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Alleviation of salinity stress *via* seed priming in tomato (*Solanum lycopersicum*) with *Bacillus paralicheniformis*

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Abstract

The present study was undertaken to assess the efficacy of biopriming of tomato seeds with different dilutions of *Bacillus paralicheniformis* suspension viz., 1:1 dilution suspension, 1:2 dilution suspension and 1:3 dilution suspension against salinity stress conditions. The assessments were made on various seed quality parameters such as speed of germination, germination per cent, length of root and shoot, dry matter production and vigour index. The results of this study revealed that the biopriming with undiluted bacterial suspension showed improvement in seedling performance when compared to control, hydropriming and other dilution suspensions of *Bacillus paralicheniformis*. Also, the increase in salinity stress decreased the physiological performance of tomato seeds. Hence, if tomato crop is to be raised in salinity stress conditions, seed biopriming with *Bacillus paralicheniformis* can be adopted to overcome the stress and to produce normal healthy seedlings.

Keywords: Biopriming, bacterial endophytes, *Bacillus paralicheniformis*, salinity stress

Introduction

Tomato is one of the most popular and frequently cultivated crops in the world, tomatoes are ranked second in significance in several nations. Tomato is consumed either as raw or cooked and is also very important crop for processing in the form of soups, juice, ketchup, puree, paste and powder. Tomato adds variety of colours and flavour to food. Tomato is very well known for lycopene pigment. Pink and yellow colours are due to presence of anthocyanin and carotene pigments. Tomato is a rich source of ascorbic acid, which varies from 15-31 mg per 100 g of fresh weight.

Area under tomato in India is 26.48 million ha and productivity is 320.47 MT, (Horticultural Statistics Division 2019-20) The major tomato producing states in the country are Madhya Pradesh, Andhra Pradesh, Karnataka, Gujarat, Odisha, Chhattisgarh, West Bengal, Tamil Nadu, Bihar, Maharashtra, Uttar Pradesh, Haryana and Telangana. These states account for about 90% of the total production of the country. Area under tomato cultivation in Tamil Nadu is 3603 ha and production are 33021q and productivity is 9.16 q/ha.

Ensuring adequate food production is the major issue in the context of increasing human population, limit to the areas of new land that can be cultivated and loss of existing cultivated lands to abiotic stresses. Now-a-days salinity is one of the major abiotic stresses that reduce seed germination, plant growth and crop productivity. Salinity consistently has the greatest impact in reducing the area of cultivated land, often due to inappropriate irrigation techniques. More than one billion hectares of the world's agricultural land area is affected by salinity. Every year there is an increase of at least 10% of saline land among which irrigated agricultural lands are severely affected In India, seven million hectares of land are under salinity stress across Indo - Gangetic plain, and in Gujarat, Rajasthan, and Madhya Pradesh. Salinity is a dominant abiotic stress that affects the productivity and quality of crop (Chintan *et al.*, 2021) ^[9].

As with other crops, the global production of tomato is threatened by certain biological stresses (pests, plant diseases and weeds) and non-biological stresses (such as salinity, drought, floods, cold and heat stress). Nowadays, the excessive use of chemical fertilizers in tomato production, in order to increase yield, has resulted in environmental pollution and danger on the health of consumers. Endophytes represent an eco-friendly option for the promotion of plant growth and for serving as sustainable resources of novel bioactive natural products. One of the alternative ways to restore normal plant growth under salinity stress may be to use endophytes to stimulate plant growth.

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Seed bio-priming is the most efficient and productive method as it is supposed to utilize bioagents judiciously for enhancing crop productivity (Singh *et al.*, 2019) [19]. Seed priming is soaking the seeds in any solution containing the required priming agent followed by redrying the seeds which results into start of germination process except radicle emergence. Biopriming is a new technique of seed enhancement in which the seeds are soaked in the bacterial suspension for precalculated period of time to allow the bacterial imbibition into the seed (Abuamsha *et al.*, 2011) [4]. It is a process of biological seed treatment that refers combination of seed hydration (physiological aspects) and inoculation (biological aspects) to protect seed (Callan *et al.*, 1997) [6].

Materials and Methods

Seed material

Pure seeds of tomato cv. PKM 1 with 8% of seed moisture and 85% germination was obtained from the Department of Vegetable Sciences, Horticultural College and Research

Institute, Periyakulam.

Bioinoculant culture and preparation of bioinoculants

The salinity tolerance bacterial endophytes culture *Bacillus paralicheniformis* was obtained from the Department of Plant Pathology, TNAU, Coimbatore. Liquid suspension of bacterial endophytes was prepared in Luria- Bertani broth for 48 hours and the concentration was adjusted to give 10⁹ CFU /mL.

Laboratory experiments

Laboratory experiments were conducted to enhance the seed quality parameters against salinity stress with bioprimed seeds in Department of Seed Science and Technology, Tamilnadu Agricultural University, Coimbatore. Tomato seeds were bioprimed with *Bacillus paralicheniformis* by soaking at the ratio of 1:2 for 12 hr. Then the seeds were dried back to original seed moisture content.

Table 1: Treatment Details

Treatments	Treatment details	Salt concentration (mM NaCl)	Soaking duration	Volume of soaking
T ₀	Control	0,25,50,75	12 hrs	1:2
T ₁	Hydropriming			
T ₂ C ₁	<i>Bacillus paralicheniformis</i> without dilution			
T ₂ C ₂	<i>Bacillus paralicheniformis</i> - 1:1 dilution			
T ₂ C ₃	<i>Bacillus paralicheniformis</i> - 1:2 dilution			
T ₂ C ₄	<i>Bacillus paralicheniformis</i> - 1:3 dilution			

With primed tomato seeds quality parameters test was carried out in which 25 (100 x 3) tomato seeds were placed in the Petri plates and roll towel paper moistened with water (control) and 25mM, 50mM, 75mM NaCl solution (salt stress). The Petri plates and roll towel containing tomato seeds were placed in the growth chamber maintained at 25±2°C, 16 hrs light cycle and 90±3% relative humidity and germination test was carried out for evaluating seed quality parameters along with control. The experiment was carried out with three replications in Factorial Completely Randomised Design (FCRD). After fourteen days the following growth parameters are recorded.

Quality parameters

Speed of germination (Maguire, 1962) [13].

It was determined from the seeds used in the germination test. From the first day after sowing to the fourteenth day, seeds germinated were counted on a daily basis. Counting the number of seeds germinated each day, the rate of germination was calculated using the following formula, and the results were expressed in number.

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \dots + \frac{X_n - X_{n-1}}{Y_n}$$

X₁ - Number of seeds germinated on first day

X₂ - Number of seeds germinated on second day

X_n - Number of seeds germinated on nth day

Y₁ - Number of days from sowing to first count

Y₂ - Number of days from sowing to second count

Y_n - Number of days from sowing to nth count

Germination (%)

Four replicates of twenty-five seeds of each treatment were

placed in roll towel medium. The roll towels were kept in a germination room with a temperature of 25±2°C and a relative humidity of 90±3% and illuminated with fluorescent light. The number of normal seedlings was recorded replication and treatment wise on the fourteenth day of planting. The mean results were computed and expressed in per cent (%).

$$\text{Seed germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds sown}} \times 100$$

Root length (cm)

After the testing period of fourteen days, ten normal seedlings were selected at random from each replication and treatment and their root length was measured between the collar and the tip of the primary root by using measuring scale. The mean length of the root was measured in centimetre.

Shoot length (cm)

The seedlings used to assess the root length were also utilized to measure shoot length. The distance between the region of collar and the tip of the primary leaf was measured. The mean values were calculated and recorded in centimetre.

Dry matter production (mg /10 seedlings)

Ten randomly selected normal seedlings used for seedling measurements were laid in a brown paper cover, shade dried and placed in a hot air oven maintained at 85±2°C for 24 h. After cooling in a desiccator containing silica gel for 30 min the dry weight of the seedling was taken using an electronic balance. The mean values were expressed in milligram per 10 seedlings.

Vigour Index (Abdul-Baki and Anderson, 1973) ^[1]

The vigour index was determined using the following formula and the values were reported as whole number.

Vigour index (VI) = Germination (per cent) × Mean length of seedlings (cm)

Statistical analysis

Data from lab experiments were analysed using analysis of variance (ANOVA), for Factorial Completely Randomised Design (FCRD) using AGRES software. Each treatment was replicated thrice.

Results and Discussion

Bacillus paralicheniformis, a Gram positive, spore - forming facultative anaerobic, rod-shaped bacterium, is used as Plant Growth Promoting Rhizobacteria and safe biocontrol agent. It has the ability to quickly colonize the root in a variety of crops. The present study was conducted in tomato seeds bioprimered with *Bacillus paralicheniformis* to alleviate the effect of salinity stress on performance of seed quality parameters such as speed of germination, germination per cent, length of root and shoot, dry matter production and vigour index. In general, as the salt stress increases, all the seedling parameters decreased and statistically significant variations were observed in speed of germination, germination percentage, shoot length, dry matter production and vigour index due to bioprimering with *Bacillus paralicheniformis* (T₂C₁) undiluted broth treatment. The results showed that bioprimered seeds have better speed of germination compared to nonprimered and hydroprimered seeds in different salinity concentration. Maximum speed of germination (8.2) was recorded in *Bacillus paralicheniformis* without dilution compare to control seeds (6.5) and hydroprimering (7.2) (Table 2). Similar observations were recorded in different salinity stress conditions. The results revealed higher germination (94%) was recorded in *Bacillus paralicheniformis* without dilution, whereas control seeds (T₀) recorded 85 per cent (Table 2). Bioprimered seeds resulted in lower reduction in germination percentage in different salinity levels when compared to control, which has higher reduction in germination percentage between different salinity levels. Similar results were reported by Agarwal *et al.* (2013) ^[2] in tomato in which the seeds germination per cent and other seed quality parameters were increased by *Bacillus spp* and it is due to the production of indole acetic acid (IAA) which is an important mechanism for plant growth stimulation in tomato seedlings. Khan *et al.* (2009) ^[11] reported that early emergence of seedlings is induced by priming treatment when compared to un-primed seeds in hot Pepper (*Capsicum annuum* L.). Similar results revealed in fenugreek that the reduction in final germination percentage can be explained by the increase of external osmotic pressure and it affects the absorption of water by the seed and can be also due to the accumulation of Na⁺ and Cl⁻ in the embryo which it may lead to an alteration of the metabolic processes of germination and subsequently causing cells death in the embryo (Maher *et al.*, 2013) ^[18]. Kaya *et al.* (2006) ^[10] stated that bioprimering of sunflower seeds have induced a range of biochemical changes which are necessary for initiating the germination process and may multiply substantially on seed during bioprimering.

The results also similar in shoot length, root length, seedling length, dry matter production of tomato seeds under different

salinity stress conditions. Seeds bioprimered with *Bacillus paralicheniformis* (T₂C₁) undiluted broth produced longest shoot length of 5.0 cm when compared to control which produced shortest shoot length of 4.4 cm (T₀) (Table 3). Root length of 14.8 cm was also maximum in seeds primered with undiluted broth (T₂C₁) in all salinity levels when compared to control (T₀) which produced root length of 9.9 cm (Table 3). Similar findings were reported by several scientists. Chakraborti *et al.* (2021) ^[5] stated that plants undergo a variety of molecular, cellular, and physiological changes to combat various abiotic stresses. Plants suffer from increased osmotic pressure outside the root due to stresses like drought or salt stress that ultimately result in reduced water availability to plant roots. In addition to morphological modifications, physiological changes such as lower leaf osmotic potential, accumulation of osmoprotectants, increased antioxidant activities, and so on occur in response to such abiotic stresses. The beneficial effect of bioprimering may be attributed to the potential of the microorganisms to proliferate, colonize and produce plant growth promoting molecules *viz.*, auxins, gibberellins, cytokinin's, ethylene and abscisic acid (Zahir *et al.*, 2009) ^[20]. Kumar *et al.* (2021) ^[8] also stated that in rice plants, *Bacillus* sp. has the potential for promoting plant growth attributes mainly plant height and root length by production of IAA and mitigating the adverse effects of salinity stress.

Dry matter production of bioprimered seeds were significantly higher when compared to control (T₀) in all salinity levels and the highest dry matter production was recorded in seeds primered with undiluted broth (T₂C₁) of 28.5 mg per 10 seedlings than all salinity levels. Significant decrease in dry matter production of tomato seedlings were observed with increase in salinity level (Table 4). Highest vigour index value of 1843 was observed in *Bacillus paralicheniformis* (T₂C₁) undiluted broth than different salinity concentration of tomato seeds, whereas control seeds (T₀) observed lowest vigour values of 1224 (Table 4). These results were similar with work done by Pan *et al.* (2019) ^[16] reported that PGPM can alleviate salt stress either by achieving ion homeostasis and by increasing nutrient uptake, reducing oxidative stress through enhanced antioxidant activity, producing osmoprotectants, ameliorating photosynthates. By synthesizing amino acids such as proline plants can be able to withstand abiotic stress, which acts as an osmoprotectant and a ROS scavenger (Kim *et al.*, 2016) ^[12]. Moretti *et al.* (2021) ^[14] stated that the microbial secondary metabolite has been reported to enhance the levels of chlorophyll, total carotenoids and net photosynthetic rate but reduces oxidative damage when the seed treatment with a bacterial consortium consisting of *Bradyrhizobium sp.*, *Azospirillum brasilense* strains. For increasing seed germination rate and seedling vigour, bioprimering in combination with hydroprimering has one of the viable treatments reported by (Zulueta *et al.*, 2015) ^[21]. *Bacillus* in association with seed and soil microbes produce IAA, which promote plant growth, by controlling the disease causing microorganism (Jothisri *et al.*, 2021) ^[7]. Moeinzadeh *et al.* (2010) ^[15] reported that in sunflower seeds quick and uniform germination as well as better plant growth recorded when bioprimering treatments with *Pseudomonas fluorescens*. Sathya *et al.* (2016) ^[17] reported in chilli seeds that bioprimering with six per cent *Bacillus amyloliquefaciens* and polymer coating @ 10 ml kg⁻¹ found higher quality parameters compared to other treatments and this may be due to better

synthesis of auxin. Bioprimered seeds with 5% *Bacillus amyloliquefaciens* showed better increase in quality parameters in maize (Anbalagan *et al.*, 2022) [3].

Table 2: Effect of bacterial culture *Bacillus paralicheniformis* in different salinity concentration on seed germination (%) of tomato

Treatments	Germination (%)					Speed of germination				
	Salinity concentration					Salinity concentration				
	S ₀	S ₁	S ₂	S ₃	MEAN	S ₀	S ₁	S ₂	S ₃	MEAN
T ₀	85 (67.52)	57 (49.27)	25 (30.20)	12 (20.26)	44 (41.81)	6.5	5.2	4.6	4.1	5.1
T ₁	89 (71.01)	60 (50.77)	33 (35.20)	13 (21.37)	48 (44.58)	7.2	6.8	6.1	4.3	6.1
T ₂ C ₁	94 (76.83.)	90 (72.29)	66 (54.74)	33 (35.25)	70 (59.77)	8.2	7.9	6.5	5.3	6.9
T ₂ C ₂	73 (58.92)	72 (58.05)	30 (33.61)	29 (32.78)	51 (45.84)	7.8	7.1	6.5	5.1	6.6
T ₂ C ₃	89 (71.01)	69 (56.38)	41 (40.00)	25 (30.20)	56 (51.94)	7.5	6.7	6.2	4.9	6.3
T ₂ C ₄	85 (67.52)	82 (65.44)	49 (44.61)	25 (30.20)	60 (51.94)	7.3	6.9	6.1	4.5	6.2
MEAN	85 (67.52)	82 (65.44)	40 (39.72)	22 (28.34)		7.4	6.7	6	4.7	
	T		S		T x S	T		S		T x S
S.Ed	0.85		0.70		1.71	0.04		0.03		0.08
CD (p=0.05)	1.72		1.40		3.45	0.08		0.06		0.16

(Figures in parantheses indicate arc sine values)

T₀ - Control, T₁ - Hydropriming, T₂C₁ - *Bacillus paralicheniformis* undiluted suspension, T₂C₂ - *Bacillus paralicheniformis* at 1:1 dilution, T₂C₃ - *Bacillus paralicheniformis* at 1:2 dilution, T₂C₄ - *Bacillus paralicheniformis* at 1:3 dilution, S₀ - Without salt, S₁ - 25mM NaCl, S₂ - 50mM NaCl, S₃ - 75mM NaCl

Table 3: Effect of bacterial culture *Bacillus paralicheniformis* in different salinity concentration on shoot length (cm) and root length (cm) of tomato

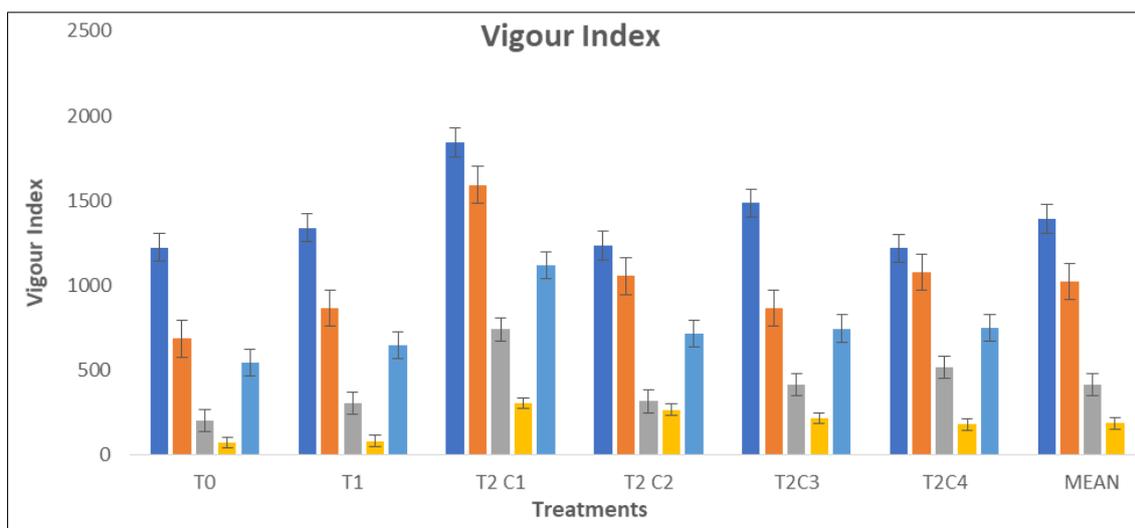
Treatments	Shoot length (cm)					Root length (cm)				
	Salinity concentration					Salinity concentration				
	S ₀	S ₁	S ₂	S ₃	Mean	S ₀	S ₁	S ₂	S ₃	Mean
T ₀	4.4	3.8	2.6	1.4	3	9.9	8.1	5.4	4.5	6.9
T ₁	4.5	4	3.1	1.7	3.3	10.4	10.3	6	4.5	7.8
T ₂ C ₁	5	4.3	3.2	2.9	3.8	14.8	13.3	7.8	6.6	10.6
T ₂ C ₂	4.6	3.9	3.2	2.5	3.5	12.1	10.6	7	6.5	9
T ₂ C ₃	4.6	4.2	3.1	2.4	3.5	11.5	8.1	6.7	5.7	8
T ₂ C ₄	3.7	4.2	2.9	2.4	3.3	10.5	8.8	7.4	4.7	7.8
MEAN	4.4	4	3	2.2		11.5	9.8	6.7	5.4	
	T		S		T x S	T		S		T x S
S.Ed	0.41		0.34		0.83	1.21		0.99		2.43
CD (p=0.05)	0.84		0.68		1.68	2.45		2		4.9

T₀ - Control, T₁ - Hydropriming, T₂C₁ - *Bacillus paralicheniformis* undiluted suspension, T₂C₂ - *Bacillus paralicheniformis* at 1:1 dilution, T₂C₃ - *Bacillus paralicheniformis* at 1:2 dilution, T₂C₄ - *Bacillus paralicheniformis* at 1:3 dilution, S₀ - Without salt, S₁ - 25mM NaCl, S₂ - 50mM NaCl, S₃ - 75mM NaCl

Table 4: Effect of bacterial culture *Bacillus paralicheniformis* in different salinity concentration on Dry matter production and vigour index of tomato

Treatments	Dry matter production (mg/10 seedlings)					Vigour Index				
	Salinity concentration					Salinity concentration				
	S ₀	S ₁	S ₂	S ₃	Mean	S ₀	S ₁	S ₂	S ₃	Mean
T ₀	21	17.5	11.7	8.8	14.7	1224	686	204	72	546
T ₁	21.9	21.1	13.4	9.1	16.3	1340	866	305	82	648
T ₂ C ₁	28.5	25.7	16.1	13.3	20.9	1843	1593	739	303	1119
T ₂ C ₂	24.6	21.4	15	13.2	18.5	1233	1054	315	265	716
T ₂ C ₃	24.3	18.2	14.6	12.6	17.4	1485	865	414	217	745
T ₂ C ₄	20.9	19	15.2	10.3	16.3	1218	1077	514	179	747
Mean	23.5	20.4	14.3	11.2		1390	1023	415	186	
	T		S		T x S	T		S		T x S
S.Ed	0.85		0.7		1.71	30.67		25.04		61.34
CD (p=0.05)	1.72		1.4		3.45	61.67		50.34		123.34

T₀ - Control, T₁ - Hydropriming, T₂C₁ - *Bacillus paralicheniformis* undiluted suspension, T₂C₂ - *Bacillus paralicheniformis* at 1:1 dilution, T₂C₃ - *Bacillus paralicheniformis* at 1:2 dilution, T₂C₄ - *Bacillus paralicheniformis* at 1:3 dilution, S₀ - Without salt, S₁ - 25mM NaCl, S₂ - 50mM NaCl, S₃ - 75mM NaCl



T₀ - Control, T₁ - Hydropriming, T₂C₁ - *Bacillus paralicheniformis* undiluted suspension, T₂C₂ - *Bacillus paralicheniformis* at 1:1 dilution, T₂C₃ - *Bacillus paralicheniformis* at 1:2 dilution, T₂C₄ - *Bacillus paralicheniformis* at 1:3 dilution, S₀ - Without salt, S₁ - 25mM NaCl, S₂ - 50mM NaCl, S₃ - 75mM NaCl

Fig 1: Influence of different bioprimering treatments on vigour index of different treatments

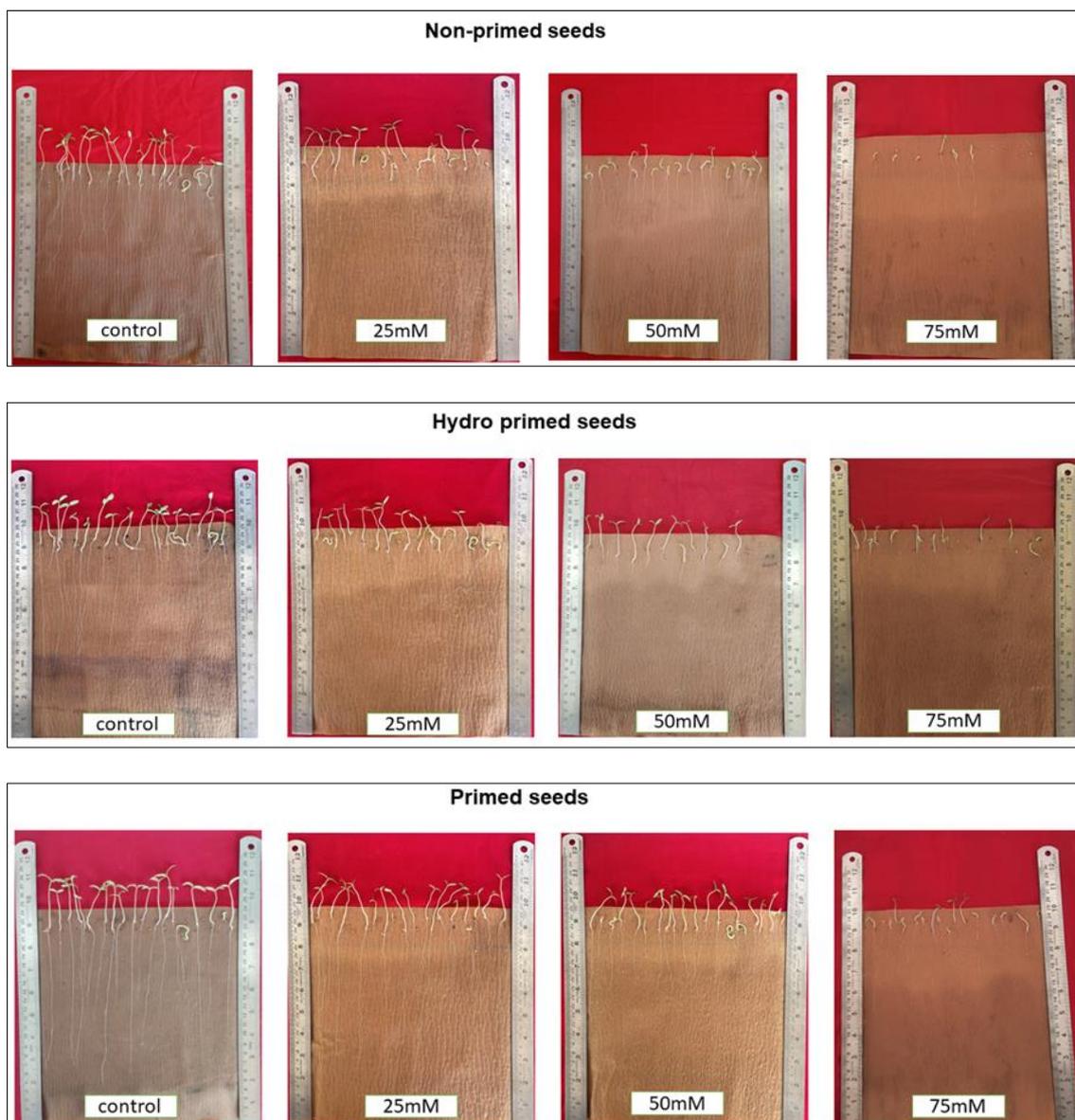


Fig 2: Quality parameters influenced by *Bacillus paralicheniformis* undiluted suspension bioprimered seeds, control and hydroprimed seeds on 14th day in different salt concentrations

Conclusion

The present conducted study showed that seeds bioprimed with undiluted suspension *Bacillus paralicheniformis* recorded maximum germination (%), shoot length (cm), root length (cm), vigour index, dry matter production (mg/10 seedlings) as compare to control and hydropriming in salinity stress condition. So, the bacterial endophyte can be used as eco-friendly instead of chemicals in salinity stress condition.

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