Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/10436
DOI URL: http://dx.doi.org/10.21474/IJAR01/10436

RESEARCH ARTICLE

TRANSCRIPTIONAL REGULATION OF PROLINE BIOSYNTHESIS

Anju Rani and Jayanti Tokas
Department of Biochemistry, College of Basic Sciences and Humanities, CCS-HAU, Hisar - 125004 (Haryana), India.

Manuscript Info

Manuscript History
Received: 01 December 2019
Final Accepted: 03 January 2020
Published: February 2020

Key words:-
Proline, Abiotic, Aba, Transcription Factors

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.

Corresponding Author:- Anju Rani
Address:- Department of Biochemistry, College of Basic Sciences and Humanities, CCS-HAU, Hisar - 125004 (Haryana), India.

In plants proline synthesis takes place through two pathways i.e glutamate pathway and orinithine 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-aminotransferase (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 proine 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 eukaryotes 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 betwwen 232-301amino 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 targeted 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 stresss. 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.

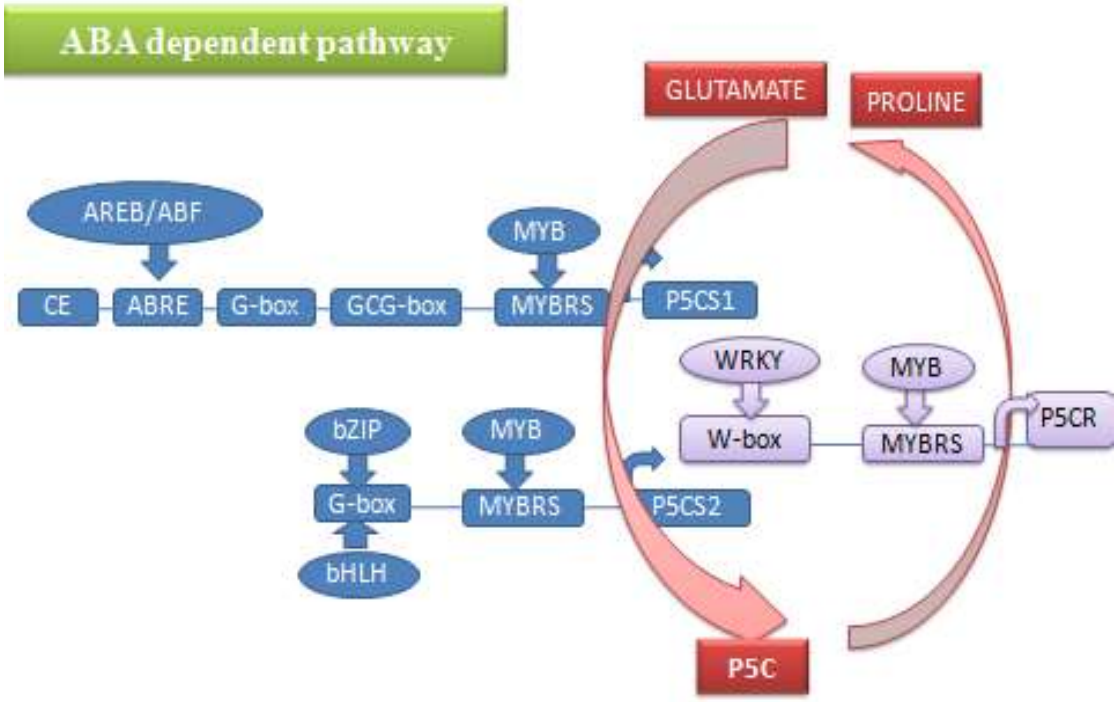

Fig:- ABA dependent pathway for proline biosynthesis regulation.

Xu et al. (2016) stated that transgenic Arabidopsis plants incorporated with soybean GmbZIP110 gene were able to accumulating significant amounts of proline in case transcription of AtP5CS1 was not apparently induced. Likewise, Zhang et al. (2015) reported overexpression of wheat TabZIP60 in transgenic Arabidopsis contained significantly higher amounts of proline. Thus, this data shows that TFs of the bZIP family plays important rolein the regulation of proline biosynthesis. Another group of TFs is the bHLH family which binds with the G-BOX (and E-BOX), 111and 162 have been found in rice and Arabidopsis. Expression of bZIP, is related to higher accumulation of proline in response to osmotic stress (Liu et al. 2014, 2015) and cold stress (Jin et al. 2016). ABA, NaCl, and mannitol treatment induced the expression of both AtP5CS isoforms which results into proline accumulation. Many GCG-box motifs presence on the synthetase transcripts support the role of transcription factors in ABA dependent signaling and proline accumulation (Liu et al. 2015).

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/CBF 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).

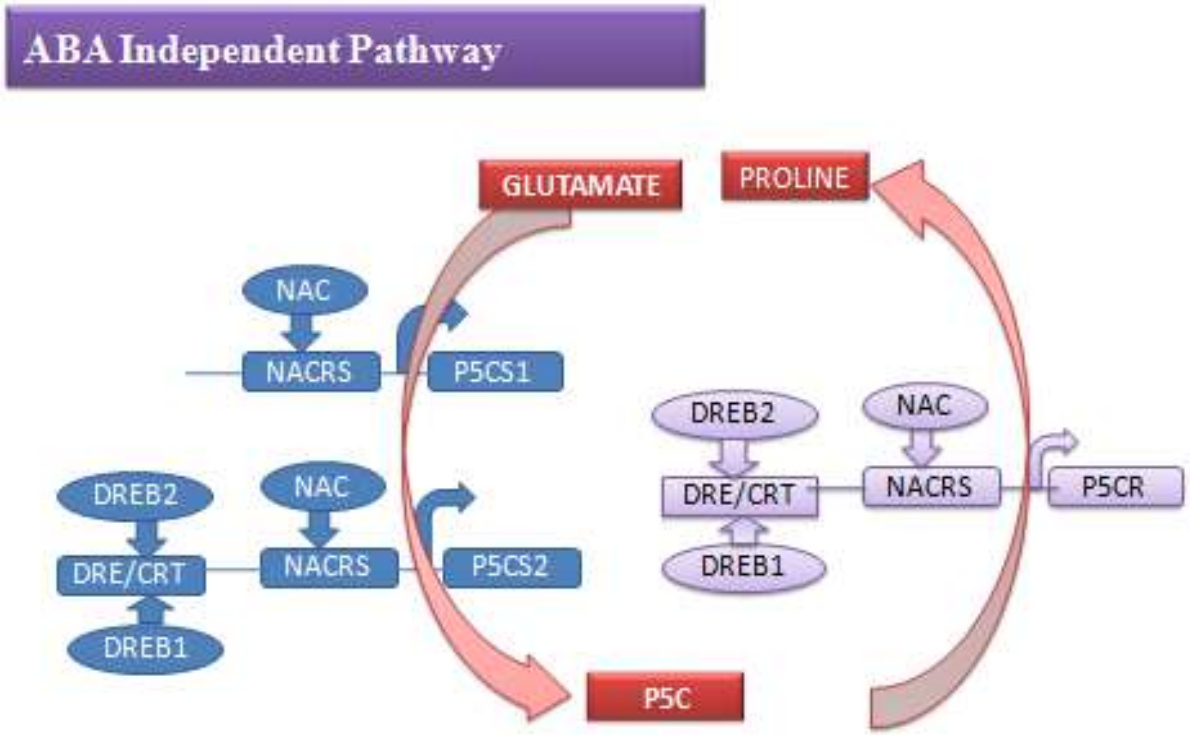

Fig:- ABA independent pathway for proline biosynthesis regulation.

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 sepuence. 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 biding site for TaPIE1 and showed its overexpression by analyzing the promoter and binding affinity.

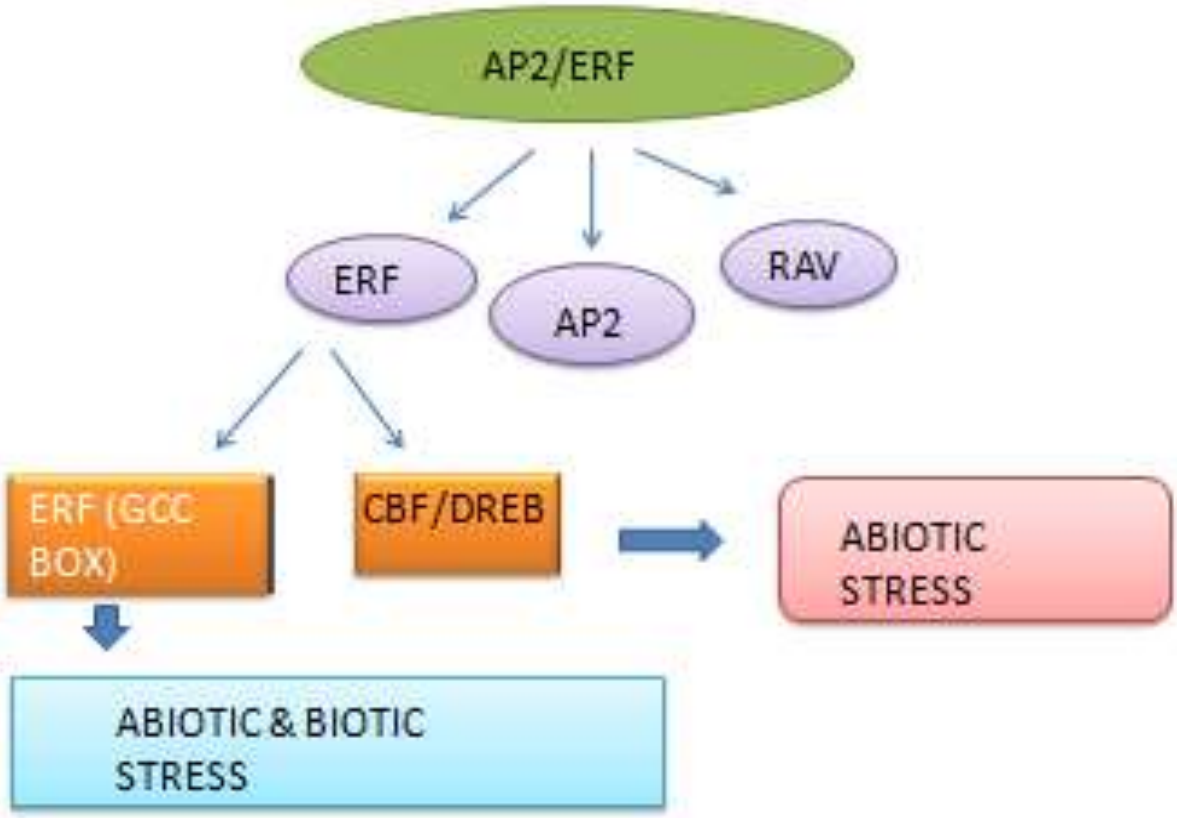

Fig:- Transcription regulation mediated by apetala2/ethylene responsive factors (AP2/ERF) in abiotic stress tolerance.

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.

References:-

1. Abraham, E., Rigo, G., Szekely, G., Nagy, R., Koncz, C., and Szabados, L. (2003). Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in Arabidopsis. Plant Mol. Biol. 51:363–372. doi: 10.1023/A:1022043000516
2. Delauney, A. J. and Verma, D. P. S. (1993) Proline biosynthesis and osmoregulation in plants. Plant J. **4**, 215-223.
3. Dey, S., and Volt, A. C. (2015). Ethylene responsive factors in the orchestration of stress responses in monocotyledonous plants. Front. Plant Sci. 6:640. doi: 10.3389/fpls.2015.00640
4. Fichman, Y., Gerdes, S. Y., Kovács, H., Szabados, L., Zilberstein, A., and Csonka, L. N. (2015). Evolution of proline biosynthesis: enzymology, bioinformatics, genetics, and transcriptional regulation. Biol. Rev. 90:1065–1099. doi: 10.1111/ brv.12146
5. Hare, P. D. and Cress, W. A. (1997) Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul. 21:79-102.
6. Hong, Y., Zhang, H., Huang, L., Li, D., and Song, F. (2016). Overexpression of a stress-responsive NAC transcription factor gene ONAC022 improves drought and salt tolerance in rice. Front. Plant Sci. 7:4. doi: 10.3389/fpls.2016.00004

7. Jin, C., Huang, X.-S., Li, K.-Q., Yin, H., Li, L.-T., Yao, Z.-H., et al. (2016). Overexpression of a bHLH1 transcription factor of Pyrus ussuriensis confers enhanced cold tolerance and increases expression of stress-responsive genes. Front. Plant Sci. 7:441. doi: 10.3389/fpls.2016.00441
8. Kavi Kishor, P. B., and Sreenivasulu, N. (2014). Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? Plant Cell Environ. 37:300–311. doi: 10.1111/pce.12157
9. Khedr, A. H. A., Abbas, M. A., Wahid, A. A. A., Quick, W. P. and Abogadallah, G. M. (2003) Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of Pancratium maritimum L. to salt-stress. J. Exp. Bot. 54:2553-2562.
10. Lata, C., and Prasad, M. (2011). Role of DREBs in regulation of abiotic stress responses in plants. J. Exp. Bot. 62: 4731–4748. doi: 10.1093/jxb/err210
11. Liu, W., Tai, H., Li, S., Gao, W., Zhao, M., Xie, C., et al. (2014). bHLH122 is important for drought and osmotic stress resistance in Arabidopsis and in the repression of ABA catabolism. New Phytol. 201:1192–1204. doi: 10.1111/nph. 12607
12. Liu, X., Liu, S., Wu, J., Zhang, B., Li, X., Yan, Y., et al. (2013). Overexpression of Arachis hypogaea NAC3 in tobacco enhances dehydration and drought tolerance by increasing superoxide scavenging. Plant Physiol. Biochem. 70:354–359. doi: 10.1016/j.plaphy.2013.05.018
13. Liu, Y., Ji, X., Nie, X., Qu, M., Zheng, L., Tan, Z., et al. (2015). Arabidopsis AtbHLH112 regulates the expression of genes involved in abiotic stress tolerance by binding to their E-box and GCG-box motifs. New Phytol. 207:692–709. doi: 10.1111/nph.13387
14. Molinaria, H., Marura, C., Darosb, E., Camposa, M., Carvalhoa, J., Filhob, J., Pereirac, L. and Vieiraa, L. (2007) Evaluation of the stress-inducible production of proline in transgenic sugarcane (Saccharum spp.): osmotic adjustment, chlorophyll fluorescence and oxidative stress. Physiol. Plant 130:218-229.
15. Qamar, A., Mysore, K. S., and Senthil-Kumar, M. (2015). Role of proline and pyrroline-5-carboxylate metabolism in plant defense against invading pathogens. Front. Plant Sci. 6:503. doi: 10.3389/fpls.2015.00503
16. Rajendrakumar, C. S., Reddy, B. V. and Reddy, A. R. (1994) Proline-protein interactions: Protection of structural and functional integrity of M4 lactate dehydrogenase. Biochem. Biophys. Res. Commun. 201:957-963.
17. Rong, W., Qi, L., Wang, A., Ye, X., Du, L., Liang, H., et al. (2014). The ERF transcription factor TaERF3 promotes tolerance to salt and drought stresses in wheat. Plant Biotechnol. J. 12:468–479. doi: 10.1111/pbi.12153
18. Savouré, A., Hua, X. J., Bertauche, N., VanMontagu, M., and Verbruggen, N. (1997). Abscisic acid-independent and abscisic acid-dependent regulation of proline biosynthesis following cold and osmotic stresses in Arabidopsis thaliana. Mol. Gen. Genet. 254:104–109. doi: 10.1007/s004380050397
19. Sripinyowanich, S., Klomsakul, P., Boonburapong, B., Bangyeekhun, T., Asamic, T., Gu, H., et al. (2013). Exogenous ABA induces salt tolerance in indica rice (Oryza sativa L.): the role of OsP5CS1 and OsP5CR gene expression during salt stress. Environ. Exp. Bot. 86: 94–105. doi: 10.1016/j.envexpbot.2010.01.009
20. Tang, N., Zhang, H., Li, X., Xiao, J., and Xiong, L. (2012). Constitutive activation of transcription factor OsbZIP46 improves drought tolerance in rice. Plant Physiol. 158:1755–1768. doi: 10.1104/pp.111.190389
21. Wang, X., Han, H., Yan, J., Chen, F., and Wei, W. (2015). A new AP2/ERF transcription factor from the oil plant Jatropha curcas confers salt and drought tolerance to transgenic tobacco. Appl. Biochem. Biotechnol. 176:582–597. doi: 10.1007/s12010-015-1597-z
22. Xu, Z., Ali, Z., Xu, L., He, X., Huang, Y., Yi, J., et al. (2016). The nuclear protein GmbZIP110 has transcription activation activity and plays important roles in the response to salinity stress in soybean. Sci. Rep. 6:20366. doi: 10.1038/ srep20366
23. Xue, X., Liu, A., Hua, X. (2009). Proline accumulation and transcriptional regulation of proline biothesynthesis and degradation in Brassica napus. BMB Reports 42:28–34. https://doi.org/10.5483/bmbrep.2009.42.1.028
24. Yao, W., Wang, L., Zhoua, B., Wanga, S., Lia, R., and Jiang, T. (2015). Overexpression of poplar transcription factor ERF76 gene confers salt tolerance in transgenic tobacco. J. Plant Physiol. 198:23–31. doi: 10.1016/j.jplph.2016. 03.015
25. Zhang, L., Zhang, L., Xia, C., Zhao, G., Liu, J., Jia, L., et al. (2015). A novel wheat bZIP transcription factor, TabZIP60, confers multiple abiotic stress tolerances in transgenic Arabidopsis. Physiol. Plant. 153:538–554. doi: 10.1111/ppl. 12261
26. Zhang, W., Yang, G., Mu, D., Li, H., Zang, D., Xu, H., et al. (2016). An ethyleneresponsive factor BpERF11 negatively modulates salt and osmotic tolerance in Betula platyphylla. Sci. Rep. 6:23085. doi: 10.1038/srep23085

27. Zhang, X.-X., Tang, Y.-J., Ma, Q.-B., Yang, C.-Y., Mu, Y.-H., Suo, H.-C., et al. (2013). OsDREB2A, a rice transcription factor, significantly affects salt tolerance in transgenic soybean. PLoS ONE 8:e83011. doi: 10.1371/journal.pone.008 3011
28. Zhu, X., Qi, L., Liu, X., Cai, S., Xu, H., Huang, R., et al. (2014). The wheat ethylene response factor transcription factor pathogen-induced ERF1 mediates host responses to both the necrotrophic pathogen Rhizoctonia cerealis and freezing stresses. Plant Physiol. 164:1499–1514. doi: 10.1104/pp.113. 229575
29. Zong, J.-M., Li, X.-W., Zhou, Y.-H., Wang, F.-W., Wang, N., Dong, Y.-Y., et al. (2016). The AaDREB1 transcription factor from the cold-tolerant plant Adonis amurensis enhances abiotic stress tolerance in transgenic plant. Int. J. Mol. Sci. 17:E611. doi: 10.3390/ijms17040611
30. Zong, W., Tang, N., Yang, J., Peng, L., Ma, S., Xu, Y., et al. (2016). Feedback regulation of ABA signaling and biosynthesis by a bZIP transcription factor targets drought-resistance-related genes. Plant Physiol. 171:2810–2825. doi: 10.1104/pp.16.00469.