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Research Article

EFFECT OF GLYCINE BETAINE ON *BRASSICA JUNCEA* GROWN UNDER HEAT AND SALT STRESS IN A HYDROPONIC SYSTEM

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ABSTRACT

Indian mustard is mostly targeted for commercial cultivation as an early-sown or late-sown crop with the expectation of higher seed yield and oil content. Indian mustard is grown under different agro-ecological situations, so exposed to abiotic stress like heat and salinity. The accumulation of osmolytes like glycine betaine (GB) in cell is known to protect organisms against abiotic stresses via osmoregulation or osmoprotection. Hydroponics systems provides an excellent tool for stress related studies. In the present study, the growth of Indian mustard was compared in a hydroponic system, soil system and cocopeat system. The effect of aeration in a hydroponic system was also assessed. It was found that the plants grew equally well in soil and hydroponic system but aeration drastically increased the growth rate in the initial days. The plants grown in hydroponic system were induced heat stress by growing them in a plant growth chamber at 45°C and salt stress was induced by adding 2% NaCl in the nutrient solution. Glycine betaine was extracted from *Bacillus subtilis* grown under salt stress. This extract was added in the nutrient solutions to alleviate the effect of stress in the plants. The control plants kept under stress conditions showed a decrease in the growth rate which was alleviated in the test plants subjected to stress and grown with glycine betaine extract. More effect was seen in the plants subjected to salt stress.

Keywords: Growth systems, Osmolyte, *Bacillus subtilis*.

1. INTRODUCTION

Nowadays, with the increasing population and various climatic changes, the traditional farming systems are unable to meet the current and future food demands [1]. Soil erosion and degradation eliminates various minerals and vitamins from the soil. Therefore, there is a need for an alternative method to grow crops. A substitute for soil that can be used for growing crops is cocopeat which has a good capacity of holding water and has some nutrients too. Hydroponics is an alternative technique that can be employed instead of traditional farming. It is a technique in which plants grow without soil in a liquid culture [2]. The nutrients provided and the growth conditions can be varied as per need and thus is a good system to study the effect of various factors in plants. Mustard (*Brassica juncea)* is one of the main economically important oilseed crop of India [3] and it's seeds have been used as condiments for many years and is also used as edible and industrial oil [4].

Due to changes in the environmental conditions, crops are now exposed to various types of biotic and abiotic stresses. Salinity is one of the major abiotic stress that each crop faces due to the contamination of various salts in the soil. The productivity of oilseed crops worldwide is greatly affected due to the problem of salinity in the soil [5]. Another major stress factor that a crop faces is heat stress due to global warming. The temperatures are no longer ideal to grow certain crops in specific weather conditions. Mustard being a cool weather crop cannot be cultivated in summers. The effect of stress on the plant can be seen with decline in photosynthesis [5].

Plants under stressful conditions require certain other compounds that can help them cope with the stress. Bacteria such as *Bacillus subtilis* and *Pseudomonas* spp. under stress such as salinity tend to produce glycine betaine. Glycine betaine (GB) or trimethylglycine is an osmoprotectant that helps the bacteria survive under stressful conditions. Micro-organisms can be cultured easily and grown in huge quantities which makes microbes a good source for extraction of glycine betaine. Exogenous application of various osmoprotectants, signalling molecules, phytohormones, trace elements and nutrients have been found to be helpful in reducing the effects of high temperature stress induced in plants [6].

This study was carried out for days to compare the germination and growth of *Brassica juncea* in soil, hydroponics and cocopeat system as well as comparison was done in an aerated hydroponic system and a non-aerated hydroponic system. This study also aimed to extract Glycine betaine (GB) from a microbial source and study its effect on salt-stress and heat-stress induced *Brassica juncea* crop grown in hydroponic system.

2. MATERIAL AND METHODS

2.1. Comparative study for different Growth system

For the study of comparison of germination and growth of *Brassica juncea* three different systems were used.

2.1.1. Soil system

In the soil system; 75 gm of garden soil was added to 3 medium sized pots. 5 mustard seeds were sowed in each pot. 30 mL of water was added to each pot every day.

2.1.2. Hydroponic system

Temporary hydroponic systems was set up using transparent rectangular plastic containers were used. Holes were made on the lid to hold the netted hydroponic cups. Each system contained 3 netted hydroponic cups. The cups were filled with gravel and 5 seeds were placed on the gravel. A media containing modified Hoagland-Arnon solution (Table 1) was used. The container was filled with the media till the top most layer of the gravel was immersed in the solution (Fig 1).
One system was aerated using all-purpose aeration pump that maintained constant air flow to the system and other was kept non aerated.

Fig. 1: Hydroponic system set up

2.1.3. Cocopeat system

The cocopeat system had 25 gm of Cocopeat added to 3 pots. 5 seeds were sowed in each pot. 15 mL of water was added to each pot every day.

2.1.4. Biometric Parameters of the plants

The root length and shoot length of the plants growing in different systems were recorded after 30 days of growth. The chlorophyll content was estimated using 100 mg of crushed leaves. 7mL of Dimethyl sulfoxide (DMSO) was added to the crushed leaves and incubated in an oven at 65°C for 60 minutes. The test tubes were cooled followed by filtration and the absorbance was read at 648 nm and 665 nm using UV-Vis Spectro-photometer. Chlorophyll concentration (a, b and total) was expressed as mg /g fresh weight and determined by the following formulae [7].

Chlorophyll a (mg/g F.W) = (14.85 A_{665} -5.14 A_{648})
Chlorophyll b (mg/g F.W) = (25.48 A_{665} - 7.36 A_{648})
Total chlorophyll (mg/g F.W)=(7.49 A_{665}+20.34 A_{648})
where: A665 = absorption value at 665 nm, A648= absorption value at 648 nm.

2.2. Induction and extraction of Glycine Betaine

Glycine betaine (GB) was extracted from *Bacillus subtilis* using modified method described by Moharramnejad et.al, 2015 for plants [8]. For inducing the production of GB, 1 mL of culture of *Bacillus subtilis* was inoculated in 5 different flasks containing 50mL of Nutrient broth supplemented with 0.5%, 1%, 1.5%, 2% and 2.5% NaCl and 50mM Choline chloride. The flasks were incubated for 24 hrs at 37°C. Each flask was separated and the contents were centrifuged. The supernatant was discarded and 5 mL of 0.05% of toluene-water mixture was added to the pellet and dissolved in it. The tubes were kept on a shaker for 24 hours. The toluene will damage the cell wall and the plasma membrane of the cells leading to permeabilization and cytoplasmic leakage [9]. 1 mL of the extract was taken in a test tube and 2 mL of 1N HCl was added to each tube. To this, 0.1 mL of potassium tri-iodide was added and kept in ice bath for 1 hour. After incubation, 5 mL of chilled isopropyl alcohol was added to all the tubes. The absorbance of the lower separated layer was checked using a UV-Vis Spectrophotometer.

2.3. Effect of Glycine Betaine on Growth of Heat and Salt Stress Induced Mustard Plants

The surface sterilized mustard seeds were allowed to germinate and then were grown in hydroponic system using Hoagland's solution as the media for 2 weeks. For salt stress the 2 week old plantlets were grown in Hoagland's solution containing 50mM NaCl and incubated at room temperature. For heat stress the plantlets growing in normal Hoagland's solution was incubated at 40°C using a plant growth chamber. Aeration was provided in both the conditions. One set of plants were maintained as control which were not induced with any stress. 1% glycine betaine extract treatment was given to one set of plants for both salt and heat stress. The growth characteristics and chlorophyll content of the plants with no stress, salt and heat stress induced and with treatment with glycine betaine were recorded.

3. RESULTS AND DISCUSSION

3.1. Comparative study for different Growth systems

Germination and growth of *Brassica juncea* was carried out in 4 different systems and based on the germination index, 85.71% of seed germination took place in the aerated hydroponic system, while 45% and 46% of germination occurred in the soil and cocopeat systems respectively which is represented in fig. 2. Whereas, non-aerated hydroponic system had the least level of germination. Studies by Arthur *et al*, 2015 also suggests that additional aeration helps in the growth of both root and shoot system [5]. Some studies also suggest that decrease in aeration reduces the mineral uptake which in turn affects the growth of the plants [10, 11].

The biometric parameters of the plants in different growth systems shows that, plants growing in the cocopeat system have the highest plant height, followed by plants growing in the aerated cocopeat system which is then followed by the plants growing in the soil system. Plants growing in the non-aerated system have the lowest plant height. Though cocopeat system grown plants have a good plant height, there is a huge difference in the shoot length to root length ratio which is represented in the graph of Fig No. 3. Similar condition was found in the early stage of plant growth as well (Fig. 4). This indicates that cocopeat system has nutrients and minerals that support the growth of root system more than shoot system. Whereas, the soil system grown plants have bigger shoot lengths than root lengths. The plants growing in the hydroponic system have approximately equal root length and shoot length which shows that the efficacy of the nutrient media used for the growth of *Brassica juncea* is suitable and supports its growth.

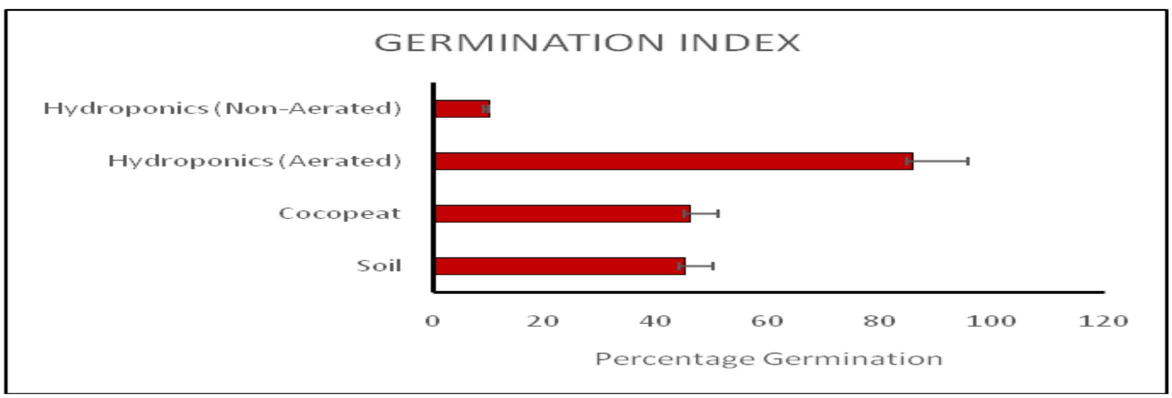

Fig. 2: Graph Depicting the germination index in different growth systems

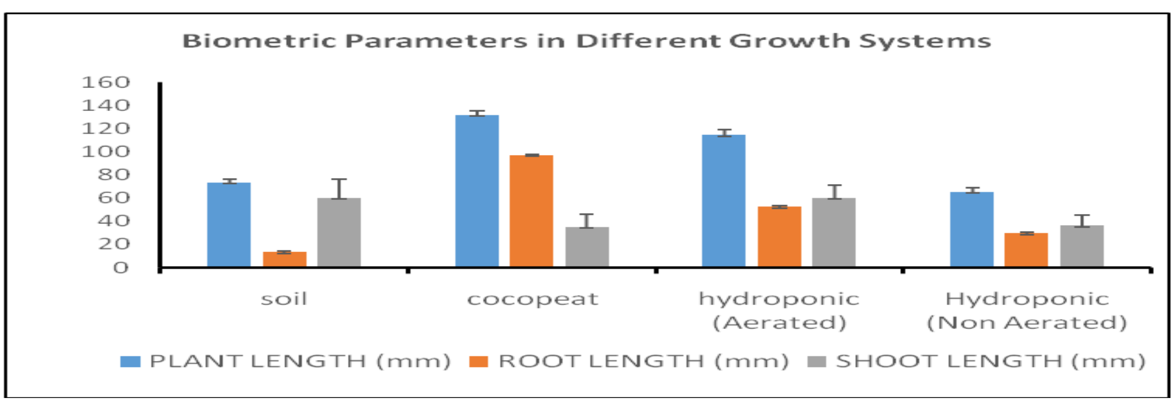

Fig. 3: Graph representing the biometric parameters in different growth systems.

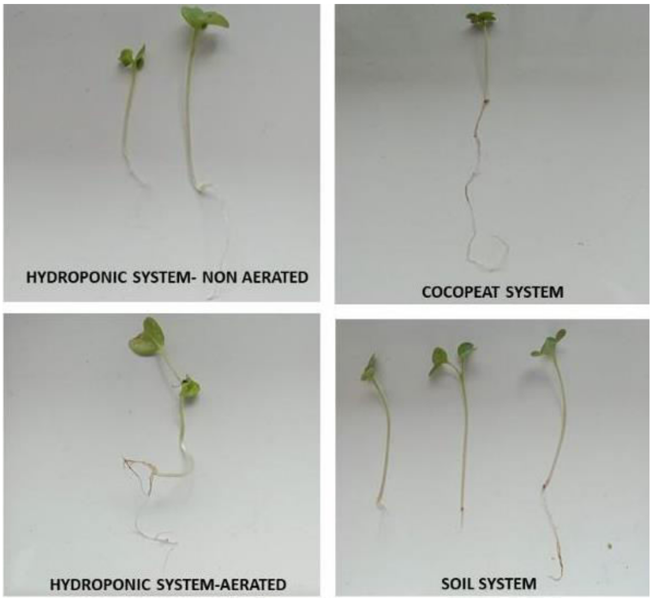

Fig. 4: Comparative picture of the shoot and root lengths at the early stage of growth

The chlorophyll content estimated using DMSO extraction and spectrophotometer readings at different wavelengths shows that Chlorophyll A, B as well as total chlorophyll content was found to be highest in the crops growing in the aerated hydroponic system while the least chlorophyll content was found in the crops growing in the cocopeat system which indicates that cocopeat system lacks nutrients necessary for optimum plant growth. The chlorophyll content in the plants growing in the soil systems were found to be approximately the same as the aerated hydroponic system which is then followed by the plants growing in a non-aerated hydroponic system. These results were in accordance with studies conducted by Padmathilake, et al 2007 in *Brassica integrifolia* where it was reported that the chlorophyll contents were not significantly different under both hydroponics and soil cultivation systems [12]. The difference in the chlorophyll content in the aerated hydroponic system and the non-aerated hydroponic system suggests that aeration is a critical factor in the plant's growth and development. Studies by R. E. Williamson have showed that aeration helps to increase the yield and crops growing in poor aeration conditions have reduced yields [13].

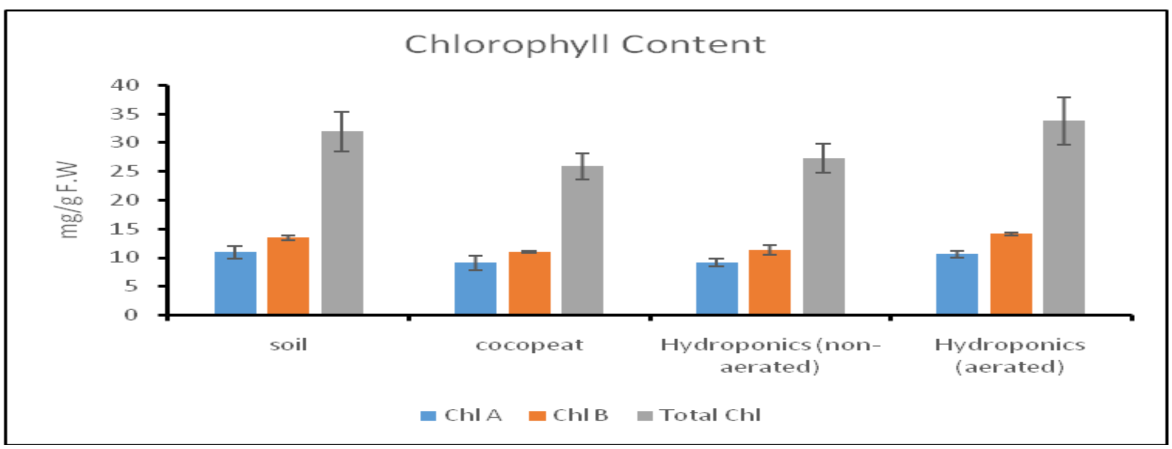

Fig. 5: The chlorophyll content represented as mg/g fresh weight of the leaves of mustard plants grown for 30 days in different growth systems

3.2. Induction and extraction of Glycine Betaine

Bacillus subtilis is a known halophile and to sustain in severe halophilic conditions, it accumulates osmolytes. Glycine betaine is one of the important osmolytes accumulated by *B. subtilis* [14]. It either takes up glycine betaine from the environment by number of systems for glycine betaine uptake or synthesize it using choline as the precursor [15]. Also, there are reports that shows glycine betaine provides thermal protection to these species [16]. In the present study, *B. subtilis* was induced to produce glycine betaine with different concentrations of NaCl and choline was provided as the precursor for the same. The cells were permeabilized using toluene and the glycine betaine was extracted using potassium tri-iodide and isopropyl alcohol. The presence of glycine betaine was confirmed spectrophotometrically at 365nm [17]. Different NaCl concentration from 0.5% to 2.5% was used for induction but as depicted in table No.1, glycine betaine was found only in the flask with 2% NaCl which was confirmed by a sharp peak at 364nm (Table 1 and Fig. 6).

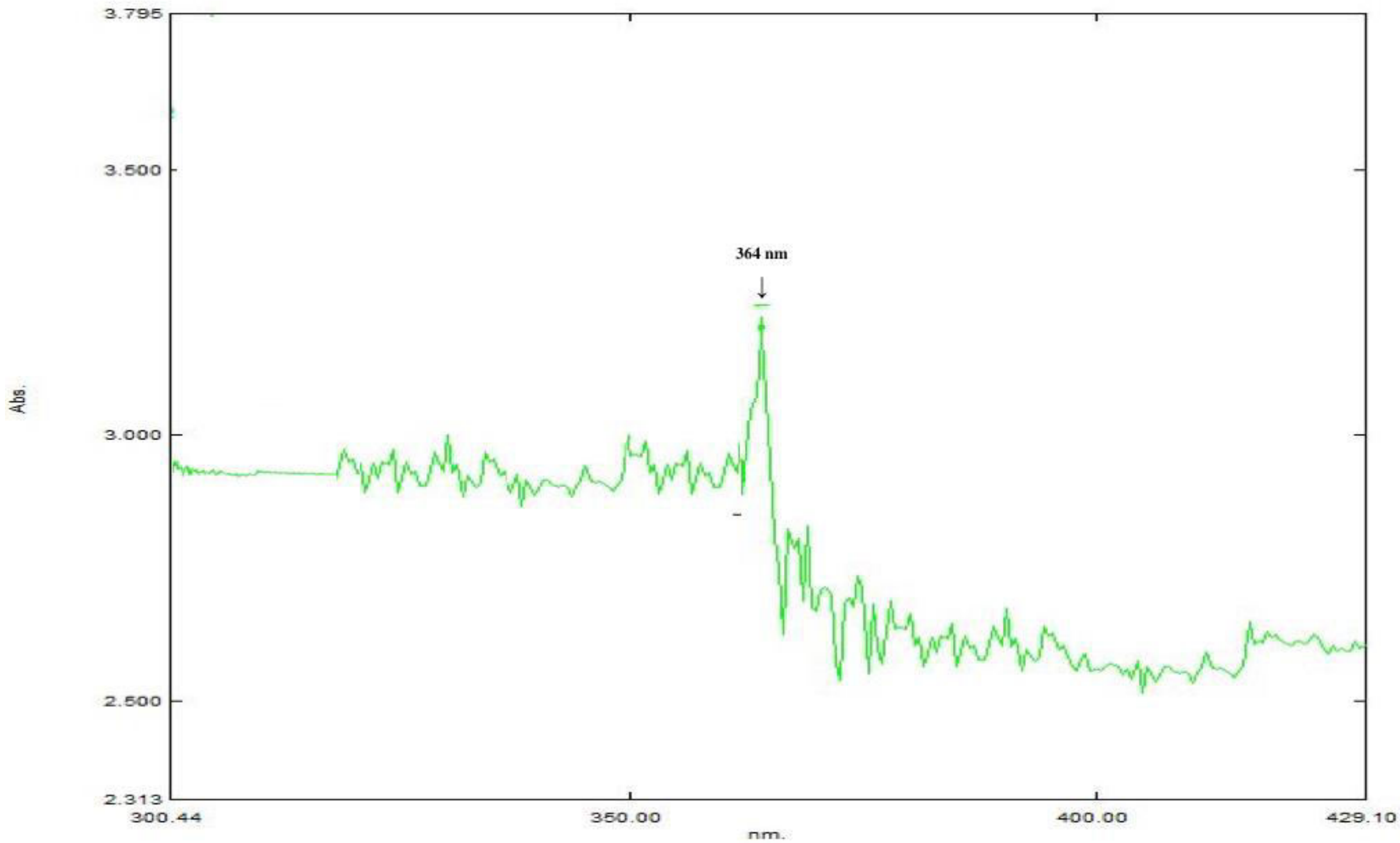

Fig. 6: Spectrophotometric analysis of the extract from *B. subtilis* grown in media containing 2% NaCl showing a sharp peak at 364nm

Table 1: Peaks obtained from extracts of *B. subtilis* grown in media containing different concentrations of NaCl

Concentration of Salt in Media	*Bacillus subtilis*
0.5% NaCl	-ve
1% NaCl	-ve
1.5NaCl	351 nm
2% NaCl	364 nm
2.5 NaCl	356 nm

3.3. Effect of Glycine Betaine on Growth of Heat Stress and Salt Stress Induced Mustard Plants

The plants were grown in Hoagland's solution containing 50mM NaCl to induce salt stress and another set was grown in Hoagland's solution at 40°C in a growth chamber to induce heat stress for 2 weeks after germination and 2 weeks growth. Glycine betaine extract (1%) was added in the test pots. Both salt stress and heat stress affected the shoot and the root length of the plantlets as well as the chlorophyll content. Similar results have been reported by many researchers [18, 19]. In this study, the results suggest that the treatment of glycine betaine extracted from *Bacillus subtilis* could counteract on the adverse effects of the heat and salt stress. Even though the treatment could not bring the growth and the chlorophyll content to the level of the plants growing without stress, the effect was considerable (Fig 7, 8 and 9).

Reports suggest that glycine betaine mediates changes in the growth parameters and also strengthens the antioxidant system which may lead to alleviation of the damages caused due to salt and heat stress [20, 21]. Studies have suggested the glycine betaine may have important role in stabilizing PSII under abiotic stresses [22]. Similar effect have been noted in not only mustard but in other plants as well like pea [23], canola [24, 25], rice [26], wheat [27] and many more. Most of these studies show that the exogenous application of glycine betaine is also effective in reducing the negative effects of the stress conditions [25, 28]. So, production of glycine betaine using bacterial sources is more suitable for larger scale which can be easily commercialized. The genetic modification of bacterial cells can give better options for increased glycine betaine production.

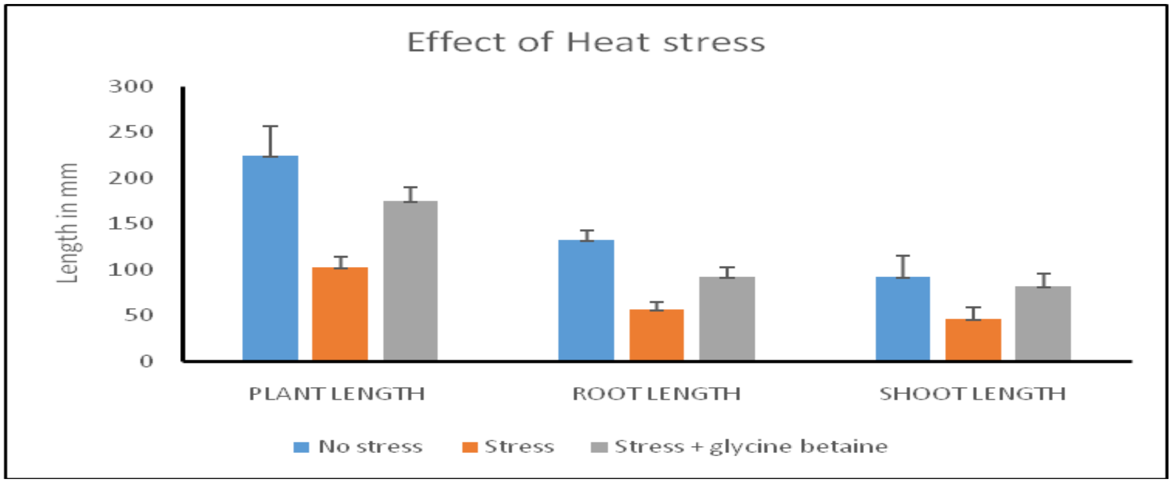

Fig. 7: Graph representing the biometric parameters of mustard plantlets growing under heat stress and after treatment with glycine betaine

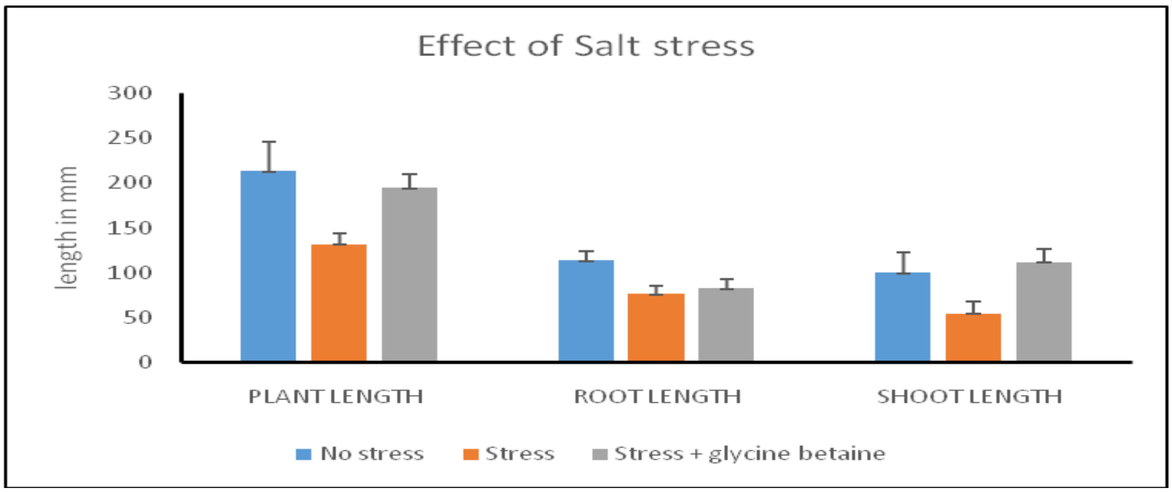

Fig. 8: Graph representing the biometric parameters of mustard plantlets growing under salt stress and after treatment with glycine betaine

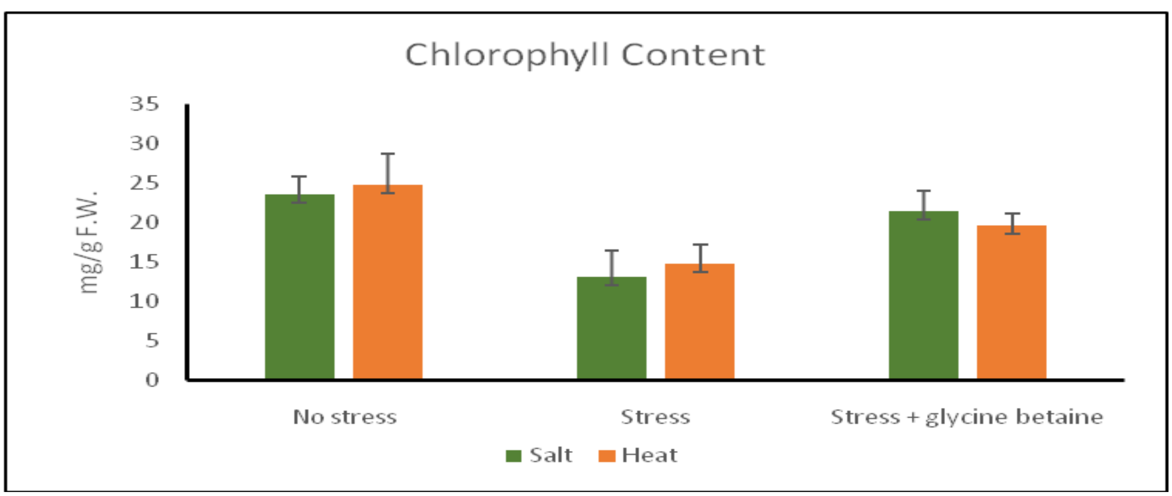

Fig. 9: The chlorophyll content represented as mg/g fresh weight of the leaves of mustard plants grown under heat and salt stress in hydroponic system

4. CONCLUSION

In the present study, the growth of *Brassica junea* was compared in three different systems: soil, cocopeat and hydroponics using Hoagland's solution. Hydroponic system was found to be good system for growing mustard and also for studying the effect of salt and heat stress. Glycine betaine was extracted from stress induced *Bacillus subtilis* and the extract was found to effective in alleviating the negative impact of heat and salt stress in mustard. From this study, we can conclude that hydroponic system can prove to be a cost effective method to grow mustard and can be an excellent system to study impact of stress. Further purification of glycine betaine can be beneficial to standardize the extraction from *Bacillus* which can scaled up for commercial purposes.

5. ACKNOWLEDGEMENTS

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Conflict of Interest

The authors declare there is no conflict of interest.

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