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Biotechnology and Drought Stress Tolerance in Plants

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Authors' contributions

This work was carried out in collaboration among all authors. Authors GAI and DTT Designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors RAB and ETA managed the literature searches. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Drought stress in plants has become one of the major abiotic stress that limits the growth and development of plants which also contributes to low yields. Biotechnology which has new and emerging techniques can be use to solve the problem of drought stress in plants. This review aimed at identifying drought stress tolerance in plants at different stages, how plants respond to drought stress using different methods and the application of different biotechnology methods to improve drought tolerance in plants. Some important parameters about drought stress in plants such as drought tolerance mechanisms, plants responses to drought stress, gene regulation for drought stress tolerance in plants, effects of drought stress at different stages of plant growth and biotechnology methods in developing drought tolerance in plants was reviewed. The use of biotechnology methods such as classical breeding, use of genetic manipulation, genes from resurrection plants and Protoplast fusion was discussed. Drought stress affects our plants seriously and it leads to wilts, reduction of yields and death of plants at different developmental stages. Plants have developed different mechanisms to respond to drought stress but these mechanisms

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are not sufficient enough without the application of biotechnology to greatly improve the growth, development and increase yield in pants. The use of biotechnology greatly improves plants ability to tolerate drought stress depending on the plant species and period of exposure. The use of biotechnology methods has become very vital in improving plants drought stress so as to overcome the major problems of plants which includes increase in population and climatic change.

Keywords: Biotechnology; classical breeding; genetic manipulation; plant drought stress; protoplast fusion and resurrection plants.

1. INTRODUCTION

Drought is the water deficit that impairs plants growth, development and yield compared with the normal water supply required for optimum growth. The drought which is an abiotic factor is one of the most common stresses that greatly hampered plants growth and development compared to other types of plant stresses [1,2]. Drought induces metabolic changes in plants, such as increased levels of free sugars and free essential amino acids, which according to the "Plant stress hypothesis" causes the plant to have a higher nutritional value for herbivores and can play an important role in herbivore outbreaks [3]. Drought stress is usually said to be an extremely dry condition beyond a threshold level which causes damage to plants. When plants lack adequate water supply, the resulting drought stress normally reduces growth more than all other plant abiotic stresses. Plant responds to lack of water by reducing the activities of photosynthesis and other plant processes. When plants experience drought stress and water loss progresses, leaves of some plant species appear to change colour (usually to yellow-brown) and drought stress also reduces crop productivity or yield [4,5]. Drought stress also plays a vital role in determining the availability of most plants species across different locations in the world. Naturally, drought stress in plants varies from species to species, a period of exposure and some environmental parameters. Plants tolerance to drought stress is a relevant issue that requires new improve techniques like biotechnology to enhance stress-tolerant [6]. The most common factors that influence plants tolerance to drought stress includes; the physiology of the plant, the extent of the plant stress, the growth stage, gene expression, the specie of the plant, etc [7].

In this review, some important parameters about drought stress in plants such as drought tolerance mechanisms, plant responses to drought, gene regulation for drought stress tolerance in plants, effects of drought stress at different stages of plant growth and biotechnology methods in developing drought tolerance in plants was discussed.

2. DROUGHT TOLERANCE MECHANISMS IN PLANTS

Plants exposed to drought stress can tolerate (adapt) to the stress depending on the plant species and period of exposure which the plants may survive under drought stress through the induction of diverse biochemical, physiological or morphological factors [8]. Phenotypic and morphological changes that often occur in plants are influenced by a spectrum of physiological and molecular interactions developed to acclimate to drought stress [9]. Drought stress tolerance is the ability of a plant to grow, develop and thrive with displayable economic yield and value under limiting or no water supply [8]. Drought stress affects not only the water relations of plants at cellular and tissue levels but also at organ levels, which may result in explicit and/or relatively ambiguous interactions that can damage or acclimatize the plant [10].

Plants often respond to abiotic stresses through the expression of stress-regulated genes and protein production. The available data on stressrelated genes is still limited as their functions have not been thoroughly established [7]. However, it has been established that plants' ability to tolerate drought stress is a complex event that involves a combination of some of the genes to express synergistically. The expression of genes may be triggered by stress-induced events or result from injury responses to the plant [8]. With the advent of genomics, some genes are known to be expressed when plants are drought-stressed to produce relevant drought stress-related proteins and enzymes including dehydrins (polypeptide), invertase, glutathione Stransferase, and late embryogenesis abundant; also, the expression of Abscisic acid (ABA) genes which is an essential phytohormone that regulates growth, development and adaptation to drought stress and the synthesis of macromolecules such as rubisco, helicase, proline, and carbohydrates are the molecular basis of drought tolerance [7]. A polypeptide (dehydrins) was observed to be the most abundant among the accumulated macromolecules in response to loss that leaves water content in some plants (pea, maize, barley, arabidopsis, etc) and under drought-induced stress LEA proteins plays the protective role of plants. In extreme cases even though they are not plant specific, LEA proteins has been associated with cellular desiccation tolerance. Osmotin which is also a stress-responsive antifungal protein accumulates under both biotic and abiotic stress in several plant species [11]. Macromolecule such as phospholipids and glycolipids are the lipid components of the plant membrane layer, while triglycerides are primarily used to store CH4 and CO2 during the developmental stages of plants [12]. 70%-80% of the total protein and lipid composition of leaf tissue are found in the chloroplasts. Lipids, which are one of the major components of the membrane, are likely to be affected by water stress [13].

3. PLANT RESPONSES TO DROUGHT

3.1 Physiological and Morphological Responses

Plant growth and development is a process that is usually accomplished through certain physiological and morphological complex interactions such as cell division, cell enlargement, and differentiation, as well as genetic interactions. The growth of a plant is regulated by these activities as well as the presence of organic and inorganic compounds required for the development of new protoplasm [14]. The quality and yield of plant growth to a reasonable degree depend on these complex interactions which can be greatly reduced by drought stress [8].

The physiological response of plants to drought stress can include; interference with photosynthetic activity, stomatal regulation, oxidative stress which eventually leads to damage of the plant, generation of toxic metabolites which can cause plant death [15], water-retention level of leaf decreases, impaired growth rate, decrease in $CO₂$ concentration, etc. Cell growth and differentiation alongside other physiological events are one of the most droughtsensitive physiological events due to a decrease in turgor pressure which is one of the major plant responses to drought stress [16]. Water is important in the maintenance of the turgor pressure which is necessary for cell enlargement, growth and for maintaining the plant as a whole. Turgor is equally vital in stomatal regulation and the motility of various differentiated plant structures [17]. In extreme drought stress, cell differentiation and elongation of some plants are repressed through the ongoing interference of water and minerals flow from the vascular tissues to the other components of elongating cells [18]. Severe drought stress mostly is accompanied by increased salt concentration which [14] defined it as osmotic adjustment. Osmotic adjustment occurs when solutes gradually accumulate in the elongating cells of developing the plant as the water retention level decreases over time. Osmotic adjustment is one of the most essential events in plant acclimatization to water-limitation, for the reason that it maintained vascular tissue metabolic activity and enables re-growth upon water availability which of course varies greatly from specie to specie [19].

3.2 Biochemical Responses

For decades, the complex interactions of biochemical pathways that arise by drought stress have become relevant [20]. Reactive oxygen species are produced in different compartments of the plant cell, both under normal and stressful conditions. When plants are stressed by drought or other abiotic stresses, reactive oxygen species are generated as a result of the inhibition of photosynthesis and the preeminence of photorespiration. The generation of reactive oxygen species is one of the earliest biochemical responses of eukaryotic cells to biotic and abiotic stresses. The production of reactive oxygen species in plants, known as the oxidative burst, is an early event of plant defense response to water-stress and acts as a secondary massager to trigger subsequent defense reaction in plants. Reactive oxygen species, which include oxygen ions, free radicals, and peroxides, form as a natural byproduct of the normal metabolism of oxygen and have an important role in cell signaling. However, during environmental stress such as drought, reactive oxygen species levels increase dramatically resulting in oxidative damage to macromolecules such as proteins, DNA and lipids [21]. Being extremely reactive, reactive oxygen species can severely damage plants by increasing lipid peroxidation, protein denaturation, nucleic acid fragmentation and finally cell death. Drought stress induces oxidative stress in plants by a generation of reactive oxygen species. Droughtinduced high production of reactive oxygen species and it increases the content of malondialdehyde. The content of malondialdehyde has been considered as an indicator of oxidative damage [22].

Reactive oxygen species are found to have a dual function in plants: they are needed as signaling molecules, but a high concentration it is detrimental. High reactive oxygen species concentration is therefore, a symptom of stress and plants have to maintain the reactive oxygen species within a certain level that is required for normal cellular homeostasis. Reactive oxygen species concentration in the cell is maintained by the antioxidant system, which is made up of the antioxidant molecules ascorbate, glutathione, and α-tocopherol in addition to the antioxidant enzymes peroxidases, catalases, and dismutases [23]. In plants, reactive oxygen species are discharged through certain antioxidant molecules, polar and lipid-soluble molecule [24], and the most effective antioxidant being the process that counteracts oxidative stress [8]. Most plants that are exposed to severe environmental stresses notably as drought have developed a mechanism to reprogram their metabolic pathways to tolerate the impending stress which often result in changes in the production and utilization of available production and utilization of available metabolites. The advent of metabolomics has uncovered how plants subjected to abiotic stresses invest in the synthesis of essential macromolecules and metabolites that contribute to palliate stresses as osmoregulators, antioxidants and defense compounds. Droughtinduced stress can also alter the available content and composition of plants macromolecules such as proteins which eventually causes proportional changes of structural and soluble proteins [25].

3.3 Gene Regulation for Drought Stress Tolerance in Plants

The advent of whole-genome sequencing has led to several drought-related candidate genes been discovered and these genes have been characterized [26]. The sequencing of model plants such as *Arabidopsis thaliana* and *Oryza sativa* marked a new era in plant biotechnology. This post-genomic era is geared towards establishing the functions of the entire genes thought to be found in plants and hence, their expression profiles. Genetic manipulation

associated with drought tolerance and other abiotic stresses has become possible. Some genes thought to be induced by abiotic stresses have been reported [27] and some of these drought-responsive genes were cloned and characterized by a range of plant species [28]. The expression of these drought-responsive genes to stress can be subjected to regulation and coordination by modifying a gene for tolerance under stress [29]. For efficient tolerance and restoration of cellular activities in plants under abiotic stresses, a gene that encodes a stress-inducible transcription factor that regulates other genes should be considered [30]. The recent candidate genes for abiotic stress used in plant genetic modification switch on transcription factors for regulation and expression of a range of genes for stress tolerance [28]. These transcription factors interact with cis-acting elements in the promoter region of related genes and act synergistically to enhance plant tolerance to a range of environmental stresses. Majority of these transcription factors that are induced by stress can be broadly categorized into these families, AP2/ERF, bZIP, NAC, MYB, MYC, Cys2His2, zinc-finger and WRKY [31] DREB2s and DREB1A are the largest subgroups of genes that are involved in two different ABA-independent pathways [32,33] involved in drought-responsive gene expression in a transgenic plant.

4. EFFECTS OF DROUGHT STRESS AT DIFFERENT STAGES OF PLANT GROWTH

4.1 Seed and Seedling

Drought stress is one of the environmental stresses in tropical regions that have a severe limitation on plants growth, development and yield. Plant responses to stress have become one of the most research fields [52]. Studies revealed that the most sensitive stage for drought stress in a plant is the seed germination and seedling stages [53] Drought stress has been reported to greatly interfere with germination and seedling phase [8]. Research on physiological and biochemical responses under water-limiting stress in the stages of seed germination and early seedling growth becomes relevant in full degree in reconciling and identifying trends in early-stage and, to a certain extent, in understanding the interior reasons for low seedling establishment under natural conditions [54]. These early growth stages are important considering the effect of drought stress. The germination of the viable seed depends to a large extent on the level of moisture available in the soil for a physiological and metabolic activity to breakdown the dormancy of the dry seed. In plants, the developmental stages which may involve germination, seedling establishment, vegetative growth and development of reproductive growth stages can easily be impaired by limited water supply [55]. Eck and Musick 1979 [56] reported the effect of drought stress exposure on irrigated sorghum seed at different stages of development such as early boot, heading, and early grain filling. According to their study, 35-42 days of water stress exposure at boot stage significantly decreased yield by 43% and 54% respectively. Inuyama et al. [57] in a separate study for a period of 16 days and 28 days under water stress reported 16% and 36% reduction in yield respectively at the vegetative stage of sorghum development. Poor water supply reduces the viability of seed and is directly linked with seedling and post seedling crisis in plants [58]. The duration and exposure to drought stress are directly linked with poor moisture intake, germination and seedling establishment in maize [55]. Roots and shoots elongations are elements of seedling and their proliferation are subject to drought stress [59]. Drought stress at seedling

stage will reduce seed endosperm weight, the growth of the coleoptile, mesoctyl, radicle, shoot, and root of sorghum [60]. Rizza et al. [61] reported that drought stress can be related to early maturity, small plant size and reduced leaf area.

4.2 Growth and Development of Plant

Growth is an irreversible increase in size, volume, and/or weight, which often involves the spectrum of stages such as cell division, cell elongation, and proliferation and cell differentiation [62]. Growth and development of plants may be greatly affected under drought stress as a result of inhibition of enzyme activities, reduced cell size and cell division, reduced water potential and gradual decline in energy supply [63]. The growth and development of plants is vital for establishing proper and normal plant structure that can carry out effective physiological and metabolic interactions which ultimately determines and give potential yields of plants [22]. Water limitation severely interferes with plant phenology where the plant phases of growth are considerably reduced with a few exceptions. Prolong drought stress initiate a signal to cause an early switch from normal plant developmental stages to vegetative reproduction

Gene	Gene expression	Plant	Nature	Reference
Adc	Production of polyamine	Rice	Drought resistance	[34]
AtTPS1	Trehalose-6-phosphate synthase	Tobacco	Drought resistance	[35]
betA	Choline dehydrogenase	Maize	Drought resistance	[36]
mt1D	Mannitol synthesis	Wheat	Drought and salinity tolerance	$[37]$
P5CS	Proline synthesis	Petunia	Drought resistance and high proline	[38]
P5CS	Proline synthesis	Soybean	Drought resistance	[39]
PPO	Polyphenol oxidases suppression	Tomato	Drought resistance	$[40]$
HVA1	LEA protein	Rice	Drought and salinity	$[41]$
ME-leaN4	LEA protein	Chinese cabbage	Drought and salinity resistance	$[42]$
Apx3	Ascorbate peroxidase	Tobacco	Drought resistance	$[43]$
ABF3	Transcription factor	Rice	Drought resistance	$[44]$
Alx8	APX2 and ABA	Arabidopsis	Drought resistance	$[45]$
AREB1	ABRE-dependent ABA signaling	Arabidopsis	Drought resistance	$[46]$
DREB	Transcription factor	Arabidopsis	Drought tolerance	$[47]$
DREB1	Transcription factor	Rice	Drought, salt and cold tolerance	[48]
DREB1A	Transcription factor	Tobacco	Drought and cold tolerance	[49]
DREB _{2A}	Transcription factor	Arabidopsis	Drought tolerance	[50]
OsDREB1A	Transcription factor	Arabidopsis	Drought, salt and freezing tolerance	[51]

Table 1. Some drought-related responsive genes in plants

stage which generally results to reduce yield [63]. In Table 2, yield reduction in crops as a result of drought stress which depends upon the degree and period of stress has been reported. McMaster and Wilhelm 2003 [64] report that growth period of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) get reduced under drought stress which ultimately affects yield.

5. BIOTECHNOLOGY METHODS IN DEVELOPING DROUGHT TOLERANCE PLANTS

5.1 Use of Classical Breeding

In light of critical global scenarios related to water availability for agricultural purposes, techniques such as conventional breeding and markerassisted selection are employed to develop drought tolerance in crop plants for human consumption [71].

Conventional breeding for developing drought tolerance in plants involves the art of hybrid cross to develop new and improved cultivars. Retrospectively, filed crop breeding approaches have increased yields through selection and combination of identifiable characteristics. The breeding program requires the identification of genetic variants to drought stress and other abiotic stress among crop cultivars where the different genetic traits are introduced into varieties with the required features [72]. This method has been used for ages in breeding programs of cereal crops. Due to the existence of tolerance variability to a large extent among plants to environmental stress, the existence of stress regulatory genes in plants to abiotic and biotic stress has been long accepted worldwide. Traditional breeding techniques have

demonstrated the fact that heritable traits conferring stress tolerance are regulated by a spectrum of genes expression synergistically, which may explain why genetic engineering of plants with drought tolerance are cumbersome [71]. The expression of single gene encoding functional proteins like late embryogenesis abundant proteins, antifreeze proteins, and molecular chaperones, would normally confer some level of tolerance to stress but do not completely give sustained tolerance to the majority of environmental stresses. Nevertheless, as the plants develop and evolve, a composite of molecular interactions may lead to their sustenance in water limitation alongside other environmental stresses and in this way, a set of regulatory genes encoding regulatory proteins have been established. The expression of regulatory proteins among others is central to the expression of genes for defense [73].

Marker-assisted selection is also a technique used in improving drought stress tolerance/resistance. In this technique, relevant quantitative trait locus for drought stress traits are usually added into plants with high yielding potential and thus developed mutant enhanced varieties that have only the major quantitative trait locus. Commonly known molecular markers such as random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism has helped to bring about the development of drought tolerance traits that their expression is independent of environmental effects [72].

5.2 Use of Genetic Manipulation

The response of a plant to drought stress has been studied at different levels such as the

ecological, cellular, physiological and molecular levels and research in these areas has established a bone technological basis now in use for developing plants with drought tolerance through genetic engineering. The limiting factor to this novel approach of plant modification for improvement is the availability of relevant genes and regulatory elements directly involved in tolerance to drought stress [71]. Increasing crop yield and value that are exposed to abiotic stress requires novel techniques to augment classical approaches which are often unable to a large degree prevent damage to a crop [26]. One such novel approach is genomics where a whole genome sequence is analyzed to discover novel and functional genes. Loosely, with the aid of microarrays, 130 genes that are sensitive and may respond to drought stress have been established [74] and these genes are directly or indirectly involved with transcription modulation, ion transport, transpiration control, and carbohydrate metabolism. With the advent of this technique, genes have been uncovered and functional genes for stress tolerance are established [26]. The discovery paves room for another novel technique known as recombinant DNA technology where the genetic makeup of plants can be modified with relevant genes to tolerate environmental stress. Plant transgenesis in contrast with conventional breeding approaches ensures the incorporation of genes of interest into the target plant. de Campos et al. reported the transgenic 'Swingle' citrumelo induced with P5CSF129A gene that encode the key enzyme for proline biosynthesis and accumulation that is crucial in promoting drought tolerance in crops with higher osmotic adjustment [75]. With the era of recombinant DNA technology, the development of genetically engineered plants with improved value seems to be a viable approach of crop improvement in contrast to classical or marker-assisted breeding
approaches [73]. The development of The development of transformation methods has resulted in an efficient generation of genetically modified plants to sustain crop productivity against abiotic stresses [76].

5.3 Through the Genes from Resurrection Plants

Resurrection plants are unique in that they can survive almost complete dehydration from their vegetative parts. They shut down their metabolic systems to tolerate dehydration and the plants are lifeless [77]. Plants species whose seeds and vegetative parts can survive severe water loss or

Ishaku et al.; APRJ, 5(2): 34-46, 2020; Article no.APRJ.56829

are desiccation-tolerant are regarded as resurrection plants (poikilohydric), as opposed to dehydration sensitive plants (homohydric) [78,79]. Resurrection plants make up an outstanding group within the flowering plants. The plants have a unique ability to withstand thorough dehydration of their vegetative components [80]. The changes in water levels and cellular responses associated with dehydration in seeds are shown to be similar in resurrection plants that are exposed to metabolic stresses as a result of severe water loss [81]. For plants to successfully withstand complete dehydration needs the concerted expression of thousands of genes that involved 63 metabolic pathways and the utilization of 64 biochemical defense mechanism to protect cellular biological integrity. The gene family of early light-induced proteins (ELIPS) is generally over-expressed during dehydration in all studied resurrection plants and may play a central role in safeguarding against photo-oxidative stress of the photosynthetic machinery during extreme dehydration [82]. One such dehydration associated gene (dsp-22) in resurrection plant Craterostigma plantagineum codes for a mature 21 kDa protein which accumulates in the vegetative parts and contrasts to other dehydration associated genes, light is crucial in regulating the expression level of dsp-22 [83]. Systemic studies of drought stress in the resurrection plant involve identifying a larger number of genes, metabolites, and proteins that usually respond to desiccation or drought stress. Some of these mechanisms that help cellular protection during extreme dehydration are peculiar to desert species [84]. VanBuren et al. [85] Reported that desiccated plant evolves from a combination of gene duplications and networklevel rewiring of existing seed desiccation pathways.

5.4 Through Protoplast Fusion

Protoplasts are cells without cell walls and cytoplasmic membrane forms the outermost layer in such cells. They can be obtained through the activity of some specific lytic enzymes such as cellulose, pectinase or macerozyme to degrade cell wall [85]. Through protoplast fusion, scientists can circumvent mating type and
incompatibility group limit to investigate incompatibility group limit to mitochondrial genetics, performed inter-generic protoplast fusion [86]. The fusion of isolated plant protoplasts by electrical stimulation has been studied and routinely employed as an experimental method. Nevertheless, the user of this method is faced with constraints by the composition of the suspension medium [87]. The protoplast fusion technique has remarkable possibilities for genetic variability and strain enhancement [88]. Hennig et al. [89] Reported the use of protoplast fusion lines of poplar hybrids under drought stress. Protoplast technology is one of the promising techniques that can be used by plant breeders to improve crop varieties [90]. Hawkes [91] Reported the use of protoplasts fusion to develop drought tolerance in explant-derived tissue cultures of Colt cherry (*Prunus avium* x pseudocerasus)

5.5 *In vitro* **Selection Technique**

The *in vitro* tissue culture approach which employ the use of a selective medium containing selective agents to select and improve plants with specific features. The technique has offer opportunity to regenerate and induce stress tolerance in plants through the use of selective agents such as NaCl, polyethylene glycol or mannitol, etc which allow preferential growth and survival of desirable features [92-94]. The explants are either exposed in a stepwise
manner with gradual increase in the manner with gradual increase in the concentration of the these selecting agents or are exposed to shock treatment where the culture medium contain high concentration of the agents [95]. Plants that survived such environmental exposure are eventually selected. These approach induces genetic variation among the exposed explants in cultured medium and regenerated plants called somaclonal variation which can result in genetically stable traits useful in crop improvement [96,97]. *In vitro* selection technique for explants demonstrating increased drought tolerance has been reported. Polyethylene glycol (PEG) has been utilized to induce drought stress in plants and the determination of plants that withstand water stress is based on accumulation of consistent solutes primarily proline as well as the presence of antioxidative enzymes such as peroxidases, catalases, and dismutases [95].

6. DISCUSSION

The development of plants with the ability to establish and withstand/tolerate water limitation and remain productive in marginal soils is one of the major goals of crop and forage breeding programs worldwide [98].

In a world where population growth exceeds food supply, overcoming environmental stresses that affect crop yield and quality becomes relevant. Drought is the most important abiotic stress that can affect plant growth and development and efforts have been recorded down the line to improve crop yield under water–limiting state. The drought tolerance mechanism is a complex process that involves a wide range of physiological, morphological and biochemical interactions at various levels of plant growth and development which may include stomatal regulation, synthesis of osmoprotectants, generation of osmolytes, reduction in water loss and increased water uptake, etc. Understanding the effect of drought stress like other abiotic stresses becomes relevant to ensure food supply.

7. CONCLUSION

Plant drought stress is a major problem in the growth and development of plants. One of the major challenges of the plant biotechnologist is solving the problem of plant drought stress so that it can combat the problem of climatic change and increase in population growth. Recent advances in plant biotechnology has seen remarkable progress in molecular markers selection processes and in developing transgenic plants with increased drought stress tolerant.

These approaches have facilitated our understanding of underlying processes in plant responses to drought induced stress. Through plant genetic engineering and molecular marker techniques, drought stress induced genes have been identified and cloned.

It therefore means that the applications of biotechnological and molecular approaches such as genomics, proteomics, and transcriptomic that can enhance a better understanding of plant water use efficiency and tolerance to improve yield under drought stress is very promising.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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