International Journal of Botany Studies
ISSN: 2455-541X
Impact Factor: RJIF 5.12
www.botanyjournals.com
Volume 3; Issue 1; January 2018; Page No. 44-46

Identification of partial genomic DNA sequence coding for *Glyoxalase I* gene in the white mangrove and its associated plant *Ipomea Macrantha* Roem & Schult: A casual interrelationship

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Abstract
Plant response to salt stress and the mechanism of salt tolerance have been the major focus of plant biology research. The mangrove plant *Avicennia officinalis* and associated woody climber *Ipomea macrantha*, observed northern mangrove regions of Kerala. Most abiotic stress causes significant increase in MG level and *Gly I* gene activity. In the present study a partial genomic DNA sequence coding for *Gly I* was PCR amplified by using gene specific primers in the mangrove plant *A. officinalis* (250 bp.) and its associated plant *I.macrantha* (300 bp.). The presence of multi stress responsive *Gly I* gene indicates its role in the protection against MG, formed under various stresses and its future utility in developing various stress tolerant crop plants. The current study also reveals the interrelationship between mangroves and its associated plants at the molecular level and adaptive mechanism of mangroves and associated plants under saline conditions.

Keywords: salt stress, salt tolerance, methyl glyoxal (MG), polymerase chain reaction (PCR), glyoxalase I (*Gly I*), mangroves

1. Introduction
Salinity is one of the most significant environmental challenges, limiting plant productivity, particularly in arid and semiarid climates [1]. Salinity tolerance involves a complex of responses at molecular, cellular, metabolic, and whole plant level. Nowadays plant biology research works give an attention towards plant responses to salt stress and mechanism of salt tolerance [2]. The various responses of the plant towards salinity have been utilized in genetic engineering to generate transgenic stress resistant crop plants either by transferring stress responsive genes or altering the expression of existing genes [3]. Mangroves and associated plants are among the most salt tolerant plants and are ideal models for studying the salt tolerant mechanisms because of their ability to tolerate extremely high salinity [4].
Methylglyoxal (MG) is an unavoidable byproduct of several metabolic pathways. MG considered being a cytotoxic at high concentration. The first line of defense against MG is the glyoxalase system. The system comprises the enzymes glyoxalase I (*GlyI*: Lactoglutathionelyase) and glyoxalase II (*GlyII*: Hydroxyacylglutathionehydrolase) and are ubiquitious in nature [5]. Over expression *Gly I* resulted in improved tolerance against MG and the transgenic plants can tolerate higher level of salinity as compared with non transgenic plants. Over expression of *Gly I* conferred improved salinity tolerance thus offering another effective strategy for manipulating salt stress tolerant crop plant [6].
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 [7, 8]. Increased *Gly I* activity is reported in the non differentiated rapidly dividing cells [9, 10] and hormones and blue light stimulated cell growth [9]. *Gly I* from tomato and *Brassica sp.* were shown to be upregulated under salt, water and heavy metal stresses [11, 12]. 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 [13].
In the present study, *Gly I* gene taken as a candidate gene and made an attempt for the isolation of genomic DNA and partial expression pattern of *Gly I* gene was amplified by PCR using specific primers in a mangrove plant and its associated climber as an indication to salinity tolerance.

2. Materials and Methods
2.1 Plant materials
Matured leaves of the mangrove plant *Avicennia officinalis* L. (White Mangrove/ Indian mangrove, Family-Avicenniaceae) and a woody climber *Ipomea macrantha* Roem & Schult. (Beach moon flower, Family-Convolvulaceae) were randomly collected from Payangadi mangrove region, in Kannur district of Kerala.

2.2 Isolation of genomic DNA
Total cellular genomic DNA was isolated and purified from the leaves of plants *A.officinalis* and *I.macrantha* by the CTAB (Hexadecyl trimethyl ammonium bromide) extraction procedure [14].

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 (Lark Company, Chennai) 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. Result and Discussions

In the present study genomic DNA of *A. officinalis* and *I. macrantha* are isolated according to CTAB extraction procedure in a good quality and concentration (Fig. 1).Under optimal PCR condition a prominent band of expected size (between 200bp. and 300bp.) was amplified from genomic DNA(Fig. 2).
In plants glyoxalase pathway considered to be associated with tolerance to various abiotic stresses. An increase in the *Gly I* was observed due to drought stress, followed by saline, chemical and heavy metal stresses [7].Under optimal PCR conditions a prominent band of 440bp. fragment amplified with cDNA template with *Gly I* specific primers in *Haevea brasiliensis* [6], in *Brassica juncea* amplicon at about 558bp [12,8] and in pumkin at (975bp.) [13]. Although glyoxalase genes have been conserved throughout evolution, some structural variation in terms of active site, position and number are exhibited. This correlates with the studied mangrove *A.officinalis* (250bp.) and associated plant *I. macrantha* (300 bp.) 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.

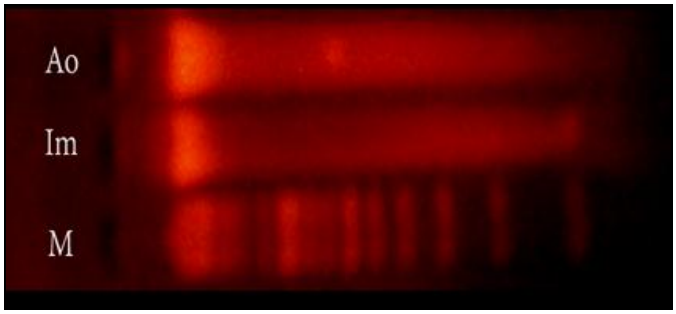

Fig 1: Genomic DNA. M= Molecular weight marker; Genomic DNA isolated from *Avicennia officinalis* (Ao), *Ipomea macrantha* (Im)

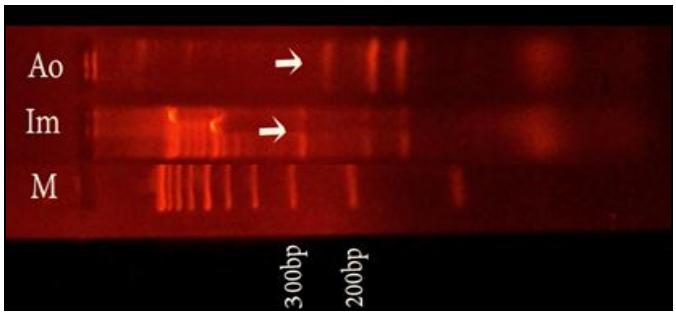

Fig 2: PCR amplification partial *Gly I* from *Avicennia officinalis (Ao)* and *Ipomea macrantha* (Im) M= Molecular weight marker; amplicons shown by arrows.

4. Summary and Conclusion

The presence of multi stress responsive *Gly I* gene indicates its role in the protection of the plants against MG that is formed under various stresses. 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. The current study also reveals the inter relationships between mangroves and its associated plant at the molecular level adaptive mechanism of mangroves and associated plant under saline conditions.

5. Acknowledgements

The authors thank Department Of Biotechnology, Sir Syed Institute for Technical Studies, Thaliparamba, Kannur for technical support and infrastructural facilities provided.

6. References

1. Munns R, Tester M. Mechanism of salinity stress. Annu. Rev. Plant. Biol. 2008; 59:651-681.
2. Zhu Z, Juan C, Hai-Lei Z. Physiological and Proteomic characterization of salt tolerance in a mangrove plant, *Bruguiera gymnorrhiza* (L.) Lam. Tree physiology. 2012; 32:1378-1388.
3. Turan S, Cornish K, Kumar S. Salinity tolerance in plants: Breeding and Genetic engineering. AJCS. 2012; 6(9):1337-1348.

4. Tomilson PB. The botany of Mangroves. Cambridge University Press, Cambridge, 1986.
5. Thornalley PJ. The glyoxalase system: New developments towards functional characterization of metabolic pathways fundamental to biological life. Biochem J. 1990; 269:1-11.
6. Siraj MVP, Thulaseedharan A, Anu Augustine, Glyoxalase I. expression pattern in *Hevea brasiliensis* seedling under varied stress conditions. Afr. J. Biotechnol. 2016; 15(11):376-383.
7. Yadav SK, Singala-Pareek SL, Ray M, Reddy MK, Sopory SK. Methyl glyoxal levels in plants under salinity stress are depend on glyoxalase I and glutathione. Biochem. Biophy. Res Commun. 2005; 337(1): 61-7.
8. Singla-Pareek SL, Reddy MK, Sopory SK. Genetic engineering of the glyoxalase pathway in tobacco leads to enhanced salinity tolerance. Proc. Natl. Acad Sci. U.S.A. 2013; 100:14672-14677.
9. Chakravarty TN, Sopory SK. Blue light stimulation of cell proliferation and glyoxalase I activity in callus cultures of *Amaranthus paniculatus.* Plant. Sci. 1998; 132:63-69.
10. Ramaswammy O, Pal S, Guha-Mukherjee S, Sopory SK. Correlation of Glyoxalase I activity with cell proliferation in *Datura* callus culture. Plant cell Rep. 1984; 3:121-124.
11. Espartero J, Sanchez-Aquayo I, Pardo M. Molicular characterization of glyoxalase I from higher plant;up regulation by stress. Plant Mol Bio. 1995; 29(6):1223-1233.
12. Veena, Vanga SR, Sudhir KS, Glyoxalase I. from *Brassica juncea*, molecular cloning, regulation and its expression confer tolerance in transegenic tobacco under stress. Plant J. 1999; 17:385-395.
13. Hossain MA, Hossain MZ, Fujitha M. Stress induced changes of methylglyoxal level and glyoxalase I activity in pumpkin seedlings and cDNA cloning of glyoxalase I gene. Aus. J Crop. Sci. 2009; 3:53-64.
14. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus. 1990; 1:13-15.