RESEARCH ARTICLE

TRANSCRIPTIONAL REGULATION OF PROLINE BIOSYNTHESIS

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Manuscript Info

Abstract

Plants are subjected to various kinds of abiotic and biotic stresses throughout their life cycles which include salinity, drought, temperature extremes, infection by pathogens, nutrient deficiency and UV radiation. A general response of plants to various kinds of stresses is the accumulation of compatible osmolytes such as proline, glycine betaine, proline betaine, glycerol, mannitol and sorbitol etc. which protect cells against damage caused by stress. Among them, proline plays a pivotal role and accumulates in a large number of species under salinity, drought, cold, nutrient deficiency, pathogen attack and high acidity. The core enzymes in this reaction are pyrroline5-carboxylate synthetase (P5CS) and pyrroline5-carboxylate reductase (P5CR). In another pathway, proline synthesis occurs via deamination of ornithine which is transaminated to P5C by ornithine-delta-aminotransferase (OAT). Plant cells have a potential to accumulate proline rapidly and break it down quickly when needed. Considerable evidence confirmed that proline synthesis under osmotic stress is driven by both ABA-dependent and ABA-independent signaling. Emerging data suggest that the expression of proline biosynthetic genes is regulated by many TFs that are related to almost all plant hormones. Several unique predicted elements were found in AtP5CR, including putative bZIP, HD-HOX, MYB and C2C2 (Zn) DOF binding sites. Thus, it could be concluded that proline regulation takes place through complex interrelation of different TFs and helps in generating tolerance in plants against abiotic stress.

Introduction:

Proline accumulation is one of the foremost metabolic responses of plants to osmotic stress (Delauney and Verma, 1993), and it plays positive roles under stressful conditions such as a component of antioxidative defense system (Molinaria et al. 2007), regulator of cellular redox potential (Hare and Cress, 1997), stabilizer of subcellular structures and macromolecular structures (Rajendrakumar et al. 1994), or active component of signal transduction pathways that helps in regulation of stress responsive genes (Khedr et al. 2003). Furthermore, during the incompatible plant–pathogen interaction, proline metabolism seems involved in the induction of the hypersensitive response (Qamar et al., 2015). Although many gene overexpressing in transgenic line have been found playing role in stress tolerance, but it is not properly elucidated how this tolerance develops (Kavi Kishor and Sreenivasulu, 2014). Therefore, the role of proline in tolerance generation in transgenic plants through metabolic engineering still remains an open question.

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In plants proline synthesis takes place through two pathways i.e glutamate pathway and ornithine pathway by action of different enzymes. A bifunctional enzyme, Δ1 -pyrroline-5-carboxylate synthetase (P5CS) catalyze the conversion of Glutamate to glutamic-γ-semialdehyde (GSA) by oxidation of NADPH+ H⁺, while ornithine is converted to GSA by action of ornithine δ-amino transferase (OAT). GSA spontaneously cyclizes into pyrroline-5-carboxylate (P5C), which is further reduced to proline by P5C reductase (P5CR). Proline degradation is the reverse process of proline biosynthesis catalyzed by two mitochondrial enzymes, proline dehydrogenase (PDH) which converts proline to P5C and this is converted to glutamate by enzyme P5C dehydrogenase (P5CDH). Proline accumulation in plants in response to stress is observed commonly but its regulation varies among and depends on lot of factors. Proline accumulation is regulated through two pathways, one is Abscisic acid (ABA) dependent and the second is ABA independent pathway (Savouré et al., 1997; Ábrahám et al., 2003). But till now, it is not well understood how the enzymes of proline biosynthesis and degradation are regulated in stress tolerance. Savouré et al., 1997 reported that under cold and osmotic stress ABA-independent P5CS1 expression has been shown In Arabidopsis, while under the same conditions P5CR expression did not correlate to proline content. ABA and NaCl treatment induced both OsP5CS1 and OsP5CR in rice (Sripinyowanich et al., 2013). In euakaryotes gene expression is regulated by a different set of transcription factors (TFs) which binds at TF binding sites (TFBS) of promoter region and modulate gene expression. The cis-regulatory elements (CREs) analysis in a given promoter may therefore represent an important tool to understand the signal transduction pathway against the response to a particular stress.

To understand the molecular mechanism underlying proline accumulation in B. napus, cDNA was isolated and characterized for BnP5CS, BnOAT and BnPDH enzymes and the relationship between proline accumulation and the transcript level of these genes was studied at both seedling and plant stage. The 2551 bp BnP5CS1 cDNA bears a 2154 bp open reading frame encoding 717 amino acids protein with a predicted approximately 77.8 kDa molecular weight and isoelectric point of 5.96. BnP5CS1 protein sequence analysis showed that BnP5CS1 have sequence identities of 96% with A. thaliana P5CS1, 77% with M. truncatula P5CS1, and Actinidia (Ac) deliciosa, 76% with Mesembryanthemum (Me) crystallinum, 75% with Vitis (Vi) vinifera and V. aconitifolia, 74% with Oryza sativa and 72% with M. sativa P5CS1 with A. thaliana P5CS1 (96%), Ac. deliciosa (77%), Me. crystallinum (76%), M. truncatula P5CS1 (77%), Vi. vinifera (75%), V. aconitifolia (75%), M. sativa P5CS1 (72%) and O. sativa (74%) (Xue et al., 2009). One open reading frame of 1431 bp is present in 1615-bp-long BnOAT cDNA, which encoded a protein of 476 amino acids with calculated molecular weight of 52 kDa and isoelectric point of 7.17. Protein sequence analysis revealed high similarity with OAT cloned from B. rapa (96%), A. thaliana (91%), M. truncatula (68%), O. sativa (69%) and V. aconitifolia (45%). OAT cofactor pyridoxal phosphate binds at the putative site positioning between 232-301 amino acid. The high amino acid similarity with OAT from M. truncatula and A. thaliana suggests that BnOAT is also a δ-form OAT (Xue et al., 2009). BnPDH is 498 amino acids with a molecular weight of 55 kDa and isoelectric point of 6.77, it has a single open reading frame of 1497 bp in BnPDH cDNA. Protein sequence comparison showed various identities with Arabidopsis thaliana PDH1 (89%), M. sativa (55%), A. thaliana PDH2 (74%), Nicotiana tabacum PDH1 (54%) and N. tabacum PDH2 (55%) (Xue et al., 2009).

In B. napus various experimental showed that salt stress-induced proline accumulation results into activated biosynthesis and inhibited proline degradation using the reciprocal pathways and during prolonged osmotic stress it the ornithine pathway possibly contribute to the proline accumulation. up-regulation of both BnP5CSs and BnPDH in flower buds and flowers during development suggests an important role of proline during flower development(Xue et al., 2009). Fichman et al. (2015) analyzed 1,000 bp upstream the translation start site (TSS) of proline regulating genes AtP5CS1, AtP5CS2, AtP5CR, and AtOAT using a specific database in Arabidopsis and found a number of putative CREs recognized by different classes of TFs. Here, multiple alignments of 50 regulatory regions of 48 plants P5CS1 showed great degree of divergence. A comparison of A. lyrata and A. thaliana showed homogeneity for P5CS2 genes and the comparison of promoters showed the identification of several CREs which were targetted by different class of promoters includes AP2/EREBP, HD-HOX, WRKY, MYB, and bZIP. For P5CR no conserved TFBS were identified in the sequences analysis of 27 plants due to their high diversity. For AtP5CR unique sequence were found for putative bZIP, MYB, HD-HOX and C2C2(Zn)DOF binding sites (Fichman et al., 2015). Similar results were reported in rice for the presence of putative CREs and dozens of possible TFBS as their binding sites. However, several different sites were identified in comparison of Oryza sativa and A. thaliana genes. 24 different classes of TFs were identified to have a binding site in the promoter of OsP5CS1, OsP5CS2, and OsP5CR in addition to the sites for the MYB, bZIP, and AP2/ERF TF families in CREs of promoter region. TFBS for TCR and WRKY were detected only in OsP5CR whereas IDEF1 was unique for the OsP5CS2 promoter and E2F and BES1 families were detected only in the OsP5CS1 promoter. Total 24 TF families were identified in rice and 15
in Arabidopsis showed that in rice proline biosynthesis is regulated in a complex way than Arabidopsis. As more than one TF can bind to TFBS further analysis of proline biosynthesis regulation is required.

**ABA Dependent Pathway:**
In ABA regulated genes, Abscisic acid-responsive elements (ABREs) act as the major cis-acting sequences belongs to the G-BOX family (ACGTGG/TC) which has ACGT as core sequence and at least one copy of ABRE with a coupling element (CE) is required to respond to ABA mediated stress. In rice, OsP5CS1 promoter region have two sequences containing a G-box element, 73 and 481 bp upstream of TSS and 300 bp upstream have core sequence CCACC for CE1. TFs belonging to bZIP family bind core sequence ACGT. Tang et al. (2012) and Zong et al. (2016) reported that various transgenic lines ectopically overexpressing bZIP proteins are more sensitive to ABA treatment and more resistant to drought and salinity.

**ABA Independent Pathway:**
In ABA-independent pathway major CREs of transcriptional regulation are Dehydration-responsive elements (DRE), DRE-related motifs such as C-repeats (CRT) and low-temperature-responsive elements. A single copy of DRE is sufficient for inducing expression. TFs belonging to the ERF/AP2 family are known as DREB1/CFB and DREB2 and able to bind DRE/CRT elements. Here, DREB2-type genes play a role in osmotic-responsive pathways and the DREB1-type genes are involved in cold-responsive pathways. Several studies reported that the overexpression of either DREB1 or DREB2 genes improved plant tolerance to salt, drought and freezing (Lata and Prasad, 2011).
Various studies supported the role of these TFs in P5CS regulation (Zhang et al. 2013, 2016). Zhang et al. 2013 reported that soybean plants overexpressing OsDREB2A showed higher GmP5CS expression, but the DRE sequence in GmP5CS promoter was absent. Moreover, the AaDREB1 protein overexpression in rice from the coldtolerant plant Adonis amurensis caused a two-fold increase of free proline under both permissive and cold stress conditions (Zong et al. 2016).

In rice, only a partially identical DRE sequence (tCCGAC) is identified 421 bp upstream of the OsP5CR TSS, and a sequence identical the DRE core ACCGAC is found 72 bp downstream of the ATG start codon of OsP5CS2. Another class of plant-specific TFs, involved in proline regulation is the NAC (NAM, ATAF, and CUC) proteins, is involved in the ABA independent pathway under stress. These TFs constitutes a wide family with CAGG core-DNA binding motif, with almost 151 members in rice and 110 members in Arabidopsis. In several cases, the NAC genes over expression resulted into increased drought/salt tolerance and higher free proline levels (Liu et al. 2013; Hong et al. 2016). OsP5CS2 and OsP5CR promoters region entails the CACG NAC-core motif but several other NAC binding motifs have been found in all three promoters analyzed suggesting that this TF family might regulate proline accumulation under both permissive and stressful conditions. On the whole, the transcriptional regulatory network of proline biosynthesis as mediated by ABA-dependent and ABA-independent pathways.

Transcription Regulation Mediated By Apetala2/Ethylene Responsive Factors (AP2/ERF) In Abiotic Stress Tolerance:
The Apetala2/Ethylene Responsive Factors (AP2/ERF) are a superfamily of TFs characterized by the AP2 DNA binding domain. This superfamily is further divided into three families i.e. ER, AP2 and RAV based on the number of repeat of AP2 sequence. ERF family is further subdivided into two sub families, one is ERF and second is CBF/DREB which regulates proline level in response to abiotic and biotic stress. CBF/DREB regulates in response to abiotic stress via activation of these TFs belonging to different families (Dey and Volt, 2015). Zhu et al. 2014 reported the presence of a GCC-box within the promoter of TaP5CR which has binding site for TaPIE1 and showed its overexpression by analyzing the promoter and binding affinity.
Several other studies also showed positive correlation between the overexpression of ERF members and increased osmotic stress tolerance due to proline accumulation (Rong et al. 2014; Wang et al. 2015; Yao et al. 2015). Moreover, both in overexpressing ERF2 Jatropha curcas showed P5CS transcripts in abundance than in wild type plants (Wang et al. 2015). On the other hand, some ERF genes negatively regulate stress tolerance. Interestingly, its overexpression resulted in decreased osmotic stress tolerance in connection with both downregulation of the proline biosynthetic genes BpP5CS1 and BpP5CS2 and upregulation of the proline catabolic genes BpProDH and BpP5CDH (Zhang et al. 2016). Two GCC-box sites were also found in OsP5CS1 promoter. However, due to the complexity of the roles of ERF proteins, no conclusion can be drawn on their possible significance.

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