Role of Chlorella Vulgaris as A Salt Resistant Barrier for Salt Compromised Crops

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Abstract: Chlorella Vulgaris is a commonly present green alga that is present in freshwater in the form of a single-celled organism with greater photosynthetic efficiency. It is sufficiently rich with macro and micronutrients include carbohydrates and proteins. Previous reports suggested that *Chlorella Vulgaris* has the potential to survive in a great saline environment. The current research focused on the application of *C. Vulgaris* at two crop plant includes cherry tomato and chilli pepper. These plants are usually sensitive against salt, while *C. vulgaris* enhanced the ability of such plants to bear excessive salinity. One-month plant seedlings were treated with 100mM and 1M concentration of *NaCl* along with *C. Vulgaris* and observed for two weeks' period. The plant growth and gene expression of *rbcl* and *sos1* were collected. Gene expression analyses revealed that plants showed high salt-resistant along with better growth of plants. It is suggested that the growth of plants was enhanced and promote the presence of cultivation media and *C. Vulgaris*. It was also observed that sole salt suppresses the growth of plants. The Bold Basal Media was more promising as compared to CHU 10 media. The current finding also suggested that *C. Vulgaris* could be applied as biofertilizer in salt-resistant crops for the enhancement of their productivity.

Key-Words: - Chlorella Vulgaris, Salt tolerance, Biofertilizer, Photosynthesis, CT value.

1 Introduction

Salinity is the major concern and risk which compromised the growth of plants present in certain environment. Different types of salts and their ions (Ca^{+2} Cl^{-1} Na^{+1} K^{+1}) are responsible for barren the soil. The major contaminating ions are of Cl^{-1} and Na^{+1}. The salinity of soil could also be increases due to agricultural practices and it also due to the global warming which eventually increases the temperature. It reduces the productivity hence result in low yield of plants and less place for agricultural activities [1]. Land of United Arab Emirates (U.A.E) majorly consists of deserts with greater amount of salt. It was reported that 8000 ppm salt present in groundwater in U.A.E which make the agricultural activities almost impossible [2].

The recommended solutions for the reduction of salinity are crop rotation, enrichment of soil with chemicals to promote the growth of crops in such fields but there are certain limitations of the process. The alternative of chemicals is biological agent which is considered as safe and environmental friendly as compare to chemicals. Such biological agents incorporated into soil for promoting the plants growth and have no reported adverse effects. In last few decades' chlorophyte were explored as bio fertilizer due to their nutritional values and components which include greater quantity of carbohydrates, proteins, lipids, growth hormones and are also reported for their antimicrobial activity [3].

Chlorella is an algae present in fresh water. It was reported that few species of Chlorella are salt resistant and has ability to bear temperature up to 40ºC [4,5]. It was suggested that *Chlorella pyrenoidosa has ability to apply as* bio fertilizer in agricultural set up for the growth of vegetables such as lettuce, rice, eggplant and cucumber. The bio fertilizers induced seed germination in rice plants, enhanced chlorophyll content in cucumber and promotes growth of lettuce in water scarcity [6,7,8].

Chlorella vulgaris is a type of algae which contains greater quantity of different macro and micronutrients include carbohydrates and proteins [9]. Few bacteria also exist in the rhizosphere and could also promote soil fertility [10].

The current study aims to explore the potential of *Chlorella vulgaris* as a salt tolerant bio fertilizer on cherry tomatoes and chilli pepper plant. Gene expression of few genes related to photosynthesis, salt tolerance and water retention were suggested to crucial for the growth of plants in high salinity areas. This study also evaluates the effect of such genes. The gene expression of *rbcl*, *pdh* and *sos1*

were also studied in order to determine the effect of *Chlorella vulgaris* on photosynthesis, water retention and salt tolerance of these plants, respectively.

2 Material and Methods

In the current research, chilli pepper and cherry tomato species were subjected to the treatment with *Chlorella* followed by salinity stress and observe their gene expression. The culture of *Chlorella Vulgaris* was acquired from Shalimar Biotechnology LLC, Dubai, UAE. Two different commercially available cultivation media were used include CHU 10 and Bold's Basal media and applied to both plants. Sodium chloride was applied in 100mM and 1M to the plant.

2.1 Cultivation of *Chlorella vulgaris* for Cell Morphology Pattern

Chlorella vulgaris was cultivated in CHU 10 and Bold's Basal media. After the incubation period, 100 µL of cell supernatant was withdrawn and observed under light microscope using haemocytometer. The cells before exposure to the media were considered as control. The following formula was used for counting the number of cells:
Number of cells in sample= Number of cells counted/Portion of chamber counted × Volume of square counted × 10^4

2.2 Cultivation of Plants in Media containing *Chlorella vulgaris* with Sodium Chloride

Cells of Chlorella were cultivated in growth media containing different concentration of NaCl followed by introduction of 20 ml of each media in the plant pots. The effect of growth was observed and compared with control plants.

2.3 Estimation of Gene Expression through Conventional and Real Time PCR

After overnight addition of cultivation media in plant, 18S rDNA gene analysis was performed. RNA was extracted using Accu Vis Bio total RNA Purification Kit. The extracted RNA was further used for the synthesis of cDNA using Norgen Transcript First Stand Synthesis Kit. PCR was performed by using primers specific (forward primer 5' CGA CCT CTG GAA GGG ACG TA 3') (reverse primer 5' GAA TCA ACC TGA CAA GGC AA 3') for *sos 1, rbcL* and *pdh* genes followed by gel electrophoresis. While, quantitative analysis of gene expression was carried out through Real Time PCR.

3 Result and Discussion

3.1 Effect of Cultivation Media on Cell Morphology of *Chlorella vulgaris*

Morphological analysis of *Chlorella vulguris* suggested that cells size was enhanced after cultivation in growth media and the colour of the cells become more prominent. In Fig 1 it was clearly seen that cell size increases after cultivation in media.

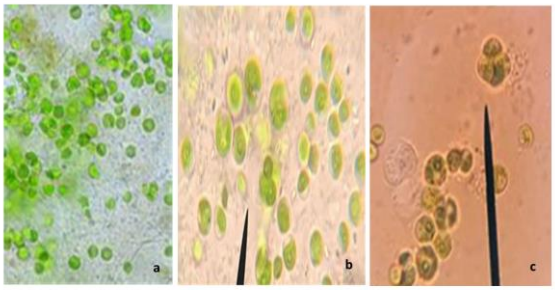

Fig 1: Effect of cultivation media on *Chlorella vulgaris.* a. Cells without any cultivation media; b. Cells in CHU 10 Media; c. Cells in Bold Basal Media

In Fig 2 it was clearly observed that cell density promotes after addition of growth media as compared to cells without any media. This suggested that this alga has ability to showed growth under-controlled environment. The media composition support of alga and hence provide a better alternative.

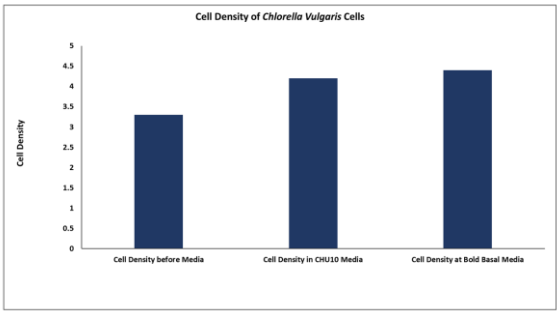

Fig 2: Growth of *Chlorella vulgaris* in nutrient media.

3.1Effect of Cultivation Media on Growth of Selected Plants

The growth of plants decreases in the presence of sodium chloride (100mM and 1M) but cultivation media possess positive effect and promote the growth of plant in the presence of salt and help the plants to bear salinity.

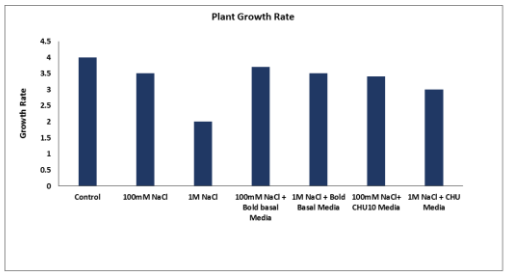

Fig 3: Effect of Sodium Chloride on the Cultivation of Plants in the presence of Media

3.3 Effect of *Chlorella vulgaris* on Gene Expression of Selected Plants

The 18S rDNA analysis was performed to find the transfer of *Chlorella vulgaris* genetic material into the plant after overnight growth. It was observed in gel electrophoresis that genetic material was successfully transferred into plants and hence their gene expression was analysed. Fig 4 showed that genetic material transfers in all control and test samples.

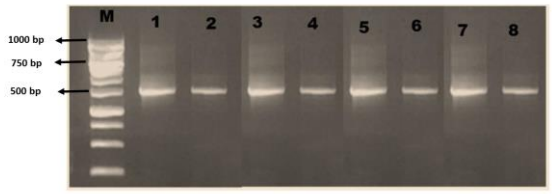

Fig 4: Transfer of 18S rDNA into of *Chlorella vulgaris* in selected plants. M: Markers; 1: Tomato in CHU10+ 1M NaCl; 2: Chilli Pepper in CHU10+ 100mM NaCl; 3: Tomato in Bold Basal's Media + 100mM NaCl; 4: Chilli Pepper in Bold Basal's Media + 100mM NaCl; 5: Tomato in CHU 10 + 100mM NaCl; 6: Chilli Pepper in Bold Basal's Media + 1M NaCl; 7: Chilli Pepper Plant Control; 8: Tomato Plant Control

Real time PCR was performed in order to determine the expression of different genes induced after the cultivation of plants in the presence or absence of *Chlorella vulgaris.*

Salt tolerance gene *sos1* expression in the presence of salt was checked in Fig 5. It was suggested that salt stress has not showing prominent impact on the growth of plant as low amplification of *sos1* gene was observed when only salt was introduced. The CT value or threshold value was observed as:

1. Ct value was 15 for chilli pepper and tomato plant control
2. Ct value was 17 for tomato in 1M salt and chili pepper in 100mM salt
3. Ct value was 18 for tomato 100mM and chilli pepper 100mM
4. Ct value was 22 for chilli pepper in 1M salt
5. Ct value was 24 for tomato in 100mM salt

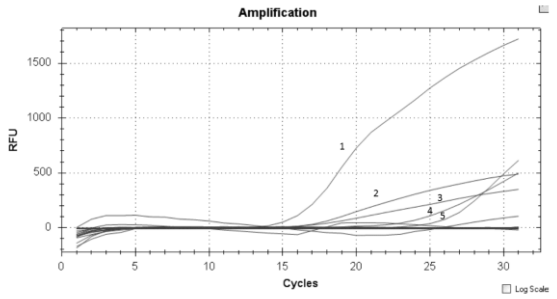

Fig 5: Real Time PCR of Plant *sos1* Gene Expression in the Presence of NaCl

Effect of media induction on different gene expression of plant was studied through real time PCR. It was suggested that induction of media with *Chlorella vulgaris* showed positive effect on plant growth. It also enhanced the potential of plant to bear salt stress. The amplification rate of *sos1* gene was observed in sample which were treated with media contain salt and *Chlorella vulgaris.* The ct value or threshold value was observed as:

1. Ct value was 12 for tomato plant control
2. Ct value was 14 for chilli pepper control
3. Ct value was 18 for tomato in CHU 10 + 1M salt
4. Ct value was 18 for chili pepper in CHU10 + 100mM salt
5. Ct value was 20 for chilli pepper in Bold Basal media + 100mM salt
6. Ct value was 20 for tomato in Bold Basal media + 100mM salt
7. Ct value was 24 for chilli pepper in Bold Basal media + 1M salt
8. Ct value was 28 for tomato in CHU10 + 100mM salt

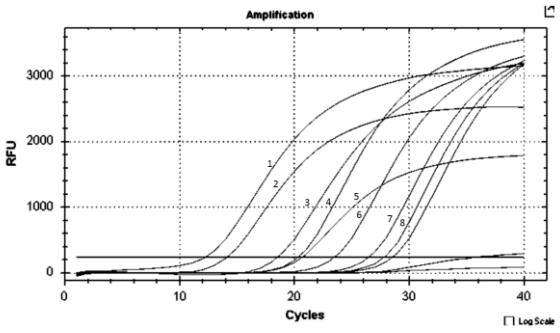

Fig 6: Real Time PCR of Plant *sos1* Gene Expression in the Presence of NaCl along with Media and *Chlorella vulgaris*

Effect of Salt stress on plant photosynthesis ability was studied and gene expression of *rbcl* gene was observed. It was revealed that salt stress causes negative effect on photosynthesis of plant. Therefore, low amplification of *rbcl* gene was monitored when different concentration was salt introduced in the system. While, after incorporation of media photosynthesis has positive impact on it and raise in photosynthetic level was observed. High amplification rate of photosynthetic gene *rbcl* was seen when different concentration of salt was incorporated in the system in the presence of media and *C. vulgaris.*

The CT value or threshold value was observed as:

1. Ct value was 9 for tomato and chilli pepper plant control
2. Ct value was 12 for tomato in CHU 10 + 1M salt
3. Ct value was 12 for chili pepper in CHU10 + 100mM salt
4. Ct value was 19 for chilli pepper in Bold Basal media + 100mM salt
5. Ct value was 20 for chilli pepper in Bold Basal media + 1M salt
6. Ct value was 20 for chilli pepper in Bold Basal media + 1M salt
7. Ct value was 20 for tomato in CHU10 + 100mM salt

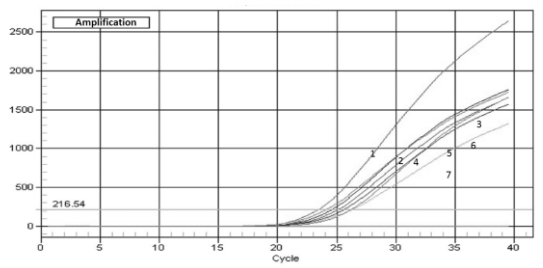

Fig 7: Real Time PCR of Plant *rcbl* Gene Expression

Effect of water stress on plant growth was observed and *pdh* gene expression was monitored for this purpose. It was revealed that media has positive effect on plant ability to bear the stress of water. High amplification of *pdh* gene expression was seen when different concentration of salt was incorporated in the system in the presence of media and *C. vulgaris.*

The CT value or threshold value was observed as:

1. Ct value was 9 for tomato plant control
2. Ct value was 9 for chilli pepper plant control
3. Ct value was 12 for tomato in CHU 10 + 1M salt
4. Ct value was 12 for chili pepper in CHU10 + 100mM salt
5. Ct value was 19 for tomato in Bold Basal media + 100mM salt
6. Ct value was 19 for chilli pepper in Bold Basal media + 100mM salt
7. Ct value was 20 for chilli pepper in Bold Basal media + 1M salt
8. Ct value was 20 for tomato in CHU10 + 100mM salt

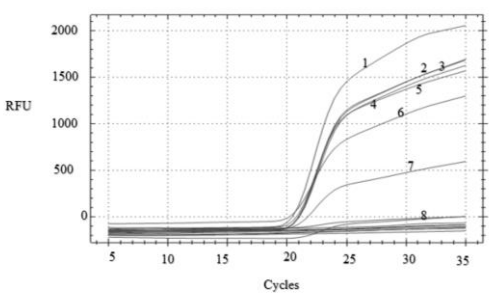

Fig 8: Real Time PCR of Plant *pdh* Gene Expression in the Presence of NaCl and Media

4 Conclusion

This study suggested that *Chlorella vulgaris* has potential to apply as bio fertilizer in the salt bearing land for enhance the fertility of tomato and chilli pepper plant sown in salt containing soil. The enhance growth rate were observed. This approach is considered as environmental friendly approach due to no harmful chemical consumption. It suggested a better technique to overcome the salinity of land. Bold basal media showed more promising results as compared to CHU10 media. The gene expression study reveals the enhance expression of *rbcl*, *sos1*and *pdh* genes which are responsible for the photosynthesis, salt tolerance and maintain the water stress in plants. The current study reveals the ability of *C. vulgaris* as promising and farmer friendly source. This study should also be implemented on other crops for getting more productivity and reducing the salinity generated issues. It should also be implemented on agricultural fields of U.A.E so, could provide a better alternative of chemical methods

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