IMPLEMENTING GENETIC ENGINEERING TO STUDY DROUGHT TOLARANCE IN PLANTS

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Abstract:

Drought is an environmental factor that posses a detrimental impact on plant growth and production. Because of climatic unpredictability, the frequency of drought-related problems during agricultural growth phases is turning into a major hurdle to improving production. New approaches are required to enhance productivity and drought adaptability. The expression of particular stress-related genes is required in order to increase drought resistance through genetic engineering, which is highly desirable. In plants with transgenic DNA, genes that pass on drought resistance and enhance the survival and development of plants have been identified. In this overview, we concentrated on creating transgenic plants that can withstand drought. Using genes associated with environmental stress or additional transcription factors, as well as other stress-related genes, most cultivated Plants have become drought-resistant and resistant to other abiotic stresses. It causes accurate manure to change the DNA with little to no effect on the growth and development of plants.

Keywords: *Transgenics, Genetic Engineering,* and *Drought*

INTRODUCTION:

Stress for plants is defined as external variables that influence a plant's growth, development, and production. The cost of plant development, crop yields, gene expression, cellular metabolism, etc. are all affected by stress. The primary reason driving agricultural decline worldwide is drought, which poses a serious risk to the availability of food. Regarding the development of plants that produce abundant yields in environments with scarce water resources, the biotechnology of plants is among the most fascinating fields of research. Research on complete Arabidopsis thaliana plants has revealed the main pathways for drought stress response, and a number of drought-tolerant genes have already been inserted into crops. It affects particular metabolic alterations as well as their growth and development. Changing gene expression has taken place to help the plants develop in these conditions. The drought may have a major impact on grain productivity and quality.

Crops have already incorporated a number of drought resistance genes, and studies on complete Arabidopsis thaliana plants have uncovered the primary pathways involved in drought stress responses. In order to help the plants survive in these circumstances, specific metabolic changes and gene expressions impact their growth and development. Soil moisture fluctuations that go above this level can have an impact on grain output and quality. Insufficient amounts of rain or a reduction in rainfall create low soil moisture levels. When a plant with low water potential has aerial structures like leaves and stems that cause shortages of water, outbreaks occur. When this occurs during a drought, the rate of water absorption by roots is surpassed by the proportion of transpiration from absorbing leaves.

These drought stress events, as well as the presence of additional stress elements in plants, are indicated by the rolling of leaves, stunning of plants, yellowing of foliage, leaf torching, and chronic wilting.. The main reasons for a lower photosynthetic rate are stomatal closure or metabolic dysfunction. Reduced photosynthetic electron transport components build up during reactions emanating from drought stress and low intercellular Carbon dioxide concentrations. Reactive oxygen species (ROS), which are dependent on molecular oxygen, have the potential to seriously damage the photosynthetic system.

The xanthophyll cycle, water-water cycle, separation of light-harvesting complexes from photosynthetic reaction centers, and thermal dissipation of light energy are specific adaptive processes that plants developed to lessen the harm that drought-induced photosynthesis causes. Changes in the metabolism of photosynthetic carbon are the major source of metabolic loss during drought. The metabolic efficiency of photosynthesis is mostly influenced by the synthesis of ribulose-1, 5-BisPhosphate (RuBP) and the activities of Ribulose-1, 5-Bisphosphate Carboxylase/Oxygenase (RuBisCO). Significant advancements have been made in the stomatal mechanisms for CO2 diffusion, photosynthetic light response, and metabolic adjustments, such as the expression of genes involved in photosynthesis, which controls photosynthesis during drought circumstances

CAUSES OF PLANT STRESS DURING DROUGHT:

Since the temperature of the atmosphere and CO2 levels continue to rise, affecting precipitation patterns and their distribution, future predictions indicate that the progress of climate change will increase. Although insufficient rainfall tends to be the primary cause of drought stress, other factors, such as high temperatures, intense sunlight, and dry winds, may aggravate an already serious situation by causing soil water to evaporate. Globally, climate change frequently leads to drought conditions that have a significant regional impact. Along with drought, salt stress represents one of the key factors thought to contribute to water shortages in plants.

EFFECTS OF PLANTS DURING DROUGHT:

Drought stress causes variable consequences; it can happen at any point in a crop's development cycle. It can vary in difficulty, and it commonly happens together with other environmental stresses like heat and salt. During times of peak photosynthetic activity, different plant tissues or organs will react to drought and high stress in different ways. The symptoms that are most prevalent of drought stress in plants are an acceleration of leaf wilting and drooping, scorching and weak leaves, rolling and cracking of the leaves, closed blooms and sagging flowers, etiolating, becoming wilted, turgidity, premature fall, senescence, and yellowing of the leaves. Some less noticeable symptoms of drought in plants include twig cracks, branch dieback, necrosis, stunted growth, bark cracks, the canopy of shrubs, and tree thinning. Drought-resistant plants, such as xerophytes, halophytes, and resurrection plants, possess morphological and physiological adaptations to deal with scarce water sources.

Plant ADAPTATION TO DROUGHT:

Drought-adopted plants, such as xerophytes, halophytes, and resurrection plants, exhibit physiological and morphological adaptations to deal with low water availability. These adaptations include halting growth until favorable conditions are once again present or shortening growth cycles, which include restricted vegetative growth followed by flowering and seed production during a little time. Most plants use strategies such as fast-growing plants adoptations, shortening their life cycle, self-reproduction, and seasonal growth before the start of a dry section of the year to avoid the negative effects of drought stress on plant production. Abscisic Acid (ABA), which is primarily involved in stress, is the main hormone and osmotic signal that dehydration typically activates. From these signals, a response occurs that may be sorted into three categories:

(i) Drought escapes
(ii) Dehydration avoidance and
(iii) Dehydration or desiccation tolerance

(i) Drought escapes:

A plant tries to escape drought by hastening the flowering process before the conditions make it difficult for it to survive. Breeders of cereal plants take advantage of this response, which is typical of annual plants and even occurs in the model species Arabidopsis thaliana. To escape the drought before it comes.

(ii) Dehydration avoidance:

In order to resist dehydration, the plant can continue to possess a high relative water content even when there is a scarcity of water (RWC% = [fresh mass dry matter]/[water saturation mass dry mass] = 100). This is accomplished through physiological and morphological processes, including a shortened plant life cycle and decreased transpiration because of ABA-mediated stomata closure. Avoiding dehydration often enhances the probability of survival by postponing vegetative growth, which in turn delays senescence and mortality.

(iii) Drought tolerance:

By controlling the metabolic processes of the plant in order to boost the synthesis of sugars, osmoprotectants, antioxidants, and ROS scavengers, a plant with dehydration tolerance, on the other hand, is able to sustain its activities in a dehydrated condition. Gibberellic Acid

(GA) signaling often activates these responses by connecting multiple hormone- and stress-related pathways and modulating the GA-signaling protein DELLA.

GENETIC ENGINEERING IN DROUGHT:

For the transmission of gene information and knowledge to crop development, genetic engineering is an essential tool.The most common approach for finding potentially useful genes involves focusing on particular pathways or processes that are known to be connected to drought responses, such as altering the expression of transcription factors or protein kinases related to drought, increasing the expression of genes related to the production of osmoprotectants, or increasing protection from drought resistant varieties to increase yield.

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At the cellular level, the majority of their gene products could be related to stress tolerance and response. Significantly, plant stress tolerance was increased as a result of the insertion of several stress-inducible genes by gene transfer (Zhang et al., 2004; Umezawa et al., 2006a). Using microarray research, a number of stress-inducible genes have recently been discovered in a variety of plant species, including rice and Arabidopsis. Now that we have a greater understanding of the molecular processes driving plant stress response and tolerance, measuring the roles of these genes is essential. This will eventually lead to genetically modified crops with improved stress tolerance.

GENES USED FOR DROUGHT RESISTANCE IN GENETIC ENGINEERING:

Protective protein genes (like HVA1, BIP, AyHSP17.6A, and LEA), osmolyte metabolism genes (like sacB, TPS1, IMT1, P5CS, GSMT+DMT, SPDS, and TPSP), involved genes in ABA biosynthesis (like AtNCED3), and involved genes in ion transferring (like AVP1) are frequently used in genetic engineering for drought resistance.

TRANSGENICS TO INCREASE CROP TOLERANCE TO DROUGHT:

Through the use of methods from genetic engineering, drought tolerance has been improved.

(i) Efficiency of water use by plants
(ii) ROS defense systems in cells

(iii) Changing the growth and development of organisms through hormonal balance to prevent drought
(iv) Modify the transcription factors that are related to drought that serve as master switches for a large number of downstream processing genes involved in the drought response.

TOOLS FOR TRANSGENSICS AND GENE EDITING:

Transgenic techniques can be used to introduce significant genes into breeding stock and can be helpful in the effective investigation of genes regulating responses to stress and adaptability. Modified plants by expressing the transcription factor ZmNFYB2, which enhanced drought tolerance and yield. The lines of plants generated by genetically modifying them to overexpress the potential transcription factor ZmAsr1 utilized water more effectively and produced more dry weight phosphatase (TPP), which raised the concentration of sucrose in the ear spikelets, kernels, and harvest index. This resulted from overexpressing the putative transcription factor ZmAsr1 in plants. This improved yield in both mild and severe droughts led to the development of transgenic maize that increased the ARGOS1 (ZAR1) gene, improving grain production, drought-stress tolerance, and maize organ growth.

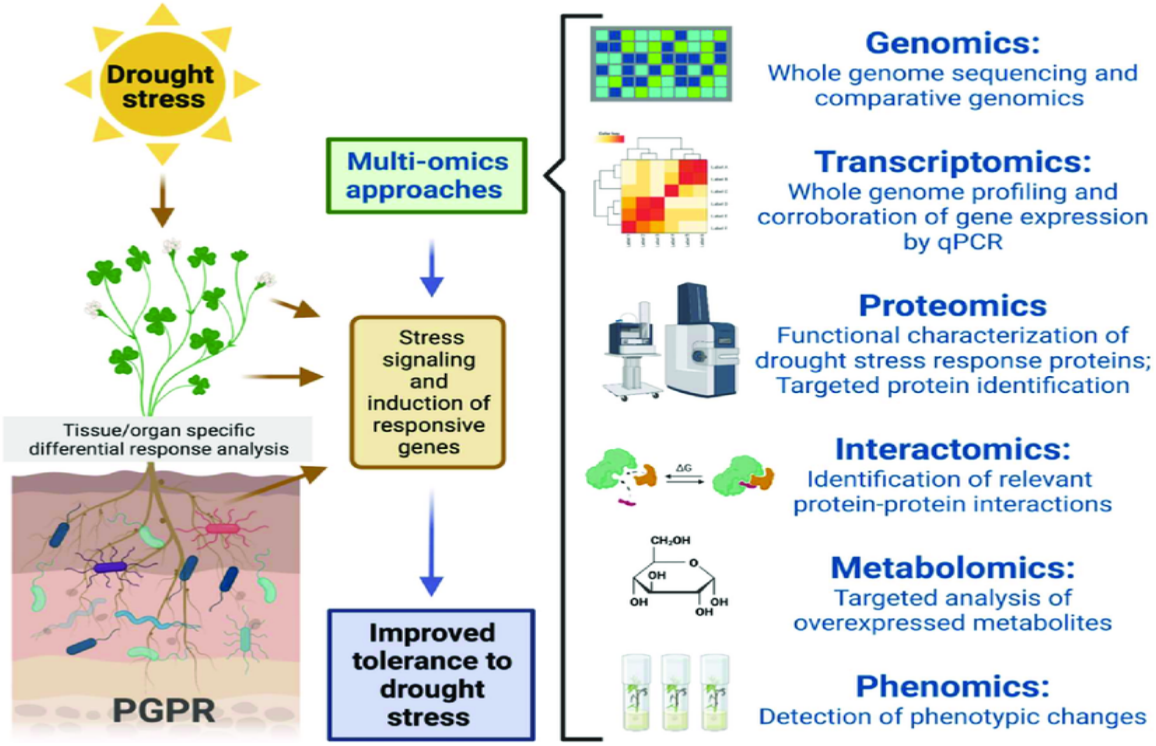
These are barriers for their implementation in released cultivars, regardless of the transgenic approach's help in the identification of genes and pathways that promote drought tolerance. The influence on the transgene may not produce an advantage in the TPE for transgenic events assessed under laboratory circumstances due to environmental interaction. Due to attenuation and compensation from other processes, an individual gene's expression in agronomic germplasm may not appreciably affect the final phenotype. Despite these challenges, some progress has been made in using transgenic cultivars to reduce the effects of drought stress on crops. In order to increase yields under drought conditions by 11% to 21%, a cold-shock protein gene (cspB) from the soil bacterium "Bacillus subtilis" was isolated and transferred. Under normal circumstances, there were no negative effects. The chaperone protein cspB is thought to aid in the separation of RNA that has folded improperly as a result of drought.

Increased kernel production per plant is the main cause of the yield gain brought on by cspB expression (Castiglioni et al., 2008).

Additionally, increased drought resistance has been achieved by using novel genome editing technologies like CRISPR-Cas9. Employed CRISPR-Cas9 to substitute and match the native ARGOS8 promoter with the natural maize GOS2 promoter. This led to variations with altered ARGOS8 expression, a suppressor of ethylene responses. Three separate homozygous lines for the i1, d2, and d35 alleles that are resistant to drought stress have been established by gene knockout studies of the AHB2 gene in maize using CRISPR/Cas9 (Liu et al., 2020). Since the crops produced using gene editing technology can be viewed as non-genetically changed and therefore more acceptable, they are likely to be more appealing.

For the purpose of evaluating crop abiotic stress tolerance responses, a broad review of the stages required in multi-omics data integration is offered here. "Experimental design" refers to the starting step of putting up and generating studies that subject crops to particular abiotic stresses and put into view suitable control groups to provide an immediate comparison. It has to ensure the collection of samples for several omics platforms, including metabolites, proteins, RNA, and DNA. With high-throughput omics technologies like whole genome sequencing (WGS) for genomics, RNAseq for transcriptomics, MS for proteomics, and metabolomics profiling for metabolomics paradigms with statistical inference from big, complex data, data production generates massive datasets for each omics layer. These methods look for interactions and connections among molecules across several omics levels.

The final stage in "experimental confirmation and proof" is selecting potential genes, proteins, or metabolites that were discovered in the integrated analysis for experimental validation. The outcomes may be validated, and their involvement in abiotic stress tolerance can be confirmed using methods like qPCR, Western blotting, or targeted metabolomics. Additionally, using this method will reveal the post-transcriptional and post-translational processes that control gene expression.



Shown in Fig. 1. Integrated multi-omics pipeline for plants' adaptation to abiotic stress.

When omics integration may benefit young plants, i.e., seedlings, it is significantly more beneficial. It is now confirmed that the viability of using a limited integration strategy for early drought response in Quercus ilex, a non-model plant, is now confirmed.

DROUGHT STRESS REGULATION OF PLANT DEVELOPMENT REGULATORS:

Major phytohormones, including auxin, ethylene, gibberellic acid, cytokinin, abscisic acid, and endotherms,regulate a variety of mechanisms that allow plants to adjust to drought stress. Almost all hormones perform their functions when a plant is under stress, but plant growth hormones are particularly vital. Gibberellic acids (GA) can be utilized to reduce the length and weight loss of the hypocotyl in seedlings brought on by water stress and aid in maintaining the internal equilibrium of water and protein synthesis in drought-stressed plants.

It is suggested that GA may positively regulate extrinsic treatments of abscisic acid, uniconazole, brassinolide, and jasmonate, which can help enhance crop yields in droughts. The hormone benzyladenine, another active cytokinin, regulates the tolerance to drought mechanisms in a variety of plants. Under drought conditions, the amount of soluble sugar and soluble protein and the activity of catalase, peroxidase, and superoxide dismutase were all increased by unconazole and brassinolide. Auxin has been found to generally negatively influence plants' ability to respond to drought. It has been proven that a drop in Indole-3-Acetic Acid (IAA) concentration causes an increase in the expression of genes that code for abundant late embryogenesis (LEA) proteins, which causes plants to adapt to drought. Auxin has recently been found to adversely influence the DEEPER ROOTING 1 (DRO1) gene, which determines a quantitative trait locus (QTL) governing root development angle. It is hypothesized that GA can effectively control CKs, which are known to delay early leaf senescence and death.

GENETIC CHANGES OF ABA (abscisic acid) STRESS RESPONSE:

This is the main hormone generated in the way of adjusting plant responses to drought, which involves closing the stomata, as a result of which less water is lost by transpiration from the leaves. In plants, ABA levels significantly increase in response to water-related stress. The response is reversible; as soon as water is once again accessible, the amount of ABA decreases and the stomata open once more. To improve drought tolerance, it became crucial to boost the plant's sensitivity to ABA.

The Arabidopsis gene ERA1 encodes a farnesyl-transferase's ß-subunit and participates in abissic acid signaling. While plants without ERA1 activity are more tolerant of drought, their yield is substantially reduced. In order to achieve constrained, variable, and decreased levels of ABA11, a team of Canadian researchers used a drought-inducible promoter to stimulate translational expression of ERA1 in Arabidopsis and canola cultivars. Under drought conditions, transgenic plants performed noticeably better and consistently produced higher yields than normal kinds. Importantly, in the presence of enough water, there was no variation in productivity between transgenic and control plants.

Drought stress gene regulation independent of ABA:

The ABA-independent drought-tolerant pathways, which induce the expression of genes that are responsive to stress, depend on the transcription factors DREB1 and DREB2. The natural form of DREB1 and a constitutively active version of DREB2 are overexpressed, which boosts the transgenic Arabidopsis plants' tolerance to cold, salt, and drought conditions. Although these genes were first discovered in Arabidopsis plants, they have since been found in numerous other significant crops, including rice, tomato, barley, canola, maize, soybean, rye, wheat, and maize. This suggests that this stress defense mechanism is common and conserved in plants. The DREB genes are significant candidates for crop development for drought resistance through genetic engineering because of their functional stability.

ABA-induced senescence, particularly when the drought occurs at the reproductive stage. OsNAP, OsNAC5, and DSM2 are ABA signaling genes that encourage increased yield under physiological drought. To increase grain output during reproductive drought, it is possible to take advantage of these non-stomatal changes that ABA causes in plants under stress due to drought.

SALICYLIC ACID IN DROUGHT STRESS:

Salicylic acid is an artificially given chemical; it can also promote drought tolerance and plant development, ultimately leading to crop harvest when there is a lack of water. When plants are under stress from drought, salicylic acid is crucial. Wheat's catalase production was shown to rise after salicylic acid treatment under less water-available conditions. Salicylic acid and its metabolites strengthened the drought adaptation mechanism in plants that were stressed by drought when applied topically and as seed treatments. According to research, adding salicylic acid to wheat caused the abscisic acid level to rise, which in turn enhanced the formation of proline. Additionally, salicylic acid is essential to plant defense against pathogens.

Brassinolide (BR), ethylene, and other plant hormones play a significant role in helping plants adapt to changing environmental challenges, particularly drought stress. By activating the BZR1/BES1 transcription factors, it improves plant adaptation to both biotic and abiotic stressors through a complicated mechanism to control the plant defense system. Additionally, it controls the activation of reactive oxygen species (ROS) in stressed plants. Plant-harming oxidative outbursts occur when ROS scavenging is out of equilibrium.

OSMOPROTECTANTS:

Osmoprotectant signaling controls plant stressors that can lower plant growth and production. The maintenance of internal physiological activities that guarantee plant life under ideal situations, such as shortages of water, depends on the accumulation of these compounds during the period when generating situations are unsuitable for plant growth and development. Proline, trehalose, mannitol, futon, and glycine betaine are a few significant osmoprotectants found in plants under water stress. These substances safeguard the subcellular structure of established crops, boost the activity of antioxidant enzymes, and mediate osmotic change in drought-stressed plants. These are commonly utilized as seed treatments or given externally at various growth stages of existing crops. Proline applied topically improves free proline levels in plants, developing resistance to drought.

DEVELOPING CROPS THAT CAN WITHSTAND DROUGHT:

To introduce this resistance into generations with suitable traits for agriculture, standard breeding needs the discovery of genetic variability for drought within Variety of crops and other species that can coexist peacefully. Even though normal breeding for drought resistance has had some success and still does, it is a laborious process that suffers from a lack of desirable genes. The Center for Soil Salinity Research Institute in Karnal, India, has created varieties of wheat, rice, and Indian mustard that are able to stand salt and acidic soils as instances of traditional breeding efforts for drought tolerance. Efforts to add drought resistance as a trait and the introduction of salt tolerance to grain from closely related wild species

Seed Priming :

Seed priming allows seeds to regulate their hydration in order to absorb water and go through the initial stage of germination, but it prohibits radical penetration through the seed coat.

Uses of seed priming in drought-stress situations:

The most important short-term approach for reducing the negative effects of drought on plants has been referred to as seed priming. Initiating the germination process in the seed's metabolic system and preparing the pre-sowing process strives for radicle protrusion without radicle emergence in the seed. When compared to non-primed seeds, primed seeds have a more effective germination process, which leads to better germination rates and uniformity.

Arabidopsis drought-inducible gene functions

Two distinct groups can be made up of the by-products of the drought-inducible genes discovered by recent microarray investigations in Arabidopsis:

Functioning proteins and
Regulation-related proteins

Functional proteins: This group of proteins consists of those that most likely help the organism tolerate abiotic stress. Chaperones, late embryogenesis abundant (LEA) proteins, osmotin, antifreeze proteins, mRNA-binding proteins, essential enzymes for osmolyte production, water channel proteins, sugar and proline transporters, detoxifying enzymes, and various proteases are some of the molecules that fall under this category.

Regulatory proteins: The second category consists of protein components that are involved in further regulating signal transmission and the expression of genes that respond to stress. Several protein kinases and protein phosphatases, enzymes involved in the metabolism of phospholipids, along with other signaling molecules like calmodulin-binding proteins. Numerous transcription factor genes were induced by stress, indicating that different transcriptional regulatory mechanisms may be responsible for controlling stress caused by drought, cold, or excess salt.
These routes are for transmitting signals. These transcription factors may make up gene networks in Arabidopsis and may control the expression of stress-inducible genes cooperatively or independently.

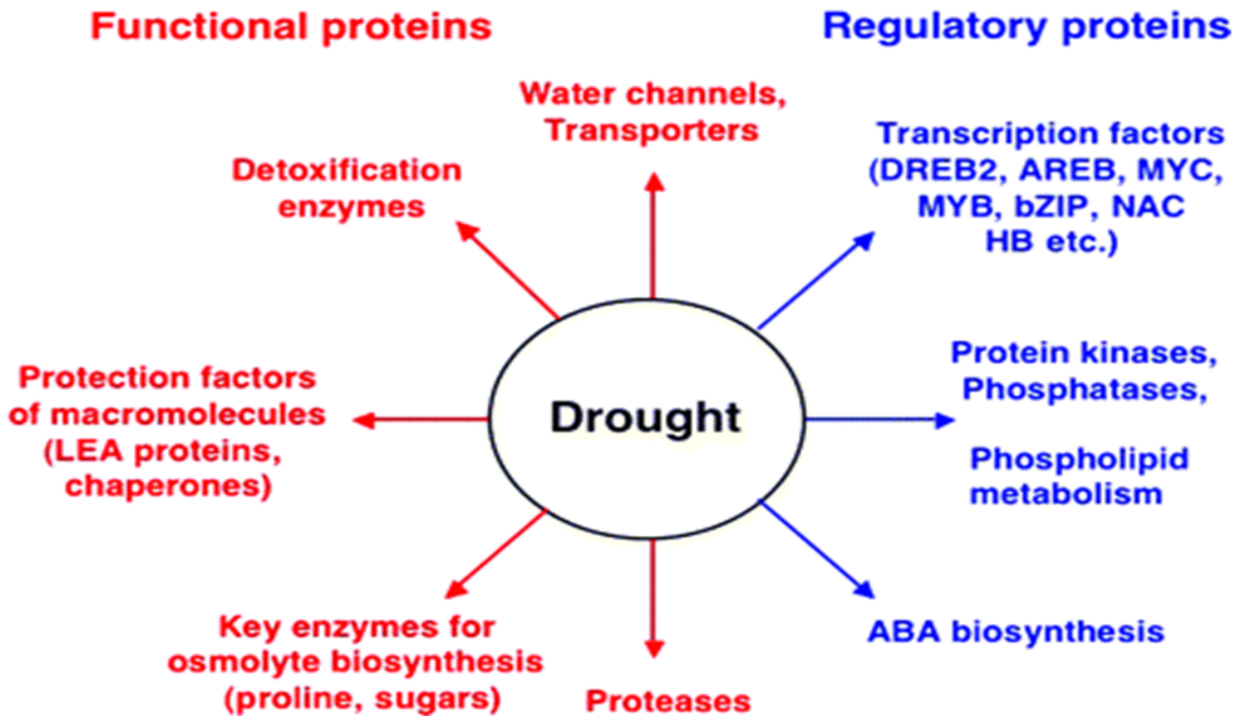


Fig. 2: The roles of genes that are induced by drought stress in coping with and responding to stress There are two categories for gene products. Proteins that are likely to play a role in stress tolerance are classified into two groups: functional proteins and regulatory proteins, which are likely to play a role in further regulating signal transduction and gene expression.

Additionally, we go through how modern genome editing tools may be used to find and control new genes that could confer resistance to drought stress. We expect that working together in the following years will lead to the identification of sustainable strategies for improving agricultural productivity in water-restricted regions.

CONCLUSION:

Our understanding of the genetic methods that cause drought tolerance has significantly advanced, but additional research is still needed. Since crops are exposed to varying degrees of diverse pressures in the field, the physiological response that plants have to challenges is a subject under investigation that requires much more research. In order for the total yield to rise and the tolerance to be evaluated, it is necessary to subject newly developed varieties to a variety of stressors and to perform substantial fieldwork in a variety of circumstances.

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