



Strategies For Crop Improvement Against Water And Salt Stress: An Overview

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Abstract

Agricultural production and quality are adversely affected by multiple abiotic stresses worldwide and this will be exacerbated by the deterioration of global climate. Therefore, it is urgently needed to develop tolerant cultivars to ensure yields under such adverse conditions. In this scenario, it is widely urged that strategies showed be adapted which may be used to get maximum crop stand and economic returns from abiotic stresses (drought and salinity). There are various strategies reported for abiotic stresses tolerance but two main strategies have been employed to this process. In this literature, we take five large families of TFs as examples and review the recent progress of TFs involved in plant various environmental stress responses and their potential utilization to improve multiple stress tolerance of crops in the field conditions and also explain some technological advances and different approaches of crop improvements that may help in developing cultivated stress tolerant plant.

Keyword: Drought, Salinity, Abiotic stress, Biotic stress, Salicylic acid

Introduction

1- Current scenario of world population and food insecurity

In view of different projections it is expected that, the deterioration of global climate will inevitably cause an increased frequency of drought, high or low temperature, salinization and other abiotic stresses (Chauhan *et al.*, 2015; Bohra and Sanadhya). This means that agricultural productivity will face a greater challenge in fighting against major abiotic stresses such as drought and salinity. Meanwhile, the growing world population will reach close to approximately nine billion by the year 2050 and then almost two times of current agricultural productivity is needed to feed the large population (United Nations, 2015). It is assumed by FAO (2015), that at least 10 billion people will be hungry and malnourished in the world by the end of this century. Thus to reduce the food insecurity, crop production will have to be doubled. For this goal, there is an urgent need to develop varieties that can maintain optimum

yield levels under environmentally sustainable ways. Where is the way to solve this problem? Many scholars and plant breeders (Newton *et al.*, 2011; Liu J.-H. *et al.*, 2014) worldwide have reached a consensus that breeding stress-tolerant crops with higher yields and enhanced qualities against multiple environmental stresses is an effective strategy, as well as one of the greatest challenges faced by modern agriculture. In the past few decades, a great deal of efforts has been devoted to breeding crop cultivars with drought and salinity stress-tolerant traits. There are various strategies reported for environmental stresses tolerance but two main approaches have been employed to this process. One is traditional breeding methods such as mutation breeding and wide-cross hybridization, which often brings about unpredictable results. Second is a modern transgenic approach by introducing novel exogenous genes or altering the expression levels of endogenous genes to improve stress tolerance. According to current opinion of Wang



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et al., 2016, since conventional breeding approaches have marginal success due to the complexity of stress tolerance traits, the transgenic approach is now being popularly used to develop transgenic crops tolerant to abiotic stresses. Therefore, many experts and breeders have well addressed that, deciphering the molecular mechanisms by which plants perceive and transduce stress signals to cellular machinery to initiate adaptive responses is an essential prerequisite for identification of the key genes and pathways to engineer stress-tolerant crop plants (Sanchez *et al.*, 2011).

1.1- Crop productivity as affected by abiotic stresses

In view of current situation of food insecurity, plants growing under field conditions are subjected to various environmental stresses which are responsible for crop performance (Petrovic *et al.*, 2016). Obviously, availability of agricultural land, fresh water resources, ever increasing abiotic and biotic stresses and low economic activity in plant breeding sector are the most important factors. It is estimated by many researchers that potential yield losses are 17% due to drought (Kalina *et al.*, 2016; Sanadhya *et al.*, 2013), salinity (Gupta and Huang, 2014), heavy metals (Cd stress) (Bohra *et al.*, 2015) and other abiotic stresses (Wanget *al.*, 2016).

1.2- Drought stress

According to the current climate prediction models, the 21st century agriculture is facing a daunting challenge of attaining nearly up to 70% increase in crop productivity by 2050 (Wang *et al.*, 2016b). Also, crop productivity needs to witness up to 40% increase by 2015 in view of the agricultural area being increasingly affected by environmental stresses (Pennisi, 2008). Frequent changes in climatic conditions also affect the crop yield (such as flooding after high temperature and water scarcity during drought and salinity), which led scientists to undertake research on this aspect in recent time around the world (Nakashima *et al.*, 2014a). Among the various environmental factors challenging crop production worldwide, drought stress is increasingly playing a crucial role. According to Singh and Laxmi, 2015, drought is a

meteorological term and is commonly defined as a combined interplay of reduced rainfall, decreasing ground water table, limiting water availability with rise in temperature. Singh B. *et al.*, 2015 reported that with global climatic vagaries registering escalated frequency, all over the world drought stress incidence is likely to swell beyond 20% by the end of this century, especially in Central and South American corn belt, and Central and Western European regions. Wang *et al.*, 2016 reviewed that similarly in South Asia, where more than 75% of the farmers are dependent on rainfed agriculture, a damage of about US\$ 84 billion is predicted owing to the global climate change. Most of the crops are susceptible to water deficit conditions, resulting in more than 50% yield losses (Singh B. *et al.*, 2015; Sanadhya *et al.*, 2013). It is also estimated by many plant breeders and experts that in South and Southeast Asia, drought stress causes about 40% annual loss in productivity, which results in 58% of income loss (Li *et al.*, 2015 and Wang *et al.*, 2016). Similarly, regional climate change models showed Mediterranean regions and Middle East among the 'Hot-spots' of severe water deficit, directly affecting crop unsuccessful and livestock death (Saad *iet al.*, 2015).

1.3- Salinity stress

Like water scarcity, high concentration of soluble salt is another means for cultivation and human lives. It is assumed that 7% of the world's land area is affected by salt stress (Rozema and Flower, 2008). In addition out of 230 million hectares of irrigated land, 45 million hectares (~20%) are affected by saline water or salt. Salt stress affected soil, arising either from long term natural accumulation of salt or improper land irrigation are generally occupied by excessive Na⁺ ion at exchange sites and a high concentration of carbonate/ bicarbonate ions (Zhang *et al.*, 2015). Generally they have a more than 7.5 pH and poor soil structure therefore, significantly reduced the yield of most crops.

The intensity of soil salinity stress varies from place to place. Low salinity (ECe 2-4 dS/m), moderate salinity (ECe 4-8 dS/m) and high salinity (ECe>8 dS/m) these are three different types of dry land salinity. Naturally, plants are

affected by soil salinity in two common ways dehydration and toxicity. Salt on the outside of the roots make it harder to extract water and have an immediate effect on cell growth and related metabolic process; toxicity only takes tardily when accumulation of salts inside plants is beyond a certain threshold. Furthermore Purtyet *al.*, 2008 stated that during the long processes of biological evolution and natural selection, plants have developed three strategies for the maintenance of a low cytosolic sodium concentration, compartmentation and secretion. One example is given by Shi *et al.*, (2000) the mechanism for sodium transport out of the cell is through operation of plasma membrane bound Na^+/H^+ antiports, putative plasma membranes encoded by SOS1 (Salt Overly Sensitive 1) gene in *Arabidopsis thaliana*.

2- Objectives of this review

In total, drought and salt affected soil cover more than 10% of arable land which result in rapid increase in desertification and salinization world-wide. Ultimately, average yield of major crops reduced by more than 50% (Bray *et al.*, 2000). For this purpose, there is an increasing demand for new plant cultivars that have a potential for higher yield under drought and salinity. Although there is a reasonable consensus of various strategies of enhancing degree of stress tolerance in crops for example, screening for stress tolerance individual, identification of promising traits conferring stress tolerance in plants, and development of stress tolerant plant through breeding or genetic engineering, there is still no consensus on physiological traits that confer salt tolerance in plants.

According to current view of plant breeders and experts, in this review discussed different strategies have been employed to improve plants water use efficiency, drought and salinity tolerance and also explain some technological advances and different approaches that may help in developing cultivated stress tolerant plant.

In this review, first highlighted the different strategies have been employed to improve plants water use efficiency, drought and salinity tolerance. Second water efficiency based on

some recent advances in basic plant biology has been mentioned in this chapter that will eventually help plant breeders to develop stress tolerance cultivars of different crops.

3- Strategies for improving crops against drought and salinity

According to Vaurasi and Kant (2016), mostly environmental stresses are created by combination of water deficiency and sodium toxicity due to sodium, chloride ions and create negative effects on crop productivity. Plant water stress tolerance involves changes at whole plant, tissue, physiological and molecular level. Naturally, plants adapted to arid environments possess inherent drought escape or drought avoidance mechanisms and can be grown in drought stressed areas. Drought escape is a phenological phenomenon of plants is attained through a shortened life cycle or growing season, allowing plants to reproduce before the environment becomes dry. Araus *et al.*, 2008 stated that flowering time is an important trait related to drought adaptation, whereas sort life cycle can lead to drought escape. However, it is occurs when phenological development is successfully matched with periods of soil moisture availability, where the growing season is shorter and terminal drought stress predominates. Drought avoidance consists of mechanisms that reduce water loss from plant, due to deeper and dense root system, greater root penetration ability, higher stomatal conductance and higher cultivar resistance to prevent water loss, higher pre dawn leaf water potential and avoid leaf rolling for longer intervals (Peng *et al.*, 2009). In naturally dry habitat, some plant species such as agave (*Agave deserti*) and cactus species store water in their buds, stems or leaves during drought stress period. These plants utilize this stored water under water deficit conditions. On the other hand, drought tolerance refers to the extent to which plants maintain their metabolic function when leaf water potential is notably low. If we analyze all these traits for drought tolerance, it appears that this tolerance in crops usually depends on one or more of the following points include avoidance (1) the capacity of plant roots to extract water from soil (2) osmotic

capacity (3) water use efficiency (Reynolds *et al.*, 2005). These crop plants or wild plants having these traits are capable to tolerate drought stress and thus they can be grown on drought affected areas.

3.1- Selection and breeding for drought tolerance

The classical strategy to obtain crops more tolerance to water stress is through selection and breeding. In this approach the genetic variability underlying stress resilience is recognized by screening germplasm collections. Although some success in improving drought stress tolerance has been obtained so far using selection and conventional breeding, as review by Ashraf (2010); Varshney *et al.*, 2011. The screening method under natural drought stress conditions in the target environments is difficult because of the irregular and erratic drought response. But it is under controlled stress environments and rain out shelter is more manageable. According to Venuprasad *et al.*, 2009 the selection method response in the target population of environments under natural stress can be considered a correlated response to selection in the managed stress environment. On the other hand, classical breeding is an effective approach for developing drought tolerance. Which relies upon multi location testing of progenies in environments representing a random selection of the variation in water stress in target environment (Babu *et al.*, 2003). Under of this strategy involves selection for putative drought adaptive secondary traits, either alone or as part of a selection index. It is also well proved by many breeders and researchers that various agronomic, physiological and biochemical selection criteria for drought tolerance are being employed to select drought tolerant plans, such as seed yield, harvest index, fresh and dry weight of shoot, leaf water potential osmotic adjustment, accumulation of compatible solutes, water use efficiency, stomatal conductance and also chlorophyll fluorescence (Ashraf *et al.*, 2010).

3.2- Molecular and functional genomics approaches

Drought tolerance in plants is identified as a quantitative trait conditioned by many genes through various pathways. The regions of chromosomes or the Loci controlling traits are called Quantitative Trait Loci (QTL). When water deficit condition is perceived by the plant, expression patterns are changed in genes. Including these involved in water transport, osmotic adjustment, damage repair and oxidative stress (Zhang *et al.*, 2014). In recent year, various laboratory and field studies have shown that transgenic expression of some of the stress regulated genes results in increased tolerance to osmotic stress or other stresses. Furthermore, the transgenic approaches are currently the mainstream method to bioengineer water stress tolerance in crop plants. Many studies have attempted to elucidate the molecular mechanism of drought tolerance in plants using various molecular marker techniques such as RFLP, RAPD, AFLP, and SNPs. Hussain, 2006 reviewed that use of these marker to identify QTLs for physiological traits responsible for stress tolerance has helped to identify some potential sub traits for drought tolerance. The molecular marker for a trait QTLs are linked to specific sub traits of drought tolerance, it would be possible to transfer these various traits into other adaptive cultivars with various agronomic backgrounds under specific targeted environments by the using of marker assisted breeding approach.

Therefore, identification of areas of a genome that have a major influence on water stress tolerance or QTLs for water stress tolerance traits could allow to identify the particular gene for drought tolerance. Further Hussain (2006) addressed that with the help of molecular marker assisted selection (MAS) seems to be a more promising approach because it enabled us to dissect quantitative traits into their single genetic components thereby helping in selecting and breeding plants that are tolerant to drought stress.

Through the next generation sequencing technology, (Yu *et al.*, 2012) conducted a pilot genome wide analysis of gene expression in plant leaves during a drought tolerant responsive genes were well recognized, among

which 37 were transcription factors. 28 genes were involved in water stress and also in osmo-sensing responsive pathways. Similarly Dong *et al.*, 2012 identified 308 down regulated genes and 248 up-regulated genes water stress conditions in *Sinapsisalba*. Differentially expressed gene was found to be involved in cell division and catalytic and metabolic processes, as revealed with the help of gene ontology (GO) analysis.

3.3- The key role of regulatory sequences to express transgenes

The transgenic approaches are currently the mainstream method to bioengineer drought and salinity tolerance in crop plants. However, enhanced expression of these genes is frequently associated with retarded growth and thus may limit its practical applications. Arising from breeding or bioengineering, the next generation of drought and salinity tolerant crop plants requires better understanding of molecular and genetic basis of drought and salinity resistance (Xiao *et al.*, 2012). Under drought stress condition, the majority of transgenic plants tolerant have been obtained through the use of strong constitutive promoters such as Cauliflower mosaic virus 35S (CaMV35S) (Odell *et al.*, 1985) or plant promoters like ubiquitin (Holtorf *et al.*, 1995) actin (McElroy *et al.*, 1990) and cytochrome (Jang *et al.*, 2002). Wang *et al.*, 2003 categorized these genes into 3 major groups (1) genes involved in signaling pathways and also in transcriptional control (2) genes involved in protection of cell membranes and proteins e.g. heat shock proteins (Hsps and chaperones, late embryogenesis abundant (LEA), proteins, osmo protectants and free radical scavengers. Last point (3) genes involved in water, ion uptake and transport such as aquaporins and ion transports. Further, Vinocur and Altman (2005) included one more group i.e., genes involved in metabolism. Under this included group, they discussed the role of osmo protectants in stress tolerance such as- amino acid, amines, proline, sugars, sugar alcohols, glycine betaine. On the other hand, a similar strategy consists in expressing a gene of interest in organs such as-

tissue or cell specific manner. Both target tissues for such gene mix- expression approach for drought stress tolerance can be envisaged: these are roots and guard cells. Whereas roots were traditionally difficult to study. But recent researches have made the manipulation of root architecture and physiology a feasible strategy to produce crops with better production. There are so many microarray studies of root gene expression responses to environmental stresses, such as high salinity in *Arabidopsis thaliana* (Jiang and Deyholos, 2006) or tomato (Ouyang *et al.*, 2007) or drought in maize plants (Ouyang *et al.*, 2007). Constitute a source of information to identify new root- specific promoters to allow root gene expression modulated by abiotic stresses.

4- Strategies for improving crop efficiency against salinity

There are following reasons for salt tolerance evaluation because salinity tolerance is very complex trait-

- (a) Salt tolerance can only be evaluated under stress condition, which can influence multiple physiological responses of the plant.
- (b) It is also a quantitative trait which requires an efficient and effective means to quantify the tolerance level.
- (c) The "salt" in " salt stress" is often ambiguous as it can be different minerals salt, such as-NaCl, MgCl₂, and CaCl₂; uptake and assimilation of mineral nutrients including Na⁺ and Cl⁻ ions are genetically controlled and can be manipulated (munns *et al.*, 2006).
- (d) Other physiological stresses (water scarcity, extreme acidity and alkalinity) are often associated with salt stressed plants, which also make this trait more difficult to evaluate or study. Thus, efficient and effective methods, including plant culture under high soil salinity conditions, trait detection and grading to evaluate the salt tolerance degree. It should be employed in the

primary stage of study because we cannot ignore the harm of other ions.

Although all biological and bio physiological strategies for crop improvement against salinity are same as for drought tolerance strategies such as - screening and selection, molecular and functional genomics approaches and also transgenic approach. According to above mentioned literature, the adverse effect of salinity on plant growth is mainly due to its toxic and osmotic stress. Therefore, major focus is on selective ion accumulation or exclusion, control of Na⁺ ion uptake and its distribution within the plant, compartmentation of ions at cellular or at whole plant level (Munns and Tester 2008).

4.1- Screening and selection for salt tolerance

Recently, there has been much interest in the development of salt tolerance crop varieties. As is well documented from the literature on the existence of inter and intra specific genetic variability for salinity tolerance. It could be exploited judiciously for screening and breeding for higher salt tolerance for example- Wu *et al.*, 2012 observed that *Thellungiella salsuginea* grows in an environment of saline water with a life cycle and also genome background similar to its close relative *A. thaliana*, therefore this species can be used as a model plant for conducting salt tolerance research. Furthermore, reviewed by Orsini *et al.*, 2010, He collected 11 wild relatives of *A. thaliana* from different environments and identified four categories of tolerance (1) halophytic (2) high tolerant (3) moderately tolerant (4) marginally better than *A. thaliana* based on the overall growth performance.

In another study, while screening 100 genotypes of sorghum at the seedling stage, Krishnamurti *et al.*, 2007 found 46 genotypes as salt tolerance, that were further confirmed as salt tolerant at the later growth stage using Na⁺ exclusion as a potential selection criterion. Zhang *et al.*, 2014 addressed that salt tolerant lines in *B. juncea* crops have been screened by various methods. Some varieties (lines) were approved as salinity tolerant genotypes and used in some studies (Ashraf and McNeill, 2004). It is also important that the performance

of the screened germplasm in real salinity fields be used to confirm their salt tolerance ability in plants. Munns *et al.*, 2002 reported that screening for a trait associated with different mechanisms of salinity tolerance is a preferable method, as measuring the effect of salt on biomass or production of a large number of lines is not feasible. Recently, the knowledge of physiological mechanisms of salt tolerance should be used to identify traits that can be employed for rapid and cost effective selection techniques. Thus there is an urgent need to develop an effective evaluation approach for screening salt tolerance genotypes, which should be reliable, quick, easy, practical and economic.

4.2- Conventional breeding and biotechnological approaches

The classical strategies to obtain crops more tolerant to salinity are through conventional breeding. In this tolerance plants have closed genetic mapping to molecular characterization of salt included genes. Biotechnological approaches such as molecular breeding and genetic engineering offer the possibility to receive better results in a shorter time whereas, genetic engineering may also allow to overcome the reproductive barrier among different plant species. To identify the less obvious genetic networks that respond to salt stress, more straight forward and sensitive methods are necessary. Bruce *et al.*, 2002 addressed that the advent of whole genomics and related technologies to providing the necessary tools to identify key genes that respond to salt stress and relating their regulation to adaptive events occurring during stress conditions. Since, transcription factors (TFs) are good candidates for genetic engineering to breed stress tolerant crop because of their role as master regulators of various stress responsive genes. Various TFs belonging to families AP2/EREBP, MYB, WRKY, NAC, bZIP have been found to be involved in multiple environmental stresses and some TF genes have also been engineered to enhance stress tolerance in model and crop plants as shown in figure 1.

On the other hand, molecular breeding combines the processes of genetic improvement

of conventional breeding with DNA markers. Cominelli *et al.*, 2013 reviewed that molecular breeding increases the genetic gain by enhancing the selection efficiency and reducing the length of breeding cycles. With the help of

these strategies few important results have been obtained in the field of high soil salinity tolerance. The genome sequences are also available for different crops including species with large and complex genomes.

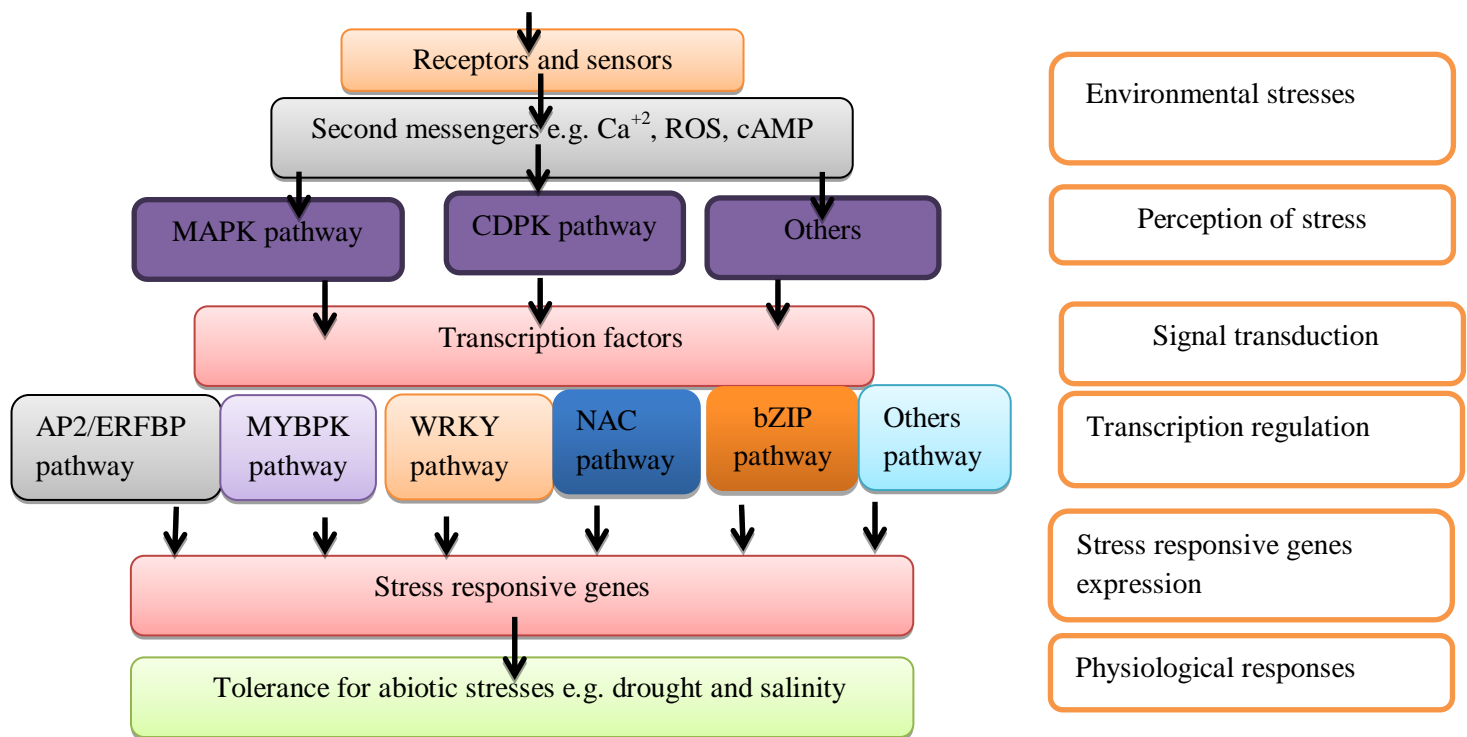


Figure 1- Generic signaling pathway involved in plant environmental stress responsive
 4.3- Molecular and functional genomics approaches

As proved earlier that salinity tolerance in plants is determined by a number of physiological and biochemical traits (Gupta and Huang, 2014). Ashraf *et al.*, 2010 well evident that successful screening and selection of salt tolerance cultivars in conventional breeding program is limited by the significance influence of environmental factors. In view of this arguments, it is suggested by Yu *et al.*, 2016 that to identify the molecular markers tightly linked to the genes governing salinity tolerance and could be used to select plants in segregating population because molecular markers are unaffected by the environmental stresses. Thus, with the help of QTLs has improved the efficiency of selection, in particular for those traits that are controlled by

several genes and are highly influenced by the factors of environments. However, most of the mapped QTLs controlling the salt tolerance were different from each other, since the mapping populations were different and the investigated traits were not all the same as reviewed by Zhang *et al.*, 2015. Although, Munns (2008) also reported that QTLs for salt tolerance have been identified in a number of potential cereals crop such as rice, barley and wheat, robust markers that can be used across a range of germplasm are very few. On the other hand, some QTLs for different traits were overlapped: for example, QTLs for radicle length and chromosome on chromosome 1 and 3, indicating that these two loci may contain genes controlling radical length

and those for salinity tolerance exhibited by root growth. It is also reported by DeRose-Wilson and Gaut, 2011 those QTLs for PC (percent cotyledons fully emerged at 21 days) and PG (percent germination) were also overlapped on chromosome 1,3 and 4, in consistence with results of phenotype assessments, in which PC and PG were highly correlated across 96 accessions ($r^2=0.69$) of *A. thaliana*.

5- Conclusion and future prospective

Among various strategies, biotechnological approaches allowed the generation of different crops tolerant to water stress and high soil salinity stresses but the transfer of these achievements to agriculture is still bagging behind. Under environmental stresses, plants are often subjected to various stresses, varying intensity, duration and differentially affecting plant performance depending on the plant developmental stages. Therefore, stress tolerance trait should be evaluated in more realistic conditions, mimicking what really happens in the agricultural land. In order to transgenic approach can be integrated with conventional and molecular breeding but also with more innovative strategies, taking advantage of the most recent technologies in the field of plant research. With the advancement in functional genomics, it is possible to identify key genes and their immediate functions at cellular as well as at whole plant level. After a series of studies, detailed analysis of underlying physiological and molecular mechanisms for drought and salinity tolerance using functional genomics is most important area of future research, which will eventually assist in developing transgenic plants for stress tolerance. Therefore, the improvement in environmental stress (drought and salinity) tolerance in agricultural crops can only be achieved practically by combining traditional and molecular breeding approaches.

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