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## Transgenic Wheat for Drought Stress Tolerance: A Review

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### ABSTRACT

Wheat occupies the first place in terms of strategic and nutritional importance globally, as it represents the main food for all the peoples of the earth, as well as being a high nutritional value fodder for animals and birds alike. In light of drought stress crisis and the limited irrigation water, we find that the areas specified for wheat cultivation are in constant decline besides, the problem of increasing population, it is noted that the process of providing wheat necessary for the bread industry takes on the priority of the strategic challenges facing the Egyptian state. In this regard, water stress is considered one of the most important and dangerous environmental obstacles that greatly limit and destroy agricultural production not only at locally but also at the globally. Also, drought stress reduces the final output of any crop by up to 50 % besides, the highly average temperatures at the same time especially in areas classified as scarce or water-poor like the Arab Republic of Egypt which exacerbates the problem. Therefore, these two environmental factors are enough to eliminate the future of agriculture in generally and in wheat in particular. This a review attempts to shed light and focus on the most important genetically, physiological and biochemical responses related to water stress tolerance in transgenic wheat. Further, these changes are considered as the main gateway on which studies and research in the production of genetically modified wheat are based to confront the problem of water shortage.

**Keywords:** Wheat, water stress tolerance, transgenic crops, molecular breeding, biotechnology.

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### 1. Introduction

Wheat (*Triticum aestivum* L.) is the first important and strategic crop for the majority of the world's population, as it feeds nearly two billion people, or 36% of the world's population. The wheat crop is considered an integrated food basket, as it contains 20% of the total calories consumed globally and 55% of carbohydrates (Breiman and Graur 1995; El-Mouhamady *et al.*, 2011 & 2014 & 2017). The global area of wheat exceeds the area of both rice and maize and this extinguishes and emphasizes the great importance and vital role that this crop provides as an essential food artery for humankind. However, the factors of climate change, especially the limited irrigation water, the excessive rise in temperatures, global warming and the high salinity level in soil, especially that Egyptian wheat is growing in winter of areas nearing from sea water due to the lack of water needed for soil washing operations. Thus, this in turn leads to the accumulation of salt levels, which negatively affects growth, physiological processes, final productivity and grain quality for wheat crop (Sairam *et al.*, 2002; Abbas *et al.*, 2013; El-Mouhamady *et al.*, 2016 & 2019; 2021a & b). In the same context, it is noted that water stress has serious and destructive effects on all stages of crops growth and productivity in general, especially wheat. Further, water stress is considered an imminent and extremely harmful threat to the

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wheat crop. It is expected that by 2025 1.8 billion people will face the threat of drought stress and that 65% of the world's population will live in environments that suffer from severe water deficit conditions (Nezhadahmadi *et al.*, 2013). There are a lot of some physiological attributes negatively affected by water stress in wheat plants such as root length, grain number per spike and grain weight per spike (Moustafa *et al.*, 1996; Plaut *et al.*, 2004; Blum 2005). Also, wheat is grown in rainy conditions, and since the rainy season occurs in which many variations occur, if the problem of limited irrigation water intended for agriculture, especially in the flowering and the grain filling stages, it is found that the final output of the grain yield is constantly decreasing (Bassi and Sanchez-Garcia 2017). Therefore, the aggravation of the problem of water shortage in parallel with the excessive increase in the world's population may lead beyond any doubt in the events of a real famine that may wipe out the lives of millions people. From this standpoint, thousands of local and international papers were launched to find practical solutions as an attempt to reduce drought stress crisis and to devise resistant lines to water stress in various crops (Esmail *et al.*, 2016 and El-Mouhamady *et al.*, 2022). This will only be achieved by following novel plant breeding methods such as genetic engineering and biotechnology through elicitation genetically modified wheat in order to save the time needed to reach the best genetic models of wheat with high productivity and tolerant of climate change factors, especially water deficit conditions and this is what we will discuss in detail in this regard. Principles, water stress can be defined as a significant and clear shortage of water necessary for the plant's life cycle, starting with growth, germination, flowering stages and seed fullness, which automatically affects the survival of the plant and its final productivity. This occurs when the rate of transpiration or water loss is much higher than the rate of water absorption (Bray 1997). Drought stress causes a significant hindrance to the growth, development and production of plants by altering physiological processes as well, altering biochemical pathways (Bayoumi *et al.*, 2008; Afzal *et al.*, 2017). Further, water stress, especially in areas close to the Nile Valley and the Delta region, leads to the accumulation and build-up of salt levels to an unsafe level which impedes the cultivation of various crops and negatively affects their growth and productivity (El-Mouhamady *et al.*, 2021 A & B and Khatab *et al.*, 2021 a & b). Biotechnology techniques, including genetic engineering and omics method, have been integrated into plant breeding programs in order to accelerate the genetic benefits and expected gains in increasing water stress tolerance in crops (Khan and Iqbal 2011). MAS or Marker assisted selection will help plant breeders and those in charge of genetic improvement of drought stress resistance in wheat through reducing the breeding cycle because it depends on the selection of resistance attributed regardless of the stage of plant growth and without interaction between genotype and environment (Acquaah 2012). The means of plant breeding in order to confront and bear the risk of water stress is a method characterized by an economic approach, especially in a strategic crop such as wheat, with the aim of raising its productivity in arid and semi-arid areas (Ray *et al.*, 2013). Genetic engineering has made a major leap in improving plants' tolerance to environmental stresses such as salinity, drought stress and pathogens through transferring genes responsible for enduring these stresses from their wild genetically distant sources to local accessions that are highly sensitive to such stresses (Bakhsh and Hussain 2015). Thus, Transformational techniques were used to identify regulatory guides and pathways that would inoculate different crops with tolerance genes for water stress, salinity, extreme cold and other stresses, whether environmental or biological. (Liang 2016) emphasized that the improvement of crops tolerant to water stress using biotechnology programs may secure sources of global food security because this scientific technology contributes significantly to increasing the area of arable land as well as increasing the degree of growth and productivity of plants. Biotechnology is one of the most famous applications of genetic engineering, which had the greatest credit for raising, improving and increasing crop productivity in light of the scarcity of water sources available for agriculture (Martignago 2020). They detected that *Arabidopsis thaliana* plant was a fertile material for study by using it to modify a large number of genetically engineered genes for water stress tolerance in different crops. However, the productivity was not at the satisfactory level and this confirms that scientists still need new ways to eliminate or reduce the impact of water stress on crops. Water stress affects the wheat crop significantly, as this effect includes all stages of wheat plant growth, especially during the stages of germination, seedling formation, tillering, flowering and grain filling which reflects a great damage on the final output (Baloch *et al.*, 2012; Zhang *et al.*, 2018; Khadka *et al.*, 2020). (Bapela *et al.*, 2022; El-Mouhamady *et al.*, 2022 A & B) reported that the use of genetic resources and genetic engineering techniques, especially biotechnology, has led to an acceleration of the pace of molecular breeding and genetic improvement

of various crops in order to adapt to biotic and abiotic stresses. Thus, the technology of genetic transfer or genetic modification between the same genera, species, or foreign species to the wheat crop added a big leap in the path of genetic modification through the transfer of genes for adaptation to water stress or high temperature. After all that has been mentioned, we can briefly say that the only and last hope to save humanity from the real threat of famine, which threatens the lives of two thirds of the world's population, especially in light of climate change, unfavorable environmental conditions and limited agricultural and drinking water is genetically modified and engineered crops especially wheat because of this crop of strategic importance at all levels whether human or animal. In order to achieve this strategic objective, it is necessary first to know and study aspects of the genetic, morphological, physiological and biochemical changes of plants in general and in transgenic wheat plants in particular, immediately upon exposure to water stress and this will be discussed in some detail in this regard. Further, mentioning the most important research and studies results that focused on genetic improvement in wheat crop to face water stress through genetic transfer technology.

## 1. Review Methods

As part of its procedural methodology, this review conducted a larger literature search and synthesis of relevant peer-reviewed journal articles, workshop papers, books, thesis works, and symposia.

## 2. Main Text

### 2.1. Influence of Drought Stress on Plants

Water stress is one of the most difficult environmental constraints that significantly limit the global agricultural area where it greatly affects all stages of plant growth, improvement, metabolism and productivity ultimately destroying the final output. Thus, the final destruction reaches approximately 50% of the final economic grain harvest. Therefore, drought is the main environmental stress that threatens all plants especially in drought-prone areas (Anjum *et al.*, 2011; Abo-Hamed *et al.*, 2016 and Diatta *et al.*, 2020). The negative effects of drought stress escalate and are directly reflected on agriculture due to the depletion and limited water needed for agriculture in light of the increasing global demand for food, especially with the excessive increase in the world's population (O'Connell 2017). Thus, this devastating effect of water stress comes in the first place in the destruction of crop productivity, followed in the second place by biological stresses like *Fusarium oxysporum* in lupine (Zian *et al.*, 2013). Figure (1) shows the impact of water deficit conditions on adventitious roots of plants where it was observed that plants grown under good irrigation conditions gave a root system that was far superior to their counterparts grown under water stress.

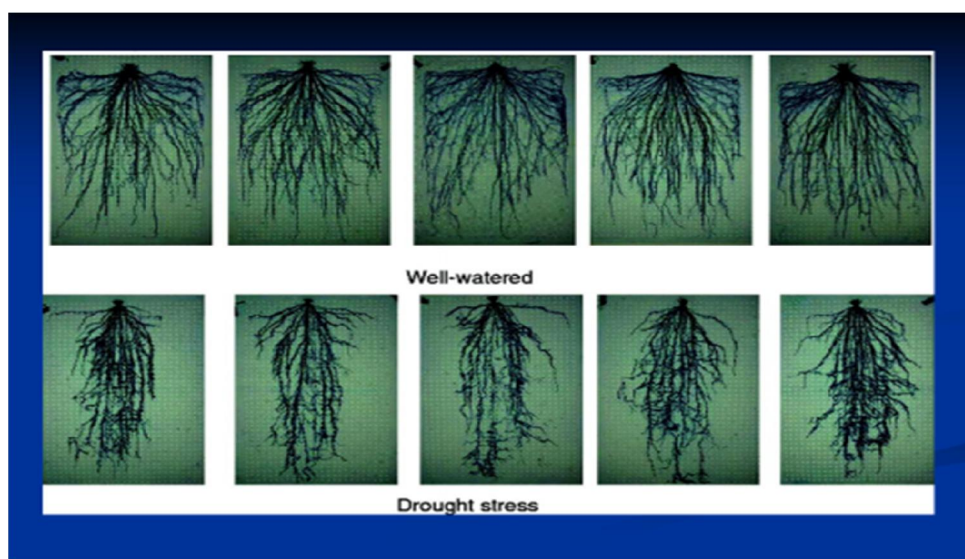


Fig. 1: Comparison of root system growth under both irrigation treatments (Normal and Water Stress).

## 2.2. Impacts of Water Stress on Agriculture Soil

For example, drought stress greatly affects the soil ecosystem, where with the intensification of temperatures in parallel with the sharp decrease in the levels of arable water a large crack occurs in the agricultural soil. Further, the agricultural soil ecosystem is also severely affected with the increasing frequency of drought, as the carbon content resulting from microbial biomass increases with the decrease in soil moisture content (Geng *et al.*, 2015; El-Mouhamady *et al.*, 2015 and El- Demardash *et al.*, 2017) in (Fig. 2). This of course leads to a clear crack in the plant roots area entrusted with the absorption of water, which accelerates the inevitable destruction of agricultural crops. The greatest credit for the aggravation of the problem of drought stress, desertification and the collapse of the structural system of agricultural soils comes due to global warming and global climatic changes such as excessive rise in temperatures and their deviation from the ideal area necessary for growth and agriculture. Where, it was observed that an increase in the temperature of one degree Celsius above the ideal limit necessary for growth and germination leads to a decrease in the final yield of any plant by a percentage that may range from 3 to 5 %, (Fig. 3.). From another angle, water deficit conditions is a severe stress on the hydrological cycle, which greatly affects society, the economy, and the environment. Also, it affects the function (Liu *et al.*, 2010; Balsler and Firestone 2005), structure (Zak *et al.*, 2003) and productivity (Lal *et al.*, 2013) of the soil ecosystem.

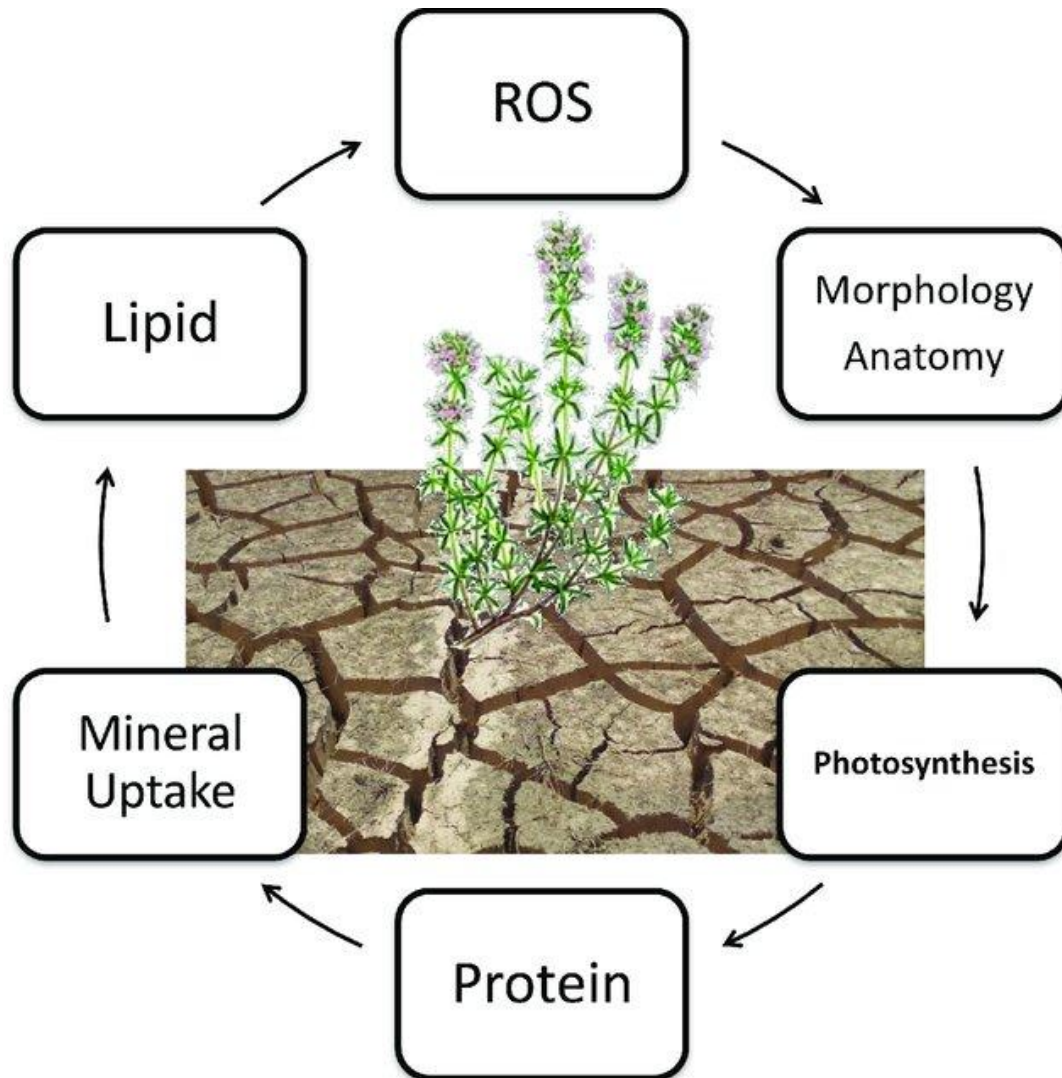
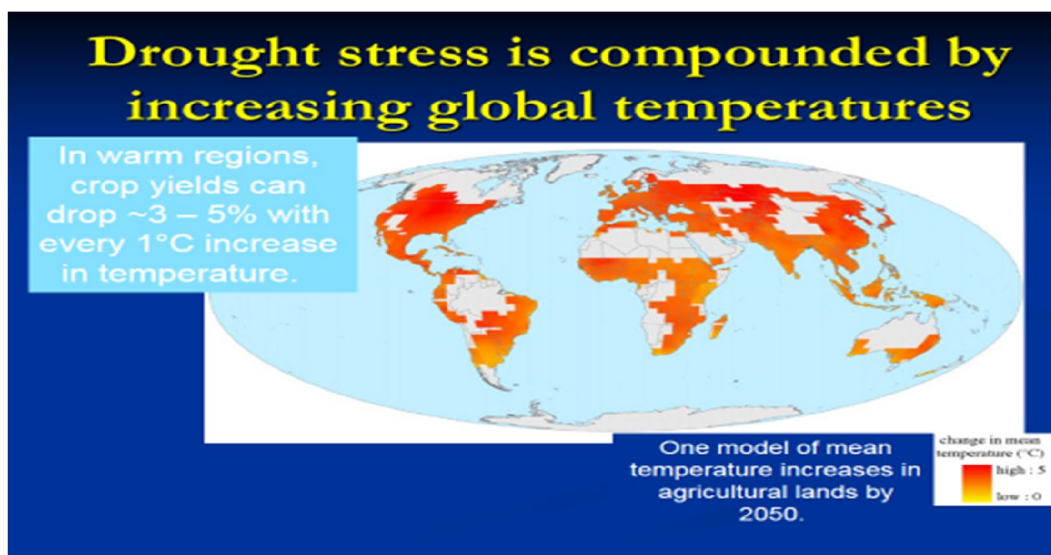


Fig. 2: The influence of drought stress on soil agriculture.





**Fig. 3:** The rise in temperature and its reflection on the final output.  
Source: Abbas S. R., 2015. Lec # 10Plant biotechnology and food biotechnology. 9-2.

### 2.3. Changes Associated with Water stress Tolerance in Transgenic Wheat

There are many responses that appear clearly for wheat plants immediately after exposed to an environment of water stress. These responses are translated as signals of tolerance or sensitivity and below is a detailed presentation of the most important results through studies and papers that explained these scientific facts.

### 2.4. Genetically Changes and Quantitative Trait Locus (QTL) Analysis

The genetically modified wheat strategy is very important for a good number of reasons according to (Wilson *et al.*, 2003; William and Wilson 2004). Further, salinity and water stress responsible for highly rank of damage on the final output in all crops like rice, (El-Mouhamady 2003 & 2009). For a thorough and constructive discussion of the points related to this topic, it is necessary to know that genetically modified wheat is one of the first cereal crops that has been genetically modified through introducing a large number of important attributes into its genome whether by genetic engineering or by some chemical mutagens such as ethyl methane sulfonate, especially to raise wheat tolerance to abiotic stresses (Heiba *et al.*, 2016). From another angle, the wheat crop, due to its great importance, is traded between different countries of the world including the producing and importing countries. Also, each of its have independent laws to regulate the process of trade in genetically modified crops and for the marketing of foods produced from those crops. Moreover, the demand for these products will be different with or without genetically modified components with the legalization of a mechanism to provide all scientific and technical information (database) to all consumers from all over the world. The process of integration between traditional plant breeding methods (MAS) and genetically modified crops led to a successful leap in the path of crop improvement. It is simply the process of combining new genes or genetic traits and merging them with the rest of total genome represented by the rest of promising germplasms for improving field crops through the gene expression of these new genes in the genetically modified plant. This can only be done by discovering new genetic genes and introducing them to plant cells, which in turn express themselves genetically in the new genetically modified plant. This does not mean that transferring and inserting new genes into the genome of the plant that is required to be genetically modified by biotechnology does not diminish the importance of the genetic origins and the distinguished old genetic structures but it is an integrated link of a group of sciences, each complementing the other (Kaehler 2006). Moreover, two previous studies have already used real options for analyzing of genetically modified traits values of wheat crop (Furtan *et al.*, 2003; Carter *et al.*, 2005; Flagg 2008) besides, (Al-Kordy *et al.*, 2019) in rice and (Tawfik and

El-Mouhamdy 2019) in sorghum. Also, (Edmeades 2006) confirmed that the genetically modified maize has outperformed its non-modified counterparts, as it gave the former a higher yield than the normal yield by 8-22 %, with an average of 15%, under water stress conditions knowing that drought destroys 50% of the final output of any crop. Where, competition took place between companies producing genetically modified crops based on the efficiency of genetic traits. This point in particular is considered a very important milestone in the analysis of values in the context of genetic competition. However, the comparison does not distinguish the efficiency of the genetic trait between genetically modified crop technology and the other crops produced by the plant breeding market. In the same track (Kirigwi *et al.*, 2007) analyzed (QTL) responsible for yield attributes in wheat crop under water stress conditions. They revealed that a hybridization among local accessions 'Dharwar Dry' (drought resistance) and 'Sitta' entry was producing 127 crosses. These wheat genotypes were grown for two years in a field under various levels of irrigation in Ciudad Obregon, Sonora, Mexico. Among the most important achievements of this investigation, a genetic map or marker called (SSR/EST-STs) was identified for grain yield trait close to chromosome 4AL. This marker was proved highly rank of relationship for improving the final output and performance under water deficit conditions. Also, the cultivar; Dharwar Dry' succeeded in giving alleles, which are an indicator for improving the performance under water stress conditions. Further, the microsatellite marker wmc89 will be very important for MAS to improve water stress tolerance in wheat. Climatic changes and fluctuations in the Mediterranean region will lead to a radical change in temperatures. This dangerous matter will definitely lead to an increase in the frequency of drought and this will be followed by a decrease in the productivity of some crops such as wheat. Also, they explained that the phenomenon of genetic diversity had the greatest impact on plant breeding through selecting of plants with attributes of environmental stresses. Further, the integration of genomic tools, modern techniques, diverse genetic resources and MAS are very importance thing to enhance wheat plant breeding to mitigate the consequences of climate change, especially water stress tolerance (Habash *et al.*, 2009). Further, genetically and physiologically attributes responsible for drought tolerance in wheat were discussed through using 152 recombinant wheat inbreed lines by (Peleg *et al.*, 2009). They reported that the group modified wheat (RILs) were highly genetic diversity for all studied traits. Also, this study focused on identifying the genetic loci responsible for high productivity traits, especially on chromosomes 2B, 4A, 5A and 7B. One of the most important achievements of this research is to prove that QTLs of higher yield was linked to QTLs of water stress resistance in wheat. Therefore, QTLs maps may be an easy and distinctive tool for using wild mechanisms to develop drought resistance in wheat genotypes. Some genes responsible for water stress resistance, salinity tolerance and quantitative trait loci (QTLs) were discovered by (Nevo and Chen 2010) in both of wheat and barley entries; *T. dicoccoides* and *H. spontaneum* which have proven greatly contributing in improving and enhancing the wheat and barley crops to face salinity and drought stresses. Accordingly, the point of molecular control of drought and salinity tolerance became easy after analyzing the backcrossing QTLs and the available database of wild wheat and barley as plant lines donating salinity and water stress tolerance genes.

## 2.5. Morphological Changes

One of the most famous morphological mechanisms that plants resort to face and bear water stress is a reduction in the length and number of internodes that make up the stem, which results in a shorter plant length. This enables plants to do two things. The first one is increasing the length of roots, especially the adventitious ones to reach the water stored in the deep layers. The second benefit is the ability to carry a large number of fertile spikes without breaking the stem of the plant during drought stress (Seleiman *et al.*, 2021). When water stress crisis worsens, especially with limited irrigation water and high temperatures, plants resort to accelerating the process of growth and germination through shortening the period of vegetative growth at the expense of maturity growth. This behavior leads to reach the maturity stage as quickly as possible with the aim of saving the plant cells from the collapse achieved in all physiological processes such as the process of metabolism and biochemical processes. Eventually the plant grows rapidly and reaches maturity while thirst is still in its infancy (Seleiman *et al.*, 2021). Among the defensive methods and techniques that plants resort to in order to resist water stress and maintain their life is the noticeable decrease in leaf area index. This leads to a reduction in the specific surface area corresponding to sunlight, which reduces the rate of transpiration and water loss and water preservation in order to use it in physiological and biochemical processes that have a

strong relationship with growth and germination (Seleiman *et al.*, 2021). Also, Plants resort to leaf rolling to reduce the specific surface area facing the sun to reduce transpiration and water loss, and this mechanism is among the mechanisms that bear water stress, (Tardieu 2005 and Cal *et al.*, 2019). Plants resistant to water stress are often characterized by the fact that the number of stomata on the upper surface of the leaf is less than their counterparts on the lower surface. This helps to reduce the rate of transpiration and water loss especially in the upper surface facing the sun and this is due to the high degree of genetic specialization that these plants enjoy (Seleiman *et al.*, 2021 and El-Mouhamady *et al.*, 2021 a & b). Drought-resistant plants, especially desert plants, tend to form a thick waxy layer that covers the leaves. This layer has a great benefit in that it prevents water from leaving the leaf during water stress with high temperature, especially at noon. This, of course, leads to maintaining an appropriate level of water to maintain the freshness of the leaf and to reuse this water when needed (Seleiman *et al.*, 2021; El-Mouhamady *et al.*, 2021 a & b). Increasing the length of the root system is one of the most important things and morphological changes that water stress-resistant plants characterized to reach the water stored in the deep layers of the soil during thirst, especially the embryonic adventitious roots, according to (Martignago 2020), (Fig. 4).

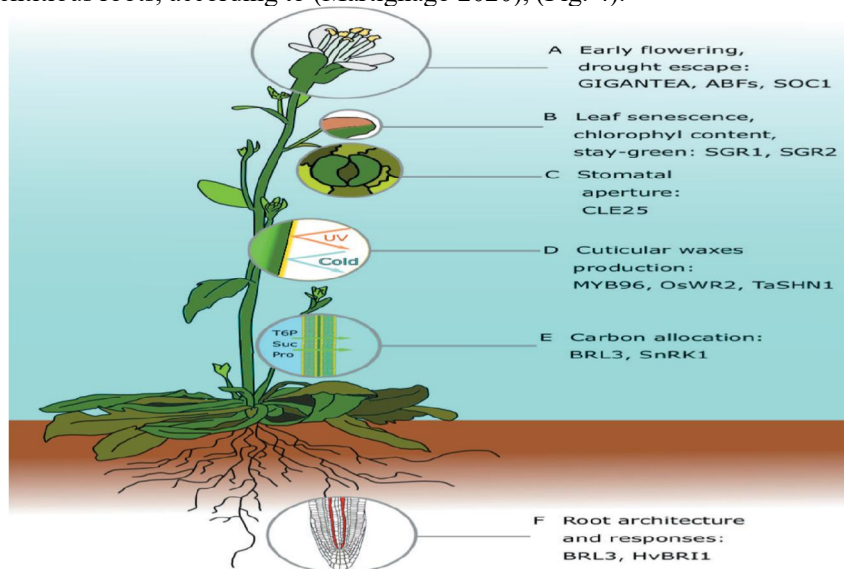


Fig. 4: The role of Root System for Drought-Stress Tolerance

## 2.6. Physiological Changes

A set of physiological changes occur to plants that are exposed to salinity and water stresses and these changes reflect the extent of plants' resistance to negative climate conditions and the limited availability of arable water. At noon, plants resort to high temperatures and increasing drought stress to give the order to the guard cells to close the stomata, especially the upper stomata of the leaf, which are more exposed to sunlight. This mechanism helps to reduce the rate of transpiration and water loss and preserve it to complete the process of photosynthesis. Thus, this mechanism is considered one of the most important famous physiological defenses to face the threat of water stress, especially in desert plants (Seleiman *et al.*, 2021 and El-Mouhamady *et al.*, 2021 a & b). Photosynthesis is the most important and dangerous physiological process that plants need in order to continue their life and survival because this process ends with the production of sugar needed for energy, metabolism and all biochemical processes. Accordingly, this process mainly needs water to be carried out successfully and here it is noted that the plants that are tolerant or resistant to water stress tend to reduce the frequency of this process or stop it temporarily in order to preserve the water needed for the freshness of the leaves (Seleiman *et al.*, 2021). Temporarily stopping the growth rate is one of the most important mechanisms that plants use to simulate water stress in order to reduce the rate of transpiration and water loss and preserve it for physiological and biochemical processes such as flowering and grain filling. Reducing the water stress of the leaf is one of the most important mechanisms that help to retain leaf water for as long as possible in order to reuse it in the photosynthesis process and all the strategic processes associated with the growth and elongation of the plant and its reaching the final stages of maturity. This

will only be achieved by producing some osmotic compounds, which would raise the leaf water potential or equalize the osmosis. Plants resistant to water stress may resort to reducing the rate of water associated with root cells with two aims. The first objective is to reduce the amount of water subject to loss by transpiration during photosynthesis and to preserve it for other vital processes. The second aim is to force the embryonic adventitious roots to reach the water stored in the deep layers of the soil by working to increase the maximum root length. This mechanism is considering the peak of water stress tolerance (Seleiman *et al.*, 2021 and El-Mouhamady *et al.*, 2021 a & b). There is no doubt that one of the biggest negative damages caused by salinity and water stresses is raising the rate of osmotic pressure. This leads to the inability of plants sensitive to salinity and drought stress to absorb water and nutrients in the soil. The reason for this is due to the higher osmotic pressure outside the root system, especially in the soil solution than inside the root cells. Accordingly, drought and salinity stresses-resistant plants may turn largely to a kind of physiological simulation through the formation of some organic compounds and acids that raise the internal osmotic pressure so that plants can absorb water. These compounds are called osmotic compounds such as proline, proline betaine, glycine, glycine betaine, trehalose, and both mannitol and pentol sugars, On the other hand, some studies have confirmed that the accumulation of proline in plant cells may be a sign of tolerance or a sign of toxicity that leads to an increase in the degree of sensitivity, especially in the case of salt stress (Mansour and Ali 2017 and Seleiman *et al.*, 2021), (Fig. 5).

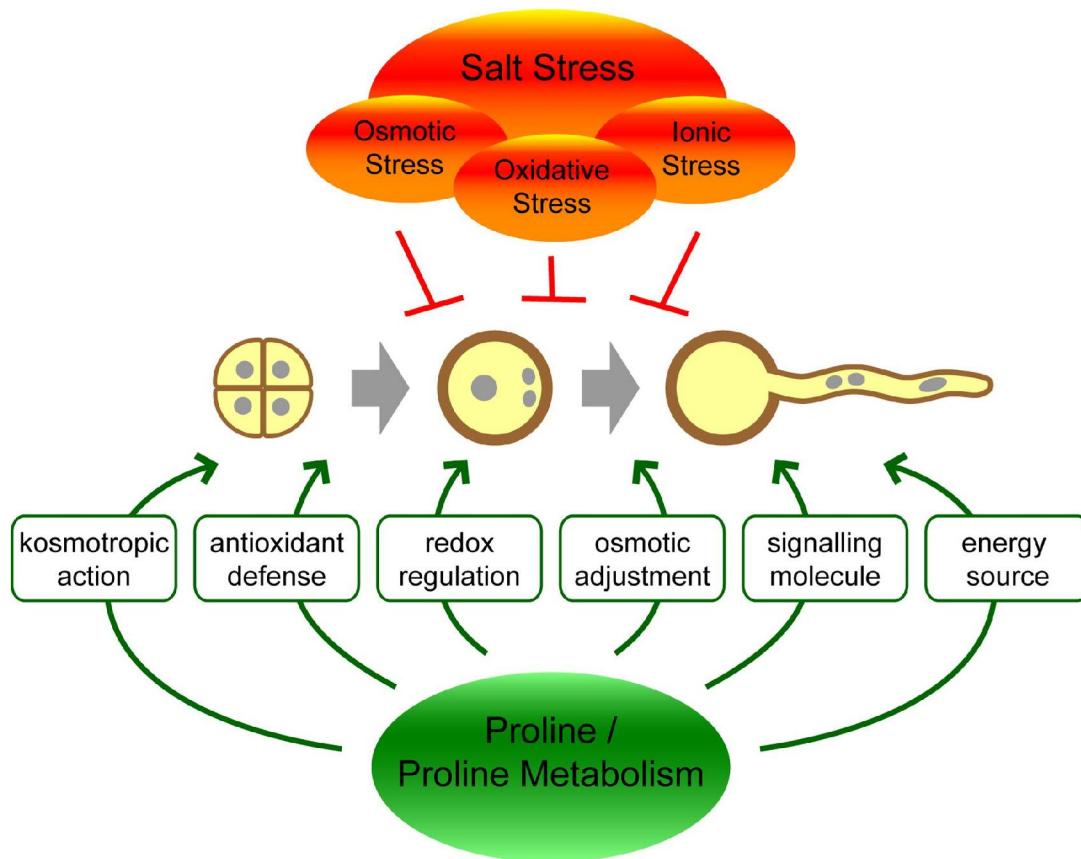


Fig. 5: The role of proline content for Abiotic stress tolerance

Salinity and drought stresses-resistant plants tend to create a state of hormonal balance between beneficial hormones that encourage growth and regulate osmotic pressure, such as auxins, cytokinins and gibberellins with harmful hormones that inhibit growth and increase stresses, especially water stress, such as ethylene, abscisic and jasmonic acids (Dobra *et al.*, 2010). Desert plants, especially those that live in arid environments such as the tropics and subtropics, exhibit very important genetic behavior by storing water in the leaves, especially the upper leaves. This helps to reuse it in physiological and



metabolic processes, as well, using it to maintain the vitality and freshness of cells for as long as possible (Seleiman *et al.*, 2021).

Plants resistant to salinity and water stresses seek to modify the osmotic pressure by producing a group of osmotic compounds which would equalize, increase or decrease the true osmotic pressure, which physiologically stressed the plant and made it unable to absorb water and convert it to osmotic adjustment. In this case, the plant can absorb water and they continued all processes Biological, physiological and biochemical (Miranda *et al.*, 2020). Increasing the rate of root production to the vegetative system helps plants to increase the degree of water stress tolerance through increasing the ability of absorbing irrigation water from the deeply layers in the soil, (Seleiman *et al.*, 2021).

## 2.7. Biochemical Changes

Abiotic stress such as drought, salinity and temperature stresses causes a high degree of oxidative stress. This stress results from an increase and stimulation in the activity of oxidative enzymes and that cause the release of free radicals. Free radicals lead to a state of complete destruction of the biological system of plant cells and tissues. As, it leads to oxidation and decomposition of tissues and cells, damage and destruction of the plasma membrane, lipid membranes, the harmful effect on the products of food transformations and the accumulation of photosynthetic products. As well, causing the most severe types of damage to important biological molecular molecules such as DNA and all of these devastating effects happen thanks to the launch of (ROS) or reactive oxygen species. On this basis, the strongest lines of plants defense against environmental stresses mentioned above center in antioxidants production which would reduce or eliminate the harmful effects of free radicals. These compounds may be enzymatic like catalase, peroxidase, and super oxide dismutase besides, mono-dehydro-glutathione reductase. While, the non-enzymatic antioxidant such as ascorbic acid, glutathione, Carotenoids, tocopherols, flavonoids and citric acid, respectively. In the context of clarifying the vital and effective role of antioxidants and the formation of osmotic substances besides, heat shock proteins in increasing the tolerance of plants to stress as a natural reaction upon exposure to it, the genetic expression of some of these enzymes such as HvAPX1, HvMT-2, and HvGST1 in the face of free radical damage, especially reactive oxygen species (ROS) was revealed by (Guo *et al.*, 2009 and Witzel *et al.*, 2009). Also, transcription factors activating increasing environmental stress tolerance in barley plants such as MYB, HvDRF1, HvDREB, HvABF1 genes besides, the over gene expression of HvCBF and VRN-H1 genes that gave barley the ability to frost tolerance to a large extent (Stockinger *et al.*, 2007 and Seleiman *et al.*, 2021). The most famous components of organic acids accumulated, unreduced sugars and osmotic substances were proline, amines, polyamines, glycine betaine and trehalose. All of these compounds modify osmosis by raising osmotic pressure and scavenging free radicals, thus reducing the negative effects of water stress (Seleiman *et al.*, 2021). Accumulation of Abscisic acid (ABA) in plants is a hormone that is directly responsible for the growth, development of plants, the embryo and seeds reveal that it is an important source of protein and fats that cells need for various metabolic processes. Thus, it has a very effective and influential role in the face of water stress. As, this hormone gives the order to the guard cells to close all leaves stomata to prevent the exit of water to reduce its losing during the transpiration process besides, preserving it for the photosynthesis process. Also, this hormone helps protect chlorophyll during photosynthesis, transmit cellular signals to and from the cell, raise the ability of plants to adapt to water deficit conditions and tolerate salinity as well. Therefore, sometimes acts as an antioxidant and repellent to free radicals. Figure (8) shows the impact of antioxidants and ABA hormone for improving water stress tolerance in plants according to (Seleiman *et al.*, 2021 and Ghosh *et al.*, 2021).

## 2.8. Molecular Breeding and Strategy of Gene Transfer Technique

Molecular breeding is one of the most prominent trends and scientific solutions for engineering plants to confront environmental stresses, especially after the exacerbation of the phenomenon of climate change and the rise in global temperatures to unprecedented levels, which led to the growing problem of water stress and its devastating impact on the productivity of strategic food crops. Accordingly, the attention of scientists and researchers turned to genetic breeding at the molecular level with the aim of engineering plants to resist environmental drought where (Fleury *et al.*, 2010; El-Mouhamady *et al.*, 2014 A, B, C & D and El-Mouhamady & Habouh 2019) succeeded in reviewing recent developments in genetics and genomics of water stress tolerance in wheat and barley with the

aim of reusing it as a basis for analyzing drought tolerance trait in wheat. This extinguishes and confirms the importance of traditional plant breeding represented by crossbreeding and selection in order to reach the best genetic structures suitable for environmental changes such as water stress crisis, which has become a priority and that deserves research work to save humanity and secure sources of food security, (Fig. 6). Also, plant breeding and molecular genetics played a major role in studying the genetic and environmental stability of a large number of imported or foreign genotypes with the aim of improving all yield components of Egyptian crops especially in soybean (El-Mouhamady and El-Metwally 2020 a) and in wheat (El-Mouhamady and Ibrahim 2020 b). Within this framework, it is necessary to know the morphological, physiological and molecular mechanisms for tolerance trait in the parents entering the production of hybrids. All of this information has created a database to improve models for QTLs analysis and positional cloning. At first, a huge group of papers were launched, aimed to improve abiotic stress tolerance in different crops not only in wheat, but also in barley through plant breeding (El-Mouhamady *et al.*, 2012 a). Further, improving some quality traits in promising rice accessions (El-Mouhamady *et al.*, 2012 b). In the same context, (Irada and Rustamova 2010) reported that understanding in genetics and genome regulation using molecular markers was of great value and fruitful thing for the goals of plant breeding. In this investigation, a large number of tests were determined to examine the ability of water stress tolerance during using 12 genetic attributes in wheat, including tolerant, medium-tolerant and sensitive accessions. Initially, RABD markers with highly related to drought tolerance were used as an attempt to know the genetic diversity in wheat genotypes under study. The results were promising that P6 (TCGGCGGTTC) primer determined that tolerant and semi-tolerant genotypes occurred at the molecular weight 920 bp. (Morran *et al.*, 2011) confirmed that transcription factors have a great ability to control the activity of a number of different stress response genes in an orderly manner. Therefore, they represent important targets in the molecular breeding of plants. This research succeeded in producing genetically modified wheat and barley, including constitutive (double 35S) and water-stress inducible (maize Rab17) expression of the TaDREB2 and TaDREB3 transcription factors isolated from wheat grain. Nevertheless, the transgenic plants (TaDREB2 and TaDREB3) gave a good appearance in terms of growth and survival vitally under water shortage conditions, which is in its highest states compared to the same non-genetically engineered genotypes. The wheat genotypes that have a high phenotype of productivity and adaptation to facing water stress are an essential thing in testing and knowing the efficiency of the modified genes in wheat crop. This is related to the point of availability of biosafety for such experiments. The genetically modified wheat (DREB1A) accession was a fertile material to talk about, especially under global warming conditions, especially as this variety is characterized by growth well and giving productivity under severe drought conditions (Pierre *et al.*, 2012 & El-Mouhamady and El-Seidy 2014). It is possible to develop and improve drought-stress tolerance in novel wheat accessions through the process of transferring and inserting tolerated genes for this trait, after locating these genes on QTLs map. Accordingly, it is necessary to identify these genes, markers, or genetic locus that contribute and participate in water stress tolerance using omics technology and QTLs maps. Among the achievements of this investigation is to reveal the great potential of the wild wheat variety (*T. dicoccoides*) which possesses genes of high tolerance to water stress, before transferring it to the local wheat cultivars. Further, genetic modification in wheat has specific steps. First, drought-tolerant genes are identified and characterized at the molecular level and their effectiveness is confirmed through a number of genetic modification tests. Secondly, the process of verifying the integrity of the bearing loci is done by conducting several tests and experiments targeting a specific environment. Besides, analyzing the morphological and physiological attributes of the genetically modified accessions to ensure the stability of the water stress tolerance in these modified genotypes with the aim of testing and evaluating their efficiency under water scarcity conditions. Further, determining the degree of their contribution to the final output in certain test regions (Budak *et al.*, 2013). (Rana *et al.*, 2016) studied the gene expression of (TaSOS1) gene which considering responsible for one of the highly sensitive salts associated with removal  $\text{Na}^+$  and accumulated of proline under both saline stress conditions and standard experiment. This investigation confirmed that the noticeable increase in the regulation and gene expression of sodium exclusion genes from root and leaf cells in parallel with the observed increase in proline content achieve together a defense network that ensures wheat plants to salt stress tolerance. Further, this information will be very useful for breeding to bear wheat genotypes to salinity stress. Osmotic adjustment helps to increase drought tolerance in wheat entries through the enlargement and expansion

of the cell size and keeping the stomata partially open to maintain plant growth and maintain carbon dioxide fixation under severe stress conditions. Further, the wheat plant collects osmotic substances such as organic and inorganic substances, which help to control the rate of osmosis and maintain the swelling or dilation of cells. Accordingly, the genetically modified wheat accessions are a mixture between all these genetically and physiologically mechanisms which eventually lead to the production of genetically modified wheat lines that are water stress tolerant and high yielding, (Ahmad *et al.*, 2018). The process of genetic modification in order to increase the ability of drought resistance is very important. Therefore, one of the most important requirements for genetic engineering is the expression of water stress-tolerant genes. Also, one of the most important achievements in this regard is discovering and identifying of genes responsible for drought tolerance and improving the growth and productivity process in genetically engineered wheat. Despite this, the number of papers conducted to produce genetically modified wheat is much less than those conducted in both rice, maize and other foods. Further, the trait to water stress tolerance without any negative impact on the final yield is an essential and fruitful role of genetic engineering, (El-Mouhamady *et al.*, 2013 A, B, C & D and Khan *et al.*, 2019). The ability of water deficit tolerance in wheat entries is related to its content of the amount of water associated with cells (RWC) and its close relationship with some future amino acids such as serine, asparagine, methionine and lysine. Further, the accumulation of methionine and lysine and their vital role in genetic modification of wheat and an effective tool in selecting of wheat accessions that are highly tolerant to water stress (El-Seidy *et al.*, 2013; Heiba *et al.*, 2016 A and Yadav *et al.*, 2019). The process of producing new accessions for wheat and barley with excellent specifications related to water stress resistance during traditional breeding programs through the process of crossing to produce promising hybrids tolerant to drought. Thus, the tolerance trait is confirming by simple selection in the early segregation generations (El-Mouhamady *et al.*, 2014; Eldessouky *et al.*, 2016 & Ramadan *et al.*, 2016 and El-Mouhamady *et al.*, 2020 c). Moreover, the success of this matter, it is necessary to identify the genes responsible and contributing for water stress tolerance, because this trait is considering a complex quantitative trait and controlling by an unlimited number of genes. In the meantime, the results of previous studies confirmed that drought tolerance trait is polygenic and that the genetic background will help the breeders in screening out a number of importance genes that controlling drought tolerance in wheat and barley, (Sallam *et al.*, 2019). Water deficit stress leads to great damage to the growth of roots and leaves, and both the dry and fresh weight of plants, which leads to severe damage to the final output of any crop (Kishk *et al.*, 2017, Khatab *et al.*, 2017; Mehmood *et al.*, 2020, Khatab *et al.*, 2022). However, studies have proven beyond any doubt that drought stress leads to activation and increased efficiency of a number of genes, causing the mechanism of tolerance to this serious environmental obstacle. In this investigation, the process of genetic transformation was conducted in wheat accession Lasani-08 through transferring the gene DREB1A to it by *Agrobacterium tumefaciens* bacteria. The best combination to bring about a good degree of genetic transformation in wheat plants was 3.0 mg/l 2, 4-D and 6 h soaking. Also, results obtained using DNA fingerprinting analysis showed the number, copies and database of genes that produced different degrees of expression in the first transformation generation. The T2 transgenic plants was the fertile field to evaluate the efficiency of the modified wheat plants to water stress resistance, through agronomical and biochemical attributes. The final results confirmed that the DREB1A gene had a pivotal and very important role in creating a state of drought stress tolerance in the modified wheat plants by maintaining the levels of growth and productivity under these conditions (Mehmood *et al.*, 2020).

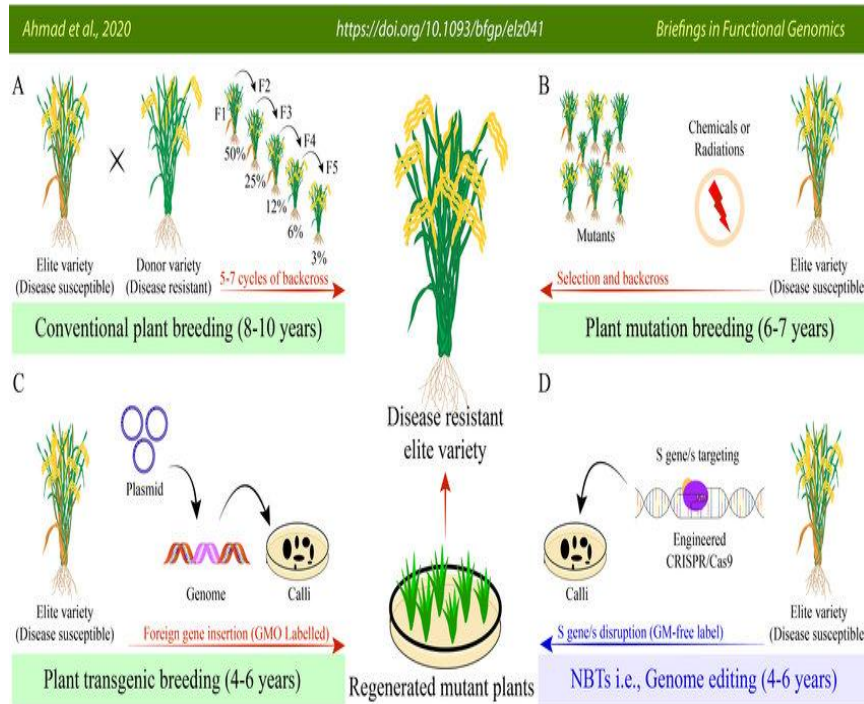


Fig. 6: Plant Breeding Method for improving Crops for Abiotic Stress Resistance

### 3. Conclusion

After all that has been mentioned in this review, it can be said that the technology of genetically modified or engineered crops is one of the most important achievements of the twenty-first century in the field of advancing global agriculture, especially in the course of improving field crops to face adverse environmental conditions. Also, this lecture reviewed modern methods of genetic modification of plants, especially wheat crop because of its strategic nutritional importance at all levels and its excessive sensitivity to water stress under Egyptian conditions. Further, this lecture mentioned the most important mechanisms that plants resort to defend against the dangers of drought stress and its relationship to the degree of acclimatization in coexistence with water scarcity conditions. It is worth mentioning that one of the most important achievements of the Egyptian agricultural research centers especially the Agricultural Genetic Engineering Research Institute (AGIRI) within the framework of using genetic engineering programs in the advancement of wheat crop to produce resistant cultivars for water stress is producing of the Egyptian wheat variety Giza 168. This was done by transferring HAVA 1 gene from barley to the Giza 178 wheat variety which is classified as sensitive to drought stress. Further, this gene succeeded in achieving water stress resistance because of this gene gives the wheat germ the ability to be complete fullness and form the grain in the last stages of maturity, especially during drought conditions. This is a new scientific dimension and direction in the field of transgenic wheat production to water stress tolerance.

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