Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci. (Jan–Mar 2015) 85(1):63–69
DOI 10.1007/s40011-014-0333-8

RESEARCH ARTICLE

Effect of Biopriming with *Enterobacter* Strains on Seed Germination and Seedling Growth of Tomato (*Solanum lycopersicum* L.) Under Osmotic Stress

Ravindra Mohan Bhatt · Govindan Selvakumar · Kaushal Kishore Upreti · Pavithra Chikkakadaluru Boregowda

Received: 27 June 2013 / Revised: 10 September 2013 / Accepted: 25 February 2014 / Published online: 3 April 2014

Abstract A study was conducted to evaluate the effect of seed treatment with bacterial strains of *Enterobacter* spp. on seed germination and seedling growth of two tomato (*Solanum lycopersicum* L.) cultivars (cv. Arka Meghali and cv. Pusa Ruby). The cultivar Arka Meghali is recommended for rainfed conditions, while cv. Pusa Ruby is grown under irrigated conditions. Seeds were treated with osmotolerant plant growth promoting bacterial strains belonging to the genus *Enterobacter* (P-39, P-41 and P-46), for a period of 24 h and subsequently incubated at 25 °C under different mannitol induced osmotic stresses (0, −0.2, −0.4, −0.6, −0.8, −1.0 MPa). Seed treatment with bacterial strains influenced the germination and seedling vigour index of both cultivars as compared to the untreated and hydro-primed seeds, up to −0.6 MPa. The response of *Enterobacter* strains to water stress was better in cv. Pusa Ruby as compared to cv. Arka Meghali as indicated by higher germination percentage and germination rate. The seeds of both cultivars treated with *Enterobacter* P-39 performed better under osmotic stress (up to −0.6 MPa in cv. Arka Meghali and −0.8 MPa in cv. Pusa Ruby), indicating the significance of this strain as compared to other bacterial strains studied. The bioprimed seeds that failed to germinate at osmotic potentials beyond −0.4 MPa, when transferred to water (0 MPa), recorded improved germination and seedling vigour. These results indicated that the treatment of seeds with osmotolerant plant growth promoting bacterial strains improved the germination and enhanced seedling growth under osmotic stress conditions.

Keywords Biopriming · *Enterobacter* · Germination · Osmotic stress · Tomato

R. M. Bhatt (✉) · K. K. Upreti · P. C. Boregowda
Division of Plant Physiology and Biochemistry, Indian Institute of Horticultural Research, Bangalore 560 089, India
e-mail: bhattrm@yahoo.com

G. Selvakumar
Division of Soil Science and Agricultural Chemistry, Indian Institute of Horticultural Research, Bangalore 560 089, India

Introduction

Drought or limited moisture condition is considered to be one of the main environmental factors that strongly limits the growth and yield of plants worldwide [1]. Global climate change is expected to exacerbate water limitations in semi-arid areas [2]. The relative performance of an individual plant during its early stages of life i.e. germination and seedling establishment, can have important effects on its subsequent growth and fitness [3]. Since seed germination is sensitive to environmental conditions it is considered as an important event in determining the plant density in vegetable crops. Though, the domesticated tomato can grow under a wide range of climatic conditions, it is sensitive to drought and temperature, thus limiting its adaptation in tropical areas. It requires a different climatic range for seed germination, seedling growth, flower, fruit set, and fruit quality [4]. Under such conditions, although soil moisture may be adequate for the growth of a plant, the surface soil often dries rapidly and prevents germination and seedling establishment. Therefore, seed treatment with water (priming) may be used as an important tool to improve seed performance and plant stand in the field, especially during the summer [5].

Priming is a process which helps to accelerate germination and improve seedling establishment in many horticultural crops, particularly under unfavourable soil conditions [6–8]. Seed treatment with microbial agents (biopriming)

may serve as an important means of managing many soil and seed-borne diseases besides improving overall plant performance [9, 10]. The potential advantages of the biopriming process include rapid and uniform seedling emergence which may be useful under adverse soil conditions. Use of microbes as biopriming agents can also improve seedling growth under stress conditions [9]. Application of beneficial microorganisms to seeds during the priming process is commercially realistic, as suspensions of microorganisms can easily be incorporated into the water used for seed priming. However less work has been reported on the effect of biopriming in vegetable crops and more particularly in tomato under water stress. This prompted the present study on the effect of seed priming with osmotolerant plant growth promoting bacterial strains (*Enterobacter* spp.) on seed germination and seedling growth of two tomato cultivars under different osmotic stress conditions.

Material and Methods

Screening and Identification of Osmotolerant Bacteria

One hundred and seven bacterial isolates, capable of growth at an osmotic potential of −2.92 MPa, attained by PEG 6000/mannitol enrichment of nutrient agar [11], were isolated, purified and maintained in slants. Auxin production by the isolates was determined initially in PEG 6000 enriched Luria–Bertani broth containing 100 µg/mL of L-tryptophan (osmotic potential −2.1 MPa), by the colorimetric method of Gordon and Weber [12]. Based on their in vitro auxin producing potential, eight isolates producing auxin concentrations ranging from 9.8 to 27.6 µg/mL were short listed for quantifying indole-3-acetic acid (IAA) content by HPLC. Growth hormone (IAA and GA_3) production, by the individual isolates was determined under normal and osmotic stress conditions, by incubating individual cultures in Luria–Bertani broth (−0.9 MPa) and Luria–Bertani broth containing 25 % PEG (−1.92 MPa), with an overnight grown bacterial suspension containing 10^7 cfu/mL for a period of 7 days under dark conditions at 27 °C. At the end of the incubation period, the broth was extracted with diethyl ether, and the growth hormone production was determined in a HPLC (Prominence, Shimadzu Japan), using a photo diode array (Shimadzu, Japan, model: SPD-M20A) detector and 4 µm-Fusion RP-C18 column (Phenomenex, USA, 250 × 4.6 mm) [13] with modifications. Acetonitrile:water (pH 4.0 adjusted by 1 M orthophosphoric acid; 30:70 v/v) at 0.8 mL/min was used as the mobile phase. The GA_3 and IAA were detected at retention times of 6.5 (200 nm) and 13.2 min (222 nm), respectively. The IAA and GA_3 contents were quantified using external standards (Sigma-Aldrich, MO, USA).

Characterization of the Bacterial Isolates

Based on GA_3 and IAA production, three bacterial isolates viz., P-39, P-41 and P-46, were selected for the study. Individual isolates were identified by the sequencing of a partial fragment of the 16S rRNA gene and subsequent BLAST analysis with the NCBI database which confirmed their identity as *Enterobacter* species.

Seed Treatment with Bacterial Strains

Seeds of tomato cultivars Arka Meghali and Pusa Ruby were surface sterilized with 0.01 % $HgCl_2$ for 30 s, and thoroughly rinsed with sterile water under aseptic conditions. Seeds were soaked (bioprimed) in exponentially grown broth suspensions of individual bacterial cultures viz., *Enterobacter* strain P-39, *Enterobacter* strain P-41 and *Enterobacter* strain P-46 for 24 h. The population of the individual cultures in the broth suspensions was adjusted to 10^7 cfu/mL of broth. The second set of seeds was treated with sterile water for 24 h (hydroprimed), while the third set was not subjected to any treatment (unprimed) and served as control.

Seed Germination

The treated and untreated seeds were placed in petri dishes (110 mm dia.), on a layer of Whatman number 1 paper and subjected to mannitol induced osmotic stress (0, −0.2, −0.4, −0.6, −0.8, −1.0 MPa). Each petri dish contained 25 seeds and each treatment was replicated thrice. The petri dishes were kept in a BOD incubator in the dark at 25 ± 1 °C. The number of germinated seeds was counted at 24 h intervals for 10 days. The emergence of radicle was considered as a germination event for the calculation of the percent germination. The root and shoot lengths were measured at the end of the experiment. The rate of germination (R_G) was calculated using following formula.

$$R_G = \Sigma N_i / D_i,$$

where N_i is the number of germinated seeds in a given time, and D_i is the time unit (days). The seedling vigour index was calculated by the following formula [14]

$$\text{Seed vigour index (SVI)} = \text{percent germination} \times [\text{seedling root length} + \text{shoot length}]\ (\text{mm}).$$

The seeds which did not germinate at higher osmotic stress viz., −0.6, −0.8, −1.0 MPa were subsequently transferred to deionized water (0 MPa) and incubated at 25 ± 1 °C. The experiment was replicated thrice with 25 seeds per replicate. The germination was recorded at 24 h interval for a further period of 10 days and the germination percentage was calculated. Observations were also

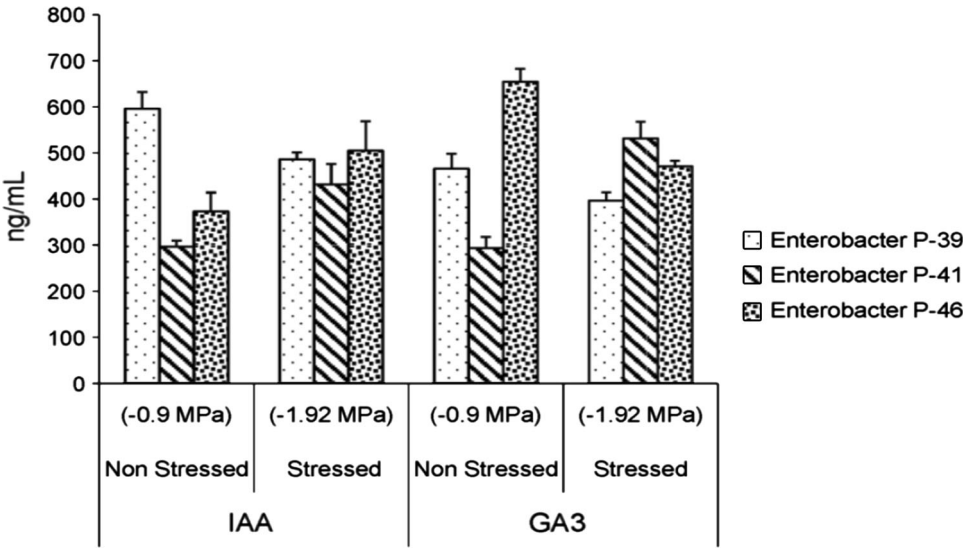

Fig. 1 Growth hormone production by the bacterial strains under normal and osmotic stress conditions

Table 1 Effect of *Enterobacter* strains on germination of tomato seeds under different osmotic concentrations (10 DAS)

Treatments	Germination (%)					
	Osmotic concentrations (MPa)					
	0	−0.2	−0.4	−0.6	−0.8	−1.0
cv. Arka Meghali						
Unprimed	75	52	17	0	0	0
Hydroprimed	87	70	47	25	0	0
Enterobacter P-39	87	80	50	10	0	0
Enterobacter P-41	70	73	20	10	0	0
Enterobacter P-46	67	73	30	10	0	0
		Treatment		Sub treatment		'Interaction
SEM		2.16		2.36		5.29
CD ($p = 0.05$)		6.48		7.1		15.88
Treatments	0	−0.2	−0.4	−0.6	−0.8	−1.0
cv. Pusa Ruby						
Unprimed	93	87	68	30	0	0
Hydroprimed	93	89	83	30	0	0
Enterobacter P-39	100	80	73	60	25	0
Enterobacter P-41	100	100	73	57	0	0
Enterobacter P-46	100	80	70	65	0	0
		Treatment		Sub treatment		Interaction
SEM		2.75		3.01		6.75
CD ($p = 0.05$)		8.26		9.05		20.25

Data represent the means of an experiment with three replicates

DAS days after start

Table 2 Seedling growth and vigour index of tomato as affected by *Enterobacter* strains under different osmotic concentrations (10 DAS)

Treatments	Osmotic concentrations (MPa)											
	Root length (cm)				Shoot length (cm)				Seed vigour index			
	0	−0.2	−0.4	−0.6	0	−0.2	−0.4	−0.6	0	−0.2	−0.4	−0.6
cv. Arka Meghali												
Unprimed	4.0	2.3	2.2	0.0	1.6	1.0	1.1	0.0	4,267	1,736	585	0.0
Hydroprimed	2.9	2.9	2.1	0.0	1.3	0.5	1.0	0.0	3,697	2,415	1,522	0.0
Enterobacter P-39	4.4	3.2	2.4	0.0	2.5	1.7	1.3	0.0	6,003	3,920	1,858	0.0
Enterobacter P-41	5.4	4.7	3.5	0.0	4.4	4.0	1.7	0.0	6,860	6,351	1,040	0.0
Enterobacter P-46	4.5	3.1	2.6	0.0	2.1	1.5	1.0	0.0	4,422	3,358	1,080	0.0
	Treatment	Sub treatment	Interaction		Treatment	Sub treatment	Interaction		Treatment	Sub treatment	Interaction	
SEM	0.30	0.23	0.52		0.27	0.20	0.46		472	365	819	
CD ($p = 0.05$)	0.91	0.70	1.57		0.81	0.62	1.40		1,415	1,096	2,456	
Treatment	Osmotic concentrations (MPa)											
	Root length (cm)				Shoot length (cm)				Seed vigour index			
	0	−0.2	−0.4	−0.6	0	−0.2	−0.4	−0.6	0	−0.2	−0.4	−0.6
cv. Pusa Ruby												
Unprimed	8.5	4.4	2.6	1.2	6.3	2.0	1.3	0.4	13,801	5,585	2,697	507
Hydroprimed	5.6	4.2	2.6	1.6	4.9	1.9	1.3	0.7	9,718	5,491	3,276	699
Enterobacter P-39	7.9	3.0	2.8	1.0	6.0	1.4	1.2	0.6	13,900	3,520	2,920	996
Enterobacter P-41	5.7	4.4	3.0	2.2	5.3	2.8	1.0	0.9	11,000	7,200	2,920	1,784
Enterobacter P-46	7.9	4.0	2.2	1.6	6.1	2.3	0.8	0.7	14,000	5,040	2,100	1,501
	Treatment	Sub treatment	Interaction		Treatment	Sub treatment	Interaction		Treatment	Sub treatment	Interaction	
SEM	0.17	0.15	0.35		0.18	0.16	0.37		338	302	676	
CD ($p = 0.05$)	0.53	0.47	1.06		0.55	0.49	1.11		1,014	907	2,028	

Data represent the means of an experiment with three replicates

DAS days after start

recorded on root and shoot length at the end of the experiment. The data were tabulated and analyzed statistically with the Agris Stat software. The bacterial strain treatments were considered as the main treatments and osmotic potentials as sub-treatments.

Results

Growth Hormone Production by the Bacterial Isolates

HPLC analysis of IAA and GA_3 production by individual isolates revealed that all the isolates retained their ability to produce the growth hormones under water stress conditions (Fig. 1). Interestingly the enterobacterial strains P-41 and P-46 produced higher concentration of IAA under in vitro osmotic stress conditions, while a reduction in the GA_3 concentrations was observed in the strains P-39 and P-46 under osmotic stress conditions.

Effect of Bacterial Strains on Seed Germination, Seedling Growth and R_G

At 0 MPa, a significant difference in seed germination was not observed between the primed and unprimed seeds (Table 1). However with an increase in the osmotic stress beyond −0.2 MPa, there was a considerable reduction in the percent germination in unprimed seeds. Seeds of cv. Arka Meghali bioprimed with bacterial strain *Enterobacter* P-39 recorded the highest percent germination ranging from 50 to 80 % at osmotic concentrations up to −0.4 MPa as compared to other treatments. The cv. Pusa Ruby seeds bioprimed with *Enterobacter* P-41 strain recorded 100 % germination at −0.2 MPa while seeds bioprimed with

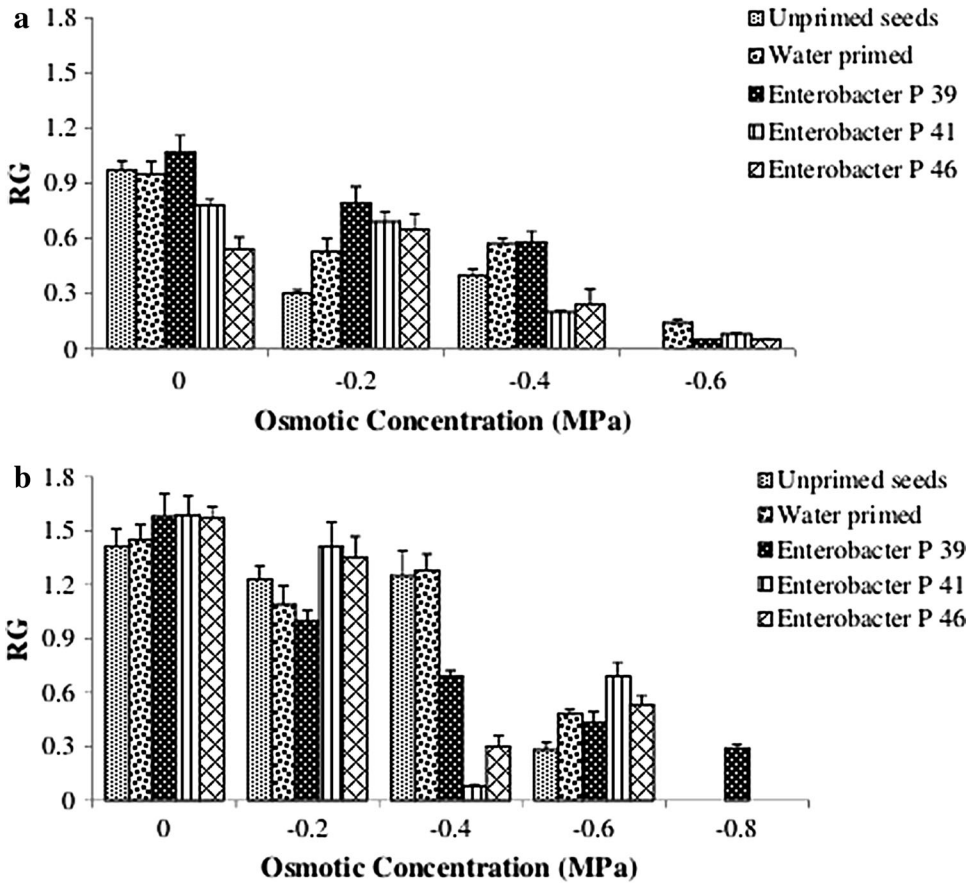

Fig. 2 Effect of *Enterobacter* strains on rate of germination (R_G) of cv. Arka Meghali (**a**) and cv. Pusa Ruby (**b**) seeds under different osmotic concentrations

Enterobacter P-39 recorded highest germination values of 60 and 25 % at −0.6 and −0.8 MPa, respectively.

The vigour index of bacteria primed seedlings was higher than the untreated and water treated seeds in most treatments (Table 2). At −0.2 MPa, seeds of cv. Arka Meghali primed with *Enterobacter* P-41 resulted in more vigorous seedlings as compared to the seedlings raised from untreated and water treated seeds, while seeds bioprimed with *Enterobacter* P-39 were superior at −0.4 MPa. Similarly in cv. Pusa Ruby, the seedlings bioprimed with *Enterobacter* P-41 were more vigorous at −0.2 and −0.6 MPa, while hydroprimed seedlings showed better vigour at −0.4 MPa. The effect of biopriming treatments on the rate of seed germination is depicted in Fig. 2. It could be observed that the application of osmotic stress delayed the germination of unprimed seeds of both cultivars and priming had a positive influence on the rate of seed germination of both cultivars. The biopriming influence was better evident in seeds of cv. Pusa Ruby where bioprimed seeds performed consistently over the unprimed and hydroprimed seeds.

When seeds of cv. Arka Meghali that failed to germinate at −0.6, −0.8, −1.0 MPa and seeds of cv. Pusa Ruby that did not germinate at −0.8, −1.0 MPa, were transferred to deionized water (0 MPa), an improvement in the percent germination was observed in both cultivars. The improvement was significant when bioprimed and un-germinated seeds of both cultivars were transferred from −0.8 and −1.0 to 0 MPa (Table 3). Similarly the bioprimed seeds recorded a higher SVI as compared to the untreated and hydroprimed seeds.

Discussion

Enterobacter is a commonly occurring rhizospheric bacterium and the beneficial effects of seed priming with

Table 3 Percent germination and seedling vigour index of ungerminated seeds from different osmotic concentrations on transfer to non osmotic conditions (10 DAS)

Treatments	Germination (%)			Seedling vigour index		
	−0.6 MPa	−0.8 MPa	−1.0 MPa	−0.6 MPa	−0.8 MPa	−1.0 MPa
cv. Arka Meghali						
Unprimed	85	70	65	7,412	6,496	5,317
Hydroprimed	73	83	80	8,081	7,212	6,680
Enterobacter P-39	77	87	63	7,222	8,908	6,457
Enterobacter P-41	77	80	67	7,900	9,728	7,437
Enterobacter P-46	80	73	70	7,456	6,548	7,770
	Treatment	Sub treatment	Interaction	Treatment	Sub treatment	Interaction
SEM	4.93	3.81	8.05	549	425	951
CD ($p = 0.05$)	14.79	11.45	25.62	1,647	1,276	2,853
Treatments	Germination (%)			Seedling vigour index		
	−0.6 MPa	−0.8 MPa	−1.0 MPa	−0.6 MPa	−0.8 MPa	−1.0 MPa
cv. Pusa Ruby						
Unprimed	Not performed	93	86	Not performed	7,499	6,991
Hydroprimed		93	96		8,787	9,974
Enterobacter P-39		93	96		6,949	10,656
Enterobacter P-41		100	100		10,650	10,440
Enterobacter P-46		90	100		10,393	9,790
	Treatment	Sub treatment	Interaction	Treatment	Sub treatment	Interaction
SEM	0.03	0.02	0.04	540	341	764
CD ($p = 0.05$)	0.10	0.06	0.14	1,621	1,025	2,293

Data represent the means of an experiment with three replicates

DAS days after start

enterobacterial strains have been reported earlier [15]. The beneficial effect of biopriming may be attributed to the potential of the microorganisms to proliferate, colonize and produce plant growth promoting molecules viz., auxins, gibberellins, cytokinins, ethylene and abscisic acid [16]. The results of the present study showed a reduction in germination percentage and seedling growth of both the cultivars under induced osmotic stress. Similar observations that water stress decreases seed germination percentage and the length of radicle and plumule were reported earlier [17, 18]. Under water deficit conditions, the reduction in the germination percentage may be associated with the lower diffusibility of the seed coat, while the reduction in growth has been attributed to the decrease in the cellular expansion [19]. Delayed germination has been attributed to the absence of energy to start the germination process, since energy is obtained by increments in the respiratory pathway, and water absorption is impaired under low water potentials [20]. In the present study, the effect of biopriming on seed germination was insignificant at higher osmotic potential (0 MPa) in both the cultivars, but significant under osmotic stress conditions.

The bioprimed seeds of both cultivars germinated faster, with a higher vigour index and percent germination. It is a well established fact that bioprimed seeds rapidly imbibe and revive the seed metabolism, resulting in a higher germination percentage [21]. However, the germination response of seeds subjected to osmotic stress varied with the bacterial strains used for seed treatment in both the varieties. The response to biopriming was better in cv. Pusa Ruby as compared to cv. Arka Meghali, which is indicated by the higher percentage of seed germination and the R_G. The present study supports the earlier findings that seed priming increases percent germination and R_G [22]. The probable reason for early emergence of the treated seeds may be the completion of the pre-germination metabolic activities, thereby making the seed ready for radicle emergence [23]. Biopriming may have induced a range of biochemical changes which are required for initiating the germination process. Though it has been reported that the

biopriming agent may multiply substantially on seed during biopriming [24], the effect on seed germination and seedling growth depends on the type of bacterial strain used for the seed treatment. The results obtained in the present study indicated that the seeds bioprimed with *Enterobacter* P-39 performed better under osmotic stress in both the varieties (up to −0.6 MPa in cv. Arka Meghali and −0.8 MPa in cv. Pusa Ruby), indicating the superiority of this bacterial strain.

The rapid germination of the un-germinated bioprimed seeds and vigorous seedling growth at 0 MPa (water), on transfer from higher osmotic stress, indicated that biopriming could promote rapid germination and improved seedling growth. A similar observation has been reported earlier [9]. The observation that biopriming with selected bacterial strains significantly increased the growth indices including root and shoot length may be attributed to the better establishment and adherence of bacteria to the seed coat of osmotically stressed seeds, before being transferred to water, and their subsequent revival and plant growth promotion under normal hydrated conditions. This hypothesis finds support in an earlier study that the bacterial agent may multiply substantially on the seed surface during biopriming process [24].

Conclusion

The results of the present investigation indicated that the reduction in tomato seed germination under high osmotic stress conditions can be overcome by the biopriming of seeds with osmotolerant plant growth promoting *Enterobacter* strains. However, the preferential choice of cultivars to microbial strains is to be investigated further.

Acknowledgments This work was carried out under the National Initiative on Climate Resilient Agriculture (NICRA), Project, Indian Council of Agricultural Research, New Delhi. The authors are grateful to the Director, Indian Institute of Horticultural Research for providing facilities during the course of this study.

References

1. Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant responses to drought from genes to the whole plant. Funct Plant Biol 30:239–264
2. IPCC (2001) Climate Change 2001: impacts, adaptation and vulnerability. In: McCarthy JJ (ed) Working Group II Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, p 1032
3. Roach DA (1987) Variation in seed and seedling size in *Anthoxanthum odoratum*. Am Midl Nat 117:258–264
4. Maiti RK, Padmavathi V, Devi MU, Amitava R, Sarkar NC (2008) Research advances on physiological basis of crop growth in tomato: a review. Int J Agric Environ Biotechnol 1:94–106
5. Nascimento WM, Pereira RS (2007) Preventing thermo-inhibition in carrot by seed priming. J Seed Sci Technol 35:504–507
6. Halmer P (2000) Commercial seed treatment in seed technology and its biological basis. In: Black M, Bewley JD (eds) Seed technology and its biological basis. Sheffield Academic Press, Sheffield, pp 257–286
7. Rush CM (1991) Comparison of seed priming techniques with regard to seedling emergence and *Pythium* damping-off in sugar beet. J Phytopathol 81:878–882
8. Taylor AG, Harman GE (1990) Concepts and technologies of selected seed treatments. Annu Rev Phytopathol 28:321–339
9. Bennett MA (1998) The use of biologicals to enhance vegetable seed quality. J Seed Technol 20:198–208
10. Walia A, Mehta P, Chauhan A, Shirkot CK (2013) Effect of *Bacillus subtilis* strain CKT1 as inoculum on growth of tomato seedlings under net house conditions. Proc Natl Acad Sci India B 1:11
11. Sandhya V, Ali SZ, Grover M, Reddy G, Venkateshwarlu B (2009) Alleviation of drought stress effects in sunflower seedlings by exopolysaccharides producing *Pseudomonas putida* strain P45. Biol Fertil Soil 46:17–26
12. Gordon AS, Weber RP (1951) Colorimetric estimation of indole acetic acid. Plant Physiol 26:192–195
13. Kelen M, Demiralay EC, Sen S, Ozkan G (2004) Separation of abscisic acid, indole-3-acetic acid, gibberellic acid in 99 R (*Vitis berlandieri* × *Vitis rupestris*) and rose oil (*Rosa damascena* Mill.) by reversed phase liquid chromatography. Turk J Chem 38:603–610
14. Hosseini SZ, Jafari M (2002) Investigation on effect of salinity stress on germination of three accessions of tall wheat grass (*Agropyron elongatum*). In: 17th WCSS, Thailand, p 2289
15. Kubik KK (1995) Seed priming and survival of desiccated *Enterobacter cloacae* inoculated during seed priming. Dissertation, University of Nebraska, Lincoln
16. Zahir ZA, Arshad M, Frankenberger WT Jr (2004) Plant growth promoting rhizobacteria: applications and perspectives in agriculture. Adv Agron 81:97–168
17. Daveman I (2006) Effects of seed priming with PEG or K_3PO_4 on germination and seedling growth of lettuce. Pak J Biol Sci 11:923–928
18. Hadas A (1976) Water uptake and germination of leguminous seeds under changing external water potential in osmoticum solution. J Exp Bot 27:480–490
19. Kramer PJ (1974) Fifty years of progress in water relations research. Plant Physiol 54:463–471
20. Mayer AM, Poljakoff-Mayber A (1989) The germination of seeds, 4th edn. Pergamon Press, Oxford, p 270
21. Rowse HR (1995) Drum priming—a non-osmotic method of priming seeds. Seed Sci Technol 24:281–294
22. Kaya MD, Okcu G, Atak M, Cikli Y, Kolsarici O (2006) Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). Eur J Agron 24:291–296
23. Arif M (2005) Seed priming in maize for improving emergence and seedling growth. Sarhad J Agric 21:539–543
24. Callan NW, Mathre DE, Miller JB (1990) Bio-priming seed treatment for biological control of *Pythium ultimum* pre-emergence damping off in sweet corn. Plant Dis 74:368–372