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## Research article

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## Germination test, seedling growth, and physiochemical traits are used to screen okra varieties for salt tolerance

## Hayat Ullah, Tour Jan

Department of Botany, University of Malakand, Chakdara, Dir (L), Khyber Pakhtunkhwa, Pakistan

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#### ABSTRACT

Excess soil salinity is a major stress factor that inhibits plant growth, development, and production. Among the growth stages, seed germination is particularly susceptible to salt stress. Okra, a nutraceutical vegetable, has a low germination percentage. Literature has revealed genetic diversity in okra, which can be studied to develop salt-tolerant varieties. This study examined the salt tolerance of 13 okra varieties using germination tests and then tested five varieties in pot experiments with different NaCl levels (75, 100, and 125 mM NaCl). Results showed that salt levels affected all varieties, with differential variations in stress response. Salt stress reduced agronomic, and physiochemical traits in the studied varieties. In variety "MALAV-27", the highest salt concentration significantly reduced the shoot length (68.12 %), root length (65.11 %), shoot fresh weight (78.73 %), root fresh weight (68.32 %), shoot dry weight (75.60 %), and root dry weight (75.81 %), along with different physiochemical traits. Variety "NAYAB-F1" performed the best, and maintained the highest shoot length (57.12 %), root length (58.72 %), shoot fresh weight (68.26 %), and root fresh weight (58.34 %), shoot dry weight (69.23 %), root dry weight (62.50 %), and numerous physiochemical traits such as sugar (0.74  $\mu$ g/g), proline (0.51 µmol/g), and chlorophyll 'a' (7.97 mg/g), chlorophyll 'b' (9.56 mg/g). The study recommended 'NAYAB-F1', 'Arka anamika', and 'Shehzadi' as salt-tolerant varieties suitable for selection in salt-tolerant okra breeding programs.

## 1. Introduction

Soil salinization is a significant abiotic stress that disrupts plant growth and development globally [1,2]. It affects 19.5% of irrigated land and 2.1% of arid land [3] with NaCl being the common salt hence the focus of most research [4,5]. It is expected that increasing salinization levels could result in a loss of 50% of cultivated land by 2050 in Asia [6]. Soil salinization continues to grow in size because of excessive irrigation [7]. Salinization occurs in arid and semi-arid regions because of high evaporation, and inadequate amounts of precipitation for considerable leaching [8].

Supersaturated salt concentrations can be toxic to plant cells [9], disrupting ionic balance and increasing osmotic pressure [10]. It also inhibits carbon fixation, thereby reducing leaf vigor and crop productivity [11,12]. Plants have defensive responses to salt [13]. Such as the uptake of osmoregulators and specific ions [14], which protect them from excessive ion toxicity and osmotic stress [15]. Despite osmotic stress and ionic toxicity, plants have to adapt and maintain balanced water absorption [16–18].

Soil salinization can affect seed germination, viability, and chloroplast pigments in most crops like melon and tomato [19,20].

\* Corresponding author. *E-mail address:* tour\_jan@yahoo.com (T. Jan).

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(4)

Salinity can negatively impact the germination of plants such as *Oryza sativa*, *Triticum aestivum*, *Zea mays*, and *Brassica* spp. [21–25]. Salinity extremely affects plant growth and development depending on the plant growth stage, variety, and salinity level [26,27]. Okra, a nutritious vegetable crop [28] is a source of calcium, potassium, vitamins, carbohydrates, and unsaturated fatty acids [29]. The viscose fiber of the okra pods manages the level of cholesterol in the human body and thus helps with weight loss [30]. Okra is sensitive to salt stress [31], and its low yield can be partially attributed to salinity in the soil [32,33]. Therefore, identifying and screening okra varieties suitable for growth under salt stress is of great ecological and economic significance. It is incredibly challenging to enhance okra's salt resistance through variety breeding since little is known about the cultivars that confer this tolerance. This study evaluated the responses of thirteen okra varieties to salt levels and their impact on seed germination, seedling growth, and synthesis of biochemical compounds, with the aim of increasing okra vield in saline soils.

### 2. Materials and methods

## 2.1. Initial screening using germination test

The study involved the collection of healthy seeds of thirteen okra varieties from the Tarnab Agricultural Research Institute in Peshawar, Pakistan (Table-1). Seeds were decontaminated with mercuric chloride solution (0.05 %) for 1 min and germinated in Petri dishes (12 cm diameter), using five replicates of 10 seeds. Seeds were irrigated with different levels of NaCl and received varying doses (50, 75, and 100 mM) of salt (5 mL per Petri dish), and the same amount of distilled water was supplemented to control seeds. The number of germinated seeds was recorded daily for up to ten days after 72 h of sowing. The emergence of plumule and radical from the seed was used as an index of germination. Germination percentage (GP) [34], germination index (GI) [34], and germination energy (GE) [35] were calculated.

$$Germination Percentage = \frac{\text{total no of germinated seed}}{\text{total no of sowing seed}} \times 100$$
(1)

Germination Index (GI) = 
$$\frac{\sum diNi}{S}$$
 (2)

Germination Energy = 
$$\frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \frac{X_3 - X_2}{Y_3} + \dots + \frac{X_n - X_{n-1}}{Y_n}$$
 (3)

## 2.2. Salinity tolerance test at early growth stage

The study aimed to identify salt-tolerant, salt-sensitive, and salt-tolerant okra varieties. Five varieties that showed germination at all salt levels in the initial Petri dish experiment were selected and further examined in a saline environment with increased salt levels (75, 100, and 125 mM). Pot experiments were conducted in plastic pots containing five seeds of the selected variety (Table-2). After 45 days of recording seedling growth data, five seedlings were selected to measure root and shoot length. The plants were dried at 70 °C to constant weight and weighed.

#### 2.3. Vigor index (VI)

The study aimed to determine the vigor index (VI) of different varieties by calculating seed germination and measuring root and shoot length. The vigor index was calculated using the formula of [36].

Vigor Index = Germination % 
$$\times$$
 (RTL (cm) + STL (cm)

#### 2.4. Physiochemical assessment

The physiochemical evaluation involved the calculation of the sugar content, Proline content, and Chlorophyll contents.

Table 1										
List of thirteen	okra	varieties	used	in	the	petri	dishes	exp	erime	nt.

S. NoVarietiesS. NoVarieties1NAYAB-F18Sabz Pari2Arka anamika9Feveeri Green3NALAV-2710Shehzadi4Punjab selection11Hunza5Local Multani12Green star6Sarhad green13Annol7051-F1		1 1		
1NAYAB-F18Sabz Pari2Arka anamika9Feveeri Green3NALAV-2710Shehzadi4Punjab selection11Hunza5Local Multani12Green star6Sarhad green13Annol7051-F1HunzaHunza	S. No	Varieties	S. No	Varieties
2Arka anamika9Feveeri Green3NALAV-2710Shehzadi4Punjab selection11Hunza5Local Multani12Green star6Sarhad green13Annol7051-F1Sarhad starSarhad star	1	NAYAB-F1	8	Sabz Pari
3NALAV-2710Shehzadi4Punjab selection11Hunza5Local Multani12Green star6Sarhad green13Anmol7051-F1	2	Arka anamika	9	Feveeri Green
4Punjab selection11Hunza5Local Multani12Green star6Sarhad green13Anmol7051-F155	3	NALAV-27	10	Shehzadi
5Local Multani12Green star6Sarhad green13Anmol7051-F1	4	Punjab selection	11	Hunza
6 Sarhad green 13 Anmol 7 051-F1	5	Local Multani	12	Green star
7 051-F1	6	Sarhad green	13	Anmol
	7	051-F1		

m-11.0

Table 2						
List of selected	okra fi	ive va	rieties	for	seedling	growth.

S. No	Varieties	S. No	Varieties
1	NAYAB-F1	4	051-F1
2	Arka anamika	5	NALAV-27
3	Shehzadi		

## 2.4.1. Sugar

Sugar content was determined by heating fresh leaves (0.2 g) with ethanol (80 %) for 10 min, adding 1 mL of phenol (18 %), and then incubating the solution at room temperature for 1 h. After incubation, 2.5 mL  $H_2SO_4$  was added to the mixture, and vortex. The absorbance of the supernatant was read at 490 nm on a UV Spectrophotometer.

## 2.4.2. Proline

Proline content of each variety was determined by the method of [37]. 0.2 g of fresh leave was put in 10 mL of 3 % sulfosalicylic acid in test tubes for 48 h, and then 0.2 mL of glacial acetic acid and ninhydrin reagent were added. The mixture was heated at 100  $^{\circ}$ C for 1 h and allowed to cool before toluene was added. The absorbance of the supernatant was measured at 520 nm on a UV spectrophotometer.

## 2.4.3. Chlorophyll

The chlorophyll content of each variety was determined by the methods of [38]. 0.2 g of fresh leaves were ground and then dipped in 80 % ethanol contained in the test tube. The test tube was heated in a water bath at 80 °C for 15 min. After that the test tube was centrifuged at 10,000 rpm for 6 min and the absorption of the supernatant was measured with a spectrophotometer.

$$Chl.a \left( 12.7 \left[ \left( mg \, g - 1 f.wt = [12.7(OD663) - 2.69(OD645)] \times \frac{V}{1000} \times W \right] \right] \right)$$
(5)

$$Chl.b[(12.7[(mgg - 1f.w)] = [22.7(OD645) - 4.49(OD663)] \times \frac{V}{1000} \times W$$
(6)

where, V = Volume of the extract (mL) and W = Weight of the fresh leaf tissue (g).

#### 2.5. Data analysis

Average data from three replicates were analyzed using one-way analysis of variance (ANOVA) using SAS statistical software according to germination percentage over different salt concentrations.

## 3. Results

#### 3.1. Initial seed germination test

The study evaluated the salinity tolerance of 13 okra varieties by exposing them to different salt concentrations (0, 50, 75, and 100 mM). The results showed that as salt levels increased, germination percentage (GP), germination index (GI) and germination energy (GE) decreased in all varieties (Table 3). Five varieties —"NAYAB-F1," "Arka anamika," "MALAV-27," "Shehzadi," and "051-F1"— were chosen based on their germination performance under all salt conditions. These cultivars were suitable to test for seedling growth and physiochemical characteristics under stress conditions.

#### 3.2. Varieties assessment for seedling growth

The study also found that the varieties showed substantial variation in seedling growth, with 'NAYAB-F1' showing the highest values in terms of shoot length (12.56 cm), root length (5.83 cm), fresh and dry weight of shoots (0.290 and 0.024 g), and fresh and dry weight of roots (0.068 g and 0.006 g). "Malav-27" shows the highest decrease in agronomic characteristics, shoot length (7.49 cm), root length (3.57 cm), fresh and dry weight of shoots (0.168 and 0.012 g), and fresh and dry weight of roots (0.043 and 0.002 g), respectively. The vigor index (VI) values also decreased with "Malav-27" (243.2 %) and increased for 'NAYAB-F1' (283.7 %) Table-4.

## 3.3. Seedling growth assessment under salinity stress

The study evaluated the effect of salinity levels on the growth of okra seed seedlings, at different salt concentrations (0, 75, 100, and 125 mM). The results showed that the effect of salt stress was proportional to the stress level applied, leading to severe impacts at high levels for all growth traits measured (Table-5). As salinity levels increased, shoot length (SL) and root length (RL) decreased, with the highest decrease observed in variety "Malav-27" with a shoot length (68.12 %), and root length (65.11 %), followed by variety "051-F1 " with a shoot length (72.97 %), and root length (60.48 %). NAYAB-F1 was observed to have the lowest decrease in shoot length (57.12

	Varieties— 0	) mM NaCl											
Traits	NAYAB-F1	Arka anamika	MALAV-27	Punjab selection	Local Multani	Sarhad green	Sabz Pari	051-F1	Feveeri Green	Shehzadi	Hunza	Green star	Anmol
GP(%)	$93.3\pm$	$100.0\pm0.0$	83.0±	73.3±	80.0±	66.7±	$60.0\pm$	$86.7\pm$	73.3±	$93.3\pm$	$80.0\pm$	66.6±	76.6±
	3.55		3.02	2.09	2.02	1.55	1.20	1.55	2.55	3.55	1.02	2.09	2.09
GE(%)	$1.50\pm$	$2.95\pm$	$0.92\pm$	$0.68\pm$	$0.37\pm$	$3.29\pm$	$2.86\pm$	$2.33\pm$	$3.62\pm$	$0.43\pm$	$0.97\pm$	$1.58\pm$	$3.49\pm$
	1.75	1.29	0.03	0.49	0.46	0.50	1.00	1.50	0.54	0.57	0.82	0.44	1.36
GI(%)	$9.3\pm$	$10.0 \pm 0.$	$\textbf{8.0} \pm \textbf{2.0}$	$7.3\pm$	$8.0\pm$	$6.7\pm$	$6.0\pm$	$6.7\pm$	$7.3\pm$	$9.3\pm$	$8.0\pm$	$8.7\pm$	$8.7\pm$
	1.16			2.05	2.04	1.16	2.05	1.16	0.58	1.15	2.04	2.31	2.31
	Varieties— 5	50 mM NaCl											
	NAYAB-F1	Arka anamika	MALAV-27	Punjab selection	Local Multani	Sarhad green	Sabz Pari	051-F1	Feveeri Green	Shehzadi	Hunza	Green star	Anmol
GP(%)	66.7±	60.0±	53.3±	33.3±	33.3±	$20.0\pm$	$13.3\pm$	56.7±	26.7±	74.0±	$13.3\pm$	46.7±	19.0±
	3.55	2.03	2.09	3.09	3.09	2.64	1.55	3.51	6.19	2.02	3.09	3.55	2.4
GE(%)	$2.87\pm$	$2.39\pm$	$2.34\pm$	$2.25\pm$	$1.57\pm$	$1.00\pm$	$0.67\pm$	$2.01\pm$	$1.29\pm$	$2.93\pm$	$0.67\pm$	$2.34\pm$	$1.67\pm$
	1.29	0.93	0.78	1.09	0.87	1.03	0.58	1.73	2.23	1.68	1.16	2.30	0.55
GI(%)	$6.7\pm$	$6.0\pm2.0$	$5.3\pm$	$5.3\pm$	$\textbf{3.3} \pm \textbf{2.3}$	$2.0\pm$	$1.3\pm$	$4.7\pm$	$2.7\pm$	$\textbf{8.0} \pm \textbf{2.0}$	$1.3\pm2.3$	4.7±	$\textbf{3.0} \pm \textbf{2.0}$
	3.05		2.31	1.12		3.46	1.15	3.05	4.62			3.06	
	Varieties— 7	75 mM NaCl											
	NAYAB-F1	Arka anamika	MALAV-27	Punjab selection	Local Multani	Sarhad green	Sabz Pari	051-F1	Feveeri Green	Shehzadi	Hunza	Green star	Anmol
GP(%)	43.7±	36.4±	33.3±	7.3±	$10.0\pm$	6.5±	_	$34.4\pm$	$11.3\pm$	$36.2\pm$	6.7±	$12.0\pm4.64$	_
	4.54	3.09	2.09	3.50	2.06	3.51		3.54	1.46	2.65	1.54		
GE(%)	$2.54\pm$	$2.33\pm$	$2.34\pm$	$0.29\pm$	$0.95\pm$	$1.33\pm$	-	$1.33\pm$	$1.33\pm$	$1.33\pm$	$0.33\pm$	$0.35\pm$	-
	2.07	1.16	1.15	0.02	0.93	1.53		0.58	1.53	1.53	0.58	0.60	
GI(%)	4.7±	$4.71\pm$	$5.3\pm$	$0.67\pm$	$2.0\pm$	$2.7\pm$	-	$2.7\pm$	$2.7\pm$	$2.7\pm$	$0.67\pm$	$\textbf{2.0} \pm \textbf{3.46}$	-
	1.16	2.31	2.31	1.15	2.04	3.05		1.15	3.06	3.05	1.15		
	Varieties— 1	00 mM NaCl											
	NAYAB-F1	Arka anamika	MALAV-27	Punjab selection	Local Multani	Sarhad green	Sabz Pari	051-F1	Feveeri Green	Shehzadi	Hunza	Green star	Anmol
GP(%)	$20.0\pm$	$16.13\pm$	$13.33\pm3.1$	-	-	-	-	$14.0\pm$	-	$16.66 \pm 2.1$	-	-	-
	2.02	1.04						1.04					
GE(%)	$1.0\pm$	$0.29\pm$	$0.67\pm$	-	-	-	-	$0.33\pm$	-	$0.67\pm$	-	-	-
	0.03	0.50	1.16					0.59		1.16			
GI(%)	$2.0\pm$	$0.67\pm$	$1.3\pm$	-	-	-	-	$0.67\pm$	-	$1.3\pm$	-	-	-
	0.04	1.15	2.31					1.55		2.31			

 Table 3

 Initial germination test in petri dishes for selection of okra varieties under different salt levels.

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Effects of the okra varieties on seedling growth, regardless of salt levels.

Varieties	SL cm	RL cm	VI%	SFW(g)	SDW(g)	RFW(g)	RDW(g)
NAYAB-F1 A. anamika MALAV-27	$\begin{array}{c} 12.56\pm 0.3^{a}\\ 11.68\pm 1.28^{ab}\\ 7.49\pm 1.04^{d}\end{array}$	$5.83 \pm 0.15^{a} \ 5.17 \pm 0.15^{b} \ 3.57 \pm 1.67^{abc}$	283.7 260.6 243.2	$\begin{array}{l} 0.290 \pm 0.3^{abc} \\ 0.285 \pm 0.2^{abc} \\ 0.168 \pm 0.23^{a} \end{array}$	$egin{array}{l} 0.024 \pm 0.02^{ m b} \ 0.023 \pm 0.06^{ m a} \ 0.012 \pm 6.2^{ m ab} \end{array}$	$\begin{array}{c} 0.068 \pm 0.01^{a} \\ 0.062 \pm 0.03^{abc} \\ 0.043 \pm 0.05^{a} \end{array}$	$\begin{array}{c} 0.006 \pm 7.81^a \\ 0.005 \pm 2.19^{ab} \\ 0.002 \pm 1.32^a \end{array}$
051-F1 Shehzadi CV%	$\begin{array}{l} 9.74 \pm 0.75^c \\ 11.27 \pm 1.31^{ab} \\ 37.29 \end{array}$	$\begin{array}{l} 4.87 \pm 0.06^{ab} \\ 5.20 \pm 0.20^{b} \\ 64.21 \end{array}$	254.9 267.6 29.55	$\begin{array}{c} 0.227 \pm 0.23^{b} \\ 0.238 \pm 0.3^{abc} \\ 86.5 \end{array}$	$\begin{array}{l} 0.014\pm 0.09^{ab}\\ 0.021\pm 0.02^{ab}\\ 102.04 \end{array}$	$\begin{array}{l} 0.047 \pm 0.03^{ab} \\ 0.056 \pm 0.03^{bcde} \\ 60.82 \end{array}$	$\begin{array}{c} 0.004 \pm 1.92^a \\ 0.003 \pm 2.49^a \\ 73.26 \end{array}$

Mean in the similar column tracked by the equal letter are not significantly different at p < 0.05, according to Duncan's multiple range test.

 Table 5

 Seedling growth assessment of okra varieties under salt stress.

	Varieties	NaCl levels	(mM)					
Trait		0	75	Change%	100	Change %	125	Change%
SL	NAYAB-F1	14.53	13.17	-9.36	10.60	-27.05	6.23	-57.12
	Arka anamika	13.83	12.37	-10.55	8.76	-36.65	5.52	-60.09
	MALAV-27	10.87	8.93	-17.84	6.46	-40.57	4.07	-68.12
	051-F1	12.77	11.20	-12.29	7.91	-38.06	3.53	-67.52
	Shehzadi	13.33	11.75	-11.85	8.43	-36.75	5.27	-60.46
SFW	NAYAB-F1	0.542	0.462	-14.76	0.252	-53.50	0.172	-68.26
	Arka anamika	0.515	0.428	-16.89	0.237	-53.98	0.207	-59.81
	MALAV-27	0.475	0.378	-20.42	0.166	-62.52	0.101	-78.73
	051-F1	0.527	0.427	-18.97	0.241	-54.26	0.162	-69.25
	Shehzadi	0.463	0.381	-17.71	0.223	-51.83	0.149	-67.81
SDW	NAYAB-F1	0.052	0.043	-17.30	0.029	-46.15	0.016	-69.23
	Arka anamika	0.061	0.050	-18.03	0.031	-49.18	0.018	-70.37
	MALAV-27	0.041	0.029	-29.26	0.018	-56.09	0.010	-75.60
	051-F1	0.027	0.021	-22.22	0.017	-37.03	0.008	-72.97
	Shehzadi	0.037	0.030	-18.91	0.021	-43.24	0.010	-70.49
Rl	NAYAB-F1	4.87	4.16	-14.57	3.37	-30.94	2.01	-58.72
	Arka anamika	3.72	3.14	-15.59	2.53	-31.99	1.47	-60.48
	MALAV-27	3.87	3.08	-20.41	2.12	-45.21	1.35	-65.11
	051-F1	4.76	3.86	-18.90	2.68	-43.69	1.68	-64.70
	Shehzadi	5.63	4.68	-16.87	3.73	-33.74	2.11	-62.52
RFW	NAYAB-F1	0.084	0.074	-11.90	0.052	-38.09	0.035	-58.34
	Arka anamika	0.083	0.070	-15.66	0.048	-42.16	0.030	-63.51
	MALAV-27	0.079	0.064	-18.99	0.041	-48.10	0.025	-68.32
	051-F1	0.073	0.061	-16.43	0.044	-39.72	0.026	-64.38
	Shehzadi	0.074	0.062	-16.21	0.045	-39.18	0.027	-63.58
RDW	NAYAB-F1	0.064	0.057	-10.93	0.038	-40.62	0.024	-62.50
	Arka anamika	0.071	0.063	-11.26	0.039	-45.07	0.024	-66.19
	MALAV-27	0.062	0.050	-19.35	0.028	-54.83	0.015	-75.81
	051-F1	0.065	0.053	-18.46	0.034	-47.69	0.021	-67.69
	Shehzadi	0.076	0.064	-15.79	0.041	-46.05	0.026	-66.78

%) and root length (58.72 %). A similar trend was recorded in the other growth traits evaluated, including shoot fresh weight (SFW), and shoot dry weight (SDW) for the studied varieties.

## 3.4. Physiochemical assessment

## 3.4.1. Sugar

The evaluations of varieties for sugar content revealed that "NAYAB-F1" had the highest sugar concentration, while "MALAV-27" had the lowest. With increasing levels of salinity, NAYAB-F1 showed a progressive increase in sugar content 0.36, 0.54, 0.68, and 0.74  $\mu$ g/g at 0.0, 75, 100, and 125 mM NaCl but other varieties' sugar content increased with 100 mM NaCl and decreased with 125 mM NaCl (Table 6).

### 3.4.2. Proline

Variety and salt content also affected the proline value. "MALAV-27" had the lowest proline values of 0.29, 0.22, and 0.10 µmol/g, and "NAYAB-F1" had the highest values of 0.48, 0.49, and 0.51 µmol/g across all salinity levels (Table 6).

## 3.4.3. Photosynthetic pigments

Consequently, the values of chlorophyll "a" and "b" were influenced differently by the variety and salt content. Chlorophyll "a" and "b" values were reduced in stressed seedlings. At the highest concentration of NaCl (125 mM), "NAYAB-F1" demonstrated the highest

#### Table 6

Responses of okra varieties to salt stress to the content of sugar, proline, and chlorophyll "a" an
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Varieties	Salt levels (mM)	Sugar µg∕g	Proline µmol/g	Chl "a" mg/g	Chl "b" mg/g
NAYAB-F1	0	$0.36\pm0.02$	$0.30\pm1.13$	$15.66\pm0.59$	$16.78\pm2.06$
	75	$0.54\pm0.01$	$0.48 \pm 2.51$	$12.30\pm0.91$	$14.57\pm0.54$
	100	$0.68\pm0.01$	$0.49 \pm 1.01$	$9.80\pm0.18$	$12.42\pm0.57$
	125	$0.74\pm0.02$	$0.51\pm0.01$	$\textbf{7.97} \pm \textbf{0.26}$	$9.56 \pm 1.49$
Arka anamika	0	$0.37\pm0.08$	$0.32\pm0.85$	$14.91 \pm 1.36$	$15.41\pm0.60$
	75	$0.52\pm0.46$	$0.40\pm0.02$	$11.13\pm0.96$	$13.38\pm1.86$
	100	$0.58\pm0.64$	$0.45\pm0.05$	$7.69\pm0.75$	$10.60\pm1.30$
	125	$0.36\pm0.53$	$0.28\pm0.49$	$5.25 \pm 1.49$	$7.81 \pm 1.14$
MALAV-27	0	$0.26\pm0.02$	$0.11\pm0.24$	$11.93\pm0.91$	$11.72\pm0.13$
	75	$0.30\pm0.02$	$0.29\pm0.01$	$7.89 \pm 1.22$	$8.32\pm0.79$
	100	$0.32\pm0.52$	$0.22\pm0.28$	$5.83 \pm 1.89$	$5.97 \pm 0.381$
	125	$0.20\pm0.53$	$0.10\pm0.43$	$2.60 \pm 1.81$	$3.18\pm2.50$
Shehzadi	0	$0.35 \pm 1.50$	$0.28\pm0.61$	$14.13\pm0.28$	$14.84\pm0.91$
	75	$0.46\pm0.31$	$0.38\pm0.24$	$10.29 \pm 1.90$	$12.63\pm2.76$
	100	$0.48\pm0.33$	$0.42\pm0.36$	$6.50\pm0.63$	$9.45 \pm 1.94$
	125	$0.32\pm0.51$	$0.21\pm0.50$	$4.14\pm0.72$	$6.55\pm0.41$
051-F1	0	$0.33\pm0.03$	$0.27\pm0.21$	$14.97\pm0.16$	$13.75\pm1.78$
	75	$0.40\pm0.01$	$0.32\pm9.16$	$8.02\pm0.38$	$10.43\pm0.56$
	100	$0.41\pm0.01$	$0.38\pm0.61$	$5.05\pm0.87$	$7.97 \pm 0.36$
	125	$\textbf{0.29} \pm \textbf{0.44}$	$0.20\pm0.46$	$3.06 \pm 1.29$	$\textbf{5.94} \pm \textbf{1.81}$

values of chlorophyll "a" (7.97 mg/g) and chlorophyll "b" (9.56 mg/g) and "MALAV-27" exhibited the lowest values of chlorophyll "a" (2.60 mg/g) and chlorophyll "b" (3.18 mg/g) compared to the control (Table 6).

## 4. Discussion

This study aimed to identify salt tolerance levels in thirteen okra varieties during germination and early seedling growth. The results showed that salt stress reduced germination percentage (GP), germination index (GI), germination energy (GE), and vigor index (VI) in okra varieties. The varieties which germinate at a high salinity level can be used as potential contributors to salinity tolerance. One of the most sensitive stages to salt during plant growth and development is the seed germination stage. The germination of seeds is greatly inhibited by salt stress [39]. When the seeds of okra are exposed to adverse conditions, such as salt and low temperatures, their GP decreases further [40]. In a salty environment, seed germination can be suppressed for various reasons. Salinity delays seedling growth, disrupts important basic cellular processes, and affects morphology throughout the seedling stage by changing the rate of emergence and growth in most glycophytes [41]. The study also assessed varying levels of stress tolerance among the varieties, with the 'NAYAB-F1' variety being the tolerant variety with 66.7 %, 43.7 %, and 20.0 % GP at 50, 75, and 100 mM salinity levels, respectively. The GPs of six okra varieties were 25.33 %–88.0 % [42]. The varieties that are minimally affected by salt stress may be probable sources of genes for salt tolerance, such varieties are significant in plant breeding programs as confirmed in tomato [43]. The results indicate that saline stress affects all the traits studied differently, the effects being in most cases proportional to the level of stress applied. These results are consistent with previous studies examining the effect of salt stress on seed germination in *Cucurbita maxima* and rice seeds [44,45], similar results were also highlighted in *Cicer arietinum* [46].

Salt stress was evident early in seedling growth, with a decrease in the fresh and dry weight of roots and shoots of the varieties studied. High-stress levels of 125 mM significantly affected seedling growth, but the rate of the effect varied among different varieties. 'NAYAB-F1' showed the highest length, fresh and dry weight of roots and shoots, providing evidence for its salt tolerance ability, whereas the lowest values were recorded in variety 'MALAV-27' indicating susceptibility to salinity. Related to the above mentioned results, all the noted agronomic traits were greatly affected by salinity stress, particularly at high levels. The negative effect of salt stress on agronomic traits has been previously evidenced in maize variety subjected to drought stress [47], previous reports underline the significant effect of the genotype on the response to salt stress for several crop species [48–50].

The study also found that 'NAYAB-F1' varieties had the highest proline content in leaves under salt stress, while 'MALAV-27' had the lowest. Our research identified NAYAB-F1 as a salt-tolerant variety and confirmed earlier findings of high proline content in salt-tolerant varieties of plant species such as tomatoes, melons, and potatoes [51–53]. The study also found that salt stress produced an increase in sugar content in stressed varieties. Plants utilize sugar as a crucial osmotic regulator to withstand stressful conditions, and their accumulation in plants under salinity stress serves as an adaptive mechanism [54]. The results of this work are consistent with reports of high sugar content in salinity-stressed okra and fava beans, respectively [55–57]. The response to high salinity could involve a decrease in chlorophyll content in various plant species, including the pepper and winter squash plants [58].

### 5. Conclusion

The experiment revealed a strong link between salt stress and reduced germination percentage and fresh and dry weight of okra variety plants, which can be used to calculate salt tolerance potential. The overall data indicate the possibility of conducting the early selection of a salt-tolerant okra variety depending on germination and seedling growth performance under salt stress. According to the

results of our study, 'NAYAB-F1', 'Arka anamika', and 'Shehzadi' varieties are recommended as salt-tolerant varieties.

#### CRediT authorship contribution statement

Hayat Ullah: Writing – original draft, Investigation. Tour Jan: Writing – review & editing, Supervision.

#### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Hayat Ullah reports equipment, drugs, or supplies was provided by The University of Haripur. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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