



## Promotion of vegetable seed germination by soil borne bacteria

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### ABSTRACT

The objective of the study was to find out the effect of soil borne bacteria on the germination of different plant seeds, soaking time on seed germination and to recommend the best germination inducer bacteria. Six different soil borne bacterial species such as *Pseudomonas* sp, *Bacillus coagulans*, *Serratia* sp, *Klebsiella* sp, *Bacillus* sp and *Escherichia coli* and four crop seeds of *Phaseolus vulgaris*, *Vigna sinensis*, *Hibiscus esculentus* and *Beta vulgaris* were used. Surface sterilized seeds were soaked in different bacterial inocula prepared with phosphate buffer (pH-7) and allowed to colonize. After ten minutes of soaking, equal number of seeds were randomly transferred into sterile petridishes containing sterile moisten filter papers and the plates were kept in the dark for five days and subsequently subjected to light condition for another five days, at room temperature. The germination of seed and other related attributes were measured. There was a significant difference in the mean length of germ tubes ( $p = 0.05$ ) of soaked seeds when compared with non infected control. Germination of *H. esculentus*, *P. vulgaris* and *V. sinensis* seeds were promoted by *Pseudomonas* sp and *B. Coagulans*. Germination of *H. esculentus* and *P. vulgaris* seeds could be promoted in an optimised manner by soaking with in *Pseudomonas* for 10 minutes and 20 minutes, respectively where as seeds of *V. sinensis* could be promoted by soaking with in *Bacillus* sp inocula for 20 minutes. *Serratia* sp, *Klebsiella* sp, *B. coagulans* and *E.coli* had no effect on seed germination.

**Key words:** Soaking time; promotion; soil borne bacteria; seed germination

### INTRODUCTION

Soil is the habitat of a great variety of microorganisms than any other environment. Any soil sample will consist of a variety of microorganisms such as fungi, bacteria, cyanobacteria, algae and nematodes belonging to innumerable genera and species. Soil microorganisms interact with plants and bring about various effects such as beneficial, harmful and neutral or variable [2, 4]. Germination testing is the most important quality in evaluating the growing value of the seed lot. Seed is targeted because of its potential as a transporting vehicle for transmitting beneficial or deleterious bacteria [7]. Testing of seeds under field condition is generally very unsatisfactory as the results cannot be reproduced with reliability and the growth can be disturbed by un-controlled factors [2]. The external factors can be controlled to provide the most uniform, rapid and complete germination environment, if the experiment is done in the laboratory under controlled environment. However, the laboratory conditions should be standardized to enable reproducible results within limits.

Most soil borne bacteria contribute to the process of decomposition and also to improve the physical structure of soil. Not only fungi but bacteria are also able to stimulate root growth, secondary root initiation, seed germination etc. Beneficial mycorrhizal associations are to be found in many of our edible and flowering crops. These associations include at least 80% of the *brassica* and *solanum* families (including tomatoes and potatoes), as well as the majority of tree species, especially in forest and woodlands [1]. Here the mycorrhizae create a fine underground mesh which extends greatly beyond the limits of the tree's roots, thus greatly increasing their feeding range and actually causing neighbouring trees to become physically interconnected. The benefits of mycorrhizal relations to their plant partners are not limited to nutrients, but can be essential for plant reproduction. Bacteria from all four

groups perform important services related to water dynamics, nutrient cycling, and disease suppression [7, 8]. Some bacteria affect water movement by producing substances that help to bind soil particles into small aggregates (those with diameters of 1/10,000-1/100 of an inch or 2-200 $\mu$ m). Stable aggregates improve water infiltration and the soil's water-holding ability. In a diverse bacterial community, many organisms will compete with disease-causing organisms in roots and on above ground surfaces of plants [9].

Modern crop production technology includes increasing concern about the time management, environmental impact of vegetable crop farming, and the yield. This concept leads to the development of more integrated approaches together for the induction of seed germination using soil borne micro organisms [10, 11]. Before that, it is necessary to investigate basic ecological interactions between different bacterial media and seed germination of economically important, especially the famous locally grown vegetable crop species. Therefore the objective of the study was to find out the effect of soil borne bacterial genera on different vegetable seed germination, to study the effect of soaking time on seed germination and to recommend the bacterial species that could be used for plant growth promotion.

## MATERIALS AND METHODS

### Plants and Microorganisms

Six bacterial genera such as *Pseudomonas* sp, *Bacillus coagulans*, *Serratia* sp, *Klebsiella* sp, *Bacillus* sp and *E.coli* and seeds of four different plants such as *Phaseolus vulgaris*, *Vigna sinensis*, *Hibiscus esculentus* and *Beta vulgaris* were used in this study.

### Inoculum preparation and transfer

From the 24 hour old bacterial cultures, suspensions (inocula -  $8 \times 10^4$  cells/mL, SD=1.02) were prepared in sterile phosphate buffer (pH -7.0) separately. Seeds were surface sterilized with 0.01% HgCl<sub>2</sub> and rinsed three times thoroughly in sterile distilled water. Then seeds were transferred into each bacterial inoculum separately and mixed well and allowed to soak. After ten minutes of soaking, fifteen seeds were transferred into sterile petridishes containing sterile moisten filter papers (Whatman #102).

### Germination condition

The petri dishes that have seeds inoculated with the appropriate microbial inocula, were kept in the dark for five days and subsequently subjected to light condition for another five days under room temperature, for germination.

### Germination trait parameters

Germination rate was calculated according to the method explained by Krishnaswamy and Seshu [6]. Measurement of shoot and root length was carried out as follows; five seedlings were randomly selected from each Petri dish and measured with a measuring tape and expressed in centimetres [6]. Number of seeds germinated and length of germ tubes of the germinated seeds were measured. This procedure was followed to each species of plant seeds with the tested bacterial genera separately. Controls were also maintained with sterile distilled water instead of inocula.

### Statistical analysis

Data were subjected to be analysed statistically by the "t" test using R 2.15.3 statistical software [8] at  $\alpha = 0.05$  confidence level.

## RESULTS AND DISCUSSION

Different bacterial treatments had diverse effects (stimulative or inhibitory) on seed germination. Statistical analysis showed that there was a significant difference in the mean length of germ tubes of soaked seeds when compared with control (Table 1).

Table 1: Presence (+) / Absence (-) of significant difference in the mean length of germ tubes of soaked seeds compared with the control.

	<i>Hibiscus</i>	<i>Phaseolus</i>	<i>Vigna sinensis</i>	<i>Beta vulgaris</i>
<i>Pseudomonas</i> sp	+	+	+	+
<i>Bacillus coagulans</i>	+	+	+	-
<i>Serratia</i> sp	-	-	-	-
<i>Klebsiella</i> sp	-	-	-	-
<i>Bacillus</i> sp	-	-	-	-
<i>E.coli</i>	-	-	-	-

There was a promoting effect of bacterial inocula on seed germination when soaking was done by using *Pseudomonas* and *Bacillus coagulans* with the seeds of *Hibiscus esculentus*, *Phaseolus vulgaris* and *Vigna sinensis*. But the degree of promotion varied among plant seeds and among the different bacterial species used. *Pseudomonas* showed high promoting effect on *Hibiscus esculentus* and *Phaseolus vulgaris* and *Bacillus coagulans* showed high promotion on *Vigna sinensis* than the others. This variation in growth promotion might be due to the variation in the rate of colonization of different bacterial species into the seeds of different plants.

But analysis showed that there was no significant difference ( $\alpha=0.05$ ) between the mean length of germ tubes of soaked *Beta vulgaris* seeds and the control. So there was neither promoting nor inhibiting effect by these bacteria on *Beta vulgaris* seed germination. Soaking by *Serratia* sp, *Klebsiella* sp, *Bacillus* sp and *E.coli* had no effect on the germination of all the selected plant seeds and this may be due to the poor colonization rate of these bacteria into the seed or the substance concentration is not enough to promote / inhibit germination or these bacteria don't produce any substances that affect seed germination.

Variation in the degree of promoting and inhibiting effect on seed germination by Soaking depends on the variety of plant seeds, bacterial species used, rate of colonization, amount of substance causing the effect and the internal physical factors of seeds [4, 6, 9].

**Table 2: Minimum time (Minutes) taken for the seed to soak to get the optimum effect on germination**

	<i>Hibiscus</i>	<i>Phaseolus</i>	<i>Vigna sinensis</i>	<i>Beta vulgaris</i>
<i>Pseudomonas</i>	10	20	30	-
<i>Bacillus coagulans</i>	20	30	20	-
<i>Serratia</i>	-	-	-	-
<i>Klebsiella</i> sp	-	-	-	-
<i>Bacillus</i> sp	-	-	-	-
<i>E.coli</i>	-	-	-	-

Table 2 shows the minimum time taken for seed soaking to get optimum promoting effect during germination, in different bacterial inocula. Above the minimum time, there was no increase in the promotion of germination. This helped to save the time of soaking. Generally *Bacillus* species have variable effects on the seed germination. There are positive effects of *Bacillus* on seed germination and plant growth as a result of their production of plant growth-promoting substances. *Bacillus* species generally have inhibitory effect on germination of *Cuscuta. campestris* and alfalfa [9].

The association between bacteria and plant roots acquire time depending on several factors. The optimum induction or inhibition depends on the rate of bacterial colonization into the seed, seed coat property and the type and amount of antibacterial substances in the seed [9]. Here some physical properties of the seed and some environmental factors may influence in different level. Soil borne bacterium *Acinetobacter* sp. can colonize and form a biofilm within 3 days of seedling growth. This microbe is known to improve the crop yield through solubilising the phosphorus or applied phosphate, secreting organic acids and/or enzymes, thus improving the availability of nutrient to host plant [2]. Usage of poor quality seeds results in low seedling vigor and poor growth of germ tube, mixed varieties differing in height and maturity and contribution of plant diseases [3]. To increase local production, good quality seeds were chosen [5].

## CONCLUSION

Germination of *Hibiscus esculentus*, *Phaseolus vulgaris* and *Vigna sinensis* could be induced by soaking of their seeds using *Pseudomonas* sp and *Bacillus coagulans*. Germination of *Hibiscus* and *Phaseolus* seeds could be promoted in an optimized manner by soaking with *Pseudomonas* for 10 minutes and 20 minutes respectively where as seeds of *Vigna sinensis* could be promoted by soaking with in *Bacillus coagulans* for 20 minutes. Promotion of germination by soaking was not possible in *Beta vulgaris* by these bacteria due to poor colonization ability, higher amount and hard substrates, thickened seed coat properties etc. *Serratia*, *Klebsiella*, *Bacillus* sp and *E.coli* had no effect on seed germination. Minimum soaking time was found out to save the time. Very large scale multicentre studies should be done to find out the effect of diverse soil bacteria on the germination of different plant seeds in order to promote germination and to determine the minimum time needed for soaking to get optimum promotion.

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