed.

**Germination Index:** In Table 4, the data for germination Index after priming under salt stress is presented with the mean value of 2.27. The highest germination Index (2.72) was significantly reported in T<sub>5</sub>, where seeds were primed with salicylic acid at 100 ppm for 12 hours. Following closely, T<sub>3</sub> showed a germination Index of 2.64, with priming involving GA<sub>3</sub> at 100 ppm for 12 hours. Notably, the lowest germination Index of 1.4 was observed in the control (T<sub>0</sub>) unprimed

**Germination rate:** In Table 4, the data for germination rate after priming under salt stress is presented with the mean value of 6.37. The highest germination rate 7.165 was significantly reported in T<sub>5</sub>, where seeds were primed with salicylic acid at 100 ppm for 12 hours. Following closely, T<sub>6</sub> showed a germination rate of, with priming involving Salicylic acid at 150 ppm for 12 hours. Notably, the lowest germination rate of 5.853 was observed in the control (T<sub>0</sub>) unprimed.

**Table 4. Mean performance of hormonal priming for seedling parameters of EC-492596 under 150mM salt stress**

Treatments	Germination (%)	Root length(cm)	Shoot length(cm)	Seedling length(cm)	Seedling Fresh weight(mg)	Seedling Dry weight(mg)	Vigour Index I	Vigour Index II	Germination Rate	Germination Index
<b>T0</b>	44	2.86	3.19	6.05	50.75	24.5	266.59	1079	5.853	1.4
<b>T1</b>	53	4.02	4.54	8.56	61.5	29	453.66	1538	6.643	2.22
<b>T2</b>	48	3.89	4.1	7.99	59.5	26.75	383.5	1285	6.500	2.34
<b>T3</b>	47	3.72	3.94	7.66	58.5	26.25	360.19	1234	6.188	2.64
<b>T4</b>	47	3.56	3.88	7.45	58	26.5	350.33	1250	6.098	2.57
<b>T5</b>	58	4.15	4.73	8.89	62.5	30.75	515.7	1783	7.165	2.72
<b>T6</b>	46	3.43	3.65	7.09	57.5	25.75	326.03	1186	7.008	1.66
<b>T7</b>	50	4	4.18	8.18	60.75	28	409.63	1402	6.180	2.47
<b>T8</b>	47	3.79	4.12	7.91	59	27	372.38	1267	6.130	2.18
<b>T9</b>	47	3.68	3.94	7.62	57.75	25.25	358.36	1187	6.085	2.23
<b>T10</b>	50	3.90	4.05	7.95	60	28	397.77	1400	6.560	2.61
<b>T11</b>	49	3.89	3.99	7.89	59.5	28.5	386.51	1395	6.093	2.30
<b>Grand Mean</b>	<b>48.83</b>	<b>3.74</b>	<b>4.02</b>	<b>7.77</b>	<b>58.770</b>	<b>27.187</b>	<b>381.72</b>	<b>1333.83</b>	<b>6.375</b>	<b>2.27</b>
<b>Min</b>	44	2.86	3.19	6.05	50.75	24.5	266.59	1079	5.853	1.4
<b>Max</b>	58	4.15	4.73	8.89	62.5	30.75	515.7	1783	7.165	2.72
<b>S (Em)</b>	0.88	0.5	0.03	0.09	1.01	0.67	14.9	60	0.03	0.04
<b>S (Ed)</b>	1.2	0.08	0.04	0.07	0.64	0.44	21.08	84.9	0.04	0.02
<b>CD 5%</b>	5.10	0.17	0.17	0.23	2.48	1.8	42.7	174.35	0.082	0.07

## 4. DISCUSSION

**Response of various chilli genotypes to different salinity levels during germination and seedling growth under salt stress:** In this study, it was observed that salt stress had a significant effect on germination and seedlings characters of chilli genotypes which differed significantly in terms of characters studied. The results of the germination percentage and seedling parameters of chilli genotypes as influenced by NaCl revealed significant differences i.e all characters of chilli investigated decreased gradually with the increasing of salt concentration and the interaction of genotypes and salinity predominantly impacted the germination and seedling characters of chilli genotypes.

Irrespective of genotypes and salinity treatments, the germination percentage of chilli was highly influenced by salt stress at higher concentration (150 mM of NaCl). The mean of germination percentage ranged from 14.00 % to 35.00 % in (150mM of NaCl) and 83.50 % to 94.00 % in (control). Under high salt concentration level (150Mm NaCl) the shoot and root length were reduced in all the genotypes studied. Decrease in the external osmotic potential due to salinity causes reduction in physiological and morphological growth of plants [17]. Similar results were also found by Mohammad et al. [18] in chilli and and Keshavarzi et al. [19] in spinach. It was also confirmed in tomato [20] and in cucumber. Yildirim et al. [21].

Among the all genotypes it was observed that the genotype EC 495632 and CMB 15 had maximum germination in the control.

In highest salt concentration of 150mM genotype EC 495632 surpassed other genotypes and had highest germination percent, root length, shoot length, seedling length, seedling fresh weight dry weight vigour index I and II and salt tolerance index compared to all other genotypes. The probable reason of the differences in these characters must be due to the genetic make-up of the cultivar which is primarily influenced by heredity. Salt stress exercised a remarkable effect on germination and growth characters of chili genotypes [18].

**Effect of different hormonal priming treatments on the seedling parameters of chilli genotype (EC 495632) Under 150mM salt**

**stress:** In this study, we investigated the impact of various priming treatments on the germination and seedling growth of chilli seeds under 150 mM salt stress. Our findings provide valuable insights into the potential of hormonal priming to enhance the tolerance of chilli seeds to salt stress, with implications for agriculture and crop production in saline soils.

Among the priming treatments evaluated, it is evident that salicylic acid at 100 ppm emerged as the most effective priming agent in terms of improving seedling parameters under salt stress conditions. This result is consistent with previous research indicating the positive influence of salicylic acid on plant stress responses and seedling vigour. Salicylic acid is known to induce the expression of stress-responsive genes and enhance antioxidant activity, which could explain its beneficial effects on germination and seedling growth under salt stress [22].

Following salicylic acid, gibberellic acid at 50 ppm demonstrated a significant improvement in seedling parameters compared to the control group (UNPRIMED - T1). Gibberellic acid is involved in cell elongation and division processes, which might have contributed to the increased root length and shoot length observed in treated seedlings [23].

It is noteworthy that the control group, represented by unprimed seeds, exhibited the lowest performance across all measured parameters under salt stress conditions. This reaffirms the importance of priming treatments in alleviating the adverse effects of salt stress on seed germination and seedling growth.

The improvement in germination percentage, germination rate, and germination index under salt stress conditions with salicylic acid and gibberellic acid priming treatments highlights the role of these hormones in enhancing the seed's ability to break dormancy and initiate growth even in challenging environments. This can be particularly advantageous in regions where salinity is a limiting factor for crop production. Muhammad et al. [24] and Khan et al. [25] noted that maximum shoot length of hot pepper (*Capsicum annuum* L.) was related to salicylic acid priming under salinity treatments. Ghoohestani et al. [26] also stated that seed priming with salicylic acid significantly increased the plumule length of tomato in comparison with control [27-32].

## 5. CONCLUSION

From the first objective, it was concluded that the response of 14 chilli genotypes to varying salinity levels revealed that NaCl negatively affects the physiological processes involved in germination and seedling growth. Increasing salinity levels resulted in reduced growth, germination, and seedling parameters. Among the genotypes tested, EC-492596, followed by Kashi Sindhure, demonstrated the highest tolerance to salinity, while CMB-15 exhibited the lowest tolerance, performing poorly across all three salt concentrations (75 mM, 100 mM, and 150 mM). The second objective, focused on hormonal priming under 150 mM salt stress, concluded that different concentrations of hormonal priming significantly improved seed germination and seedling vigour. Notably, the treatment with salicylic acid (100 ppm) for 12 hours (T5) produced the most positive effect on seedling parameters. These findings hold broader implications for agricultural practices and future research. The identification of salt-tolerant genotypes, such as EC-492596, and the demonstrated effectiveness of hormonal priming, particularly with salicylic acid, can inform breeding programs aimed at developing more salt-tolerant chilli varieties. This knowledge could be applied to enhance crop productivity in saline-affected regions, contributing to sustainable agricultural practices and improved food security.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Ibn Maaouia-Houimli S, Denden M, Bouzlama M. Effects of salinity on the yield, essential oil composition, and mineral nutrient contents of basil (*Ocimum basilicum* L.). *J Agron.* 2008;7(1):1-10.
2. Horticultural statistics. Horticulture Statistics at a Glance 2020. New Delhi: Ministry of Agriculture & Farmers Welfare, Government of India; 2020.
3. International Seed Testing Association (ISTA). International rules for seed testing 2021. Bassersdorf, Switzerland: ISTA; 2021.
4. Yuan F, Leng B, Wang B. Progress in studying salt secretion from the salt glands in recretohalophytes: how do plants secrete salt? *Front Plant Sci.* 2015;6:67.
5. Arora S, Sharma SK. Status and management of salt-affected soils in India. *Soil Sci Annu.* 2017;68(1):35-45.
6. Mandal AK, Sharma RC, Singh G. Salt-affected soils of India: Current assessment and management options. *J Soil Salinity Water Qual.* 2018;10(1): 51-8.
7. Daszkowska-Golec A, Szarejko I. Open or close the gate – stomata action under the control of phytohormones in drought stress conditions. *Front Plant Sci.* 2013;4:138.
8. Lee KP, Lopez-Molina L, Coupland G. Phytochrome signaling and control of seed germination. *J Exp Bot.* 2012;63(11):3849-63.
9. Cipollini DF, Enright S, Traw MB, Bergelson J. Salicylic acid inhibits jasmonic acid-induced resistance of *Arabidopsis thaliana* to *Spodoptera exigua*. *Mol Ecol.* 2003;12(4):493-9.
10. Hussain S, Hussain S, Qamar Z, Aslam M. The role of seed priming in improving seed germination and seedling growth under saline conditions. *Environ Sci Pollut Res.* 2015;22(5):3281-93.

11. Paparella S, Araújo SS, Rossi G, Wijayasinghe M, Carbonera D, Balestrazzi A. Seed priming: state of the art and new perspectives. *Plant Cell Rep.* 2015;34(8): 1281-93.
12. Benincasa P, Falcinelli B, Lutts S, Pace R. Seed priming with antioxidant agents enhances germination and physiological responses of forage legumes exposed to severe water stress. *Seed Sci Res.* 2016;26(3) :157-67.
13. Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria. *Crop Sci.* 1973;13(6):630-3.
14. Scott SJ, Jones RA, Williams WA. Review of data analysis methods for seed germination. *Crop Sci.* 1984;24(6): 1192-9.
15. Association of Official Seed Analysts (AOSA). Seed vigor testing handbook. East Lansing, MI: AOSA; 1983. Contribution No. 32 to handbook on seed testing.
16. Goudarzi M, Pakniyat H. Evaluation of wheat cultivars under salinity stress based on some agronomic and physiological traits. *J Agric Sci Technol.* 2008;10(2):287-98.
17. Radhouane L. Response of Tunisian autochthonous pearl millet (*Pennisetum glaucum* (L.) R. Br.) to drought stress induced by polyethylene glycol (PEG) 6000. *Afr J Biotechnol.* 2007;6(9): 1102-5.
18. Mohammad A, Ahmed M, Ahmed F. Effect of salinity stress on germination and seedling growth of chilli (*Capsicum annum* L.) genotypes. *J Agric Sci.* 2018;10(5):23-30.
19. Keshavarzi M, Babazadeh H, Fahmi M. Effects of different salinity levels on the germination and seedling growth of spinach (*Spinacia oleracea* L.). *J Environ Stud.* 2011;37(2):117-26.
20. Kulkarni M, Deshpande U. Salt stress-induced changes in germination, growth, and total soluble sugar content in cultivars of tomato (*Lycopersicon esculentum* Mill.). *Indian J Plant Physiol.* 2007;12(2): 151-5.
21. Yildirim E, Taylor AG, Spittler TD. Germination and seedling emergence of cucumber (*Cucumis sativus* L.) seeds in response to salt stress. *Scientia Hortic.* 2008;118(1):1-4.
22. Gupta B, Huang B. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *Int J Genomics.* 2020; 2020:8456208. DOI:10.1155/2020/8456208.
23. Koornneef M, Veen JH, Hanhart CJ. Gibberellin biosynthesis mutants and the involvement of gibberellins in germination and early seedling growth in *Arabidopsis thaliana*. *Planta.* 1982;155(2):196-203.
24. Muhammad S, Anwar S, Shahbaz M. Salicylic acid-induced improvement in growth, photosynthesis, and mineral nutrients in salt-stressed wheat (*Triticum aestivum*). *J Plant Physiol.* 2007;164(6): 811-20.
25. Khan MA, Ungar IA, Showalter AM. Effects of salinity on the growth and ion content of salinity-sensitive plants. *Plant Physiol.* 2009;94(2):504-9.
26. Ghoohestani A, Esmaeili A, Kamyab M. Effect of seed priming with salicylic acid on germination characteristics of tomato under salt stress. *J Seed Sci Technol.* 2012;40(3):484-90.
27. Abdul-Baki A, Anderson JD. Vigor determination in soybean seed by multiple criteria. *Crop Sci.* 1973;13:630-3.
28. Association of Official Seed Analysts (AOSA). Seed vigor testing handbook. East Lansing, MI: AOSA; 1983.
29. National Horticulture Board. Indian Horticulture Database. New Delhi: Ministry of Agriculture and Farmers Welfare, Government of India; 2021. Available:<http://nhb.gov.in/>
30. Afzal M, Hindawi SES, Alghamdi SS, Migdadi HH, Khan MA, Hasnain MU, Sohaib M. Potential breeding strategies for improving salt tolerance in crop plants. *Journal of Plant Growth Regulation.* 2023;42(6):3365-3387.
31. Johnson R, Puthur JT. Seed priming as a cost effective technique for developing plants with cross tolerance to salinity stress. *Plant physiology and biochemistry.* 2021;162:247-257.
32. Sghayar S, Debez A, Lucchini G, Abruzzese A, Zorrig W, Negrini N, Morgutti S, Abdelly C, Sacchi GA, Pecchioni N,

Vaccino P. Seed priming mitigates high salinity impact on germination of bread wheat (*Triticum aestivum* L.) by improving carbohydrate and protein

mobilization. Plant Direct. 2023 Jun 4;7(6):e497.

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