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MECHANISM OF DROUGHT STRESS TOLERANCE IN MAIZE

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Abstract: *Drought stress greatly threatens agricultural productivity, particularly in arid and semi-arid regions. Maize is a key crop globally, and understanding its mechanisms of drought stress tolerance is of utmost importance for sustainable food production. This paper reviews the literature on the molecular and biochemical mechanisms governing maize's response to water scarcity. Further, epigenetic plasticity, transcription regulation, metabolic reprogramming, and gene expression are discussed in detail as adaptive strategies. Additionally, conventional techniques, such as cross-breeding and mutation breeding, as well as biotechnological approaches, like QTL mapping, molecular marker-assisted breeding, transgenic approach, and CRISPR-Cas9, are reviewed as strategies to enhance maize's drought tolerance. This paper concludes by emphasizing the need for additional research to develop advanced crop varieties with improved drought tolerance, contributing to greater sustainability and food security worldwide.*

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Introduction

Climate change, characterized by the alteration of global and regional weather patterns, has a major impact on the productivity of crops. Various human activities, such as urbanization, industrialization, deforestation, and changing land-use practices, are responsible for the emission of greenhouse gases into the atmosphere, accelerating the rate of climate change. Common consequences of climate change include sudden temperature increases, increased levels of carbon dioxide, and fluctuating rainfall patterns. According to a report from the Intergovernmental Panel on Climate Change (IPCC) in 2018, global temperatures have been raised by almost 1.0 ℃ due to industrial waste in the environment (Masson-Delmotte *et al.*, 2018). If anthropogenic activities are continued at the same rate, global temperatures are projected to rise 1.5 ℃ by 2052. As a result of increased temperatures, low soil moisture levels have become a major challenge to crops, particularly in arid and semi-arid regions.

Growing crops in open atmospheres exposes them to biotic and abiotic stresses throughout their life cycle. These stresses can significantly impact their growth potential and yield. Drought, particularly in rainfed areas or those with inadequate irrigation systems, is a global concern that can cause reduced yield and stunted growth. This is due to the lack of water needed to complete the plant's normal life cycle, which can alter its metabolic activities and cause a decrease in light-absorbing ability due to the reduced production of photosynthetic pigments. Temperature, pollutants, heavy metals, soil pH, and chemicals are some of the other abiotic factors that limit the productivity of our agricultural sector. Studies by Ludlow & Muchow (1990) and Lawlor & Cornic (2002) have highlighted the detrimental effects of these stresses on crop plants. Meena *et al.* (2017) further discuss the impact of drought stress on crop growth and yield.

The global population is growing exponentially, resulting in increasing levels of drought stress which threaten the proper functioning of agricultural systems (Vurukonda *et al.*, 2016). To cope with these low water conditions, plants have evolved certain adaptive mechanisms such as epigenetic plasticity, transcription regulation, metabolic reprogramming, and gene expression (Chinnusamy & Zhu, 2009; Zhu, 2016). Intensive research has been conducted to understand the drought stress tolerance mechanism in the model plant Arabidopsis thaliana (Clauw *et al.*, 2016; Provart *et al.*, 2016; Shinozaki & Yamaguchi-Shinozaki, 2007; Zhu, 2016). However, the application of this research to crop species has yet to yield much progress (Joshi *et al.*, 2016; Li & Cui, 2014). In contrast to Arabidopsis, plant species have developed various other strategies to deal with stress, including artificial selection, metabolic pathways, evolutionary processes, and drought-responsive gene regulation systems. Consequently, further research is required to gain a greater understanding of such stress-related mechanisms in crop species, which can be used to facilitate the production of advanced crop varieties that can withstand drought and provide increased food security.

Status and Effect of Drought Stress in Maize Crop

Maize originated in Mexico around 8700 years ago and has become a major food crop worldwide (Vallebueno-Estrada *et al*., 2016). Not only is maize a staple cereal crop, but it is also used as animal feed and a source of biofuels. In terms of production, maize surpasses rice or wheat (McCann, 2005). However, its yield potential is limited by water scarcity in many parts of the world. Most of the world's maize production comes from the United States, producing more than 30% of the total (https://www.statista.com/). Despite its global production, maize cultivation faces serious issues such as drought and water stress (Webber *et al*., 2018). Sustainable production technologies and drought-resistant cultivars of maize must be implemented to increase the economic value of maize. Zea mays L., originating from the tropics, plays a major role in the global agricultural system and is one of the most commonly grown crops (Tesfaye *et al*., 2018). Although cultivated worldwide, most maize is cultivated in semi-arid areas and is thus exposed to water deficiency, high temperatures, or both (Zhao *et al*., 2016). Drought and heat stresses can affect maize crops at all stages, with the greatest impact occurring during the reproductive growth stages and when the plants reach the 8th leaf stage (Chen *et al*., 2010). Water scarcity during the growing season can cause a decrease in maize production of up to 15% (Ziyomo & Bernardo, 2013). In the major maize-producing areas of China, almost 60% of the crop is exposed to water and heat stress, resulting in a 30% reduction in the annual yield (Zhao *et al*., 2016). Considering the rapid climate change and changing weather patterns worldwide, water and heat stress are anticipated to further reduce the global maize supply by 15-20% each year (Chen *et al*., 2012). It is clear from previous studies that the coexistence of heat and water stress can cause severe damage to the productivity and growth of maize crops. Moreover, individual stresses can significantly impact the yield (De Boeck *et al*., 2016; Suzuki *et al*., 2014). Temperature above 35℃ can visibly affect maize crops' reproductive and vegetative growth, from seed germination to grain filling, the last stage (Hatfield *et al*., 2011). Nevertheless, when both water and heat stress occurs during the maize's reproductive stages, the crop is even more susceptible. On the other hand, individual stresses can produce different effects on the reproductive traits of plants (Suzuki *et al*., 2014). The most notable effects of drought stress include photosynthetic activity, membrane stability, leaf area, stomatal conductance, water use efficiency, and oxidative metabolism (Zheng *et al*., 2016).

Maize cultivation involves several critical growth stages, such as seedling emergence and development, vegetative growth, flowering and pollination, grain filling, and maturation. Water scarcity during any of these stages can negatively affect the yield and quality of the maize crop. Research has demonstrated that water stress during the vegetative growth stage can cause a reduction in growth rate, redirect root systems, prolong the vegetative growth phase, and alter the distribution of CO_2. A shorter period of water scarcity can result in a 28-32% reduction in dry weight during the vegetative growth phase and 66-93% during the tasselling/ear formation phase (Cakir, 2004). Prolonged drought stress before flowering has been found to reduce leaf size and internodal distance and delay silk emergence tasselling ling, leading to a 15-25% decrease in overall yield. Moreover, drought stress lasting even a few days during the pollination/fertilization stage may cause abnormal embryo formation and fewer kernels per plant. Drought stress in the pre-and post-pollination stages has also been linked to a significant reduction in kernel set. The photosynthetic activity carried out by the maize plant's five- or six-ear leaves is responsible for most of the plant's biomass (Liu *et al*., 2015). However, drought stress can reduce the photosynthetic rate by shrinking the size of the ear leaves, resulting in a slowed crop growth (Aslam *et al*., 2015).

Adaptations of Maize to Enhance Drought Tolerance

Crop plants possess natural molecular and physiological features that allow them to cope with various biotic and abiotic stresses. These adaptations have developed over time through both natural and artificial selection. Different plant species and varieties have varying responses to water stress, depending on the plant's development stage when the drought takes place, the rate of dehydration, and the intensity and length of the stress. The capacity of a crop plant to implement drought defense strategies when presented with water-deficient conditions is known as drought tolerance or resistance. Drought resistance is a complex character in crops and is accomplished through three distinct approaches: drought escape, drought avoidance, and drought tolerance (Figure 1).

Drought Escape

To avoid the damaging effects of drought and water stress, crops have developed the capacity to reduce their growing period to complete their life cycle before the start of the drought. Through selection procedures, genetically heritable traits such as germination, inflorescence, and maturity can be manipulated based on moisture availability. This will be determined by the genotype of the plant and the degree of moisture present in the soil, ultimately allowing the crop to finish its cycle ahead of the drought. Maize crops are particularly vulnerable to drought stress, so adopting an escape mechanism is especially important. This can be achieved by cultivating early maturing or short-duration cultivars (Kumar & Abbo, 2001). However, this will result in reduced yields, as the crop's duration directly impacts the yield (Turner *et al*., 2001).

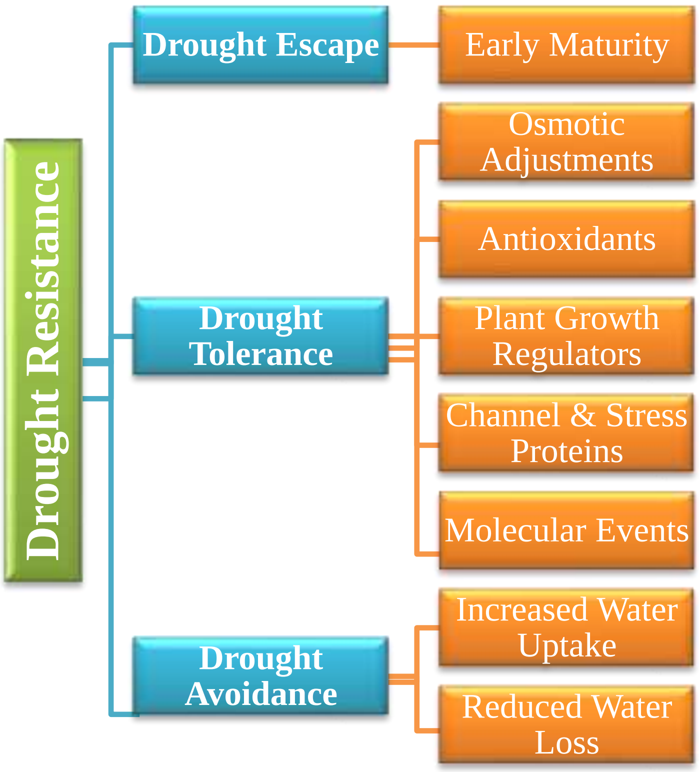


Figure 1: Physiological, morphological, and biochemical mechanisms to drought resistance in maize crop

Drought Avoidance

Drought avoidance is the ability of plants to maintain an adequate turgor pressure/tissue water level when water availability in the natural environment or soil is limited. This process involves sustaining metabolic and physiological functions without being affected by water stress or drought (Blum *et al*., 2011). Drought avoidance can be described as measuring the water content in plant tissues during water stress, usually expressed in terms of turgor pressure. To regulate the water status in their tissues, crop plants increase their water uptake or reduce the transpiration rate when there is a lack of water. In maize, some of the most common features of drought avoidance are leaf firing, leaf attributes, stomata closure, leaf rolling, canopy temperature, and root attributes. Stomata are essential for respiration and photosynthesis as they are the gaseous exchange and transpiration points. However, when the water supply is limited, plants close their stomata to avoid excessive water loss, leading to decreased yield due to its effects on respiration and photosynthesis. To combat this, maize plants have two attributes or traits they use to avoid drought: leaf firing and leaf rolling. Leaf firing results from a reduced transpiration rate, where the leaves become dry. This is accompanied by the developing of an extensive root system to take up water from the ground. The root attributes for effective drought avoidance are waxy or hairy root texture, stomatal conductance, and functional and phenotypic traits that promote water uptake. Both of these mechanisms help to avoid drought in maize.

Drought Tolerance

Plants' ability to withstand drought conditions and grow normally is known as drought tolerance. Along with sustaining normal physical growth, drought tolerance is also associated with yield stability under water-stressed conditions, a complex process in which crops have developed various natural mechanisms to adapt and tolerate drought stress. These include adaptative mechanisms at the molecular, physiological, and economic levels. A high economic yield in water-stressed conditions is crucial for successful drought tolerance. At the seedling stage, survival is key, but when they reach the reproductive growth stage, farmers and breeders become more interested in the yield of their crops. To improve drought tolerance in maize crops, attention should be paid to growth, grain filling, phenology, transfer of photoassimilate reserves (Edmeades, 2013), and other mechanisms such as plant growth regulators, transcription factors, antioxidant defense processes, stress, and water channel proteins, and signal transduction pathways.

Current Status of Breeding Strategies for Drought Tolerance in Maize

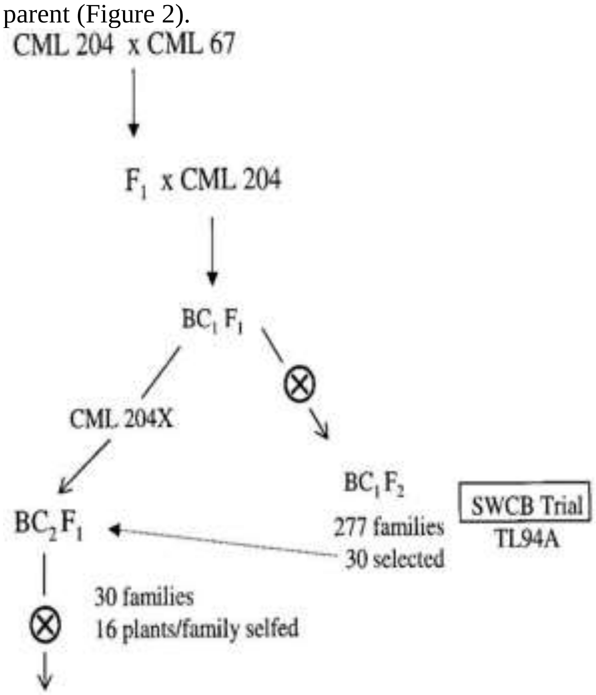
Utilizing conventional breeding and biotechnological strategies effectively improves crop productivity and resilience to abiotic stress, such as drought. Breeding plants with desirable traits, such as drought tolerance, through conventional methods requires crossing and selecting plants over several generations (Ahmar *et al*., 2020). On the other hand, biotechnological techniques involve the genetic engineering and manipulation of plants to introduce desired genes or traits (Martignago *et al*., 2020). Both approaches have successfully developed drought-tolerant crops and, when used in conjunction, can produce even more impressive results. By combining the advantages of conventional breeding and biotechnology, researchers and farmers can work together to create crops that can withstand increasingly frequent and severe droughts caused by climate change.

Conventional Breeding

Conventional breeding strategies for maize cultivars with improved drought tolerance involve selecting and cross-breeding plants with desirable traits that allow them to survive and thrive under drought conditions. This process requires time, expertise, and access to diverse genetic resources (Rosero *et al*., 2020); yet, it offers a promising pathway to create cultivars that can withstand the more frequent and intense droughts caused by climate change. Examples of these strategies are outlined below:

Backcrossing

Backcrossing is a widely employed breeding strategy for creating maize cultivars with improved drought tolerance. This method involves crossing a drought-tolerant donor parent with a recipient parent with desirable traits, such as high yield potential, to generate an F_1 generation (Hospital, 2005). Subsequent generations are then crossed back to the recipient parent, and each is screened for drought tolerance as well as the other desirable traits of the recipient parent. This process enables the transfer of the drought-tolerance trait from the donor parent to the recipient parent while preserving the other desirable features of the recipient parent. The progeny of the initial cross that exhibits the desired drought-tolerant trait is chosen and crossed with the recipient parent for several generations until the resulting maize cultivar has a genetic makeup similar to the recipient parent except for the particular drought-tolerant trait acquired from the donor parent. Backcrossing has been used effectively in numerous studies to create drought-tolerant maize cultivars. For instance, Indian researchers used backcrossing to transfer a drought-tolerant gene (DREB1A) from wild maize relative to an elite maize cultivar, resulting in a novel cultivar that displayed remarkable drought tolerance (Sarkar *et al*., 2019). Similarly, a Chinese study employed backcrossing to move a drought-tolerant QTL from a drought-tolerant maize line to an elite maize cultivar, creating a new cultivar that showed increased yield under drought conditions. Overall, backcrossing is a powerful approach for conventional breeding strategies for drought tolerance in maize and facilitates the transmission of desirable traits while retaining the other desirable qualities of the recipient parent (Figure 2).



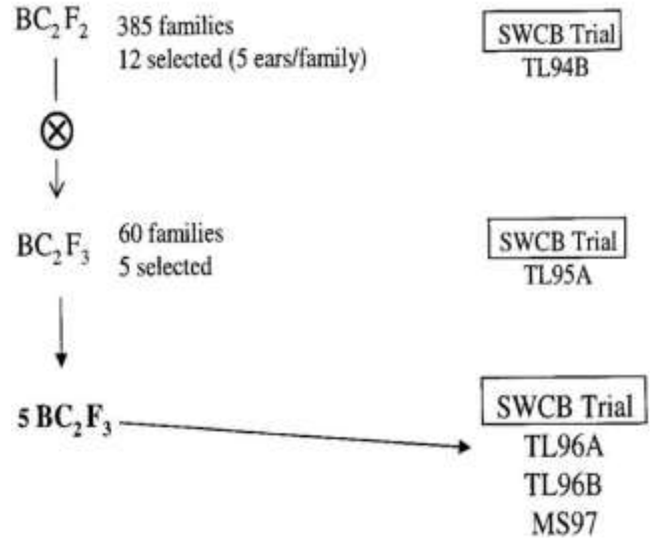


Figure 2: Diagram of the marker-assisted backcrossing procedure

Mutation Breeding

Mutation breeding is a useful conventional strategy for developing drought-tolerant maize cultivars (Gedil & Menkir, 2019). The process involves exposing maize plants to radiation or chemicals to induce random mutations in their DNA. This can produce a broad range of genetic variability in a relatively short period. Gamma-ray irradiation is one approach to mutation breeding, where maize seeds are exposed to gamma radiation to induce mutations. Another approach is to use chemical mutagens like ethyl methane sulfonate (EMS) to cause mutations in the DNA by soaking maize seeds in a solution of EMS (Varshney *et al*., 2021). The mutated plants are then screened for desirable traits, such as drought tolerance, and the most promising ones are selected for further breeding. It is important to note that mutation breeding can also introduce undesirable mutations or affect non-targeted genes. Therefore, carefully screening and selecting mutated plants are key to ensuring that the desired trait is successfully introduced without negatively impacting other characteristics.

Biotechnological Breeding

Tolerance or resistance to drought is a quantitatively-inherited trait in maize crops, with many genes controlling its expression or phenotype. Consequently, drought tolerance is a complex trait to characterize. Genomics offers a more suitable approach for studying this complex quantitative trait than traditional breeding techniques. This approach has enabled the identification of quantitative trait loci (QTL) to facilitate marker-assisted selection (MAS) for cloning these loci and manipulating them through genetic engineering. Consequently, significant advancements have been made in improving drought tolerance in maize by locating the QTL controlling various drought responses (ZHU *et al*., 2011). The following section provides an overview of the key advances in improving drought tolerance in maize.

QTL Mapping

QTL mapping is a commonly used technique to decipher the genetic basis of quantitative traits controlled by multiple genes. It is also the basis for a marker-assisted selection (MAS) selection process. This technique is usually used for populations derived from a cross between two parents (biparental progeny). QTL mapping helps to identify if a particular region of the chromosome is linked to a certain trait or phenotype. In the last century, several molecular markers have been used for QTL analysis, such as simple sequence repeats (SSRs), random amplified polymorphism DNA (RAPD), sequence characterized amplified regions, and restriction fragment length polymorphism (RFLPs) (Kim *et al*., 2000). The early seedling stage of maize is essential for its overall development and grain yield, and it is also suitable for identifying QTLs related to drought tolerance. According to a research study, seven QTLs controlling the survival rate of maize were located on different chromosomes (3, 4, 6, 7, and 9) using 93 SSR markers (Hao *et al*., 2009). Additionally, nine QTLs associated with leaf temperature were identified in 248 SSR markers on chromosomes 1, 2, 9, and 10 through recombinant inbred lines (Liu *et al*., 2011). Due to its complex genetic basis and low heritability for crop yield, individual trait components such as ASI and KNR are investigated more often than drought tolerance (Monneveux *et al*. 2008; Xue *et al*. 2013). QTLs related to grain yield and flowering time have been reported, some of which have been identified under normal and water-stressed conditions. Drought tolerance genomic segments are responsible for yield component traits were identified in 142 RILs derived from a cross between drought-tolerant and drought-sensitive maize genotypes, while 80 F4 random families from a cross between high and low L-ABA parent genotypes (Landi *et al*. 2005) revealed QTLs controlling bulk-leaf ABA concentration. Moreover, two QTLs for ear setting were found to explain about 19.9% of the phenotypic variance under well-watered conditions (Zhao *et al*., 2023). However, despite identifying many QTLs, few studies have focused on fine mapping of QTLs, which would make it possible to locate genetic positions and clone candidate genes precisely. This is due to the need for large secondary populations.

Transgenic Approach

Transgenic approaches offer a biotechnological breeding strategy for drought tolerance in maize, utilizing genes from other species known to confer drought tolerance (Anwar & Kim, 2020). For example, the insertion of foreign genes encoding proteins that control stomatal closure or enhance water uptake has been shown to improve drought tolerance in maize (McMillen *et al*., 2022). Additionally, genes from other species can be inserted into maize to target pathways involved in drought stress response, such as ABA signaling and aquaporin regulation, which control water movement in plant cells (Yang *et al*., 2021). These genes can be introduced into maize using various techniques, such as biolistic or Agrobacterium-mediated transformation, and the resulting transgenic plants can then be screened for improved drought tolerance. Transgenic approaches offer the benefit of precise manipulation of specific genes or pathways involved in drought tolerance, which can result in more efficient improvements in maize productivity under drought conditions (Sheoran *et al*., 2022). However, using transgenic approaches can raise regulatory and public perception issues related to introducing foreign genes into food crops. Several practical steps must be taken to use transgenic approaches to improve drought tolerance in maize. Researchers must identify candidate genes from other plants or organisms that confer drought tolerance (Wang *et al*., 2021). These genes can be used to construct gene expression vectors, which are plasmids containing regulatory elements and can introduce foreign DNA into maize cells (Nora *et al*., 2019). Subsequently, transgenic events can be generated through Agrobacterium-mediated, biolistic, or protoplast transformations (Du *et al*., 2019). To identify successful events, selectable marker genes can be linked to the gene of interest to confer resistance to antibiotics or herbicides (Du *et al*., 2019). Once grown, the transgenic plants can be screened for improved drought tolerance under controlled conditions, such as water deficit or simulated drought stress (Mahmood *et al*., 2019). Ultimately, successful transgenic events can be used in breeding programs to develop drought-tolerant maize varieties for farmers to cultivate in water-limited environments.

Molecular Marker-Assisted Breeding

First, molecular markers associated with drought tolerance are identified. These markers may consist of DNA sequences, single nucleotide polymorphisms (SNPs), or other markers associated with drought tolerance traits, such as water use efficiency or root architecture. Next, the identified markers are used to screen large populations of maize plants for the presence of the desired traits. Plants with the desired markers are then selected for further breeding, increasing the likelihood that the offspring will also have the desired traits. Molecular marker-assisted breeding can also be used with other biotechnological approaches, such as transgenic approaches or genome editing techniques, to improve drought tolerance in maize further. This combination of methods can help breeders to achieve higher drought tolerance in maize varieties, as well as provide breeders with the opportunity to incorporate specific traits into elite maize cultivars. Molecular marker-assisted breeding can save time and resources compared to traditional breeding methods, allowing breeders to select plants with desired traits at the initial stages of development. It also increases the efficiency and precision of breeding programs by reducing the need for phenotypic evaluation of large populations (Hasan *et* *al*., 2021). Molecular marker-assisted breeding is an effective tool for developing maize cultivars with improved drought tolerance. This approach combines genetic and phenotypic analysis to allow for the early selection of plants with desirable traits, thus saving time and resources compared to traditional breeding methods. Finally, phenotypic evaluation is conducted on the selected plants to evaluate their performance in different drought conditions. The results of this evaluation are then used to select the best-performing plants for further breeding (Figure 3).

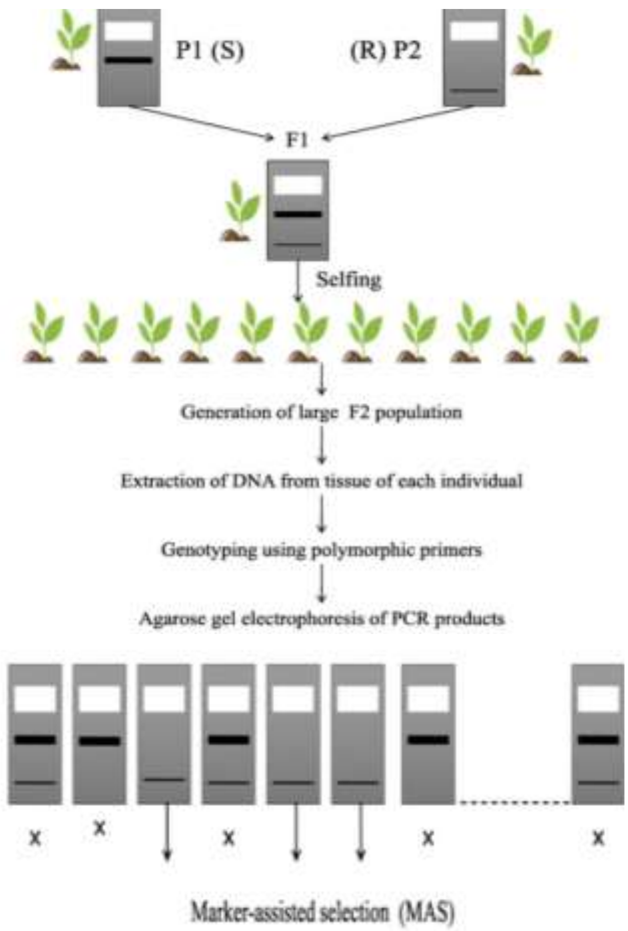


Figure 3: The figure explains the basic procedure of marker-assisted selection

CRISPR-Cas 9

CRISPR-Cas9 is a revolutionary biotechnological tool that can create drought-resistant maize cultivars. It is a precise genome editing technique that enables modifications to be made to the DNA of plants, such as maize. Using CRISPR-Cas9, researchers can identify and modify genes associated with drought tolerance, like those that control water uptake, water-use efficiency, or the production of Osmo protectants like proline (Shelake *et al*., 2022). One of the major benefits of using CRISPR-Cas9 for drought tolerance is that it eliminates the need for long backcrossing or other conventional breeding strategies (Zhu *et al*., 2021). This can significantly reduce the time and resources required to develop new drought-resistant maize cultivars. However, it should be noted that using CRISPR-Cas9 in crop breeding is still a relatively new and rapidly evolving technology. There are concerns about the potential unintended effects of genome editing, including off-target effects, unintended mutations, and the impact on the safety and regulatory aspects of the resulting crops (Jaganathan *et al*., 2018). In conclusion, CRISPR-Cas9 can be a powerful tool for developing drought-tolerant maize cultivars; it should be used with other conventional breeding strategies to ensure the safety, effectiveness, and widespread acceptance of the resulting cultivars (Rosero *et al*., 2020).

Conclusion

To sum up, drought stress is a significant obstacle to crop production, particularly in areas with limited rainfall or inadequate irrigation systems. Maize is an entire crop worldwide, and in-depth knowledge of its mechanisms of drought stress tolerance is essential for sustainable food production. This article has looked into the current understanding of maize's tolerance to drought or water stress, focusing on different molecular and biochemical mechanisms which account for the plant's response to water stress. These include epigenetic plasticity, transcription regulation, metabolic reprogramming, and gene expression. Further research is required to improve our investigation and comprehension of the mechanisms that underlie the drought tolerance in various crop species and speed up the production of advanced crop species that can withstand drought stress more effectively. This could result in higher sustainability and food security worldwide, ultimately helping to address the issues caused by climate change.

Conflict of interest

The authors declared the absence of a conflict of interest.

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