Chapter

Breeding for Drought Resistance

Pamirelli Ranjith and Madasu Srinivasa Rao

Abstract

Drought is the most severe abiotic stresses in many parts of the world and is one of the major problems in present-day climatic scenario. Drought tolerant varieties are with high demand which seems to be a great challenging task to plant breeders however difficulties are combined by the difficulty of crop yield on the genetic and physiological bases. Drought resistance may be defined as the mechanism(s) causing minimum loss of the yield in a drought environment relative to the maximum yield in a constant-free of optimal environment for the crop. Several researchers explained the plant reaction to drought through drought escape, dehydration avoidance, and/or dehydration tolerance mechanisms. Drought stress decreases size of the leaves, stem extension and root proliferation inside the soil, it also disturbs plant water relations and reduces water-use efficiency ultimately reduces the yielding ability of the plant so, breeding for Drought resistance is a good approach, following different breeding strategies and approaches to develop a drought resistant variety combining both conventional and molecular approaches. Considering the parameters like root morphology studies, proline estimation, leaf rolling etc., Selection based on a comprehensive approach of testing might be more effective in breeding better drought-tolerant cultivars.

Keywords: drought, proline, resistance, abiotic stress, avoidance and tolerance

1. Introduction

Many crops grown in diverse environmental conditions and are subjected to different stress conditions among them drought is the major yield limiting factor in major crops. Food for future generations is challenged by increasing demand and threatened by deteriorating water availability, nearly 23 million hectares of rainfed rice in South and Southeast Asia are drought prone areas. In some states of India, due to severe drought conditions nearly 40% yield loss, amounting to $800 million is affected. As the Southwest monsoon are irregular it results in moderate to severe drought in rainfed regions, particularly in eastern India, as a result many morphological, physiological and phenological traits have been reported to improve the performance of many crops to challenge the drought condition Drought Tolerance is defined as the capacity of plants to uphold a certain level of physiological activity over the regulation and well fine-tuning of thousands of genes and numerous metabolic pathways to decrease the resulting damage [1, 2].

Various biotic and abiotic environmental factors interfere with the complete genetic potential of crop plant are called stress. Moisture stress occurs when plants are unable to meet evapotranspiration demand. Drought is induced by absence of water due to irregular rainfalls or insufficient irrigation but it can be impaired by other factors like soil salinity and physical properties and high air or soil temperature. Drought is insufficiency of water availability, including precipitation and soil moisture storage capacity, in quantity and supply the life cycle of a crop to restrict the maximum genetic grain yield possibility of the crop.

2. Effects of drought on plant growth and development

Water stress has significant effect on plant cellular processes, growth, development and economic yield. At cellular level, it effects structure of membranes and organelles, hydration and structure of proteins and nucleic acids, pressure differential across the membrane cell wall complex. Water stress is usually measured as leaf water potential since leaves are directly involved with production of assimilates for growth and yield. Osmotic adjustment or Osmoregulation is active accumulation of solutes in the cells during stress period. Different solutes like sugars, fructans and most importantly inorganic ion K^+. Osmotic adjustment is finite and sufficient time is required for solute accumulation.

The development of water stress in the field and its effects on the crop is as follows. Initially, a moderate level of stress develops at which leaf expansion ceases but photosynthesis continues a part of photosynthate is used for osmotic adjustment. Thus plant growth is inhibited by moderate level of stress that reduce cell enlargement, increased root/shoot ratio, leaf area prevention. Osmotic adjustment would occur which will protect cell from extreme desiccation and allow a continued gas exchange. As water stress increases, older leaves senesce to various degrees, reduced leaf area, decline in water use, increased hydraulic resistant, stomata remain open and photosynthesis may continue as long as bulk leaf turgor is maintained in leaves due to osmotic adjustment. The level of water stress in reproductive meristems is lower than that in the transpiring leaves of a plant at any given time. During increased water stress, turgor is lost, stomata close fully, growth ceases, leave roll up, gas exchange drops to zero, carbon is lost by respiration, tissue water continues to decrease slowly, plant enters the pre lethal, non-reproductive stage of survival, leaves temperature increases to lethal levels, and the meristem dies then plant is considered as died. Time scale of plant processes which may influence their drought tolerance includes turnover of some proteins, stomatal movement within minutes of stress. In hours, production of heat shock proteins (hsp) or dehydrins, leaf movement, wilting, osmotic adjustment response to ABA. Cellular hardening, induction of housekeeping genes, floral initiation in 1–2 days of stress.

3. Drought resistance

Drought resistance is mechanisms causing minimum loss of yield in a drought environment. Different mechanisms through which a crop is capable of minimize the loss in yield due to drought stress are grouped in to three categories: drought escape, dehydration avoidance and dehydration tolerance.

3.1 Drought escape

Drought escape describes the situation where drought susceptible variety performs well in a drought environment simply by avoiding the period of drought.

Early maturity is an important attribute of drought escape, suitable for environments subjected to late season drought stress. Early varieties usually contain lesser leaf area index, minor total evapotranspiration and inferior yield potential.

3.2 Dehydration avoidance

It is the ability of a plant to retain a relatively higher level of hydration under conditions of soil or atmospheric water stress. Dehydration avoidance can be achieved either by reducing transpiration (water savers) or increased water uptake (water spenders). The following plant characteristics are responsible for dehydration avoidance. i) Reduced transpiration by closure of their stomata in water saving xerophytic species in response to water deficit well before wilting. In less irrigate plants, stomata possibly will remain open during morning hours and close as solar radiation increases. In CAM plants stomata closes during the night, when CO_2 is fixed by them. ii) Osmotic adjustment is an important mechanism positively affects growth and yield under stress. iii) Increased concentrations of Abscisic acid a stress hormone plays an important role in water stress avoidance by effecting stomata closure, reduction in leaf expansion and promotion of root growth.

Many of stress responsive genes encodes such proteins e.g., dehydrin, osmotin, Lea proteins etc. also produced in response to ABA; genes encoding such proteins are called ABA responsive (ABAR) genes. iv) Deposition of wax within and over the cuticle reduces transpiration during water stress. v) Leaf characteristics like leaf pubescence, erect leaf angle, leaf rolling etc. vi) increased water uptake by deep root system.

3.3 Dehydration tolerance

Dehydration tolerance of a genotype means that a significantly lower level of changes in it than those in another genotype when both of them are subjected to the same level of dehydration. Various measurements of dehydration tolerance are as follows. i) maintenance of membrane integrity by low levels of solute leakage. ii) seedling growth parameters like seedling survival or recovery, seedling growth, seed germination under osmotic stress created by Polyethylene glycol (PEG), stem reserve mobilization, presence of awns in cereals, high levels of proline accumulation. Plant drought response mechanisms and main related traits (**Figure 1**).

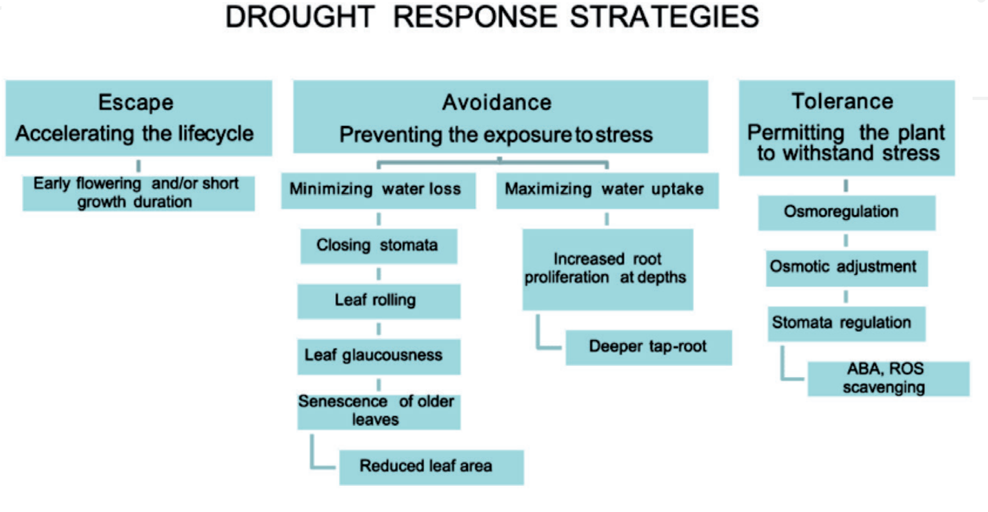

Figure 1.
Plant drought response mechanisms and main related traits.

4. Characterization of drought resistance traits

4.1 Traits associated with drought tolerance

Sl.no	Category	Traits
1.	**Morphological & Anatomical:**	Yield; More Root length, Root Volume, Root Dry Weight, Root Thickness; Root surface area, More Plant Biomass; Harvest index; Leaf drying; Leaf tip firing; Delay in flowering.
2.	**Phenological:**	Early to maturity, Late Flowering; Anthesis, Silking Interval; Seedling vigor; Weed competitiveness; Photosensitivity; perennially.
3.	**Physiological & Biochemical:**	Osmotic Adjustment; Carbon Isotope Discrimination; Stomatal conductance; Remobilization of stem reserves; Specific leaf weight; ABA; Electrolyte leakage; leaf rolling, tip firing, Stay-green; Epicuticular wax; Feed forward response to stress; Heat shock proteins; Cell wall proteins; Leaf water potential; Water use efficiency; Aquaporins; Nitrogen use efficiency; Dehydrins.

Despite many decades of research on drought tolerance in several crops, little progress has been reported in terms of genetic enhancement of crop productivity under water-deficits environments. Breeders and crop physiologists need to work closely in testing the viability/validity of the trait-based approaches for drought tolerance. This has not happened to any great extent previously, but a few success stories have been recently reviewed [3].

Identification of simple to observe morphological and phenological traits, reflective of mechanisms and processes that confer drought tolerance is a priority activity in drought research. An appropriate screening trait for drought stress tolerance should fill the following criteria: (i) a strong link with higher or more stable grain yield in the target stress environment, (ii) a high level of heritability, and (iii) the expression of tolerance must be easily measurable, with adequate replication (**Table 1**).

S. No.	Instruments/techniques used Screening for the purpose of	References
1.	Infrared thermometry used to measure the moisture uptake efficiency	[4]
2.	Banding herbicide metribuzin by deep in the soil, and usage of iodine-131 and hydroponic culture in stress at 15 bar towards growth of the roots	[5, 6]
3.	Adaptation of psychometric process Evaluation of osmotic	[7, 8]
4.	Diffusion porometry method Leaf water conductance	[9]
5.	Mini-rhizotron method Root penetration, supply and thickness in the field	[10]
6.	Infrared aerial photography Dehydration postponement	[11]
7.	Carbon isotope discrimination Improved water-use efficiency	[12]
8.	Drought index measurement Total yield and number of fruits	[13, 14]
9.	Visual scoring or extent Maturity, leaf molding, leaf length, angle, orientation, root morphology and other morphological traits	[2]

Table 1.
Screening procedure for drought tolerance.

5. Breeding approaches for drought resistance

To improve crop at genetic level, plant breeders are usually more concerned in the use of intra specific variation, easily exploitable without any genetic barriers. Intra-specific crosses follow normal Mendelian segregation and selection of F2 at future generations to identify the suitable pant progenies including pure lines. Many breeding methods like mass selection, pure line recurrent selection, methods can be applied, in stress or non-stress conditions besides some other methods like in-vitro selection and usage of somaclonal variations can also be employed.

1. **Mass selection:** Mass selection, the easy method of selection and is used to develop the large population by combining positive or negative mass selection method. Mass selection is employed within populations for characters with high narrow sense heritability. Environment has major influence in mass selection it is a main disadvantage towards development, phenotypical expression of single plants. It also helps in selecting the varieties appropriate for local performance.

2. **Pure Line Selection:** In this method a parent with drought tolerance is crossed with high yielding parent to develop segregating individuals. New combination of alleles obtains due to recombination and segregation of traits. In F2 generation best performing plants are selected in both drought stress and irrigated situations. Seeds from the chosen plants are collected to raise new plant progenies. New plant types are grown to screen best progenies and superior plants among the population to develop pure lines. Disadvantages of this methods very few recombinants are formed F_1 to F_3 generation there is a very less probability to change the genotypes in further generations because homozygosity is achieved nearly 87.5% in F4 generation.

3. **Recurrent Selection:** In this method, the individual plants are selected from original population and are subjected to Progeny testing further the individuals are to cross among each individual in all possible combinations to produce seeds to generate the new base population. The selfing of F_2 plants results heterozygosity, and creates novel genetic variation in each population and generates new recombinants and help to involve promising alleles into a single genotype. Among these individual's gene linkage is slowly removed and new recombination are introduced. The process is repeated for many times and novel genetic recombination's are formed in each cycle of selection. Recurrent selection is widely used to enhance yield ability and resistance to drought. After each cycle precise selection is subjected to increase the genetic variability. Many studies have proven that using recurrent selection in drought increase in gain yield is observed [15].

4. **Manipulation of Somaclonal Variation**: Variations are phenotypic variations occur due epigenetically changes occur in cells derivative from somatic or gametic explants. These variations are usually not desirable because they decrease the homogeneity nature of the restored plants. However, these variations are spontaneous provides the plant breeders to identify the new variations among the individuals among the emerged plants. These variations can be permanent or temporary. The variations which caused due to physiological effects within the plants which are not heritable are temporary in nature. Permanent variations may arise due to mutations, polyploidy, endopolyploidy, chromosomal aberrations.

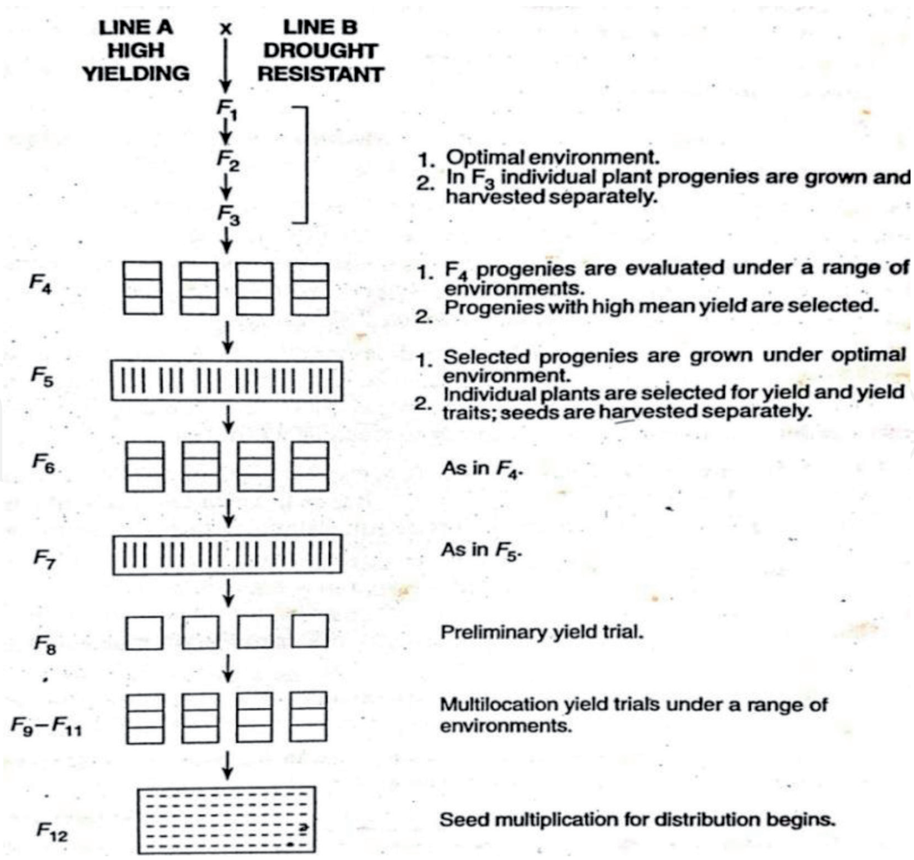

Figure 2.
A breeding approach to develop drought resistant variety for different environmental conditions.

It is significant that drought resistance is combined in to materials with more yield potential. Drought tolerance ability can be seen in improved cultivated, land races, related wild species or varieties developed using molecular breeding techniques. Breeding for drought resistant varieties is difficult to creation drought stress situations and selection of individuals is a difficult task which has to be carried out in rainout shelter conditions.

To identify drought resistance varieties and with high yielding ability both yield and yield related characters are evaluated in both irrigated and water stress conditions. Stability in yield for selected individuals are evaluated over different environments in both irrigated and stress conditions. The breeding approach should be able to combine high yielding with wide adaptability. Selection of individual progenies is selected by considering the average performance over wide range of environmental conditions under stress. The selected individuals are grown under best environments and in progeny selections are supported out for yield and yield characters. The breeding material evolved from breeding environments are lastly assessed in a wide range of environments and those showing high mean performance with high stability may be released for cultivation. A breeding approach to develop drought resistant variety for different environmental conditions (**Figure 2**).

6. Difficulties in drought resistance breeding

1. Identifying drought prone areas in which the variety should be developed is a difficult task because it differs from place to place and time to time.

2. It is difficult to maintain the plant population in controlled conditions like moisture stress and temperature in both field and green house conditions.

3. The plant breeder should use combined selection devices with other useful resources

4. Estimating of all drought resistant characters is a difficult task and not simple.

5. There is an inverse relationship between drought resistant characters and yield potentiality of the crop so breeder has to make additional breeding efforts to increase the yielding ability of the plant.

6. Combined breeding scheme is to be developed by plant breeder to enhance yield and drought resistance characters.

7. Usage of primitive or wild cultivars will be a source of drought resistance will be difficult.

6.1 Drought hardening

Drought hardening refers to an agronomic practice to improve the resistance of genotype to drought as a consequence of seed/seedling treatment. Pre-sowing and post sowing treatments improve drought hardening. In pre-sowing treatments, seed is soaked in water for 24 hr. and dried in sun before they are sown in the main field. In post sowing treatment, a slight moisture stress applied to young seedlings to recover their drought resistance during later stages of growth.

7. Proline content

All plants are capable of detecting and responding to [16, 17]. To overcome the effect of stress, plants have advanced using adaptive mechanisms which may be classified into four categories. Three of these adaptations are developmental traits (e.g., time of flowering), structural traits (e.g. leaf waxiness) and physiological mechanisms (e.g. ability to exclude salt while maintaining the absorption of water and the capacity to sort ions with in vacuoles) involve complex interaction. The fourth one is the metabolic responses such as alteration in photosynthetic metabolism and accumulation of organic osmolytes, most commonly proline. One mechanisms utilized by the plants for overcome the water stress effects might be via accumulation of compatible osmolytes, such as proline and soluble sugars. Production and accumulation of free amino acids, especially proline by plant tissue during drought, salt and water stress is an adaptive response. Proline has been proposed to act as a well-suited solute that regulates the osmotic potential in the cytoplasm. Thus, proline can be used as a metabolic marker in relation to stress. Moreover, under drought stress, the accumulation of total soluble sugars in different plant parts would be increased. However, the rate of additional production or accumulation of proline and soluble sugar is different in different plant parts. Proline content increases in a large variety of plant under stress up to 100 times the normal level, which makes up to 80% of the total amino acid pool. Proline accumulation is maximum during the flowering stage and minimum at vegetative stage. Proline source can be either from the synthesis from glutamate or hydrolysis of proteins. The proline accumulated in response to drought stress or salinity stress in plants is primarily restricted in the cytosol (**Figure 3**) [18–20].

Proline biosynthesis path way undergoes by plant in drought stress condition.

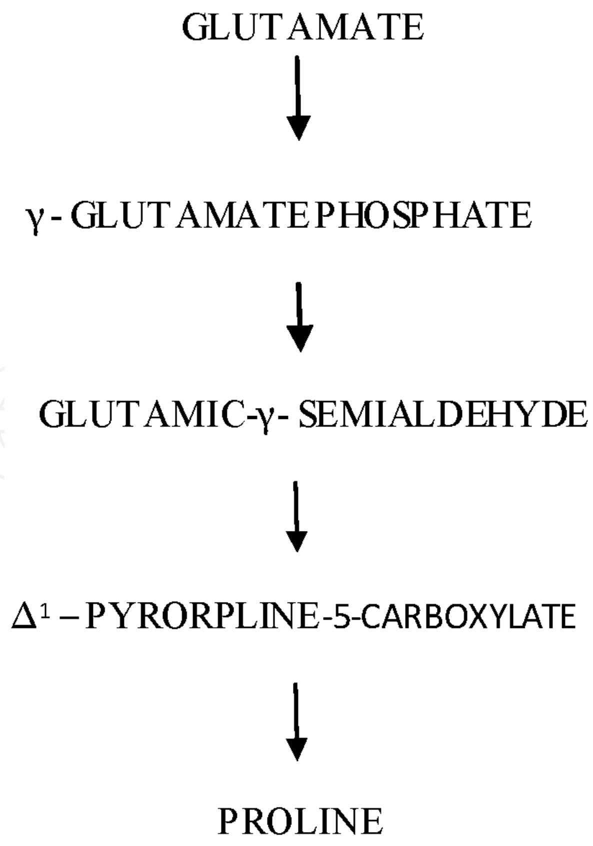

Figure 3.
Flow chart showing proffered rout of proline biosynthesis in plant in stress full condition [21, 22].

7.1 Estimation of proline

Proline content was assessed for the leaves exposed to control and water-stressed conditions. Leaves (100 mg) from control and water-stressed plants were separately standardized in 10 mL of 3% sulphosalicylic acid using mortor and pestle and centrifuged at 5000 rpm for 10 min and the supernatant was collected to estimate the Proline. Ninhydrin (1.25 g) was liquified in 30 mL of glacial acetic acid and then 20 mL of 6 M phosphoric acid was added and kept for 24 h at 40°C. To 2 mL of plant extract, 2 mL of acid Ninhydrin and 2 mL of glacial acetic acid were added and the mixture was boiled at 100°C for 1 h in a water bath. At that time, the solution continued to cool and the reaction was completed. About 4 mL of toluene was added to the contents and mixed vigorously for few sec and OD values for the colored component was restrained at 520 nm using toluene as the blank. From the OD values, proline content (μmoles/g fresh wt.) was calculated individually.

8. Conclusion

Finally, studying all traits related to shoot and root its morphological, physiological, biochemical, phenological, anatomical or responses to environment shows additional opportunities to increase drought resistance in crops. The characters of the plant have to be synchronized with the suitable agronomical practices or better expression of diverse characters. This is a best approach to plant breeding for drought resistance.

Conflicts of interest

The authors have no conflict of interest to declare.

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