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Molecular Breeding for Abiotic Stresses in Maize (*Zea mays* L.)

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Abstract

Abiotic constraints resulting from climate changes have widespread yield reducing effects on all field crops and therefore should receive high priority for crop breeding research. Conventional breeding has progressed a lot in building tolerant genotypes but abiotic stress tolerance breeding is limited by the complex nature of abiotic stress intensity, frequency, duration and timing, linkage drag of undesirable traits/genes with desirable traits; and transfer of favorable genes/alleles from diverse plant genetic resources limited by gene pool barriers giving molecular breeding a good option for breeding plant genotypes that can thrive in stress environments. Molecular breeding (MB) approaches viz., marker-assisted selection (MAS), marker-assisted backcrossing breeding (MABB), marker assisted recurrent selection (MARS) and genomic selection (GS) or genome wide selection (GWS) offer opportunities for plant breeders to develop high yielding maize cultivars with resilience to diseases in less time duration precisely. For complex traits (mainly abiotic stresses) where multiple QTLs control the expression, new strategies like marker assisted recurrent selection (MARS) and genomic selection (GS) are employed to increase precision and to reduce cost of phenotyping and time duration with disease resilience. This review discusses recent developments in molecular breeding for developing and improving abiotic stress resilience in field crops.

Keywords: cold, drought, waterlogging, climate change, salinity

1. Introduction

Even though climate change is one of the major current global concerns, it is not new. Several climate changes have occurred before, with dramatic consequences. Among them is the decrease in CO_2 content, 350 million years ago considered responsible for the leaf

appearance. It took nearly 40–50 million years for leaves to appear [1]. The massive volcanic eruptions were the second climatic change during the end-permian age in Siberia when lava erupted over 4 million km^3 onto the surface of earth [2] and today the volcanic eruption remnants cover an area of 5 million km^2. This volcanic eruption resulted in accumulation of organohalogens causing depletion of the ozone layer worldwide. Consequently, UV radiation burst was one of the cause of mass extinction resulting in wiping out 0.95 of all the species [2]. The end of the last ice age came to an end was the third major result of climatic changes causing long dry seasons. Hence, the annual plants survived dry seasons either as tubers or as dormant seeds leading to birth of agriculture in Fertile Crescent and then in other areas. The fourth climate change induced the Holocene flooding, ago which is now believed to be associated with collapsing of the ice sheets, resulting in rise of global sea level up to 1.4 m [3]. Rising sea levels caused massive migration towards the North Western areas which explained the domestication of plants and animals, which reached modern Greece, Balkans and Europe. During the last 5000 years, drought has historically been the main factor limiting crop production. Water availability has led to rise of multiple empires, while drought caused collapse of various civilizations viz., Mesopotamia, (6200 years ago), Yucatan Peninsula (1400 years ago), coastal Peru, (1700 years ago) and early bronze society in the south of Fertile Crescent [4, 5].

Climate changes have adverse impacts on food production, quality security [6]. The number of undernourished people would increase by 150% in the areas like, north of Africa and Middle East by year 2080 compared to 1990 and 300% in sub-Saharan Africa [7]. Agriculture is extremely vulnerable to climate change. Higher temperatures eventually reduce crop yields without discouraging weed, disease and pest challenges. Long-term production declines and short-term crop failures result from changes in precipitation patterns. Overall negative impact of climate change on agriculture is expected to threaten the global food security [8] which would probably increase unless early warning systems and breeding strategies are developed [9]. Climate change is reducing production while increasing hunger among populations. High temperatures with less precipitation over semi-arid regions would reduce yields of crops in the next two decades causing negative impacts on global food security and calorie consumption causing malnutrition [10]. Thus, agricultural productivity investments are needed to tackle the negative impacts of climate change on the health scenario and food security [8].

The most likely stresses within which plant breeding targets need establishing are: [11]

- High temperatures.
- Drought.
- Salinity.
- Biotic stresses.
- Increase in CO_2 concentration.

There is a three-fold relationship between climate change and agriculture. Firstly, agriculture contributes indirectly to climate change by emitting methane from rice fields, N_2O from fertilizers & manure and CO_2 emissions from field work, machinery, fertilizers and pesticides.

Second relation is the impact of these climate changes on agriculture caused by increased weather variability (extremes in temperature and precipitation), sea level rise and surge thus, inundating & ruining coastal agricultural lands, pathogen and pest pressures and decreased plant biodiversity. The third relation is that agriculture can itself become a potential moderator of climate change by mitigating climate change by carbon sequestration by having agroforestry, rotations with cover crops, green manure, conservation tillage, by changing inputs like going for organic farming, reducing fertilizers, using bio-fuels and by adapting to climate changes by breeding crop varieties with resilience to climate change by selective breeding and developing genetically modified organisms (GMOs) [12, 13].

To increase the efficiency of breeding pipelines, a combination of conventional, molecular, and transgenic breeding approaches will be needed. Breeding approaches are not mutually exclusive and are complimentary under most breeding schemes [14].

Plant breeders respond to climate related stresses in multiple ways:

- Selection and backcross breeding.

- Extensive managed stress screening experiments to develop superior tolerant germplasm via recurrent selection.

- Exploitation of alien genetic variation (Conserved Wild Relatives).

- Breeding for earliness and varieties with specific adaptation to specific ecologies.

One of the effective ways for crop production to grow or to stay stable under new challenges from climate change is through improved varieties developed by plant breeding. The genetic diversity of crop plants is the foundation for the sustainable development of new varieties for present and future challenges. For example, common beans biodiversity has been used by plant breeding to develop both heat and cold tolerant varieties grown from the hot Durango region in Mexico to the cold high altitudes of Colombia and Peru. Similar is the case with other crops too. Resource-poor farmers have been using genetic diversity intelligently over centuries to develop varieties adapted to their own environmental stress conditions.

Biotechnological tools: The tools of modern plant breeding include following:

- Molecular breeding (marker-assisted selection (MAS), marker-assisted backcrossing breeding (MABB), marker assisted recurrent selection (MARS), genome wide selection (GWS)).

- Genetic engineering.

1.1. Molecular breeding (MB)

The MB approach involves first identifying quantitative trait loci (QTLs) for tolerance to abiotic stresses. After identifying the markers associated with QTLs or genes for traits of interest, the candidate QTLs or genes can be introgressed in elite lines through marker-assisted backcrossing (MABC). Until recently, QTLs were identified by linkage mapping [15], but now association genetics has started to supplement these efforts in several crops [16, 17]. Nested association mapping, which combines the advantages of linkage analysis

and association mapping in a single unified mapping population, is also being used for the genome-wide dissection of complex traits in maize [18]. Association mapping, compared with linkage mapping, is a high-resolution and relatively less expensive approach. In the near future, it is likely to be routinely used for identifying traits associated with abiotic stresses [16], particularly given the availability of high-throughput marker genotyping platforms [19]. An example of the systematic use of association mapping for drought tolerance is the collaborative project between Cornell University and CIMMYT (http://www.maizegenetics.net/drought-tolerance).

MABC helps in developing crops that are drought and heat tolerance, adapted to low rainfall and high temperature conditions. In rice, molecular breeding was used for one major effect QTL for submergence tolerance Sub1 QTL [20] and drought tolerance [21]. One of the difficulties of developing superior genotypes for abiotic stresses such as drought or heat is that these traits are generally controlled by small effect QTLs or several epistatic QTLs [22]. Incorporating QTLs by MABC has been limited, mainly because of the large sizes of the backcross populations. Therefore, marker-assisted recurrent selection (MARS) and genome wide selection (GWS) or genomic selection (GS): are used to overcome this problem of pyramiding several QTLs in the same genetic background [19, 23].

The estimated genetic gain by MARS or GWS is greater than obtained by using MABC for transferring QTLs /gene alleles for complex abiotic stress traits in one genetic background [24, 14]. The MARS approach is used routinely in private sector breeding programs [14, 25]. MABC and MARS require information on marker trait associations which is not necessarily required for GWS [26, 27]. GWS studies both phenotyping data as well as genome-wide marker profiling of a 'training population' and predictions of the genomic-estimated breeding values (GEBVs) of progeny GEBVs are calculated based on phenotyping and marker datasets. These values are used to select the superior progeny lines for advancement in breeding cycle [27, 28]. Several computational tools are available or are being developed to calculate GEBVs, such as the Best Linear Unbiased Prediction method and the geostatistical mixed model [29], (http://genomics.cimmyt.org/#Software).

2. Few case studies

2.1. Drought tolerance in rice

Birsa Vikas Dhan 111, an upland rice cultivar released in Jharkhand was bred by utilizing MABC for improved root growth QTLs towards improved performance under drought in a collaborative partnership programme between Birsa Agricultural University, Ranchi, Jharkhand, and CAZS-NR; Gramin Vikas Trust, Ranchi, Jharkhand. This variety is high yielding (out yielding recurrent parent by 10% in rainfed conditions) with good grain quality and matures early with tolerance towards. This specific QTL was identified by Adam Price in first instance (Aberdeen University, UK) and Brigitte Courtois (CIRAD, France/IRRI, Philippines). Here marker-assisted back-crossing breeding and marker assisted pyramid crossing was

conducted to improve the morphological and root traits for drought tolerance of Indian rice variety, Kalinga III (*indica*) used as recurrent parent and Azucena, an upland japonica variety from Philippines as donor parent.

Five segments each from different chromosomes were targeted for introgression; four segments out of five carried the QTLs for root length and root thickness while as fifth segment had a recessive QTL for aroma. 24 NILs (Near isogenic lines) were evaluated in five field experiments in UAS Bangalore for root traits Dr. Shashidhar. The segment on chromosome number 9 with flanking markers viz., RM242-RM201 increased root length significantly both under drought & irrigated treatments thereby confirming the QTL from Azucena cultivar expressed well [21].

Significant number of QTLs associated with drought tolerance have been reported for drought tolerance. A QTL located on chromosome 9 has been found associated with spikelet fertility under drought stress and for root and shoot traits [30–32]. 'Teqing' a *indica* cultivar used as recurrent parent in a study with 'Lemont' as donor (*japonica*) several alleles from Lemont were found associated with improved drought tolerance [33]. Detection of qtl12.1 QTL for tolerance towards drought accounting for 51% of the genetic variance and located on chromosome 12 was reported by [34] localized to a 10.2-cM region (RM28048 and RM511).

NERICA rice varieties are promising for Africa. These varieties mature early and escape drought. Rice varieties hardier than NERICA are being, developed by maximizing the diversity of the African rice germplasm pool consisting of *Oryza glaberrima*, its wild relatives (*Oryza barthii*, *Oryza longistaminata*) and *Oryza sativa* landraces using both conventional breeding and biotechnology.

2.2. Drought tolerance in maize

One of the major limiting factors for maize production and productivity is inadequate soil moisture particularly during flowering and grain filling stages [35]. Studies on drought tolerance have focused on identifying the genetic basis of yield and its components and secondary traits viz., including anthesis-silking interval (ASI), root architecture and stay green. Stable genomic regions associated with flowering, maturity and yield components identified more than 1080 QTLs [36]. For narrow ASI, five QTLs were introgressed from a drought-tolerant donor Ac7643 through MABC to CML-247 an elite, drought-susceptible line. The selected lines out yielded the control under drought conditions while decreasing the yield advantage from mild to moderate drought stress [37].

In India several QTL mapping experiments on drought stress has been undertaken [38] and in China [39, 40]. In India, QTL mapping for maize drought tolerance identified major effect QTLs on chr. 1, 2, 8 and 10 after assessing a set of 230 CIMMYT developed RILs at Hyderabad and Karimnagar. A significant digenic epistatic QTL effect for kernel number ear^{-1} under drought stress was detected. A major QTL for ASI (anthesis-silking interval) and ear number per plant under drought stress was detected on chr. 1 (bin 1.03) and chr. 9 (bins 9.03–9.05) [39, 40] from a cross between X178 (tolerant line) and B73 which corresponded to several QTLs

identified in different experiments carried on drought worldwide [41]. Several such identified 'consensus QTLs' would serve as good candidates in marker-assisted breeding to improve maize production under drought.

Drought resilient maize product pipeline:

Over 80% maize is grown as rain-fed crop, with avg. yield less than half of irrigated maize. Following are the few projects for developing drought resilient maize:

• Drought tolerant maize for Africa (DTMA),

• Water efficient maize for Africa (WEMA),

• Affordable accessible Asian drought tolerance maize project (AAA),

• Asian maize drought tolerance project (AMDROUT).

Drought tolerance maize varieties developed:

Variety	Trait + selection strategy	Developed By
ZM 309, 401, 423, 521, 623, 625 and 721	Conventional breeding	South Saharan Africa
KDV1, 4, 6	Conventional breeding	South Saharan Africa
WS103	Conventional breeding	South Saharan Africa
Melkassa 4	Conventional breeding	South Saharan Africa
WH 403, 502, 504, and ZMS402, 737	Conventional breeding	South Saharan Africa

2.3. Cold tolerance in rice

Tolerance of low temperature at both the vegetative and the reproductive stage is an important breeding objective for improving rice cultivars in the temperate and high altitude areas of the tropics and subtropics. Low temperatures during booting stage reduce yields by causing cold-induced male sterility. Cold prevents sugar accumulation in the pollen causing no starch build-up and hence no energy for pollen germination hampering grain production. Enzyme invertase regulated by hormone abscisic acid (ABA) transports sugar to tapetum before moving to the pollen and cold decreases the invertase levels in susceptible cultivars [42] lowering pollination and hence grain development. Several QTLs for cold tolerance were identified at booting stages on chromosomes 4 (Ctb1) and 8 (qCTB8) in Silewah (a javanica cultivar). Significant number of markers have been used by several workers [43, 44] to transfer cold tolerant gene (Ctb1) into japonica rice cultivars. Eight QTLs for booting-stage cold tolerance were identified in a RIL (recombinant inbred line) population derived from a cross between japonica and indica cultivars [45]. A QTL for cold induced wilting and necrosis tolerance has been fine mapped & identified on chromosome 12 [46, 47]. q*CTS4* fine mapped to 128-kb region on chromosome 4 associated with tolerance to stunning and yellowing of seedlings under cold contributed 40% of the phenotypic variation [48].

2.4. Salinity tolerance in rice

"White Leaf tip" is first symptom at vegetative stage in rice caused due to salinity stress followed by "Tip burning" which extends towards base. At reproductive stage papery sterile spikelets is another symptom resulting in huge losses and ultimately extreme high Salt Stress kills the rice plants. Central Soil Salinity Research Institute, Karnal is a pioneer institute in breeding for salinity resistant varieties. Few varieties developed by different approaches are as:

- Conventional:
  - **Pureline Selections** from local traditional cultivars **Pokkali, Nona Bokra and Kala-rata**:
  - **Damodar (CSR1), Dasal (CSR2), CSR3**.
  - **Pedigree: CSR10, 13, 23, 27, 30, 36.**
- Nonconventional:
  - Anther Culture: **CSR-21** for salinity.
  - **CSR: 28** for salinity and alkalinity.
- Other salt-tolerant rice varieties
  - Usar dhan 1, 2 & 3 (India);
  - BRRI dhan 40, BRRI dhan 41 (Bangladesh);
  - OM2717, OM2517, OM3242 (Vietnam).

MABC is being employed to efficiently transfer the Pokkali seedling stage salinity tolerant *Saltol* QTL into popular varieties such as IR64, BR11, BR28, Swarna, etc. *Saltol* QTL has been fine mapped on Chr. 1 shirt arm associated with the Na-K ratio (high K^+ & low Na^+ adsorptions) [49]. SKC1, a QTL for salt tolerance, maintains K^+ homeostasis in the tolerant cultivar and encodes HKT-type transporters [49]. QTLs for reproductive-stage salt tolerance are yet to be reported.

2.5. Submergence tolerance in rice

QTL *Sub1* fine mapped on chromosome 9 contributes 70% of the phenotypic variation for survival under submergence [50]. Two of the three ethylene-response factor (ERF) like genes induced by submergence were identified at this locus. [51] reported gene *Sub1A* gene responsible for submergence tolerance which has been integrated into Swarna by marker assisted backcross breeding [52] which demonstrated that QTLs controlling tolerance of abiotic stresses can be used to improve mega varieties in the target regions [53].

2.6. Waterlogging tolerance in maize

Over 18% of the total maize production area in South and Southeast Asia is frequently affected by floods and waterlogging problems, causing production losses of 25–30% annually [54]. Many QTLs for waterlogging tolerance at seedling stage have been reported [55]. A

F2:3 mapping population comprising 288 lines derived from HZ32 × K12 (sensitive) inbred lines studied under flooded and nonflooded conditions helped in identifying 25 and 34 QTLs accounting for between 4 and 37% of the genotypic variation to waterlogging tolerance. QTLs associated with plant height, root and shoot dry weight, total dry weight were identified in different experiments on chromosomes 4 and 9. In a F2 mapping population of B64 and teosinte (*Z. mays* ssp. Huehuetenangensis) QTLs associated with adventitious root formation under flooding were identified on chromosomes 3, 7 and 8 [56] confirming the potential use of teosinte as donor for waterlogging tolerance. A cross between *Z. mays* spp. Nicaraguensis (a different teosinte accession) and inbred line B73 helped in identifying QTLs for aerenchyma formation located on chromosomes 1, 5 and 8 [57]. These QTLs from different donors hence, provide a valuable genetic resource for breeding waterlogging tolerant maize.

2.7. Wheat drought and heat tolerance

Markers associated with a QTL for grain yield in wheat under drought has been identified at 4AL. 127 RILS were developed from a cross between Dharwar dry drought tolerant and Sitta drought susceptible [58]. XBE637912, Xwmc89, and Xwmc420 SSR markers were found linked to Grain Yield QTL.

3. Genetic engineering

Plant adaptation to environmental stresses is controlled by cascades of molecular networks. These activate stress responsive mechanisms to re-establish homeostasis and to protect and repair damaged proteins and membranes [59]. Abiotic stresses are multigenic, and hence difficult to control and engineer. Therefore, strategies like plant genetic engineering for building tolerance rely on gene expression involved in signaling pathways and regulatory pathways. Consequently, engineering genes that protect and maintain the function and structure of cellular components can enhance tolerance to stress [60].

4. Few case studies

4.1. Heat-tolerant basmati rice developed by over-expression of hsp101

Heat-tolerant basmati rice was developed by introducing *Arabidopsis thaliana hsp101 (Athsp101)* cDNA into the Pusa basmati 1 by *Agrobacterium* mediated transformation [61]. Transgenic lines were compared for survival after exposure to different levels of high-temperature stress {45°C for 3 h and then were placed at 28°C} with the untransformed control plants. It was reported that transgenic lines (15 and 43) survived heat stress as compared to the untransformed ones and the optimum temperature for rice growth throughout its life cycle is 25–31°C [61].

4.2. Barley gene in rice for drought tolerance

Barley gene *HVA7* was introduced into rice suspension cells using the Biolistic-mediated transformation method in rice for drought tolerance [62], *HVA7* is a late embryogenesis abundant (LEA) protein gene, from barley and this gene was regulated by the rice actin 1 gene promoter leading to high-level, constitutive accumulation of the HVA 7 protein in both leaves and roots of transgenic rice plants.

4.3. Yeast gene in tomato for salinity tolerance

In yeast (*Saccharomyces*) overexpression of *HAL 1* gene confers tolerance to salinity. So, introduction of this *HAL1* gene (using Plasmid pPM5 contained an *EcoRI: HindIII* fragment of 1.75 kb with the reinforced 35 S promoter, the *HAL1 ORF*, and the *nos* terminator) was done in Tomato (*Lycopersicon esculentum* cv P-73) [63]. Transgenic tomato (TG$_3$) was reported tolerant to salinity by maintaining K uptake in the presence of external Na.

4.4. Increased glycine betaine (GB) synthesis for salinity tolerance in cotton

Choline mono-oxygenase (CMO) is a major catalyst in glycine betaine (GB) synthesis. Glycine betaine is a osmolyte and overexpression of this osmolyte confers tolerance to salinity. This CMO gene cloned from *Atriplex hortensis* (AhCMO) was introduced into cotton (*Gossypium hirsutum* L.) via Agrobacterium mediation for development of Cotton plant having introduced CMO gene for glycine betaine (GB) [64].

4.5. Alteration in fatty acids: for cold stress tolerance

Plants such as squash and arabidopsis having high proportion of cis-unsaturated fatty acids are chilling resistant. Hence, the degree of unsaturation of fatty acids is closely related to chilling tolerance among the plants. Enzyme *glycerol-3-phosphate acetyl transferase* determines the phosphatidyl glycerol fatty acids unsaturation and hence cold tolerance.

5. Conclusions

Plant Genetic diversity and Plant Breeding are key elements in tackling climate change, and integration of plant breeding in climate change strategies is one of the best paths to sustainable food production by developing climate smart crops: Development of abiotic and biotic resistant crop varieties which cope with climatic vagaries, Varieties suited to new agricultural areas resulting due to shift in climatic pattern, Varieties with reduced total pesticide and fungicide consumption and hence, their reduced ill effects on environment which indirectly contribute to Climate Change. "It is not the strongest of the species who survive, nor the most intelligent, but the one most responsive to change." Let us be the difference we want to make to the world: Charles Darwin.

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