BIOTECHNOLOGY AND ABIOTIC STRESS TOLERANCE IN PLANTS

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All biological systems essentially depend on their environment for survival. Animals, being heterotrophs depend on autotrophs i.e. plants for food. Human survival is no exception. We depend on agricultural production for food. In turn, agricultural production is highly influenced by the climatic factors such as temperature, rainfall, light, etc. and edific factors such as nutrients, etc. Plant productivity depends on the availability of these essential components in optimum. Excess or low availability of any of these will make plants feel "stressed". Any change in environmental conditions that may reduce or adversely affect the plant growth and development are called "Abiotic Stress". The stressed plants are adversely affected with regard to growth and development and ultimately yield. All plants do not show same response or vulnerability to abiotic stresses. Some plant species are relatively more tolerant to abiotic stresses. Even within a plant species, some varieties show greater tolerance than others. Different species of plants evolved different mechanisms to overcome or withstand the abiotic stresses. Some plants "escape" stress period by completing their life cycle within short favorable period. They are called "evaders". Some plants avoid stress by adopting themselves for stress period e.g., deep root penetration for water uptake to avoid water stress. They are called "avoiders". However, some plants are able to withstand stress owing to their physiological, biochemical, anatomical, morphological and phenological adaptations. These are called "tolerant" types. The abiotic stresses and their effects on plant growth and development are summarized below.

a) Drought stress : This is the most prevalent abiotic stress which affects the plant growth and crop productivity. In fact, 1/7 of global surface is occupied by deserts. According to FAO, among the cultivated land world wide, only 16% of land is irrigated. In India, about 70% of agricultural land is under rainfed condition. Inspite of the development of irrigation facilities, it is expected that still 60% of land has to remain under rainfed condition. Inadequate water availability for plant growth due to low rainfall is termed "drought stress" and those low rainfall years are called "drought years". The region or a period is considered drought affected if it receives 20% or less of normal rainfall for that area or period. However, more than 50 definitions are in use for drought. From agriculture point of view, drought can be defined as "the inadequacy of water availability, including precipitation and soil moisture storage capacity, in quantity and distribution during the life cycle of the crop to restrict expression of its full genetic potential". Over generations plants developed mechanisms to overcome drought stress. Thus the drought resistance is defined as "the mechanisms causing minimum loss of yield in water deficit environment relative to the maximum yield in a water constraint-free management of crop".

Some crop species like sorghum, castor, millets. etc. are more resistant to drought than others (e.g., wheat, maize, sunflower, etc.). Variability for drought resistance was observed even within a crop species e.g., in wheat C 306, in rice N 22 are drought resistant genotypes.

Drought and plant growth and development : Water stress is the main factor which affects plant growth and development due to drought stress, secondary factor is non uptake of nutrients, essential for various metabolic activities, by roots. Lack of adequate water in cells lead to loss of turgor thus causing closure of stomata which in turn adversely affects the photosynthetic rates. This affects growth in general, decreases root growth, increases respiration and denaturates enzymes and decreases Hill activity. These result in the formation of superoxide radicals which damage the membrane integrity. All these adverse effects on physiology and cell structure of plant leads to poor growth and lower yields.

To overcome water stress plants have certain adaptive mechanisms at different levels i.e., molecular, biochemical, physiological, anatomical, morphological and phenological levels. As mentioned earlier, some plant species complete their life cycle within favorable period and remain dormant till favorable conditions restored. These are called "evaders". Some plant varieties postpone the occurrence of sensitive stage during stress period to overcome it. Some plants have special structural modifications to reduce water loss by having thick cuticle on leaf surface, sunken stomata, rolling of leaves, reduced leaf size, deep root system, etc. These are called "drought avoiders". Some types of plants also possess C4 or crussulacian acid metabolism (CAM) types of photosynthetic pathways to withstand the water deficit arid conditions. Plants which can withstand the water stress are called "drought tolerant" types. These drought tolerant types are of importance in agricultural production point of view. These plants mainly posses the following drought tolerant mechanisms.

1. Osmotic adjustment - in which plants lower osmotic potential by accumulating the compatible solutes like sucrose, mannitol, etc.

2. Antioxidant capacity - to detoxify the active oxygen radicals by certain enzymes like super oxide dismutase, catalase, peroxidase, etc.

3. Desiccation tolerance *per se* - capacity of the cells to survive low leaf water status through mechanisms other than osmotic adjustment and anti oxidant capacity e.g., by accumulating/synthesizing certain drought induced proteins like late embryogenesis (LEA) proteins, etc.

b) Flooding stress: Excess water affects plant growth because i) roots disfunction due to nonavailability of oxygen (anoxia or hyperoxia) and thus the water and mineral uptake is adversely affected. Crop plants are particularly intolerant to flooding (e.g., maize). Aquatic plants adopt to excess water by having large air spaces in their body and they have less developed root system and mechanical tissue. Rice adopted to flooding by having poration in stalk which supplies air to roots. Deep rice withstand flooding in late monsoon by releasing ethylene gas which help in fast growth of plant. This keeps the plants floating. Flood tolerance mechanism also involves decrease in production of ethanol due to anaerobic respiration.

c) Salinity/ alkalinity stress : Excess irrigation without proper drainage facilities cause salinity/ alkalinity. It is estimated that India is losing about 10 thousand hectares annually due to salinity and alkalinity. Effect of excess salts on osmotic pressure of soil solution resulting in reduced availability of water (physiological drought) is called salinity stress. This is caused due to chlorides, sulfates and nitrates of Na, Ca, Mg, K, etc. The dispersive effects of excess exchangeable Na resulting in poor soil physical properties, increase in soil pH and nutritional imbalances causing toxicity of specific ions like Na, B, Mo, etc. are called alkalinity stress. Both salinity and alkalinity stress together are called salt stress. Certain plant species like apple, orange, pulses etc. are sensitive to salt stress whereas rice, sugarcane, sugarbeet, barley are relatively tolerant.

Salt stress causes harmful effects on plant growth and development due to i) dehydration stress ii) toxic action of ions of the salt. Due to this, plant cell membranes are damaged, proteins are denatured (as in case of drought stress) and also plants face toxicity of certain ions leading to accumulation of nitrate, amino acids, amides, ammonia, etc. This leads to damage of cell integrity and metabolism. Due to changes in pH of soil and thus decreased soil microbial activity, plants suffer deficiency of K, P and Ca. All these lead to decreased leaf area, necrosis of leaves and leaf fall, adversely affecting plant growth and development resulting in lower yields.

In nature plants overcome the salt stress by various means. Certain plant species can grow well in presence of high concentration of Na salts. They are called "halophytes" e.g., *Atriplex*. Some plants avoid salt stress by i) excluding the salt passively (root exclusion) ii) extruding the salts actively and diluting the entering salts (Na extrusion by salt glands) and iii) tolerate high concentration of ions in protoplasm and periplasm to avoid concentrating of salts (succulence). Some plants withstand excess salt tolerance through osmotic adjustment by accumulating compatible solutes like glycerol, sucrose, proline, etc. These are strong water structure formers. They also possess Na+ extrusion mechanism and water channels. Ion chaperones like citric and malic acid avoid ions act freely. These plants also have antioxidant capacity by possessing super oxidase dismutase, catalase, peroxidase activities.

d) Temperature stress : Temperature stress may be due to low temperatures (chilling and freezing stress) or due to high temperature.

Low-temperature stress : Stress caused to plants during periods of low but above freezing temperatures is called "chilling stress". Whereas, "freezing stress" is a condition when water inside plant tissue is in the solid frozen state. These cause direct damage to the plant system as low temperatures cause metabolic reduction, membrane lipid solidification and mechanical damage due to ice crystal formation. Another important stress caused is due to water deficit (osmotic stress). They cause oxidative radical formation and damage proteins and membranes. All these adversely affect plant growth and development. Some plants are chilling sensitive e.g., rice, rye, barley, etc.

Plants possess chilling tolerant mechanism by having ability to increase ratio of unsaturated to saturated lipids in cell membranes, thus maintaining cell membrane integrity. Ability to detoxify O_2 free radicals and accumulating compatible solutes and LEA like proteins are some of the mechanisms.

Freezing resistance is the capacity of plants to survive instances or long periods when their internal liquids are in a solid frozen state. Freezing tolerance is acquired by accumulating compatible solutes and other antifreeze proteins. They lower the freezing threshold temperature of cytoplasm. These plants also have capacity to initiate ice formation in intercellular space rather than in cytoplasm and they accumulate protein chaperones which protect proteins from denaturation. Some plants avoid ice formation by having 'supercooling' mechanism (up to –40° C).

High temperature stress : Plants that grow in hot conditions have to face high temperatures some times as high as 70° C e.g., *Tidestromia, Stripa, Carex,* etc. High temperatures damage protein structures and membranes. The enzyme activities are modified. Photoinhibition of photosystems forms active oxygen radicals and also increase photorespiration. Apart from these, at high temperatures plants also suffer dehydration stress.

To overcome high temperature stresses, plants become succulent and some plants adopt crassulacian acid metabolism (CAM). During stress period, plants produce some specific proteins called heat shock proteins (HSPs). These protect essential metabolic enzymes and nucleic acids from heat denaturation. Plants also accumulate compatible solute and possess antioxidant capacity.

e) Ion stress : In soil, mineral and metallic ions are present which provide plants with essential macro and micro nutrients. However, presence of certain metallic ions in higher concentrations cause stress to plants e.g. Cu, Al, Co, Th, Ar, Mn, etc. These metallic ions at toxic level inhibit cell division, cause hormonal imbalances and adversely affect enzyme activity.

Ion resistance is acquired by plants by i) avoidance - complete exclusion of these toxic ions at root zone and/or ii) tolerance to high accumulation of ions by tightly binding with in plant (forming metal chelates) by certain proteins or accumulation of ions in vacuoles or secretion out by specific glands.

f) High light intensity : High light intensities cause photo-oxidation thus inhibiting photosynthesis and low light intensities lower photosynthetic rates due to lack of sufficient light.

Plants tolerate high light intensities by developing mechanisms like thick cuticle (to reflect the light), thick leaves (so that the light intensities at the chloroplast level reduces), modifying the photosynthetic pigment concentrations, possessing antioxidant capacity, etc.

From the brief account on abiotic stress and their impact on plant growth and development it is evident that some plants species or genotypes in a species possess certain advantageous adaptive mechanisms/characters which make them tolerant to stress. In classical plant breeding approach, efforts were made to increase the stress tolerance of high yielding varieties which are susceptible to abiotic stress by breeding with stress tolerant varieties (which are generally low yielders). However, success in this approach was very less due to several limitations like i) this approach can use the genetic variation with in species and rarely intergeneric ii) the selection mainly depends on phenotypic variation (morphological characters) and iii) it is time consuming (5-10 years). To overcome these limitations, a new approach is now followed. This is based on the knowledge that these abiotic stresses induce synthesis of certain plant products (like proteins, etc.) which make the plants to resist or tolerate the stress. It is well known that the synthesis of any new product in biological systems is governed by genes. That means abiotic stresses induce some genes (Table 1). It is also known that the abiotic stresses cause almost similar damages to plants and also that the resistance or tolerance mechanisms involve almost similar responses like production of certain proteins (stress proteins), osmotic adjustment, etc. Moreover in natural conditions two or three abiotic stresses occur simultaneously (e.g. drought stress associated with high temperature stress). However, the degree, nature and time of occurrence of a particular stress itself is highly variable. Uncertainties still persist in the knowledge of causes and effects of many stress resistance mechanisms. The situation is further complicated by the knowledge that most of the abiotic stress resistance mechanisms are controlled by more than two genes (polygenic-quantitative traits). Thus it is a uphill task to make a genotype completely tolerant to abiotic stresses and at the same time high yielder.

Inspite of all these challenges, scientists are making efforts to increase the stress tolerance of a susceptible high yielding variety by genetic transformation (inserting the desirable gene/genes into a plant - 'transgenic'). The genetic transformation is done by the following steps.

⇒ Identification of physiological and biochemical markers which are related to specific stress tolerance.

⇒ Establishing close relationship between these molecular markers and genes encoding them.

⇒ Finding the placement of these genes in genome (QTL - quantitative trait loci for a given trait)

⇒ Cloning desirable gene/genes/isolation of gene/genes.

⇒ Construction of proper vector to carry the gene along with an assayable marker gene into plant.

⇒ Transformation of desirable gene/genes in to the target plant site by suitable mechanism of transformation. There are about ten types of methods by which the gene transformation can be done. Among them *Agrobacterium* mediated transformation method is most successful one and is widely used.

⇒ Regeneration of whole transgenic plant from the transformed cell

⇒ Test for stable integration and expression of gene to confirm heritability.

Molecular genetic maps for some of the chromosomes are already available for some crop plants like rice, wheat, maize, cotton, tomato, potato, tobacco, etc. Another important factor is once a gene is cloned, it can be inserted in any targeted plant. The details about the some of known QTLs for abiotic stress tolerance are listed in Table 2 .

Already some lead is made in inserting the desirable gene/genes into a genotype for specific stress tolerance. Table. 3 gives some of such examples. These transgenics are so far tested essentially under controlled conditions for stress tolerance However, in field conditions, the nature and degree of abiotic stresses are complex. So, it is desirable to have more than one gene to combat stress. In future, the biotechnological research may emphasize on accumulating as many desirable genes as possible in one genotype (*gene pyramiding*) in order to get a much dreamt "Ideotype". Hopefully we may come across in near future a rice or a tomato or for that matter any important food crop plant withstanding all types of abiotic and biotic (pests and diseases) stresses and yielding high at the same time.

Table 1: Typical examples of genes (cDNA) induced by different types of abiotic stresses in higher plants

Type of stress , Genes/ cDNA	Characters/functions	Species
Osmotic stress		
1. Genes coding for LEA proteins Groups 1 to 6: D19,11,7,113,29	Enhanced water binding capacity, Ion sequestration, membrane structure preserves	Cotton, barley, rice, tomato
2. Genes encoding enzymes for osmolyte accumulation		
pVAB2, pProC1	Proline synthesis related enzymes	Mothbean , Soybean
pBAD, BADH, BADH1 & BADH15	Betaine synthesis related enzymes	Barley, spinach, sugarbeat, sorghum
3. Genes encoding enzymes for removing active oxygen		
SOD2, SODCc1	Super oxide dismutase	Pea, rice
Apx 1	Ascorbate peroxidase	Pea
c DNA	Glutathione reductase	Pea
Catl-Cat3 & CAT1-CAT3	Catalase	Maize, *Arabidopsis*
4. Genes encoding water channel proteins 7a, RD 28	Water channel	Pea, *Arabidopsis*
5. Genes whose products have other physiological functions		
PKABA1	Signal transduction (protein kinase)	Wheat
15a	Thiol protease	Pea
6. Genes whose functions are not clear RD 20, Sal T		*Arabidopsis*, rice
Low temperature stress		
1. Lea cor, cap, rab		
2. Antifreeze encoding genes		
3. Proteins related to other stress Osmolyte gene, lipid transfer protein gene	HSP 70, Aldehyde dehydrogenase	
4. Other cold induced genes	Genes related to actin binding protein and signal transduction proteins, etc.	
High temperature stress Genes related to HSPs	HSP17-6	Soybean
Flood stress Genes related to metabolism	Anarobic stress proteins, glycolytic enzymes and sugar phosphate metabolism and transition proteins.	
Mineral stress Genes related to metallothionine like proteins	Chaparones	
HSPs	HSP 70, small HSPs	

Table 2: Some examples of QTLs already identified for abiotic stress tolerance

Type of stress / quantitative character	QTL location in genome	Species
Phenology	3 QTLs : one Flr Avg D1	Sorghum
Drought tolerance		
Leaf ABA accumulation	QTL on long arm of 5A chromosome	Wheat
Osmotic adjustment	QTL on chromosome 7A	Wheat
Stress induced genes	QTL on chromosome 5	Triticeae
Anthesis-silking interval	6 QTLs on 1, 2, 5, 6, 8, 10 chromosomes	Maize
Root morphology	QTL	Rice
Root hairs	QTL	Rice
Root penetration ability	QTL	Rice
Submergence tolerance	QTL for (Sub 1) on chromosome 9	Rice
Salt tolerance	Major gene on chromosome 7 identified 5 genomic regions	Rice Tomato
Thermo tolerance	6 QTLs	Maize
Cold tolerance	QTL	Barley
Frost tolerance	QTL on long arm of 5A chromosome	Wheat
Al toxicity	QTL on chromosome 4D	Wheat

Table 3: Selected examples of transgenic plants for improved stress tolerance

Type of stress	Gene/ protein	Source	Transgenic plant
Drought tolerance	Fructan Sac B Proline P5C synthetase gene LEA (hva1)	*Bacillus subtilis*	Tobacco Tobacco Rice
Salinity tolerance	Mannitol-1-phosphate dehydrogenase (mtl-1D) D1-pyrroline-5-carboxylate synthetase	*E. coli* *V. aconitifolia*	Tobacco Tobacco
High temperature tolerance	Heat shock factor (HSF) (A thsf1)	*Arabidopsis*	Rice
Chilling tolerance	Antifreeze protein (afa 3) Glycerol-3-phosphate acetyl transferase	Chemically synthesized sequence Squash and *Arabidopsis*	Tobacco Tobacco