

System Perspective Analysis for Molecular and Genetic Source of Salt Tolerance in Cotton

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Abstract

Sources of tolerance salinity stress in plants are the results of sets of the simple effect and interaction among genes. A systematic perspective at the polygenic trait process of resistance to environmental stresses, including salinity stress, and the interactions among genes and their inheritance, may provide new insights into the process of integrating beneficial genes into genotypes. In this study, we aimed interaction of candidate genes include *NHX1*, *TPS1*, *ERF2*, *SOD*, *CIPK*, *PP2C*. Salinity was selected and their correlation was calculated using their pathway. The results showed that the effect of antioxidant gene alignment including SOD, the most direct effect and the genes most effective in regulating sodium / potassium channels and antiporters were the second most effective factor. The relationship between these two groups of genes and their protein activity was positive and highly significant (Pvalue <0.001). This result showed that, strengthening of antioxidant systems in cotton either directly or indirectly through environmental induction or through trans activators can be effective in salt stress tolerance of genotypes. If we ignore the effects of environmental induction regulated by agro-ecophysiological conditions, most transcriptional factors study focused on ERF1. It was through binding to CIS elements that were effective in resisting to salt stress.

Keywords: pathway studio, cotton, salt stress, genetic tolerance

Introduction

Three categories of soil affected by salt was classified in: saline soils, alkaline soils and salt-alkaline soils (Novo *et al.*, 2006). Saline soils comprise excessive amount of neutral salts, which include NaCl and Na₂SO₄, as a major part, resulting in salt stress. NaOH and Na₂CO₃ are responsible for the saline and alkalization of soils by creating a high pH value, with a destructive effect on plants growth (Shi *et al.*, 2005). Stress resulted from alkaline soils causes several issues of osmotic pressure stress, different types of ionic injuries and high pH stress. Plants under salt-alkaline conditions suffer from both salt stress caused by excessive salt ions and alkaline stress caused by high pH (Zhang *et al.*, 2013). There are different genes for biological processing, cellular component and molecular function in the cotton under salt stress (Figure 1).

In general, most of the genes and proteins related to Na⁺ stress (treated with NaCl) and high pH (treated with NaOH) are also involved in the pathways against Na₂CO₃ stress. High pH leads to oxygen deprivation stress, which causes cotton organs nigrities. High pH also increases the synthesis of pectin-related enzymes, that strengthens cell wall to defense damage of high pH. In the process of the hydrolysis of ATPase, extra H⁺ produced help to neutralize OH⁻ within the cytoplasm (Figure 2).

Besides, genes and proteins related with ion homeostasis under Na⁺ stress were also found in our study, such as protein kinases, transcription and transporters. These genes and proteins have been reported in previous studies. MYBs that regulate genes of the anthocyanin pathway were up-regulated under Na₂CO₃ stress, which always cause leaves to turn red or orange in apple (Min *et al.*, 2016). Here, our study provides some candidate genes, particularly responding to high pH and Na⁺ stresses. For instance, Hexokinase (HXK) acted as a sugar sensor in eukaryotic cells (Min *et al.*, 2016), being found to be up-regulated under high pH stress, which indicates that genes encoding HXK may be related to high pH stress (Bingeli *et al.*, 2018).

There are several different genes on the Na⁺ tolerance mechanism and other stress resistant, which had complicity interaction between.

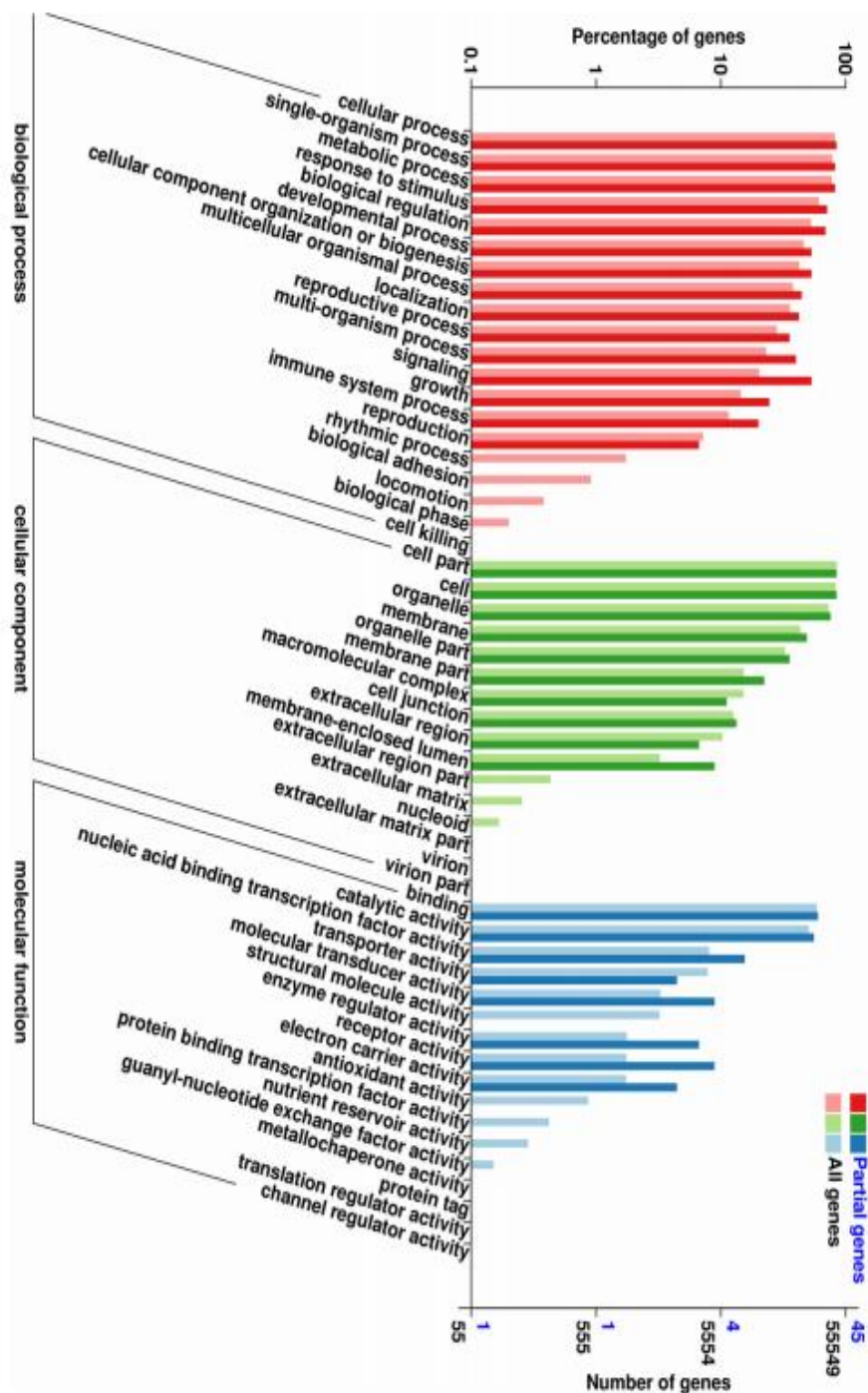


Figure 1. Different genes for biological processing, cellular component and molecular function in the cotton under salt stress (Bingeli *et al.*, 2018)

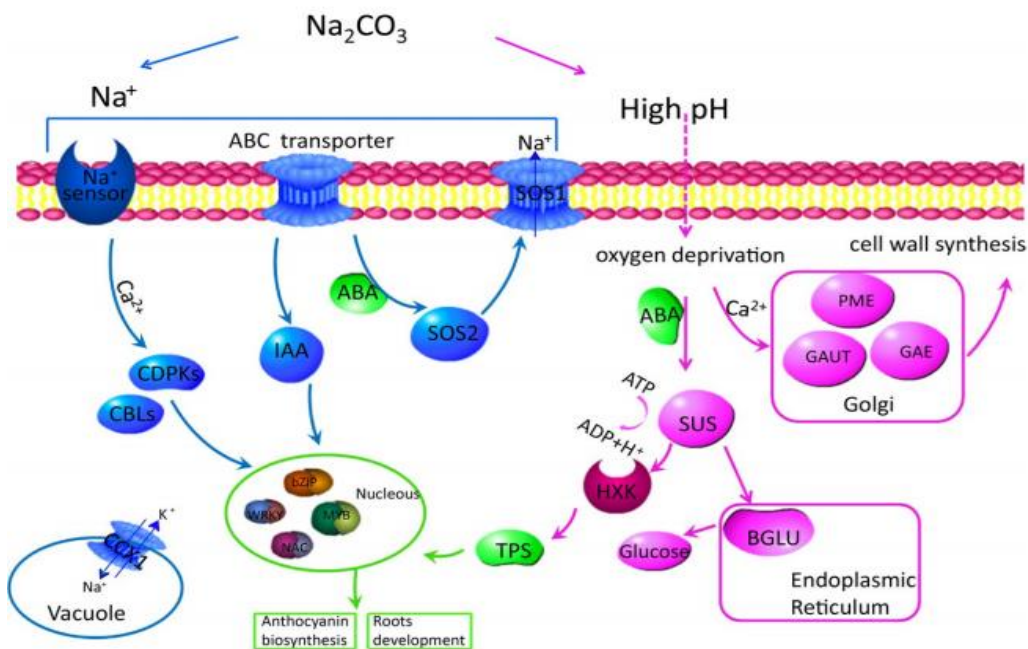


Figure 2. Model of the regulatory networks to Na⁺ stress and high pH [Bingeli et al., 2018]

Trehalose-6-phosphase synthase (TPS)

Trehalose, a non-reducing disaccharide, is composed of two glucose molecules that are connected by α , α -1, 1- glycosidic linkage and exist in bacteria, fungi, algae and plants (Elbein *et al.*, 2001). TPS was synthesized in the high osmolarity cell condition. Trehalose protects bioactive substances and cell structures, such as proteins, nucleic acids, and biological membranes, under adverse environmental stresses, such as drought, freezing, oxidation, high salt, high temperature and low temperature (Garg *et al.*, 2002). Trehalose synthesis in plants is a two-step process: first, TPS catalyzes UDP-glucose and glucose-6-phosphate to generate trehalose-6-phosphate (T6P); second, trehalose-6-phosphatase catalyzes the dephosphorylation of trehalose-6-phosphate to trehalose. The structure of TPS proteins in plants contains two domains: TPS and TPP; however, many studies have shown that the TPP domain in TPS proteins appears to have lost enzymatic activity during evolution (Vandesteene *et al.*, 2010). The 3d structure of TPS on the figure 3 was shown.

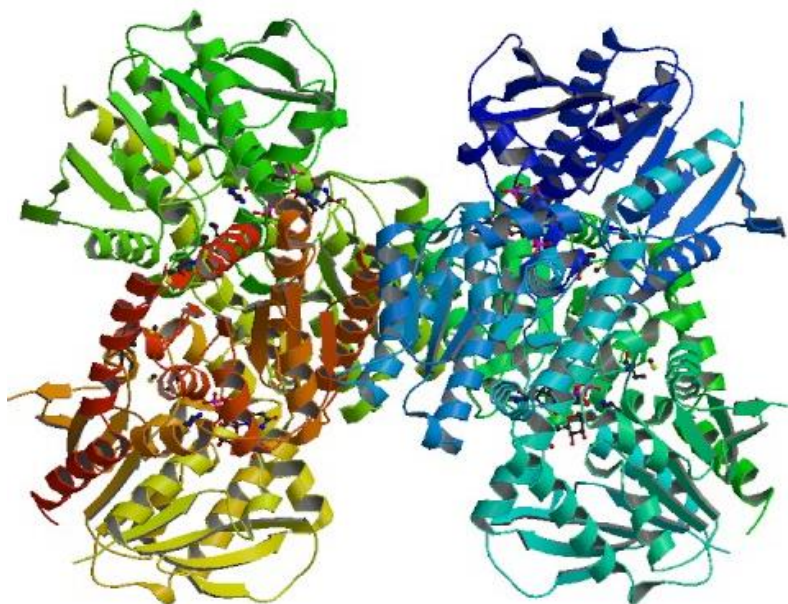


Figure 3. The 3D structure of Trehalose-6-phosphase synthase (TPS)

A TPS gene was cloned named as AtTPS1 which had the trehalose-6-phosphase synthetase function from higher plants (Blazquez *et al.*, 1998). The AtTPS1 mutant TPS1 was a recessive embryonic lethal gene [Eastmond *et al.*, 2002]. Even so, AtTPS1 played an important role in the process of vegetative growth and transition to flowering (WGomes *et al.*, 2006). Studies showed that TPS expression levels in cotton increased under drought stress (Kosmas *et al.*, 2006) and TPS genes in maize were also found upregulated in response to both salt and temperature stress (Jian *et al.*, 2010). OsTPS1 might enhance the abiotic stress tolerance of rice by increasing the trehalose and proline content (Li *et al.*, 2011). Many studies have suggested that TPSs play a vital role in plants adjusting to environmental stresses.

Profiling of Protein Phosphatases (PP2C):

The protein kinases (PKs) and protein phosphatases (PPs) are known to regulate the protein function, and are the fundamental molecular mechanism, by reversing protein phosphorylation during cellular signaling (Hamna *et al.*, 2019). Thus, it is involved in many biological processes, such as signal transduction, development, and environmental stimuli. The PKs phosphorylate largely serine (Ser), threonine (Thr), and tyrosine (Tyr), whereas PPs can reverse this function by eliminating the phosphate group (Li *et al.*, 2014). Mg²⁺-dependent protein phosphatase (PP2C) is evolutionarily conserved from Archaea to higher plants that pointedly modulate stress signaling pathways and reverse the stress-induced PK cascades to complex

environmental stimuli (Cao *et al.*, 2016). From various literature, several key stress-responsive protein kinases genes have been extensively studied and proven to respond in diverse stress conditions including biotic and abiotic factors (Boudsocq *et al.*, 2004).

In *Arabidopsis*, several members of PP2c such as PLL4 and PLL5 (POL-like gene) are known to adjust leaf development, though with no obvious functions within the meristem (Song *et al.*, 2005). Cotton, as an oil crop and an important source of natural textile fiber, plays a crucial role in agriculture and industry all around the world. However, its production is mainly constrained due to various abiotic and biotic stress conditions. The release of *Gossypium* whole-genome data in four different cotton species, such as *Gossypium arboreum* (Li *et al.*, 2014), *Gossypium barbadense* (Yuan *et al.*, 2015), *Gossypium hirsutum* (Zhang *et al.*, 2015) and *Gossypium raimondii* (Wong *et al.*, 2012) and their publicly available database allows us to comprehensively characterize the PP2C gene family based on bioinformatic tools.

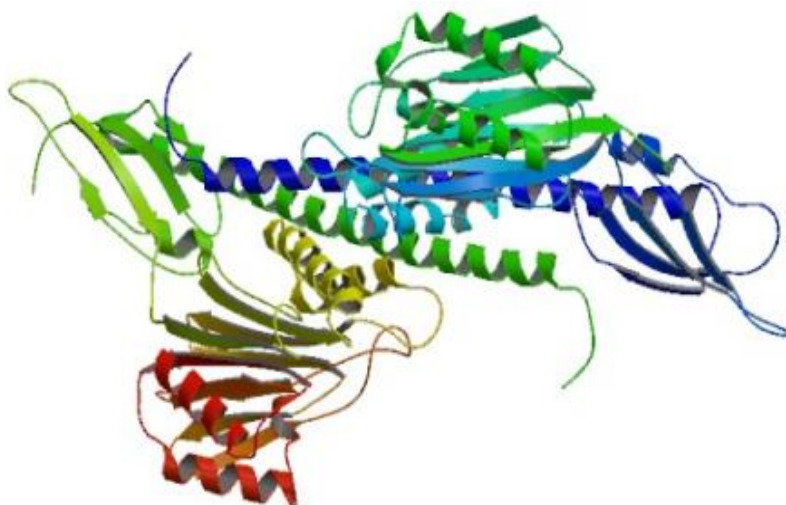


Figure 4. The 3D structure of Profiling of Protein Phosphatases (PP2C)

Following the gene structure organization analysis, conserved protein motifs, and cis-elements, we traced the duplication gene pairs and their evolutionary divergence that likely resulted in the widespread extension of the PP2C gene family. The protein phosphatase (PP2C) gene family, known to participate in cellular processes, is one of the momentous and conserved plant-specific gene families that regulate signal transduction in eukaryotic organisms. Recently, PP2Cs were identified in

Arabidopsis and various other crop species, but analysis of PP2C in cotton is yet to be reported.

NHX1

Protein of NHX1 with molecular function potassium and sodium antiporter activity contend potassium ion transmembrane transport source and regulation of intracellular pH. Sodium ion import across plasma membrane can be established resistance to salt stress in plant.

Plants have developed a complex mechanism to prevent damages caused by environmental changes. ABA is a pivotal element in this mechanism. ABA functions in seed germination inhibition, growth regulation, fruit abscission, and stomatal closure (Raghavendra *et al.*, 2010). These better salt-tolerant accessions could be selected as parents to accelerate the progress of cotton tolerance breeding by molecular design (Rai *et al.*, 2011).

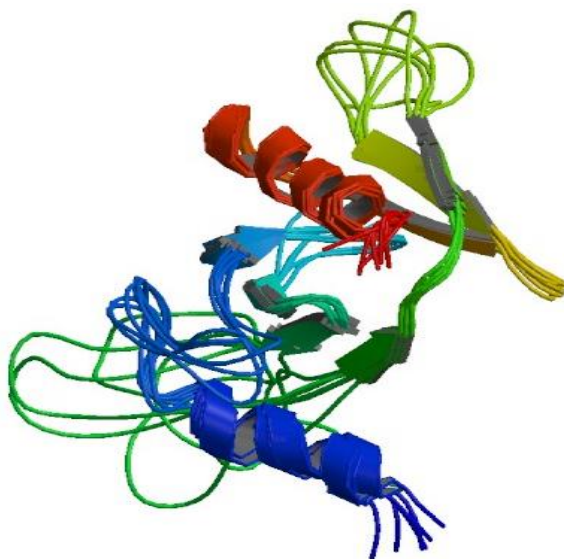


Figure 5. The 3D structure of NHX1

Salt tolerance is a genetically and physiologically complex trait controlled by multiple small effect genes (Flowers, 2004). With the fast development of SNP arrays and next-generation sequencing technology, GWAS is becoming a novel and effective method for determining useful genes in crop plants. Association analysis has been successfully used in mining candidate genes of important agronomic traits

in cotton, such as fiber quality, yield and its components, and Verticillium wilt resistance (Wang *et al.*, 2017). The cotton accessions consisting of diverse germplasms worldwide showed large variations in RSR and STL under salt stress. A total of 10 and 15 SNPs significantly associated with RSR and STL were identified, respectively, of which the two SNPs i46598Gh and i47388Gh on D09 were simultaneously associated with the two traits (Sun *et al.*, 2018).

CBL-interacting protein kinase (CIPK)

A few studies have indicated that the CIPKs function in plant development (Held *et al.*, 2011), nutrient uptake (Qingchen *et al.*, 2017), and pollen tube elongation (Zhou *et al.*, 2015). The CIPKs perform major functions in stress responses. The first characterized CBL-CIPK network is the SOS network. Plasma membrane-localized SOS3 (AtCBL4) recruits SOS2 (AtCIPK24). Subsequently, the CBL-CIPK complex activates a Na⁺/H⁺ antiporter SOS1 (AtNHX7), enhances sodium export, and promotes salt tolerance (Qiu *et al.*, 2002). The *atcipk3* mutant shows a hypersensitive phenotype when treated with salt and ABA (Kim *et al.*, 2007). The *atcipk23* mutant exhibits enhanced drought tolerance by regulating leaf transpiration (Cheong *et al.*, 2007), whereas the *atcipk21* mutant presents impaired salt and osmotic tolerance (Pandey *et al.*, 2015). Similar CBL-CIPK networks are also found in other plant species including wheat. TaCIPK25 negatively regulated salt tolerance in transgenic wheat (Jin *et al.*, 2016). The over expression of the constitutively activated form of BnCIPK6 enhances salt and low-KC tolerances, as well as ABA sensitivity in *Arabidopsis* (Chen *et al.*, 2012).

ETHYLENE RESPONSE FACTORS

Cotton is native to tropical and subtropical zones and is relatively resistant to stress (Zhou *et al.*, 2016). Its long growth period makes it suffer from a variety of abiotic stresses that reduce production or quality. During the reproductive growth stage, yield reduction and fiber quality compromises are inescapable when drought stress conditions override the plant's protective mechanisms (Loka & Oosterhuis, 2014). Cotton is also susceptible to salt and cold stresses during seed germination (Shannon & Francois, 1976). Therefore, improving the comprehensive tolerance of cotton to numerous stresses for instance drought, salt, and extreme temperature is very fruitful and vital for cotton breeding. ROS are oxygen-containing substances of metabolites and their derivatives are generated through oxidation in plants directly or indirectly (Mettler *et al.*, 2011). ROS play a role in stress signal transduction, but excessive

active oxygen oxidation harms the plants. Additionally, fourteen genes were identified as responsive to stresses, such as heat, dehydration and phosphate stress. Examples of these genes are *late embryogenesis-abundant protein 2*, *late embryogenesis-abundant protein* and *dehydrin*. (Zhou *et al.*, 2016).

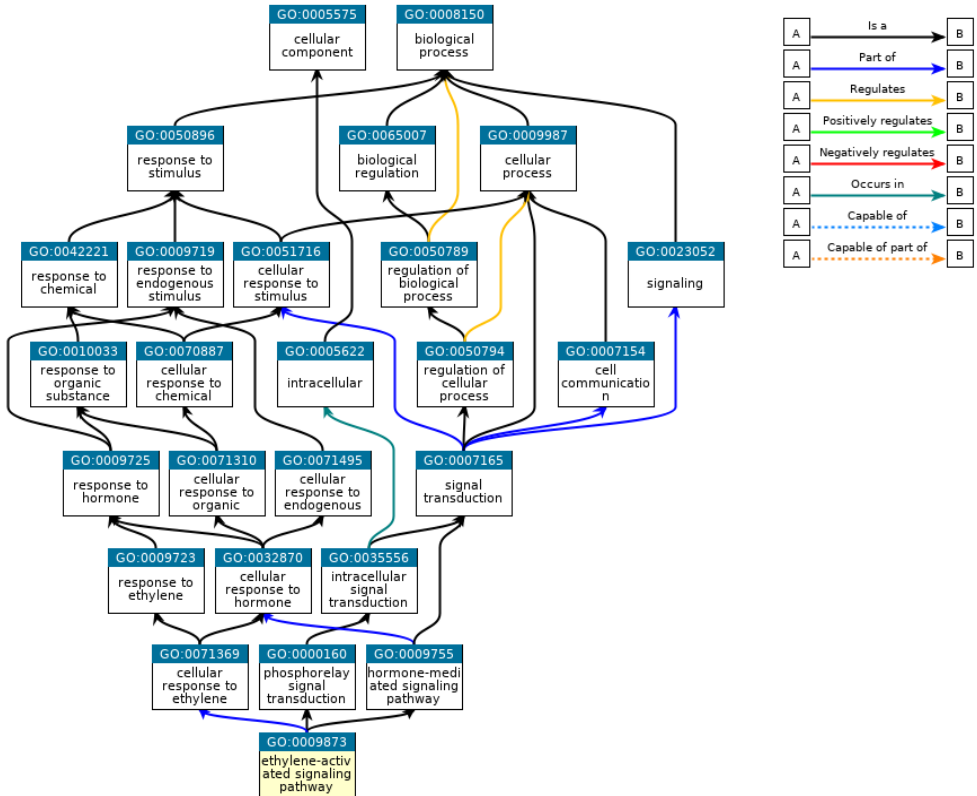


Figure 6. Ethylene-activated signaling pathway

Ethylene response factors (ERFs) are transcription factors that play crucial roles in plant immunity (Na Song *et al.*, 2019). In *Arabidopsis*, several ERFs have been identified as important regulators in *Botrytis* resistance, such as ORA59, ERF1, and RAP2.2 (Zhao *et al.*, 2012). Most ERFs are able to bind specifically to DNA sequences containing a GCC (GCC box) and/or a dehydration-responsive element/C-repeat (DRE/CRT box, A/GCCGAC) (Na Song *et al.*, 2019).

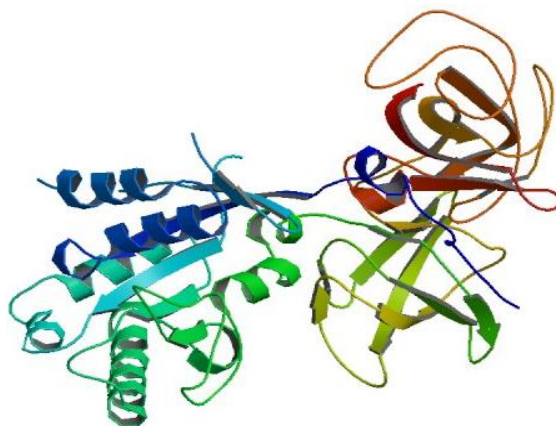


Figure 7. The 3D structure of ERF2 (Source: PDB)

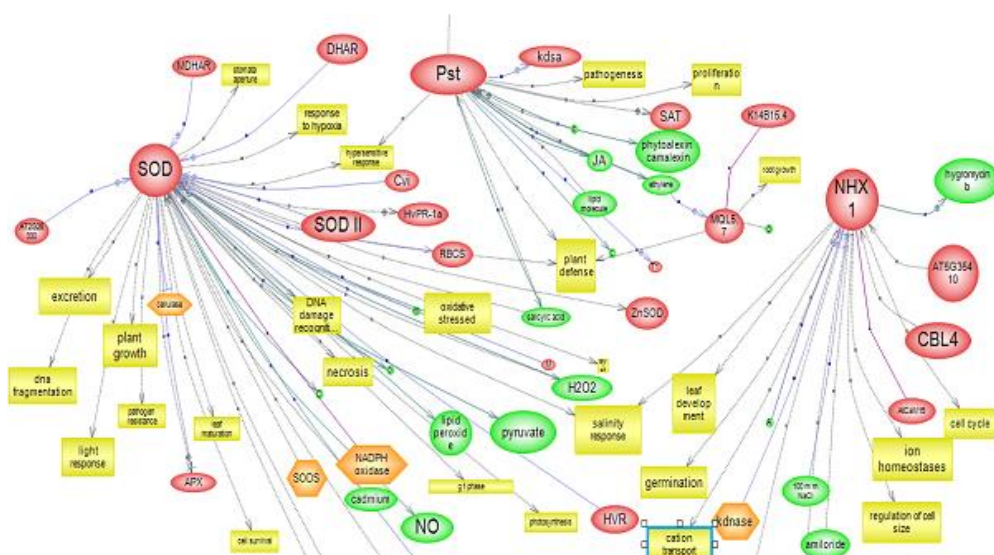


Figure 8. The relationship between genes and proteins to effect on the cotton salt tolerance stress

The analysis of the relationship between genes and proteins to effect on the cotton salt tolerance stress with pathway studio showed that, ABA-induced AtNHX1 expression was also decreased in *abi1-1* but not in *abi2-1*. Accumulation of JA after inoculation with different Pst strains. Activities of enzymes involved in scavenging of reactive oxygen species such as SOD and ascorbate peroxidase also increased during leaf maturation and showed significant fluctuations in mature leaves. After inoculation with Pst / *avrRpt2*, the accumulation of SA in *pad4* mutants was similar

to that in WT plants, in accordance with the observation that pad4 does not display an enhanced susceptibility to this pathogen strain. Although total SOD activity slightly increased in chloroplasts of salt-treated Lem plants, differentiation between SOD types revealed that only stromal Cu/ZnSOD activity increased (Mittova *et al.*, 2000).

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