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Review on Integrated Plant Breeding Approaches for Moisture Stress Tolerance in Maize (*Zea mays* L.)

B. R. Mani
Ph.D. Scholar, Department of GPB, College of Agriculture, U.A.S., Dharwad, Karnataka, India
S. K. Deshpande
Professor and Head, Department of GPB, College of Agriculture, U.A.S., Dharwad, Karnataka, India

Abstract:
Maize being grown throughout the world due to its adaptability with range of environments and occupies third largest area under cultivation. It can be used as fodder, poultry feed and human staple food crop. It also serves as raw material for more than five hundred food products. Therefore, maize has huge demand in both national and international markets. At the same time, it faces serious problems viz., abiotic and biotic stresses which reduces production greatly. Among the stresses, moisture stress is the one that can decrease yield up to 79-80% (Monneveux et al. 2005). Maize varieties or hybrids have to be developed to meet the present demand by applying suitable breeding approaches.

Studies are being conducted, to explore germplasm, develop breeding material, understand genetics of moisture stress tolerance and to determine the physiological, biochemical, molecular, agronomical as well as phenotypic traits associated with tolerance to moisture stress. There are many reports confirming, moisture stress tolerance a complex trait, controlled by poly genes and highly influenced by environmental factors. Researchers employed different breeding approaches and physio-biochemical along with agronomic parameters to assess the moisture stress tolerance and to develop a variety tolerant to stress.

Mahmood et al. 2013, evaluated six maize cultivars for tolerance based on physiological traits associated with cell wall plasticity and found two (EV-1097 & Agaiti 2002) as tolerant, based on higher values of LGR, Chlorophyll, TSP, Proline, SLW and TSS. In addition to above physiological traits, Canopy Temperature is also a trait which is directly associated with stress tolerance as reported by Abbas et al. 2014. Root is the organ which senses the water limitation and signals the above ground canopy to run adoptive mechanisms thus, study of root features is essential to develop a drought tolerant cultivar as suggested by Rangyao Li, 2015.

Drought tolerant indices are mathematical ratios calculated based on yield data from stress and non stress field. Researchers have concluded the significant and positive association of tolerant indices (DTI, MP, GMP, TOL, SSPI, K1STI and K2STI) with grain yield in stress condition. Zahra& Jahad (2012) evaluated the maize cultivars using tolerant indices and find out best indices and cultivars for determining tolerance and moisture stress tolerant respectively. Yanli Lu et al. 2011 screened 551 lines for drought tolerance by considering multiple selection criteria among which NDVI, chlorophyll content and leaf senescence are the major traits. This study confirmed the NDVI as an effective parameter to determine stress tolerance.

Identification of morpho-physiological traits and QTLs linked with tolerance and stay green can serve as effective strategy to improve stress tolerance via MAS, as confirmed by Zhu et al. (2011) and Ai-Yu et al. 2012. MABC breeding could be employed to select and develop hybrids tolerant to moisture stress as confirmed by jean and Mic 2007. Among various breeding approaches described, integrated breeding approach would be the best technique to develop variety resistant to moisture stress.

Keywords: *Moisture stress, physiological, indices, roots, selection, hybridization, yield*

1. Introduction

Maize (Zea mays L) is one of the staple food crop grown under varied agro-climatic conditions across the world. It is cultivated on nearly 150 mha in about 160 countries having wider diversity of soil, climate and management practices that contributes 36 % (782 mt) in the global grain production. In India, maize is the third most important food crops after rice and wheat. It contributes nearly 9% to the national food grain production. In addition to staple food for humans and quality feed for animals, maize serves as a basic raw material for nearly thousands of food products some of which are, starch, oil, protein, alcoholic beverages, food sweeteners, pharmaceutical, cosmetic, film, textile, gum, package and paper industries etc. A major shift in global cereal demand is underway and by 2020, demand for maize in developing countries is expected to exceed demand for both wheat and rice (Pingali and Pandey, 2001).

Maize being grown under rainfed conditions throughout the world thus, it experience insufficiency soil water to carry out normal metabolic activities. Drought a worldwide phenomenon and is a major production constraint, reducing crop yields (Toker et al. 2007).Drought stress particularly damages grain yield (Table 1) if it occurs at flowering and grain filling stage (Heisey and Edmeades, 1999). The most critical period for water stress in maize is ten to fourteen days before and after flowering Grain yield reduction is 2-3 times more when water deficit coincides with flowering compared with other growing stages (Grant et al. 1989).The extent of climate change over the next 20 years and its impact are difficult to predict but it is essential to put research in place now that will be needed in the longer term. The unpredictability of drought, geographically and across seasons, has emphasized the importance of drought tolerance as a maize breeding objective.

Sl. No	Crop	Growth Stage	Yield Reduction	References
1	Barley	Seed filling	49–57%	Samarah (2005)
2	Maize	Grain filling	79–81%	Monneveux et al. (2005
3	Maize	Reproductive	63–87%	Kamara et al. (2003
4	Maize	Reproductive	70–47%	Chapman and Edmeades (1999)
5	Maize	Vegetative	25–60%	Atteya A,M. (2003)
6	Maize	Reproductive	32–92%	Atteya A, M. (2003)
7	Rice	Reproductive	53–92%	Lafitte et al. (2007)
8	Rice	Reproductive	48–94%	Lafitte et al. (2007)
9	Rice	Grain filling	30–55%	Basnayake et al. (2006)
10	Rice	Grain filling	60%	Basnayake et al. (2006)
11	Rice	Reproductive	24–84%	Venuprasad et al. (2007)

Table 1: Yield loss status due to moisture stress among the major cereal crops

To address the complexity of plant responses to drought, it is vital to understand the physiological and genetic basis of stress response. Failure to understand the molecular mechanismsof seed yield stability has hampered the traditional breeding and the use of modern genetics in the improvement of drought tolerance of crop plants (Passioura 2010; Sinclair 2011). The present paper will overview both conventional and molecular breeding approaches towards development of maize cultivars for limited water condition and will briefly describe a breeding strategy applied to screen moisture stress tolerance in maize inbreds conducted at University of Agricultural Sciences, Dharwad, Karnataka, India, as a post doctoral research objective.

1.1. Physiology of Drought Tolerance

The physiological dissection of complex traits like drought is a first step to understand the genetic control of tolerance and will ultimately enhance the efficiency of breeding strategies. Moisture stress affects cell elongation, cell division, modify root morphology, reproductive tissues, biomass production and ultimately grain yield. Drought is often accompanied by relatively high temperatures, which promote evapo-transpiration and affects photosynthetic rate, thus intensifying the effects of drought and further reducing crop yields. Physiological response of plant to water stress are, leaf wilting, reduction in leaf area, leaf abscission and stimulation root growth.

1.2. Effect of Moisture Stress on Photosynthetic Rate and in Turn Grain Yield

Drought stress is perceived by unknown mechanism which activates signaling cascades such as, ABA, H_2O and Calcium. These cascades activates synthesis of specific protein kinases which activates different downstream responses like, a) ABA signaling lead to closure of stomata and decreased CO_2 influx that directs the production of Reactive Oxygen Species (*Peroxidases, Superoxides, Hydroxyl radicals, hydrogen peroxides* & *Singlet oxygen*) which attack on membranes and disturb ATP synthesis pathway thus, photosynthetic rate. b) Reduced tissue water potential due to moisture stress increases Rubisco binding inhibitors thus limits activity of *Rubisco, PEP corboxylase, NADP malic enzyme, Fructose 1,6, bisphosphate* and *Pyruvate orthophosphate* thus photosynthetic rate and grain yield (Fig 1).

1.3. Genetics of Drought Tolerance

Drought tolerance is a complex genetic and physiologic trait. Most plant processes which are critical in drought tolerance have little inheritance and show continual variation and are also under the influence of environmental conditions (Tuberosa and Salvi 2006).Tolerance to drought is a complex quantitative trait controlled by several small effect genes or QTLs and is often confounded by differences in plants phenology (Barnabas et al. 2008; Fleury et al. 2010). Previous genetic studies revealed that both additive and dominance gene effects in inheritance are included in almost all traits related to drought (Shri et al. 2010). In maize, about 148 QTLs for grain yield have been detected. However, fewer QTLs were identified (Table 2) under water-stressed conditions (Sharp 2001). To maximize the impact of using specific traits, breeding strategies requires a detailed knowledge of the environment where the crop is grown, genotype X environment interactions and fine tuning the genotypes suited for local environments.

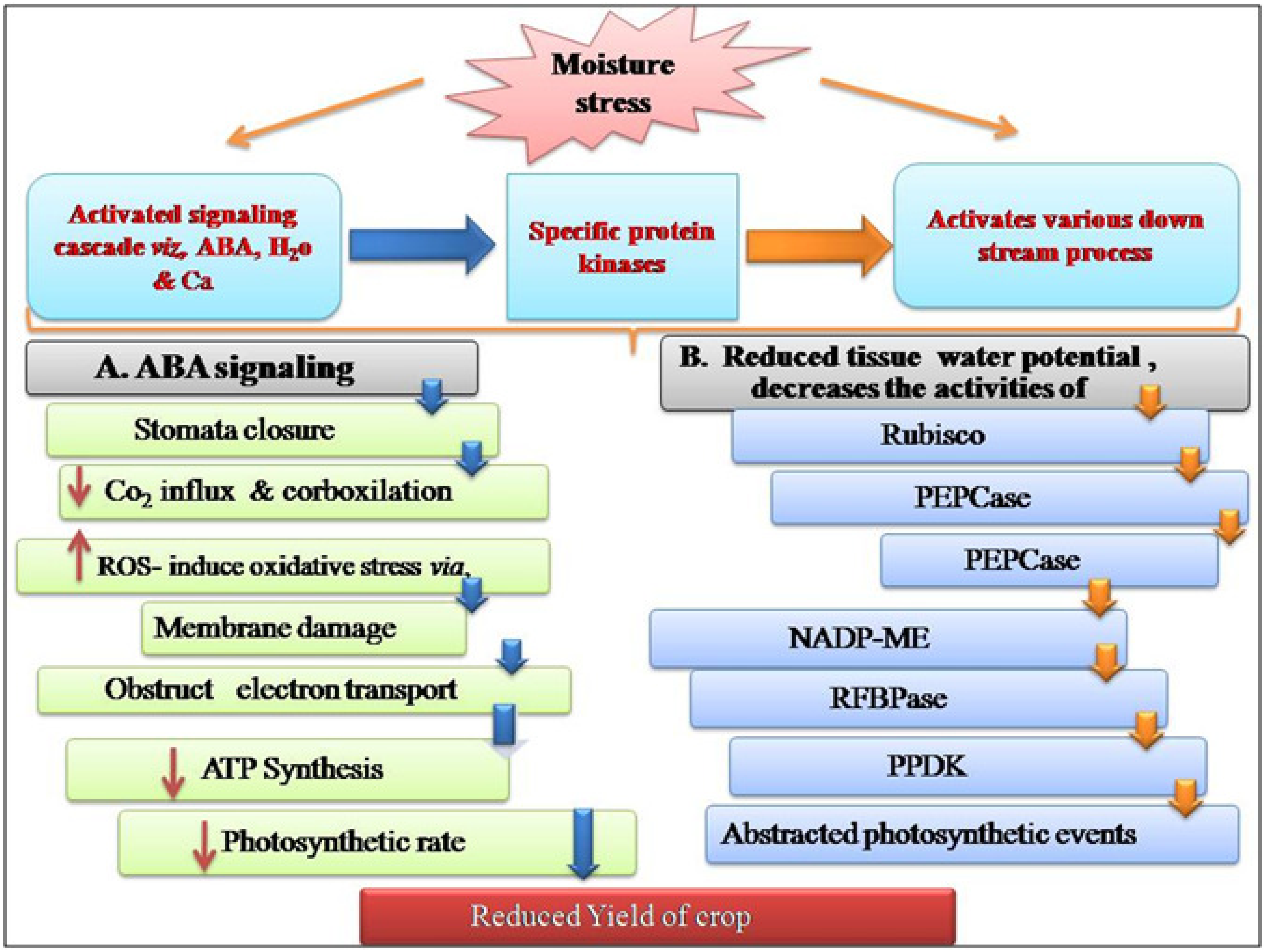

Figure 1: Physiological changes occurred during moisture limited situations which lead to reduction in grain yield

Traits studied	No. of QTLs	Linkage group	(PVE %)	Reference
Yield components and secondary traits	81	1-10	0.1–17.9	Messmer et al. (2009)
Grain yield and yield components	20	1, 2, 3, 5, 7, 8, 9	4.1–31.3	Xiao et al. (2005)
Root characteristics, drought tolerance index and yield	56	All chromosomes	6.7–47.2	Tuberosa et al. (2002)
Leaf ABA	1	2	32	Landi et al. (2005)
Grain yield and yield components	46	All, except 10	4.0–12.9)	Ribaut et al. (1997
Anthesis–silking interval	6	1, 2, 5, 6, 8, 10	48 (total)	Ribaut et al. (1996)

Table 2: QTLs for the traits associated with moisture stress tolerance in maize

1.3.1. The Various Levels at Which Moisture Stress Tolerance Is Associated

Tolerance to moisture stress is combined phenomenon exhibited at whole plant, cellular and metabolic level as well as at genetic level. Leaf rolling or reduced leaf area, stomata closure, cuticular wax formation, adjustment to sink-source allocation through altered root depth and density of root hair development are the traits contributing to tolerance at whole plant level. At cellular and metabolic level, osmotic adjustments through production of proline and glysine, cell membrane stability, turgor maintenance, protoplasmic resistance and dormancy are the mechanisms contribute to tolerance. Expression of complex array of drought specific genes or the genes associated with, a) signal transduction pathway and transcription control, b) membrane and protein protection function and c) water, ion uptake and transport functions play a major role in importing tolerance to moisture stress at genetic level. Thus, conventional breeding approaches should concentrate on selection, improvement and identification of the traits associated moisture stress tolerance whereas, molecular breeding approaches should identify and introgress the genes/QTLs responsible for moisture stress tolerance.

1.4. Environment for Moisture Stress Tolerance Screening

Screening for drought resistance could be conducted in the managed field or Rain out shelter (ROS). Field selection can be undertaken in very low rainfall environments (<100 mm), where water-stress conditions can be more readily controlled by the use of supplementary irrigation. Artificially induced moisture stress conditions that is, withholding the irrigation or maintaining soil moisture at <45% of field capacity during critical growth periods (10 days before and after Silking) would also be a recommended for screening. Selection under managed levels of drought stress at one location together with multi location testing may be desirable in breeding maize for moisture stress tolerance.

Early work in maize suggested that selection under dry-land conditions may significantly reduce selection gains (Arboleda-Rivera and Compton 1974) whereas, selection under irrigated conditions may have some spill-over to dry-land conditions (Johnson and Geadelmann 1989). As a consequence, many breeders adopted selection under high potential conditions followed by extensive multi-environment testing as the most effective approach to maize improvement. Theoretical considerations suggest that testing sites should be representative of production conditions and selection decisions weighted according to the relative economic value of the crop produced under stress and non-stress conditions (Rosielle and Hamblin 1981).

1.5. Phenotyping for Moisture Stress Tolerance

Field based managed environment provide a practical method for phenotyping for moisture stress tolerance (Weber et al. 2012; Rebetzke et al.2013). Recent advances in crop physiology, systematic phenotyping and genomics have led to new insights in drought tolerance, thus providing crop breeders with greater knowledge of the gene networks and providing new tools for plant improvement to increase crop yield (Tuberosa and Salvi 2006). Plant physiology improves our understanding of the complex network of drought tolerance- related traits thus improving selection efficiency. Molecular approaches identify the candidate genes and QTLs associated with these traits. Candidate genes are the prime targets for generating transgenics using genetic engineering (Varshney et al. 2011).

1.6. Secondary Traits Associated with Moisture Stress Tolerance

Selection based on grain yield alone is inefficient due to decline in heritability under stress (monnereax et al. 2005) therefore; secondary traits are used for selection under drought. The traits such as, deep root systems, long upright leaves, medium sized tassel, short anthesis-silking interval, early maturity, waxy cuticle, heavy glaucousness, dense pubescence, stay-green characteristics and high harvest index (Slafer et al.2005) were considered as secondary traits. These traits can be modified through conventional breeding techniques, such as pedigree breeding, backcross breeding, bulk-population breeding, recurrent selection, and gene transference using biotechnology. There has been some success using conventional breeding for improved drought resistance by selection for one or more secondary traits conferring drought tolerance (Zaidi et al. 2004).

2. Breeding Approaches for Identification and Development of Moisture Stress Tolerance

2.1. Screening for Moisture Stress Tolerance Based on Physio-Biochemical Parameters

Physiological traits associated with moisture stress tolerance (Table 3) in maize are Relative Water Content (Ability of a plant to retain cellular water under water deficit stress can be referred to as RWC, Barr and Weatherly, 1962), Specific Leaf Weight (SLW), Chlorophyll content, Total Soluble Protein (TSP), Total Soluble Sugars (TSS), Prolline, Glycinebetaine, accumulation of ABA, cell wall Plasticity, Turger maintenance, Protoplasmic resistance, dormancy, canopy temperature and stomata closure. In maize, moisture stress alters sugar level such as Raffinose, which plays an important role in maintaining cell wall plasticity. Cellular structures will get damaged if cell wall lost its plasticity due to moisture stress therefore, ability of cell wall to maintain its shape in such a manner that enhance cell wall turger and improves plants ability to tolerate drought. Various researchers utilized above parameters to screen maize inbreds for tolerance to moisture stress and some of which are overviewed below.

Sl.No	Parameter	Formulae
1	Total Chlorophyll Content mg/ml	$=20.2 \times \text{Chlorophyll a} + 8.02 \times \text{Chlorophyll b}$ or SPAD 502 readings
2	Total Soluble Protein Content (mg/g)	$= \frac{\text{Absorbance of sample} \times \text{K Value} \times \text{Dilution Factor}}{\text{Weight of Sample} \times 100}$
3	Total Soluble Sugar Content (mg/g)	$= \frac{\text{Absorbance of sample} \times \text{K Value} \times \text{Dilution Factor}}{\text{Weight of Sample} \times 100}$
4	Proline Content (mg/g):	$= \frac{\text{Absorbance of sample} \times \text{K Value} \times \text{Dilution Factor}}{\text{Weight of Sample} \times 100}$
5	Specific Leaf Weight (mg/cm^2)	= Dry Weight / Unit Leaf Area
6	RWC (%)	$= \frac{\text{WF} - \text{WD}}{\text{WT} - \text{WD}} \text{ x } 100$
7	Excised leaf water retention	$= 1 - \frac{\text{WF} - \text{W3}}{\text{WF}}$

8	Excised leaf water loss	$= \frac{\mathrm{WF} - \mathrm{W3}}{\mathrm{WF} - \mathrm{WD}}$
9	Leaf water loss	$= \frac{\mathrm{WF} - \mathrm{W1}}{\mathrm{WF}}$
10	Normalized Difference Vegetation Index	$= \frac{\mathrm{NIR} - \mathrm{Red}}{\mathrm{NIR} + \mathrm{Red}}$

Table 3: Physiological parameters for moisture stress tolerance screening

Where,

- → WF - leaf fresh weight,
- → WD -leaf dry weight (by leaving the leaves in the oven with 80˚ C for 24 hours),
- → WT - turgidity weight (by immersing the leaves in distilled water for 18 to 20 hours),
- → W1, W2, and W3 -are weight of the leaf after 2, 4, and 6 hours of being shed from the plant
- → NIR-Reflectance at blue channel
- → Red- Reflectance at red channel

Measuring the temperature of plant green cover is an effective criterion for discovering water stress situation. Temperature of plant crown increases along with severity of drought stress due to, closure of stomata and cessation of respiration it lead to moisture stress tolerance therefore, inbred lines showing high canopy temperature considered to be tolerant. Considering the canopy temperature as criteria, Abbaset al. 2014, screened maize hybrids SC400, ZP434, SC524, ZP599, BC66, SC704 for tolerance by taking observations at three different stages like stem elongation stage, taselling stage and blistering stage. They identified SC704 as tolerant since it showed high canopy temperature at blistering stage. Chen et al,2012, identified tolerant inbred lines Tx205, C2A554-4, and B76 as they maintained relatively high leaf relative water content and also showed significantly greater ability to maintain vegetative growth and alleviate damage to reproductive tissues under drought conditions compared to the sensitive lines (B73 and C273A) when subjected to drought stress.

Cultivars EV- 1097 and Agaiti-2002 were the best performers, showing maximum cell wall plasticity, having the highest leaf growth rate, proline, protein, sugar and relative water contents, as well as the highest specific leaf weight, leaf water potential, and chlorophyll content compared with other cultivarsSawaan-3,Islamabad Gold and EV-1098 for tolerance to water deficit based on their cell wallplasticity characteristics by Mehmood et al.2013.

2.2. Screening for Moisture Stress Tolerance Based on Root Parameters

Roots are the first organs to sense water shortage, maize respond to drought stress by redirecting dry matter accumulation away from the shoot to root, it lead to increase in root cell wall extensibility that is mediated by increased levels of *Xyloglycan Endotransglucosylases* and other cell wall loosening factors at the root tip. These modifications result in sustained growth of the root and inhibited growth of the shoot in the face of decreased water potential (Ober and Sharp 2007). Avoidance of water stress by effective root water uptake is considered a promising approach to yield stability in water limiting environments. Water uptake efficiency is the result of multiple plant root traits that dynamically interact with site hydrology.

Rongyao Li, 2015 compared root architectures of 103 maize lines under well-watered and water-stressed conditions using *WinRhizoPro 2007a root analyzer* system.Traits such as Total root length (TRL) and totalroot surface area (TSA) had high phenotypic diversity, and TRL was positively correlated with TSA, root volume, and root forks. The first two principal components (TRL & TSA) explained 94.01% and 91.15% of total root variation in well-watered and water-stressed conditions, respectively. Thus, TRL and TSA, major contributors toroot variation, can be used as favorable selection criteria for drought tolerance at the seedling stage.Abdul et al. 2012, considered number of crown roots, number of seminal roots, primary root length, number of lateral roots, fresh root weight and dry root weight as parameter to assess the moisture stress tolerance using hybrids of tropical yellow of which, H3, H4, H8, H11, H15, H19 and highland yellow H27, H29 showed best performance under the drought conditions.

2.3. Selection for Moisture Stress Tolerance Based on Drought Tolerant Indices

Drought tolerance selection is not easy due togenotypes X environment interactions and restricted knowledge about the function and role of tolerance mechanisms. Various researchers have used different methods to evaluate genetic differences in drought tolerance. According to Fernandez (1992) the best measure for selection in drought condition could be able to separate genotypes which have desirable and similar yield in stress and non-tress condition from other groups and also, the best indices are those which have high correlation with kernel yield in both conditions. Several selection indices have been proposed to select genotypes based on their performance in stress and non-stress environments. Indices are calculated using individual and over all mean yield data from stressed and non stressed field. (Table 4)

Rosielle and Hamblin (1981) demonstrated that lower the stress tolerance index (STI), hybrid yield in normal irrigation and drought condition is close to each other. According to Blum (1988) drought resistance index (DI), used to identify genotypes producing high yield under both stress and non-stress conditions. Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield between stress and irrigated environments and mean productivity (MP) as the average yield of genotypes under stress and non-stress conditions. The geometric mean productivity (GMP) is often used to predict relative performance. Fischer and Maurer (1978) suggested the stress susceptibility index (SSI) for measurement of yield stability that apprehended the changes in both potential and actual yields in variable environments. The yield index (YI) suggested by Gavuzzi et al.1997 and yield stability index (YSI) suggested by Bouslama and Schapaugh (1984) in order to evaluation the stability of genotypes in the both stress and non-stress conditions. To improve the efficiency of STI a modified stress tolerance index (MSTI) was suggested by Farshadfar and Sutka (2002). Moosavi et al. (2008) introduced stress susceptibility percentage index (SSPI) for screening drought tolerant genotypes in stress and non-stress conditions.

Sl. No	Indices	Formulae
1	Stress susceptibility index (SSI)	$= (1-(Ys/Yp))/(1-(\bar{Y} s/\bar{Y} p))$
2	Relative drought index (RDI)	$=(Ys/Yp)/ (\bar{Y} s/\bar{Y} p)$
3	Stress tolerance index (STI)	$=(Ys *Yp)/(\bar{Y} p2)$
4	Geometric mean production (GMP)	$=\sqrt{}Ys*Yp$
5	Tolerance index (TOL)	$=Ys-Yp$
6	Mean production (MP)	$=(Ys+Yp)/2$
7	Golden mean (GOL)	$=Yp+Ys/Yp-Ys$
8	Harmonic mean (HARM)	$= 2(Yp)(Ys)/Yp+Ys$
9	Yield index (YI)	$=(Ys)/(\bar{Y} s)$
10	Drought resistance index (DI)	$=(Ys*(Ys/Yp))/\bar{Y}s$
11	Yield stability index (YSI)	$=Ys/Yp$
12	Stress susceptibility percentage index (SSPI)	$=(Yp-Ys/2(\bar{Y} p))*100$
13	Modified stress tolerance (KiSTI, K1)	$=Yp^2/\bar{Y} p^2$
14	Modified stress tolerance (KiSTI, K2)	$=Ys^2/\bar{Y} s^2$

Table 4: Drought tolerant/Resistant indices for moisture stress tolerance screening

Where,
Ys, and Yp, represent yield under stress and yield under non-stress for each cultivar,
$\bar{Y}$ *s* and $\bar{Y}$ p yield mean in stress and non-stress conditions for all cultivars.

Zahra and Jahad, 2011, considered maize cultivars MO17, K19, K18, A679, K3651/1, K166A, K166B to evaluate for tolerance based on MP, TOL, SSI, STI and GMP and confirmed, MP, GMP and STI indices as the more accurate criteria for selection of drought tolerant and high yielding inbred lines. The positive and significant correlation of STI and grain yield under all conditions revealed that this index is more applicable and efficient for selection of parental inbred lines. Based on the STI, GMP and MP indices, K166B proved to be the most drought tolerant line. Moradi et al. 2012, identified KSC704 and H4 as tolerant hybrids based on high mean values of MP, GMP, STI and HM

Mohammad et al. 2013, identified the best indices to screen drought tolerance based on their positive and significant correlation with yield in stress and non stress condition that is, STI, GMP, MP, YI, TOL, DI, RDI, YSI, SSPI, K1STI and K2STI with yield. Thus, cultivars KSC720, KSC 710GT and 'KSC 700' were considered as drought tolerant. Similarly Masoud Kiani, 2013, stated K104/3, K760/7 and K126/10 as tolerant genotypes based on drought tolerant indices.
A study conducted to screen moisture stress tolerance of ninety eight maize inbreds under induced moisture stress conditions, based on drought tolerant indices at University of Agricultural Sciences, Dharwad, during *Summer* 2015 showed that, of the ninety eight inbreds onlythirty one were tolerant based on minimum yield reduction (TOL) in stress condition. Among 31 inbreds, MLB34 (0.28), BLSB7-1 (0.31), PDM 6572 (0.37), PDM 6541 (0.22) and PDM 6529 (0.29) were highly tolerant. Tolerant indices viz.,STI (91.5%), GMP (92.8%) and MP (92.8%) showed high heritability coupled with high genetic advance over percent mean are most appropriate indices which determine drought tolerance (Table 5).

2.4. Selection for Moisture Stress Tolerance Based on Agronomic Traits

Moses and Abebe 2014, evaluated ninety-six elite maize hybrids alongside four hybrid checks for grain yield and other agronomic traits under managed stress conditions over two seasons, hybrids differed significantly for grain yieldand other measured traits under both drought stress and well-watered conditions. Three hybrids namely, ADL47 X EXL15,ADL41 X EXL15 and EXL02 X ADL47, produced competitive yields under both irrigation treatments had least ASI and long duration of leaf greenness or stay green trait. As discussed by Campos et al, 2004, Selection based on performance in multi-environment trials (MET) has increased grain yield under drought through increased yield potential and kernel set, rapid silk exertion, and reduced barrenness, though at a lower rate than under optimal conditions.

INBRED	GYPP(WW)	GYPP(MS)	SSI	RDI	STI	GMP	TOL	MP	GOL	HARM	YI	DI	YSI	SSPI	K1	K2
PDM-4061	80.50	118.50	1.17	0.94	2.80	97.67	38.00	139.75	05.24	95.87	1.38	0.94	0.68	23.63	2.17	1.90
PDM-4191	85.00	96.50	0.44	1.21	2.41	90.55	11.50	133.25	17.58	90.34	1.46	1.28	0.88	07.15	1.44	2.13
PDM-4201	57.00	88.00	1.28	0.89	1.47	70.80	31.00	101.00	04.78	69.13	0.98	0.63	0.65	19.27	1.20	0.95
PDM-4211	78.00	97.00	0.71	1.11	2.22	86.92	19.00	126.50	10.30	86.35	1.34	1.08	0.80	11.81	1.46	1.79
PDM-4491	62.00	89.50	1.12	0.95	1.63	74.48	27.50	106.75	05.55	73.23	1.06	0.74	0.69	17.10	1.24	1.13
PDM-4791	67.50	83.50	0.69	1.12	1.65	75.04	16.00	109.25	10.37	74.58	1.16	0.94	0.81	09.95	1.08	1.34
PDM-6505	59.50	99.50	1.47	0.82	1.73	76.68	40.00	109.25	04.30	73.99	1.02	0.63	0.60	24.87	1.53	1.06
PDM-6508	54.50	63.00	0.49	1.19	1.01	58.59	8.50	86.00	14.04	58.44	0.93	0.81	0.87	05.28	0.62	0.88
PDM-6515	76.00	102.00	0.93	1.02	2.28	88.04	26.00	127.00	06.87	87.08	1.30	0.97	0.74	16.17	1.61	1.70
PDM-6516	82.00	122.50	1.21	0.92	2.94	100.19	40.50	143.25	05.12	98.17	1.40	0.94	0.67	25.18	2.32	1.98
PDM-6518	62.00	70.68	0.45	1.21	1.28	66.19	8.68	97.34	15.82	66.04	1.06	0.93	0.88	05.39	0.77	1.13
PDM-6528	112.00	136.00	0.64	1.13	4.47	123.42	24.00	180.00	10.33	122.84	1.92	1.58	0.82	14.92	2.86	3.68
PDM-6529	96.50	104.50	0.29	1.27	3.00	100.41	8.00	148.75	27.57	100.32	1.65	1.53	0.92	04.97	1.71	2.78
PDM-6541	87.00	92.50	0.22	1.29	2.36	89.71	5.50	133.25	33.00	89.66	1.49	1.40	0.94	03.42	1.32	2.22
DM-6547	131.00	148.50	0.42	1.22	5.70	139.36	17.50	205.25	27.75	138.98	2.24	1.99	0.89	10.88	3.42	5.03
PDM-6547	116.50	131.50	0.41	1.22	4.51	123.77	15.00	182.25	16.70	123.54	1.99	1.77	0.89	09.33	2.68	3.99
PDM-6550	53.88	72.00	0.94	1.02	1.16	62.19	18.13	89.88	07.54	61.46	0.92	0.70	0.74	11.27	0.81	0.88
PDM-6554	81.67	108.50	0.89	1.04	2.60	94.09	26.84	135.92	07.47	93.11	1.40	1.06	0.76	16.68	1.83	1.96
PDM-6563	94.50	110.50	0.49	1.19	3.08	102.07	16.00	149.75	22.83	101.65	1.62	1.40	0.87	09.95	1.92	2.62
PDM-6567	74.50	85.00	0.45	1.21	1.86	79.52	10.50	117.00	21.31	79.30	1.28	1.12	0.88	06.53	1.12	1.64
PDM-6571	77.50	90.00	0.50	1.19	2.04	83.47	12.50	122.50	16.68	83.19	1.33	1.15	0.86	07.77	1.25	1.76
PDM-6572	118.00	131.50	0.37	1.24	4.56	124.55	13.50	183.75	20.39	124.36	2.02	1.81	0.90	08.39	2.69	4.09
PDM-6573	83.50	105.00	0.75	1.09	2.58	93.63	21.50	136.00	08.76	93.02	1.43	1.14	0.79	13.37	1.71	2.05
PDM-6576	48.00	97.00	1.84	0.68	1.36	68.20	49.00	96.50	02.98	64.17	0.82	0.41	0.50	30.47	1.46	0.68
MLB-28-1	68.00	96.50	1.08	0.97	1.93	80.97	28.50	116.25	05.90	79.71	1.16	0.82	0.70	17.72	1.44	1.37
MLB33-1	73.50	110.50	1.22	0.92	2.39	90.12	37.00	128.75	05.00	88.27	1.26	0.84	0.67	23.00	1.90	1.59
MLB34	110.50	119.50	0.28	1.27	3.89	114.88	9.00	170.25	38.57	114.75	1.89	1.75	0.92	05.60	2.21	3.62
BLSB7-1	65.67	72.00	0.31	1.26	1.39	68.74	6.34	101.67	31.52	68.65	1.12	1.03	0.92	03.94	0.81	1.27
BLSB8-1	100.00	117.50	0.55	1.17	3.45	108.29	17.50	158.75	16.76	107.83	1.71	1.47	0.85	10.88	2.14	2.97
HKI-163	110.00	131.00	0.51	1.18	4.32	119.87	21.00	175.50	23.90	119.25	1.88	1.60	0.86	13.06	2.77	3.59
H^2 (bs)	0.91	0.91	0.55	0.548	0.91	0.93	0.58	0.93	0.35	0.92	0.91	0.85	0.55	0.58	0.90	0.90
GAM	95.50	74.98	72.60	28.209	171.98	85.40	89.74	86.42	72.04	87.87	95.44	111.91	28.13	89.74	149.11	187

Table 5: Drought Tolerant Indices of top 31 out of 98 inbreds screened under stress and non stress conditions during Summer -2016

3. Molecular Breeding Approaches for Improvement and Development of Drought Tolerance

3.1. Marker Assisted Selection and Back Crossing to Transfer Qtls Associated with Tolerance

Identifying the markers linked with target gene and mapping its chromosome locus is important goal in plant breeding for Marker Assisted Selection (MAS). If the selection is made based on genotype by DNA markers, the efficiency of selection will increase considerably. In a genetic evaluation program, the combination between the data from the linkage between marker position and quantitative traits loci (QTL) as well as the phenotypic data can be used to increase the accuracy of the assessments and thereby the accuracy of selection.

Earlier reporters suggested that stay-green is a trait directly associated with drought tolerance in maize. Identification of markers and QTLs linked to stay green characters useful in MAS to transfer them into a high yielding but susceptible drought cultivars. Ai-yu Wang 2012,studied on the F_2 population derived from the cross A150-3-2 (a stay-green inbred line) and Mo17 (a normal inbred line) and identified14 QTLs for three stay-green related traits like, green leaf area per plant at 30 DAF, green leaf area per plant at the grain-ripening stage, and left green leaf number per plant at the grain-ripening stage. Single QTL explained from 3.16 to 12.50% of the phenotypic variance. These results will be helpful to the maize breeders for marker-assisted selection.Similarly QTLs associated with the traits contributing to yield such as ASI, plant height, grain yield, ear height, and ear setting were studied for two successive years (2008 and 2009) by Zhu Jing-jing, 2011 using the F_2 population derived from a cross between D5 (resistant parent) and 7924 (susceptible parent). On an average for each trait four different QTLs were identified. The universal QTLs information generated in this study will aid inundertaking an integrated breeding strategy for further genetic studies in drought tolerance improvement in maize.Jean and Michel, 2007, reported a successful story about introgression of favorable alleles involved in the expression of yield components and flowering traits increased grain yield and reduced the ASI under water-limited conditions. Selected MABC-derived BC_2F_3 families were crossed with two testers and evaluated under different water regimes. Mean grain yield of MABC-derived hybrids was consistently higher than that of control hybrids under severe water stress conditions.

3.2. Candidate Genes for Drought Tolerance in Maize

Jie Xu et al.2014, surveyed maize reference genome (B73) and 15 inbred lines to determine the SNP diversity and used three each of extremely tolerant and susceptible lines to identify candidate genes associated with drought tolerance. A total of 524 nsSNPs that were associated with 271 candidate genes involved in plant hormoneregulation, carbohydrate and sugar metabolism, signaling molecules regulation, redox reaction and acclimation ofphotosynthesis to environment were detected by CV and cluster analyses. Changes of expression level in these candidate genes fordrought tolerance were detected using RNA sequencing for fertilized ovary, basal leaf meristem tissue and rootscollected under drought stressed and well-watered conditions. Instead of QTLs candidate gene alone can be transferred to cultivar of interest to import drought tolerance.

3.3. Transgenic Approach for Tolerance to Moisture Stress in Maize

Maize transgenic event MON810, bears resistance to European corn borer, is the widely cultivated GM event in the European Union. It contains a stable, genome-integrated plant expression cassette comprised of the cauliflower mosaic virus 35S promoter and HSP70 maize intron sequence, driving the expression of a synthetic CryIAb gene. MON810, variety DKC6575, and the corresponding near-isogenic line Tietar were studied in different growth conditions, to compare their response to drought. Main photosynthetic parameters were significantly affected by drought stress in both GM and non-GM varieties but extent of reduction is high in isogenic line compared to MON810 suggesting suitability of it to moisture stress situation (Mariolina et al. 2015).

4. Conclusion

Germplasm, genetics, physiology, phenotyping and selection, combined with a clear definition of target product, are the foundation for maize crop improvement. Maize undergoes moisture stress during its growing period since majority of its acreage is under rainfed condition. Compared with other members of cereals, maize is more sensitive to moisture stress if, occurred during reproductive stage and can lead to 70-80% yield loss. Tolerance or resistance to moisture stress is a complicate phenomenon controlled by many genes, influenced by environmental effects and associated with various physio-biochemical parameters. To address the effect of moisture stress on the crop understanding the physiology and genetics of tolerance is essential. Breeding approaches considering primary (yield), secondary, physiological traits and based on molecular and transgenic techniques have identified and developed breeding materials or parents for hybridization or introgression programme to develop a cultivar that perform stably under moisture limited conditions. Considering the experimental findings of various researchers in the field of maize moisture stress tolerance, it is clearly understood that, integrated breeding approaches are valuable techniques to develop breeding material and to derive cultivars tolerant/resistant to moisture stress situation (Fig 2).

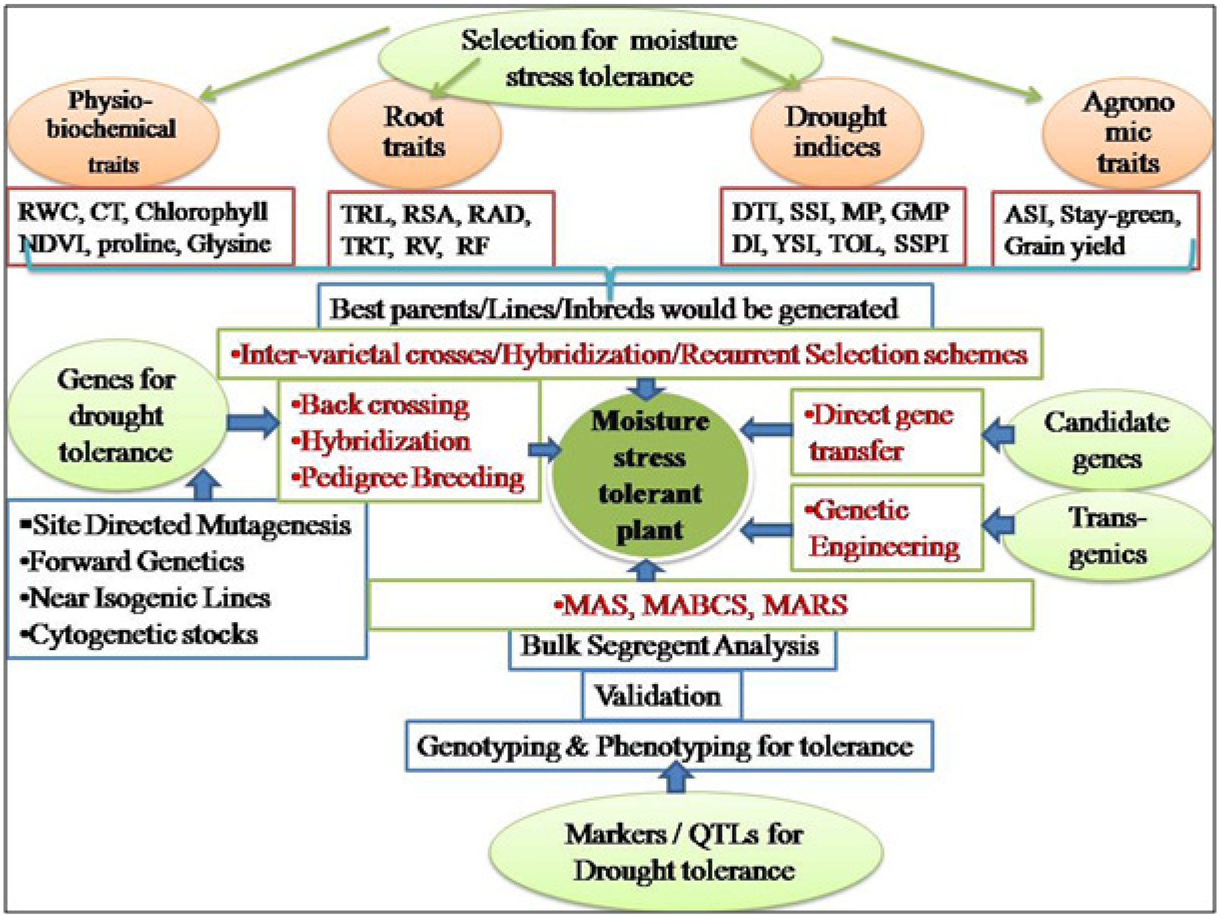

Figure 2: An integrated breeding approaches for improvement and development of moisture stress tolerance in maize

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