Advances in Agriculture and Biology (2023) 6(1): 1-10

REVIEW PAPER

A discussion on maize transformation during the last two decades (2002–2022): An update on present trends and future prospects

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Received: 17 June 2023
Accepted: 25 October 2023

Abstract

Over the past decades, improvements in plant biotechnology have been used to produce genetically modified maize cultivars that have the potential to tolerate various stresses and as a result maize production has increased in the world. GM maize varieties having various traits i.e. disease resistance and abiotic stress tolerance denote 30% of the world's maize cultivated area. Genetic transformation in maize has been successfully achieved for the development of trait specific genotypes. Traditionally, some barriers in maize tolerance to certain traits still exist that can never be accomplished and to fill this gap, maize biotechnology has developed and commercialized GM maize varieties as an alternative to conventional breeding methods. Though various stresses i.e. biotic and abiotic can affect maize production but among other abiotic stresses, drought is a major issue that directly disrupts maize yield and productivity across the globe. Drought tolerant gene isolation and their transformation into maize are the key steps towards technological improvements. Agrobacterium mediated gene transformation in maize is widely used for the stable integration and transient expression pattern through optimized tissue culture protocols. This review mainly represents updated success in gene transformation in maize in the last two decades in terms of achievements, their uses and future prospects of GM maize cultivars. © 2023 The Author(s)

Keywords: Gene transformation, Genotypes, GM maize, Optimized protocols, Prospects, Traits

Citation: Zia, M. A., Shoukat, S., Arif, M., Ahmad, B., Nawaz, A. F., Bahadur, A., Zakria, M., Khan, H. S., Khan, S., Suleman, M., & Ali, S. (2023). A discussion on maize transformation during the last two decades (2002–2022): An update on present trends and future prospects. *Advances in Agriculture and Biology*, *6*(1), 1-10.

Introduction

General importance of maize genome under drought stress

Zea mays is diploid having 2n = 20, and x = 10. Thus, 10 chromosomes are present in the maize genome. The maize genome also contains jumping genes, called transposons. Transposons cause many genetic changes in the genome of maize, which can be beneficial in the field of corn production. According to the maize genome annotations, there are 39000–42000 protein coding genes representing as a whole about 800–1700 genes taking part in the selection of domestication. Further improvement could be possible to find more related genes in maize through NGS and other related advanced biotechnology applications (Springer et al., 2018; Sun et al., 2018; Yang et al., 2019a). Maize after wheat and rice is the third most important cereal crop in the world (Batool et al., 2019; Noor et al., 2021; Aslam et al., 2021; Iqbal et al., 2022; Raza et al., 2023). Globally maize is a major crop but some abiotic stress factors like drought had significantly affected their yield (Campos et al., 2004). Drought stress losses 20–25% of maize production across the world as maize is very susceptible to drought conditions. Likewise, long exposure to drought stress at tasseling can never recover plant growth even after irrigation (Jiang et al., 2018). Gibbon and Larkins (2005) stated that several organic compounds are found in corn. Too much/rich/ abundant protein termed as zeins. Zein is deficient in lysine and tryptophan amino acids. These amino acids are of good quality and low nutritional value. Maize seeds have low zein proteins, so some modification should improve their quality. All of these are the result of modern biotechnology techniques for optimum level of lysine and tryptophan expression.

Role of transcription factors in regulation of abiotic stresses

Hussain et al. (2011) mentioned that plants are highly affected by abiotic stresses. Among all of the abiotic stresses, drought is the most important foremost boundary to crop yield in the world for its multigenic nature in the development of transgenic crops as a demanding hope. To produce plants that can tolerate drought stress is a very crucial step for the enhancement of all biological activities essential for plant growth and productivity. Drought stress signals in plants and as a result the expression of different genes responsible fordrought stress is activated. Transcription factors (TFs) bind to the stress related genes that have to control various metabolic pathways in response to drought stress in plants. One of the most famous classes of transcription factors is DREB/CBF. These factors have been used against drought tolerance in transgenic plants. In recent times the roles of TFs are being improved by considering their controlling response in drought stress that has guided to useful approaches for transgenic plants development. The exploitation of these factors in plant regulation in drought stress shows a new period for engineered tolerant crops. Liu et al. (2010) isolated the complete cDNA encoding EREBP domain-having AoDREB transcription factor from *Asparagus officinalis* L. through RACE-PCR. It has homology with *DREB* protein and is further classified into subgroups and families of this transcription factor. AoDREB is a transcription factor that binds to the DRE element and activates other related genes in abiotic stresses. Results revealed that genes responsible for this protein are activated under 20% of PEG and at high salt stress conditions. This protein had no harmful effects in transgenic *Arabidopsis* plants when overexpressed. AoDREB induced the expression of other related genes resulting in augmented tolerance to drought and salinity in *Arabidopsis* plants. It has been proved that the application of exogenous AoDREB into the plants can increase tolerance to abiotic stresses and ultimately plant yield can be improved.

Uniqueness of *Agrobacterium* method of gene delivery

Gene transformation through Agrobacterium mainly refers to foreign gene successfully transfer to the host plant. Agrobacterium mediated transformation mainly rely on the use of its Ti plasmid whereby the T-DNA is integrated into the host plant genome to the resultant progeny (Li et al. 2017b). The basic process of agrobacterium mediated gene transformation into plants is to infect the desired plant tissue and gene construct may enter their genome. The success rate of gene transformation frequencies have been improved by using crop specific tissue culture optimized protocols, use of super-binary vectors, induction of vir genes (Komori & Komari, 2011). Pitzschke and Hir (2010) said that soil dwelling bacteria *Agrobacterium* when infecting plants causes crown gall formation in these plants. As a result, inside the *Agrobacterium* the activation of vir genes and their expression may result in the formation of various vir proteins that help in the gene transfer into plants. By adhering to plant cells, the T-DNA of *Agrobacterium* is transferred into the plant cells through the help of virulence protein T4SS. Through contact with a lot of Vir proteins repress the plant defense system and thus the T-DNA integrates into the plant genome. It is fully understood that the interaction of host-*Agrobacterium* and their mutual coordination can enhance the desired gene transfer into the host plant. The mechanism of plant uptake of the foreign gene is very complicated as compared to *Agrobacterium* and it involves various steps like transportation and their stable integration into the chromosomes of the plant cells.

Gene transformation into maize through Agrobacterium method

Takavar et al. (2010) described genetic transformation with the help of *Agrobacterium* in maize. Genetic transformation through *Agrobacterium* method/ mediated strain has better results as compared with other techniques. In this study several factors were studied similar to tissue culture and gene transformation. The majority of embryogenic calli collected in maize lines S61 and A188 were 75% and 71%, respectively. Infection from embryos with LBA4404 harboring a standard binary vector pCAMBIA3301 has been conducted through transgenic plant development. Regenerated plant results were confirmed by Polymerase chain reaction in front of *bar and gus* genes in these two lines of maize. The best line S61 for maize transformation frequency of 6.45% was detected. Zhang et al. (2010) investigated that DREB and CBF transcription factors played a significant role in plant abiotic stresses. Transformation of *TsCBF1* gene from halophyte *Thellungiella halophila* to monocot-like maize. The results show that the *TsCBF1* gene has increased stress tolerance in maize plants. Assem et al. (2009) mentioned that biotechnology has its own importance by developing inherent tolerance to abiotic stresses. Recently, the transformation of *NPK1* gene through *Agrobacterium* has been successfully incorporated in the genome of maize. Molecular analysis shows that this gene is present in the maize genome. Transgenic plants progeny (R1) for osmotic stress tolerance were reviewed. *NPK1* gene has reached the conclusion that it will create stress tolerance in maize plants.

Effect of drought stress on maize

Like other abiotic stress factors, drought events in the 21st century will continue to happen across the globe (Dai, 2013; Spinoni et al., 2020). It is necessary and need of the day to develop drought tolerant maize cultivars to overcome this major issue in the world and to fulfill the requirements of maize consumption. Due to the vast genetic variability potential of maize breeding for drought tolerance to enhance their yield (Fisher et al., 2015). There are two growth stages of maize to which drought stress causes severe damage (i) from late stage of plant growth period until to flowering (ii) and full grain stage of development (Sayadi Maazou et al., 2016).

Jaleel et al. (2009) reviewed that biotic and abiotic stresses have negatively affected plant growth. Drought is one of the foremost abiotic stresses, which badly affects crop productivity. Drought has primarily disturbed the metabolic pathways and as a result crops lose their normal functions. The major problem is that of photosynthesis that is badly affected and ultimately plants lose their normal functions. The plant biomass is also concerned with photosynthetic pigments and in water deficient conditions their validity is lost. Drought perturbs all sorts of activities including plant physiology, anatomy, morphology and nutritional composition that have direct impact on poverty and hunger on humans as well.

Sajedi et al. (2009) reported the maize under drought stress conditions and subsequently the nutrients effect on drought stress. In this experiment various factors were studied. Selenium levels and micronutrients were applied at different stages of maize growth in the form of foliar spray. The results at the 1% level showed a significant water stress on maize in different developmental stages. Drought decreased compared to control grain production up to 33% of the grain filling stage. The selenium and micronutrients analysis showed antagonistic effects in traits. Because of drought stress, maximum grain production 8159.33 kg ha-1 was calculated at eight-leaf stage. These results show that the use of micronutrients in water and selenium during drought stress has increased reaction characteristics compared to control. Shou et al. (2004) described that among the abiotic stresses, drought and cold are the most important stresses that affect maize crop. Transcription factors have its role to regulate gene expression in these stress conditions. In this study, the genes responsible for the activation of mitogen kinases provided protection against freezing tolerance by the elimination of ice crystals in transgenic maize. The mitogen-activated kinases played their role in oxidative stress signaling. We exhibit that *Nicotiana PK1* gene under strong constitutive promoter increases stress tolerance in transgenic maize lines that are very much sensitive to low temperature. Our findings propose that new inventions need to be designed through biotechnological approaches towards stress tolerance in different crops. Jeanneau et al. (2002) mentioned that genetic manipulation has tremendously increased the endogenous biochemical pathways that have direct impact on drought tolerance in maize under dry conditions. Water is one of the basic requirements for the survival of plant growth. Monocots like maize is very susceptible to drought stress at early flowering stages of their growth. It was observed that various genes like *Asr1* played their roles in drought tolerance when overexpressed and their transcription factors stimulate other related genes in abiotic stress conditions. The overexpression of *C4-PEPC* has increased endogenous water up to 30% with respect to moderate drought stress. Negative effects of proteins were recorded in transgenic maize when it was expressed at low level. Various genes that have been transformed to maize in response to drought stress are illustrated in Table 1.

Use of embryos explant in genetic transformation experiments in maize

Martinez and Wang (2009) studied that ripe maize seeds, which are used in direct media regeneration, have been shown to be 98% successful in all of essential nutrients and their effects in the aseptic environment. Various steps have been taken to sterilize the maize embryos. Seeds were soaked in distilled water and then disinfection was applied for surface sterilization. Abebe et al. (2008) used as a starting material to practice and effectively regenerate the immature embryo of maize in vitro culture. The use of mature embryos compared to mature embryos may indicate response to tissue culture. Optimizing and implementing the protocol of mature embryos improves the regeneration of maize inbred lines. Mohammad Hani and Heidari (2008) reported that various drought treatments have been made by PEG and the water potential was measured by considering control and different MPa treatments after one day. The complete soluble proteins were analyzed for two maize cultivars through SDS-PAGE. Initially the total soluble protein contents were increased in roots and leaves and then decreased by the application of water deficient potential. It has been concluded that the protein contents had no differences in the roots of both the varieties but has increased in the leaves of 301 as compared to 704 varieties of maize. While in drought stress, the root and shoot fresh weight in 704 has been increased having water potential –1.76 MPa, with addition of dehydrin like proteins in leaves and root were induced in these two varieties of maize. The levels of Dehydrin-like proteins were increased in the 704 cultivar of maize (Fig. 1).

Effect of drought stress on maize physiological responses

Campos et al. (2004) investigated that maize yield decreased due to drought problems. Modern biotechnology and genetic engineering techniques have contributed to better crop development in plant breeding experiments for drought tolerance. This new science has increased the crop yields in drought stress. Strong promoter genes for drought tolerance are used to reduce the damage in drought prone areas. Kellos et al. (2008) reports the drought stress has altered the activity of reactive oxygen species (ROS) and antioxidants in maize. Under drought stress, the amount of ABA acid increased in maize. Bhatnagar-Mathur et al. (2008) studied the abiotic stress, plant produces different osmoprotectants like GlyBet and trehalose which stabilizes the membrane and proteins (quaternary) structures. Mannitol acts as free radical scavenger and Proline serves as a component of nitrogen, carbon and a free radical hunter. Furthermore, these osmoprotectants maintain the subcellular structures, buffers and redox potential during abiotic stresses. It is believed that all crop plants are not able to produce naturally all these osmoprotectants under stress conditions. It is suggested that regulation in osmosis would be the most excellent approach for abiotic stress tolerance if genes responsible for the osmoregulation could be used in response to all abiotic stresses. Thus, an extensively adopted plan to build up different stress tolerant crops has been to overexpress various genes responsible for the osmolytes in plants. Aslam et al. (2006) tested sixty varieties of maize to test drought tolerance at four different humidity levels: 40%, 60%, 80% and 100%. Relative cell membrane damage (RCI %) and stomatal function were examined. Drought resistance in maize was found to be RCI %. NC-9 selection criteria show drought tolerance compared to drought susceptible T-7.

Table 1 List of key genes involved in drought tolerance in maize

Gene name	Function	Reference
ZmVPP	Enhanced activity of root growth and development	Wang et al. (2015)
ZmNAC111	Enhances drought tolerance in transgenic maize	Mao et al. (2015)
ZmPP2C-A10	Induces in ABA signaling	Xiang et al. (2017)
ZmWRKY40	Regulate drought stress-related genes	Wang et al. (2018)
MYB gene, ZmMYB3R	Increased CAT, POD and SOD enzyme activities, increased sensitivity to ABA, and stomata regulation	Wu et al. (2019)
ASR gene ZmASR3	Lower MDA levels and higher relative water content and proline content	Liang et al. (2019)
Maize glossy6 (gl6)	Involved in trafficking intracellular cuticular waxes	Li et al. (2019)
Late embryogenesis abundant (LEA) gene ZmLEA14tv	Enhanced the seed germination and survival rate	Minh et al. (2019)
ARGOS8	Increased grain yield	Shi et al. (2017)
ZmVP14	Biosynthesis of abscisic acid	Cura et al. (2017)
ZmCIPK8	Involved in ABA and H2O2 signaling	Tai et al. (2013)
tb1	TCP transcription factor	Studer et al. (2011)
tga1	SBP-domain transcription factor	Wang et al. (2005)
UB3	SBP-box transcription factor	Liu et al. (2015)
ids1/Ts6	AP2 transcription factor	Wang et al. (2019)
ZmSh1-1, ZmSh1-5.1+ZmSh1-5.2	YABBY transcription factor	Lin et al. (2012)
ra1	Transcription factor	Sigmon and Vollbrecht (2010)
ZmRap2.7	AP2-like transcription factor	Castelletti et al. (2014)
ZmCCT10	CCT transcription factor	Wang et al. (2017)
ZmCCT9	CCT transcription factor	Huang et al. (2018)

Optimization of factors affecting genetic transformation in maize

Lee & Gelvin (2008) conducted a research study on traits improvement in maize through genetic engineering. Two genotypes in corn have been transformed by *Agrobacterium*. Various hormonal compounds have been produced in the media. Transformation protocols have been optimized. The maize genotype chalok-2 showed 0.16% transformation frequency in optimized conditions. The best results were calculated on H99 genotype with 1.8% transformation success. Nelson et al. (2007) investigated that under drought stress conditions the constitutive expression of transcription factor from that of nuclear factor of the Arabidopsis AtNF-YB1 binds to the family of CCAAT factor which showed better resistance in drought stress. The gene responsible for transcription factor in maize ZmNF-YB2, and their constitutive expression has obtained good results as compared to others pattern of expression system in maize. Maize transgenic plants were tested under drought stress conditions in open fields and glasshouse-based treatments. Maize transformed plants showed lower levels of wilting and rapid growth than wild type plants in the glasshouse-based drought treatment. Field-based transgenic lines under drought stress showed higher vegetative stage, better health and photosynthesis and more yield as compared to non-transformed maize lines. It has been concluded that ZmNF-YB2 performs their function in the form of high yield of transgenic maize plants under drought stress environments. Wang et al. (2007) studied the use of different techniques to bring about effective genetic change in maize. The seeds were cleaned and damaged in the meristem tissues and cultivation of two of two types of tissues in the same medium for 2-3 days. Plant selection markers such as hygromycin were performed for transgene confirmation. Transgenic plants have been confirmed through PCR and southern blotting. T0 seedlings were approximately 29% transgenic. In another experiment, a mature embryo of maize inbred line SM5-4 as a starting material through *in vitro* culture. All the culturing conditions were optimized for the SM5-4 inbred maize line to obtain *in vitro* regenerated plants. Optimization of all these conditions is necessary for efficient plant regeneration to control all the problems related to tissue culture response. Mature embryos of maize were subjected for surface sterilization with 50% Clorox for 20 minutes and their subsequent washing with sterilized water to remove the traces of clorox. By applying this concentration 90% control of contamination was achieved. Among all these tested medium N6 was the better one for maize tissue culture viewpoint. Phytohormones like IAA, 2, 4-D and BAP showed the best performance in this study.

Mechanism of stress related genes in crop improvement

Cassells and Doyle (2003) reported that genetic engineering has improved crop tolerance. This method is widely used in the gene pool. Several genes that control drought, pesticides and disease have been successfully incorporated into crop genetic engineering. Stress mechanism, and the control in plants are too complex to control their metabolic pathways in response to biochemical stresses. Proteins in stress responses gene code must have a strong binder that helps in the cassettes expression to improve tolerance in abiotic stresses and resist the destruction within the plant cells (Fig. 2).

Future perspectives of maize genetic transformation

This review mainly highlighted genetic transformation advances in maize in the last two decades. Though upto some extent remarkable success has been achieved in genetic transformation in maize but still some more attention is required to transform more trait specific achievement through maize biotechnology. Problem oriented research in the field of maize genetic transformation is required to address the major issue and according to that new genes should be selected and transformed into maize genome to produce fruitful results. More sophisticated molecular biology techniques are required to be applied in maize transgenic plant development that could possibly cover all biological mechanisms, their process and underlying genetic regulation. High-throughput automation, NGS, and finding of key biochemical pathways involved in the improvement of stress related factors are mandatory to improve maize molecular genomics approaches. More transformation methods and their applicability are required that have tissue and genotypes independent roles to get gigantic success. Similarly more explants of maize should be tested to report their transformation efficiency. Moreover, in order to improve the efficiency of maize transformation, it is necessary to use infection response genes. The use of Gemini viruses in the transformation of maize should also be considered as a new viral delivery system. Genetics improvement in maize through single cell techniques, such as protoplast transformation should be applied to achieve maximum potential of automation. Furthermore, genome editing technology through CRISPR/Cas9 must be applied on maize to retain valuable results that are normally not possible. Genome editing strategy will further strengthen maize genomics due to their immense use having no restrictions in regulatory framework across the countries and borders as well. For developing countries like Pakistan, more improvement in maize functional genomics and other biotechnological innovative tools are prerequisite to maintain the agricultural benefits. But on the other hand, simple, cheaper and robust technology for genetic transformation in maize will be more authentic and reliable to apply in government research institutes of plant biotechnology in developing countries.

Conclusion

Maize transformation has become possible in the modern era of biotechnology and genetic engineering and as a result progressive achievements were made. Biotic and abiotic stresses abruptly disturbed maize productivity enhancement and to combat with these stresses. GM maize with various useful traits is helpful in maintaining daily use consumption for the world. Though various transformation methods are routines for the transfer of novel genes into the maize genome, the utility through agrobacterium mediated transformation is very dominating as compared to others. In this way, various successful stories were achieved using Ti plasmid of this bacterium. Drought stress has drastically affected maize yield across the globe. Hence efforts were carried out to transform drought tolerant genes in maize to cope low to extreme stress conditions in the last two decades. In this review, we have highlighted the importance of GM maize transformation steps optimization, drought stress effect on maize and plant response under drought stress. Besides these, agrobacterium mediated transformation method and transcriptional factor’s role in portraying drought tolerance in maize and some other key factors accelerating benefits to tolerate drought stress mechanism of action. Some key drought tolerance genes were highlighted in this review that could be useful for the readers to take help in conducting some more advancements through more innovative technological progress to tolerate hazardous effects of drought stress.

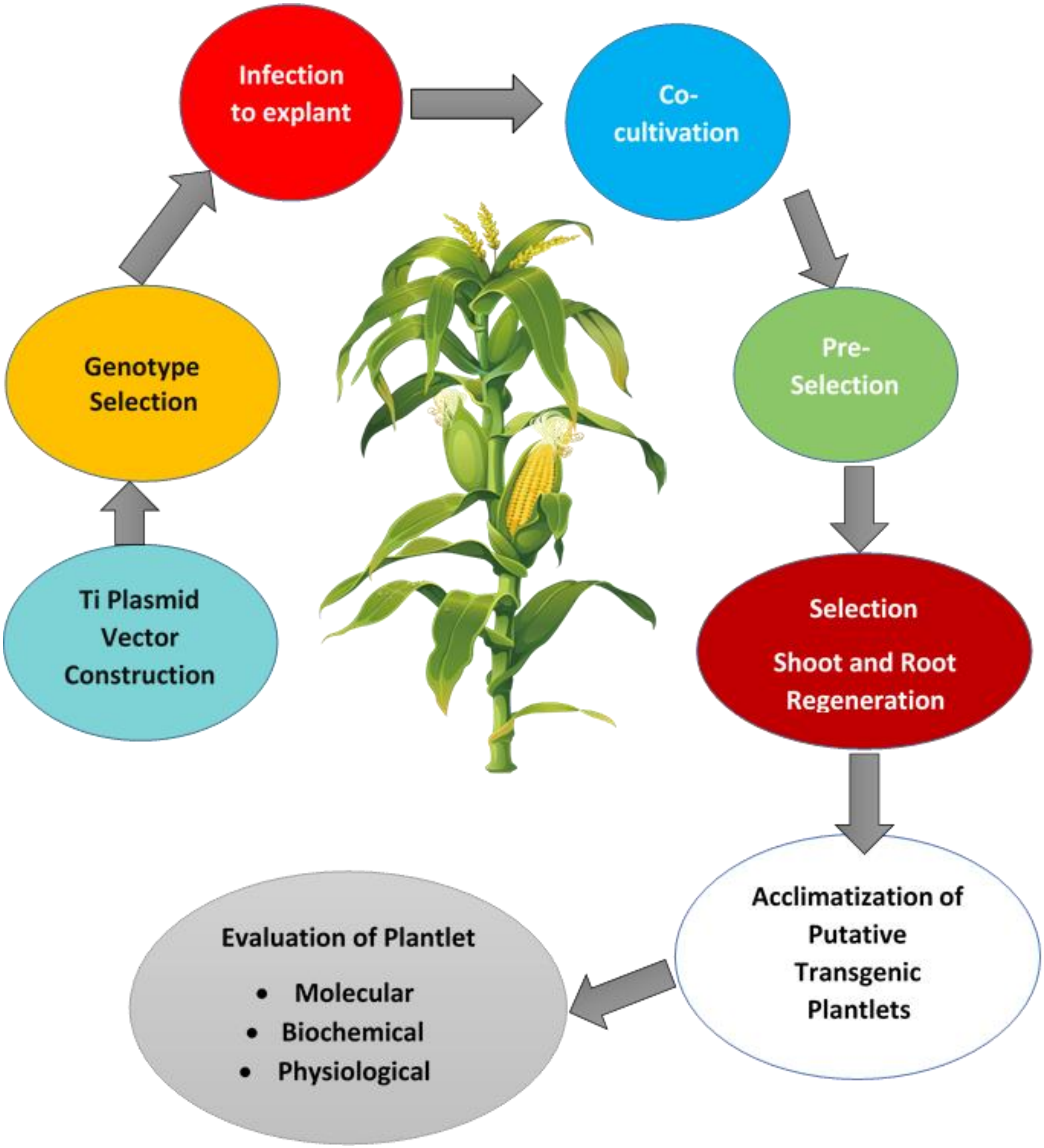

Fig. 1 Diagrammatic workflow of *Agrobacterium* mediated transformation in maize

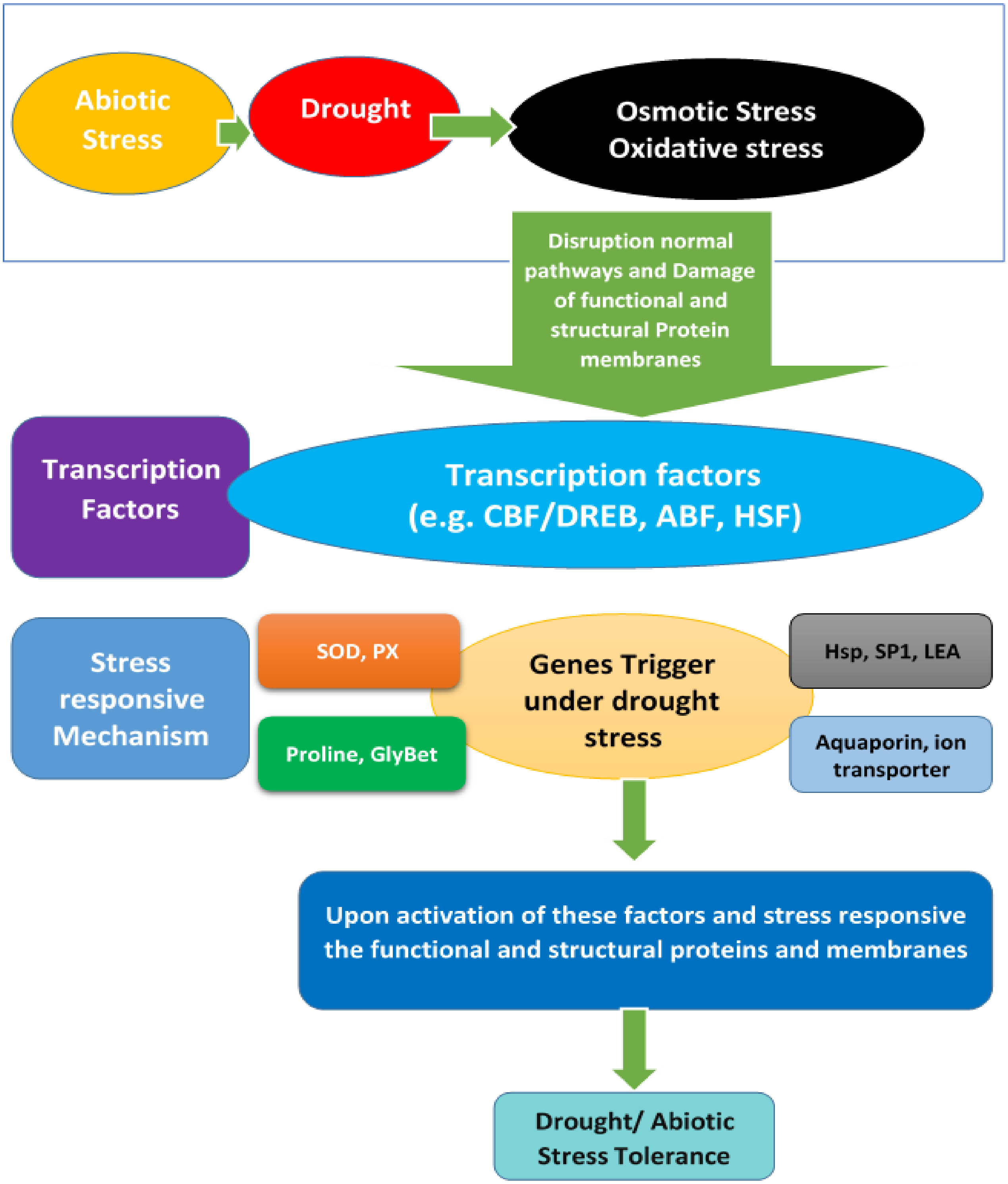

Fig. 2 Mechanism of drought stress tolerance in plants

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