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# Seed Priming with Chitosan for Enhanced Plant Growth under Salt Stress

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## Abstract
Chitosan is a polysaccharide biopolymer derived from Deacetylation of chitin using microbes. Chitosan not only stimulates growth and increases the crop yields but also alleviates the harmful effect of abiotic stress on plant growth as well as preserves enzymatic activity of the seeds. The objective of this study was to investigate the effects of chitosan coated seeds on germination, seed vigor index, root and shoot length of rice seeds using mangrove soil. The chitosan was dissolved in 0.5% and 1% acetic acid and subjected to seed priming. The parameters measured after seed priming is as follows:

| Isolate | Seed Vigor Index | | Shoot Length (cm) | | Root Length (cm) | | Germination Index | | Germination Percentage | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.5% | 1.0% | 0.5% | 1.0% | 0.5% | 1.0% | 0.5% | 1.0% | 0.5% | 1.0% |
| IS 5 | 3610 | 3420 | 16.1 | 14.03 | 17 | 18.03 | 5.5 | 6.1 | 79.2 | 87 |
| IS 12 | 3145 | 3240 | 12.8 | 16.3 | 15.1 | 16.4 | 5.6 | 6.1 | 80 | 87 |

From the above result, the seeds coated with chitosan obtained from IS 5 were found to be efficient in enhancing the seed vigor and germination of the coated seeds. The IS 5 was subjected to double pass FASTA for genus level identification and belongs to Bacillus Spp. Hence, from this investigation was proved that chitosan coating enhances the seed germination, preserves the enzyme activity and protects the seed from salt stress.

**Keywords**
Chitosan, Deacetylation, mangrove soil, seed vigor index, enzyme activity

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## INTRODUCTION
Environmental pollution is a natural consequence of human activities. In the course of human development, industrialization has made possible higher standards of living in our modern society. Such "progress" has created increased problems with wastes from processing operations and their ultimate disposal creating water pollution, air pollution and land pollution. The nature of wastes is wide and varied, being broadly classified into agricultural, municipal and industrial. (Aranaz I., *et al.,* 2009). Salinity is one of the major abiotic stresses limiting crop production, particularly in arid and semi-arid regions. Salt stress affects plant physiology at both whole plant and cellular levels through osmotic and ionic stress. Germination and seedling

growth of plant are decreased in salinity condition due to an external osmotic potential that prevents water uptake or due to the toxic effects of $Na^+$ and $Cl^-$ ions or both on the germinating seed. Salinity also reduces the plant growth and development through specific ions effects, nutritional imbalance, low osmotic potential of soil solution and combinations of these factors. All of these factors caused by high salt contents can affect various major plant processes like photosynthesis, protein synthesis and also energy and lipid metabolisms. The negative effects of salinity on plant is being tried to be improve by new research methods. Application of chitosan is one of the methods to decrease the negative effect of abiotic stress. Chitosan is a linear β-(1, 4)-glucosamine polymers produced by deacetylation of chitin and is an important structural component of several plant fungi cell walls. The positive traits of chitosan give to this polymer numerous and unique physiological and biological properties with great potential in a wide range of industries such as pharmacology, medicine, and agriculture. One of the most important bioactivities of chitosan on plants is stimulation of seed germination. (Mahdavi B. *et al.,* 2013) This paper investigates the effectiveness of seed priming (coating) with chitosan dissolved in acetic acid for enhanced plant growth under salt stress.

## MATERIALS AND METHODS

The chitosan was dissolved in 0.5% & 1% acetic acid solution. Then the rice seeds were soaked in the 0.5% & 1% acetic acid solution for one hour at 25°C and the control seeds was not subjected to chitosan treatment. The chitosan coated seeds sowed in mangrove soil. The moisture content was monitored regularly. At an interval of 7 days, seed vigor index, germination percentage & index, shoot and root length was measured.

**Seed vigor index**

This was calculated by determining the germination percentage and seedling length of the same seed lot. Twenty seeds each in four replications are germinated in towel papers as prescribed for the crop species in germination test. Seed vigor index is calculated by multiplying germination (%) and seedling length. The seed lot showing the higher seed vigor index is considered to be more vigorous.

**Germination rate**

According to the seed test procedures methods of Inter-National Seed Association (ISTA), germination percentage (GP) and germination index (GI) were as follow:

**Germination Percentage (GP) = D/E x 100%**

D is the number of germinated seeds on the seventh day,

E is the number of total seeds investigated

**Germination Index (GI) = ∑ (F/G) x 100%**

F is the number of germinated seeds one day,

G is the days of seeds germination

## Results & Discussion

The chitosan was obtained by Deacetylation of chitin by chitin deacetylase positive isolates (IS5, IS6, IS12, IS13 and IS14). Then the chitosan was dissolved in 0.5% and 1.0% acetic acid solution followed by seed coating. The growth parameters under salinity conditions were evaluated. This helps in studying the seeds with a greater tolerance to salt stress and their survival in those environments.

**Seed Vigor Index**

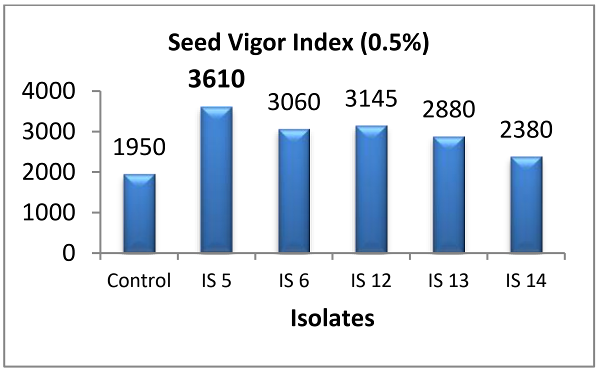
Vigor testing does not only measure the percentage of viable seed in a sample, it also reflects the ability of those seeds to produce normal seedlings under less than optimum or adverse growing conditions similar to those which may occur in the field. The seed vigor index was measured with a maximum of 3610 for IS5 and the minimum of 1950 for control seeds using 0.5% chitosan coated seeds. Similarly, for 1.0% chitosan coated seeds with the maximum seed vigor index were 3420 for IS5 and 1560 for control was minimum. Seed vigor testing is also used as indicator of the storage potential of a seed. Hence, chitosan protects the seed and helps in vigoring it under the salt stress. Chitosan obtained from IS5 was found to be effective in enhancing the vigoring of the seed.

| Isolate | Total seeds | Germinated Seeds | % | Seed Length (mm) | SVI |
|---|---|---|---|---|---|
| **Control** | 20 | 13 | 65 | 30 | 1950 |
| **IS 5** | 20 | 19 | 95 | 38 | 3610 |
| **IS 6** | 20 | 17 | 85 | 36 | 3060 |
| **IS 12** | 20 | 17 | 85 | 37 | 3145 |
| **IS 13** | 20 | 18 | 90 | 32 | 2880 |
| **IS 14** | 20 | 17 | 85 | 28 | 2380 |

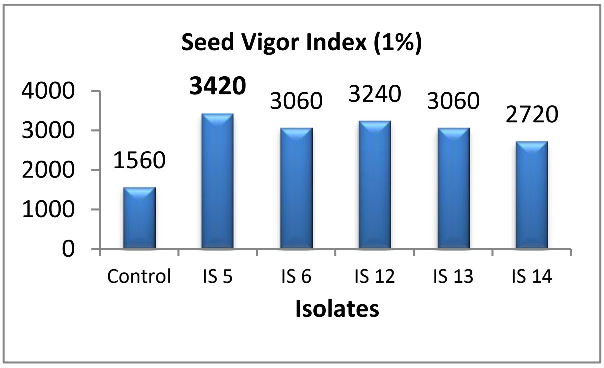
**Table 1:** Seed vigor index (SVI) of seeds coated with chitosan dissolved in 0.5% acetic acid

| Isolate | Total seeds | Germinated Seeds | % | Seed Length (mm) | SVI |
|---|---|---|---|---|---|
| **Control** | 20 | 12 | 60 | 26 | 1560 |
| **IS 5** | 20 | 18 | 90 | 38 | 3420 |
| **IS 6** | 20 | 17 | 85 | 36 | 3060 |
| **IS 12** | 20 | 18 | 90 | 36 | 3240 |
| **IS 13** | 20 | 18 | 90 | 34 | 3060 |
| **IS 14** | 20 | 17 | 85 | 32 | 2720 |

**Table 2:** Seed vigor index (SVI) of seeds coated with chitosan dissolved in 1.0% acetic acid



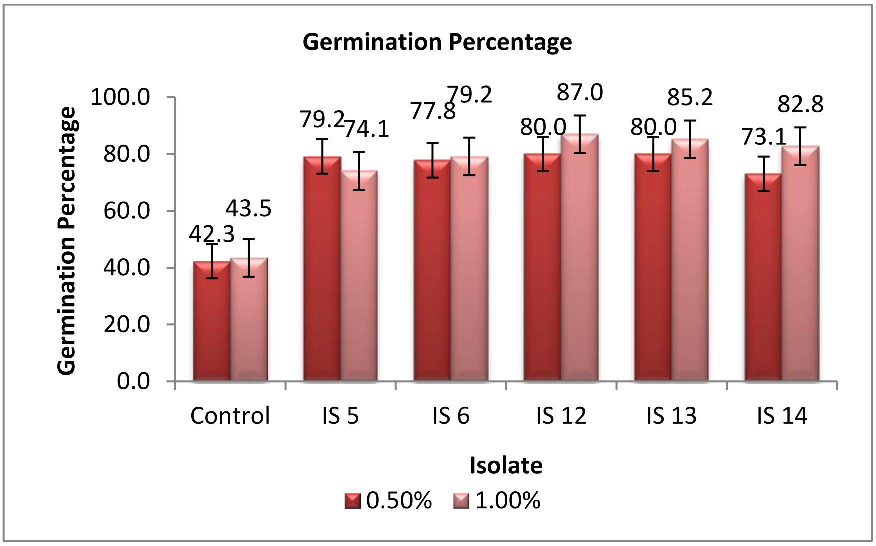

**Graph 1**: Seed vigor index (0.5%)



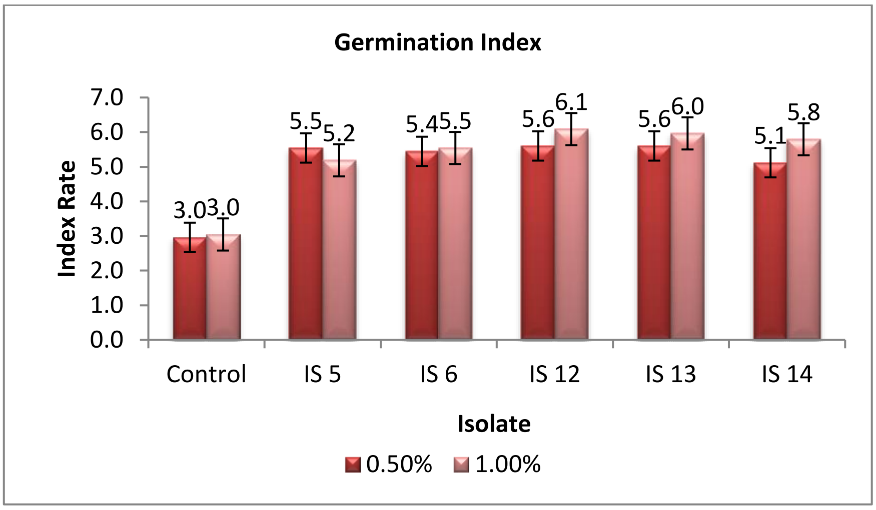

**Graph 2:** Seed vigor index (1.0%)

**Germination Rate**

Germination is the growth of a plant contained within a seed; it results in the formation of the seedling; it is also the process of reactivation of metabolic machinery of the seed resulting in the emergence of radical and plumule. One of the most important bioactivities of chitosan on plants is stimulation of seed germination. Germination rate of chitosan coated seeds was measured. The chitosan treatment significantly increased germination percentage by 46.06 % as compared to control. Chitosan seed pretreatments significantly affected shoot and root length. The results of germination percentage and germination rate were represented in graph 3 and 4.




**Graph 3:** Results of Germination Percentage of chitosan coated seeds

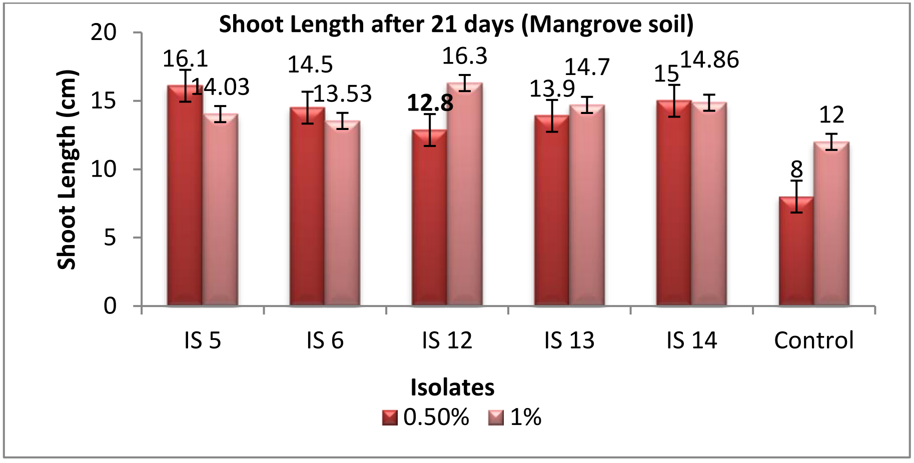



**Graph 4:** Results of Germination index of chitosan coated seeds

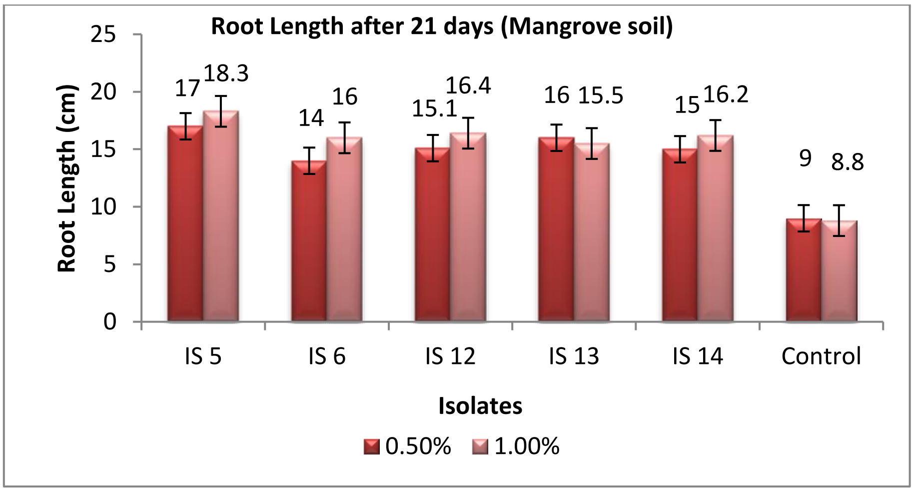
The chitosan obtained on deacetylation of chitin by IS5 was found to be consistent in enhancing the properties viz., seed vigoring, germination of the seeds.

**Growth Promotion**

The root and shoot lengths are the most important parameters for salt stress, because roots are in direct contacts with soil and absorb water from soil and supply it to the rest of the plant. For this reason, root and shoot length provides an important clue plant response to salt stress. The results of chitosan coated seeds for the promotion of plant growth (root & shoot length) is represented in the graph 5 & 6.




**Graph 5:** Results of shoot length of chitosan coated seeds




**Graph 6:** Results of root length of chitosan coated seeds

The chitosan treatment was significant for increasing the root and shoot length. The shoot length of seeds coated with chitosan obtained from IS5 was 50% more effective in enhancing the shoot length than the control. While in case of root length, there was a significant increase than the control. In addition, other studies also supported a role of chitosan in modulating the plant response to several abiotic stresses including salt and water stress. Chitosan obtained from IS5 was proved to e effective in elevating the properties of seed vigor index, germination rate and root & shoot length. Hence, chitosan protects the seeds and preserves the enzyme activity under salt stress.

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