Environmental stresses represent the most limiting factors for agricultural productivity worldwide. During their development, plants may be exposed to multiple stresses. In recent years, advances in physiology, molecular biology, and genetics have greatly improved our understanding of how plants respond to these stresses and the basis of varietal differences in tolerance. However, abiotic stress is still the subject of intense research.

Plants respond to the various stresses in differential manner depending upon the nature and duration of stress. Abiotic stresses induce signal transduction cascades leading to the activation of stress responsive genes. Plants are equipped with an array of defence mechanisms to protect themselves when exposed to abiotic stresses. Some of these defence mechanisms pre-exist, whereas others are activated only upon the exposure of plants to abiotic stresses. Out of these mechanisms, there are two key defence pathways which get activated and protect plants. One pathway involves the scavenging of ROS produced as a result of stress, as plant cells are particularly prone to environmentally induced oxidative stress (Asada et al. 1999). The other line of defence is through the detoxification of the toxic products like methylglyoxal (MG) which are produced in plants as by-products. In this review, we will discuss the role of the two major proteins viz. RCD1 and TPI which are involved in oxidative stress and MG stress respectively.

**Oxidative Stress**

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. Earliest signals in many abiotic stresses involve ROS accumulation which modifies enzyme activity and gene regulation (Wilkinson et al. 2009). Abiotic stresses such as drought, salinity, flooding, heat and cold disrupt the metabolic balance of cells, resulting in enhanced production of ROS such as O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub> and HO (Mittler, 2002). Enhanced ROS production is kept under tight control by a versatile and
cooperative antioxidant system that modulates intracellular ROS concentration and adjusts the redox status of the cell. Moreover, ROS accumulation under stress also functions as a signal which triggers various responses by specific signal transduction pathways. Thus oxidative stress may be broadly defined as an imbalance between oxidant production and the antioxidant capacity of the cell to prevent oxidative injury.

Defence against Oxidative Stress

Anti-oxidative defence systems comprise several non-enzymatic and enzymatic components to scavenge ROS. In plant cells, specific ROS producing and scavenging systems are found in different organelles such as chloroplasts, mitochondria, and peroxisomes which are well coordinated (Pang et al. 2008). Under normal conditions, potentially toxic oxygen metabolites are generated at low levels. An appropriate balance between production and quenching of ROS is perturbed by a number of adverse environmental factors, giving rise to rapid increases in intracellular ROS (Noctor et al. 2002; Sharma et al. 2010), which can induce oxidative damage to lipids, proteins, and nucleic acids. In order to avoid the oxidative damage, there is an increase in the level of endogenous antioxidant defence (Sharma et al. 2010).

Non-enzymatic components of the anti-oxidative defence system include the major cellular redox buffers such as ascorbate (AsA) and glutathione (γ-glutamyl-cysteinyl-glycine, GSH) as well as tocopherol, carotenoids, and phenolic compounds. These interact with numerous cellular components and play crucial roles in defence and also as enzyme cofactors. The antioxidants influence plant growth and development by modulating processes from mitosis and cell elongation to senescence and cell death (Pinto et al. 2004). Mutants with decreased non-enzymatic antioxidant contents have been shown to be hypersensitive to stress (Gao et al. 2008; Semchuk et al. 2009).

The enzymatic components of the anti-oxidative defence system comprise of several enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), enzymes of ascorbate-glutathione (AsA-
GSH) cycle, ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). SOD rapidly scavenges superoxide, one of the first ROS to be produced, dissociating it to oxygen and $H_2O_2$ (Bowler et al. 1992). However, this reaction only converts one ROS to another i.e $H_2O_2$ which also needs to be destroyed since it promptly attacks thiol proteins (Noctor et al. 1998). SOD is present in most of the subcellular compartments that generate activated oxygen. Three isozymes of SOD, copper/zinc SOD (Cu/Zn-SOD), manganese SOD (Mn-SOD), and iron SOD (Fe-SOD) are reported in plants (Fridovich, 1989; Racchi et al. 2001). SOD activity has been reported to increase in plants exposed to various environmental stresses, including drought and metal toxicity (Sharma et al. 2005; Mishra et al. 2011). It was suggested that SOD can be used as an indirect selection criterion for screening drought-resistant plants (Zaefyzadeh et al. 2009). Overproduction of SOD has also been reported to result in enhanced oxidative stress tolerance in plants (Gupta et al. 2009).

Plants contain several types of $H_2O_2$ degrading enzymes, of which catalases are unique as they do not require cellular reducing equivalent. As the peroxisomes are major sites of $H_2O_2$ production, catalase scavenges $H_2O_2$ generated in this organelle during photorespiratory oxidation, β-oxidation of fatty acids, and other enzyme systems such as XOD coupled to SOD (Scandalios et al. 1997; Río et al. 2006; Corpas et al. 2008).

The Ascorbate-Glutathione (AsA-GSH) cycle also referred to as Halliwell-Asada pathway, is the recycling pathway of ascorbate and glutathione regeneration which also detoxifies $H_2O_2$. The AsA-GSH cycle involves successive oxidation and reduction of ascorbate, glutathione, and NADPH catalyzed by the enzymes APX, MDHAR, DHAR, and GR. The AsA-GSH cycle is present in at least four different subcellular locations, including the cytosol, chloroplast, mitochondria, and peroxisomes (Nasrabadi et al. 2011). AsA-GSH cycle plays an important role in combating oxidative stress induced by environmental stresses (Sharma et al. 2005; Jiménez et al. 2009). Over the course of evolution, plants have developed several other elaborate and efficient network of scavenging mechanisms that allow them to overcome...
ROS toxicity and using some of these toxic molecules as signal transduction mediators (Mittler et al. 2004; Bailey-Serres and Mittler, 2006). Thus ROS play a dual role in plants as both toxic compounds as well as key regulators of many biological processes such as growth, cell cycle, programmed cell death, hormone signalling, biotic and abiotic cell responses and development (Foyer and Noctor, 2005; Mittler et al. 2004). The delicate balance between ROS production and scavenging allows this duality in plants which is regulated by a large network of genes termed as ‘ROS gene network’.

ROS are reported to be involved in the regulation of the closure of stomata, gravitropism in the root tip, and root branching (Joo et al. 2001; Foreman et al. 2003; Kwak et al. 2003). Genetic data indicate that Arabidopsis thaliana ATGPX3 functions as a transducer that relays a H$_2$O$_2$ signal in the ABA pathway of stomatal guard cells that controls drought stress tolerance. In addition to the usual Halliwell-Asada pathway, there are other proteins which act as regulators of ROS. One such protein is Radical induced Cell Death (RCD1).

Role of RCD1 in Plant Defence

Radical induced Cell Death or RCD1 protein belongs to a plant specific SIMILAR TO RCD ONE (SRO) gene family present in all land plants. There are several genes identified as ROS scavenger including RCD1 and SRO. RCD1 has now been established as an important regulator of stress and hormonal and developmental responses in Arabidopsis thaliana.

RCD1 was first isolated by Belles-Boix et al (2000) based on its ability to complement ROS sensitivity in yeast cells. It was first identified in Arabidopsis thaliana as a peculiar plant protein and was named as CEO (Cell differentiation protein). It was reported that the production of the plant-specific protein CEO1 in yeast results in a cross-tolerance of the engineered yeast cells against hydroperoxides (Belles-Boix et al. 2000). The production and the putative protein-protein interaction of CEO1 with other plant proteins was also characterized which revealed that both CEO1 and its close homologue CEO2 have three conserved monopartite NLS sequences and, therefore, a nuclear localization for these proteins was predicted (Belles-Boix et al. 2000).
Additionally, CEO1 was found to interact physically in the yeast two-hybrid system with DNA-binding-like proteins, which are putative transcription factors from *Arabidopsis thaliana* (Belles-Boix et al. 2000). The first isolated interacting partner of CEO1 was found to be STO that contains two putative zinc fingers similar to those found in the transcription regulators GATA-1 (Putterill et al. 1995) and CO (Orkin et al. 1996). Expression analyses show that CEO1 and STO share similar features and are ubiquitously synthesized in *Arabidopsis thaliana*. It was also found that CEO1 and STO mRNA levels do not increase on exposure to oxidative and salt stress conditions, respectively. The second CEO1 interacting protein is a putative protein similar to members from the Ethylene Responsive Element Binding Protein (EREBP) subfamily of AP2/EREBP plant transcription factors. Members of this protein subfamily are known to be involved in the induction of defence genes in response to biotic and abiotic stress (Zhou et al. 1997; Fujimoto et al. 2000). It was also shown that the plant specific protein CEO1 may constitute a cofactor of transcription factors involved in responses to biotic and abiotic stress conditions. RCD1 has also been shown to interact with EREBP-like protein via its carboxyl terminal (Belles-Boix et al. 2000).

**RCD1- A Specific Plant Protein**

RCD1 belongs to a putative novel gene family with 5 unknown genes encoding proteins distinctively similar to RCD1 (SRO1-SRO5; SIMILAR TO RCD-ONE 1-5). A genome duplication event connects RCD1 to SRO1, SRO2 to SRO3 and SRO4 to SRO5, after which all except SRO3 have remained as expressed genes. Both RCD1 and SRO1 have nuclear localization signals and a WWE-protein-protein interaction domain implicated to ubiquitination and ADP-ribose conjugation systems (Fig.1). However, these conserved domains are lacking from SRO2-SRO5.
Figure 1. The protein Structure of RCD1 and SRO1. Percentage indicates the proportion of identical amino acids between the two proteins. Abbreviations: NLS-Nuclear localisation Signals; WWE-WWE Domain; PARP-poly (ADP-ribose) polymerase catalytic region; RST- RCD1-SRO-TAF4 Domain (Jaspers et al. 2009).
RCD1 has been classified as a novel subfamily of proteins, involved in ubiquitin and ADP-ribose conjugation systems, that contain a conserved globular domain called the WWE domain (PF02825; IPR004170) predicted to mediate specific protein–protein interactions (Aravind, 2001). It was also proposed that the ubiquitin ligase and PARP-related WWE proteins could bind the same targets and facilitate them with alternative modifications, resulting in different functional consequences (Aravind, 2001). WWE domains have also been demonstrated in Drosophila deltex protein; where it interacts with ankyrin repeats of the Notch receptor (Zweifel et al. 2005). RCD1 was the only plant protein that was originally used by Aravind (2001) to define the WWE domain. The WWE domain is present in RCD1, SRO1, and the rice ortholog but is missing from the shorter (SRO2 to SRO5) proteins. Although RCD1 are the only known WWE domain containing proteins in plants, similar WWE and PARP domain architecture are found in other eukaryotes, even as distant as humans (Otto et al. 2005; Hassa et al. 2006). PSI-BLAST analyses also identified nine mammalian proteins of unknown function that have a low, 15 to 20% overall amino acid identity with RCD1, but all have both WWE and PARP domains with similar domain architecture as in RCD1 (Ahlfors et al. 2004).

RCD1 proteins also contain a region (amino acids 317 to 416) similar to the catalytic domain of poly (ADP-ribose) polymerase (PARP signature, pfam00644) (Ahlfors et al. 2004). Poly(ADP-ribose) polymerases are involved in a wide variety of cellular functions, including response to DNA damage, programmed cell death, regulation of gene expression and telomere length (Ame et al. 2004; Diefenbach and Burkle, 2005). PARPs and the role of poly (ADP-ribosyl)ation have not been as well studied in plants as in animal systems. Studies have demonstrated the involvement of PARPs in abiotic stress (Amor et al. 1998; De Block et al. 2005) and defense responses (Berglund et al. 1996; Adams-Phillips et al. 2008). A role for PARP activity in seeds has also been identified against genotoxic stress (Hunt and Gray, 2009).

It has now been validated that the domain composition of all the RCDs is unique within plants. While two Arabidopsis thaliana RCD1 family members contain an N-terminal WWE domain (PS50918) (Aravinda, 2001), all of them are characterized by the possession of the core of the poly(ADP-ribose)
polymerase (PARP; PS51059) domain and a conserved C-terminal RCD1-SRO-TAF4 domain (RST domain; PF12174) (Jaspers et al. 2009). The RST domain-bearing C-termini of RCD1 and SRO1 are suggested to be critical for the interaction with several, mostly plant specific transcription factors (Jaspers et al. 2009). This RST domain is also present in TAF4 which is a component of several multimeric protein complexes including general transcription factor TFIID involved in transcriptional initiation (Fig.2). It was found that RST domain is a highly conserved domain in all the proteins including RCD1, SRO1, TAF3 and TAF4 (Teotia et al. 2010). It was further confirmed that RST domain is present in all known SRO family members (Jaspers et al. 2010). The promoter regions of RCD1 and SRO genes contain several transcription factor-binding sites, for instance cis-acting elements involved in abscisic acid, salicylic acid, defence and stress responses as well as light responsive elements. However, the promoter structures within the RCD1-SRO gene family vary which indicates that they may response differently to environmental stimuli.

**RCD1 and Stress Response**
Kangasjärvi and group isolated a co-dominant Arabidopsis mutant, radical-induced cell death1 (rcd1), in which ozone (O₃) and extracellular superoxide (O₂⁻), but not hydrogen peroxide, induce cellular O₂⁻ accumulation and transient spreading lesions (Overmyer et al. 2000). It was shown that ethylene biosynthesis was activated in rcd1 to a higher degree, as compared to the parent ecotype (Overmyer et al. 2000). It is also hypothesized that RCD1 may be involved in processes related to regulation of ethylene biosynthesis or in communication between the ethylene, JA, and SA pathways, which are known to interact with each other (Reymond and Farmer, 1998). Arabidopsis mutant rcd1 was also found to be hypersensitive to apoplastic superoxide and ozone, but more resistant to chloroplastic superoxide formation and also exhibited reduced sensitivity to ABA, ethylene, and methyl jasmonate (Overmyer et al. 2005) and showed higher stomatal conductance than the wild type (Ahlfors et al. 2004).
Figure 2. The RST domain of the plant SRO protein family contains a strongly conserved amino acid pattern. (A) Domain structure of AtRCD1 and TAF4s from multiple species (Saccharomyces cerevisiae, Schizosaccharomyces pombe, Homo sapiens, Drosophila melanogaster). All TAF4s have the conserved TAF4 superfamily domain (TAF4; PF05236). Yeast TAF4s lack an N-terminal extension while metazoan TAF4s have an extension bearing an ETO domain (ETO/TAFH domain; PF07531). Plant TAF4s also have an N-terminal extension that lacks the ETO domain but bears the structurally unrelated plant-specific RST (RCD1-SRO-TAF4; PF12174) domain. AtRCD1 also bears PARP-like (PS51059) and WWE (PS50918) domains. (B) The C-terminal RST domain of the different groups and subgroups (Ia, Ib, Ic, IIa, IIb) of the plant SRO protein family were aligned using ClustalW and Boxshade. Under the sequence, alternatives for AAs are shown. AAs with similar chemical properties are indicated using colored bars (Jaspers et al. 2009).
Later, one more allele of rcd1 mutant was isolated as methyl viologen resistant, recessive mutant from Arabidopsis, which was also found to be more ozone sensitive (Fujibe et al. 2004). SRO2 is found to be up-regulated in chloroplastic ascorbic peroxidase mutants (Kangasjarvi et al. 2008). However, the SRO5 function was assigned to endogenous RNA silencing mechanism due to sequence overlap with neighbouring P5CDH gene involved in proline catabolism and induction of SRO5 by oxidative stress (salt/H$_2$O$_2$) results in degradation of P5CDH transcript and therefore proline accumulates to protect plants from the stress (Borsani et al. 2005). It has also been reported recently that rcd1-3 and sro1-1 mutants are resistant to osmotic stress and also display different responses to oxidative stress and salt stress (Teotia et al. 2010). The same group showed that rcd1-3 and sro1-1 double mutant also display pleiotropic developmental defects (Teotia et al. 2009). rcd1 co-dominant mutants have also been isolated from Arabidopsis mutant, in which ozone (O$_3$) and extracellular superoxide (O$_2$$^-$$^-$), but not hydrogen peroxide, induce cellular O$_2$$^-$$^-$ accumulation and transient spreading lesions (Overmyer et al. 2000). It was also reported that O$_3$ and superoxide-induced cell death in the O$_3$ sensitive radical-induced cell death1 (rcd1) mutant exhibited typical morphological characteristics of the hypersensitive response and PCD. Double-mutant analyses indicated a requirement for salicylic acid and the function of the cyclic nucleotide-gated ion channel AtCNGC2 in cell death (Overmyer et al. 2005).

Although rcd1 was found sensitive to O$_3$ and extracellular superoxide, but not to H$_2$O$_2$ or chloroplastic superoxide generated by paraquat treatment, ROS sensitivity of rcd1 was not the result of decrease in the expression of antioxidant genes. O$_3$ sensitivity in rcd1 is a co-dominant feature, while other phenotypes such as paraquat tolerance are recessive. Thus loss of RCD1 can be defined as a pleiotropic effect. The sro5 mutant is also reported to be sensitive to salt and oxidative stress (Borsani et al. 2005) while overexpression of RCD1 or/and rcd1 in wild type plants conferred rcd1 phenotype (Ahlfors et al. 2004; Fujibe et al. 2006). Interestingly, decreased expression of RCD1 has been shown to contribute the higher O$_3$ sensitivity in the wild type plant (Li et al. 2006).
Two other mutants were isolated for both RCD1 and SRO1 namely rcd1-3 and sro1-1, respectively (Teotia et al. 2009). rcd1-3 plants displayed phenotypic defects as reported for previously isolated alleles, most notably reduced stature. In addition, rcd1-3 mutants also displayed a number of additional developmental defects in root architecture and maintenance of reproductive development. While single mutant sro1-1 plants are relatively normal, loss of a single dose of SRO1 in the rcd1-3 background increases the severity of several developmental defects, implying that these genes do share some functions (Teotia et al. 2009). However, rcd1-3 and sro1-1 mutants behave differently in several developmental events and abiotic stress responses, suggesting that they also have distinct functions. Remarkably, rcd1-3; sro1-1 double mutants display severe defects in embryogenesis and postembryonic development which showed that RCD1 and SRO1 are at least partially redundant and that they are essential genes for plant development (Teotia et al. 2009). It was also observed that most rcd1-3; sro1-1 individuals could not survive embryogenesis and die with defects in the SAM, RAM, and hypocotyl, demonstrating that the function(s) encoded by these genes are critical for plants (Teotia and Lamb, 2009). Those that could not survive have pleiotropic phenotypes including short stature, short roots, and reduced apical dominance (Jaspers et al. 2009; Teotia and Lamb, 2009).

Several studies suggest that the expression of AtRCD1 and AtSRO1 is developmentally regulated and only slightly stress responsive (Jaspers et al. 2009; Teotia et al. 2009; Ahlfors et al. 2007), whereas AtSRO5 has previously been indicated as stress responsive gene (Ma et al. 2008). To confirm this phenomenon, Jaspers et al. (2010) mined publicly available Affymetrix microarray chip data and showed that AtSRO5 was the transcriptionally most responsive member of the SRO family, though AtRCD1 and AtSRO1 exhibited only subtle regulation in response to stress treatments (Jaspers et al. 2010). However, this result was in contrast to the previous finding where Bechtold et al. (2008) reported a strong increase in AtRCD1 transcript abundance in response to excess light stress.
Interaction property of RCD1

An interaction between the predicted cytoplasmic tail of SOS1 sodium transporter and RCD1 was proposed as a regulator of oxidative stress responses (Katiyar-Agarwal et al. 2006). It also revealed a function for RCD1 in salt-stress tolerance. Furthermore, it was found that salt stress causes a change in the subcellular localization of RCD1. It is localized in the nucleus under control conditions while it was found in the cytosol as well as in nucleus under stress conditions (Katiyar-Agarwal et al. 2006). Jaspers et al. (2009) established that full-length RCD1 and SRO1 proteins can interact with several proteins and the majority of them were transcription factors belonging to the DREB, NAC, basic helix–loop–helix and like zinc finger-containing families. Other than above, RCD1 was also found to interact with several transcription factors like PIF5, PIF7, COL9, COL10 and STO which belong to families known to play a role in light responses (Castillon et al. 2007; Indorf et al. 2007). It was also observed that these genes were significantly enriched in the rcd1 gene expression data (Jaspers et al. 2009). This suggests a possible role of RCD1 in light response also. An expression analysis was also performed which showed that the expression of both RCD1 and SRO1 was highest in young developing tissues (Jaspers et al. 2009).

A recent report demonstrated that RCD1 and SRO1 genes function to control division and differentiation in the root. They are necessary to maintain QC identity within the RAM and to support the population of dividing cells of this region; subsequently they are required for proper differentiation, both at an organ level and at an individual cell level (Teotia et al. 2011).

It is now established that RCD1 could participate, alone or presumably in combination with other regulatory proteins, in the mechanism of adaptation to stress conditions. Therefore, the association of RCD1 with different transcription modulators would serve to regulate the transcription of specific genes in response to different environmental stimuli. It can be concluded that there is an optimum balance required for RCD1 function. RCD1 can also be an integral part acting as an integration molecule between hormone signalling.
Triose Phosphate Isomerase: Structure, Role and Functions

Triose phosphate isomerase (TPI, TIM, D-glyceraldehyde-3-phosphate ketol-isomerase, EC 5.3.1.1) is one of the well studied enzyme which is present in all the organisms including animals such as mammals and insects as well as in fungi, plants, and bacteria and is described as the “evolutionarily perfect enzyme” (Knowels et al. 1991). However, some bacteria that do not perform glycolysis, like ureaplasmas, lack TPI. By contrast, some green algae possess only chloroplast isoenzyme (cpTPI), and they lack the cytosolic isoform (cTPI) (Henze et al. 1994).

The structure of TPI (also known as TIM) was first solved by Greg Petsko in the mid-1970’s, which revealed that TPI is a functional dimer, composed of two identical subunits of approximately 250 residues. The tertiary structure of TPI is composed of a single domain, the so-called “TIM barrel” which is composed of eight central strands that form an infinite sheet, wrapped in a barrel (Branden et al. 1999). The active site of TPI is located in the centre of the barrel at the C-terminal end of the strands. However, the crystallographic structure of TPI was first determined at 2.5 Å resolution in chicken (Banner et al. 1975). It was found that each subunit of TPI is composed of alternate segments of polypeptide chain in the α- and β-conformations and the residues participating in the subunit interface were also identified (Banner et al. 1975). Crystal structure of TPI has also been depicted in yeast (Lolis et al. 1990), Thermotoga maritima (Maes et al. 1999) and Escherichia coli (Noble et al. 1993). The structure for Trypanosoma brucei (TbTIM) (Lolis et al. 1990), human (HuTIM) Noble et al. 1993), Plasmodium falciparum (PfTIM) (Mande et al. 1994), Leishmania mexicana (LmTIM) (Williams et al. 1999) and Trypanosoma cruzi (Maldonado et al. 1998) has also been described.

In the glycolytic pathway, TPI catalyzes the conversion of DHAP to GAP so that the breakdown products of fructose-1,6-bisphosphate (F-1,6-P) are two molecules of GAP, which is the substrate of the next enzyme in the pathway (Reider et al. 1959). The equilibrium constant between DHAP and GAP is 0.05 (Veech et al. 1969). In terms of thermodynamics, DHAP
formation is favored 20:1 over GAP production (Harris et al. 1998). However, GAP is rapidly removed by GAP dehydrogenase in the next step of glycolysis, assuring that the reaction catalyzed by TIM flows essentially in a single direction, from DHAP to GAP (Knowels et al. 1991). The direction from DHAP to GAP (thermodynamically unfavourable) has particular biological relevance also as DHAP accumulation disrupts the regulation of normal cell functions, leading to chronic hemolytic anaemia and neuromuscular disorders (Schneider et al. 2000).

The role of TPI is not confined to glycolytic pathway only, but TPI also plays an important role in other metabolic pathways such as gluconeogenesis, fatty acid biosynthesis, pentosephosphate pathway, and photosynthetic carbon dioxide fixation (Miernyk et al. 1990). Triose-phosphate isomerase is usually present in all diploid homozygous plants as two isozymes with different sub-cellular localization (Wendel and Weeden, 1989). The product of one nuclear gene is active in the cytosol and the other one, after translation, is actively transported into plastids (Pichersky and Gottlieb, 1984). It is not known if the gene encoding pTPI is also expressed at high levels in non-photosynthetic tissues. However, TPI activity is found in non-green plastids also (Eastmond et al. 2000).

Genes encoding TPI have been cloned and sequenced from 11 species, including chicken (Straus and Gilbert, 1985), human (Brown et al. 1985; Maquat et al. 1985), maize (Marchionni and Gilbert, 1986), mouse (Cheng et al. 1990), Aspergillus nidulans (McKnight et al. 1986), Coptis japonica (Sato et al. 1990), Escherichia coli (Pichersky et al. 1984), Macaca mulatta (Old and Mohrenweiser, 1988), Trypanosoma brucei (Swinkels et al. 1986), Saccharomyces cerevisiae (Alber and Kawasaki, 1982) and Schizosaccharomyces pombe (Russell et al. 1985).

The high degree of sequence conservation of TPI suggests a low rate of evolutionary divergence, a common feature of all the glycolytic enzymes (Fothergill-Gilmore et al. 1986). Highly purified preparations have been obtained from rabbit muscle (Meyerhof et al. 1944). The properties and kinetics of the yeast and animal enzymes have also been investigated (Meyer et al. 1934; Meyerhof et al. 1943, 1944; Oespfer et al. 1950). The kinetic properties for TPI was first described in chicken breast muscles by Putman et
al (1972) where they described the characteristics of the enzyme catalysed reaction in each direction. The pH-dependence of the kinetic parameters $k_{\text{cat}}$ and $K_m$ for the TPI reaction was also determined in each direction (Plaut et al. 1972). The kinetic properties of *Trypanosoma brucei brucei* TPI was measured and compared with the commercially available rabbit muscle, yeast enzymes and chicken muscle enzyme (Lambier et al. 1987). Purification and the kinetic properties of TPI from the psychrophilic bacterium *V. marinus* is also reported (Alvarez et al. 1998). TPI from rabbit muscle (Hartman et al. 1975) and rabbit liver (Krietsch et al. 1975) was also purified and analysed for kinetic parameters. Later in 1978, TPI from human skeletal muscle was purified, crystallized and kinetic properties were described (Dabrowska et al. 1978). Recently, TPI activity and kinetic parameters have also been determined for yeast TPI (endogenous), human TPI (expressed in yeast), and rabbit TPI (purified from muscle) (Gruning et al. 2011).

The first report of studies on TPI from higher plants revealed the presence of TPI in extracts of pea seeds (Hatch et al. 1958) though TPI extract was also reported to be present in pea roots (Gibbs et al. 1955). An active preparation of TPI was obtained from pea seeds and kinetic parameters for TPI were also determined (Turner et al. 1965). The two TPI isozymes in flowering plants are coded by independent nuclear genes (Gallez et al. 1982; Pichersky et al. 1983). The cytosolic TPI isozyme acts primarily in glycolysis and gluconeogenesis whereas the plastid TPI functions in these pathways and also has an essential role in photosynthesis (Dennis et al. 1982; Robinson et al. 1981). However, both the TPI isozymes were first purified to the homogeneity by Pichersky and Gottlieb (1984). They also reported the structural and immunological studies of both purified isozymes from spinach, lettuce, and celery. Isolation and characterization of a cDNA clone encoding the cytosolic form of TPI from rice has also been reported (Xu et al. 1993). It was also suggested that the chloroplastic and cytoplasmic forms of TPI in higher vascular plants were derived from common ancestral forms of TPI (Anita et al. 1974). However, in case of cherimoya (*Annona cherimola*), a primitive angiosperm, three sets of TPI bands were observed (Patty et al. 1988). It was also found that the level of TPI is strongly dependent on the age and stage of tissue development. In particular, the
activity of cTPI was found to be significantly higher in expanding leaves compared to mature senescing ones, because the growing leaves need energy and C skeletons from glycolysis and respiration for biosynthetic purposes (Dorian et al. 2005).

TPI was first cloned and characterized in plants from maize roots and it was found that the gene is interrupted by eight introns ((Marchionni and Gilbert, 1986). TPI cDNA sequences have also been published earlier for *Coptis japonica* (Sato et al. 1990). Rice cDNA was used as a probe to show that the cytosolic TPI gene is present as a single copy per haploid rice genome, whereas multiple TPI gene copies exist in maize (Marchionni and Gilbert, 1986; Xu and Hall, 1992). However the isolation and characterization of cytosolic form of TPI from rice was first reported by Xu et al (1993) where they also found that the gene encoding cytosolic TPI from rice contains eight introns whose positions are well conserved with those in maize. The nine exon sequences are identical with the cDNA (Xu and Hall, 1992) and share 74 to 94% similarity with those of maize TPI genes.

An important role of TPI came into light in mid 1960s (Schneider et al. 1965) when TPI deficiency was found responsible for a unique glycolytic enzymopathy with autosomal recessive inheritance that is characterized by chronic haemolytic anaemia, cardiomyopathy, susceptibility to infections, severe neurological dysfunction, and, in most cases, death in early childhood because of a marked decrease in TPI activity and an accumulation of DHAP (Schneider et al. 2000). Interestingly, mice studies demonstrated that mutations resulting in catalytically inactive TPI variants (null alleles) led to early prenatal lethality in the homozygous state, an incidence that might also arise in humans (Mohrenweise et al. 1982; Mohrenweiser et al. 1981; Eber et al. 1979; Merkle et al. 1989). The most prominent missense mutation detected in TPI deficiency patients occurs at codon 104 in the TPI gene encoding aspartic acid instead of glutamic acid within the enzyme (Glu104Asp variant) (Daar et al. 1986; Arya et al. 1997). It has also been reported recently that TPI deficiency is caused by altered dimerization and not because of catalytic inactivity of the mutant enzymes (Ralser et al. 2006).
Response of TPI in Abiotic Stress

There are several genes encoding proteins whose functions do not seem to be directly related to stress but were shown to be expressed at greater levels in response to abiotic stress. These include several enzymes involved in glycolysis (Umeda et al. 1994; Velasco et al. 1994) and in the synthesis of Met (Glaser et al. 1993), SAM (Espartero et al. 1994; Chang et al. 1995), peroxidases (Botella et al. 1994), nonspecific lipid transferases (Torres-Schumann et al. 1992; Ouvrard et al. 1996), and early light-induced proteins (Bartels et al. 1992; Ouvrard et al. 1996). TPI and few other glycolytic enzymes have also been found to be induced under various stresses. Although the simultaneous changes in gene expression and physiological responses strongly suggest that induced proteins play a role in these responses, the correlation between their expression and the level of stress tolerance in the different genotypes has been rarely studied (Ramagopal, 1987; Hurkman et al. 1989; Moons et al. 1995). Remarkably, TPI is highly up-regulated under stress conditions in mammalian cells also (Yamaji et al. 2004).

It was in late 90s when transcript levels of several enzymes of glycolytic and alcohol fermentation pathway were examined in shoot and root tissues of rice seedlings subjected to various abiotic stress and it was found that TPI was induced in response to desiccation (Minhas et al. 1999), while there was no alteration in the activity of TPI under water stress in cucumber (Todaka et al. 2000) and about 1.4 fold increase in transcript level of TPI was reported under water stress in maize (Riccardi et al. 1998). A genetic analysis of the maize opaque-2 mutant has revealed that the high levels of free amino acids phenotype associated with this mutation could be linked to a cTPI locus (Wang et al. 2001).

TPI has also been shown to be induced upon saline and water stress in cultured cells of rice (Umeda et al. 1994) where the coordinated induction is thought to be essential for activation of energy producing pathways and in-turn maintaining the homeostasis in stressed cells (Yan et al. 2005). TPI has also been reported to be induced by drought stress in rice and maize based on 2-DE (Riccardi et al. 1998; Salekdeh et al. 2002). It was also reported that TPI is also induced by salt stress in rice.
Rice cytosolic TPI also showed higher transcript level under submergence (Masaaki et al. 1994) and salt stress (Kawasaki et al. 2001). Most of the other glycolytic enzymes also show higher activity under hypoxia and anoxia stress in rice (Mustroph et al. 2003) while no significant change has been observed in activity of TPI under salt stress in pea (Hernandez et al. 2001). It has also been investigated how the accumulation of glycine betaine in vivo affects several key enzymes in the Calvin cycle under salt stress conditions; however, there were no significant changes in the activity of, TPI in both wild type and transgenic plants (Yang et al. 2008). Though the Calvin-Benson cycle appears to be a major target of glutathionylation in Chlamydomonas except TPI (Zaffagnini et al. 2011) but was earlier identified as a target of glutathionylation in Arabidopsis (Ito et al. 2003). It was found that under drought stress there was a decrease in the TPI activity in pea (Pelleg et al. 1992) TPI was also reported to be ABA responsive protein in rice leaves (Ho et al. 2008). Minhas et al (1999) reported significant alterations in the transcript levels of various glycolytic and fermentative enzymes, including TPI, induced by desiccation, salt, and high temperature. It was also demonstrated how some ATP generating enzymes, TPIs among them, were induced by saline and water stress conditions in rice cells (Umeda et al. 1994) where a coordinated induction of these enzymes for the production of energy leads to maintain the homeostasis in stressed cells. Another study showed that, in a resistant genotype of Brassica carenata, TPIs and other enzymes of the Calvin cycle were significantly more induced upon pathogen infection than in a susceptible genotype (Subramanian et al. 2005).

TPI was also cloned and expressed in potato where TPI activity and protein levels appeared to be regulated during the development of potato leaves, with highest levels of expression in expanding leaves suggested that TPI may be important in the glycolytic pathway for the supply of C to respiratory and biosynthetic pathways during active growth (Dorian et al. 2005).

Analyses of the responses of Arabidopsis thaliana root cells to different gravitational conditions using an agravitropic mutant, pin2 and its wild type shows that cytosolic TPI, insensitive to altered gravity in pin2 roots (Tan et al. 2009). Effect of herbicides on TPI activity in Italian ryegrass (Lolium
multiflorum) has been demonstrated (Del et al. 2009). Up-regulation of TPI was also observed in wheat (Triticum aestivum) under salt stress (Gao et al. 2011). It is recently reported that the rice transgenic overexpressing sucrose non-fermenting 1-related protein kinase2 (SnRK2) shows upregulation of TPI under salt stress as compared to wild type plant (Nam et al. 2012).

**Methylglyoxal: A Cytotoxic Component of Biological System**

It is well established now that during glycolysis, TPI interconverts DHAP and GAP, enabling DHAP to be metabolically used. Loss of TPI activity, therefore, results into the excess accumulation of DHAP (Fig.3). Indeed, the most apparent biochemical alteration reported in TPI-deficient patients is the striking (20-fold) increase in cellular concentration of DHAP (Schneider et al. 2000, Tanaka et al. 1990). Triose phosphates are very unstable metabolites (Richard, 1984, 1993) and therefore, spontaneous production of MG is an unavoidable consequence of the glycolysis pathway during stress. Although there is no indication that DHAP itself is toxic, it decomposes non-enzymatically to MG (Kalapos et al. 1999) which is a highly reactive oxoaldehyde that can modify both proteins and DNA to form AGEs (Lo et al. 1994). The deleterious consequences of accumulation of glycation adducts include protein inactivation, denaturation and crosslinking, altered gene expression, increased oxidative stress, DNA damage, and apoptosis. In humans, MG and AGEs have been linked with a variety of conditions, including diabetes, uremia, cancer, and aging. Most important with respect to the phenotypes, MG is toxic to neurons (Ramasamy et al. 2005). It has also been suggested that the defects in the function and survival of TPI-deficient neurons result from the toxic effects of elevated MG levels and not because of the alteration in the concentrations of ATP levels (Gnerer et al. 2006). It has been reported that in foodstuffs and beverages, MG can be formed during processing, cooking, and prolonged storage. Consumption of even low doses of MG over a longer period of time can cause degenerative changes in different tissues (Nemet et al. 2006).
Endogenous production of MG increases during various stresses in yeast *(Saccharomyces cerevisiae)*, and bacterial systems (Cooper et al. 1984; Abordo et al. 1999; Kalapos et al. 1992) and recently in plant systems also (Yadav et al. 2005a; Singla-Pareek et al. 2006). A high level of MG accumulation is toxic to cells as it inhibits cell proliferation (Ray et al. 1994) and results in a number of adverse effects such as increasing the degradation of proteins and inactivating the antioxidant defence system (Martin et al. 2001).

The role of MG in plants was first described by Yadav et al. (2005). It was also shown that the MG levels increase significantly in plants in response to salinity, drought, and cold stress conditions. MG accumulation was also reported in tobacco when exposed to zinc metal stress (Singla-Pareek et al. 2006). The mechanism of MG accumulation under stress is not yet well understood. However, increase in the rate of glycolysis under stress could lead to higher MG level as it is produced mainly from triose phosphates (Sommer et al. 2001). These observations suggested that increase in MG levels is a general response of plants upon exposure to abiotic stresses.

Normally the glyoxalase pathway protects cells from excess MG (Thornalley, 2003). Glyoxalase I and II detoxify MG by converting it to lactic acid, using catalytic amounts of glutathione as a cofactor. It has also been observed that plants can be protected from toxic effect of MG by overexpressing enzymes of the glyoxalase pathway. In transgenic plants overexpressing glyoxalase enzymes, MG level is maintained during abiotic stress similar to the level in non-transgenic and transgenic plants under non-stress conditions (Yadav et al. 2005b). However, under conditions of oxidative stress, glutathione levels are reduced, resulting in elevated levels of MG. This effect can be further magnified in a positive feedback loop because AGEs themselves contribute to the production of ROS (Ahmed et al. 2003). It has also been demonstrated that different kind of stress conditions, such as salt and metal stress, results in enhanced expression of gly I (Veena et al. 1999).
Figure 3. A model of how mutations in TPI lead to neuronal dysfunction and neurodegeneration. Relevant enzymatic steps in glycolysis are shown. Double-headed arrows represent reactions that can flow in either direction. Loss of TPI activity causes accumulation of excess DHAP, which converts to MG nonenzymatically (Gnerer et al. 2006)
Transgenic tobacco plants overexpressing gly I and gly II genes individually or together in the same plant indicated their role in salt tolerance (Single-Pareek et al. 2003). It was also reported that tobacco plants overexpressing gly genes could efficiently detoxify MG beyond a certain level thus preventing its accumulation to toxic level under NaCl stress (Yadav et al. 2005a). Reduction of intracellular MG by overexpression of aldose/aldehyde reductase in transgenic tobacco has been shown to enhance stress tolerance (Oberschall et al. 2000). Recently, it is reported that MG induces stomatal closure accompanied by ROS production and cytosolic free calcium concentration oscillations in Arabidopsis guard cells and that MG-induced stomatal closure does not require endogenous ABA and MeJA (Hoque et al. 2012).

Though universal degradation of MG appears to be predominantly through glyoxalase system, in some other organisms, α-oxoaldehyde dehydrogenase [2-oxoaldehyde:NAD(P)-oxido-reductase, EC. 1.2.1.23] has also been found in MG degradation (Vander Jagt, 1992, 1995; Inoue and Kimura, 1995). This enzyme was purified from sheep liver (Monder, 1967). Later its existence in microorganisms was also shown (Inoue and Kimura, 1995). Both NADP and NAD have been shown as coenzymes for this enzyme and the $K_m$ value for MG with respect to NADP was less than with respect to NAD (Monder, 1967). However, this enzyme has not been reported from plants yet.

There is a possibility of MG detoxification by other unknown methods. According to a recent report, exogenous selenium pre-treatment protects rapeseed seedlings from cadmium-induced oxidative stress by upregulating antioxidant defense and MG detoxification systems (Hasanuzzaman et al. 2012). It is also found that cytosolic ascorbate protect the cells from oxidative damage by scavenging reactive oxygen species in *Nicotiana tabacum* (Hoque et al. 2012). Another glycolytic enzyme Aldose reductase has also been shown to confer enhanced drought and salinity tolerance to transgenic tobacco plants by scavenging MG (Kumar et al. 2012).

MG has also been considered as a signalling molecule. In *Saccharomyces cerevisiae*, possible signalling pathway for the induction of gly I gene under high osmotic stress condition has been suggested (Inoue et
al. 1998). The gly I gene has been shown to be expressed via the HOG-MAPK pathway to scavenge MG that may be increased by the osmotic stress. It has been shown that Hog1p is one of the mitogen-activated protein kinases (MAPKs) in *S. cerevisiae*. It can be inferred that several defence mechanisms for plant survival under abiotic stress has been reported from various sources. There is a need to work out other unknown pathways and mechanisms.