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USE OF BIOTECHNOLOGICAL TOOLS FOR DEVELOPING DROUGHT RESISTANT PLANTS

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Abstract

There are many challenges that come in the path of sustainable food production among which abiotic stress is one that is responsible for reducing the potential yields by 70% in crop plants. There are many abiotic stress factors that are responsible for this reduction but drought is regarded as the most damaging factor among them. Various advances in the field of biotechnology have made us understand about the process by which the plant respond to drought at the molecular and whole plant level. The objective of this review is in particular to identify drought stress tolerance in various plants at different stages, and how different biotechnological tools can be used to improve plant drought tolerance.

Keywords: Drought tolerance, abiotic stress, biotechnology, QTL (Quantitative trait loci).

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Introduction

The existence of life on earth is dependent on certain essential factors. Water being one of those essential factors is required by every living being for its survival on the planet Earth and its scarcity can be very lethal (Amara et al 2013). Stress can be defined as the process that restricts crop productivity by affecting the plant metabolism. Biotic stress is defined as the stress caused by insects and diseases whereas abiotic stress may include drought, flooding, salinity, metal toxicity and mineral deficiencies (Basu *et al* 2016).

Drought is explained as an extended dry period that leads to crop stress and decrease in the yield. The lack of sufficient precipitation, either snow or rain, can cause reduced soil moisture or groundwater, resulting in drought (Basu et al 2016). Drying stress is a multidimensional strain and generally leads to physiological, morphological, ecological, biochemical and molecular effects on flora. Plant responses to drought conditions depend upon the time period and severity of water deficiency as well as their species, age and their developmental stage (Chen et al 2010). The production of ABA hormone in plants is regarded as a result of stress in the root tip (Diab et al 2004).

Drought Tolerance Mechanism in Plants

The ability of species or crops for growth and development under conditions of drought can be defined as drought tolerance (Diab et al 2004). The mechanism by which plants get exposed to drought stress is directly proportional to the duration of its exposure and the species to which it belongs. The effect of biochemical, physiological or morphological factors is known as mechanism of drought tolerance in plants. For making the plants habitual to this stress, they are influenced by physiological spectrum and molecular interactions. Moreover, this is the ability for the complete development of the plant system mechanism which enhances the economic yield with reduced water supply.The drought stress influences the water level of the plant cell mechanism at tissues and organs which corresponds with the adaptation on ambiguous interaction. Plants reciprocate to the stress caused by abiotic factors by means of expression of genes formulating protein formation. Though the data which commemorates with protein functions is not yet traceable to vast extent, the drought stress establishment is perplexed which has synergistically expressedgenes which get triggered due to some injury or stress caused to the plants (Fang and Xiong 2015).

For decreasing the rate of transpiration, the plants close theirstomata which leads to reduction in absorption and reduced sweating or a combination of all three levels. Drought stresses causewaxy leaves in certain plants leading to reduced water loss (Diab et al 2004). Plant drought resistance has four major mechanisms: the avoidance of drought (DA), drought tolerance (DT), drought escape and drought recovery (DE) in some of the literature. DA and DT are two vital mechanisms for the resistance to drought conferred on plants, among the four elements of dryness. DA is the ability of plants to maintain under-slight or moderate drought stress conditions by modifying some morphological structures and/or growth rates to avoid the adverse effects of drought stress. In the presence of water deficiency, DA is primarily characterized by the preservation of high plant water potential. Generally, plants adopt three DA strategies: (1) Reduce water losses from the quick stomata closure; (2) improve water uptake through a thorough root system (especially increased rooting depth, rooting density or roots-shoot-to ratio) and improve water holding capability in specific organisms; (2) promote water uptake in many of the species of plants such as alfalfa, tobacco and rice; and (3) increasing or decreasing conversions to reproductive growth, in the face of severe drought stress, in order to prevent complete abortions. DT means that plants are able, under conditions of severe drought, to maintain a certain degree of physiological activity by regulating thousands of genes and metabolism in order to reduce or repair damages caused by stress (Amara et al 2013).

Effect of Drought Stress on Photosynthesis

Reduction in photosynthesis is also a known effect of drought stress which occurs due to the reduction of both leaf area and rate of photosynthesis per unit leaf area. Decreased photosynthesis is mainly caused by stomata closure or metabolism. During a limited intercellular CO_2 concentration, continuous photosynthetic light reactions during drought stress leads to accumulation of photosynthetic transport components of electron transport chain, which can potentially decrease molecular oxygen, resulting in the production of reactive oxygen species (ROS) (Huang et al 2010).

Biotech Approaches for Crop Improvement

Agricultural biotechnology includes application of scientific techniques to modify and enhance crops production. Advanced agricultural biotechnology has been under development for the past four decades that can help to overcome major bottlenecks of classical plant breeding like the lack of natural sources of resistance (resistance to insect pests, fungal pathogens and viral diseases) and abiotic constraints (tolerance to drought, salinity and temperature) to crop productivity. However, successful application of biotechnology to address these constraints needs a good biological knowledge of the target plant species and the mechanisms underlying tolerance to these stresses. Different approaches involving genomics and transgenics have been developed to understand the biological mechanisms and ultimately to enhance the crop yield and quality (Fathi and Tari 2016).

Development of Drought Resistance

In plant architecture, lateral root development isconsidered as an important agronomic character that discover the crop productivity and environmental stress adaptability. Thus, it is highly controlled by the intrinsic developmental cues, like the abscisic acid (ABA) and auxin, and by the diverse environmental growth conditions, that involves water deficit and high salinity in the soil (Goel et al 2010). Research attempts have been made to make use of conventional breeding methods, to involve the techniques of inter-specific, inter-generic hybridizations and mutagenesis, but they ended up with a small amount of success(Basu et al 2016).

Drought resistance being a complex trait, less genetic variations for yield component under drought stress, and effective selection procedures were some of the reasons that were considered responsible for this limited success. The advancement of innovations in the field of biotechnology have really helped us to know better about what are the different processes by which a particular plant responds to drought conditions at the molecular level.After a thorough study, hundreds of genes have been identified that can be introduced in the plants to overcome the drought stress and some of these have been cloned. For a better understanding of the drought tolerance mechanism, a wide range of different tools from the gene expression pattern to the transgenic plants have been discovered and made available. With the innovation of new techniques such as stable isotopes role, genome wide association techniques and tools, thermal or fluorescence imaging and interaction of various set of proteins in plant proteins, can prove helpful to overcome or tackle the difficultieswhere other tools are lacking. Some biotechnological approaches such as Plant Genetic Engineering and molecular -marker technology can prove helpful for the development of drought tolerant germplasm (Basu et al 2016).

Due to the variations in plant phenology and the drought traits being maintained by multiple quantitative trait loci (QTLs), the mechanism of drought tolerance is considered as complex. Unless detailed study of the physiological and genetic foundations is done, the way in which plants respond to drought strain is somewhat complex and difficult to understand. If the molecular mechanisms related to the grain yield stability are not sufficiently considered, then neither the traditional breeding and nor the modern genetic techniques can affectively enhance the drought tolerating mechanism of crop plants (Guo et al 2008).

Various new discoveries in crop drought tolerance are the results of the various advancements in plant physiology, phenotyping and plant genomics. As a result of the new gene discovery for plant development, crop breeders will be able to boost crop yields. Increased understanding of the complexity and relation of the dry-tolerant mechanisms with different characteristics will result in the identification of QTLs and traits associated genes in terms of selection efficiency using molecular approaches and genomics. To develop transgenic crops with improved drought stress tolerance, the identification of candidate genes which are responsible for plant tolerance under different abiotic stresses is important. Once the gene controlling drought tolerance has been identified through QTL mapping, these genes can then be inserted into the genetic background of any desirable cultivar using genetic engineering (Agrobacterium tumefaciens or gene gun) and hybridization using marker assisted selection (Guo et al 2008).

Breeding for Drought Tolerance through Molecular Markers

At present, the molecular markers are extensively used to find the position of drought-induced genes. Various characters which are important for Marker-assisted breeding (MAB) method in wheat under stress conditions also require Molecular markers for their genome mapping and tagging. The main purpose behind using this technique is to develop stress – tolerant lines in various crops. Marker-assisted selection (MAS) can be defined as the process in which the selection is done by the DNA markers that are linked to QTLs that are very powerful. Therefore, DNA markers can trace the existence of QTLs for drought tolerance. Marker-assisted selection (MAS) is considered as the best process for the development of drought tolerance in plants that use molecular linkage maps (Gosal et al 2009).

In winter wheat, with the application of amplified fragment length polymorphism (AFLP) and the sequence repeat (SSR) markers, QTL mappings for senescence of flag leaf

Table 1: QTLs of Physiological Responses to Drought Stress Identified In Wheat and Barley

Crop	***Wheat***	***Durum wheat***	***Barley***	***Barley***	***Barley***	***Barley***	***Barley***
Trait	Water soluble carbohydrate	Carbon isotope ratio Osmotic potential Chlorophyll content Fag leaf rolling index	Grain carbon isotope discrimination	Relative water content	Leaf osmotic potential Osmotic potential	Chlorophyll fluorescence parameters	Relative water content
Condition of drought	Rainfed field	Rainfed field	Mediterranean rainfed field	Mediterranean rainfed field	Water-deficit in growth chamber	Post-flowering drought	Water-withholding
Chromosomal location	1A, 1D, 2D, 4A, 6B, 7B, 7D	2B, 4A, 5A, 7B	2H, 3H, 6H, 7H	6HL	6HL	2H, 4H, 6H, 7H	1H, 2H, 6H
Reference	(Ishaku et al 2020)	(Nezhadahmadi et al 2013)	(Oladosu et al 2019)	(Peleg et al 2009)	(Salehi-Lisar and Bakhshayeshan-Agdam 2016)	(Sharma et al 2014)	(Teulat et al 2001)

Table 2: Resistance to Drought Stress Exhibited by Various Transgenic Plants through Expression of Genes

Plant	**Gene involved**	**Function**	**Tolerance**	**Growth conditions**	**Reference**
Tomato	CaXTH3,ahotpepper xyloglucanendo-transglucosylase/hydrolase	Maintainedsufficientchlorophyllevenat100mM NaCl	Droughtand salinity	Controlledconditions	(Teulat et al 2003)
Maize	Rab28LEA	IncreaseRWC,leafandrootgrowth,lowerMDA	Drought	Greenhouse	(Tirado and cotter 2010)
Tobacco	PtrABF,abZIPtranscriptionfactor	DecreasedROS	Drought	Growth chamber	(Yang et al 2007)

(FLS) in normal and water-stressed environments have been evaluated. The specific gene that is considered responsible for this particular characteristic is revealed and the QTL is also flecked on chromosome 2D associated with enhanced performance under water deficiency conditions. A study by Quarrie et al shows that DNA markers such as RFLP, AFLP and SSR are used to tag the QTLs for drought stress in wheat. During the past few decades, certain molecular markers like SDS-protein, isozymes, and DNA sequences have proved beneficial in selecting the quantitative traits particularly the drought tolerance one. In wheat crop, these molecular markers are also used to find out the genotype and genetic mapping and also to study about the diversity of the genes. There are various markers present in the *Triticum durum* wheat which are associated with the grain yield and morphophysiological characters that are considered responsible for drought tolerance. Leaf water potential, canopy temperature, chlorophyll inhibition, and proline content all these showed strong affinity with the molecular markers (Gosal et al 2009).

Another study by Ashraf et al developed different DNA markers to get an estimate particularly about the inheritance of stress tolerance such as PCR indels, RAPDs, RFLPs, CAPS, AFLPs, microsatellites (SSRs), SNPs and sequences of DNA. In cereal crops, RAPDs with the implementation of DNA primer were largely used. ISSRs were also used in mapping of the genome in wheat crop and other crops. Milad et al. recognized Random Amplified polymeric DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) markers which were linked to the flag leaf senescence gene in wheat crop particularly under the moisture stress conditions. RAPDs proved to be very helpful in hexaploid wheat as the genetic markers. It is observed that when the connection joining a molecular marker and a character is eminent than the heritability of the trait, the marker assisted selection can prove very helpful. Thus, these results indicate the importance of the molecular markers to enhance drought tolerance in durum wheat under drought conditions(Gosal et al 2009). Table 1 highlights the QTLs of physiological responses to drought stress identified in wheat and barley.

The first drought – tolerant aerobic rice variety in India was released in the year 2007 and was named as MAS 946-1 (Teulat et al 2002). Transgenic plants have also been developed with resistance traits for drought stress through expression of genes as highlights in Table 2.

Conclusion

Drought is one of the major abiotic stresses which is responsible for reducing the production of agricultural products not only in India but throughout the world. It is therefore essential to study the stress thoroughly and to try to overcome this problem through various progress in biotechnology. By introducing various drought resistant genes in plants and by using molecular markers, it is possible to solve the problem of drought, but at the same time, these biotechnological practices should not harm the environment, since their effect on living beings would be direct or indirect.

References

Amara I, Capellades M, Ludevid MD, Pagès M, and Goday A (2013). Enhanced water stress tolerance of transgenic maize plants over-expressing LEA Rab28 gene. *Journal of plant physiology*, *170*(9), 864-873.

Basu S, Ramegowda V, Kumar A, and Pereira A (2016). Plant adaptation to drought stress. *F1000Research*, *5*.

Chen G, Krugman T, Fahima T, Chen K, Hu Y, Röder M, and Korol A (2010). Chromosomal regions controlling seedling drought resistance in Israeli wild barley, Hordeum spontaneum C. Koch *Genetic Resources and Crop Evolution*, *57*(1), 85-99.

Diab AA, Teulat-Merah B, This D, Ozturk NZ, Benscher D, and Sorrells ME (2004). Identification of drought-inducible genes and differentially expressed sequence tags in barley. *Theoretical and Applied Genetics*, *109*(7), 1417-1425.

Fang Y, and Xiong L (2015). General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and molecular life sciences*, *72*(4), 673-689.

Fathi A, and Tari DB (2016). Effect of drought stress and its mechanism in plants. *International Journal of Life Sciences*, *10*(1), 1-6.

Goel D, Singh AK, Yadav V, Babbar SB, and Bansal KC (2010). Overexpression of osmotin gene confers tolerance to salt and drought stresses in transgenic tomato (Solanum lycopersicum L.). *Protoplasma*, *245*(1-4), 133-141.

Gosal SS, Wani SH, and Kang MS (2009). Biotechnology and drought tolerance. *Journal of Crop Improvement*, *23*(1), 19-54.

Guo P, Baum M, Varshney RK, Graner A, Grando S, and Ceccarelli S (2008). QTLs for chlorophyll and chlorophyll fluorescence parameters in barley under post-flowering drought. *Euphytica*, *163*(2), 203-214.

Huang XS, Liu JH, and Chen XJ (2010). Overexpression of PtrABF gene, a bZIP transcription factor isolated from Poncirus trifoliata, enhances dehydration and drought tolerance in tobacco via scavenging ROS and modulating expression of stress-responsive genes. *BMC plant biology*, *10*(1), 1-18.

Ishaku GA, Tizhe DT, Bamanga RA, and Afolabi ET (2020). Biotechnology and drought stress tolerance in plants. *Asian Plant Research Journal*, 34-46.

Nezhadahmadi A, Prodhan ZH, and Faruq G (2013). Drought tolerance in wheat. *The Scientific World Journal*, *2013*.

Oladosu Y, Rafii MY, Samuel C, Fatai A, Magaji U, Kareem I, and Kolapo K (2019). Drought resistance in rice from conventional to molecular breeding: a review. *International journal of molecular sciences*, *20*(14), 3519.

Peleg ZVI, Fahima T, Krugman T, Abbo S, Yakir DAN, Korol AB, and Saranga Y (2009). Genomic dissection of drought resistance in durum wheat× wild emmer wheat recombinant inbreed line population. *Plant, cell & environment*, *32*(7), 758-779.

Salehi-Lisar SY, and Bakhshayeshan-Agdam H (2016). Drought stress in plants: causes, consequences, and tolerance. In *Drought Stress Tolerance in Plants, Vol 1* (pp. 1-16). Springer, Cham.

Sharma KK, Dumbala SR, and Bhatnagar-Mathur P (2014). Biotech approaches for crop improvement in the semi-arid tropics. In *Plant Biotechnology* (pp. 193-207). Springer, Cham.

Teulat B, Borries C, and This D (2001). New QTLs identified for plant water status, water-soluble carbohydrate and osmotic adjustment in a barley population grown in a growth-chamber under two water regimes. *Theoretical and Applied Genetics*, *103*(1), 161-170.

Teulat B, Merah O, Sirault X, Borries C, Waugh R, and This D (2002). QTLs for grain carbon isotope discrimination in field-grown barley. *Theoretical and Applied Genetics*, *106*(1), 118-126.

Teulat B, Zoumarou-Wallis N, Rotter B, Salem MB, Bahri H, and This D (2003). QTL for relative water content in field-grown barley and their stability across Mediterranean environments. *Theoretical and Applied Genetics*, *108*(1), 181-188.

Tirado R, and Cotter J (2010). Ecological farming: Drought-resistant agriculture. *Exeter, UK: Greenpeace Research Laboratories*.

Yang DL, Jing RL, Chang XP, and Li W (2007). Identification of quantitative trait loci and environmental interactions for accumulation and remobilization of water-soluble carbohydrates in wheat (Triticum aestivum L.) stems. *Genetics*, *176*(1), 571-584.