EFFECT OF ZINC GLYCINATE ON SEED PRESOWING TREATMENT IN GREEN GRAM (*Vigna radiata)*

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Abstract

Seed presowing treatment was conducted in Sacred Heart College, Tirupattur, and Vellore. This study is aimed evaluate the effect of different seed hardening or priming treatment on the growth and morphological attributes of Green gram. Seeds were fully soaked in normal water, 1%, $CaCl_2$, 1% Zinc glycinate and 1% $CaCl_2$ plus 1% Zinc glycinate for 3 hours. The results showed that seed hardening treatment with dilute chemicals enhances seed germination, increases their morphological parameters and also increases the photosynthetic activity of plants when compared to the control. In conclusion, seed pretreated with 1% $CaCl_2$ plus 1% Zinc glycinate recorded the highest growth and morphology when compared to other treatments.

Key words: *Green gram, seed presowing, Zinc glycinate, $CaCl_2$ and Chlorophyll*

INTRODUCTION

Pulses, the legume crops contribute chief proteins part of diet throughout the world and are crucial for the conserved of good health. Besides providing rich proteins, it plays a vital role in sustainable agriculture through a distinct mechanism called nitrogen fixation and maintains the fertility of soil and also they grow well in the integrated forming farming in the paddy fields. It also provides healthful food for the livestock's (Christopher *et al*., 2017). Green gram is one of the most important pulse crop grown in India. The green gram is widely cultivated in India, Pakistan, Bangladesh, Sri Lanka, and Southeast Asia. It is used as an ingredient for the preparation of sweet dishes. There are more than 70% of the world's green gram production comes from India and which accounts for about 10-12% of the total pulse production in the country (Ministry of Agriculture, Government of India, 2014) it is quite versatile in nature which is the crop grown for seeds, green manure and it is also considered as "Golden Bean" said because of its nutritive values and suitability for increasing the soil by the addition of nitrogen to the soil. Green revolution which made our nation self-sufficient in cereals but we are still deficit in pulse production.

India still imports green gram from countries like Myanmar, Australia and Africa to fulfil the pulse requirements. Hence there is an urgent need of increasing pulse production in India to combat the shortage of pulse grains (Bhattacharjya *et al.*, 2017). The effective formation of the crop is mostly governed by the good quality of seeds. In order to enhance or improve the quality of the seed for the farmer, many seed companies have been established the new skills which are called as Seed Enrichment Techniques. These technologies are the key inter-phase between the two highly specialized industries namely Crop Production and Seed Industry.

Through the pre sowing treatment in the seed, one can enhance the quality of seeds. The seed can protect the stress which is caused by the abiotic stress and also produce equal germination, thus overall crop yields might be greatly enhanced. It is also reported that seed hardening can be useful to develop the rapid and uniform germination and emergence of the seeds and it also increases the seeds tolerance to adverse environmental conditions (FAO, 2012).

Amino acids are called building block of protein and play vital role in the plants and human wellbeing. Among the amino acids Glycine is the smallest amino acid, pass readily through plant tissues. Once inside the plant, the minerals/metals (e.g. calcium, zinc and manganese,) is released, and the leftover amino acids that formed the protective shell are either used by the plant directly as amino acids or further broken down into water-soluble nitrogen. Glycine boost up root cells to open up calcium ion channels and stimulate the plants to take up calcium ions several times faster than simple osmosis. Calcium is an important secondary messenger. As soon as plants are under attack from external biotic agent including insects, microbes and other pathogens, it induces the chain reaction and making various secondary metabolites to repel the attackers. Therefore, supplementing plants with amino acid especially, glycine could chelate calcium. Thus the combination of calcium and amino acids help strengthen the plants' natural immune system. It has been shown that administration of amino acids with plants can reduce the stress of High temperature, frost, low humidity etc., thus amino acids are directly related to the stress physiology. Further, the glycine converts the potentially harmful substance in to the harmless substance through it modulatory effect on the xenobiotic metabolizing enzymes. It provides the basic nitrogen source needed for the production of many amino acids. Glycine has the important role in the formation a natural antioxidant substance, called Glutathione. Zinc is one of the essential mineral for the synthesis of growth hormone. Hence, the present study was made an attempt made to find the combined effect Zinc-glycinate and $CaCl_2$ in seed pre-soaking treatments with low concentration of chemicals in promoting the growth and morphological parameters of green gram.

MATERIALS AND METHODS

Genetically pure seeds of Green gram obtained from the Tamil Nadu Pulse Research Station, Vamban-2, and Tamil Nadu. The bulk seeds were manually cleaned to remove unwanted material from the lot and were graded using 8 x 8 sieves for uniformity. Experiment was conducted in Sacred Heart College (Autonomous), Department of Biochemistry, Tirupattur, Vellore.

Chemicals

Zinc, Glycine and $CaCl_2$ were purchased from Himedia, Pvt. Mumbai. All other chemicals used for this study were standard and of analytical graded.

Seed source

The breeder seeds of green gram were obtained from Tamil Nadu Pulse Research Station, Vamban-2 Tamil Nadu

Steps involved in seed hardening

For the soaking of green gram, the dilute chemical solutions were prepared by weighing 1g of each chemical in 100ml of distilled water. The seeds were soaked in the solution. The green gram was soaked up to 3 hours in dilute chemical solution then it is allowed to dried under shade condition for a period of 3 hours and finally it dried under sun condition which bring back to its original moisture condition the hardened seed is germinated in the field and observed the growth parameters after 10 days. The seeds were divided into four groups which was soaken in the four treatment groups in dilute chemicals as follows

T_0 - Control (Water alone)

T_1 - 1% Calcium Chloride

T_2 - 1% Zinc Glycinate

T_3 - 1% Calcium Chloride + 1% Zinc Glycinate

Figure-1, 10th day after germinated

After the plant has grown it was observed under various field evaluation studies which includes germination percentage(%) , morphological characters such as root length, shoot length and number of leaflets and also the chlorophyll content of control and seed treated with chemicals are determined in that following results are obtained.

Germination Percentage (%)

Germination test was conducted in a standard size petri – dishes using blotting paper technique. Germination counts were taken after 7 days and the same were calculated using the following formula.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds put for germination}} \times 100$$

Root length (cm)

Ten normal seedlings were selected randomly in each treatment from all the replications upto tenth day from germination. The root length was measured from the tip of the primary root to base of hypocotyle with the help of a scale and mean root length was expressed in centimetre.

Shoot length (cm)

The experimental seedlings were used for root length measurement. The shoot length was measured form the tip of the primary leaf to the base of the hypocotyle and mean shoot length was expressed in centimetre.

Number of Leaves per Plant

The number of leaves per plant was counted and recorded a specific time intervals from overall plants in each replication.

Estimation of Chlorophyll a, b and total chlorophyll content

For different biochemical estimation the irradiated and non-irradiated plantlets were frozen in liquid nitrogen, ground to a powder with a mortar and pestle under chilled condition and kept in a freezer for further analyses. Lyophilized leaf (0.1 g) powder were homogenized in 80% acetone and centrifuged at 10,000×*g* for 10 min. The supernatant was subjected to spectrophotometer determination of chlorophyll *a* and *b* at 646 and 663 nm, respectively. Chlorophyll *a* and chlorophyll *b* content was determined according to the following equation and expressed in milligram per gram fresh weight of plant material.

RESULTS AND DISUSSION

Table 1& 2 shows the effect of effect amino acids, Zinc and $CaCl_2$ in different treated groups. A steady increase in germination percentage (Fig.2), height of the plants (Fig.3), root length, shoot length and number of leaflets were absorbed in the individuals chemicals treated groups. When compared to the control group. In T_3 group treated with $CaCl_2$+Zinc-glycinate treated groups shown the highest height of plants over control and other treatments groups

Table-1: Shows the effect of $CaCl_2$ and Zinc-Glycinate on % germination in control and treated groups.

Groups	% germination
T_0- Water Soaking	50
T_1- $CaCl_2$	65
T_2- Zinc-glycinate	65
T_3- $CaCl_{2+}$ Zinc-glycinate	95

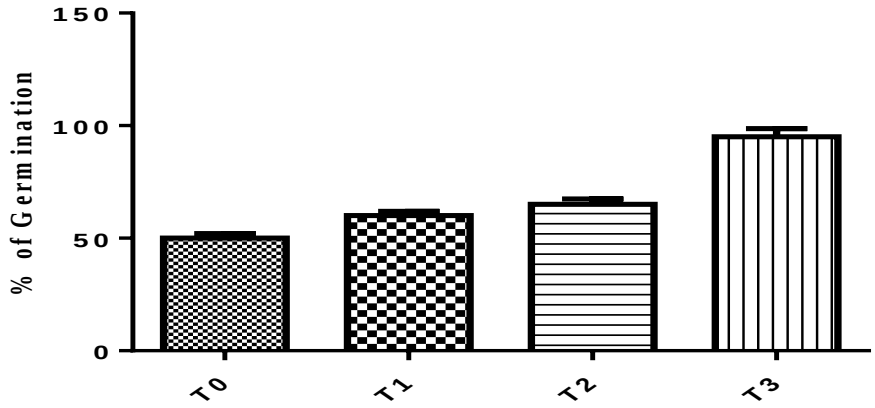

Figure -2: Shows the effect of $CaCl_2$ and Zinc-Glycinate on % germination in control and treated groups.

Among the treated groups seed pre-treated with combine effect of zinc-glycinate with chemicals shown highest plant height. In the previous study has shown that Plant growth promoter scan take part in a helpful function of improving physiological characters in plants. Movement of plant growth regulating substance, such gibberellic acids to the aerial parts of the plant might also one the reason the increased plant height in plants treated with combined effect of glycine, amino acids and $CaCl_2$ (Ananthi *et al.*, 2017). Plant height is one of among the important physiological parameters. The improved plant height can be due the availability of high energy compound, ATP and various important macromolecules in the aerial parts.

Table-2: Shows the effect of water, $CaCl_2$, Zinc-Glycinate and $CaCl_2$, on the Plant height, No. of branches/plant and Dry Matter Production.

Groups	Plant height(cm)	No of Braches/plant	Dry Matter Production (g/plant)
T_0– Water Soaking	30	2	9.40
T_1 –$CaCl_2$	35.5	3	11
T_2-Zinc-glycinate	36	3	11.20
T_3- $CaCl_2$+ Zinc-glycinate	40	4	13.5

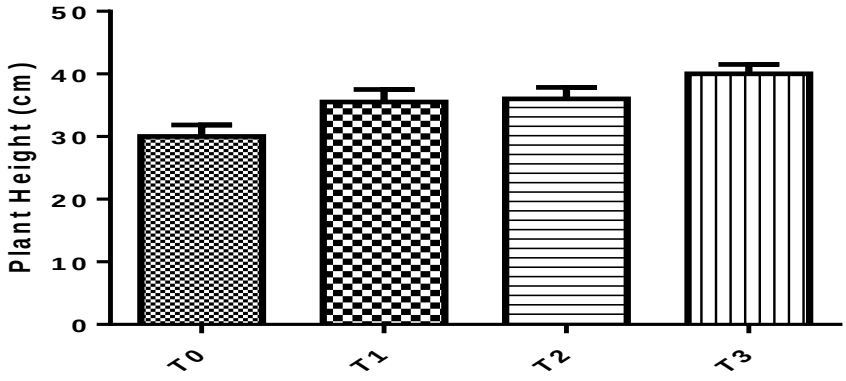

Figure 3: Effect water, CaCl2, Zinc-Glycinate on plant Height

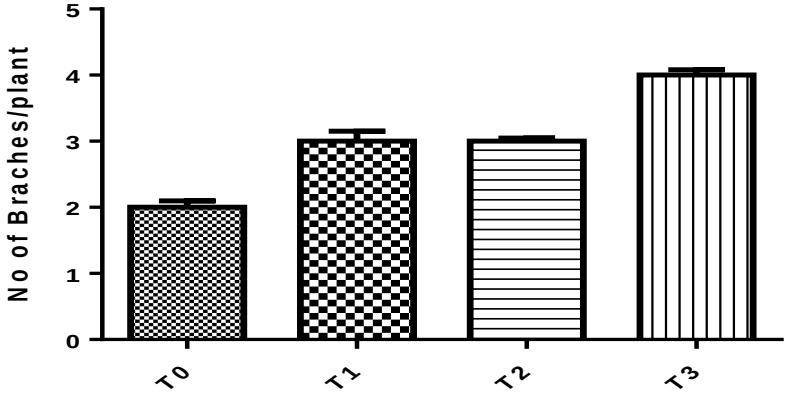

Figure 4: Effect water, $CaCl_2$, Zinc-Glycine and CaCl2 plus Zinc-Glycine in Number of branches production/plants in pre-sowing treated groups.

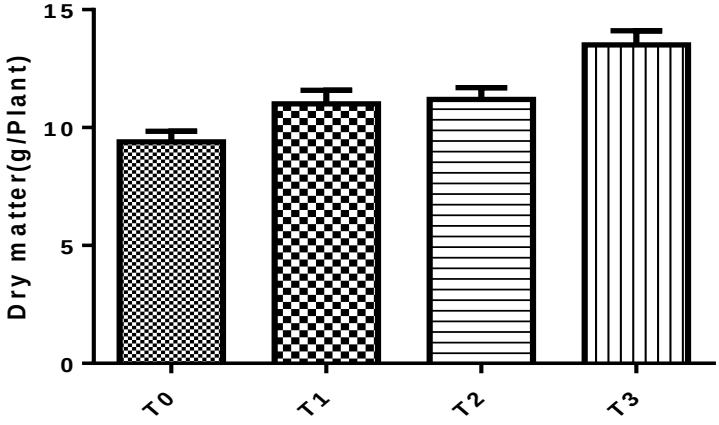

Figure 5: Effect water, $CaCl_2$, Zinc-Glycine, $CaCl_2$ plus Zinc-Glycine in dry matter production

Among the amino acids Glycine is the smallest amino acid, pass readily through plant tissues. Once inside the plant, the minerals/metals such as calcium, zinc and manganese are released, and the leftover amino acids that formed the protective shell are either used by the plant directly as amino acids or further broken down into water-soluble nitrogen. Glycine boost up root cells to open up calcium ion channels and stimulate the plants to take up calcium ions several times faster than simple osmosis. Calcium

chloride used in this study may activate the enzymes namely ATPase and amylase, further participate in the starch metabolism, the events required to energy to the growing plants (Forde and Roberts, 2014).

Table- 3: Shows the effect of water, $CaCl_2$, Zinc-Glycinate and $CaCl_2$ plus Zinc-Glycinate on Total chlorophyll.

Groups	Total Chlorophyll content (mg g^{-1})
T_0– Water Soaking	1.80
T_1 - $CaCl_2$	1.95
T_2-Zinc-Glycinate	1.95
T_3- Zinc-Glycinate plus $CaCl_2$	2.50

The data presented in Table-3 shows the effect of Zinc-glycinate, and $CaCl_2$ on total chlorophyll content in leaves. The chlorophyll content was gradually increased as follows 1.80 to 2.50. Among the treated groups highest values were noticed in combined treatment of Zincglycinate and calcium Chloride treated groups (T_3.2.50).on the other hand, control recorded lowest values (1.80). The other such groups have shown significant lesser that the other combined treatment

For the harvesting of light and formation of reducing energy Photosynthetic pigments such as chlorophyll, carotenoids etc. are very important to the plants. Among these Chlorophyll content is the main factors affecting photosynthetic capability. Plants allowed to environmental varying stress show a prompt loss of leaf chlorophyll content with improved intensity of stress (Kiani *et al.*, 2008). In the present experiment, total chlorophyll content was found to increase in the seed pre-treated with combination of Zinc-glycine and $CaCl_2$. Whereas, less amount of total chlorophyll contents were found in the control, and low concentrated individual solutions alone. This could be maintenance of the level of total chlorophyll by the glycine and Zinc and further might enhance the chlorophyll formation. Environmental stress either biotic or abiotic stress can induces the alteration the structure of chlorophylls and its content resulting in the inhibition of light harvesting and photosynthetic process. This reduction in leaf chlorophyll content under abiotic stress could be due to the extreme bulge of chloroplast membranes, alteration of the lamellae vesiculation and the formation of lipid droplets (Zaeifizade and Goliov, 2009). Further, various free radicals such as O^{2-}, H_2O_2 are produced during the stressed conditions, which leads to increased formation of lipid peroxidation. Previous report has shown that the glycine converts the potentially harmful substance in to the harmless substance through it modulatory effect on the xenobiotic metabolizing enzymes. It provides the basic nitrogen source needed for the production of many amino acids. Glycine has the important role in the formation a natural antioxidant substance, called Glutathione. In addition to this glycine is nontoxic compound and helps in protection against various oxidative stress arises during the metabolic process (Younis *et al*., 2009).

CONCLUSION

In conclusion, among the different seeds pre-treated groups, seed treated with the combination of Zinc-glycinate, and $CaCl_2$ solution showed significant improved plant growth height, increased number of branches/plant, elevated total chlorophyll contents were observed when compared to the control; amino acids and chemicals individually. It is stating that seed pretreatment with Zinc-glycine and $CaCl_2$ could improve the quality of seeds. It is the simple and inexpensive method to enhance the seed enactment and farm productivity mostly in dry land of poor rural farmers and can maintain the water balance in plant tissue and increased the phosynthetic activity. The net results could increase the yield.

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