

MOLECULAR MARKER TECHNOLOGY FOR ABIOTIC STRESS MANAGEMENT IN RICE

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ABSTRACT:

The green revolution of the 1970s resulted in remarkable increase in rice production. However since then the rate of production in most rice growing countries has slowed down and now has reached a plateau. Contributing factors include a higher population growth rate and the conversion of some highly productive rice lands for industrial and residential purpose. These developments have pushed rice cultivation to less productive land including saline and drought prone areas. Rice is highly important for human beings. Over half of the world's population depends on rice as a staple crop; in Asia, rice supplies 30 – 80% of the daily calories consumed. For rice important stresses include water deficit, submergence, salinity, and deficiencies of P and Zn. In recent years, advances in genetics, biochemistry, physiology and molecular biology have greatly improved our understanding of how rice responds to these stresses and the basis of varietal differences in tolerance. A lot of QTLs /genes linked to various highly reproducible molecular markers have been identified. The objectives of this paper are to briefly review various abiotic stresses affecting global rice production, recent efforts to better understand rice adaptation to several common abiotic stresses and to highlight efforts to integrate results of advances of Molecular Marker Technology (MMT) into rice breeding programs for effective management of various abiotic stresses in rice.

Key words: Abiotic stress, Drought, Submergence, Salinity, Molecular Markers

INTRODUCTION:

Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment. The non-living variable must influence the environment beyond its normal range of variation to adversely affect the population performance or individual physiology of the organism in a significant way. Abiotic stress factors, or stressors, are naturally

occurring, often intangible, factors such as intense sunlight or wind that may cause harm to the plants and animals in the area affected. Abiotic stress is essentially unavoidable. Abiotic stress affects animals, but plants are especially dependent on environmental factors, so it is particularly constraining. Abiotic stress is the

most harmful factor concerning the growth and productivity of crops worldwide.

Various abiotic stresses limit rice production in rainfed environments, which comprise about 45% of the global rice area. Important stresses include water deficit, submergence, salinity, and deficiencies of P and Zn. Rice has the smallest genome among the cultivated cereals, and it conserves much of the gene content and, to some extent, gene order present in other species [1]. The full rice genome has now been sequenced [2] allowing the identification and localization of genes related to stress tolerance. In recent years, advances in physiology, molecular biology, and genetics have greatly improved our understanding of how rice responds to these stresses and the basis of varietal differences in tolerance. Progress has relied on the application of rather specific phenotypic screens that allow the effects of stress to be distinguished from general differences in adaptation of diverse parents. QTLs have been identified that explain a considerable portion of observed variation, and in some cases, the genes underlying specific QTLs.

MOLECULAR MARKER TECHNOLOGY (M.M.T):

The science of plant genetics traces back to Mendel's classical studies on garden peas. Since then researchers have been identifying, sorting and mapping single gene markers in many species of higher plants. In early part of twentieth century, scientist discovered that Mendelian factors controlling inheritance which we now call genes were organized in linear fashion on chromosomes. Actually the first genetic map was produced by Sturtevant with segregation data derived from studies on drosophila. The markers on first genetic map were phenotypic traits scored by visual observation of morphological characteristics of the flies. Generally a marker must be polymorphic that is it must exist in different forms so that the chromosome carrying the mutant gene can be distinguished from the

chromosome with normal gene by the form of marker it also carries. This polymorphism in the marker can be detected at three levels i.e phenotypic level (by Morphological markers), difference in proteins (by Biochemical markers) or difference in the nucleotide sequence of DNA (by Molecular markers)

MORPHOLOGICAL MARKERS:

Morphological markers generally match to the qualitative traits that can be scored visually. They have been found in nature or as the result of mutagenesis experiments. These markers are highly influenced by environmental factors.

BIOCHEMICAL MARKERS (e.g.Isozymes):

Biochemical markers are proteins produced by gene expression. These proteins can be isolated and identified by electrophoresis and staining. Isozyme the different molecular form of same enzyme, that catalyzes the same reaction, are proteins. They are revealed on electro phoregram through a coloured reaction associated with enzymatic activity. They are the products of various alleles of one or several genes .The first molecular markers that initially were used extensively to study systematic of plants, animals and insects were isozyme (or isoenzyme) [3]. Isozyme analysis was initially utilized to study the taxonomy of plant pathogenic fungi [4, 5]. The method involves extraction of crude proteins and separation by electrophoresis on starch, non-denaturing poly acrylamide gels (PAGE) or isoelectric focusing. Isozyme zones are visualized after supplying the appropriate substrate necessary for the specific activity of each enzyme. The resulting Isozyme banding patterns (zymograms) are used to infer genetic relationships based on interpretations of banding polymorphisms assuming that isozyme zones correspond to equivalent loci.

Because Isozymes are post transcriptional markers, their expression is influenced by environmental changes leading to polymorphisms that might not reflect real differences at the

molecular level. This was the basic reason that application of isozyme in studying fungal plant pathogen variation was rather limited although in general they may provide satisfactory levels of polymorphic loci.

D.N.A BASED MOLECULAR MARKERS:

Molecular markers are also called DNA markers. It is a DNA sequence that is readily detected and whose inheritance can be easily monitored. The use of molecular markers is based on naturally occurring DNA polymorphism, which forms the basis for designing strategies to exploit applied purpose. A molecular marker has some desirable properties like

- It must be polymorphic
- Co-dominant inheritance
- Should be evenly and frequently distributed
- Should be reproducible
- Should be easy fast and cheap to detect.

No single marker meet all these requirements so there is need to develop a wide range of molecular markers. Molecular markers are also reported to explore genetic diversity in rice [6, 7] which may also assist in management of Abiotic stress in rice.

CLASSIFICATION OF MOLECULAR MARKERS:

Table No. 01:

Class	Marker system	Abbreviation	Remarks	References
First Generation Molecular Markers	Restriction Fragment Length Polymorphism	RFLP	Based on restriction digestion and hybridization with probe	[8]
	Sequence Tagged Sites	STS	RFLP probes sequenced and converted in to PCR based STS markers	[9]
	Random Amplified Polymorphic DNA	RAPD	Random primers for PCR amplification	[10]
	Arbitrary Primed PCR	AP-PCR	RAPD primers of 10-15 bases in length for discrete amplification	[10]
	Sequence Characterized Amplified Regions	SCAR	RAPD marker termini sequenced for designing longer primer	[11]
	DNA Amplification Fingerprinting	DAF	Single random primer of 5 bases short length	[12]
Second Generation Molecular Markers	Simple Sequence Length Polymorphism	SSLP	Based on tandem repeat flanking sequence	[13]
	Variable Number of Tandem Repeats	VNTRs	Based on tandem repeat sequence hybridization by probe	[14]
	Random Amplified Micro satellite Polymorphism	RAMPO	Random primers used for amplification and then hybridized with micro satellite oligonucleotides probe	[15]
	Cleaved Amplified Polymorphic Products	CAPs	PCR amplified products digested by restriction enzymes	[16]
	Inter Simple Sequence Repeat	ISSR	Single primer based on SSR motif	[17]
	Amplified Fragment Length Polymorphism	AFLP	Detection of genomic restriction fragment by PCR amplification	[18]
Third Generation Molecular Markers	Allele Specific Associated Primers	ASAP	Specific allele sequenced and primers designed for amplification	[19]
	Expressed Sequence Tag markers	ESTs	Sequencing of random DNA clones	[20]
	Single Nucleotide Polymorphism	SNP	Non-gel based marker system and DNA sequence differs by single base	[21]
	Miniature Inverted Repeat Transposable Elements	MITE	Non autonomous transposable elements with strong target site preference	[22]

TYPES OF ABIOTIC STRESSES AND THEIR MANAGEMENT:

1. Drought (Water deficit): Drought tolerance refers to the ability of the plant to grow and complete its life cycle under water limited conditions. In rice drought at vegetative stage decreases yield via a reduction in leaf area and tiller number. At the reproductive stage heading and consequently crop maturity is delayed. Poor panicle exertion leads to production of sterile spikelet and reduction in grain quality. Drought is generally avoided in irrigated rice production systems, but it is a consistent feature across much of the 63.5 million hectares of rainfed rice sown annually, most of which is in tropical Asia, Africa, and Latin America [23]. Farmers have been selecting those plants that survived drought events for centuries, and there is a wealth of genetic variation for response to water deficit among traditional cultivars [24]. There are few examples, however, of improved cultivars that combine acceptable yield potential and drought tolerance. The immediate difficulty lies in reliably measuring drought tolerance. Like other seed-producing crops, rice is more susceptible to damage from water deficit at particular growth stages. Rice varieties differ greatly in their ability to tolerate aerobic soil and moisture deficit. The greatest ability to grow and produce some grain with chronic moderate water deficit is found in japonica varieties from upland ecosystems such as those found in hilly Southeast Asia and Africa [24]. Examples include Azucena from the Philippines and Moroberekan from Guinea. Notable levels of drought tolerance are also observed in the early-maturing aus and indica varieties traditionally grown in the plateau region of Eastern India, such as N22 and Dehula [25]. Rice researchers are currently seeking QTLs that are stable across environments and that co-segregate with improved yield. Lafitte *et al.*, 2004 [26] found co-segregation between QTLs for yield components under drought and for traits

such as leaf rolling and drying in a population from Azucena/Bala. In a Vandana/Wayrarem population, there was co-location for QTLs for grain yield under drought with QTLs for maturity, panicle number and plant height [27]. Much genetic analysis has been done in the past 20 years to identify QTLs for traits related to drought resistance [28]. A high-density map for a cross between an upland (CT9993) and a lowland variety (IR62266) revealed QTLs across the genome for osmotic adjustment (OA) and root physiological and morphological traits. This map has been used to locate expressed genes and identify putative candidates for these traits [29, 30]. Roots have been the focus of many physiological and QTL mapping studies aimed at improving drought tolerance [31]. In rice, some of the many QTLs for roots are common across different genetic backgrounds [32]. A QTL for root length and thickness on chromosome 9 has been mapped in several populations and is expressed across a range of environments. It was the only one of the four target root QTLs that significantly increased root length when introgressed into a novel genetic background [33]. Root density and root thickness QTLs show poor alignment with QTLs for non-root-related drought avoidance traits; however, root penetration QTLs do co-segregate with them, particularly leaf drying QTLs [34, 26]. Steele *et al.*, 2004 [35] tested an alternative method to simultaneously identify molecular markers linked to agronomic performance under stress and transfer them to elite rice varieties termed marker-evaluated selection (MES), this approach used a very large segregating population derived from a wide cross between the upland variety Kalinga III and the irrigated variety IR64. There is no unified abiotic stress resistance mechanism for drought at the level of the whole plant or the single gene [36]. The traits associated with avoidance and tolerance can be constitutive (differing between genotypes) or adaptive (vary with the stage of the life cycle). Drought

avoidance and drought tolerance involve different mechanisms and processes, and phenology is the single most important factor influencing whether a plant avoids drought. Drought stress is highly variable in its timing, duration and severity, and this result in high environmental variation and G x E variation. Nguyen and Buu (2010) [37] have reported SSR technique combined with selective genotyping was used to map quantitative trait loci (QTLs) associated with drought tolerance in rice. Degenkolbe *et. al.*, 2013 [38] recently have identified the metabolic and transcript marker candidates for drought tolerance in a highly diverse population of cultivars. These markers may be used to select for tolerance in a wide range of rice germplasms.

2. Submergence (Excess water):

Water logging is one of the most hazardous natural occurrences, which can also be called as flood, submergence, soil saturation, anoxia, and hypoxia, which are generally used to describe water logging conditions depending upon the moisture or water level on the field. Generally, two types of flooding are present in the field: (i) water logging, in which root and some portion of the shoot goes under water, and (ii) complete submergence, where the whole plant goes under water. Excess water is a common constraint throughout the rainfed rice production areas as in South and Southeast Asia and tropical Africa. While rice is adapted to waterlogged conditions because of the well developed aerenchyma that facilitates oxygen diffusion and prevents anoxia in roots, complete submergence can be lethal. Out of 40 million ha in Asia grown under rainfed lowlands, about 15 million ha are frequently damaged by submergence [39]. The annual average yield loss from submergence is estimated at about 80 kg/ha [40]. In general, two types of flooding cause damage to rice: flash flooding that results in complete inundation of short duration, and long-term flooding, where water stagnates for up to few months (deepwater and tidal flood

areas). Most existing rice cultivars are seriously damaged if they are completely submerged for more than 3 d; however, a few tolerant cultivars can withstand complete submergence for 10 to 14 d, such as FR13A, FR13B, Goda Heenati, Kurkaruppan, BKNFR76106-16-0-1-0 and Thavalu. FR13A was released in the 1940s in Orissa, India, as a pure line selection from the local variety Dhullaputia [24]. Genetic studies suggested both simple and quantitative inheritance for submergence tolerance [41]. Sub1 gene has been reported quite helpful in generating submergence tolerance in rice by various workers [42,43,44].

3. Salinity (Salt stress): Salinity is one of the major impediments to enhancing production in rice growing areas worldwide. One-fifth of irrigated arable lands in the world have been reported to be adversely influenced by high soil salinity [45]. As per the report of FAO, 2010 [46], over 800 million ha of worldwide land are severely salt affected and approximately 20% of irrigated areas (about 45 million ha) are estimated to suffer from salinization problems by various degrees. This is more serious since irrigated areas are responsible for one-third of world's food production. In Asia, 21.5 million hectares of land areas are affected by salinity and estimated to cause the loss of up to 50% fertile land by the 21st midcentury [47]. Salt stress is a major constraint to cereal production worldwide. In Asia alone, 21.5 million ha are affected, of which 12 million ha are saline and 9.5 million ha are alkaline/sodic. Rice is a salt-sensitive crop, but it is the only cereal that has been recommended as a desalinization crop because of its ability to grow well under flooded conditions. Rice is comparatively tolerant of salt stress during germination, active tillering, and towards maturity and is sensitive during early seedling and reproductive stages.

Few attempts have been made to identify QTLs associated with salinity tolerance in rice. For

example, seven QTLs for seedling traits associated with salt stress were identified and were mapped to five different chromosomes [48]. A major gene for salt tolerance was mapped on chromosome 7, using an F₂ population derived from a salt-tolerant japonica rice mutant, M-20 and the sensitive original variety 77-170 [49]. The QTLs associated with different mechanisms of salinity tolerance in rice independently govern the uptake of Na and K and Na: K selectivity and are mapped on different chromosomes [50]. A major QTL designated ‘*Saltol*’ was mapped on chromosome 1 using a population generated from a cross between the sensitive variety IR29 and a tolerant landrace, Pokkali. This QTL accounted for more than 70% of the variation in salt uptake in this population [51] and is now being mapped to within 1 cM using a large set of NILs. Candidate BAC clones from the physical map have also been identified. Marker assisted backcrossing is currently being used to incorporate this QTL into popular varieties sensitive to salt stress. M. R. Islam *et al.*, 2011 [52] has reported QTL mapping for salinity tolerance at seedling stage in rice. Le Hung Linh *et al.*, 2012 [53] have reported that MABC has accelerated the development of superior qualities in the genetic background of BT7.

4. Phosphorus deficiency: Phosphorus (P) deficiency has been identified as a major factor limiting grain yield in rice worldwide. In general, rice varieties with tolerance to P deficiency exhibited the root elongation under P deficient conditions, and the relative root length (RRL) were determined as a reliable parameter for tolerance to P deficiency in rice [54]. The lack of locally available P sources and the high cost of importing and transporting fertilizers prevent many resource-poor rice farmers from applying P. Some rice soils can quickly fix up to 90% of the added P fertilizer into less soluble forms [55]. An attractive, cost-effective and sustainable strategy is to develop rice cultivars capable of

extracting higher proportion of fixed P. Genetic variability among lowland and upland rice cultivars in their ability to exploit soil and fertilizer P were observed. Variation in uptake in the range of 0.6 to 12.9 mg P plant⁻¹ was reported, and with the traditional landraces being superior to modern varieties. Hence, genetic variation in tolerance to P deficiency could effectively be exploited for rice improvement.

However, tolerance to P-deficiency is quantitatively inherited with both additive and dominant effects [56] and as with other quantitative traits, progress through conventional approaches will be slow. More rapid progress in breeding may be achieved through the application of modern molecular approaches. Attempts have been made to detect QTLs controlling P-deficiency tolerance in rice, and four QTLs have been identified for P-uptake. One major QTL (*Pup1*) was mapped on chromosome 12 [57]. *Pup1* was found to triple P uptake under P-deficient soils, with no apparent effect when P was not limiting [58]. At present, *Pup1* is fine-mapped in a 0.4 cM interval, which, on the rice physical map, spans 3 BACs that have been fully sequenced. Physiological studies suggest that the *Pup1* gene is expressed in root tissue, where it either leads to higher root growth per unit P uptake (higher internal efficiency) or improves P uptake per root surface. Work is currently ongoing to identify and characterize the putative candidate genes in this region and to develop a MAS strategy for its incorporation into modern varieties. Quantitative trait loci (QTLs) for P deficiency tolerance had been identified in a rice population derived from a cross of the intolerant japonica cultivar Nipponbare and the tolerant indica landrace Kasalath [59] and the cross between tolerant indica cultivar IR20 and indica sensitive variety to P-deficiency IR55178-3B- 9-3 [60]. Recently Nguyen and Bui 2006 [61] have mapped QTLs for phosphorus deficiency tolerance in rice. *Pup1* has also played important role in phosphorus deficiency control [62].

5.Zinc deficiency: Zn deficiency causes multiple symptoms that usually appear 2 to 3 weeks after transplanting (WAT) rice seedlings; leaves develop brown blotches and streaks that may fuse to cover older leaves entirely, plants remain stunted and in severe cases may die, while those that recover will show substantial delay in maturity and reduction in yield [63]. Zinc (Zn) deficiency was first diagnosed in rice (*Oryza sativa*) on calcareous soils of northern India [64]. It was subsequently found to be a widespread phenomenon in lowland rice areas of Asia, and, next to nitrogen (N) and phosphorus (P) deficiency, Zn deficiency is now considered the most widespread nutrient disorder in lowland rice [65]. Zinc (Zn) deficiency is the most common nutrient problem for rice next to nitrogen and phosphorus, with as much as 50% of all lowland rice soils being affected [66]. Deficiency is normally associated with continual soil wetness and occurs particularly in alkaline, organic and poorly drained soils [67]. It is also associated with high bicarbonate content and high levels of available phosphate and silica. The use of high levels of fertilizers with antagonistic effects on Zn availability and intensive cultivation of modern rice cultivars has exacerbated the deficiency problem over the past several years. Noticeable differences between rice cultivars in ability to extract Zn and grow under low Zn conditions have been observed [68]. Limited efforts are now underway to map this trait and to pave the way for further physiological and molecular exploration. In this sequence Three QTLs linked with HvSSR 01- 80 , HvSSR 01-87 and RM 499 markers identified on chromosome 1, two QTLs linked with RM 135 and RM 232 markers located on chromosome 3, one QTL linked with marker Hv SSR 05-31 present on chromosome 5, two QTLs linked with RM 242 and RM 296 on chromosome 9 and one QTL linked with marker RM 26334 located on

chromosome 11 are contributing zinc deficiency tolerance based on the genotypic data [69].

CONCLUSIONS AND OPPORTUNITIES:

Millions of hectares in the humid regions of south and south East Asia are technically suited for rice production but are left uncultivated or give very poor yield because of salinity and Abiotic stresses .To narrow the yield gap between areas susceptible to Abiotic stresses and irrigated fields, multiple Abiotic stress tolerance traits should be considered aside from high yielding ability and disease resistance. The development of submergence tolerant cultivars has required the use of stress-specific screens, because direct evaluation of tolerance is not as simple as it might seem. Results depend strongly on the depth and duration of submergence, age of seedlings, and water temperature. Because of these complexities, alternative indirect screening approaches have been developed using traits known to be associated with submergence tolerance such as extent of underwater shoot elongation, shoot carbohydrate storage and extent of chlorophyll retention, all of which are correlated significantly with seedling survival. Our knowledge of how rice genes respond to stress is increasing daily. Our understanding of how these changes translate into plant growth and crop level differences in performance under stress lags behind, and will require a focused effort at synthesis in order to convert the exciting results of genomics into tools and guidelines that plant breeders can use. The most direct application will be in marker-aided selection, though novel high-throughput trait-based screens for use in breeding programs may also emerge from this effort. Marker-assisted selection of progeny from crosses between tolerant, low-yielding cultivars and susceptible, high yield-potential lines theoretically allows for much greater efficiency in a breeding program, because extensive unreliable phenotypic screening can be eliminated, and linkage drag can be effectively

reduced. For crop improvement, the identification of useful allelic variation for genes in the second group may be the most promising approach. Once such genes or gene combinations are identified, either molecular approaches or trait-specific physiological screens can be used to search for these superior alleles. Marker-assisted backcrossing can then be applied to incorporate these alleles into agronomically superior germplasm.

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