

Chapter 2

Recent Advances in Rapid and Sensitive Screening For Abiotic Stress Tolerance

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Abstract Traditionally, screening for abiotic stress tolerance at field level was based on necrosis scores and shoot biomass reduction on stress exposure, relative to unstressed controls. However, such a measure of tolerance screening is laborious, destructive, and time consuming, and results are subjected to environmental variation. Recently, noninvasive, high-throughput screening techniques have been developed for screening abiotic stress tolerance in crops. In this direction, some physiological, biochemical, and/or molecular indicators/markers have been identified for rapid and sensitive indirect screening of germplasm. Physiological markers like membrane damage based on electrolyte leakage, stomatal conductance, chlorophyll content and so on are currently available. In addition, quick and sensitive screening in crop plants is possible with biochemical markers like status of reactive oxygen species and oxidative damage to biological macromolecules like lipids, proteins, and nucleic acids. Identification of molecular markers associated with the tolerance response has also made rapid and sensitive indirect selection possible in a few crop species. Thus, development of such methods is valuable in breeding for abiotic stress tolerance in plants.

Keywords Abiotic stresses · Screening · Indirect selection · Physio-biochemical and molecular markers

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1 Introduction

Plant growth, productivity, and distribution are greatly affected by environmental stresses such as high and low temperature, drought, and high salinity. In response to abiotic stresses, plants undergo a variety of changes at the molecular level (gene expression) leading to physiological adaptation (Zheng et al. 2010; Patade et al. 2011a, b; Mantri et al. 2012). Salinity and drought are the major abiotic stresses which severely affect yield and quality in many regions of the world endangering the food security. The situation has become more serious with concerns of global climate change. Therefore, studies on abiotic stress tolerance have become one of the main areas of research worldwide. In this direction, efforts are being made to breed tolerant varieties using conventional breeding and contemporary biotechnological tools. Recent advances in this area include unraveling the physiological, biochemical, and molecular mechanism of abiotic stress tolerance and corresponding development of tolerant cultivars through transgenic technology or molecular breeding (Ashraf 2010; Patade et al. 2011a, b, c; Niu et al. 2012). Desired tolerant genotypes/varieties so developed need to be screened at laboratory as well as field levels for functional validation. Numerous physio-biochemical indicators for tolerance screening have been suggested (Chen et al. 2007; Smethurst et al. 2009). In addition, indirect selection using molecular markers linked to desired loci is being deployed for accelerating the production of stress-tolerant varieties (Ribaut and Ragot 2007; Wei et al. 2009; Ashraf and Foolad 2013). Here, advancement in various abiotic stress tolerance-screening techniques is discussed in light of recent publications in this area.

2 Field Screening For Abiotic Stress Tolerance Based On Conventional Techniques

Earlier, screening the breeding population/developed varieties for abiotic stress tolerance at field level was based on visual symptoms and/or shoot/root biomass reduction on exposure to the stress(es). Further, to quantitate the tolerance level, necrosis scores on stress exposure relative to unstressed controls were proposed for tolerance screening (Coram et al. 2007; Mantri et al. 2010a). Moses et al. (2008) screened 600 accessions of chickpea (*Cicer arietinum* L.) for salt tolerance under greenhouse conditions based on necrosis scores and shoot biomass reduction compared to unstressed controls at harvest stage. The results indicated wide variation in salinity tolerance determined by both measures. In addition, increase in grain yield on exposure to stress has been commonly used to screen for tolerance in the field. However, these measures of screening for tolerance are laborious, destructive, and time consuming, and the results are subject to environmental variation. Therefore, nondestructive biomass measurement techniques based on satellite remote sensing have been recently developed (Heiskanen 2006; Masuka et al. 2012).

3 Indirect Screening for Abiotic Stress Tolerance in Laboratory and Field

Indirect screening of breeding population/varieties for stress tolerance may be performed at the laboratory level (in vitro or in greenhouse) or in open field conditions. Various physio-biochemical indicators for tolerance screening have been suggested including photosynthesis rate based on CO₂ assimilation, stomatal conductance, chlorophyll fluorescence, and various electrophysiological characteristics (Munns and James 2003; Chen et al. 2007; Smethurst et al. 2009). Plant phenotyping greatly helps the genetic analysis of abiotic stress tolerance to further elucidate the stress tolerance mechanisms. However, conventional methods of plant phenotyping are laborious and destructive as compared to the recently developed high-throughput, nondestructive imaging technologies (reviewed by Roy et al. 2011; Yang et al. 2013). The recent phenotyping techniques, being nondestructive, enable acquiring quantitative data on plant growth, health, and water use under abiotic stress by taking multiple images of the same plant at different time points and at different wavelengths (Morison et al. 2008; Jones et al. 2009). Therefore, these technologies are being routinely applied to quantify traits related to salt and drought tolerance in a number of crop plants (Berger et al. 2010; Rajendran et al. 2009; White et al. 2012).

3.1 *In Vitro* Tolerance Screening

The major advantages of in vitro screening are: controlled environment, large population that can be handled in a lesser space within a short span of time, and the plant material being kept disease free (Patade et al. 2008; Patade and Suprasanna 2008). Patade and Suprasanna (2009) in vitro screened radiation-induced sugarcane cv. Co 86032 mutants for salt tolerance by exposing embryogenic calli to different salt (NaCl) concentrations. The screening was performed based on relative growth rate, cell viability, and membrane damage. The results suggested in vitro mutagenesis–selection as a powerful tool for efficient screening for salt tolerance in sugarcane to enable commercial cultivation in saline areas. Recently, Sorkheh et al. (2011) screened wild almond species based on root growth characteristics using sorbitol and polyethylene glycol (PEG) as an osmoticum and concluded in vitro screening as an effective system for screening drought tolerance.

Screening for aluminum (Al) tolerance using nutrient solution culture is the most common method as it allows nondestructive measurement of tolerance, and provides easy access to root systems and tight control over nutrient availability and pH (reviewed by Wang et al. 2006; Arenhart et al. 2013). In nutrient solution culture, root length measurement and root staining are the major criteria for evaluation of Al tolerance.

3.2 *Physiological and Biochemical Markers For Tolerance Screening*

Chlorophyll fluorescence and thermal imaging are well-established, powerful, non-destructive, and rapid techniques for detecting and diagnosing plant stresses in the field by providing information on both stomatal and photosynthesis-related parameters, the key factors that determine plant yield (West et al. 2005). Studies comparing the chlorophyll fluorescence with the conventional techniques indicated that in vivo chlorophyll fluorescence can be a useful tool for screening biotic and abiotic stress tolerance in various crops (Matous et al. 2006; Chaerle et al. 2007a; Mishra et al. 2011). Chaerle et al. (2007b) reviewed the relative advantages and disadvantages of thermal and chlorophyll fluorescence imaging for the study of spatial and temporal heterogeneity of leaf transpiration and photosynthetic performance. The combined thermal and chlorophyll fluorescence imaging can highlight presymptomatic responses before appearing in visual spectrum images and thus may increase the power of disease diagnosis and the potential for screening of stress-tolerant genotypes. Each thermal and spectral sensor detects a different basic physiological response; therefore, combining information generated from a broad range of sensors may enhance sensitivity of diagnosing and quantifying different stresses. Jones and Schofield (2008) reviewed the potential applications of multi-sensor imaging in diagnosis and quantification of both abiotic and biotic stresses in plants. The multi-sensor imaging for stress diagnosis and monitoring may be a simple combination of thermal and reflectance sensors, or visible reflectance and fluorescence sensors, through to combined fluorescence, reflectance, and thermal imaging sensors. Jiang et al. (2006) screened barley genotypes for salinity tolerance by measuring net photosynthesis, stomatal conductance, gas exchange, chlorophyll fluorescence parameters, dry matter, and carbon isotope discrimination in saline conditions relative to control plants. Among the various attributes measured, stomatal conductance was the best to screen in barley genotypes for absolute performance on exposure to salinity stress. Salinity susceptibility indices (SSI) used to estimate the relative salinity tolerance also varied considerably between the parameters and could not provide useful information on performance under saline conditions. However, according to Chen et al. (2007), only the chlorophyll fluorescence method has often been the most attractive tool for rapid and sensitive screening with fully automated fluorimeters. Positive correlation of grain carbon isotope discrimination under post-anthesis drought stress with economic yield has been established in wheat (*Triticum aestivum* L.); therefore, these indices may be used as indirect selection criteria for wheat grown under stress environments (Monneveux et al. 2005, 2006; Zhu et al. 2009). However, a study conducted to investigate the relationships between seed cotton yield and carbon isotope discrimination concluded that the leaf physiological traits could not be reliably used for yield selection in cotton (*Gossypium hirsutum* L.) due to site-specific effects on the yield–physiological trait relationship (Tsiatas et al. 2008).

El-Shabrawi et al. (2010), based on biochemical analysis on redox homeostasis and antioxidant defense in salt-tolerant and salt-sensitive rice cultivars, suggested that the status of reactive oxygen species and ascorbate and glutathione homeostasis can serve as quick and sensitive biomarkers for screening salt tolerance in crop plants. A recent study using salt-tolerant and salt-sensitive genotypes (Gomathi and Rakkiyapan 2011) identified reliable indices, viz. higher membrane stability, and maintenance of high chlorophyll fluorescence ratio (fv/fm) and lower lipid peroxidation for salt tolerance screening in sugarcane at various stages of crop growth. Biochemical analyses in response to root zone salinity at various growth stages in salt-tolerant and salt-sensitive genotypes indicated that lower lipid peroxidation and higher phenolic contents were associated with tolerance response in hexaploid bread wheat (Ashraf et al. 2010); hence, they may be used for tolerance screening. Thakur (2004) screened fruit crops for drought tolerance based on indices, namely xylem water potential, relative water content, chlorophyll stability index, drought injury index, and rapid test for drought tolerance. The results indicated that fruit crops may be rapidly screened for drought tolerance based on these simple, cost-effective, and reliable indices at all phenological phases. Siddiqi et al. (2009) screened ten accessions of safflower (*Carthamus tinctorius* L.) for salt tolerance based on biomass (shoot and root dry mass) and other physio-biochemical parameters, viz., photosynthesis, transpiration, stomatal conductance, and chlorophyll *a* and *b* at the vegetative stage. Positive association with biomass was observed only for net photosynthetic rate among the various parameters examined. Hence, it may be used as an effectual indicator of salinity tolerance in safflower.

Mishra et al. (2011) studied cold tolerance based on electrolyte leakage and chlorophyll fluorescence in *Arabidopsis* accessions. The results indicated easy applicability of the fluorescence technique over the conventional electrolyte leakage methods. Further, it can be employed to detect cold tolerance at mild subzero temperatures by including the resolving power of several fluorescence features, thus avoiding plant freezing to the largely damaging temperatures of around -15°C for screening. Root length measurement and root staining are the major criteria for evaluation of Al tolerance. Stodart et al. (2007) screened 250 accessions of bread wheat (*Triticum aestivum* L.) for Al tolerance based on hematoxylin staining of root tips and root regrowth measurement. The accessions classified as tolerant based on the root tip staining test also exhibited increased root length on exposure to Al. Thus, the results indicated hematoxylin staining of root tips as a simple technique to screen large number of accessions for Al tolerance. Measurement of change in stomatal conductance is a reliable and useful screening technique for abiotic stress tolerance. Results of the experiment in durum wheat indicated that stomatal conductance could be a means of screening for osmotic stress tolerance in cereals (Rahnama et al. 2010). Results of the high-temperature screening of common bean genotypes in greenhouses and fields indicated that a superior heat-tolerant genotype may be identified based on geometric mean and stress tolerance index (Porch 2006).

3.3 *Molecular Marker-Assisted Indirect Selection For Stress Tolerance*

Molecular marker-assisted selection (MAS) is desirable, if visual selection is difficult and cost/time ineffective. MAS is a strategy for accelerating the crop breeding for biotic and abiotic stress tolerance (Ribaut and Ragot 2007; Wei et al. 2009; Mantri et al. 2010b). Identification of molecular markers linked to the desired traits has made it possible to examine their usefulness in crop improvement (Ashraf 2010; Delannay et al. 2012). Indirect selection using molecular markers linked to desired loci is highly regarded as an efficient selection tool. In the recent past, efforts were made to develop molecular markers such as restriction fragment length polymorphism (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphism (AFLPs), and simple sequence repeats (SSRs) for efficient MAS in breeding programs. Lang et al. (2008) investigated the genetic basis for salinity tolerance using SSR markers in rice and tagged the locus imparting salt tolerance (RM223) for marker-assisted salt tolerance screening. Huseynova and Rustamova (2010) screened drought-tolerant, semi-tolerant, and sensitive wheat genotypes using RAPD primers associated with drought tolerance. The results indicated amplification of a specific product of 920 bp only in the drought-tolerant and semi-tolerant (absent in sensitive) genotypes. This molecular marker may be useful in screening for drought tolerance in wheat. Wang et al. (2006) reviewed the identification of molecular markers linked with the Al tolerance gene(s) in barley (*Hordeum vulgare* L.). Identification of tightly linked RFLP (Tang et al. 2000), SSR (Raman et al. 2003, Wang et al. 2007), and AFLP (Raman et al. 2002) markers to a major Al tolerance locus has enabled fast-tracking of the tolerance alleles in different breeding programs (Wang et al. 2006). However, these markers are usually not developed from the target genes. On the contrary, functional markers (FM) are usually designed based on polymorphism for transcribed regions of the functional target genes. Therefore, these markers completely correlate with gene function and may facilitate accurate selection of target genes (Andersen and Lübberstedt 2003; Wei et al. 2009).

Indirect selection based on molecular markers has been mostly limited to improving traits with marker-assisted backcrossing (MABC) of major genes (Nataraj-kumar et al. 2010). Improvement of abiotic stress tolerance and other quantitatively inherited traits involves introgression of many genes; therefore, it may not be logically feasible for most breeding programs (Wang et al. 2007; Xu and Crouch 2008). Further, the requirement of prior mapping of significant marker–trait associations across breeding pools, in different environments, or after several cycles of selection is another drawback of MAS strategies. A recent marker-assisted recurrent selection (MARS) strategy, which involves multiple cycles of the indirect selection, is suggested for obtaining the desired frequency of target quantitative trait locus (QTL) alleles (Bernardo 2008). Further, genome-wide selection (GS) is another recent strategy that uses the combined effect of genome-wide markers on a trait, to pyramid favorable alleles for minor-effect QTLs (Bernardo 2009; Heffner et al. 2009;

Bernardo 2010). The major advantage of GS is that it does not require any prior knowledge about the QTL controlling the target traits. GS predicts the breeding values of lines in a population by analyzing their phenotypes and high-density marker scores. Unlike MABC or MARS, in GS, the marker effects across the entire genome that explain the entire phenotypic variation are calculated. The genome-wide marker data on the progeny lines are used to calculate genomic breeding values as the sum of the effects of all QTLs across the genome, thereby potentially exploiting all the genetic variance for a trait (Heffner et al. 2009). Thus, once a marker–trait association is developed, performance of progeny from selected cross may be predicted even before phenotyping, enabling indirect selection of the desired traits. Recently, single nucleotide polymorphisms (SNPs), a new-generation marker, are rapidly taking over the conventional molecular markers owing to their abundance, stability, and cost-effectiveness (McNally et al. 2009). In addition, these markers are amenable to automation and efficient in screening large population (Tung et al. 2010). Efforts are being made to discover SNPs, to develop functional SNPs for foreground selection, and to develop high-resolution SNP chips through deep sequencing for association genetics studies (Duran et al. 2009; McNally et al. 2009; McCouch et al. 2010). To this end, the availability of cost-effective, next-generation sequencing platforms and high-throughput marker genotyping may greatly enhance genome-wide selection for crop improvement in the near future (Varshney et al. 2009; Bernardo et al. 2010; Akpinar et al. 2013).

4 Conclusion and Future Perspectives

Multiple physio-biochemical traits contribute to the abiotic stress tolerance in plants. The success of the breeding (conventional and/or molecular) program for abiotic stress tolerance was previously limited by the availability of rapid, nondestructive, sensitive, and efficient screening techniques. Further, the ability to screen large sample population was challenging in abiotic stress tolerance screening using the conventional techniques. Recent advances have led to identification of highly predictive, simple, low-cost techniques for abiotic stress tolerance screening. Further, recently developed molecular markers have improved efficiency of plant breeding through precise and rapid foreground and background selection. Thus, the recent breakthroughs in the development of physio-biochemical–molecular indicators/markers for rapid and sensitive tolerance screening have revolutionized a new variety of development process.

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