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Pragati Mardi
Plant Biotechnology, I.Ag. Sc, RGSC, Banaras Hindu University, Uttar Pradesh, India

Akanksha Gupta
Plant Biotechnology, I. Ag. Sc, RGSC, Banaras Hindu University, Uttar Pradesh, India

Anshuman Kumar
Department of Animal genetics and Breeding, Faculty of Veterinary and Animal Sciences, RGSC, Banaras Hindu University, Uttar Pradesh, India

Corresponding Author:
Pragati Mardi
Plant Biotechnology, I.Ag. Sc, RGSC, Banaras Hindu University, Uttar Pradesh, India

Role of PIMT in plant: A perspective towards abiotic stress

Pragati Mardi, Akanksha Gupta and Anshuman Kumar

Abstract
The yield loss due to abiotic stress is an agonizing issue in agriculture mutilating its protein makeup, function and structure which can impact its survivability in stressful circumstances by formation of abnormal L-isoaspartyl residues through spontaneous covalent modification. To overcome this serious problem PIMT, a protein repairing enzyme, transforms abnormal L-isoaspartyl residues to normal L-aspartyl residues and re-establishes the protein's innate structure and function. PIMT has a key role in preserving seed vigor and viability for prolong periods of time. PIMT role has also been reported in vegetative organs in few plant species.

Keywords: Abiotic stress, PIMT, Plant, Aspartyl residues

Introduction
Plants undergo many unfavourable climatic damages during their growth cycles and the damage due to abiotic stress is a perturb issue leading to 50-70% reduced crop yield in world agriculture annually [1]. Plant population face a large number of physical and chemical environmental factors that are hostile to them, including high temperature, salinity, heavy metals, ultraviolet (UV) radiation, collectively referred as abiotic stress [2]. Abiotic stress impacts the food quality, security and the geographical distribution of plants in nature. Being sessile in nature plants encounters the stresses and to grapple with it plants have developed potent adaptive organized proteolytic system pathways to avoid their harmful effects. Plants have established many physiological and biochemical mechanisms to combat the abiotic stress conditions. Stress affected plants exhibit response in three phases firstly the alarm phase (stress initiation), second the resistance phase (activation of defense) establishing cellular, physiological and morphological defense, such planned structured pathway at molecular levels restores the deformed amino acid at elevated stress conditions and third the phase of exhaustion (damage due to stress) [3]. Stress has destructive effects on plant protein structure and function including cellular localization, alterations in protein relative abundance, protein expression, post translational modifications (PTMs) and protein interaction.

Effects of Abiotic stress on proteins
Proteins are susceptible to abiotic stress and are crucial for cell's well-being as it is directly involved in novel phenotype shaping. Plant's stress proteomics reveals the protein biological functions in plants and functions when exposed to elevated abiotic stress [4]. Plants induce stress response either by stress avoidance and escape or stress resistance. On grounds of intrinsic chemical activities of amino acid side chains, proteins are accountable to various spontaneous modifications that perniciously affect the functionality of the protein. These modifications include deamidation, racemization and oxidative damage [5, 6]. If not processed for long it may accumulate in protein over time. Proteins degradation can occur due to deamidation of aspargine (Asn) and (Asp) aspartate [7]. Cysteine and methionine are most prone to oxidative damage formed due to elevated level of reactive oxygen species [8] while aspargine and aspartate being the most vulnerable to deamidation and residues of Aspartic acid (Asp) are most prone to racemization [9]. The protein degradation rate and racemization rate depend on the different structural layer and Aspartic acid is considered to have the fastest racemization rate causing covalent modifications due to accumulated damaged abnormal protein.
Plants consequently use proteolytic machinery system and repair enzymes to deal with structurally damaged proteins. Much of the mutilation incurred by proteins is irrevocable and the annihilation of damaged proteins by proteolytic systems is well recognized, as opposed to only a very small number of protein repair enzymes have been identified.

PIMT: an emerging enzyme for sustaining abiotic stress

The center of attention here is one of these repair enzymes, that catalyzes the S- adenosylmethionine (AdoMet)-based on methylation of L-isoAsp and (D-Asp) residues in age damaged proteins. The objective of this literature is to review the biochemistry of PIMT-catalyzed methylation, structural study of PIMT, the regulation and distribution of PIMT activity in living organisms, and understanding the biological importance of protein isoAsp methylation. Besides that, the transcript focuses on PIMT, the review covers information attained from high-throughput genomic research. Considering of the loads of published manuscripts, it has not been feasible to include all of the studies whose findings have contributed to our prevailing knowledge of PIMT function.

Physical properties of PIMT

PIMT activities were first characterized and purified in mammalian cells, but have proven to be commonly found throughout all walks of life. In mammals, PIMT activity can be identified in all tissues, although the quantified specific activities alter markedly between tissues. The enzyme has been purified to homogeneity from numerous tissues, compromising, spleen, brain and erythrocytes. With few exceptions, PIMT fractionates as a cytosolic enzyme with a quantified molecular weight of 24,000 27,000 Dalton. The chromatographic behaviour of PIMT on gel filtration columns is consistent with that of a globular, monomeric protein.

PIMT catalyzes the formation of protein carboxyl methyl esters

PIMT catalyzes the transfer of a methyl group from AdoMet to either the - carboxyl group of IsoAsp residues or the - carboxyl group of D-Asp residues in peptide and protein substrates. Stereo chemically, D-Asp and L-IsoAsp residues are surprisingly similar in functional group distribution, explaining the ability of PIMT to recognize both classes of substrates. The cytoplasmic reaction occurs through the formation of a cyclic succinimide intermediate and produces IsoAsp or Asp from succinimide. PIMT converts IsoAsp to succinimide, raising the possibility of later conversion to Asp PIMT are unstable [10, 11]. The mechanism of PIMT mediated repair is shown in (Figure 1). The processing of protein carboxymethyl esters appears to be entirely non-enzymatic. At present there is no convincing evidence for protein demethylases or other enzymatic activities that might be involved in the processing of esters. Studies with synthetic peptide substrates show that the immediate product of demethylation is a succinimide structure that forms spontaneously when methanol is released. The chemical mechanism is postulated to involve nucleophilic attack of the carboxyl peptide nitrogen on the ester carboxyl group of the aspartate resulting into accumulation of Isoaspartate residue in protein due to deamidation of Aspargine (Asn) or isomerization of Aspartate (Asp) and dehydration hence affecting catalytic proficiency of the enzyme and forming a kink in the protein structure. The actual rate of succinimide formation is significantly greater than the rate of ring hydrolysis and shows a strong dependence on the nature of the carboxylic amino acid PIMT uses s-adenosylmethionine as the methyl source. Owing to these properties and since isoAsp formation represents a major source of spontaneous covalent mutilation that sequel proteins under physiological conditions, it is abriged that PIMT plays an utmost role in repairing altered isoAsp-containing proteins *in vivo* [12].

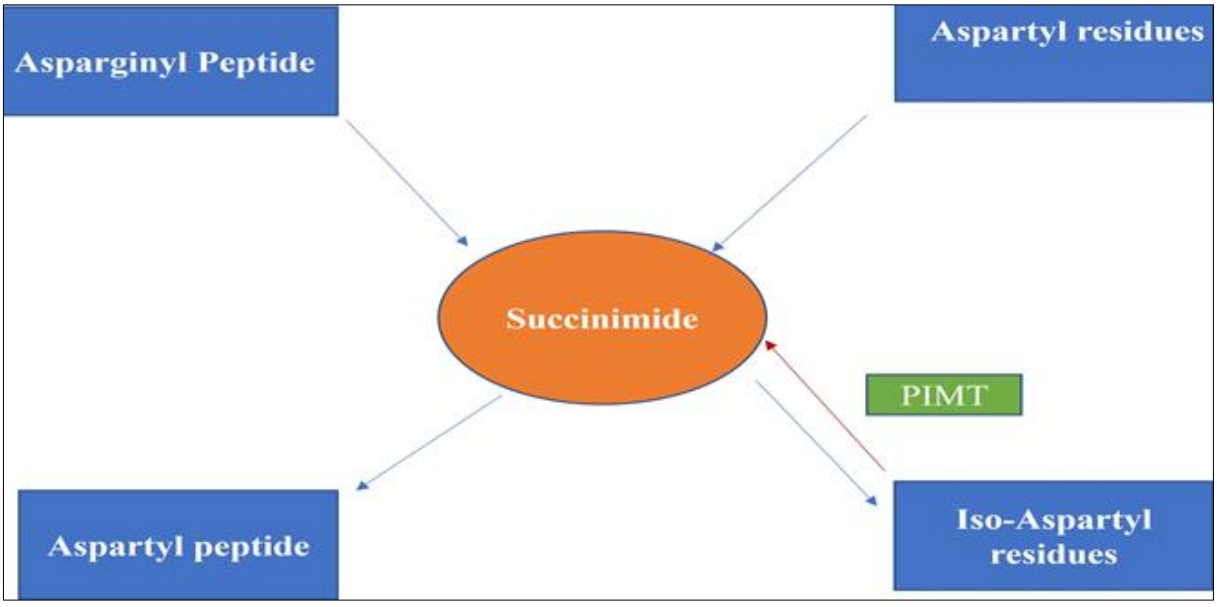

Fig 1: Mechanism of PIMT mediated repair process

PIMT and protein methylation

Protein methylation is a system of complex and diverse processes [13]. The significance of protein methylation and its job in gene regulation and signaling remains indistinct. The natural methyl emissions of numerous amino acids have been extensively delineated. In addition, methylation of amino acid residues occurs after peptide bond formation in proteins and has important implications for cell physiology. Enzymes responsible for protein methylation comprise a broad class and are termed protein methyltransferases (PMTs). In all living things, about 2% of genes comprise methyltransferase, inclusive of significant number of enzymes that use proteins as substrates. PMTs are divided into three classes based on the popular amino acids of methylation (Arg, Gln/Asp, and Lys, respectively). These enzymes use S-adenosylmethionine (SAM) as the methyl group provider. New insights have advanced the active role of PMTs in processes such as epigenetic regulation through histone transformation, sensory adaptation to bacterial chemical stimuli, cellular response to stress, aging, protein repair and regeneration, etc. Due to their

greater structural complexity, it is more difficult to demonstrate structural repair of isoaspartyl containing proteins by PIMT. PIMT is a universal enzyme that participates in the repair of different isomerized forms of proteins.

Modification of protein D-aspartyl and L-isoaspartyl residues by PIMT

The unusual nature of the substrate sites of PIMT was first determined by Steven Clarke's lab in 1982 after providential congruence of chemical and biological studies of protein carboxyl methylation reactions in the erythrocyte of human. They revealed attributes of the methylation reactions that were tough to attune with a directory role of PIMT in erythrocytes. The experiment illustrated that all the methylation reactions including membrane proteins were noticeably sub-stoichiometric. Moreover, the amount of methyl esters in an erythrocyte was positively associated with the age of the cell. Peaking up the probability that methyl-accepting sites arose from aging damage. The broad specificity illustrated by PIMT is readily demonstrated by the unconventional behavior of protein D-Asp and L-isoAsp residues, which arise intuitively as proteins age because all Asn and Asp residues in a protein is theoretically area of spontaneous sudden damage that provide increase to D-Asp and L-isoAsp residues proteins[14]. Nevertheless, that traits of a protein vigorously affect the actual incidence of protein isomerization. In general, isoAsp residues increases in flexible regions of polypeptide possibly because of steric downsides linked with succinimide formation in many structured regions of polypeptides. PIMT normally has a modified Rossmann-type fold composed of seven mixed stranded central beta (β) sheet bound by alpha (α) helices on one side. Additional proof for the establishment of protein isoAsp residues on a physiologically important time scale has come from the biotechnology industry. In many cases, the presentation of an isoAsp residue was identified to resentfully alter the stability and integrity of a recombinant protein designed for therapeutic use. Examples of recombinant proteins resentfully influenced by isoAsp formation comprise of human growth hormone, epidermal growth factor, stem cell factor, and recombinant antibodies. Sequence examination showed that isoAsp residues do not arise suddenly in protein sequences. Instead, there are apparantly hot spots for isoAsp generation in sequences. The isoAsp residue formed from deamidation of Asn-67, was found as crystal structure in a structured surface loop where it was unmanageable to reshape by PIMT [15].

Conclusion

Role of PIMT in plant protein repair is few. Further, the moon lighting action of PIMT in plant is not yet extensively studied. Considering its multifaceted role in many other strata of life forms its presence in plant must be significantly associated with various activities. Radiolabelled transfer assay with proteomic assay may shed some light on the targets of this enzyme in plants and its repair process.

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