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Evaluation of endophytic *Bacillus* spp. isolates from tomato roots on seed germination and seedling vigour of tomato

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Abstract
Rocky tomato seeds variety was treated with endophytic *Bacillus* isolates to determine their efficacy and ability to enhance seedling vigour and germination per cent as a potential plant growth promoter. Twelve screened endophytic *Bacillus* isolates were evaluated for their germination and vigour of tomato seeds in *in vitro* condition. Seed germination percent ranged between 60-95% with lowest in uninoculated control. Among them, *Bacillus velezensis* ERBS51 treated seeds had the highest germination per cent (95%) and vigour index (1073.50 and 1472.5) at both 7th and 14th day which was the best performing isolate among the twelve. *Bacillus* sp. ERBS10 also gave significant good result with 88% germination per cent along with 953 and 1287 vigour indexes at 7th and 14th days. The root length and shoot length were also found higher in both the isolates. The result indicates that *Bacillus velezensis* ERBS51 was found most effective in enhancing plant growth.

Keywords: Endophytic, *Bacillus*, germination per cent, vigour index

Introduction
Tomato is an important vegetable crop widely grown around the world because of its importance and value. In India, it is cultivated in an area of 789000 ha in different states with a production of 19759000 metric tonnes (Anon, 2018) [1]. Tomato fruit has got outstanding nutritive value which is rich in mineral, vitamins, and organic acids. Endophytes are groups of fungi or bacteria that are beneficial to the plant residing inside the tissues of a healthy plant without causing any symptoms or damage to the host (Robert *et al*., 2008; Hallmann *et al*., 1997) [2, 3]. Both gram positive and negative endophytic bacteria have been known to occur in diverse plant species internally inside leaves, roots, flowers, fruits, seeds, and stem (Kobayashi and Palumbo 2000; Bacon and Hinton, 2007) [4, 5]. They have both plant disease suppression and plant growth promotion abilities.
Endophytic bacteria can promote plant growth by different mechanisms such as phosphate solubilisation, phytohormones production, siderophore, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, biological nitrogen fixation and indole-3-acetic acid (IAA) synthesis (Yan *et al*. 2018; Feng *et al.* 2006; Bohlool *et al*., 1992) [6, 7, 8]. *Bacillus* is among the commonly occurring genera of endophytic bacteria exploited for biocontrol and plant growth promotion activity (Nandhini *et al*., 2012) [9]. *Bacillus* are rod-shaped, aerobic or facultative anaerobic and endospore-forming gram-positive bacteria (Vargas *et al*., 2004; Borriss, 2011) [10, 11] commonly used for their Plant growth promoting (PGP) activity since they can produce endospores which are heat and desiccation-tolerant making them perform well under different environmental conditions (Priest, 1993; Gardener, 2004) [12, 13]. Seed germination and seedling vigour are two important factors influencing crop yield and quality (Rudolph *et al*., 2015) [14]. There are reports of *Bacillus* spp. improving seed germination, vigour, rate of germination, seedling growth and yield of crops (Raj *et al*., 2003; Kloepper *et al*., 2004) [15, 16] thereby also protecting the seeds and germinating seedlings from seed or soil borne pathogens infection (Gowthamy and Manonmani, 2018) [17]. *Bacillus* spp. can produce gibberellins and cytokinins, which helps in root growth and also increases the number of root hairs (Araujo and Hungari, 1999) [18]. The benefit of seed inoculation is that only small amount of PGP bacteria is required and allows the bacteria to establish close intimate association between the seed and the bacteria thereby resulting in resilience of the seedlings (Ahmad *et al*., 2011) [19]. They multiply on seed surface ultimately colonizing the whole root system of the seedling (Suslow and Schroth, 1982) [20]. Seed treatment with effective PGP bacteria are known to be beneficial to both the seed and environment with plus benefit of being ecologically sound.

As the present agriculture scenario is more inclined to organic farming natural microbial products has increased its popularity. So, studies on the effect of PGP *Bacillus* on seed germination and early seedling growth is of vital important. Therefore, the present study was taken up to determine if selected endophytic *Bacillus* spp. isolated from tomato roots applied as a seed treatment could enhance seed quality such as seed germination and seedling vigour of tomato.

Materials and Methods

Microbial cultures

Twelve efficient strains of endophytic *Bacillus* isolates from the roots of tomato grown in different districts of Meghalaya, India isolated and characterized by molecular method prior to this study were used in the experiment (Table 1). The isolates were maintained on nutrient agar slants and subsequently subcultured regularly at an interval of two months and kept at 4 ±1 °C in refrigerator.

Preparation of inoculum and germination assay

The twelve isolates of *Bacillus* spp. were individually grown in 20 ml nutrient broth (10g beef extract, 10 g peptone, 5g NaCl in one litre of distilled water, pH 7.2) at 28 ± 2 ◦C for 24 hours in a rotary shaker.

Rocky tomato variety was used for the study. The seeds were surface sterilized with 0.1% mercuric chloride for 3 min and washed 4-5 times thoroughly with sterile distilled water. The seeds were transferred and soaked for 3 hrs in 48 hrs old bacterial broth cultures containing at least 1×10^6 cells/ml. 20 seeds per plate were placed in the petri dish containing sterile filter paper moistened with sterile water and replicated 3 times. The seeds treated with sterile water instead of innucula were served as control. The plates were incubated at 28±1 ºC and moisture of the petri dishes was maintained regularly. The observations for percentage seed germination were taken at 7 days after incubation. Five seedlings were randomly selected from each Petri dish and root and shoot length were measured in centimetre using a measuring tape (Krishnaswamy and Seshu, 1990) [22]. Root length, shoot length and vigour index were recorded at 14 and 21 days after incubation (Malleswari and Bagyanarayana, 2013) [21]. The vigour index was calculated as:

Vigour index (Vi) = (RL + SL) × GP

Where

RL = mean root length (cm)
SL = mean shoot length (cm)
GP = germination percentage (%)

Statistical analysis

The experiment was laid out in a Completely Randomized Design (CRD). Data were analysed using the One-way analysis of variance (ANOVA) at a significance level of 0.05 difference.

Result and Discussion

As shown in table 2 and 3 highest germination per cent of 95% at both 7th and 14th day and highest seedling vigour index 0f 1073.50 (7th day) and 1472.5 (14th day) were recorded in tomato seeds treated with T5, *Bacillus velezensis* ERBS51 followed by T2, *Bacillus* sp. ERBS10 with 88% germination per cent along with 953 and 1287 vigour index at 7th and 14th day. The highest root length of 7.67cm (7th day) and 8.67cm (14th day) was also observed in T5, *Bacillus velezensis* ERBS51 treated seeds (Fig 1 and 2). The highest shoot length of 3.94 cm at 7th day was recorded in T6, *Bacillus cereus* ERBS69 but at 14th day highest shoot length of 7.03 cm was found in T5, *Bacillus velezensis* ERBS51 treated seeds respectively. While the lowest germination per cent and seedling vigour index was recorded in control. Majority of the treatments could significantly improve the growth of tomato compared with control (fig 3). This statement is in agreement with Agarwal and agarwal, 2013 who reported 5 bacterial isolates of *Bacillus* that showed potential plant growth promoting activity by significantly improving seed germination, vigour index, shoot and root length of tomato compared to the uninoculated control. Similar germination rate was also recorded in *B. licheniformis* SV4 treated tomato seeds which induced an earlier germination of tomato seeds, by 40%, after 2 days of incubation (Sundaramoorthy and Balabaskar, 2012) [25]. The application of PGP bacteria like *Bacillus* is one of the best potential approaches to improve crop productivity in sustainable agriculture (Rammamoorthy *et al.* 2001) [24].

Table 1: List of different treatments of endophytic *Bacillus* isolates along with the place of tomato root collection used in the experiment

Treatments	Bacillus isolates	Locations of tomato root collection
T1	*Bacillus* sp. ERBS4	East Khasi Hills, Sohryngkham
T2	*Bacillus* sp. ERBS10	East Khasi Hills, Umshing-Mawkynroh
T3	*Bacillus* sp. ERBS29	Ri Bhoi District, CPGS-AS campus
T4	*Bacillus* sp. ERBS47	Ri Bhoi District, Umdihar
T5	*Bacillus velezensis* ERBS51	West Jaintia Hills, Moodymmai
T6	*Bacillus cereus* ERBS69	East Jaintia Hills, Moolasngi
T7	*Bacillus* sp. ERBS75	East Jaintia Hills, Daistong
T8	*Bacillus subtilis* ERBS80	South west Garo Hills, Bhoirakupi
T9	*Bacillus swezeyi* ERBS115	West Garo Hills, Damalgre
T10	*Bacillus* sp. ERBS118	West Garo Hills, Damalgre
T11	*Bacillus cereus* ERBS119	West Garo Hills, Damalgre
T12	Bacillus *subtilis subsp. Spizizenii* ERBS130	South West Garo Hills, Lokaichar
T13	Control	

Table 2: Effect of Endophytic *Bacillus* isolates on Germination percent, Root length, shoot length and vigour index of tomato on 7th day

Treatments	Germination %	Root length (cm)	Shoot length (cm)	Seedling Vigour index
T1	70.00 ± 2.88^{cdef}	5.24 ± 0.12^{ef}	2.81 ± 0.05^{cd}	504.93^{f}
T2	88.33 ± 3.33^{a}	7.30 ± 0.11^{b}	3.50 ± 0.05^{b}	953.96^{b}
T3	71.67 ± 3.33^{bcde}	5.57 ± 0.06^{de}	3.67 ± 0.08^{b}	661.75^{d}
T4	65.00 ± 2.88^{efg}	4.43 ± 0.08^{h}	2.37 ± 0.06^{f}	442.00^{g}

T5	95.00±2.88[a]	7.67±0.03[a]	3.63±0.08[b]	1073.50[a]
T6	78.33±1.66[b]	6.90±0.15[c]	3.94±0.04[a]	848.57[c]
T7	73.33±1.66[bcd]	4.92±0.03[fg]	2.90±0.05[c]	574.41[e]
T8	76.67±1.66[bc]	4.83±0.03[g]	2.81±0.05[cd]	585.24[e]
T9	71.67±1.66[bcde]	4.30±0.05[h]	2.77±0.06[cde]	506.47[f]
T10	71.67±1.66[bcde]	3.77±0.08[i]	2.56±0.12[ef]	453.88[fg]
T11	68.33±1.66[def]	5.70±0.05[d]	2.60±0.05[de]	567.13[e]
T12	63.33±1.66[fg]	3.83±0.13[i]	2.76±0.14[cde]	405.30[g]
T13	60.00±2.88[g]	3.73±0.28[i]	1.87±0.03[g]	336.00[h]
SE(d)±	3.39	0.16	0.11	26.03
CD (0.05)	6.98	0.34	0.22	53.53

*Values with different letters within a column are significant at p< 0.05.

Table 3: Effect of Endophytic *Bacillus* isolates on Germination percent, Root length, shoot length and vigour index of tomato on 14th day

Treatments	Germination %	Root length (cm)	Shoot length (cm)	Seedling Vigour index
T1	70.00±2.88[cdef]	5.75±0.09[d]	5.97±0.08[e]	820.63[f]
T2	88.33±3.33[a]	8.25±0.14[a]	6.32±0.05[d]	1287.54[b]
T3	71.67±3.33[bcde]	5.92±0.05[d]	6.64±0.04[bc]	900.17[de]
T4	65.00±2.88[efg]	5.08±0.05[e]	4.97±0.08[gh]	653.25[i]
T5	95.00±2.88[a]	8.47±0.08[a]	7.03±0.17[a]	1472.50[a]
T6	78.33±1.66[b]	7.43±0.14[b]	6.83±0.03[ab]	1117.50[c]
T7	73.33±1.66[bcd]	5.56±0.10[d]	6.46±0.07[cd]	880.93[e]
T8	76.67±1.66[bc]	5.59±0.03[d]	6.55±0.12[cd]	930.51[d]
T9	71.67±1.66[bcde]	4.64±0.05[f]	5.68±0.04[f]	739.87[g]
T10	71.67±1.66[bcde]	4.51±0.00[f]	5.10±0.15[g]	688.74[h]
T11	68.33±1.66[def]	6.53±0.01[c]	5.20±0.05[g]	801.51[f]
T12	63.33±1.66[fg]	4.55±0.14[f]	5.94±0.05[ef]	664.54[hi]
T13	60.00±2.88[g]	4.01±0.37[g]	4.82±0.06[h]	529.80[j]
SE(d)±	3.39	0.19	0.12	17.00
CD (0.05)	6.98	0.39	0.26	35.15

*Values with different letters within a column are significant at *p*< 0.05.

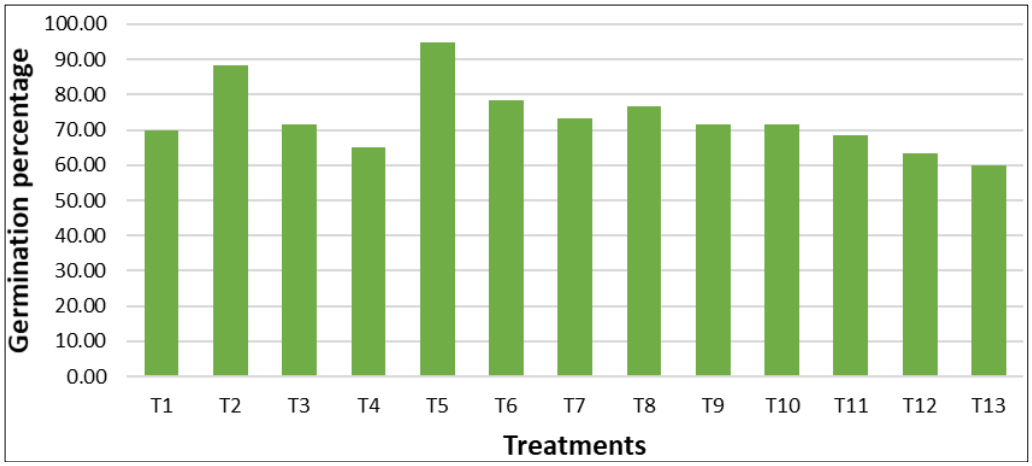

Fig 1: Germination percentage of different treatments

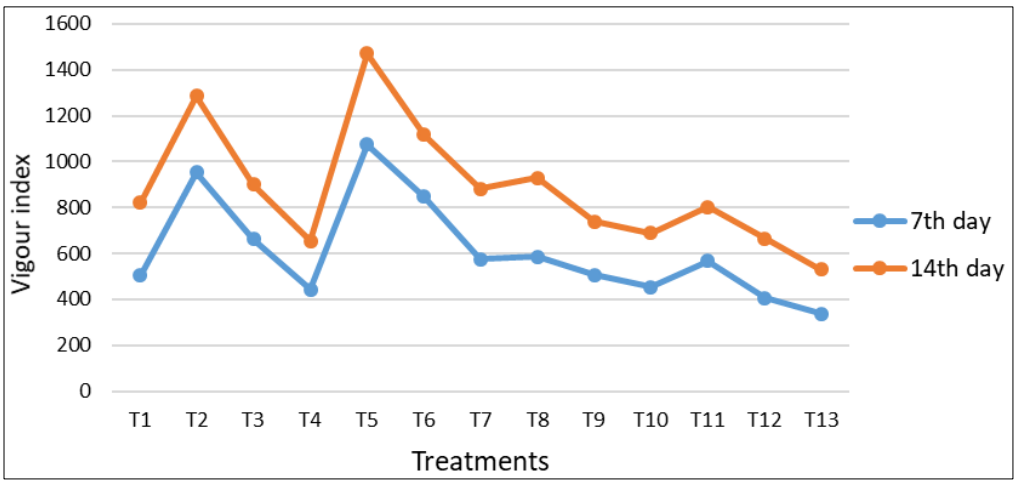

Fig 2: Vigour index of the treatments at 7th and 14th days.

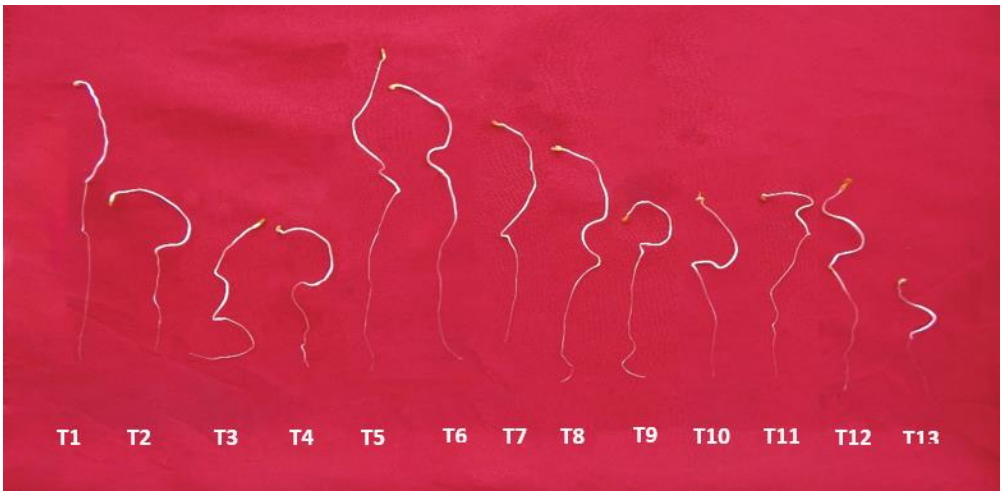

Fig 3: Growth of tomato treated with different endophytic *Bacillus* isolates

Conclusion

Treatment of the tomato seeds with potential endophytic *Bacillus* isolates significantly increases the germination percent and vigour index of tomato compared with control which proves that they have plant growth promoting potential. T5, *Bacillus velezensis* ERBS51 is the best in terms of germination and vigour followed by T2, *Bacillus* sp. ERBS10 with least in control. These may be due to production of certain enzyme, solubilisation of nutrients or production of phytohormones etc. by the *Bacillus* isolates. Hence, Endophytic *Bacillus* sp. could be further studied to develop microbial inoculant to improve plant growth and protect against plant pathogens.

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