IDENTIFICATION OF MULTISTRESS RESPONSIVE *GLYOXALASE I* GENE IN THE SELECTED MANGROVES - *AN* INDICATION TO SALINITY TOLERANCE.

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Abstract: Mangroves are characteristic intertidal plant formations of sheltered tropical and subtropical coastlines and are ideal models for studying salt tolerant mechanisms at molecular level. Plant glyoxalase genes have been primarily associated with stress responses. Their over expression is known to impart tolerance to various abiotic stresses such as salinity, drought, chemicals and heavy metal toxicity. The present study made an attempt for the isolation of the genomic DNA and identification of multistress responsive *Gly* I gene in the mangrove plants *Acanthus ilicifolius* L., A*vicennia officinalis* L. and *Bruguiera cylindrica* (L.) Blume. Total cellular genomic DNA was isolated by CTAB method and its quality was checked by agarose gel electrophoresis. PCR amplification of *Gly* I sequence was carried out under optimal amplification profile and the quality of the gene from genomic DNA was also checked. The genomic DNA from the three mangrove plants were isolated in good quality and concentration. Under optimal PCR conditions, a prominent band of expected size was obtained in *Acanthus ilicifolius* (300 bp. amplicon) and *Avicennia officinalis* (250 bp. amplicon). The study suggests that under saline conditions more amount of glyoxalase enzyme were produced in the plant to detoxify MG and ROS which lead to the increased tolerance of these plants against salinity stress.

Keywords: **Mangroves, methyl glyoxl (MG), polymerase chain reaction (PCR), Reactive oxygen species (ROS), amplicon**.

1 INTRODUCTION

Plants are subjected to a great variety of stresses that restrict their growth and survival. The insight in to the specific mechanisms of stress leading to the acquisition of resistance are of particular value for molecular-biological, ecophysiological and applied studies. The salt stress may have been the first chemical stress factor that encountered by the organisms during the evolutionary course of life on the earth (Walter Larcher, 1995). So they must evolve effective mechanisms for the successful survival in the saline habitat, from the very beginning itself. Plant response to salinity is a complex set of traits which involve morphological, physiological, cellular and molecular or the whole plant levels.

The development of enzyme system is one of the defense mechanisms in the plants for protection against toxic effect of xenobiotic and reactive oxygen species that are generated at various abiotic stresses. Methylglyoxal is unavoidable byproduct of several metabolic pathways and considers being cytotoxic at high concentration. So MG homeostasis is very important in plant cells. The glyoxalase system (*Gly*) is important for detoxification of MG (Methylglyoxal). The system comprises the enzymes glyoxalase I (*GlyI*: Lactoglutathionelyase) and glyoxalase II (*GlyII*: Hydroxyacylglutathionehydrolase) and are ubiquitious in nature (Thornalley, 1990). Over expression of *Gly I* and *II* together confer improved salinity tolerance and offer an effective strategy for manipulating stress tolerance in crop plant (Siraj *et al.*, 2016).

Glyoxalase pathway has been reported from a diverse group of organisms, including humans, mice, protozoa, fungi, bacteria and plants. It has been reported that in plants MG levels were increased significantly in response to salinity, drought and cold stress (Yadev *et al.*, 2005; Singala-Pareek *et al.*, 2003). Increased *Gly I* activity is reported in the non differentiated rapidly dividing cells (Chakravarthy and Sopory, 1998; Ramaswamy *et al.*, 1984) and hormones and blue light stimulated cell growth (Chakravarthy and Sopory, 1998). *Gly I* from tomato and *Brassica* were shown to be up regulated under salt, water and heavy metal stresses (Espartero *et al.*, 1995; Veena *et al.*, 1999). However, whether the accumulation of MG and upregulation of *Gly I* activity in plants in response to the various stresses is a common phenomenon or not, remains to be addressed (Hossain *et al.*, 2009)

Mangroves are characteristic intertidal plants of tropical and subtropical coastlines. They are salt tolerant woody plants and are the ideal models for studying salt tolerant mechanisms (Tomilson, 1994). Some progress has been achieved in understanding the mechanism of salt tolerance in mangroves on molecular levels and these results indicated that mangrove adaptation to a high saline environment is indeed tightly linked to the regulation of gene expression. (Lian *et al.* 2008). Keeping all these, in the present study *Gly I* gene has taken as a candidate gene and an attempt has done to study the isolation and expression of *Gly I* gene using specific primers in different mangroves.

2 MATERIALS AND METHODS

2.1 Plant materials:

Matured and healthy leaves of the mangrove plants, *Acanthus ilicifolius* L. (Holy mangrove, Family: Acanthaceae), *Avicennia officinalis* L. (White mangrove / Indian mangrove, Family: Avicenniaceae) and *Bruguiera cylindrica* (L.) Blume. (White Burma mangrove, Family: Rhizophoraceae) were collected randomly from the mangrove area at Payanhgadi in Kannur district of Kerala.

2.2 Isolation of genomic DNA: Total cellular genomic DNA was isolated and purified from the plants *A.ilicifolius, A.officinalis* and *B.cylindrica* by the CTAB (Hexadecyl trimethyl ammonium bromide) extraction procedure (Doyle and Doyle, 1990).

2.3 Agarose gel electrophoresis: The quality of the isolated DNA was checked by agarose gel electrophoresis. 5ul each of loading buffer was added to 10ul of DNA and the samples were loaded to 1% agarose gel prepared in 0.5X TBE buffer. The gel was visualized in a gel documentation system and was photographed under UV light.

2.4 PCR amplification of *Gly I* sequence:

2.4.1 Design of gene specific primers: A set forward and reverse primers were designed based on the already published cDNA sequences of *Gly I* from database using Megalign software.

Forward primer: 5'GATGAAGCAACTAAAGGTTA3'

Reverse primer: 5'CCAATAGCCATCAGGATCTT3'

2.4.2 PCR amplification and reaction conditions: The amplifications were carried out in 50ul reaction mixture which contained 4ul DNA sample, 4ul Taq DNA buffer (Tris with 15mM MgCl2), 4ul dNTPS mix (10 mM), 2ul each forward and reverse primer, 1ul Taq DNA polymerase enzyme and the solution was finally made up to 50ul with sterile water.The PCR amplification profile consisted of first a denatuaration at 94^0C for 4 minutes, 35 cycles of 94^0C at one minute, 45^0C for two minutes and 72^0C for 2 minutes. The final elongation was performed at 72^0C for 10 minutes.

2.5 Agarose gel electrophoresis: The quality of the amplified Gly I sequences from genomic DNA were checked by agarose gel electrophoresis. 20 ul of PCR product was loaded to 2% agarose gel prepared in 0.5X TBE buffer. The gel was visualized in a gel documentation system and was photographed under UV light.

3 RESULTS AND DISCUSSION

In the present study genomic DNA isolated were in good quality and concentration (Fig. 1). Various conditions were optimized for successful amplification of partial *Gly I* gene from the plant *A. ilicifolius, A. officinalis* and *B. cylindrica.* Under optimal PCR amplification profile, a prominent band of expected size (between 200bp. and 300bp.) was obtained from *A. ilicifolius* (300bp. amplicon) and *A. officinalis* (250bp. amplicon) (Fig.2).

Plant glyoxalase pathway is considered to be associated with stress responses and their over expression is known to impart tolerance to various abiotic stresses such as salinity, chemical, drought, heavy metal stresses, oxaldehyde toxicity etc. (Yadev *et al*., 2005). Hence *Gly I* gene have been suggested as a potential a biomarker for assaying abiotic stress tolerance in plants by various researchers (Gupta and Huang, 2014; Yan *et al*., 2016; Sankaranarayanan *et al*., 2017).

Various conditions such as purity concentration of template, effect of Taq DNA polymerase concentration, effect of annealing temperature and primers concentration were optimized for successful amplification of *Gly I* sequence from the genomic DNA. Under optimal PCR condition a prominent band of 440bp. fragment amplified with cDNA template with *Gly I* specific primers in *Hevea brasiliencis* and a significant increase of *Gly I* gene activity in the drought stress treatment in accordance with the increasing MG level (Siraj *et al*., 2016). *Gly I* in *Brassica juncea* (558bp. amplicon) was reported by Veena et al., 1999 and conferd resistance to salt stress (Veena *et al*., 1999; Singala-Pareek *et al*., 2003).

The Pumpkin *Gly I* cDNA observed at 975bp. amplicon and stress induced increase in *Gly I* activity at transcript and methyl glyoxal levels in plants was reported by Hossain *et al*., 2009. Over expression of *Gly I -3* gene (558bp.) from heat tolerant *Brassica napus* in yeast conferred resistance to heat and cold stress to a certain extent (Yan *et al*., 2016). Although glyoxalase genes have been conserved throughout evolution, some structural variation in terms of active site, position and number are exhibited. This was correlates with the studied mangroves *A.ilicifolius* (300bp.) and *A.officinalis* (250bp.) mentioned in the present study. In this study, it is assumed that the tolerance towards salinity of the mangrove plants may be due to the over expression of *Gly I* gene, in the sense that under saline conditions more amount of Glyoxalase enzyme was produced due to the increased activity of *Gly I* gene, to detoxify MG and reactive oxygen species which lead to the increased tolerance of these plants against salinity stress.

4. SUMMARY AND CONCLUSION

A partial genomic DNA coding for *Gly I* gene was amplified in the above mentioned mangroves which show that the gene has a prime role towards salinity tolerance. Also the study indicates that the occurrence of variation in the site, action and numbers may be due to the fact that glyoxalase genes exist as a multigene family and thus the molecular mechanisms, sub cellular localization and functional roles of its isoforms are yet to be uncovered.

5. FUTURE RESEARCH PERSPECTIVES

Determination of nucleotide sequences of *Gly I* in the mangroves and study of their homology with other known *Gly I* sequences of plants will further improve the tolerance of plants towards different abiotic stresses. Moreover, the existence of multiple forms of these genes in plants indicates that it may have diverse tissue specific roles .So further studies are needed to reveal complete molecular mechanisms that taking place in the saline stress tolerance of plant in correlation with glyoxalase pathway.

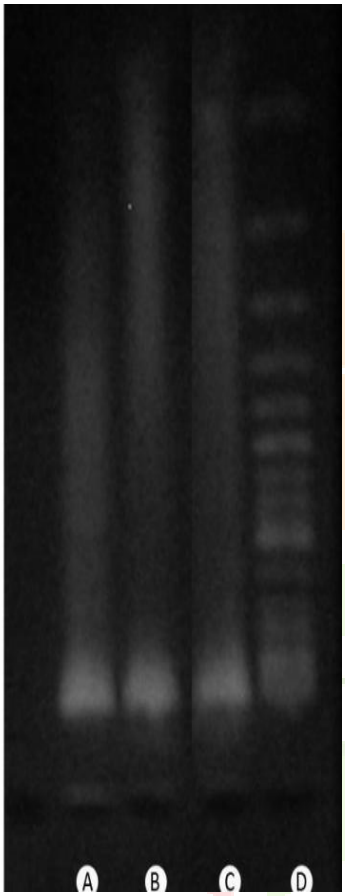

Fig. 1: Genomic DNA isolated from *B. clindrica* (A), *A.officinalis* (B), *A. ilicifolius* (C) and molecular marker (D)

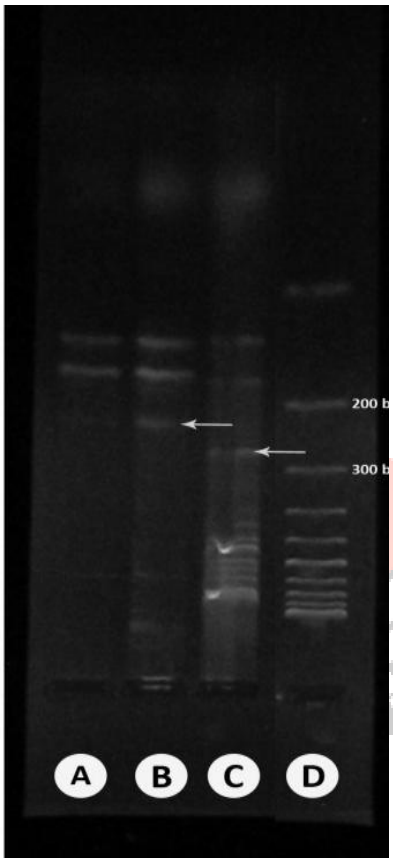

Fig.2: PCR amplification of partial Gly I from from *B. clindrica* (A), *A.officinalis* (B), *A. ilicifolius* (C) and molecular marker (D);Presence indicated by arrows.

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