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Abatement of salinity stress effect through bioseed priming in okra (*Abelmoschus esculentus*)

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Abstract
The present research was carried out to study the effect of seed priming with bacterial suspension *viz. Bacillus sonorensis* KM Root 3 (MW331689) on performance of seed quality parameters *viz.* speed of germination, germination percentage, root length, shoot length, dry matter production and vigour index under salinity stress. Results indicated that increased salinity reduced various physiological parameters *viz.* germination percentage, seedling length and vigour index; seed priming with *Bacillus sonorensis* in its undiluted form (T_2C_1) was found to abate the effect of salinity stress on seed germination traits at all salinity level and performed equally good as that of control. From this study, it was concluded that if conditions arise to raise bhendi under salinity conditions, seed priming with *Bacillus sonorensis* could be an important technique to compete the stress and make the seeds comfortable to produce normal seedlings.

Keywords: Okra, seed, biopriming, salinity stress, germination, seedling vigour

1. Introduction

Okra (*Abelmochus esculentus*) is one of the most important vegetable crop grown from tropical to subtropical and warmer parts of temperate Asia. It is commercially grown in India, Turkey, Iran, West Africa, Bangladesh, Afghanistan, Pakistan, West Bengal, Burma, Japan, Malaysia, Brazil and the southern USA. It is one of the important vegetable crop grown in India. It is a multipurpose crop meant for its tender fruits and delicious pods. The dried seeds provide oil, protein and a coffee additive or substitute. Dry okra seeds have an oil content of 18-20% and a crude protein content of 20-23%. Okra is rich in essential macro nutrients and some of the micronutrients.

The plant growth and productivity are affected by both biotic and abiotic stresses. Abiotic stresses include heat, cold, drought and salinity (Allakhverdiev *et al*., 2000) [3]. Salt stress exerts more drastic effects in terms of low productivity in arid, semiarid and coastal regions (Munns, 2002) [23]. Lack of proper irrigation and drainage management, low precipitation, high evaporation and saline water irrigation all contributes to the salinization of land (Munns and Tester, 2008) [25]. Effects of salinity comes from the complex interactions between morphological, physiological, and biochemical processes (Akbarimoghaddam *et al*., 2011) [4]. Rouhi *et al.* (2011) [29] stated that there is reduction in the germination percentage and increase in germination time due to increase in salinity. Mahajan and Tuteja (2005) [19] stated that salinity has a deleterious impact on physiological processes, nutritional balance, membrane characteristics, cellular homeostasis, enzymatic and metabolic activities, as well as seed germination and plant growth. High concentration of salt accumulation outside the roots, reduces the water potential, makes it harder for the plants to take water and it results in osmotic stress. In leaves, higher salt concentration causes stomatal closure, impairment of electron transport and reduced photosynthesis (Deinlein *et al.*, 2014) [9]. Munns (2005) [24] theorised that salinity damage occurs in two phases in plants. The first phase occurs rapidly after exposure and is due to an osmotic effect, while the second phase is a slower process, is due to the accumulation of salt ions, mainly in older leaves.

Seed priming is a pre-sowing seed enhancement technique in which the seed is soaked in some kind of solution which enables the seed to emerge fast, maintain uniformity and increase yield potential. It is a cost-effective way to enhance germination and seedling characteristics (Khanal *et al*., 2022) [13]. Seed priming has been used to shorten the time between the sowing of seeds and the emergence of seedlings and to bring uniform emergence (Parera *et al.*, 1994) [27]. Sedghi *et al.* (2010) [31] reported that priming is one of the seed enhancement techniques for improving the performance of seeds under stressful conditions like salinity, temperature and drought stress.

Among different types of priming, biopriming is one of the new seed enhancement techniques in which the seeds are pre-soaked in the bio or organic solution or suspension for definite period of time to allow the bacteria or microbes to impregnate the seed (Abuamsha *et al.*, 2011) [2]. Bio seed priming results in more rapid growth and increased seedling vigour. *Bacillus amyloliquefaciens* improved the seed quality parameters such as speed of germination, germination percentage, root length, shoot length, dry matter production and vigour index in maize (Anbalagan *et al*., 2020) [5]. *Bacillus sp*. could be an effective approach for maize seed biopriming to overcome the inhibitory effects of salinity stress and promoting seed germination and seedling growth (Li *et al*., 2021) [17]. *Bacillus aryabhattai* has the ability to augment salt stress tolerance in wheat under saline environmental conditions (Mehmood *et al*., 2021) [21]. Beneficial endophytes inoculation is an important approach to help plants withstand environmental stresses, including salinity (Lastochkina *et al*., 2019) [15]. McQuilken *et al.* (1998) [20] stated that the ideal conditions for the bacterial inoculation and colonization is created by seed priming. Many bacterial strains play an important role in salinity stress tolerance. In this study, *Bacillus sonorensis* is used as bioinoculants to know its efficacy in abating salinity stress.

This present study was aimed at optimizing the concentration of bio-inoculant *Bacillus sonorensis* KM Root 3 in bhendi to alleviate the salinity stress, based on various seed physiological parameters.

2. Materials and methods

An experiment on alleviation of salinity stress and optimization of concentration of bio-inoculants through biopriming in Bhendi Hybrid CO 4 was conducted at the laboratory of Department of Seed Science and Technology, Tamil Nadu Agriculture University, Coimbatore during 2022. Seeds were collected from Department of Vegetable Science, TNAU, Coimbatore and bacterial suspension of bio-inoculant, *Bacillus sonorensis* KM Root 3 (MW331689) from Department of Plant Pathology, TNAU, Coimbatore. Seeds of Bhendi Hybrid CO 4 were bioprimed at dilution ratios of 0(undiluted),1:1,1:2 and 1:3 for 12 hrs (Nirmala *et al*., 2006) [26]. The bioprimed seeds along with control and hydroprimed seeds were studied for its effect under different salinity levels (0mM, 20mM, 40mM, 60mM and 80mM NaCl). Hydropriming was performed for 12 hrs in a seed to solution ratio of 1:2 (Table 1). After soaking for 12 hrs, the solution was drained out and the seeds were shade dried in order to bring them back to their original moisture content. The experiment was conducted using a Factorial Completely Randomized Design (FCRD) with three replications.

Table 1: Treatment details

Treatments	Salinity levels (mM NaCl)	Duration of soaking	Volume of soaking (v:v)
Control	0,20,40,60,80	-	-
Hydropriming		12 hrs	1:2
Bacillus sonorensis - UD			
Bacillus sonorensis - 1:1			
Bacillus sonorensis - 1:2			
Bacillus sonorensis - 1:3			

The standard germination test was carried out by using between paper method (ISTA, 2019) [11]. The germination percentage was calculated 21 days after sowing and expressed in percentage. Root length and shoot length were measured for all normal seedlings and mean values were calculated and expressed in centimetre. For dry matter production, the normal seedlings were shade dried for 24 hrs and then dried in a hot air oven at 80º C for 24 hrs. Dried seedlings were weighed and mean values were calculated and reported in g per 10 seedlings. Vigour index I (Abdul-Baki and Anderson, 1973) [1] and Vigour index II (Reddy and Khan, 2001) [28] of the seedlings were computed by using the following formulae and the mean values were expressed in whole number.

Vigour index I = Germination (%) x Total seedling length (cm)

Vigour index II = Germination (%) x Dry matter production (g per 10 seedlings)

According to Maguire (1962) [18], speed of germination was calculated by using petri plate method.

3. Results and discussion

The results obtained from the study conducted in okra to alleviate the effect of salinity stress through biopriming with bio-inoculants is presented below. In general, as salt stress increases, speed of germination, germination percentage, root length, shoot length, dry matter production and seed vigour index I and II, all decreases. There is significant decrease in speed of germination with increase in salinity stress. Speed of germination of bioprimed seeds were greater than control (T_0) in all salinity levels and the maximum speed of germination was observed in seeds primed with undiluted broth (T_2C_1) in all salinity levels (Table 2). When compared to the control (non-primed), seed priming with bio-inoculant has significant difference on germination percentage of okra seeds under salinity stress. The results of seed biopriming with different concentrations of *Bacillus sonorensis* revealed that the seeds primed with undiluted broth (T_2C_1) recorded the highest germination in all salinity levels compared to control (T_0), which recorded the lower germination in all salinity levels (Table 2) (Fig. 2). Biopriming resulted in lower reduction in germination percentage between two different salinity levels when compared to control which has higher reduction in germination percentage between two different salinity levels. Seed priming with endophytic *B. subtilis* improved *Phaseolus vulgaris* L. (common bean) seed germination and plant growth under both saline and non-saline conditions (Lastochkina *et al*., 2021) [16]. Endophytes colonize the roots of cotton and helps in improving the seed germination and growth parameters by improving the physiological functions like mitigating the osmotic stress, increasing the K^+ absorption and decreasing the Na^+ absorption (Saleem *et al*., 2021) [30]. This is also similar in shoot length, root length, seedling length, dry matter production of okra seeds under different salinity stress. Shoot length of bioprimed seeds were greater than control (T_0) in all salinity levels and the maximum shoot length was observed in seeds primed with undiluted broth (T_2C_1) in all salinity levels (Table 3). Bacterial endophytes improved the seedling performance in lucerne by increasing germination percentage, root length, shoot length, seed vigour and seedling fresh weight (Ansari *et al*., 2017) [6]. Root length of bioprimed seeds were greater than control (T_0) in all salinity levels and the maximum root length was recorded in seeds primed with undiluted broth (T_2C_1) in all salinity levels (Table 3). Kumar *et al*. (2021) [14] reported that *Bacillus sp.* has the potential for promoting plant growth attributes mainly plant height and root length by production of IAA and mitigating the deleterious effects of salinity in rice. The reduction in root length may be due to the physiological drought caused by the salt deposit in root cells, which results in reduced cell division and root growth (Munns, 1993) [22]. Seedling length is the sum total of shoot and root length. Since, maximum shoot length and root length were recorded in seeds primed with undiluted broth (T_2C_1) in all salinity levels, seedling length were also maximum in seeds primed with undiluted broth (T_2C_1) in all salinity levels. Significant reduction in dry matter production of okra seedlings were observed with increase in salinity levels. However, dry matter production of bioprimed seeds were significantly higher when compared to control (T_0) in all salinity levels and the highest dry matter production was recorded in seeds primed with undiluted broth (T_2C_1) in all salinity levels (Table 5). Cicek and Cakirlar (2002) [7] also reported that there is reduction in fresh and dry weight of maize seedling under saline conditions. There is significant decrease in seed vigour index I and seed vigour index II with increase in salinity stress. This is because of that seed vigour index I is the product of germination percentage and seedling length and seed vigour index II is the product of germination percentage and dry matter production. Seed vigour index I and II of seeds primed with undiluted broth (T_2C_1) was found to be performed significantly better when compared control (T_0) in all salinity levels (Table 4) (Fig. 1). Khajeh-Hosseini *et al.* (2003) [12] reported that the reduction in germination percentage and seedling vigour might be due to toxic effects of Na^+ and Cl^- ions present in the salt solution. On the otherhand, water uptake by seed is inhibited by an external osmotic potential which is created by salt solution. Bacterial endophytes may produce IAA, which enhance the survival of seedlings, especially during the stress conditions (Egamberdieva, 2009) [10]. Conrath *et al*. (2006) [8] stated that there is faster activation of abiotic stress defence responses in bioprimed plants. Thus, endophytes act as a plant defense agent by enhancing plant growth, productivity and tolerance in saline environment.

Table 2: Standardization of concentration of *Bacillus sonorensis* and its effect on speed of germination and germination percentage in Okra

Treatments	Speed of germination						Germination percentage					
	Salinity level						Salinity level					
	S_0	S_1	S_2	S_3	S_4	Mean	S_0	S_1	S_2	S_3	S_4	Mean
T_0	13.2	11.3	8.9	7.6	6.4	9.5	81(64.16)	52(46.15)	41(39.82)	20(26.57)	7(15.34)	40(39.23)
T_1	14.8	13.4	10.6	8.0	7.0	10.7	80(63.44)	63(52.54)	53(46.72)	44(41.56)	12(20.27)	50(45.00)
T_2C_1	17.4	16.2	14.7	12.0	8.8	13.8	91(72.54)	83(65.65)	76(60.67)	67(54.94)	36(36.87)	70(56.79)
T_2C_2	16.5	15.2	13.6	11.0	8.3	12.9	83(65.65)	76(60.67)	71(57.42)	55(47.87)	27(31.30)	62(51.94)
T_2C_3	16.1	14.0	12.6	10.7	8.0	12.3	84(66.42)	79(62.73)	67(54.94)	52(46.15)	32(34.45)	63(52.53)
T_2C_4	15.4	13.2	11.9	9.9	7.7	11.6	87(68.87)	81(64.16)	73(58.70)	51(45.57)	25(30.00)	64(53.13)
Mean	15.5	13.8	12.0	9.9	7.7		84(66.42)	72(58.05)	64(53.13)	48(43.86)	23(28.66)	

	T	S	T*S
S.Ed	0.17	0.15	0.37
CD (P=0.05)	0.34	0.31	0.76

	T	S	T*S
S.Ed	1.63	1.49	3.64
CD (P=0.05)	3.26	2.98	7.29

T_0	-	Control	S_0	-	0mM NaCl
T_1	-	Hydropriming	S_1	-	20mM NaCl
T_2C_1	-	Seeds primed with *B. sonorensis* at undiluted broth	S_2	-	40mM NaCl
T_2C_2	-	Seeds primed with *B. sonorensis* at 1:1 dilution ratio	S_3	-	60mM NaCl
T_2C_3	-	Seeds primed with *B. sonorensis* at 1:2 dilution ratio	S_4	-	80mM NaCl
T_2C_4	-	Seeds primed with *B. sonorensis* at 1:3 dilution ratio			

Table 3: Standardization of concentration of *Bacillus sonorensis* and its effect on root length and shoot length in Okra

Treatments	Root length (cm)						Shoot length (cm)					
	Salinity level						Salinity level					
	S_0	S_1	S_2	S_3	S_4	Mean	S_0	S_1	S_2	S_3	S_4	Mean
T_0	13.1	10.0	8.0	5.1	3.7	8.0	15.8	13.4	8.5	6.8	5.1	9.9
T_1	13.0	9.9	7.2	5.1	3.7	7.8	16.3	12.6	9.6	7.0	5.3	10.1
T_2C_1	14.1	12.8	10.9	9.0	7.0	10.8	17.5	14.7	12.5	8.5	6.9	12.0
T_2C_2	13.5	11.9	10.0	8.1	5.8	9.9	16.1	14.3	11.1	7.7	6.1	11.1
T_2C_3	13.2	12.0	10.2	8.0	6.3	9.9	16.5	14.3	11.5	8.1	6.0	11.3
T_2C_4	13.6	11.4	10.7	7.9	6.1	9.9	15.6	14.4	12.3	7.4	6.1	11.2
Mean	13.4	11.3	9.5	7.2	5.4		16.3	13.9	10.9	7.6	5.9	

	T	S	T*S
S.Ed	0.23	0.21	0.51
CD (P=0.05)	0.46	0.42	1.03

	T	S	T*S
S.Ed	0.29	0.26	0.64
CD (P=0.05)	0.57	0.52	1.27

T_0	**-**	**Control**	**S_0**	**-**	**0mM NaCl**
T_1	-	Hydropriming	S_1	-	20mM NaCl
T_2C_1	-	Seeds primed with *B.sonorensis* at undiluted broth	S_2	-	40mM NaCl
T_2C_2	-	Seeds primed with *B.sonorensis* at 1:1 dilution ratio	S_3	-	60mM NaCl
T_2C_3	-	Seeds primed with *B.sonorensis* at 1:2 dilution ratio	S_4	-	80mM NaCl
T_2C_4	-	Seeds primed with *B.sonorensis* at 1:3 dilution ratio			

Table 4: Standardization of concentration of *Bacillus sonorensis* and its effect on Vigour index I and II in Okra

Treatments	**Vigour index I**						**Vigour index II**					
	Salinity level						**Salinity level**					
	S_0	**S_1**	**S_2**	**S_3**	**S_4**	**Mean**	**S_0**	**S_1**	**S_2**	**S_3**	**S_4**	**Mean**
T_0	2349	1214	684	238	58	909	26.4	16.2	11.4	3.0	0.30	11.5
T_1	2344	1409	896	529	105	1057	26.6	19.8	14.8	7.0	1.3	13.9
T_2C_1	2873	2275	1783	1165	506	1721	31.6	26.4	22.4	16.2	7.3	20.8
T_2C_2	2447	1994	1489	868	319	1423	27.4	24.3	19.9	13.3	5.0	18.0
T_2C_3	2493	2068	1450	837	396	1449	27.5	25.0	19.4	12.2	6.2	18.0
T_2C_4	2533	2096	1717	774	307	1485	28.6	25.6	21.3	11.7	4.5	18.3
Mean	2506	1843	1337	735	282		28.0	22.9	18.2	10.5	4.1	

	T	**S**	**T*S**
S.Ed	48.45	44.22	108.33
CD (P=0.05)	96.91	88.46	216.69

	T	**S**	**T*S**
S.Ed	0.58	0.53	1.29
CD (P=0.05)	1.15	1.05	2.58

T_0	**-**	**Control**	**S_0**	**-**	**0mM NaCl**
T_1	-	Hydropriming	S_1	-	20mM NaCl
T_2C_1	-	Seeds primed with *B.sonorensis* at undiluted broth	S_2	-	40mM NaCl
T_2C_2	-	Seeds primed with *B.sonorensis* at 1:1 dilution ratio	S_3	-	60mM NaCl
T_2C_3	-	Seeds primed with *B.sonorensis* at 1:2 dilution ratio	S_4	-	80mM NaCl
T_2C_4	-	Seeds primed with *B.sonorensis* at 1:3 dilution ratio			

Table 5: Standardization of concentration of *Bacillus sonorensis* and its effect on dry matter production in Okra

Treatments	**Dry matter production (g / 10 seedlings)**					
	Salinity level					
	S_0	**S_1**	**S_2**	**S_3**	**S_4**	**Mean**
T_0	0.324	0.312	0.277	0.144	0.042	0.220
T_1	0.332	0.317	0.276	0.160	0.114	0.240
T_2C_1	0.348	0.320	0.294	0.243	0.201	0.281
T_2C_2	0.332	0.320	0.282	0.242	0.185	0.272
T_2C_3	0.327	0.319	0.290	0.234	0.192	0.272
T_2C_4	0.329	0.315	0.285	0.230	0.173	0.266
Mean	0.332	0.317	0.284	0.209	0.151	

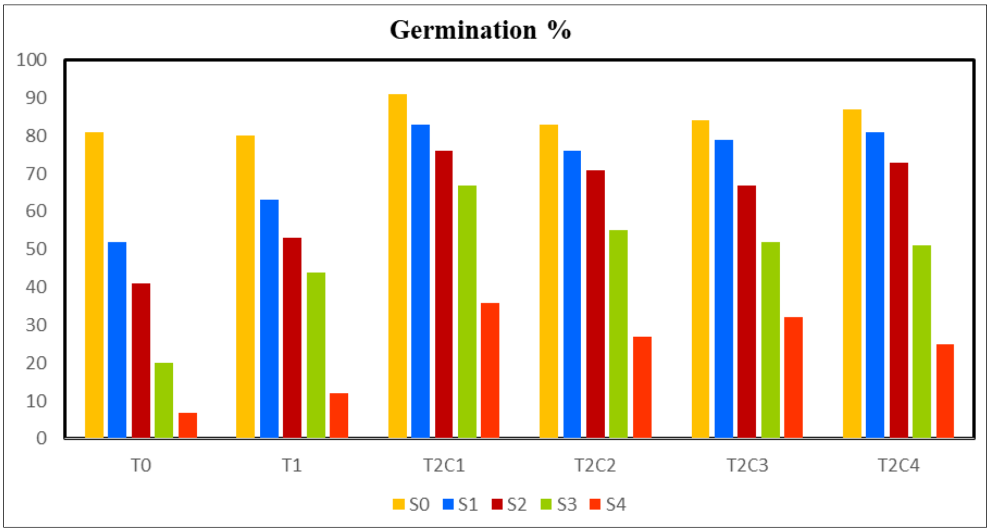
	T	**S**	**T*S**
S.Ed	0.0068	0.0062	0.0151
CD (P=0.05)	0.0135	0.0123	0.0302

T_0	**-**	**Control**	**S_0**	**-**	**0mM NaCl**
T_1	-	Hydropriming	S_1	-	20mM NaCl
T_2C_1	-	Seeds primed with *B.sonorensis* at undiluted broth	S_2	-	40mM NaCl
T_2C_2	-	Seeds primed with *B.sonorensis* at 1:1 dilution ratio	S_3	-	60mM NaCl
T_2C_3	-	Seeds primed with *B.sonorensis* at 1:2 dilution ratio	S_4	-	80mM NaCl
T_2C_4	-	Seeds primed with *B.sonorensis* at 1:3 dilution ratio			

a. Non-primed okra seedlings at different salinity levels
b. Hydroprimed okra seedlings at different salinity levels
c. Bioprimed okra seedlings with *Bacillus sonorensis* in its undiluted form at different salinity levels

S0 - 0mM NaCl S1 - 20mM NaCl
S2 - 40mM NaCl S3 - 60mM NaCl
S4 - 80mM NaCl

Fig 1: Seedling vigour influenced by *Bacillus sonorensis* bio-primed seedlings compared to control and hydroprimed seedlings on 21st day



T_0	-	Control	S_0	-	0mM NaCl
T_1	-	Hydropriming	S_1	-	20mM NaCl
T_2C_1	-	Seeds primed with *B.sonorensis* at undiluted broth	S_2	-	40mM NaCl
T_2C_2	-	Seeds primed with *B.sonorensis* at 1:1 dilution ratio	S_3	-	60mM NaCl
T_2C_3	-	Seeds primed with *B.sonorensis* at 1:2 dilution ratio	S_4	-	80mM NaCl
T_2C_4	-	Seeds primed with *B.sonorensis* at 1:3 dilution ratio			

Fig 2: Effect of seed biopriming with different concentrations of *Bacillus sonorensis* on germination percentage in Okra at different salinity levels

4. Conclusion
Seed biopriming is one of the seed enhancement technique which is of low cost, easy to conduct with minimal or no risk will improve various seed quality parameters even under different salinity stress. From this present study, it was concluded that, the seeds primed with *Bacillus sonorensis* without any dilution (T_2C_1) have expressed superior performance in all the seed quality parameters under all salinity stress level. Therefore, seed biopriming with *Bacillus sonorensis* without any dilution can be used as one of the best seed enhancement technique to abate the effect of salinity stress in okra (*Abelmoschus esculentus*). It needs further investigation in field trails, since these results were obtained from the controlled atmosphere of germination chamber. This approach can be used for commercial cultivation by farmers, if the field trails provide clarity about the enhancement of seed during salinity stress.

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