Int.J.Curr.Microbiol.App.Sci (2019) *8*(1): 1991-2000

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 8 Number 01 (2019)**
Journal homepage: http://www.ijcmas.com

Review Article https://doi.org/10.20546/ijcmas.2019.801.209

Abiotic Stress Tolerance in Legumes – Critical Approaches

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A B S T R A C T

Legumes are well recognized for their nutritional and health benefits as well as for their impact in the sustainability of agricultural systems. The threatening scenario imposed by climate change highlights the need for concerted research approaches in order to develop crops that are able to cope with environmental stresses, while increasing yield and quality. During the last decade, some physiological components and molecular players underlying abiotic stress responses of a broad range of legume species have been elucidated. Plant physiology approaches provided general outlines of plant responses, identifying stress tolerance-related traits or elite cultivars. A thorough identification of candidate genes and quantitative trait loci (QTLs) associated with these traits followed (Collins *et al*, 2008). The products of stress-inducible genes which could be directly protecting against these stresses includes transcription factors, protein kinases and enzymes involved in phosphoinositide metabolism (Knight and Knight, 2001). Crosstalk among various transduction pathways under abiotic stresses ABA biosynthesis suggested connection between cold, drought, salinity and ABA signal transduction pathways (Xiao *et al*, 2013). Targeted editing of the genomes of living organisms not only permits investigations into the understanding of the fundamental basis of biological systems but also allows addressing a wide range of goals towards improving productivity and quality of crops. These advances will support the development of legumes better adapted to environmental constraints, tackling current demands on modern agriculture and food production presently exacerbated by global climate changes.

Keywords

Gene, Enzyme, Environment, QTL, Stress

Article Info

Accepted:
14 December 2018
Available Online:
10 January 2019

Introduction

Legumes (*Leguminosae* or *Fabaceae*) belong to the second most important plant family in agriculture after the *Poaceae* or grass family. They provide the largest single source of vegetable protein in human diets and livestock feed (forages), and contribute to agriculture, the environment and human health (Grant and Cooper, 2003). In developing countries, grain legumes or pulse crops represent an important component of local food consumed and are a key source of protein in the diets. They provide an input-saving and resource-conserving alternative because they fix atmospheric nitrogen, thus reducing the need for chemical fertilizers while enhancing overall crop productivity. In farming systems, legumes are often used as an inter-crop (e.g., combined with cereals) or in crop rotation resulting in a decrease in pests, diseases and weed populations, while enhancing the overall farm productivity and income of smallholder farmers. Based on these attributes, it is tempting to claim that legumes are one of the most promising components of the Climate Smart Agriculture concept (FAO, 2013). Abiotic stresses play a major role in determining crop and forage productivity (Rao, 2013), and also affects the differential distribution of the plant species across different types of environments (Chaves *et al.*, 2003). Climate change exacerbates abiotic stress on a global scale, with increased irregularity and unpredictability, and as a result, adaptation strategies need to be developed to target crops to specific environments (Beebe *et al.*, 2011). Within a single production region, a crop may encounter both excess and deficient moisture, depending upon the year, or even within the same growing season, when rainfall distribution becomes erratic. Higher temperatures will probably accelerate mineralization of soil organic matter, making soil constraints more intense (Lynch and St. Clair, 2004), and these in turn can limit root penetration and plant development, further intensifying the effects of unfavourable climate (Beebe *et al.*, 2013). Furthermore, interactions between different stress factors will likely increase damage to crop yields (Beebe, 2012; Yang *et al.*, 2013). Depending upon the extent of stress, the plants try to adapt to the changing environmental conditions. For example, under osmotic and ionic stresses, the plants must get adequate amount of water for their growth and development of reproductive structures. Therefore, under these conditions, the adaptive mechanisms should be directed to this objective. The closure of stomata limits water loss and the integrity of the photosynthetic and carbon fixation apparatus is maintained by the initiation of a series of physiological processes (Horton *et al.*, 1996). In addition to external abiotic signals, a variety of internal signals such as hormones and solutes modify plant cell growth and development. A cascade of complex events involving several interacting components required for initial recognition of signal and subsequent transduction of these signals to the physiological response is triggered. The cascade of events is called signal transduction, which normally acts through second messengers that can trigger the molecular events leading to physiological response, often by modification of gene expression.

Products of stress-inducible genes

The products of stress-inducible genes are classified into two groups (Soki *et al.*, 2004).

(i) Those which directly protect against stresses, and these are the proteins that function by protecting cells from dehydration. They include the enzymes responsible for the synthesis of various osmoprotectants like late embryogenesis abundant (LEA) proteins, antifreeze proteins, chaperones and detoxification enzymes.

(ii) (The second group of gene products includes transcription factors, protein kinases and enzymes involved in phosphoinositide metabolism. This group of gene products regulates gene expression and signal transduction pathways. Stress-inducible genes have been used to improve the stress tolerance of plants by gene transfer (Shinozaki *et al.*, 2000). The signal transduction pathways in plants under environmental stresses have been divided into three major types:

(i) osmotic/oxidative stress signalling that makes use of mitogen activated protein kinase (MAPK) modules;

(ii) (ii) Ca+2-dependent signalling that leads to activation of LEA-type genes such as dehydration responsive elements (DRE)/cold responsive sensitive transcription factors (CRT) class of genes, and

(iii) (iii) Ca+2-dependent salt overly sensitive (SOS) signalling that results in ion homeostasis.

Osmotic/oxidative stress signalling by MAPK modules

On exposure to water deficit or salinity stresses, plants lower the osmotic potential of the cell cytosol and accumulate compatible osmolytes (Kaur *et al.,* 2003). In glycophytes, the capacity for sodium compartmentalization and osmolyte biosynthesis is limited; however, an increased production of compatible osmolytes such as proline, glycine, betaine and polyols can reduce stress damage to plant cells. This is an adaptive strategy and transgenic plants with increased osmolyte production or decreased degradation showed improved salt and drought tolerance (Nanjo *et al.,* 1996; Kiyosue *et al.,* 1996), These osmolytes may protect proteins from misfolding and alleviate the toxic effects of ROS.

MAPKs are signalling modules that phosphorylate specific serine/threonine residues on the target protein substrate and regulate a variety of cellular activities. The MAPK phosphorylation system serves as a link between upstream receptors and downstream targets, thereby regulating many important cellular functions. MAPKs are activated in response to drought and other environmental stresses. MAPK genes encode polypeptides whose sequence and function are highly conserved among eukaryotes. The MAPK cascade consists of three functionally interlinked protein kinases: MAPKKK, MAPKK, and MAPK5. In this phosphorylation module, a MAPKKK is phosphorylated directly downstream of the stimulus. The activated MAPKKK then phosphorylates and activates a particular MAPKK, which in turn phosphorylates and activates a MAPK. Activated MAPK is imported into the nucleus, where it phosphorylates and activates specific downstream signalling components, such as transcription factors to induce cellular responses (Fig. 1).

Role of ABA in signalling

Abiotic stress causes an increase in ABA biosynthesis, which is then rapidly metabolized following the removal of stress (Taylor *et al.,* 2000; Liotenberg *et al.,* 1999). Many stress-responsive genes are upregulated by ABA (Rock, 2000). ABA is a regulatory molecule involved in drought stress tolerance. The main function of ABA is to regulate osmotic stress tolerance via cellular dehydration tolerance genes and to regulate plant water balance through guard cells. ABA is also induced by salt and to a lesser extent by cold stress. ABA-inducible genes have the ABA-responsive element (ABRE) (C/T) ACGTGGC in their promoters. Basic leucine zipper factors (bZIP) function in signal transduction by binding to the ABRE element in stress-inducible genes. Many bZIP factors have now been identified, including AREB binding protein. They could activate the dehydration-responsive *RD29B* gene8 (Choi *et al.,* 2000).

Ca^{+2}-dependent SOS signalling that regulates homeostasis

Restoring ion homeostasis in plants disturbed by salt stress represents a crucial response. Plant responses in countering ionic stress caused by high salinity include restricting salt intake, increased extrusion, compartmentalization and controlled longdistance transport to aerial parts. Additionally, to avoid cellular damage and nutrient deficiency, plant cells need to maintain adequate K+ nutrition and a favourable K+/Na+ ratio in the cytosol (Fig. 2). Calcium has been observed to have a protective effect under sodium stress both in solution culture and in soils that had increased calcium supply. This effect could be due to increased availability of cytosolic Ca^{+2}. Sodium stress is sensed by an unknown receptor and calcium signal serves as a second messenger. In *Arabidopsis*, genetic studies suggested that the sensor protein for this salt-induced calcium signature is the Ca^{+2}-binding protein SOS3. A loss of function mutation in this protein renders the plant hypersensitive to salt stress. Sodium extrusion is achieved by plasma-membrane localized Na+/H+ antiporter SOS1. Mutations in SOS1 rendered the mutant plants sensitive to Na. Plasma membrane vesicles from *Arabidopsis* plants have a Na+/H+ antiporter activity, which was enhanced by pretreatment with salt stress (Qui *et al.*, 2002). Whatever is the mechanism of response of plants to abiotic stresses, a transient increase in cytosolic Ca^{+2} must be coupled with downstream signalling events to mediate stress adaptation. In *Arabidopsis* salt stress signalling, the Ca^{+2} signal is perceived by the calcineurin-*b*-like Ca^{+2} sensor96 SOS3. However, unlike the calcineurin-*b* in yeast that acts through activation of a protein phosphatase, SOS3 interacts with and activates protein kinase SOS2. Thus SOS3 resembles an adapter or scaffold protein that mediates the interaction of SOS2 with other proteins such as ion transporters. This property of SOS3 was suggested due to the requirement of its myristolylation for full action in salt tolerance.

Cross talk

When stress signalling pathways are examined in the laboratory, they are usually considered in isolation from other stresses to simplify interpretation. In nature, however, the plant encounters stress combinations concurrently or separated temporally and must present an integrated response to them. In the case of phytochrome signalling, the two pathways leading to red-light-induced *CHS* and *CAB* gene expression negatively regulate flux through one another. Seemingly separate abiotic stress signalling pathways are also likely to interact in a similar manner. In addition, several abiotic stress pathways share common elements that are potential ‘nodes’ for cross-talk. Cross-talk can also occur between pathways in different organs of the plant when a systemic signal such as hydrogen peroxide moves from a stimulated cell into another tissue to elicit a response (Fig. 3).

Specificity

In spite of considerable overlap between many abiotic stress signalling pathways, there might, in some instances, be a benefit to producing specific, inducible and appropriate responses that result in a specific change suited to the particular stress conditions encountered. One advantage would be to avoid the high energy cost of producing stress-tolerance proteins, exemplified by the dwarf phenotype of plants constitutively overexpressing the frost tolerance protein DREB1A (Liu, 1998). In some cases, the signal transduction pathways triggered by different stresses are common to more than one stress type. One possible reason for this is that, under certain conditions, the two stresses cannot be distinguished from one another. Alternatively, each stress might require the same protective action (or at least some common elements). The discovery of separate sensing mechanisms for each stress would invalidate the first suggestion but the second is true in several cases. For example, dehydration protection is required in plants undergoing either freezing or drought and the production of antioxidants and scavenging enzymes (e.g. catalase and peroxidases) that protect against oxidative damage affords protection against a variety of different abiotic (and biological) stresses5. Most abiotic stresses tested have been shown to elicit rises in cytosolic free calcium levels and to involve protein phosphatases and kinases [including mitogen-activated protein kinase (MAPK) cascades]. However, are any of these components truly specific to one stress and which of them are 'nodes' at which cross-talk occurs?

Molecular mapping and breeding of physiological traits

Development of molecular markers in the 1980s proved to be a major breakthrough in the field of plant breeding as it facilitated the selection and characterization of QTLs. Molecular markers assists the construction of linkage maps which represents the position of genes within a linkage group. This dissolved the problem of creation of multimarker lines for construction of linkage map. Using QTL analysis, linkage maps can be exploited for detection of chromosomal regions governing traits controlled by either oligogenes or polygenes. In addition, the efficiency and precision of conventional breeding can be enhanced through DNA markers which have the potential to be used as molecular tool for marker-assisted selection (MAS) in plant breeding (Fig. 4).

MAS permits for the selection of genes that control traits of interest using the presence/absence of a marker. Combined with traditional phenotypic selection techniques, MAS has become an efficient, effective, reliable and cost-effective tool compared to the more conventional plant breeding methodology. The use of DNA markers in plant breeding as marker- assisted selection has unlocked a new realm in agriculture and is a component of the new discipline called 'molecular breeding'.

How linkage map constructed ?

- Production of a mapping population
- Identification of polymorphism
- Linkage analysis of markers

Single-marker analysis, simple interval mapping and composite interval mapping are the three widely-used methods for detecting QTLs (Semagn *et al.,* 2010). Single-marker analysis is the detection of QTLs associated with single markers. Whereas, simple interval mapping (SIM) instead of analyzing single markers utilizes linkage maps taking up one marker interval at a time and analyses intervals between adjacent pairs of linked markers along chromosomes simultaneously (Lander and Botstein, 1989). SIM has become the standard method for mapping QTL as use of linked markers for analysis compensates for recombination between the markers and the QTL and has been put into practice in several freely distributed software packages (Gupts *et al.,* 2010). Once the candidate gene or the markers associated with the trait of interest has been identified the next step is their utilization in the breeding programme. Here, Marker assisted backcrossing (MABC), marker assisted recurrent selection (MARS) and genome wide selection (GWS) is few important approaches which can be taken up. MABC is the process in which the QTLs are introgressed into the recipient parent (breeding lines) without linkage drags i.e. transfer of any undesirable genes from donors.

Microbiome

Due to photosynthesis, plants can produce carbohydrates, of which a considerable fraction passes to root-associated microorganisms, commonly denoted as the rhizosphere. Plant growth also requires significant quantities of nitrate, phosphate, and other minerals which are often not available in free form or in limited quantities in the soil.

This is where root-associated beneficial microbes are important partners. The best-known beneficial microbes are mycorrhizal fungi and rhizobia.

Genome editing systems

Novel genome editing tools, also referred to as genome editing with engineered nuclease (GEEN) technologies, allow cleavage and rejoining of DNA molecules in specified sites to successfully modify the hereditary material of cells. To this end, special enzymes such as restriction endonucleases and ligase can be used for cleaving and rejoining of DNA molecules in small genomes like bacterial and viral genomes. However, using restriction endonucleases and ligases, it is extremely difficult to manipulate large and complex genomes of higher organisms, including plant genomes. The problem is that the restriction endonucleases can only "target" relatively short DNA sequences. While such specificity is enough for short DNA viruses and bacteria, it is not sufficient to work with large plant genomes. The first efforts to create methods for the editing of complex genomes were associated with the designing of "artificial enzymes" as oligonucleotides (short nucleotide sequences) that could selectively bind to specific sequences in the structure of the target DNA and have chemical groups capable of cleaving DNA.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR*)*

Novel genome editing system that has emerged recently and has become widely popular is the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated (Cas) protein system with the most prominent being the CRISPR/Cas9 (based on Cas9 protein) (Fig. 5).

Fig.1 Model of MAPK cascade depicting how MAPK phosphorylation system serves as a link between upstream receptors and downstream signalling components such as transcription factors to induce cellular response

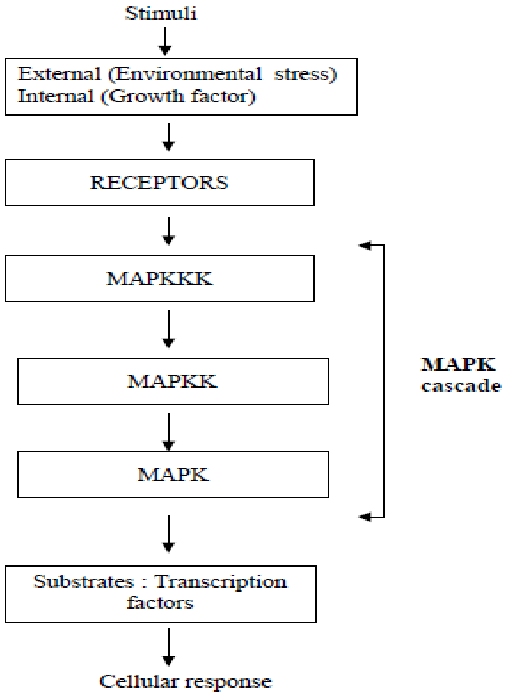


Fig.2 Pathways showing activation of SOS2 protein kinase by calcium sensor, SOS3 and regulation of ion homeostasis

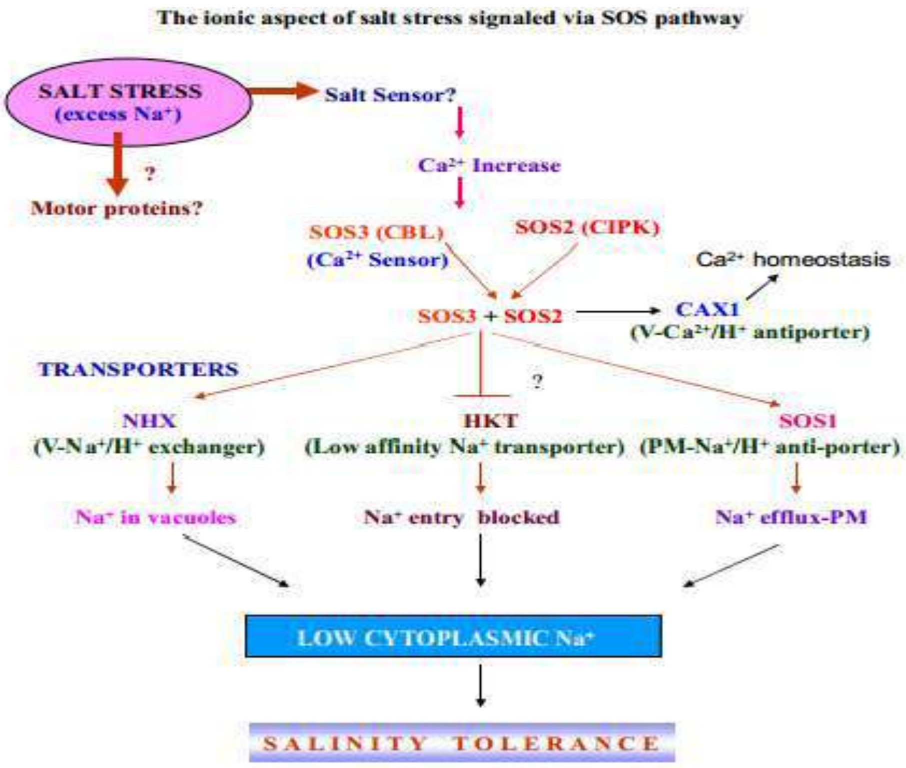


Fig.3 The DREB1 and DREB2 transcription factors, key components in cross-talk between cold and drought signalling in Arabidopsis

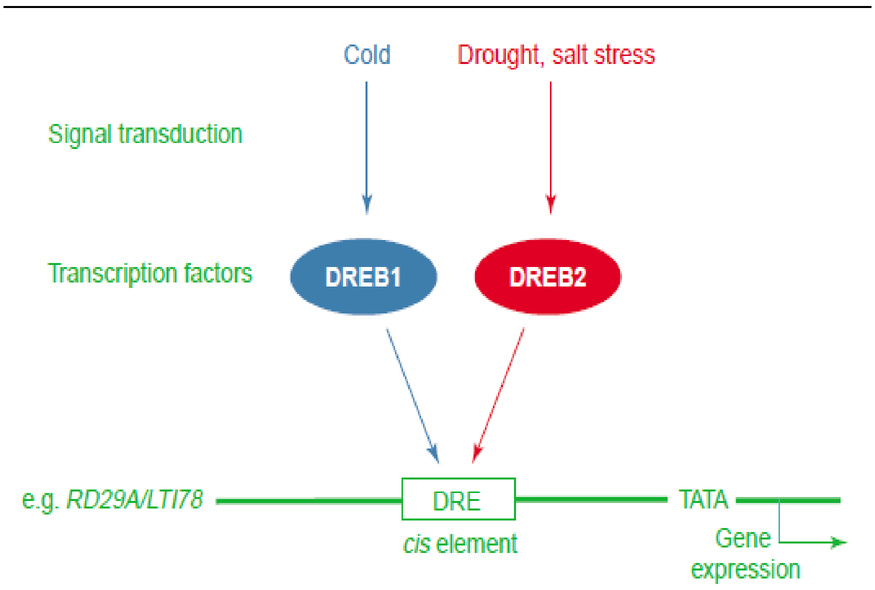


Fig.4 Construction of linkage map

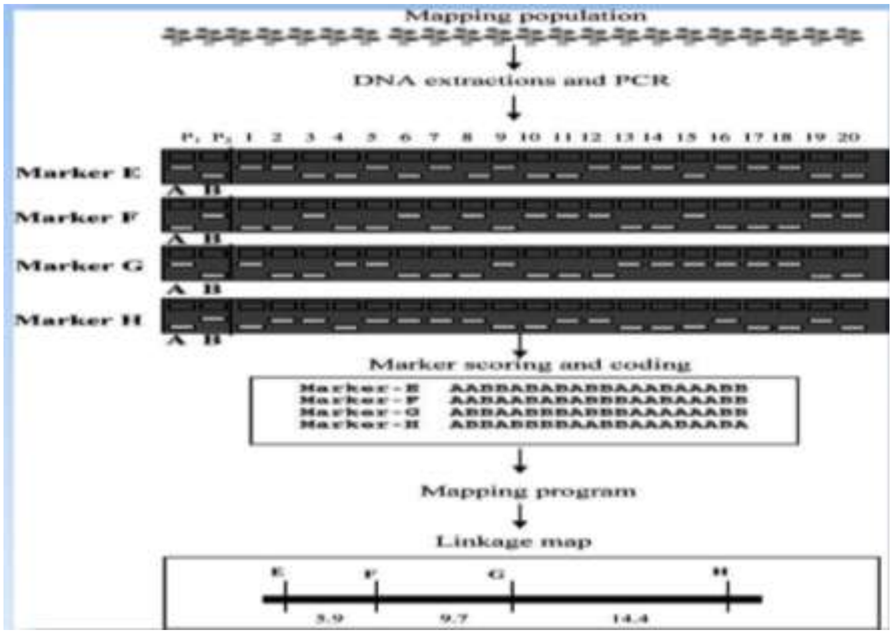
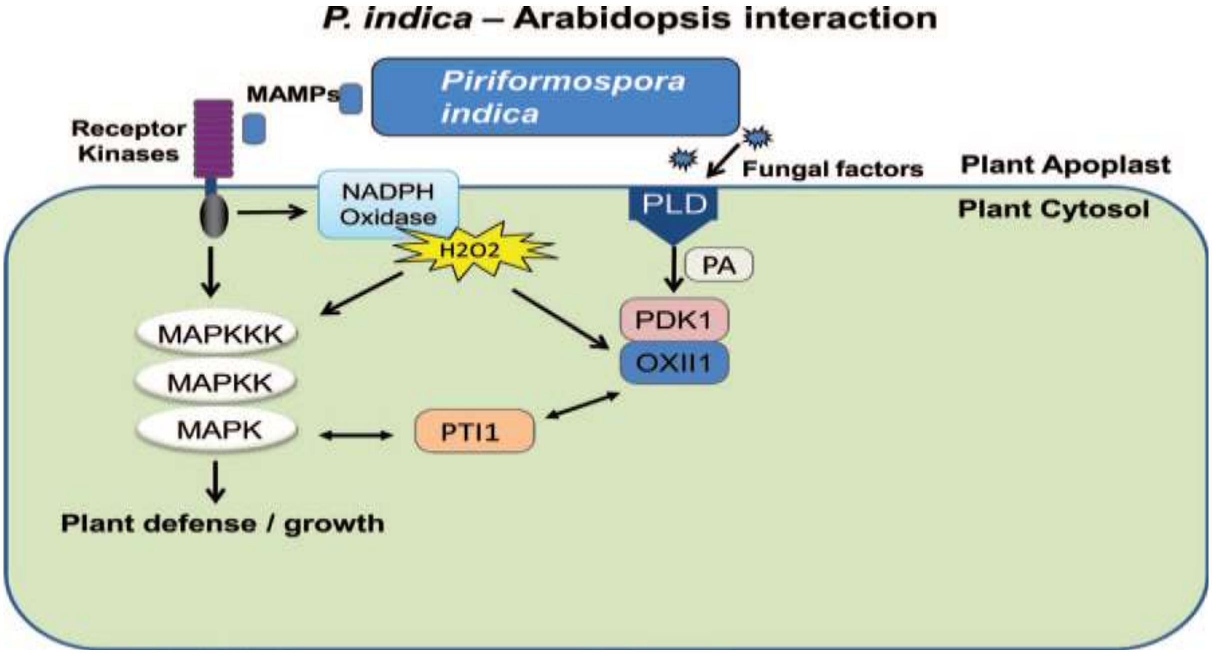


Fig.5 The beneficial fungus *Piriformospora indica* stimulates phospholipase D to synthesize phosphatidic acid (PA) which activates the protein kinases PDK1 and subsequently OXI1 and MAPKs. OXI1 and MAPKs can be activated via recognition of microbe associated molecular patterns (MAMPs) and also generate H_2O_2 to activate the OXI1–MAPK pathway. On the other hand, fungal auxin production interferes with the activation of plant defense responses, suggesting that the balance between inactivation and activation of the host defense pathways might determine whether plants go into a defense or growth mode, respectively



This is a method that utilizes adaptive bacterial and archaeal immune system, the mechanism of which relies on the presence of special sites in the bacterial genome called CRISPR loci. These loci are composed of operons encoding the Cas9 protein and a repeated array of repeat spacer sequences. The spacers in the repeat array are short fragments that are derived from foreign DNA (viral or plasmid) that have become integrated into bacterial genome following recombination.

In conclusion, the multiple stress responses on various kinds of genes and their transcribed products involved in a variety of cellular functions are important in understanding and solving the problems of drought/salt stress tolerance. Different signal transduction pathways act independently and also have a significant crosstalk among themselves. It makes their understanding under abiotic stimuli complex. Multiple genes which are affected under abiotic stresses indicate that there could not be a single marker for stress tolerance. Studying abiotic stress signalling pathways in isolation is valuable but it can be misleading because they form part of complex networks. In future, the onus will be on taking this fact into account, both intellectually and in terms of technology development. Genetic mapping through molecular markers is necessary not only for the reliable detection, mapping and estimation of gene effects of important agronomic traits, but also for further research on the structure, organization, evolution and function of the plant genome. As abiotic stress tolerance is a multi-genic trait, the identification of robust marker gene(s) conferring the traits related to enhanced tolerance might prove to be elusive. The focus of research should be given on dissecting traits that enhance adaptation to stress conditions. QTL mapping or gene discovery through linkage and association mapping, QTL cloning, candidate gene identification, functional genomics along with transcriptomics, can be used to understand crop responses to different physiological traits. Dissecting complex phenotypes into their constituting QTLs will offer a more direct access to hit valuable genetic diversity regulating the adaptive response to stress conditions (drought, salinity etc.). Candidate genes can be identified through positioning consensus QTLs with more precision through meta-QTL analysis.

References

Beebe, S. E. 2012. Common bean breeding in the tropics. *Plant Breeding Rev.* 36: 357–426.

Beebe, S., Rao, I. M., Mukankusi, C., and Buruchara, R. 2013. Improving resource use efficiency and reducing risk of common bean production in Africa, Latin America and the Caribbean. In: *Eco-Efficiency: From Vision to Reality*. pp. 117

Chaves, M. M., Maroco, J. P., and Pereira, J. S. 2003. Understanding plant responses to drought- from genes to the whole plant. *Funct. Plant Biol.* 30:239–264.

Choi, H., Hong, J., Ha, J., Kang, J. and Kim, S. 2000 ABF, a family of ABA-responsive element binding factors. *J. Biol. Chem*., 275, 1723–1730.

FAO. 2013. *Climate-Smart Agriculture Sourcebook.*

Gupta PK, Kumar J, Mir RR, Kumar A. 2010. Marker assisted selection as a component of conventional plant breeding. *Plant Breed Rev*. 33: 145-217.

Horton, P., Ruban, A. V. and Walters, R. G. 1996. Regulation of light harvesting in green plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 1996, 47, 655–684.

Kaur, S., Gupta, A. K. and Kaur, N. 2003. Effect of kinetin on starch and sucrose metabolising enzymes in salt stressed chickpea seedlings. *Biol. Plant.*. 46, 67–

72.. Kiyosue, T., Yoshiba, Y., Yamagushi-Shino-Zaki, K. and Shinozaki, K. 1996. A nuclear gene encoding mitrochondrial proline dehydrogenase, an enzyme involved in proline metabolism, is upregulated by proline but down regulated by dehydration in *Arabidopsis*. *Plant Cell*. 8: 1323–1335

Lander E, Botstein D. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*. 121(1): 185-199.

Liotenberg, S., North, H. and Marion-Poll, A. 1999. Molecular biology and regulation of abscisic acid biosynthesis in plants. *Plant Physiol.Biochem*. 37, 341–350.

Liu, Q. 1998. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low temperature- responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell*. 10: 1391–1406

Lynch, J. P., and St. Clair, S. B. 2004. Mineral stress: the missing link in understanding how global climate change will affect plants in real world soils. *Field Crops Res*. 90: 101–115.

Nanjo, T., Kobayashi, M., Yoshiba, Y., Kakubari, Y., Yamaguchi- Shihozaki, K. and Shinozaki, K. 1996. Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. *FEBS Lett*. 461, 205–210.

Qiu, Q. S., Guo, Y., Dietrich, M. A., Schumaker, K. S. and Zhu, J. K. 2002. Regulation of SOS1, a plasma membrane Na+/H+ exchanger in *Arabidopsis thaliana* by SOS2 and SOS3. *Proc. Natl. Acad. Sci*. 99, 8436–8441.

Rao, I. M., Beebe, S. E., Polania, J., Ricaurte, J., Cajiao, C., Garc´ıa, R., andRivera, M. 2013. Can tepary bean be a model for improvement of drought resistance in common bean? *African Crop Science Journal* 21: 265–281.

Rock, C. D.2000. Pathways to abscisic acid-regulated gene expression. *New Phytol*. 148, 357–396.

Seki, M. *2004. Arabidopsis* full length (RAFL) cDNA and its applications for expression profiling under abiotic stress conditions. *Journal of Experimental Bot*any 55, 213– 223

Semagn K, Bjørnstad Å, Xu Y. 2010. The genetic dissection of quantitative traits in crops. *Electronic Journal of Biotechnology*, 13(5): 119-124

Shinozaki, K. and Yamaguchi-Shinozaki, K. 2000. Molecular responses to dehydration and low temperature: Differences and crosstalk between two stress signalling pathways. *Curr. Opin. Plant Biol*. 3: 217–223.

Taylor, I. B., Burbidage, A. and Thompson, A. J. 2000. Control of abscisic acid synthesis. *Journal of Experimental Bot*any 51: 1563–1574.

Yang, Z., Rao, I.M. and Horst, W. J. 2013. Interaction of aluminium and drought stress on root growth and crop yield on acid soils. *Plant Soil* 372: 3–25.

How to cite this article:

Asmat Ara, P.A. Sofi, M.A. Rather, Munezeh Rashid and Musharib Gull. 2019. Abiotic Stress Tolerance in Legumes – Critical Approaches. *Int.J.Curr.Microbiol.App.Sci*. 8(01): 1991-2000. doi: https://doi.org/10.20546/ijcmas.2019.801.209