GM Maize for Abiotic Stresses: Potentials and Opportunities

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1 Introduction

In Africa, Asia, and Latin America, maize serves as the basis of food security but the production rate of maize in most developing countries is very low. Per unit area production of maize in developing countries is about 20% of the average production in developed countries. Over the past 10,000 years, maize has evolved from its wild grass progenitor teosinte (*Zea mays*, *Zea* spp.), which arose in Southwestern Mexico. Maize has become a major food and staple resource through the years of cultivation and substantial selection for traits favoring temperate areas. In many instances, genetic diversity has decreased because of domestication and artificial selection, and favorable genes/alleles have vanished from wild precursors that were formerly associated with environmental stress tolerance. For example, the *ZmWAK* locus, which contained resistance to head smut, has been lost from the

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teosinte ancestry. Therefore, the production rate of maize is limited by many factors, including biotic and abiotic stresses. In tropical and subtropical regions, drought is a highly significant restraint to increasing the production of maize. Fertility of soil is also a major limitation in maize production because less fertilizer is being used and toxic elements are present (Byerlee et al. 2007; Farooq et al. 2015). The changes in the global climate, amalgamation of heat, drought stress, and excess moisture, coupled with susceptibility to rising diseases and insect pests, are increased in some regions, especially in Asia and Africa. Losses from abiotic stresses as well as biotic stresses such as disease, insect pest, and weeds have reduced average yield by more than 30%. It is estimated that annually 54% of yield has been lost to insects, diseases, weeds, and animals in Africa, and 48% in Central and Southern America and 42% in Asia (Oerke 2006). The yield of maize is reduced by insect pests which directly damage stems, leaves, stalks, grains, ears, and tassels: 18% of maize production is lost annually to the stem borers, the most damaging group of insect pests (De Groote 2002).

During the past two centuries, methods of conventional breeding have been used to enhance and improve the production rate as well the quality of grain. These methods have been successful in the form of hybrid technology and increased yield many fold; however, the increasing demands for maize mean that conventional maize breeding methods need addition support to meet the desired production rate. Similarly, quality is also important in providing healthy food to the population (Barampuram and Zhang 2011). To keep pace for increasing yield and grain quality, transgenic technology was introduced in the mid-1980s and now is being improved and used extensively to enhance production and also to improve the grain quality in a short duration of time. Several methods have been used to produce transgenic plants: either the direct method of gene transformation such as biolistic transformation, silicon carbide fibers, electroporation, and native gene transfer, or by indirect methods of transformation such as through *Agrobacterium tumefaciens*-mediated gene transformation (Jones 2009). Biolistic- and *Agrobacterium*-mediated transformation are the two most popular and best studied methods of transformation. Through transgenic technology, a desired gene can be introduced to develop plants with the desired traits such as high nutritional value and resistance against herbicides, drought, and heat stress (Husaini et al. 2010).

1.1 Maize Production in the World

The global output of maize in 2017–2018 was about 1033.75 million tons, 9.8% more than that of 2016–2017 (FAO Report 2018). Worldwide, the maize production rate and areas have changed dramatically from the past 50 years. The global area of maize production has been increased by 50%, from about 100 million hectares to more than 150 million hectares. Especially in developing countries, the cultivated area of maize has been increased, almost doubled from 60 million hectares in 1961 to 120 million hectares in 2010 (Shiferaw et al. 2011).

The yield of maize has been doubled by the use of improved maize genetic architecture in the form of hybrids, fertilizers, water, and pesticides, but it is difficult to meet the needs of increased food production aggravated by climate conditions (Evenson and Gollin 2003). The prime task of the future is to attain sufficient growth in food production in such a way that health, environmental quality, and farming systems are not compromised (Tilman et al. 2002).

1.2 Role of Maize in Food Security

Maize is a major source of food security and nutrition for millions of people all over the world. In 94 developing countries, more than 4.5 billion people obtain 30% of their food calories from maize today. By 2050, in developing countries the requirement of maize will double as the population becomes 9.0 billion (Rosegrant et al. 2009).

Maize is consumed equally by human beings, livestock, and poultry. Maize has a high nutritional composition, as its grain contains starch, vitamins A and B_3, oil, protein, sugar, and fibers. In terms of productivity and industrial products (fermentation and pharmaceuticals), maize is the most important cereal crop in the world. Maize is an ideal staple food because of its low price and the high consumption rate, particularly in those areas where deficiencies of micronutrients are a serious public health problem (Laillou et al. 2012).

For the meat industry, maize is the most important element of feed, particularly in Asia where maize has a high consumption rate from the high demand as poultry and pig feed. Every year, the worldwide demand for maize has been growing at the rate of 6% for livestock feed and is estimated as a vital element of future requirement. In developed countries, 30% of maize used for human consumption whereas 70% is used as feed. Maize is used for many purposes including food and fuel for humans and feed for livestock. The high nutritional value of maize promotes the use of its grain in industries as raw material for many products (Afzal et al. 2009). Maize is grown all over the world: United States, China, and Brazil are the top three maize-producing countries. Maize contains 72% starch, 4% fat, 2.1–27% fiber, 45–70% carbohydrates, and 10% protein: 365 kcal/100 g energy is provided by maize, in comparison to wheat and rice, but it has a lower protein content. Wheat, rice, and maize are considered to be 94% of all cereal utilization as the most important human food sources. Maize can be used to manufacture many products such as starch, beverages, industrial alcohol, glue, and fuel ethanol.

1.3 Role of Maize in Biofuel Production

Maize is used for the production of biofuel, which is mostly used as motor fuel. Maize is the prime feed material used to produce ethanol, and the price of maize has been increased by the high requirements of ethanol production. The demand of

maize has been increased in industry as it used in the bio-energy sector. The demand of maize in fuel production has been increased from the past 10 years. Maize has emerged as the prime source of biofuel because it can be stored without fermenting, which is not the case in competing crops such as sugarcane. Breeders have identified genotypes that are high yielders and more responsive for biofuel production. In USA, 40.5% of the corn-growing area was being used for ethanol production in 2011 (Mumm et al. 2014).

1.4 Seed Quality of Maize

In the world economy and trade, maize has an ascendant position as an industrial grain. Consequently, it is essential to ameliorate the industrial and nutritional characteristics of the grain by acquiring knowledge of the genes that control seed quality traits of protein, starch, oil, and other compounds. The composition of maize grains could be improved for quantity as well as quality of starch, protein, and oil by exploiting genetic variation. The prolific amount of storage protein called "zeins" is present in up to 60% in developing endosperm tissues. The kernel of maize has been extensively used not only for its starch content but also for the oil that accumulates in the embryo. By industrial processing of maize grains, oil is a high-value product and also used as a high-quality source of oil for human consumption. Oil and starch are stored in distinct niches of maize kernel: 85% of oil is accumulated in the embryo and 98% of starch is accumulated in the endosperm (Motto et al. 2012). The grain of maize is highly used for the production of corn flakes, grain cake, cornstarch, lactic acid and acetone, used by many industries such as food and fermentation as well as in the textile industries.

2 Effects of Abiotic Stresses

Such abiotic stresses as heat, high salt content, heavy metal toxicity, or less availability of nitrogen greatly influence crop production (Kellős et al. 2008). Under the climate change regime, these stresses are aggravated further, especially those of heat and drought (Bänziger et al. 2006). Reduction in anthesis to stalking, principally under the drought condition, has been reported for the greater grains which are up to 9.5% per cycle. Unimproved tall tropical germplasm, however, has been accompanied by their short plant height with less barrenness (Edmeades and Tollenaar 1990). The life cycle of every cereal has a phase in which their temperature shifts between the vegetative and the reproductive growth, determining its field utility. Working on climate change-bearing crop varieties is very important in general and temperature-resistant varieties in particular. An important role has been shown by genetic engineering in maize improvement across the world for insect resistance, herbicide tolerance and currently heat tolerance. Maize has the

specificity in its genotypes that grow only in the temperate zone (Khan et al. 2008 and Iqbal et al. 2010), requiring measuring conditions suitable for crops and the water retention potential for sowing in summer and spring seasons.

2.1 Effect of Heat on Maize Grain Quality

The two most important environmental factors that affect the growth of crops, its yield and development, are a shortage of water and heat stress (Prasad and Staggenborg 2009). Increasing temperatures at the crop growing season can disrupt the crop production system. At the growing season, storage temperature can reduce the crop production in two ways as rising temperatures enhance the growth of crops such as maize that reduce the time of grain and maize development by reducing the accomplishment of potential yield. Second, during extreme heat conditions, flowering of maize such as in the silk-tassel stage, pollination is hindered and the development of grain is completely intercepted. The duration of the growth cycle is reduced by the effects of temperature; in particular, the grain-filling stage is the most significant factor that reduces yield at very hot temperature (White and Reynolds 2003).

The yield of maize is a function of the quantity of grain and its weight as there is a strong relationship between grain weight and grain-filling stage. When the duration of filling period and grain-filling rate are optimal, then the full potential weight of the grain is attained. Maximum grain filling is attained at 25–32 °C, a moderate high temperature. High temperature enhances development of the plant but reduces the time period of grain filling. At temperatures higher than 32 °C, production of starch is diminished, affecting the grain-filling rate (Singletary et al. 1994). Above 32 °C, pollen is reduced in their capability to germinate on silk (Basra 2000); as a result, few grains are available for grain filling.

3 Transformation Systems in Maize

Transformation in maize has been tried by all the possible systems. The three major systems that have acquired routine status are biolistic transformation, *Agrobacterium*-mediated transformation, and in planta transformation. These are discussed now.

3.1 Transformation Through a Biolistic Gun

A meta-analysis of 21 (1996–2016) years of field data reported through extensive publications on transgenic maize showed approximately 6% increase in yield compared to near-isogenic lines and 30% reduction in various toxins including

mycotoxins, fumonisin, and thricotecens. This result shows considerable success of the technology when seen in the context of the various controversies that were raised (Pellegrino et al. 2018). To develop transgenic maize for the first time, Coe and Sarkar (1966) injected DNA in newly developed maize seedlings, but no phenotypical change was observed. Transformation of the crop progressed significantly with the development of biolistic technology that abolished the requirement to transfer naked DNA with the capability to integrate DNA across the plant cell wall and could be used for stable transformation. In the biolistic method, metal microparticles are physically coated with the desired gene and accelerated toward the target cell through a gene 'gun' (Sanford 1990) with adequate acceleration to penetrate the cell wall but not trigger cell death. Post bombardment, DNA coated on microparticles is released slowly into the cell and integrated into the genome (Taylor and Fauquet 2002). For the first time, Gordon-Kamm et al. (1990) and Fromm et al. (1990) reported maize transformation by integration of foreign DNA into the embryogenic callus through a biolistic gun and confirmed transgene trafficking to the next generation. Later, several reports on maize transformation showed that the biolistic gun is an efficient technique for successful integration of the transgene into the genome of maize and reproductive results (Brettschneider et al. 1997; Frame et al. 2000).

3.2 Agrobacterium-*Mediated Transformation*

Agrobacterium tumefaciens is a naturally occurring, soil-borne bacterium that naturally infects dicot species and causes crown gall disease. *Agrobacterium* can transfer DNA to dicot as well as monocot species. t-DNA (transfer DNA), an ambulant part of the Ti (tumor-inducing) plasmid, is transferred to the nucleus of the plant cell during *Agrobacterium* infection and integrated into the chromosomes of the plant (Hooykaas and Schilperoort 1992).

The delivery of t-DNA is imparted by genes that are present on another part of the Ti plasmid called the vir region. These virulence genes are not transferred with t-DNA but help in transformation. The t-DNA contains a border sequence, which is a 25-bp direct repeat present on both ends of t-DNA termed the left and right borders, which is recognized and extirpated by particular endonucleases. Phenolic compounds such as acetosyringone, which is produced by wounded cells of a plant, are involved in induction of vir genes that are present on the Ti plasmid, causing a nick on the lower strand of t-DNA through endonucleases. A single-stranded copy of t-DNA is transferred to the plant cell from *Agrobacterium* (Gelvin 2003), covered by a protein that protects it from plant cell nucleases.

For maize transformation, *Agrobacterium tumefaciens* has become the ideal method for delivery of the foreign gene. *Agrobacterium*-mediated transformation was reported for the first time in maize in the early 1990s, and these protocols have since been improving because of the several advantages over other transformation

methods such as stable integration of intact transgenes, stable expression, and inheritance (Dai et al. 2001; Hu et al. 2003; Shou et al. 2004; Travella et al. 2005).

Transformation of maize through *Agrobacterium tumefaciens* depends upon various factors that relate to this transformation system, such as growth stages of explants, the genotypes, cell density and variation of strains, augmentation of phenolic compound, pH of medium, and its composition, and time duration of co-cultivation (Amoah et al. 2001).

3.3 Inplanta Transformation

In Inplanta transformation involves the transformation by reducing or eliminating the tissue culture process to exclude the negative impacts posed by in vitro conditions. In this technique, the reproductive or somatic cells are targeted, and pollination and seed development are accomplished if the reproductive cells are targeted. In the case of somatic cells, the plant is allowed to mature and the seed is harvested. The seed developed is used to obtain the next generation and screening is done by applying selection pressure. The first major report on inplata transformation came from Chumakov et al. (2006), who used maize pistils as target for *Agrobacterium* treatment with the hypothesis that pollen tubes will be going through the silks and make way for the *Agrobacterium* to reach the egg cells. Upon interacting egg cells, the single-celled egg or few-celled embryo is transformed to give rise to transformed seeds that are identified in the next generation through selection pressure. These protocols were modified and improved by many, including the author's laboratory, and Mamontova et al. 2010: Abhishek et al. 2014; Moiseeva et al. 2014, and are now becoming routine.

4 Transgenic Events Commercialized for Abiotic Stresses

The International Service for the Acquisition of Agri-Biotech Application (ISAAA) is an organization that keeps records of genetically modified crops/plants. According to their website there are seven events registered for abiotic stress tolerance, as commercialized. All these events belong to Monsanto. These events cover drought tolerance, herbicide tolerance, water stress, and insect tolerance. Details can be seen in Table 1.

5 Transformation for Abiotic Stress Tolerance

Three major stresses have been addressed extensively through transgenic technology. Some of the experiments conducted are discussed next.

Table 1 Transgenic events commercialized for abiotic stress tolerance in maize (Source, ISAAA)

Introduced gene	Gene source	Product	Function
MON87427 × MON87460 × MON89034 × TC1507 × MON87411 × 59122 (Pyramided Traits)			
cp4 epsps (aroA:CP4)	*Agrobacterium tumefaciens* strain CP4	Herbicide-tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
cry2Ab2	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry2Ab delta-endotoxin	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cry1A.105	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry1A.105 protein: comprises Cry1Ab, Cry1F, and Cry1Ac proteins	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cry1F	*Bacillus thuringiensis* var. aizawai	Cry1F delta-endotoxin	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
pat	*Streptomyces viridochromogenes*	Phosphinothricin *N*-acetyltransferase (PAT) enzyme	Eliminates herbicidal activity of glufosinate (phosphinothricin) herbicides by acetylation
cry34Ab1	*Bacillus thuringiensis* strain PS149B1	Cry34Ab1 delta-endotoxin	Confers resistance to coleopteran insects, particularly corn rootworm, by selectively damaging their midgut lining
cry35Ab1	*Bacillus thuringiensis* strain PS149B1	Cry35Ab1 delta-endotoxin	Confers resistance to coleopteran insects, particularly corn rootworm, by selectively damaging their midgut lining
cry3Bb1	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry3Bb1 delta-endotoxin	Confers resistance to coleopteran insects, particularly corn rootworm, by selectively damaging their midgut lining
dvsnf7	Western corn rootworm (*Diabrotica virgifera virgifera*)	Double-stranded RNA transcript containing 240-bp fragment of WCR Snf7 gene	RNAi interference resulting in downregulation of function of targeted Snf7 gene, leading to western Corn rootworm mortality
MON87460 (Single Trait)			
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
nptII	*Escherichia coli* Tn5 transposon	Neomycin phosphotransferase II enzyme	Allows transformed plants to metabolize neomycin and kanamycin antibiotics during selection

MON87460 × MON88017 (Pyramided Traits)			
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
cp4 epsps (aroA:CP4)	*Agrobacterium tumefaciens* strain CP4	Herbicide-tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
cry3Bb1	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry3Bb1 delta-endotoxin	Confers resistance to coleopteran insects, particularly corn rootworm, by selectively damaging their midgut lining
MON87460 × MON89034 × MON88017			
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
cry1A.105	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry1A.105 protein: comprises Cry1Ab, Cry1F, and Cry1Ac proteins	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cry2Ab2	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry2Ab delta-endotoxin	confers resistance to lepidopteran insects by selectively damaging their midgut lining
cry3Bb1	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry3Bb1 delta endotoxin	Confers resistance to coleopteran insects, particularly corn rootworm, by selectively damaging their midgut lining
cp4 epsps (aroA:CP4)	*Agrobacterium tumefaciens* strain CP4	Hrbicide tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
MON87460 × MON89034 × NK603			
cp4 epsps (aroA:CP4)	*Agrobacterium tumefaciens* strain CP4	Herbicide tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
cry2Ab2	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry2Ab delta-endotoxin	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cry1A.105	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry1A.105 protein: comprises Cry1Ab, Cry1F, and Cry1Ac proteins	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation

(continued)

Table 1 (continued)

Introduced gene	Gene source	Product	Function
nptII*	*Escherichia coli* Tn5 transposon	Neomycin phosphotransferase II enzyme	Allows transformed plants to metabolize neomycin and kanamycin antibiotics during selection
MON87460 × NK603			
cp4 epsps (aroA:CP4)	*Agrobacterium tumefaciens* strain CP4	Herbicide-tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
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cp4 epsps (aroA:CP4)	*Agrobacterium tumefaciens* strain CP4	Herbicide-tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
MON89034 × MON87460			
cry2Ab2	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry2Ab delta-endotoxin	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cry1A.105	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry1A.105 protein: comprises Cry1Ab, Cry1F, and Cry1Ac proteins	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
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5.1 Herbicide-Resistant Transgenic Maize

Chlorsulfuron is an extensively used herbicide, but because of its long residual duration in alkaline soils and its effect on shoots, as well as on the growth of further crops, the implementation of chlorsulfuron is restricted to rotation soils of maize and wheat. Haughn and Somerville (1986) acquired a mutant from *Arabidopsis thaliana* that had resistance for the chlorsulfuron herbicide. Mazur et al. (1987) observed that mutation in the *als* gene was the main cause of resistance. In the previous study of maize transformation, maize embryos, calli, and suspension cells were utilized as targets. With the exemption of limited varieties, however, it was difficult for some elite and hybrid lines to produce suspension cells and subsequently regenerated plantlets. Thus, immature embryos as a target for transformation were dependent on greenhouse conditions, and some seasonal deviations and embryogenic calli were dependent on genotype as well. Lowe et al. (1995) bombarded the meristem of immature embryos of hi-bred inbred lines and induced multiple shoot clumps. Although transgenic plantlets were attained, they could not resolve the problem of genotypic dependency in selecting material. Li et al. (2001) developed herbicide (chlorsulfuron)-resistant maize by bombardment of the isolated herbicide-resistant gene *als* from mutant *Arabidopsis thaliana* on an established multiple shoot clump system from shoot tip meristems of maize. Then, herbicide-resistant regenerated plantlets were attained through selection of herbicide (chlorsulfuron), and further, polymerase chain reaction (PCR) and Southern blot analysis were performed to confirm the transformation of transgenes *als* in some regenerants. The spray of chlorsulfuron depicted the transgenic plants and the R1 generation had an affirmative herbicide-resistant trait. Through this protocol, a genotype-free transformation system was established that enabled producing large numbers of transgenic platelets. The prime impetus of this research was not only herbicide-resistant maize plants but also the hope that chlorsulfuron could be used widely in rotation soils of wheat and maize in future.

Weeds compete by divesting other plants of light, water, nutrients, and space. These undesirable plants can produce allelopathic substances that are quite toxic to crop plants. Weeds frequently serve as hosts for several crop diseases and also offer shelter to insects and their diseases. To eradicate these harmful weeds, different kinds of herbicides have been used.

Later, Kim et al. (2009) reported herbicide-resistant hybrid maize lines using type II embryogenic calli as explants through *Agrobacterium tumefaciens* strain C58C1 taking the binary vector pTF102. Glyphosate is nonselective and is an herbicide extensively used throughout the world. 5-Enolpyruvylshikimate-3-phosphate synthase (EPSPS) has always been preferred to target glyphosate to develop transgenic glyphosate-resistant crops (Steinrücken and Amrhein 1980). Glyphosate involves the enolpyruvyl shikimate-3-phosphate (EPSP) synthase enzyme pathway and inhibits it, which in turn interferes with the growth of weed plants. The major benefit of using this herbicide is that its target, the shikimate pathway, is not present in animals; hence, this herbicide is safe for animals, humans, insects, and birds.

In 1980, scientists efficaciously isolated EPSPS from bacteria and plants (He et al. 2001; Wang et al. 2003; Funke et al. 2006; Zhou et al. 2006). The t-DNA vector contained two cassettes of EPSPs, and the bar gene conferred resistance to glyphosate and phosphinothricin, respectively. Northern blot analysis has been performed to confirm the presence of the bar gene in transgenic maize, and through this protocol 0.6% transformation efficiency was obtained.

Later, Wang et al. 2010 integrated glyphosate-resistant gene (EPSPS) in a maize hybrid line through *Agrobacterium tumefaciens* under the observation of several factors that actually affect the transformation efficiency to optimize it. Through this protocol transgenic plants were obtained that could pass the stably expressed gene to further generations. Further, attention has been given to enhance more resistance towards glyphosate by not only isolating new genes but also through optimizing transformation-effecting factors. Therefore, in 2013, Yu Gui-rong et al. reported the glyphosate-resistant gene *2mG2-EPSPS*, which has been isolated from a strain of *Pseudomonas fluorescens*, which exhibited five to tenfold more resistance towards glyphosate, transformed into immature embryos of maize through *EHA105* and *LBA4404* strains of *Agrobacterium tumefaciens* under optimal conditions. Several factors such as usage of different strains, inclusion of L-cysteine, with a long heat and resting duration, were evaluated to enhance the transformation efficiency, such as inclusion of L-cysteine along with heating of the embryos for 3 min before infection-enhanced transformation efficiency and keeping them in resting medium for a longer duration by delaying selection of transgenic embryos, leading to better survival of transgenic calli. Strains of *Agrobacterium tumefaciens* and genotypes of maize are crucial in determining the transformation efficiency. Results suggested that *EHA105* has 65% higher transformation efficiency as compared to *LBA4404*. So, through this protocol, 8.2% transformation efficiency was obtained and from 88 transgenic plants, 66 maize plants exhibited resistance towards glyphosate. Further, PCR and Southern blot analysis confirmed the integration of transgene in the genome of maize. Later, Sun et al. (2015b) transformed *2mG2-EPSPS* (glyphosate-resistant gene) in immature embryos of an inbred line of maize by *Agrobacterium* with little variation in transformation factors such as *Agrobacterium* concentration, which was OD_{600} = 0.6, and a 10-min infection time was reported. From 46 transgenic plants, five showed positive results, and their stable expression in further generations was analyzed through real-time PCR. Ascertainment of novel EPSPS is significant for the production of glyphosate-resistant crops. AM79 is a bacterial gene and AM79 EPSPS can endure a high concentration of glyphosate in *Escherichia coli*, and thus could serve as a good choice for the development of transgenic glyphosate-resistant crops (Cao et al. 2012). In 2015, Ren et al. reported formation of a synthetic *AM79 aroA* gene because the wild-type gene contained numerous motifs that could lead to instability of mRNA of the bacterial gene in transgenic plants. Therefore, synthetic *mAM79 aroA* cloned with plant expression vector pM3301UbiSpAM79 was transformed into immature embryos of maize through *Agrobacterium tumefaciens*. Approximately 79 transgenic plants were obtained, and their PCR analysis exhibited that these plants had an integration of *mAM79*. Results of RT-PCR depicted the high transcription of *mAM79* in transgenic maize,

and these transgenic maize could endure fourfold commercial glyphosate application when sprayed with glyphosate. So, this study confirmed that *mAM79* could be used for generation of transgenic maize against glyphosate.

5.2 Drought-Resistant Transgenic Maize

Maize productivity is mainly affected by drought (Boyer and Westgate 2004; Campos et al. 2004). In plants, water stress is established when the level of transpiration is increased compared to the level of water absorption. Under water stress, photosynthetic activity is reduced by the closing of stomata (Chaves 1991). Seeds and vegetative tissues develop desiccation-tolerant structures where late embryogenesis abundant (LEA) proteins accumulate. Overexpression of LEA protein, in different plants such as *Arabidopsis*, wheat, rice, tobacco, cabbage, or lettuce through transgenic approaches have exhibited an ameliorated phenotype under abiotic stress (Leprince and Buitink 2010; Yang et al. 2010). Amara et al. (2013) reported transgenic maize against water stress by integrating the LEA gene Rab28 along embryogenesis through biolistic transformation. The presence of the gene was confirmed in different cells such as axial and vascular as compared to just the scutellar cells of embryos as previously reported by Niogret et al. (1996) under water stress conditions. The Rab28 gene was constitutively expressed in maize plants, which enabled them to sustain their growth rate and development. Overexpression of LEA proteins under water deficit and salinity enhanced their phenotype by increasing crop stress resistance (Leprince and Buitink 2010; Xiao et al. 2007; Yang et al. 2010).

Drought is one of the most significant environmental stresses, reducing the growth and yield of plants and crops, respectively (Boyer 1982; Passioura 1996). Drought is the major reason of reduction in yield of maize (Maiti et al. 1996). Shortage of water, great variation in weather patterns, and the uncertain nature of drought result in a notable threat to global maize production. In consequence of its complications and hardness, drought has been considered as a “cancer” of plants. Therefore, there is an immense requirement for improving maize drought tolerance through biotechnological techniques.

Earlier studies indicated the activation of oxidative signal cascades from the expression of mitogen-activated protein kinase kinase kinase (MAPKKK), which leads to tolerance against cold, drought, and salinity in tobacco. In 2004, Shou et al. transformed the npk1 gene in maize, under a constitutive promoter through the *Agrobacterium tumefaciens* strain EHA101, to analyze the role of the tobacco MAPKK npk1 gene in crops to improve drought tolerance. Results indicated that the npk1 gene induced a mechanism that protects the photosynthesis process under drought conditions, and in this way transgenic maize maintained its photosynthesis rate compared to nontransgenic plants. Transgenic plants showed an increase in kernel weight and leaf number as compared to negative controls. Thus, this study provides a reference to study the physiological and morphological aspects of transgenic maize under stress conditions.

Phosphatidylinositol-specific phospholipase C (PI-PLC) has a significant role in various physiological processes in plants, incorporating drought tolerance. It has been noted the *ZmPLC1* gene was cloned from maize that encoded PI-PLC and was overexpressed in roots of maize under water stress (Zhai et al. 2005). The transgenic elite inbred lineYe 7922 maize was generated through the *Agrobacterium*-mediated transformation LBA4404 strain by expressing the *ZmPLC1* gene in sense and antisense orientation. Under drought stress, it was found that sense transgenic maize showed a high rate of photosynthesis, high water content, better water adjustment, and higher yield than the wild type. Antisense transgenes showed subservient characters as compared to wild type. It was inferred that enhanced expression of sense *ZmPLC1* ameliorated the drought tolerance of maize (Wang et al. 2008). This finding was the first report to exhibit the role of PI-PLC in plants against drought stress. Later, Omer et al. (2013) conducted an experiment to determine susceptibility of different tropical maize genotypes to *Agrobacterium*-mediated transformation with the drought tolerance gene *NPK1*. It has been observed that plants that have a drought-tolerant gene showed better growth under drought conditions than normal plants. Muoma et al. (2014) transformed *Nicotiana* protein kinase (npk1) in tropical maize lines through the *Agrobacterium tumefaciens EHA101* strain to analyze the role of oxidative signal cascades under stress conditions. PCR and Southern blot analysis were performed to confirm the presence of the *npk1* gene and its copy number, respectively. Further, physiological and morphological variations were assessed in these transgenic lines under drought stress. So, transgenic lines under drought stress have been accredited to lengthen days of maturity an average of 5–8 days as compared to the wild type under absence of any stress. There was no difference in kernel weight among transgenic plants under drought stress and well-watered plants, which showed that the *npk1* gene enabled the transgenic plants to withstand drought stress and also enhanced maize yield (kernel numbers). Overall, a 20–35% improvement in yield of transgenic plants under stress conditions was seen as compared to nontransgenic plants.

However, identification of genetic components that confer resistance to drought in maize is of great significance. For this purpose, Wang and Qin (2017) reported a genome-wide association study (GWAS) of maize at the seedling stage against resistance to drought in a naturally varying population. In maize seedlings, apart from 82 genetic variants, only 42 candidate genes were notably connected to drought tolerance. Five significant single-nucleotide polymorphisms (SNPs) located within the 3′-untranslated region (UTR) of a single gene residing on chromosome 9 encodes vacuolar type H^+ pyrophosphatase, having the same protein sequence homology with *Arabidopsis* AVP1. So, in maize it is designated as zmVPP1. The peak GWAS signal exhibited that ZmVPP1 is significantly related to drought tolerance in maize. Expression of zmVPP1 has been enhanced in the maize inbred line A188 through the *LBA4404* strain of *Agrobacterium*-mediated transformation method; under water deficiency, transgenic lines showed greater grain yield than the wild type. Transgenic maize with a high expression of zmVPP1 exhibited improved drought tolerance that is more probably the result of high photosynthesis efficiency as well as root development.

5.3 Chilling-Resistant Transgenic Maize

Betaine seems to be the interpretative determinant of stress tolerance in plants. Under different stress conditions, the growth and endurance of a diverse variety of plants, incorporating maize, improves with external application of betaine. It reported that with an elevated level of betain, lipid peroxidation of the cell membrane decreased and as a result chilling tolerance was enhanced in maize. Sakamoto and Murata (2002) proposed that under diverse kinds of stress conditions, betain might protect the machinery of protein synthesis and sustained conditions under which the repair processes happened more rapidly as compared to damaging processes. Later, Quan et al. (2004) reported four transgenic elite maize inbred lines by transferring the *betA* gene from *E. coli* through *Agrobacterium tumefaciens* against chilling stress. Betaine concentration in leaves of a few transgenic lines was 208–333% higher than in the wild type in pre-chilling stress, whereas post-stress betaine concentration in transgenic plants was considerably higher than in the wild type. Under chilling stress, less cell membrane damage by maintaining its stability, less cell injury, and no lowered rate of photosynthesis as compared to wild-type maize plants has been observed from betaine synthesis. Therefore, it could be determined that engineering of betaine synthesis is a possible way to enhance chilling tolerance in maize (Table 2).

Extensive studies have been conducted on the development of genetically modified maize plants for biotic and abiotic stress tolerance. Nijmeijer (2013) noted 99 events for insect tolerance and four commercialized events for drought tolerance. One of the possibilities of a large number of transgenic plants not becoming commercialized is the fact that most of the genes improving stress tolerance have alternate targets in the cell. These transgenes result in distorted and agronomically disadvantaged plants; hence, only a few could be commercialized (Nijmeijer 2013).

6 Environmental Protection and Risk Assessment

The possible risks associated with transgenic plants include transgene flow, evolution of resistance in targeted pathogen species, effects on nontargeted species, and health hazards from transgene integration and expression. Maize is a cross-pollinated crop plant, and transgene flow from transgenic to nontransgenic plants is most likely; therefore, specialized biosafety guidelines are recommended to control vertical and horizontal gene transfer. Transgene flow is reported in Mexico, Brazil, and Columbia (Chaparro-Giraldo et al. 2015), and the possible reason was avoidance of recommended biosafety rules. This problem was also seen changing with the social groups: one group of farmers did not care for biosafety guidelines, resulting in gene flow to nontransformed genotypes and landraces, including teosinte in Mexico, whereas other workers did follow the biosafety guidelines and there was no gene flow (Agapito-Tenfen et al. 2017). There is a possibility of developing herbicide-resistant teosinte and other superweeds if biosafety guidelines are not followed. Resistance against all chemical pesticides can be developed by insects according to the

Table 2 Transformation targeted to abiotic stress tolerance

Genotype	Vector	Strain	Trait gene	Agronomic trait	Reference
Hi II and inbred line B73	pSHX002 and pBAR184	*EHA101*	npk1 and phosphinothricin transferase	Drought stress	Shou et al. (2004)
Elite inbred line Ye 7922, TL08, KAT, PTL001, DH01, DLC1	pCUA	LBA4404	*ZmPLC1*	Drought stress	Wang et al. (2008)
Inbred line A188	pSHX004	*EHA101*	npk1	Drought stress	Muoma et al. (2014)
Inbred lines	pSB II	*LBA4404*	ZmVPP1	Drought resistant	Wang and Qin (2017)
Hybrid line	pTF102	C58C1	EPSPs	Glyphosate phosphinothricin resistant	Kim et al. (2009)
78599, Zong 31 and BA	pCAMBIA	*EHA105, LBA4404*	EPSPS	Glyphosate-resistant	Wang et al. (2010)
Inbred line Z31	pM3301UbiSpAM79	*EHA105*	*2mG2-EPSPS*	Glyphosate resistant	Yu et al. (2013)
Inbred line X090 and Z3	p2EPUHLAGN	*LBA4404*	*mAM79 aroA*	Glyphosate resistant	Ren et al. (2015)
Hybrid line Q31 × Z3	*pCDH1300*	*LBA4404*	*Btcry1Ah* and *2mG2-epsps*	Pest and glyphosate resistant	Sun et al. (2015a)
Elite inbred line DH4866	*P35S-als*	*LBA4404*	*betA*	Chilling resistant	Quan et al. (2004)
Inbred lines Qi319 and N10-6	*P35S-als*	Gene-Gun PDS-1000	*als*	Chlorsulfuron resistant	Li et al. (2002)
Hi-II (A-188 × B73)	*pAHC25*	Gene-Gun PDS-1000	Rab -28 gene	Drought resistant	Amara et al. (2013)

principles of evolution; therefore, the Bt corn cultivation was regulated by the United States Environment Protection Agency. As 20% of the seed must have been nontransgenic to provide refuge against evolving insects, and sometimes the rules are not followed by the citizens, so it was seen that 20% of the farmers were planting all Bt seeds in the United States in 2002 (Gewin 2003). Similarly, Dively et al. (2004) reported the unusual behavior of monarch butterfly larvae fed continuously on corn pollen expressing the Cry1AB gene. Allergies and metabolic disorders have been hypothesized over the years by the opponents of transgenic crops; therefore, allergenicity tests have been part of the risk assessment for transgenic plants. According to a report in 2017, approximately 20 proteins were seen with altered expression in transgenic and its counterpart nontransgenic lines under various biotic and abiotic stresses. This report requires the inclusion of molecular analysis of proteins and metabolites in risk assessment studies (Benevenuto et al. 2017).

7 Conclusion and Future Prospects

Because of the necessity for better crop cultivation, there is a growing interest in research for developing good-quality transgenic plants. Maize transformation started about half a century ago, and at present various technologies for gene transfer are available, although the randomness of integration sites as well as low transformation efficiency are yet restraints. *Agrobacterium*-mediated and biolistic transformation are the most efficient techniques because of their simplicity and the stable integration of the gene on the broad spectrum of plant species. An essential definition of transgenic crops is to produce genetic improvement by introducing remote genes, not only from other plant species but also from different bacteria, fungi, viruses, and animals. Using tissue culture technique, the transformed plant cells are regenerated into a complete plant. Regeneration is yet genotype dependent in most cases; therefore, for the past two decades; many efforts have been made to overcome the barrier of genotype dependence so that transformation could also be genotype independent. Inplanta transformation is proving one of the worthy efforts from extensive research during the past 10–12 years. The transformation methods developed over the years have been utilized to enhance various traits in maize with a premise to increase maize productivity, improve nutritional value, and develop resistance against biotic and abiotic stresses to fulfill the requirements of the ever-increasing human population, animal feed, and industrial sectors. However, still there is an immense requirement to explore many pathways to develop crops on a large scale with desirable characteristics that have the competence to combat major environmental restrictions and survive under severe conditions. In coming years, research will be more focused on enhancing not only the quantity of maize through transgenic crops but also towards improving nutritional value. The quality of transgenics plants itself will also be improved by such means as defined integration events, marker-free transgenics, and transgenes pyramided transgenics for quantitatively controlled characters.

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