The role of Ascorbic acid application on germination of *Vignamungo (L)* under saline stress

Dr.S.Jasminemary[1], S.Surega[2], D.Santhanabharathi[3]

[1]Lecturer in Chemistry, [2,3]M.Sc. in Chemistry
Gcw (Autonomous),
Kumbakonam – 612 001.

***Abstract*: High salt level of a germination medium may induce a reduction, delay and even complete inhibition of germination due to osmotic effect and ion toxicity. The objective of this study was to investigate the effect of salinity due to the level of NaCl solution and the effect of different composition of ascorbic acid using against the salt solution of the crop *Vignamungo (L)*. Seeds of these crop was treated with 1% NaCl solution and the five different composition of ascorbic acid against salt solution prepared and applied in petri dishes. Surface sterilized seeds were sown as 10 per petri dishes. Germination percentage, decreased in 1% NaCl solution and gradually increased with an increase in level of ascorbic acid against salt solution. Although the crop is low salt tolerant legume and the ascorbic acid is increasing the salt tolerant nature of *Vignamungo (L)*. This study could be strengthened by further work under field conditions and also at mature vegetative and reproductive stages of the crop.**

***Keywords*: Ascorbic acid, germination percentage, NaCl solution, salt tolerance.**

Introduction

Germination is the initial stage of a plant's life cycle and determines where and when a crop can be established. It is a complex metabolic process that oxidizes the lipids and carbohydrates within the seed and breaks down storage proteins in order to obtain energy and amino acids necessary for plant development (Almansouri*et al.*, 2001). Seed germination under saline condition is considered as major factors limiting the establishment of crops (Kitajima and Fenner, 2000). Interaction between seedbed environment and seed quality is also important (Khajeh – Hosseini *et al.*, 2003).

The effect of salinity on germination can be either by creating osmotic potential which prevents the uptake of water. Soil salinity is a worldwide problem hampering productivity of several agricultural crops. Many attempts have been made to alleviate the effect of sodium chloride on plants cells (HAWADA, 1994).

Germination is a critical part of plant life histories. The ability of their seeds to germinate at salt concentration in the soil is therefore of crucial importance for the survival and perpetuation of these species. In saline habitats, seed germination takes place after high precipitation, i.e., under conditions of reduced soil salinity (KHAN and RIZVI, 1994).

Although salinity stress mostly reduces the germination percentage and delays the onset of germination, its effects are modified by interactions with other environmental factors as temperature and light. Salinity can affect germination by affecting the osmotic component, which the ionic component, i.e., Na and Cl accumulation (ZIVKOVIC *et al.*, 2007).

The abscisic acid has been proposed to act as a mediator in plant responses to a range of stresses, including drought and salt stress. IAA (Indole Acetic Acid) plays a major role on regulating plant growth, it controls vascular tissue development, cell elongation and apical dominance. Gibberllins are generally involved in growth and development. They control seed germination, leaf expansion, stem elongation and flowering. Ascorbic acid is involved in the regulation of many critical biological processes such as photoinhibition and cell elongation.

In this paper, effect of 1% NaCl solution on seed germination and the effect of five different composition of ascorbic acid against salt solution on seed germination of the plant ***Vignamungo (L).*** It germinate in the presence of certain concentration of NaCl solution and the certain composition of ascorbic acid using against salt solution. By this experiment we wanted to determine which composition of ascorbic acid against the salt solution would be increased the process of germination of seeds on given plants.

The objective of this study was to evaluate the potential for mitigation of salt stress in ***Vignamungo (L)*** plants by applying the ascorbic acid. The purpose of this preliminary study is to assess the effect of treatment with ascorbic acid on the salt tolerance of ***Vignamungo (L)*** seeds.

Materials and methods

The sterilized, hand selected seeds of ***Vignamungo (L)*** was used for this study. Before beginning the experiment, solutions were made by dissolving **one gram of Sodium chloride** in **100 ml distilled water.** The salt solution was prepared

freshly and the seeds were treated for the germination. The five different compositions of ascorbic acid (i.e. 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml) against 1% NaCl solution is using for the seed germination.

Seeds of ***Vignamungo (L)*** were separated by sieving and for each type, 10 seeds were soaked in 1% NaCl solution.

Following the solution preparation, petri dishes were washed and disinfected with alcohol and air dried. Germination chambers were prepared from the sterilized glass petri dishes and filter papers (whatman no.2). The filter papers were cut into two pieces of equal size with labelled seed compartments and concentrations of salt solutions and made ready for use.

Seed priming experiment

- There were 2 seed pretreatment reagents, namely 1% NaCl solution and ascorbic acid (0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml) and unprimed seeds were used as control.
- 10 seeds were placed in two layers of filter paper in a 12cm petri dish. The filter paper was moistened with about priming reagents, ensuring that the seeds were immerged with solutions.

Germination percentage (%)

Germination is a complex phenomenon involving many physiological and biochemical changes leading to the activation of embryo. Seed germination is the most critical and sensitive stage to salinity stresses. Poor germination in saline soils leads to poor crop stand and productivity. Germinability of the black gram seeds under salt stress condition is a index of salt tolerance (Vadez*et al.,* 2005).

The germinability was recorded on the 5th day after day of sowing (DAS) and number of seeds germinated was expressed as percentage.

$$\textbf{Germination percentage} = \frac{\textbf{No. of germinated Seeds} \times 100}{\textbf{No of seeds kept for germination}}$$

Fresh and dry weight (gm)

The total fresh weight of the seeds were recorded using a monopan balance. For observing the dry weight of the seeds were dried in shadow and the readings were tabulated.

Result and Discussion

Effect of 1% NaCl solution and five different composition of ascorbic acid on the germination of black gram *(Vignamungo .L)*

Germination and its percentage

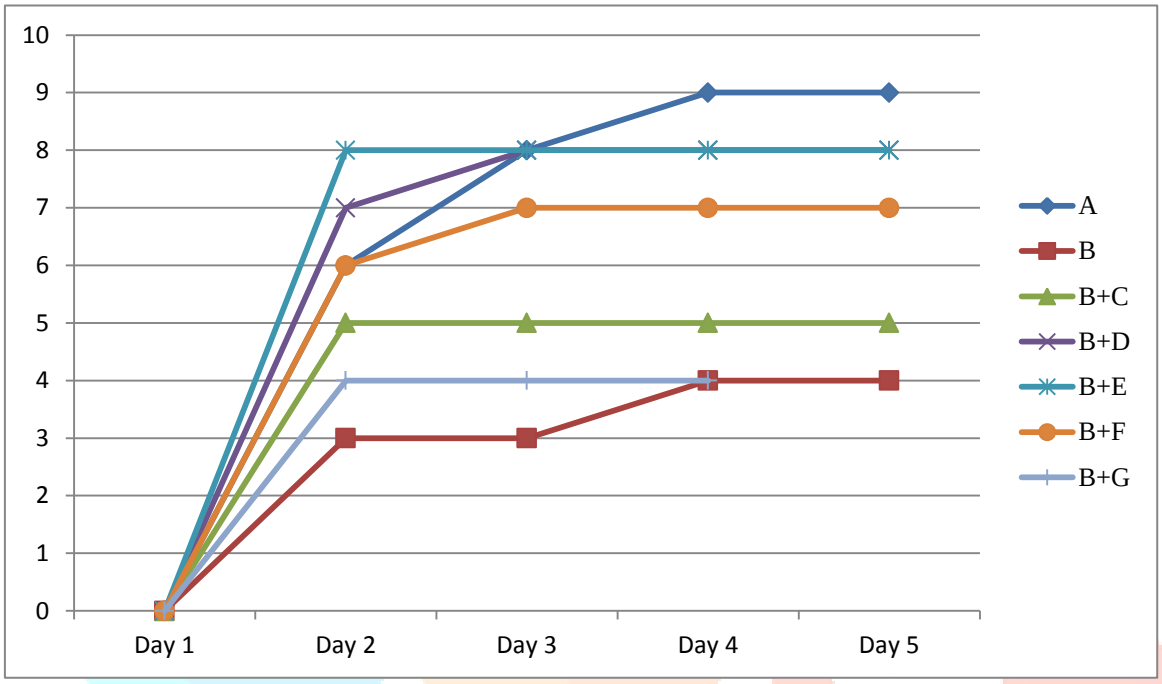
The germination of black gram seeds were observed to 5 days and the germinated seeds are calculated and presented in the Table.1

Table.1 Germination of *Vignamungo (L)*

	Day 1	Day 2	Day 3	Day 4	Day 5
Control	0	6	8	9	9
1% Nacl solution	0	3	3	4	4
1% NaCl solution + 0.1ml ascorbic acid	0	5	5	5	5
1% NaCl solution + 0.2ml ascorbic acid	0	7	8	8	8
1% NaCl solution + 0.3ml ascorbic acid	0	8	8	8	8

1% NaCl solution + 0.4ml ascorbic acid	**0**	**6**	**7**	**7**	**7**
1% NaCl solution + 0.5ml ascorbic acid	**0**	**4**	**4**	**4**	**4**

Figure.1 Germination of *Vignamungo (L)*



A-Control

B-1%NaCl Solution

C-0.1ml Ascorbic acid

D-0.2ml Ascorbic acid

E-o.3ml Ascorbic acid

F-0.4ml Ascorbic acid

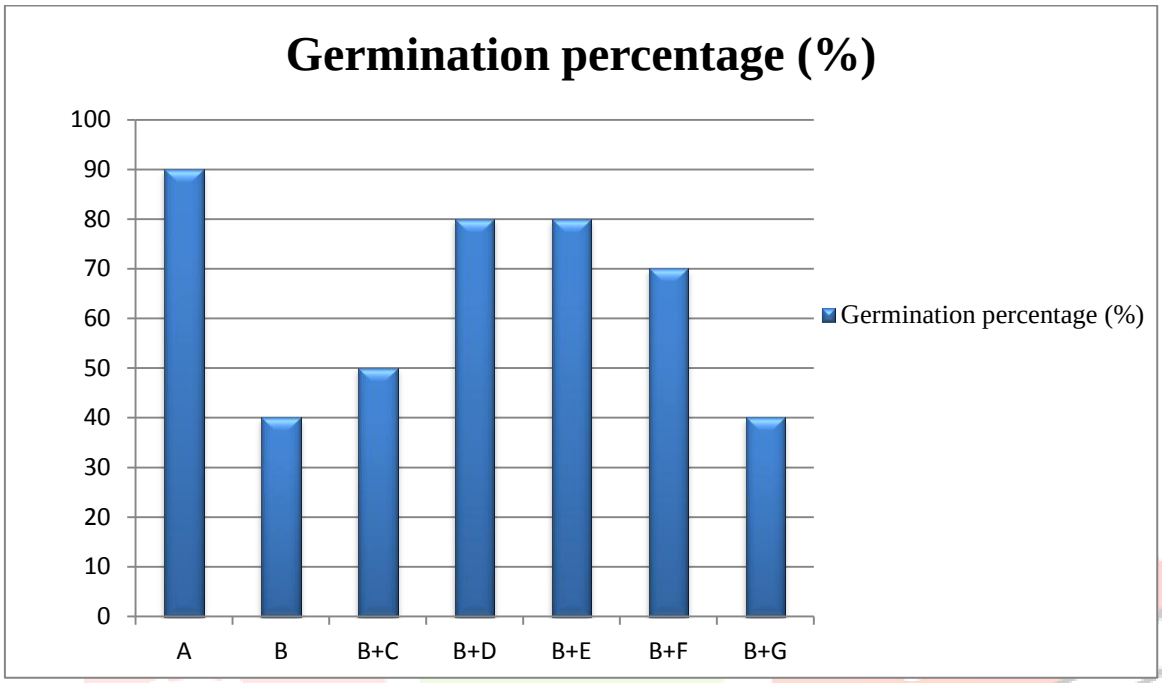
G-0.5ml Ascorbic acid

Table.2 Germination percentage of *Vignamungo (L)*

	Germination percentage (%)
Control	**90**
1% NaCl solution	**40**
1% NaCl solution + 0.1ml ascorbic acid	**50**
1% NaCl solution + 0.2ml ascorbic acid	**80**

1% NaCl solution + 0.3ml ascorbic acid	**80**
1% NaCl solution + 0.4ml ascorbic acid	**70**
1% NaCl solution + 0.5ml ascorbic acid	**40**

Figure.2 Germination percentage of *Vignamungo (L)*



A- Control

B- 1% NaCl solution

C- 0.1ml Ascorbic acid

D- 0.2ml Ascorbic acid

E- 0.3ml Ascorbic acid

F- 0.4ml Ascorbic acid

G- 0.5ml Ascorbic acid

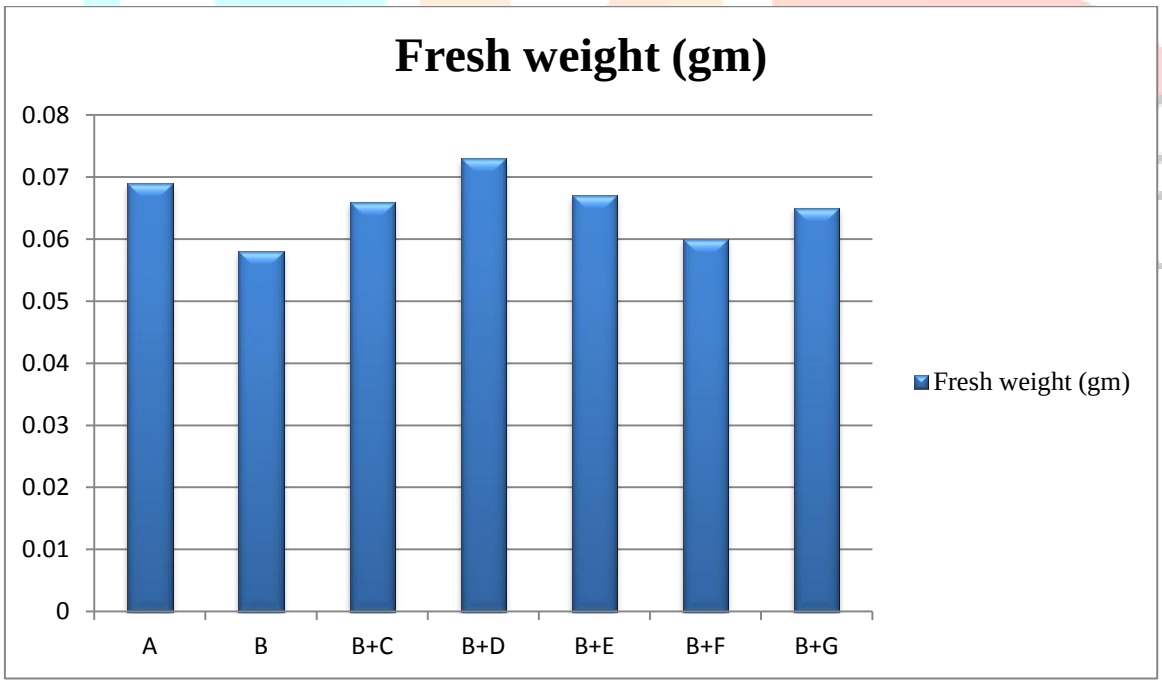
Fresh weight (gm)

The mean fresh weight of the germinated seeds in each treatment is given in Table.3. The fresh weight of the seeds increased gradually during the different treatments. On 5th day the fresh weight of the germinated seeds shows maximum at 0.2ml of ascorbic acid against 1% NaCl solution (i.e) 0.073gm

Table.3 Germination fresh weight of *Vignamungo (L)*

	Fresh weight (gm)
Control	0.069
1% NaCl solution	0.058
1% NaCl solution + 0.1ml ascorbic acid	0.066
1% NaCl solution + 0.2ml ascorbic acid	0.073
1% NaCl solution + 0.3ml ascorbic acid	0.067
1% NaCl solution + 0.4ml ascorbic acid	0.060
1% NaCl solution + 0.5ml ascorbic acid	0.068

Figure.3 Fresh weight of *Vignamungo (L)*



A- Control

B- 1% NaCl solution

C- 0.1ml Ascorbic acid

D- 0.2ml Ascorbic acid

E- 0.3ml Ascorbic acid

F- 0.4ml Ascorbic acid

G- 0.5ml Ascorbic acid

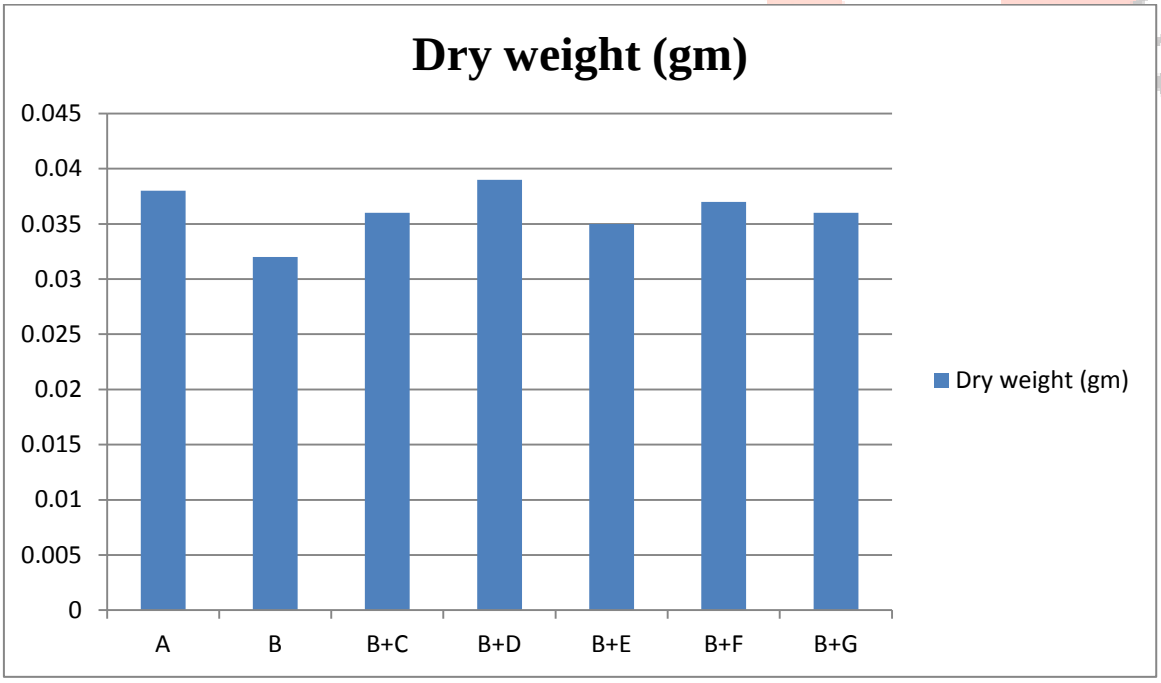
Dry weight (gm)

The germinated seeds were dried in shadow and weighed. The mean dry weight of the germinated seeds in each treatment is given in Table.4. The dry weight of the seeds increased gradually during the different treatments. On 5th day the dry weight of the germinated seeds shows maximum at 0.2ml of ascorbic acid against 1% NaCl solution (i.e) 0.039gm.

Table. 4 Germination dry weight of *Vignamungo (L)*

	Dry weight (gm)
Control	0.038
1% NaCl solution	0.032
1% NaCl solution + 0.1ml ascorbic acid	0.036
1% NaCl solution + 0.2ml ascorbic acid	0.039
1% NaCl solution + 0.3ml ascorbic acid	0.035
1% NaCl solution + 0.4ml ascorbic acid	0.037
1% NaCl solution + 0.5ml ascorbic acid	0.036

Figure.4 Dry weight of *Vignamungo (L)*



A-Control

B-1%NaCl Solution

C-0.1ml Ascorbic acid

D-0.2ml Ascorbic acid

E-o.3ml Ascorbic acid

F-0.4ml Ascorbic acid

G-0.5ml Ascorbic acid

Discussion

As predicted in our hypothesis, overall germination of receiving salt solution (i.e) 1% NaCl solution was less than the control group. Table.1 shows the overall germination of the control group of black gram seeds with an increase of **9** seeds, while seeds receiving 1% NaCl solution showed only on **4** seeds are germinated. Then the seeds receiving 0.2ml and 0.3ml ascorbic acid against salt solution showed an increase of seeds (i.e) **8** seeds.

In our experimental groups supports our hypothesis that 1% NaCl solution adversely affect the germination of black gram seeds. Limited germination is achieved in 1% NaCl solution group compared to the control group. Then the 0.2ml and 0.3ml of ascorbic acid is used to against that 1% NaCl solution, it is tolerates the salinity level on the black gram seeds. Then the highest germination is achieved in control group seeds compared to the seeds germinated in 1% NaCl solution group.

Adding ascorbic acid on black gram seeds could also increase the germination of these seeds and increase the tolerance of the black gram seeds against salinity.

Results of this experiment indicates support that different composition of ascorbic acid solution can induced the germination of black gram seeds under the saline condition (i.e) 1% NaCl solution is added.

Conclusion

From the results obtained in the present investigation, we can conclude that overall showed better tolerance to salt stress with a lesser extent of antagonistic effect of NaCl on germination and the effect of ascorbic acid against salt solution and biomass production of germination stage. All these biochemical parameters might have played an important role in salt tolerance nature of black gram seeds. In further research should be carried out to findout the producing the salt tolerance of black gram at genetic level.

References

[1] Almansouri MJ, Kinet M, Lutts S (2001). Effect of salt and osmotic stress on germination in durum Wheat (Triticum durum Desf.). Plant Soil 231:243-254

[2] BerhanuAbrahaTsegay and BerhaneGebreslassie (2014). The effect of salinity (NaCl) on germination and early seedling growth of Lathyrussativus and Pisumsativum var. Abyssinicum.

[3] BiljanaBojovic, GoricaDelic, Marina Topuzovic and Milan Stankovic (2010). Effects of NaCl on seed germination in some species from familiesBrassicaceae and Solanaceae.

[4] HAWADA, A.M. (1994): Alleviation of the adverse effect of NaCl on germination of maize grains by calcium. BiologiaPlantarum, 20, 623-627.

[5] Khajeh-Hosseni MA, Powell A, Bingham IJ (2003). The interaction between salinity stress and seed vigour during germination of soybean seeds. Seed Sci. Technol. 31:751-725.

[6] KHAN, A.M., RIZVI, Y. (1994): Effect of salinity, temperature and growth regulators on the germination and early seedling growth of Atriplexgriffthii. Can. J. Bot., 72, 475-479.

[7] Kitajima K, Fenner M (2000). Ecology of seedling regeneration. J. Plant Cell Environ. 25:239-250.

[8] ZIVKOVIC, S., DEVIC, M.,FILIPOVIC, B., GIBA,Z. and GRUBISIC, D. (2007): Effect of NaCl on seed germination in some centaurium Hill. Species (Gentianaceae). Arch. Biol. Sci., 59 (3), 227-231.