Influence of *Bacillus subtilis* bacteria with enhanced α-amylase expression on seed germination time, germination rate and plant growth of *Spinacia oleracia* plant

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Abstract :

This paper illustrates the relationship between plant seed germination time, germination rate and seedling growth in the presence of soil-dwelling *Bacillus subtilis* bacteria. *Bacillus subtilis* is a rod-shaped gram-positive and predominant bacterial community that lives in soil, especially in the tropical regions of south-east Asia due to the humid and warm climate. The optimum temperature for microbial growth of this bacteria is 20° to 38° Celsius. Many Bacillus grouped bacteria (such as *Bacillus stearothermophilus*, *Bacillus licheniformis*, *Bacillus subtilis*) are found in the soil of Bangladesh. Production of alpha-amylase enzyme by these bacteria shortens germination time and the latent period of seeds. This study shows the effect and mechanism of shortening generation time and enhancing germination rate, seedling growth by *Bacillus subtilis* bacteria.

Keywords : germination time, germination rate, seedling growth, *Bacillus subtilis*, effect, shorten

Introduction :

This research analyzes one of the soil microbiome (*B. subtilis*) involved in shortening plant seed germination time and latent period. The soil dueling *Bacillus subtilis* bacteria produce an enzyme called alpha-amylase. Plant seed stores lipid and starch inside its shell. Plant seed cells have cellular organelles called glyoxysomes, which contains beta-oxidation enzymes and alpha-amylase enzyme. Alpha-amylase enzymes play a vital role in converting starch to fructose and glucose, which help plant seed to develop their differentiated tissues in a faster way. Alpha-amylase starch converting process also stimulates gibberellin production, which promotes seedling and plant growth, and vernalin, which shortens plant seed germination time and latent period. The alpha-amylase enzyme also affects phytohormones and increases signal peptide activity in the cell, which increases cell communication and signaling, making an infection-free, healthy plant.

Materials & Method :

For easier and proper understanding, we have divided this section into 4 sub-points –

i)Selective media preparation of *Bacillus subtilis* Bacteria :For preparing culture, we have used Petri dishes, sterile inoculating needles, and PLET agar, which is used as selective media for *Bacillus subtilis* bacteria. PLET agar (Polymyxin B-Lysozym-EDTA-Thallous acetate agar) is the best selective medium for isolation and cultivation of *Bacillus subtilis* bacteria. This medium inhibits further microbial growth without *Bacillus subtilis* bacteria. Agar is a gelatinous element that creates a semi-solid, stable environment for bacterial growth. After sterilizing Petri dishes, we used PLET agar in 15g/L concentration in each Petri dish. For sterilization of experimental tools, the tools were exposed to 121°C temperature and 15 psi pressure in the autoclave for 30 minutes.

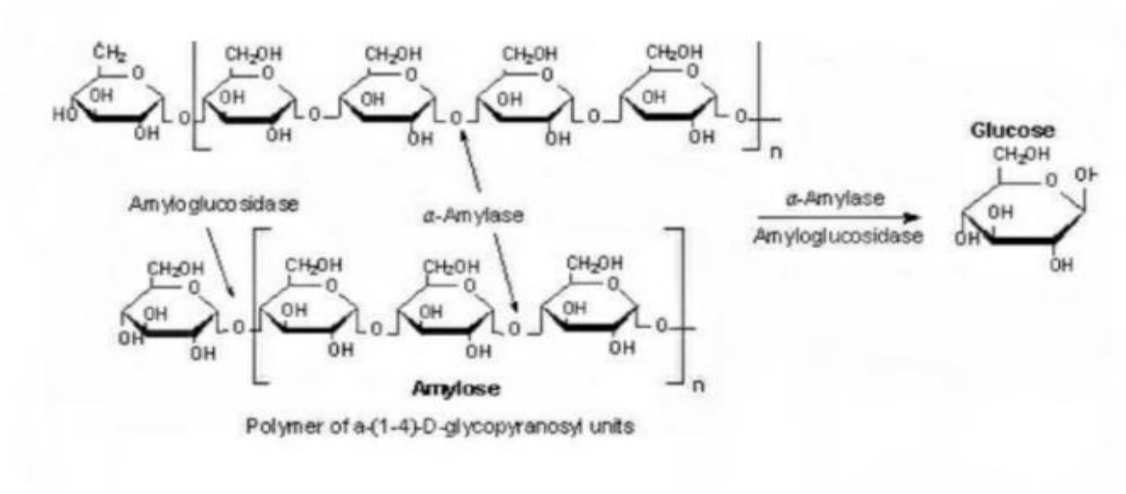
ii)Preparation of Bacillus bacterial source plates : We have used *Bacillus subtilis* bactobead, sterile inoculation loop, sterile broth, and incubation oven for this step. At first, we removed a bactobead from the vial using a sterile loop and transferred the bead to the edge of the selective agar plate. Then we dissolved the bead with 10 microliters of sterile liquid broth. We streaked the loop back and forth through the dissolved bactobead to make a primary streak at the top of the plate. Then we rotated it and streaked the loop through the primary streak to a clean part of the agar several times to create a secondary streak. We repeated this process one more time, and we prepared additional source plates using this procedure. After that, we covered all the plates with lids and incubated them inverted at 35° Celsius for 48 hours. After 48 hours, in all prepared plates, CFU (Colony Forming Unit) was visible of *Bacillus subtilis* bacteria.

iii) **Nutritionally enhancing amylase expression in B. subtilis:** *Bacillus subtilis* cultures were introduced in a nutrient medium containing Maltose and Maltodextrin for 28 hours at 33°C. Nutritional induction enhances amyR binding and RNA polymerase activity resulting in increased alpha-amylase expression rate.

iii)Infusing Seeds with Bacteria: Our experiment was conducted on pepper seed. Then we mixed up bacteria by inoculation needle in 25% 500ml sucrose and broth solution after prepping pepper seeds. From 500 ml, 50 ml of this bacterial solution was mixed in each bag of soil containing pepper seed of the Experimental group. We mixed compost fertilizers with soil in a 1:7.5 ratio for bacteria growth. We take 62 gram soil and 8 gram of compost in each seedling bag.10 seeds were infused with a bacterial solution, which was the experimental group, and 10 were not, which were the control group for our investigation. They were implanted in an individual bag and kept in an environment where each seed could get equal amounts of sunlight.

iv)Alpha Amylase Production (Starch Hydrolysis Test) : From bactobead, we grew *B.subtilis* on starch agar. Starch agar is a differential medium that tests the ability of an organism to produce certain exoenzymes such as Alpha-Amylase, Oligo-1, 6-glucosidase that hydrolyze starch. Starch agar is a nutritive medium with starch added. After growing *B.subtilis* bacteria in starch, due to secretion of Alpha-Amylase from bacterial cells, starch will be converted into glucose. Then We added iodine solution (10%) in the medium, and the color turned into pale white. After adding iodine solution, if there was no glucose or monosaccharide instead of starch, the color of the mixture would be a dark purple, but here due to hydrolysis of starch for the presence of Alpha-Amylase enzyme, starch was converted into simple sugar, so the color was seen light pink. This test proves the production of Alpha-amylase by *Bacillus subtilis* bacteria in our media.

Light pale pink or white = Alpha Amylase(+)



Dark purple = Alpha Amylase(-)

Result :

We observed the plants and collected data from both the control group and the experimental group. Plants in the experimental group had germination time, almost half of the control grouped plants.

In the experiment group, 10 among 10 seeds were germinated.

So, germination rate = (10÷10)×100% = 100%

In the control group,8 among 10 seeds were germinated.

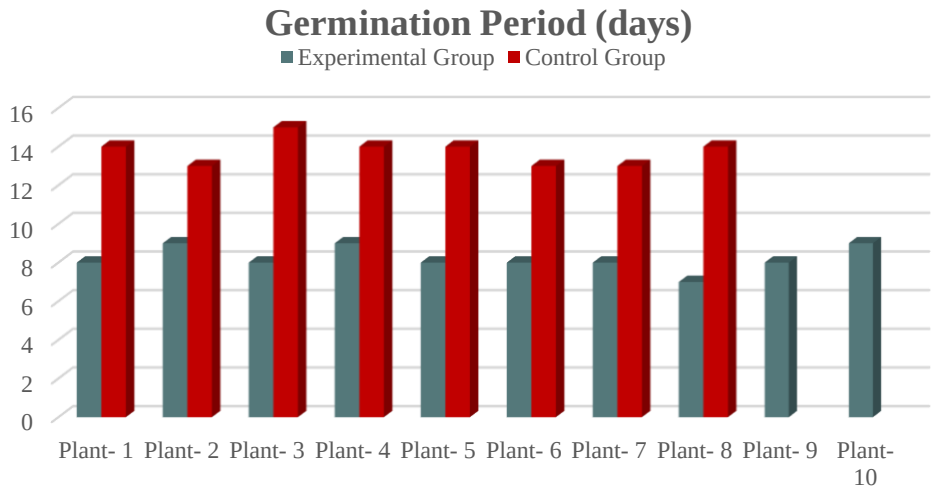
So, germination rate = (8÷10)×100% = 80%

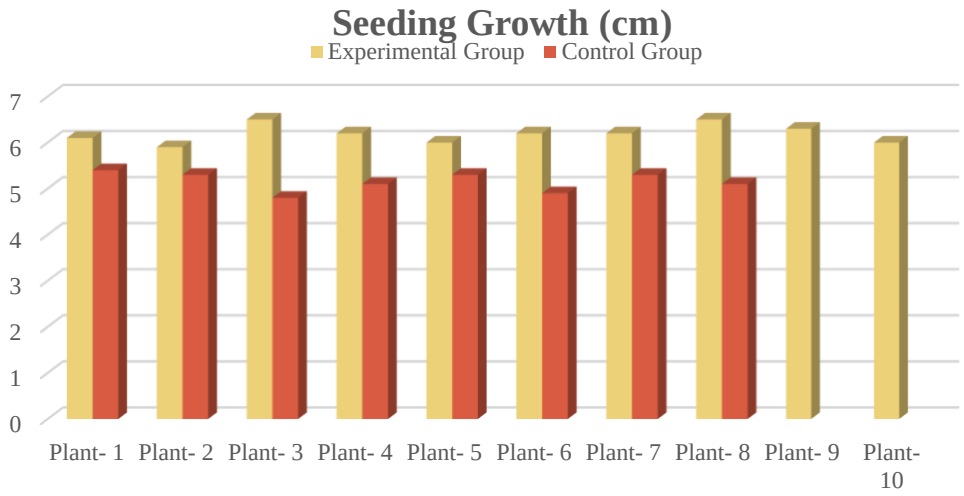
Plant sl no.	Experimental Group					Control Group				
	Germination time (days)	Average	Standard deviation	Germination rate (germinated seeds number ÷ Total number of seeds)×100%	Seedling growth 1st 2 week after germination (cm)	Germination time (days)	Average	Standard deviation	Germination rate (germinated seeds number ÷ Total number of seeds)×100%	Seedling growth 1st 2 week after germination (cm)
Plant-1	08	8.2	0.6	100%	6.1	14	12.875	3.103	80%	5.4
Plant-2	09				5.9	13				5.3
Plant-3	08				6.5	15				4.8
Plant-4	09				6.2	14				5.1
Plant-5	08				6	14				5.3
Plant-6	08				6.2	13				4.9
Plant-7	08				6.2	13				5.3
Plant-8	07				6.5	14				5.1
Plant-9	08				6.3					
Plant-10	09				6					

2 weeks after germination, the seedlings of the experimental group were taller than the germinated seedlings of the control group.

Here, we have presented the collected and analyzed data –

Alpha-amylase concentration in soil is dependent on bacterial cell number. Plants in the soil enriched with *Bacillus subtilis* bacterial community will have a short germination time, and other plants will have a longer germination time.





Discussion :

The result from our investigation shows that *Bacillus subtilis* bacteria is an essential factor for shortening seed germination time, latent period and increases germination rate and seedling growth. The findings showed a significant difference to the plants of the experimental group and plants of the control group.

The application of our experiment-

1) This method for producing fast germinating and the healthy plant is easy, cheap and can be done with some basic lab tools.
2) Secretion of Alpha-amylase from *Bacillus subtilis* bacteria not only shortens germination time and the latent period of seed but also promotes seedling growth and regulates phytohormone, causing a healthy plant.
3) Once a territorial colony formed in the soil, they can't be broken easily. Bacillus bacteria can be replicate rapidly, and they are resistant to adverse environmental conditions as well as they have a broad spectrum of bio-control abilities. So, they restrict the growth of other pathogenic microorganisms of plants.
4) Volatile Organic Compounds (VOC) produced by *Bacillus subtilis* also play an important role in plant growth promotion and activation of a plant defence mechanism by triggering the Induced Systemic Resistance (ISR). Endoscopic and enzymatic products of *Bacillus subtilis* are highly active against many fungal pathogens.
5) In this method, testing bacterial presence and determining cell number is quite easy. By adding a measured amount of iodine solution in starch agar and after reaction observing the change in colour of starch in which some portion of dark purple colour and some light pink colour is seen, we can determine the Alpha-amylase concentration. By this, we can quantify bacterial cell number.
6) *Bacillus subtilis* synthesize various types of lipopeptides based on secondary metabolites with specific activities against plant pathogens which give them unique importance in agriculture, biotechnology and pharmaceutical industries.

Conclusion :

From all of our analyzed data, we can say that Bacillus subtilis bacteria play a vital role in shortening plant seed germination time and increasing germination rate and seedling growth. But the resulted data may be changed if this experiment cannot be done in the same factors & no. of plants as ours. Before germination, the average was the illumination of sunlight 56,150 lux, and after germination, the average germination was 42,570 lux .

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