Review Paper:

Role of molecular markers in improving abiotic stress tolerance in agricultural crops

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Abstract

Molecular markers are one of the expandable inventions in agricultural science. After decades of their introduction, these still are an integral part of crop genotyping and crop improvement. The present scenario offers to choose from different molecular marker types based on their principle, methodologies and extent of applications. Several markers have been used to identify and map abiotic stress related genes and quantitative trait loci (QTL) onto the chromosomes of different crops. The genetic mapping of these identified genetic elements by using different markers provides the scope of selective breeding in the agricultural crops to tackle abiotic stresses.

Moreover, recent progress achieved in genetics, molecular breeding and genomic selection has facilitated a comprehensive understanding of the molecular markers and unlocked new insights into marker-assisted breeding. The advent of new technologies like association mapping (AM) and genome-wide association studies has unveiled the high-throughput application of molecular markers. In this review, a focused representation of the use of different molecular markers in the identification of tolerant crop genotypes and associated genes/QTLs against major abiotic stresses, including salinity, drought and temperature has been provided. Additionally, the application of molecular markers in AM and AM-based breeding of some important agricultural crops has been discussed.

Keywords: Molecular markers, Abiotic stress, QTLs, Association mapping

Introduction

The sessile nature of plants exposes them to an array of environmental stresses in their lifetime. These enduring conditions vary from attacking pathogens and pests to numerous abiotic stresses such as salinity, temperature, dehydration, heavy metals and nutrient deficiency. These abiotic stresses have huge implications in plant growth and productivity, thus affecting the global food security. For instance, salinity stress alone has caused more than 48 million hectares of agriculture land to be uncultivated[23]. On the contrary, the ever-increasing population urges to increase the yields of different agricultural produce significantly to meet the food demands. Moreover, drastic climate changes have now complicated the current scenario than ever before[39].

Some of the most affecting abiotic stress types include salinity, drought and heat stress, which tend to even intensify with every passing year. While severe salinity results in the compromised plant growth by destabilizing the water and ion homeostasis, dehydration or drought stress results in wilting of plants and can lead to eventual plant death[23,67].

Similarly, heat stress causes detrimental effects to agricultural crops via evapotranspiration flux and hampers the plant growth and productivity[27]. Therefore, mitigating the abiotic stress effects on agricultural crops is the foremost challenge in hand.

The DNA-based molecular markers have been used for the genetic assessment of agricultural crops since many years. Mostly, the use of molecular markers has been attributed to analyze the genetic identification, genetic variation, molecular mapping and marker-assisted breeding in different crops. However, depending on their usage mechanism, the markers are divided into two groups such as polymerase chain reaction (PCR)-dependent and PCR-independent markers. Although PCR-independent markers like restriction fragment length polymorphism (RFLP) were in use in the 20th century, now the PCR-dependent markers have become the first choice for their use in agricultural advances.

Some of the most widely used PCR-dependent molecular markers include simple sequence repeats (SSRs), amplified fragment length polymorphism (AFLP), inter- simple sequence repeats (ISSRs) and rapid amplification of polymorphic DNA (RAPD). Recently, markers like single nucleotide polymorphisms (SNP), sequence-tagged sites (STS) and sequence-characterized amplified regions (SCAR) are used extensively in developing genetic linkage maps and molecular mapping[53].

Furthermore, using molecular markers, valuable details about the genetic makeup of a crop can be obtained including the parental allelic variations. In addition, molecular markers play crucial roles in determining the inheritance, genetic linkage and genetic diversity in crop genotypes and hybrids[9,46]. Moreover, several markers have been reported to be linked with plant stress response in crops including onions and chili[30,37].

Thus, this review provides an exhaustive description on the roles of molecular markers in the assessment of genetic makeup, mapping and screenings of agricultural crops against some of the prevalent abiotic stresses encountered by them. Additionally, the roles of the markers in the association mapping (AM) and AM-based breeding of some important crops are discussed.

Use of molecular markers in identification of abiotic stress tolerance in agricultural crops: In the present scenario, the effects of the abiotic stresses can be observed on worldwide agriculture resulting in about 91% of agriculture land under stress and approximately 50% loss in the crop yield[23]. Conversely, to overcome this impact, the wide use of stress tolerant crop varieties with improved agronomic characters and their molecular breeding can be a solution[37]. Moreover, there is an urgency in the improvement in stress tolerance in the agricultural crops by strengthening their genetic makeup and promoting the technologies or approaches to conserve such resources. In this regard, the use and application of various molecular markers have proven to be invaluable.

One of the DNA-based markers used in the agriculture research since decades is the random amplified polymorphic DNA (RAPD). This is a simple and effective PCR-based marker with no pre-requisite gene/DNA sequence information that can screen the intra or inter-specific hybrids[50]. The RAPD-based PCR amplification of specific DNA sequences is the primary approach in detecting the stress tolerant genotypes in crops. For instance, RAPD markers were employed in identifying the salt tolerant varieties in wheat[7]. Additionally, it can help in the molecular breeding of the salt tolerant wheat lines by the crossing of the contrasting varieties. Moreover, exposure to the abiotic stresses can cause changes in DNA including structural rearrangements due to the accumulation of reactive oxygen species (ROS)[8]. Using RAPD polymorphism, these changes in the crop genetic materials can be detected. For instance, RAPD markers could successfully identify the genetic instability in cotton plants caused by salinity stress[13].

Saleh[48] reported the identification and analysis of DNA changes in cotton plants under salt stress by the use of RAPD markers. Another use of the RAPD markers is in the molecular screening of plants developed in *in vitro* tissue culture. Identification of stress tolerant and agronomical improved plants via tissue culture in the filed screening method is time consuming and laborious. However, these plants can be screened for the genetic variability using RAPD before their field screening[45].

Although, RAPD is widely exploited for its basic principle and experimental methodology, it has some associated drawbacks. Issues like dominant marker nature, less reproducibility, DNA contamination and DNA amplification competition make the other DNA-based markers, including the microsatellites preferable over RAPD[13]. On the other hand, the use of microsatellites or simple sequence repeats (SSRs) markers helped in overcoming the limitations associated with RAPDs. SSRs are powerful co-dominant markers having grater resolution power than the dominant marker types including RAPDs. Crop genetic analysis using SSR markers has been successfully employed to identify superior crop genotypes related to stress resistance and agronomic traits. For instance, SSR markers have been used to detect improved agronomic and heat stress tolerant varieties in two important cereals including rice and wheat[5,70].

Similarly, SSR-based drought resistance screening was carried out in cotton plants to find out stress tolerant hybrids[42]. Moreover, based on SSR analysis, more exhaustive and in-depth genetic analyses of agricultural crops are performed. Wang et al[63] performed the molecular diversity analysis, allele mining and marker-traits association mapping in cassava germplasms to determine superior haplotypes against drought stress. Similarly, commercial tomato genotypes were screened for salinity tolerance using the SSR markers[18]. Recently, SSR-marker based genetic screening in wheat genotypes helped in the identification and analysis of candidate gene in response to salt stress[52]. In another study, SSR markers linked to drought tolerance were validated in contrasting wheat cultivars[15].

Another PCR-based marker which is efficient in amplifying polymorphic alleles and gene sequences is the sequence-related amplified polymorphism (SRAP) markers. SRAPs are used in the evaluation of genetic diversity, DNA fingerprinting and genetic mapping in agricultural crops due to their multi-allelic and multi-locus natures[29]. Similarly, the target region amplified polymorphism (TRAP) markers, another class of efficient DNA marker based on the expressed sequence tag information of a gene which have valuable applications in crop genotyping against abiotic stresses[8]. For instance, SRAP and TRAPs have been successfully employed in wheat and peas to identify temperature tolerant genotypes and their subsequent marker-assisted breeding (MAB)[10,32].

Another type of highly efficient DNA marker is the single nucleotide polymorphic (SNP) marker, which is hugely exploited in marker-assisted selection (MAS) and MAB of agricultural crops. SNPs are even preferred over traditional DNA markers including the microsatellite markers for their robustness and high efficiency[66]. Use of SNPs can identify the differences among the germplasm of a particular crop present within the coding and non-coding regions. The detailed use of SNPs in agricultural developments is discussed further in the association mapping segment of this review.

Use of molecular markers in QTL harnessing and molecular breeding against abiotic stresses: Quantitative trait loci (QTLs) are the genetic regions of a crop genotype which is associated with a phenotypic trait. Use of molecular markers to identify and map several QTLs associated with abiotic stress responses in crops has been carried out since decades. QTL identification and mapping associated with abiotic stresses in agricultural crops have facilitated the conventional breeding approaches in achieving stress-tolerant genotypes. Thus, molecular breeding of different crops using genomic selection (GS) and MAS has been hugely beneficial for crop improvements[26]. Several QTLs associated with major abiotic stresses including salinity, drought, submergence and temperature have been identified in many important crops (Table 1). For example, 16 QTLs in the rice F2 populations generated from the crossing of two contrasting rice genotypes were identified and mapped by using polymorphic SSR markers under salt stress[21].

Likewise, 85 different QTLs were mapped to different chromosomes in rice under salinity by using the SNP markers[12]. Similarly, Babu et al[4] reported the introgression of the well-characterized *Saltol* QTL in the basmati rice variety via MAB. In maize, 15 salinity stress associated QTLs were identified on different chromosomes of F2:3 populations[20]. In another study involving the SSR-based association study in wheat varieties, a QTL was identified associated with percentage of dead leaves under salt stress[61].

On the other hand, multiple QTLs have been identified in different important crops for drought tolerance, however, their introgression into susceptible genotypes via MAB or marker-assisted backcross breeding (MABB) has not been immensely successful due to the role of minor QTL in subtle phenotypic variations[64].

In rice, microsatellite-based QTL mapping resulted in the identification of 3 QTLs on chromosome 1, 4 and 6 under drought stress[43]. Similarly, 13, 3 and 3 QTLs associated with yield and abscisic acid content, biomass and seminal root angle respectively were identified in wheat populations under drought conditions[6,34,36].

Moreover, a genome-wide association study in 5K inbred maize lines using SNP markers resulted in the identification of 354 candidate genes in response to drought stress[30].

Agricultural crops in Asia face a particular waterlogging related stress also known as submergence stress due to improper irrigated and rain-fed lands. In this regard, several QTLs have been identified under submergence stress in the major cereals like rice, wheat and maize. In rice, five QTLs were identified to aid in the submergence tolerance provided by the *SUB1* gene[19]. Similarly, several minor QTLs have been identified in wheat related to dry root, shoot and mass index under submergence stress[68]. Similarly, QTLs associated with the temperature stress (either cold or heat stress) in different crops have been identified and mapped onto different chromosomes by the use of different molecular markers. For instance, TT1 was the first QTL reported for heat tolerance in African rice[31].

Additionally, three QTLs (*qPSLht4*.1, *qPSLht7* and *qPSLht10.2*) associated with spikelet fertility under high temperatures were identified in rice genotypes by using SSR marker analysis[54]. Another novel QTL *qSTIPSS9.1* associated with spikelet sterility in rice was identified by high-resolution mapping using a 5K SNP array which can be used for the MAB of rice. Similarly, QTLs associated with chlorophyll content under heat stress in wheat were identified by marker-assisted genotyping[3,59]. Conversely, QTLs associated with cold temperature tolerance have been identified and mapped onto chromosomes in maize[2].

Table 1
QTL mapping in agricultural crops for different abiotic stress responses using molecular markers.

Crop Name	QTL(s) identified	Associated molecular marker type	Associated trait
Rice	16	SSR[28]	Salinity tolerance
	85	SNP[12]	Salinity tolerance
	3	SSR[34]	Drought tolerance
	3	SSR, SNP[39]	Submergence tolerance
	3	SSR[42]	Heat tolerance
Wheat	6	SNP[24]	Salinity tolerance
	1	SSR[32]	Salinity tolerance
	8	SSR[14]	Drought tolerance
	1	SSR[14]	Drought tolerance
	3	SNP[58]	Heat tolerance
	2	SSR[62]	Cold tolerance
Maize	29	SNP[11]	Salinity tolerance
	15	SSR[33]	Salinity tolerance
	27	SNP[46]	Heat tolerance
Barley	13	SSR[65]	Salinity tolerance
	2	SNP[49]	Salinity tolerance
Cotton	14	SSR[47]	Salinity tolerance

Table 2
Use of the molecular markers in AM studies in some agricultural crops under different abiotic stresses.

Crop Name	Associated molecular marker type	Associated trait
Rice	SNP[50]	Salinity tolerance
	SNP[41]	Salinity tolerance
	SNP[51]	Salinity tolerance
	SSR[57]	Drought tolerance
	SNP[71]	Cold tolerance
Wheat	SNP[35]	Heat tolerance
Cotton	SNP[48]	Drought tolerance
	SNP[49]	Salinity tolerance
	SSR, SNP[33]	Yield component
Sorghum	SNP[54]	Drought tolerance

Application of molecular markers in association mapping related to abiotic stress tolerance: The concept of AM estimates the abundance of polymorphisms in agricultural crops to evaluate the effects of identified QTLs on a phenotypic level. Thus, via AM, the complex phenomenon like control of a phenotypic character by QTL(s) can be dissected while achieving other important information including allelic diversity and candidate gene identification.

However, while performing AM, the crop population type and structure, sample size and marker density are the major factors that greatly influence the final outcomes. Usually, AM can be divided into two components based on their analysis approach, first genome-wide association studies (GWAS) and secondly, candidate gene identification[25]. Linked molecular markers that share an association with phenotypic traits are identified in AM while QTLs identified via interval mapping are corroborated via GWAS. Moreover, molecular markers including RAPD, SSR, EST and SNP play crucial roles in AM analysis (Table 2).

On the other hand, any existing relationship between molecular marker(s) and phenotypes can be determined by the GWAS analysis. Generally, GWAS and SNP analysis are the keys to a successful AM analysis. The AM-breeding involves the identification of superior alleles of a resistant crop and their subsequent introgression into the contrasting varieties. For instance, four candidate genes such as PIP2, PIP2c, HAT22 and RD2 were identified in cotton genotypes associated with drought tolerance[22]. Additionally, two SNP markers (i47388Gh and i46598Gh) associated with salinity stress tolerance were identified in cotton genotypes[1,56].

Similarly, in rice, GWAS analysis revealed the identification of *Saltol* QTL contributing towards salinity tolerance[28]. In wheat, QTL identification and SNP-genotyping revealed QTLs that are associated with heat tolerance traits from seedling to reproductive stages. In addition, the candidate gene analysis further identified loci contributing towards heat, drought and salinity stress responses in wheat. Moreover, GWAS analysis in the plants has resulted in the identification of thousands of SNPs associated with different phenotypic traits[55,60].

Different breeding populations used in molecular breeding involve GS that is achieved by screening crops through consecutive seasons or generations for a selected phenotype based on whole-genome haplotypes rather than individual genes. Application of high-throughput polymorphic molecular markers is imperative in both GS and MAS. GS as a part of AM offers the direct estimation of loci having major or minor phenotypic effects on the whole genome which make it advantageous over other breeding approaches[40]. For instance, 347 associations were identified in 94 peach germplasms between different SNP markers and phenotypic traits. Furthermore, candidate gene analysis within the interval revealed several genes is involved in different physiological pathways[16].

Similarly, AM analysis coupled with genomic prediction identified 1549 SNPs correlated to 12 different trait-environment combinations in 300 maizes inbred lines[69]. In another recent study, the GWAS analysis and linkage mapping resulted in the identification of 4 QTLs and 17 candidate genes correlated to 42 SNPs associated with temperature tolerance in maize germplasms[17]. Moreover, AM is still a progressive concept in agricultural improvements. Both GS and AM are associated with some challenges like selection of phenotyping method, genotyping accessibility and availability of an efficient statistical algorithm that might be addressed in the times to come. However, whatever the progress or advances might be achieved in AM or GS, the role of molecular markers shall remain indispensable.

Conclusion

Molecular markers are used in the field of agriculture since long time. Marker-based crop genotyping and marker-based crop improvements are two major areas in which the molecular markers are highly exploited. Thus, molecular marker-based analysis and improvement of crop genotypes in responses to various abiotic stresses are both necessary and beneficial for the subsequent applications in breeding programs. In earlier times, molecular markers were used to provide basic genetic information on various crops, at best identification of QTLs. However, the advent of new markers and new technological approaches such as AM, GWAS and SNP-chip can now identify much detailed information such as allelic diversity and identification of superior haplotypes of a candidate gene.

Although, these advances have opened many avenues for crop improvements and better breeding programs, some limitations associated with these still offer the scope for improvements. In the times to come, molecular markers and marker-based crop improvements will be keys to satisfy the crop produce demands and mitigating the adverse challenges brought by several abiotic stresses and the climate changes.

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