

BREEDING FOR ABIOTIC STRESS RESISTANCE

DROUGHT RESISTANCE

Drought: Scarcity of moisture (soil moisture) which restricts the expression of full genetic yield potential of a plant.

Drought resistance: The ability of crop plants to grow, develop and reproduce normally under moisture stress.

Mechanisms of drought resistance

There are 4 mechanisms of drought resistance.

Drought Escapes : It is due to ability of a genotype to mature early, before occurrence of drought. Drought escape is most common in plants grown in desert region.

Eg. Early maturing varieties of sorghum, maize, bajra, wheat, rice etc; give more yield than late maturing under drought.

Drought Avoidance (Dehydration avoidance) : It is due to the ability of plants to maintain favourable water balance even under stress. The plants which avoid drought retain high moisture content in their tissues and lose less water. This is possible either because of :

Increased water uptake (due to increase in root development) plants are called water spenders.(or)

Reduced water loss (due to reduction in growth of aerial parts are called water savers (i.e. to avoid transpiration)

Dehydration avoidance is interpreted as the ability of genotypes to maintain high leaf water potential when grown under soil moisture stress: Several traits contribute to dehydration avoidance Such as :

Leaf rolling, folding and reflectance narrow leaves, increased pubescence on aerial organs , presence of awns, osmotic adjustment of stomata, cuticular wax, increased water uptake ;

Reduced Transpiration : Increase in concentration of Abscisic Acid (ABA), closure of stomata, ABA plays role in reduction of leaf expansion, Promotion of root growth etc.

Drought Tolerance (Dehydration tolerance) : Ability of plants to produce higher yield even under 'low water potential'. In cereals drought tolerance generally occurs during reproductive phase. Tolerant cultivars exhibit better germination, seedling growth and photosynthesis. Drought tolerance may be because of

- i. high proline accumulation
- ii. maintenance of membrane integrity

Drought Resistance : It is the sum total of avoidance and Tolerance. It refers to the genetic ability of plants to give good yield under moisture stress conditions.

Various morphological, physiological and biochemical features / parameters associated with drought resistance

Morphological

Earliness

Reduced tillering

Leaf characters :Leaf rolling , Leaf folding, Leaf shedding, Leaf reflectance

Reduced leaf area : Narrow leaf, Change in leaf angle

Hairiness (presence of hairs on leaf and other parts, lowers leaf temperature and reduce transpiration)

Colour of leaves

Wax content

Awns (eg. wheat and barley)

Root system (rooting depth and intensity)

Physiological

Photosynthesis (efficient system like C4) under stress, photosynthetic efficiency is reduced due to chloroplast damage.

Reduced Transpiration and reduced respiration losses

Stomatal behavior (closure of stomata, also change in size and number of stomata)

Osmotic adjustment

Leaf enlargement (increase in thickness)

Leaf cuticle wax (increases)

Biochemical

Accumulation of proline and betaine

Increase in Abscisic acid (barley) and Ethylene (maize & wheat)

Protein synthesis (increases under stress)

Nitrate – reductase activity

Sources of drought resistance

Cultivated varieties

Land (old or desi primitive varieties), Wild relatives (reported in several crops)

For example :

S.No.	Crop	Wild sps	Resistant to
i	Wheat	<i>Aegilops. variabilis</i>	drought
		<i>Aegilops speltoides</i>	"
		<i>Aegilops umbellulata</i>	"
		<i>Aegilops squarrosa</i>	"
ii	Sugarcane	<i>Sacharum. spontaneam</i>	Drought & salinity

4. Transgenes :

Eg. 'Rab' (Responsive to abscisic acid) in

rice Screening / Evaluation

Field Env. Highly desirable

Green house Env. More precisely controlled than field

B reeding Methods and Approaches

It is important that drought resistance be incorporate in material with high genetic potential for yield.

Yield and yield components are best evaluated under non stress / optimal environments, while

Drought resistance must be evaluated under water stress.

Breeding methods : Methods are same as for yield and other economic characters. Breeding for drought resistance refers to breeding for yield under moisture stress, i.e. developing varieties which can give high yields under stress. The common methods are

Introduction

Selection

Hybridization

Mutation

Biotechnology

Limitations :

Generally resistant varieties have low yield

Do not have much wider adaptability (as abiotic resistant is location specific)

Drought resistant genes may have linkage with undesirable genes.

Transfer of resistant genes from wild types may post problem.

Drought resistance is a consequence of a combination of characters and single character can be used for selection.

Measurement of many drought resistant traits is difficult and problematic, since virtually all the useful drought resistant traits are under polygenic control. (So pedigree method most common). But if resistant genes is from agronomically inferior race then 1-2 backcrossing with cultivated type in made. If resistance gene is from wild species-go for backcrossing breeding.

Generally selection is performed on individual plant progenies instead of individual plants (i.e. similar to line breeding)

Creation of controlled moisture stress Environments

Selection require considerable resources

WATER LOGGING

As per Levitt (1980 b) flooding (i.e. water logging) is the presence of water in soil excess of field capacity. It leads to deficiency of O₂ and build up of Co₂, Ethylene and other toxic gases and this leads to reduction in aerobic respiration.

Effects of water logging:

Once soil becomes water logged, air space in soil is displaced with water, the O₂ in the soil in dissolved in water. i.e. O₂ decreases; Co₂ ethylene and other toxic gases increases.

O₂ replacement in the soil is very inefficient. Diffusion of atmospheric O₂ into the water logged soils is very inefficient (because of the slow diffusion of atmospheric O₂ to water logged soil).

Root systems are suddenly plunged into an anaerobic condition. This switching from aerobic to anaerobic respiration disrupts root metabolism.

Carbohydrates level get depleted it is due to

Dissipation of metabolism

High water temperature

Low light

Characteristics of plants in response to water logging stress :

Reduced growth / elongation.

Chlorosis, senescence and abscission of lower leaves

Wilting & leaf curling

Hypertrophy (increase in size of organ due to increase in cell size)

Epinasty (downward growth of petioles)

Mechanisms of tolerance:

Adventitious root formation on lower part of stem (close to surface so that O₂ tension is quickly restored after transient water logging) eg. Tomato

Lenticel (i.e. raised pores in the stem of plants) formation

Aerenchyma formation (soft plant tissue contains air spaces found in aquatic plants) in the cortex that provide canal parallel to the axis of the root through which gases can diffuse longitudinally (eg. rice)

Elongation capacity (In rice – best elongation response give 100% recovery from submergence and poorest elongation gives upto 49% recovery)

Scoring for elongation can be done between booting and flowering stage after flooding the crop to varying depths.

In sugarcane, *S. spontaneum* has more tolerance to flooding. Some canes gave upto 70% of their production potential when in continuous flood for 5 months (in an east at canal point Florida, USA)

Ideotype for flooded areas : The postulated ideotype for flooded areas should have the following characteristics.

Capacity to carry out functional activity at low O₂ concentration
(i.e. High cytochrome activity)

Ability for photosynthesis under low light intensity

Capacity to synthesis food rapidly

Regeneration capacity of shoots when damaged by flood

Ability to withstand drought at later growth stage

Deep root system

Narrow, medium long and dark green leaves with high sugar and protein content.

Breeding methods : Same as in other stresses.

BREEDING FOR SALT TOLERANCE

Salt Tolerance: refers to the ability of plants to prevent, reduce or overcome injurious effects of soluble salts present in their root zone.

It is a global problem as saline and alkali soils are found in almost all the countries of the world, more in Semi Arid Tropic (SAT) of world.

Problem of salinity can be overcome by two ways:

Soil reclamation : costly, time consuming and short lived

Resistant varieties : less costly, more effective, long lasting require longer period to develop.

Behavior / characteristics of plants to salt :

Land races more tolerant than High yielding varieties. Tolerant plants varieties are found in salt affected areas

Salt tolerance capacity differs from species to species. Also genetic differences exist among cultivars for their salt tolerance capacity.

Different crop plants show differential response to salinity

Salinity	Crops
a. Highly tolerant crops	Sugarbeet, sunflower, barley (grain), cotton, datepalm, asparagus
b. Moderately Tolerant crops	Barley (Forage), rye, sorghum, wheat, safflower, soybean
c. moderately sensitive	Rice, corn, foxtail millet, cow pea, peanut, sugarcane, tomato, potato, sweet potato, radish, alfalfa, cabbage
d. Extremely sensitive	Citrus, straw berry, melon, peas, other legumes, apple, rajmabeen, carrot, okra, onion (orange)

Higher ploidy level crops are more tolerant than lower ploidy level

crops. Eg. Hexaploid wheat more tolerant than tetraploid

Tetraploid Brassica more tolerant than diploid Brassica

In rice tall, coarse grained, late maturing varieties- more tolerant

In sugarcane different strains have differential tolerance Barley more tolerant than wheat.

Symptoms of plants to salt stress :

Retardation / cessation of growth

Necrosis

Leaf abscission

Loss of turgor

Ultimate death of plant

Mechanisms of salt tolerance :

2 types of mechanisms

Salt Tolerance : Plants respond to salinity stress by accumulating salt, generally in their cells or glands and roots etc.

Salt avoidance : plants avoid salt stress by maintaining their cell salt concentration unchanged either by water absorption eg. Rice, chenopodiaceae family or by salt exclusion eg. Tomato, soybean, citrus, wheat grass

Glycophytes (Non-halophytes) plants owe their resistance primarily to avoidance. Eg. Barley

Halophytes (plants that grew in salty or alkaline soils) show tolerance by ion accumulation mechanism

Breeding methods

Breeding methods are same but breeding strategies are

Breeding for yield potential should have greater emphasis than breeding for salt resistance per se (As screening is done on the basis of yield reduction in stress environment as compared to non-stress Environment.).

Selection should be done in stresses target environments (As abiotic stress resistance is an important part of Environ. Fitness & is bound to be location specific i.e. it is related to narrow adaptation.

Screening Techniques

Common methods are

1. Sand culture by using nutrient solution in sand & irrigation with saline water
2. Solution culture by using solution culture tanks (Hydroponic culture)
3. Microplot techniques by using small microplots

Microplot Techniques: By using small microplots of size 6 x 3 x 1 m (CSSRI, Karnal, Haryana) at central soil salinity Research Institute.

Then Multilocation Trial (MLT) conducted over seasons to get more reliable results.

Genotypes which survive better under salinity are considered tolerant & tested further.

Selection criteria

Germination (%) in saline medium

Dry matter accumulation (seedling / plant dry wt.) / Early vigour

Leaf senescence or death – Estimated by total dead leaf area or No. of dead leaves

Leaf necrosis

Leaf ion content

Osmoregulation (Determined as maintenance of turgor under stress) Measured as proline or CHO accumulation or accumulation of glycine, betaine etc.

Yield – Economic yield

Problems

Creation of reliable controlled salinity Env.

Scoring for salinity resistance

Genetic control – it is complex & polygenic

Mechanisms of resistance poorly understood. Salinity may have interaction with other stresses.

COLD TOLERANCE

When temperatures remain above-freezing *i.e.* $>0^{\circ}\text{C}$ to $<10^{\circ}\text{C}$ - 15°C it is called chilling. When temperature remain below freezing *i.e.* $<0^{\circ}\text{C}$ it is called Freezing.

A. Chilling Resistance:

Chilling sensitive plants are typically tropical plants. Temperate plants are generally tolerant to chilling injury.

Effects of chilling stress on plants :

- Reduced germination
- Poor seedling establishment
- Stunted growth
- Wilting
- Chlorosis
- Necrosis
- Pollen sterility
- Poor fruit set / seed formation
- Reduced root growth
- Locked open stomata
- ABA accumulation

At subcellular level

- Reduces membrane stability
- Poor chlorophyll synthesis (affected)
- Reduced photosynthesis & respiration
- Toxicity due to H_2O_2 formation

Chilling Tolerance

Ability of some genotypes to survive / perform better under chilling stress than other genotypes is called chilling tolerance. It is because of chilling hardening, *i.e.* an earlier exposure to a near chilling temperature for a specific period as a result of which chilling tolerance of the concerned plants increases.

Mechanisms of chilling tolerance:

- Membrane lipid un-saturation
- Reduced sensitivity of photosynthesis
- Increased chlorophyll accumulation
- Improved germination
- Improved fruit / seed set

6. Pollen fertility

Sources of chilling Tolerance :

Late adopted breeding populations eg. maize

Germplasm (eg. That collected from high altitude , low temperature geographic regions)

Induced mutants for cold tolerance

Cold tolerant somaclonal variants

Related wild species eg. Tomato

Selection criteria

Based on -

Germination test

Growth under stress (measured as plant dry matter accumulation)

Chlorophyll Loss under chilling stress eg. rice, cucumber, tomato (measured as seedling colour)

Membrane stability : (Assayed in terms of solute leakage from tissues)

Photosynthesis : Chilling injury to photosynthesis is assayed as variable chlorophyll fluorescence at 685 nm

Seedling mortality

Seed / Fruit set

Pollen fertility (apply during injury at PMC)

Freezing Resistance

Freezing injury / Frost injury / cryo injury

Freezing Stress : Dormant state is conducive to freezing resistance, while resistance in actively growing tissue is rare : Thus Freezing resistance largely involves surviving freezing stress in such a manner as to enable subsequent regrowth when the temperature rises.

As water in plants cools below 0°C, it may either

1. Freeze *i.e.* form ice or
2. Super cool without forming ice.

Effects of freezing stress

1. Ice formation : Two types
 - Intercellular ice formation
 - Intracellular ice formation

Intercellular Ice formation: Initiation of ice formation on plant surface is sufficient to induce freezing of the internal (intercellular & xylem vessels etc.) water in most plant species.

Intracellular ice formation: It is more lethal may be due to physical disruption of subcellular structure by ice crystals. Intracellular ice formation is the major and terminal freezing stress.

Extracellular ice formation in a cases the concentrations of extracellular solutes, the re by water is withdrawn from the cells during extracellular ice formation. This creates water-stress in the frozen tissue / plant.

Membrane disruptions :

Freezing causes disruptions is and / or alter the semipermeable properties of plasma membrane

Loss of solutes from the cells occur

Cells remain plasmolyzed even after thawing which is often called as frost plasmolysis

Cells may become highly turgid due to uptake of excess water.

SUPERCOOLING :

Cooling of water below 0°C without ice crystal formation is called supercooling

In plants water may cool down to -1 to -15°C in herbaceous sps and to -40 to -45°C in hardy trees.

This becomes possible apparently because internal ice-nucleators are absent in such cases.

This is regarded as an important mechanism of freezing avoidance

Stress due to external factors :

Consequent to freezing

Ice sheet formation below and above the ground causes reserve depletion anoxia etc. in plants.

Tissues killed during freeze-thaw are highly prone to pathogen attacks

Auto toxicity may occur

Mechanism of Freezing Resistance :

The ability of a genotype to survive freezing stress and to recover and re grow after thawing is known as freezing resistance. Freezing resistance is a complex trait involving physiological, chemical & physical processes at the tissue and cell level.

Mechanism of Freezing resistance.

Freezing avoidance : The ability of plant tissues / or genes (but the whole plants) to avoid ice formation at sub zero temperature is called freezing avoidance

Supercooling is a mechanism of freezing avoidance it is controlled by

1. Lack of ice-nucleators
2. Small cell size
3. Little or no intercellular space
4. Low moisture content
5. Barriers against external nucleators
6. Presence of antinucleators

Freezing Tolerance : Ability of plants to survive the stresses generated by extra cellular ice formation and to recover and regrow after thawing is known as freezing tolerance. The various components of freezing tolerance are as follows:

- Osmotic adjustment
- Amount of bound water
- Plasma membrane stability
- Cell wall components properties
- 5) Cold-responsive proteins Eg. ABA

Sources of freezing tolerance

- Cultivated varieties
- Germplasm lines
- Induced Mutations
- Related wild species Eg. Wheat *Agropyron* sps; rye
- Barley – *H. jubatum*, *H. brachyantherum* x *H. bogdanii*, *H. jubatum* x *H. compressum*
- Oats – *Avena sterilis*
- Transgenes : Eg. chemical Synthe sized antifreeze protein gene, ala 3, in tobacco

Selection criteria:

Based on

- Field survival
- Freezing test in laboratory
- Cryo freezing
- Osmoregulation

Problems in breeding for freezing tolerance

Freezing Tolerance is a complex trait & involves several components. So, it is not ready measurable under field conditions

Breeding work under field conditions is highly influenced by other environ factors and biotic stresses

Due to large G X E for the trait field survival shows poor heritability

Freezing tolerance also shows a large GXE interaction which limits progress under selection

