

Genetics and genetic improvement of drought resistance in crop plants

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Drought limits the agricultural production by preventing the crop plants from expressing their full genetic potential. Three mechanisms, namely drought escape, drought avoidance and drought tolerance are involved in drought resistance. Various morphological, physiological and biochemical characters confer drought resistance. Morphological and physiological characters show different types of inheritance pattern (monogenic and polygenic) and gene action (additive and non-additive), whereas the genes responsible for biosynthesis of different compatible solutes have been identified and cloned from plants, yeast, mouse and human. Different breeding approaches for drought resistance have emerged, with their merits and demerits. Efficient screening techniques are pre-requisite for success in selecting desirable genotype through any breeding programme. Genetic engineering has been successfully applied to identify and transfer different genes responsible for biosynthesis of different metabolites

such as proline, trehalose and polyamines from different organisms to crop plants through a targeted approach. Barley *hva1* gene responsible for late embryogenesis abundant (LEA) proteins has been transferred to rice to produce drought-resistant transgenics through a shotgun approach. Lack of multidisciplinary approach and precise screening techniques, incomplete knowledge about genetic basis of drought resistance, negative correlation of drought resistance traits with productivity and unavailability of appropriate genes to obtain transgenic plants are the main constraints for genetic improvement of drought resistance. Exploration of wide genetic variation of relevant characters, consideration of more genes at a time to transfer through breeding or genetic engineering method, application of antisense RNA technique, assessment of polypeptides induced under drought and multidisciplinary approach should be included in the future research programmes for drought resistance.

DROUGHT is actually a meteorological event which implies the absence of rainfall for a period of time, long enough to cause moisture-depletion in soil and water-deficit with a decrease of water potential in plant tissues¹. But from agricultural point of view, its working definition would be the inadequacy of water availability, including precipitation and soil-moisture storage capacity, in quantity and distribution during the life cycle of a crop plant, which restricts the expression of full genetic potential of the plant². It acts as a serious limiting factor in agricultural production by preventing a crop from reaching the genetically determined theoretical maximum yield³. The effect of drought on crop production and overall economy is well known⁴. The most affected states in India are Rajasthan, parts of Gujarat, Haryana and Andhra Pradesh. Most of the crops are sensitive to water deficits, particularly during flowering to seed development stage⁵. Even crops grown in arid and semi-arid regions such as pearl millet, sorghum and pigeon pea are also affected by drought at the reproductive stage.

In agriculture, drought resistance refers to the ability of a crop plant to produce its economic product with minimum loss in a water-deficit environment relative to the water-constraint-free management. An understanding of genetic basis of drought resistance in crop plants is a pre-requisite for a geneticist to evolve superior genotype through either conventional breeding methodology or biotechnological approach. This article aims at a review on genetics of drought resistance and different approaches, constraints and future strategies for its genetic improvement.

Mechanisms of drought resistance

In genetic sense, the mechanisms of drought resistance can be grouped into three categories, viz. drought escape, drought avoidance and drought tolerance⁶. However, crop plants use more than one mechanism at a time to resist drought⁷. Drought escape is defined as the ability of a plant to complete its life cycle before serious soil and plant water deficits develop. This mechanism involves rapid phenological development (early flowering and early maturity), developmental plasticity

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(variation in duration of growth period depending on the extent of water-deficit) and remobilization of pre-anthesis assimilates to grain⁸.

Drought avoidance is the ability of plants to maintain relatively high tissue water potential despite a shortage of soil-moisture, whereas drought tolerance is the ability to withstand water-deficit with low tissue water potential. Mechanisms for improving water uptake, storing in plant cell and reducing water loss confer drought avoidance. The responses of plants to tissue water-deficit determine their level of drought tolerance.

Drought avoidance is performed by maintenance of turgor through increased rooting depth, efficient root system and increased hydraulic conductance and by reduction of water loss through reduced epidermal (stomatal and lenticular) conductance, reduced absorption of radiation by leaf rolling or folding^{9,10} and reduced evaporation surface (leaf area)^{11,12}. Plants under drought condition survive by doing a balancing act between maintenance of turgor and reduction of water loss¹³. The mechanisms of drought tolerance are maintenance of turgor through osmotic adjustment (a process which induces solute accumulation in cell), increase in elasticity in cell and decrease in cell size and desiccation tolerance by protoplasmic resistance^{14,15}.

Unfortunately, most of these adaptations to drought have disadvantages. A genotype of short duration usually yields less compared to that of normal duration. The mechanisms that confer drought resistance by reducing water loss (such as stomatal closure and reduced leaf area) usually result in reduced assimilation of carbon dioxide. Osmotic adjustment increases drought resistance by maintaining plant turgor, but the increased solute concentration responsible for osmotic adjustment may have detrimental effect in addition to energy requirement for osmotic adjustment⁸. Consequently, crop adaptation must reflect a balance among escape, avoidance and tolerance while maintaining adequate productivity.

Genetics

Drought resistance is a complex trait, expression of which depends on action and interaction of different morphological (earliness, reduced leaf area, leaf rolling, wax content, efficient rooting system, awn, stability in yield and reduced tillering), physiological (reduced transpiration, high water-use efficiency, stomatal closure and osmotic adjustment) and biochemical (accumulation of proline, polyamine, trehalose, etc., increased nitrate reductase activity and increased storage of carbohydrate) characters. Very little is known about the genetic mechanisms that condition these characters.

The identification of genes responsible for morphological and physiological traits and their location on

chromosome have not been possible, but their inheritance pattern and nature of gene action have been reported. Polygenic inheritance of root characters is reported by Ekanayake *et al.*¹⁶. The long root and high root numbers are controlled by dominant alleles and thick root tip by recessive alleles¹⁷. However, leaf rolling¹⁸ and osmotic adjustment¹⁹ have shown monogenic inheritance. Tomar and Prasad²⁰ reported a drought resistance gene, *Drt1* in rice, which is linked with genes for plant height, pigmentation, hull colour and awn, and has pleiotropic effect on the root system. Similarly, in cowpea drought resistance is reported to be controlled by a single dominant gene²¹.

Though some more reports in this regard for other traits are available²²⁻²⁷, further investigation is the need of the hour to have better understanding of genetic control of morphological and physiological traits contributing to drought resistance.

In addition to morphological and physiological changes, biochemical change involving induction of compatible solute biosynthesis is one way to impart drought²⁸. Under drought, plants try to maintain water content by accumulating various solutes that are non-toxic and do not interfere with plant processes and are, therefore, called compatible solutes²⁹. Some of them are fructan, trehalose, polyols, glycine betaine, proline and polyamines. The different genes responsible for different enzymes involved in biosynthesis of these solutes have been identified and cloned from different organisms (bacteria, yeast, human and plant), which have been discussed later in the article.

Breeding approach

Three breeding approaches for drought resistance have been evolved. The first is to breed for high yield under optimum (water-stress-free) condition. As the maximum genetic potential of yield is expected to be realized under optimum condition and a high positive correlation exists between performance in optimum and stress conditions³⁰, a genotype superior under optimum level will also yield relatively well under drought condition. This is the basic philosophy of this approach.

However, the concept of expression of maximum genetic potential in optimum condition is debated³¹ as genotype environment interaction may restrict the high-yielding genotype to perform well under drought. Thus, the second approach, i.e. to breed under actual drought condition has been suggested³².

The second approach suffers from the problem that the intensity of drought is highly variable from year to year and as a consequence environmental selection pressure on breeding materials changes drastically from generation to generation. This problem compounded with low heritability of yield³³ makes for the complicated and slow breeding programme.

An alternative approach to the above two would be to improve drought resistance in high-yielding genotypes through incorporation of morphological and physiological mechanisms of drought resistance. However, transferring drought resistance in high-yielding genotypes is complicated due to lack of understanding of the physiological and genetic basis of adaptation in drought condition. In contrast, improving the yield potential of an already resistant material may be a more promising approach, provided there is genetic variation within such a material³⁴. Simultaneous selection in non-stress environment for yield and in drought condition for stability may be done to achieve the desired goal of evolving drought-resistant genotype with high yield.

As such, the breeding methodology to be applied for drought resistance is the same as that applied for other purposes. In general, pedigree and bulk method could be used for self-pollinated crops and recurrent selection for cross-pollinated crops. However, if transfer of few traits relating to drought resistance to a high-yielding genotype is the aim, then back cross is the appropriate methodology. On the other hand, biparental mating (half sib and full sib) maintains the broad genetic base as well as provides the scope to evolve the desired genotype of drought resistance³⁵. The success of any breeding programme depends on the availability of the screening technique, especially for drought resistance.

Screening techniques

Any effort for genetic improvement in drought resistance utilizing the existing genetic variability requires an efficient screening technique, which should be rapid and capable of evaluating plant performance at the critical developmental stages and screening a large population using only a small sample of plant material³⁶. As stated earlier, drought resistance is the interactive result of different morphological, physiological and biochemical traits and thus, these different components could be used as selection criteria for screening appropriate plant ideotype. A combination of different traits of direct relevance, rather than a single trait, should be used as selection criteria³⁷. Ludlow and Muchow³⁸ ranked the merit of various traits conferring drought resistance. McCree *et al.*³⁹ and Johnson *et al.*⁴⁰ provided a framework for evaluating how combination of traits influences plant water status and growth and this may usefully bridge physiology and breeding into the integrated programme of plant improvement.

The importance of developing a reliable screening technique for drought resistance has been realized very early⁴¹. The different techniques used so far are as follows:

1. Use of infrared thermometry for screening efficient water uptake⁴².
2. Banding herbicide metribuzin at a certain depth of soil⁴³, and use of iodine-131 (ref. 44) and hydroponic culture under stress of 15 bar¹⁴ for screening root growth.
3. Psychrometric procedure for evaluating osmotic adjustment^{45,46}.
4. Diffusion porometry for leaf water conductance⁴⁷.
5. Use of the mini-rhizotron technique for root penetration, distribution and density in the field with minimum disturbance⁴⁸.
6. Infrared aerial photography for dehydration postponement⁴⁹.
7. Use of carbon isotope discrimination for selecting increased water-use efficiency⁵⁰.
8. As loss of yield is the main concern for the crop plant from agricultural point of view, plant breeders emphasize on yield performance under moisture-stress condition. A drought index which provides a measure of drought based on loss of yield under drought-condition in comparison to moist condition has been used for screening drought-resistant genotype⁵¹⁻⁵³. An artificially created water-stress environment⁵⁴ is used to provide the opportunity in selecting superior genotype out of a large population. Visual scoring or measurement for maturity, leaf rolling, leaf length, angle, root morphology and other morphological characters of direct relevance to drought resistance are also taken into consideration.

Biotechnological approach

The techniques for gene transformation of crop plants have been applied for identification of genes responsible for drought resistance and their transfer⁵⁵. Mainly two approaches, namely targeted and shotgun approach facilitate genetic engineering to obtain transgenic plants conferring drought resistance.

Targeted approach

Metabolic pathways involving the synthesis of different metabolites such as polyamine, carbohydrate, proline, glycine betaine and trehalose have shown to be associated with drought resistance. This approach relies on the availability of relevant information on biochemical reaction for synthesis of these metabolites and utilizes the related genes to transfer them from different sources to crop plants. This approach is more precise and methodical, having a higher probability of success in comparison to the shotgun approach.

In recent years, introduction of drought-induced genes involved in different biochemical pathways from different sources to sensitive plants has evolved as one of the promising methods. The gene *TPS1* found in yeast encodes for trehalose-6-phosphate synthetase and

is involved in biosynthesis of trehalose. Tobacco has been transformed with the yeast *TPSI* gene^{56,57}. By determining the water loss of detached leaves⁵⁶ or by determining the effect of withholding irrigation on the death and damage of leaf⁵⁷, it has been shown that the transgenic plants have increased drought resistance. Another gene, *P5CS*, encodes for pyrroline-5-carboxylate synthetase which is involved in proline synthesis, and the over-production of proline confers drought resistance. Transgenic tobacco over-expressing *P5CS* gene transferred from mothbean exhibited a high level of enzyme and produced 10–18-fold more proline than control plant. The over-production of proline enhanced root biomass and flower development under drought condition⁵⁸.

The bacterial gene *SacB* found in *Bacillus subtilis* encodes for levan sucrose, which takes part in fructan synthesis. When this gene was transferred to tobacco, the transgenic plant produced fructan and showed better performance in comparison to control under PEG-mediated drought condition⁵⁹.

The genes *betA* encoding for choline dehydrogenase and *betB* encoding for betaine aldehyde dehydrogenase are involved in the biosynthesis of glycine betaine and accumulation of glycine betaine confers drought resistance. Holmstrom *et al.*⁶⁰ transferred *betB* gene from *Escherichia coli* to tobacco.

With the availability of genes responsible for polyamine biosynthesis such as *ADC* (encodes for arginine decarboxylase), *ODC* (encodes for ornithine decarboxylase) and *SAMDC* (encodes for S-adenosyl-methionine decarboxylase), it is now possible to manipulate polyamine content using sense and antisense constructs of these genes in transgenic plants. Transgenic tobacco plants with *ODC* gene from yeast⁶¹ and mouse⁶², *ADC* gene from oat⁶³ and *SAMDC* gene from human⁶⁴ have been reported, but sufficient studies have not so far been carried out to observe whether the transgenics show any tolerance to drought. Only expression level of polyamines in plants has been studied. However, *SOD* (superoxide dismutase) gene from pea⁶⁵ has been transferred to tobacco and transgenics were found to be drought resistant.

Shotgun approach

This approach to obtain the desired gene is indirect. A random analysis of stress-related alteration in cell process and gene expression is employed. Genes, which are expressed under drought and for which no specific role has yet been proven, are identified. Though the approach is less precise with low probability of success, the strategy can work even as there is no prior information about the gene or gene product. Thus, the shotgun approach appears to be better choice due to dearth of

sufficient information on biochemical changes in the cell for drought resistance. Transgenic rice carrying barley *hva1* gene produced through this approach has shown drought resistance⁶⁶. Gene *hva1* encodes for a group of three LEA (late embryogenesis abundant) proteins which gets accumulated in vegetative organs during drought condition⁶⁷.

The tissue culture method has a potential to create somaclonal variation for drought resistance also^{68,69}, but difficulties in selection for desired variant restrict its use⁷⁰.

Marker-assisted selection

In most breeding programmes, the genetic improvement for drought resistance is accomplished through selection for yield and because of low heritability of yield under stress and the spatial as well as temporal variation in the field environment, conventional breeding approaches are slow. Whereas molecular markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and isozyme will facilitate to develop drought-resistant genotypes more effectively as their expressions are independent of environmental effects.

After identification of the molecular markers associated with yield or other morphological traits related to drought resistance, those markers could be used as a selection criteria for drought resistance. The application of marker-assisted selection in evolving drought-resistant genotypes is in an experimental stage; more specifically just identification of RFLP markers associated with osmotic adjustment, stay green, root traits^{71–74} has been achieved.

Constraints

Though researchers have proposed many possible different characteristics related to drought resistance that could be used in selection and genetic variability for those exists in different crops^{75–80}, the success rate in obtaining drought-resistant genotypes is low. The lack of success probably results from a combination of following factors:

1. Lack of efforts through multidisciplinary approach to understand the integrated plant responses to drought and complex genetic control of different mechanisms of drought resistance.
2. Lack of repeatable and precise screening techniques.
3. Knowledge is incomplete about reliable attributes as indices of drought resistance, selection criteria and influence of environment on drought-related traits⁸¹.
4. Several adaptations reducing water loss under drought seem to have a negative effect on crop pro-

ductivity. For instance, both leaf rolling and stomata closure conserve water in plant, but reduce light interception and entry of carbon dioxide into leaf and in turn, reduce the yield. These traits are not useful in breeding drought resistance.

5. Drought reduces nutrient uptake and is associated with temperature stress and at higher elevation with cold. This association makes the breeding programme more complicated.
6. Despite the realization about the importance of water-use efficiency and the availability of its genetic variability, selection for high water-use efficiency often results in decrease in crop growth rate. Most often plants evolve to maximize water-use efficiency through reduction in transpiration. Since dry matter production is strongly associated with total transpiration, any reduction in transpiration results in reduced crop growth rate⁸².
7. Limitation in application of genetic engineering in this aspect is lack of information on availability of the most appropriate gene.

Future strategies

The future research programmes for drought resistance should consider the following strategies:

1. There is an urgent need for exploration of the plant genetic resources with attributes related to drought resistance in different crop plants and their characterization to facilitate transfer of desired traits through conventional plant breeding or biotechnological method.
2. A single trait cannot confer drought resistance satisfactorily. Therefore, breeding programme for drought resistance should aim at pyramiding a number of relevant traits in a crop.
3. Plant genetic engineering also generated transgenic plants with only one transgene in all cases. Many different genes responsible for biosynthesis of different solutes and osmolytes conferring drought resistance should be considered for transfer in a crop plant at a time.
4. Attention should be concentrated on better understanding of genetic basis of drought resistance through antisense RNA technique, observing the effect of expression level of different enzymes/proteins in different biochemical pathways on drought resistance.
5. Several stress proteins (such as LEA, dehydrin, etc.) are synthesized and accumulated in plant tissues under drought condition. A comparative assessment of various polypeptides produced in response to drought, between sensitive and tolerant genotypes may be used in identification of protein marker,

which could help in producing transgenic drought-resistant plants.

6. A multidisciplinary approach involving genetics, biochemistry, biotechnology, physiology, plant breeding and crop science will be appropriate to assess the complicated and integrated response of plants to drought and to evolve superior drought-resistant genotypes.

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