RAMA UNIVERSITY

www.ramauniversity.ac.in

FACULTY OF AGRICULTURE SCIENCES AND ALLIED INDUSTRIES

(Crop Improvement I (Kharif))

For

B.Sc. Ag (Third Year)

Course Instructor

Dr Shiv Prakash Shrivastav

FASAI(Genetics and Plant Breeding)

Rama University, Kanpur

BREEDING FOR BIOTIC STRESS RESISTANCE

DISEASE RESISTANCE

Stress: Constraining influence, force, pressure or adverse conditions for crop growth caused by biological or environmental factors.
Biotic (living): Adverse effects due to pests and diseases
Abiotic (non living): Adverse effects on host due to environmental factors eg: Drought, water logging, heat, cold, salinity, alkalinity and air pollution etc.
Host : Plant effected by a disease or which can accommodate pathogen.
Pathogen : An organism that produces the disease
Disease : an abnormal conditions in the plant caused by an organism (pathogen)
Pathogenicity : The ability of a pathogen to infect a host strain
Virulence : Capacity of a pathogen to incite a disease
Avirulence : The inability of a pathogen to cause or incite a disease
Physiological race: Strains of a single pathogen species with identical or similar morphology but differ in pathogenic capabilities.
Pathotype: Strains of a pathogen classified on the basis of their virulence to known resistance genes present in the host.
Epidemic: Severe and sudden out break of disease beginning from a low level of infection.

Variability in fungal pathogens:

a) **Hybridization:** Recombination of genes of the two parental nuclei takes place in the zygote, and the haploid nuclei or gametes resulting after meiosis are different both from gametes that produced the zygote and from each other.
Thus every diploid pathogen individual is genetically different from any other pathogen even within the same species and variability of the new individual pathogens is continued indefinitely. *e.g., Phytophthora infestans.*
b) **Heterokaryosis:** Condition in which fungal hyphae that are genetically different come together in the same cell to form heterokaryons.
c) **Parasexualism** : Parasexuality – re-assortment of genetic material both in haploid and diploid condition, ready for natural and artificial selection.
Mixtures of races grown together on a susceptible host combine genetically to produce new races e.g. *phytophthora infestans*
d) **Mutation:** The rate at which new variants of a pathogen are produced will depend on the mutation rate of the genes at a particular locus. The mutation rate varies from gene to gene and from pathogen to pathogen. e.g. *Melampsora lini* – new race produced with UV rays (Flor 1956)

e) **Cytoplasmic adaptation:** There are several examples of cytoplasmic inheritance of important characteristics such as growth rate and virulence (Jinks 1966). Virulence of *P.graminis f. sp. Avenae*, carrying gene E, is maternally inherited and may be controlled by single plasma gene (Johnson *et al* 1967).

MECHANISMS OF DISEASE RESISTANCE:

There are different ways of disease resistance *viz.,* disease escape, disease endurance or tolerance disease resistance and immunity.

1. **Disease escape**: The ability of susceptible host plants to avoid attack of disease due to environmental conditions factors, early varieties, charge in the date of plating, change in the site of planting; balanced application of NPK etc.

Eg. Early varietie s of groundnut and potato may escape 'Tikka' and 'Late blight' diseases respectively since they mature before the disease epidemic occurs. Changing planting season in sugarcane from June to October has successfully escaped leaf-rust. Virus free seed potato is produced by sowing the crop in October in Jullundher and other places instead of November, the normal planting time.

2. **Disease endurance or tolerance**: The ability of the plants to tolerate the invasion of the pathogen without showing much damage. This endurance is brought about by the influence of external characters. Generally, tolerance is difficult to measure since it is confounded with partial resistance and disease escape. To estimate tolerance the loss in yield and some other trait of several host varieties having the same amount of disease eg., leaf area covered by disease etc., is compared.

Eg. In Barley the variety Proctor shows 13% yield loss as compared to 20% loss in the varieties Zephy and Sultan.

□□Wheat varieties when fertilized with potash and phosphorus are more tolerant to the rust and mildew infection.

□□The Rice crop fertilized with silicate is resistant to blast infection in Japan.

3. **Disease Resistance**: The ability of plants to withstand, oppose or overcome the attack of pathogens. Resistance is a relative term and it generally refers to any retardation in the development of the attacking pathogen. In case of resistance, disease symptoms to develop and the rate of reproduction is never zero i.e., r? o but it is sufficiently lower than 1 (the rate of reproduction on the susceptible variety) tobe useful. The inhibition of growth of the pathogen is believed to be nutrional in nature and in some cases chemical growth inhibitors may be involved.

Resistance is largely controlled by inherited characters i) may be controlled by single dominant gene in Ottawa 770 B, Newland flax variety, wheat all rusts NP 809

4. **Immunity**: When the host does not show the symptoms of disease it is known as immune reaction. Immunity may result from prevention of the pathogen to reach the appropriate parts of the host e.g. exclusion of spores of ovary infecting fungi by closed

flowering habit of wheat and barley. It is more generally produced by hypersensitive reaction of the host usually immediately after the infection was occurred. In immune reaction the rate of reproduction in zero i.e. $r = 0$

5. **Hypersensitivity:** Immediately after the infection several host cells surrounding the point of infection are so sensitive that they will die. This leads to the death of the pathogen because the rust mycelium cannot grow through the dead cells. This super sensitivity (hypersensitivity) behaves as a resistant response for all practical purposes. Phytoalexins are specific polyphenolic or terpenoid chemicals and are produced by the host in response to the infe ction by a pathogen. More than 30 different phytoalexins have been identified. Phytoalexins are either fungicidal or fungistatic. Eg. Rust fungi and virus attack.

Factors for disease resistance (Causes of Disease resistance)
The disease resistance may be caused due to 1. Morphological, structural and functional characteristics which prevents the entrance of the pathogen *i.e.* prevents the first stage of infection. 2. Biochemical or anatomic al properties of tissue which prevent the establishment of parasitic relationship.

a. Morphological characters
Certain morphological features of the host may prevent infection. Eg. Resistance to Jassid attack in cotton has been shown to be correlated with the hariness of varieties : hairy type resists the attack more, than glabrous types. Failure to germinate rust spores on the leaves of the barley due to waxy coating. Young sugarbeet leaves practically immune to attack of the circos pora because the stomata size is very small.

b. Physiological characters Protoplasmic factors or chemical interactions :
By virtues of its chemical composition the protoplasm may exert an inhibitory influence on the pathogen bringing about the desired resistance in the plant.
Eg. : Resistance of grape to powdery mildew is highly correlated with the acidity of cell sap. Presence of toxic substance in the red pigment in the coloured onions. The outer scales resist the smudge fungus attack when the scales are removed they become susceptible.
c. Anatomical: More secondary thickening of the cell walls of resistant potato varieties which resists the mechanical puncture of the invading *Pythium* pathogen.
d. Nutritional factors : Reduction in growth and in spore production is generally supposed to be due to unfavourable physiological conditions within the host. Most likely a resistant host does not fulfill the nutritional requirements of the pathogen and thereby limits its growth and reproduction.
e. Environmental factors: In addition to the above the environmental factors have marked effect on the pathogen attack. Temperature, moisture, humidity and soil PH and fertility status of the soil effects the pathogen reaction greatly.

Genetic basis of disease resistance

The first study on genetics of disease resistance was done by Biffen in 1905. He reported the inheritance of resistance to leaf rust of wheat variety Rivet in crosses with some susceptible varieties. In F2 there were 3 susceptible : 1 resistant plants indicating that resistance was controlled by a single recessive gene. Most of the earlier studies were conducted without taking into consideration the physiological specialization (pathotype differentiation) of the pathogen which can materially influence the conclusions drawn. It is now recognized that disease resistance may be inherited in three different ways : Oligogenic, Polygenic and Cytoplasmic inheritance.

Oligogenic inheritance:

The disease resistance is governed by one or few major genes and resistance is generally dominant to the susceptible reaction. The action of major resistance genes may be altered by modifying genes in many cases. Eg. bunt resistance in Wheat. Oligogenes generally produce immune reaction. The chief characteristic of the oligogenic disease resistance is pathotypespecificity, i.e. resistant gene is effective against some pathogens, while it is ineffective against the others. In most cases, there are a number of major genes that determines resistance to a particular disease Eg. more than 20 different resistance genes are known for leaf rust of wheat, while those for stem rust resistance exceed 30. The genetics of oligoganic resistance has advanced by two events *viz.,* 1. Discovery of a resistance gene to the prevalent pathotype and 2. Evolution of a pathotype virulent to the new resistance gene. Oligogenic resistance is synonymous to vertical resistance.

Gene for gene hypothesis:

The concept of gene for hypothesis was first developed by Flor in 1956 based on his studies of host pathogen interaction in flax rust caused by *Malampsora lini.* The gene for gene hypothesis states that for each gene controlling resistance in the host, there is a corresponding gene controlling pathogenicity in the pathogen. The resistance of host is governed by dominant genes and virulence of pathogen by recessive genes. The genotype of host and pathogen determine the disease reaction. When genes in host and pathogen match for all the loci, then only the host will show susceptible reaction. If some gene loci remain unmatched, the host will show resistant reaction. Now gene-for -gene relationship has been reported in several other crops like potato, *Sorghum,* wheat etc. The gene for gene hypothesis is known as “Flor Hypothesis”.

Note: Dominant genes in the host are responsible for resistance and recessive genes in the pathogen for virulence.

Vertifolia Effect : Vander plank introduced the term vertifolia effect and refers to epidemic development in a variety carrying vertical resistance genes (oligogenes) leading to heavy economic losses. Total failure of vertical resistance leading to a disease epidemic is known as vertioalia effect. This failure occurs because of two reasons :

1. The level of horizontal resistance in varieties carrying oligogenes is usually low and

2. The pathogen is able to evolve new virulent pathotypes.

Polygenic inheritance

In this type the disease resistance is governed by many genes with small effects and a continuous variation for disease reaction is produced. The genes show additive and non additive effects and the environmental effect is also observed. The polygenic resistance does not show pathotype-specificity as against the oligogenic resistance. It is almost same as horizontal resistance. In some cases the polygenic inheritance may have a oligogenic component, the oligogenes acting in an additive manner eg. bacterial blight resistance in cotton.

Cytoplasmic inheritance :

Resistance in some cases is determined by cytoplasmic genes or plasma gene(s). Eg. The T-male sterilizy cytoplasm (cms-T) in maize is extreamly susceptible to *Helminthosporium* leafblight, while the non-T cytoblasms are resistant to this disease.

Vertical and Horizontal Resistance (Vander plank)

Feature Vertical resistance Horizontal resistance

1. Pathotype – specificity Specific Non specific
2. Nature of gene action Oligogenic Polygenic; rarely oligogenic
3. Response to pathogen Usually, hypersensitive Resistant response
4. Phenotypic expression Qualitative Quantitative
5. Stage of expression Seedling to maturity Expression increases as plant matures
6. Selection and evaluation Relatively easy Difficult
7. Host pathogen interaction Present Absent
8. Commonly used, synonyms Major: gene, race –specific seedling, monogenic, pathotype specific resistance Polygenic, race nonspecific, pathotype-nonspecific, mature plant, adult plant, field uniform resistance
9. Efficiency Highly efficient against specific races Variable, but operates against all races

Sources of Disease Resistance

Resistance to diseases may be obtained from four different sources :

1. A known variety
2. Germplasm collection
3. Related species
4. Through mutations

1. **A known variety:** Disease reactions of most of the cultivated varieties are documented and a breeder may find the resistance he needs in a cultivated variety. Resistant plants were also lated from commercial varieties as in the case of cabbage yellows in cabbage curlytop resistance etc. These provide the basis for new resistance varieties.

2. **Germplasm collection :** When resistance to a new disease or a new pathotype of a disease is not known in a cultivated variety germplasm collection should be screened. Several instances disease resistance were found from the germplasm collections. Eg. resistance to neckblotch in barley resistance to wilt in watermelon.

3. **Related species :** Often the resistance to a disease may be found in related species and transferred through interspecific hybridization. Eg. Resistance to stem, leaf & stripe rusts of wheat.

4. **Mutation :** Resistance to diseases may be obtained through mutation arising spontaneously or induced through mutagenic treatments.

Eg.

1. Resistance to Victoria blight in oats was induced by irradiation with x-rays or thermal neutrons / also produced spontaneously
2. Resistance to stripe rust in wheat
3. Resistance to brown rust in oats
4. Resistance to mildew in barley
5. Resistance to rust in linseed
6. Resistance to tikka leaf spot and stem root in groundnut

Vertical and Horizontal Resistance (Van der plank)

Vertical Resistance is generally d etermined by major genes and is characterized by pathotype specificity. Clearly immune or susceptible response in the case of vertical resistance depends on the presence of virulent pathotype. When virulent pathotype becomes frequent, epidemics are common in the cases of vertical resistance. Thus an avirulent pathotype will produce an immune response i.e. r=0 or close to 0 but the virulent pathotype will lead to susceptible reaction i.e. r=1. It is also known as race specific, pathotype specific or simply specific resistance.

Horizontal Resistance

Race non-specific, pathotype -nonspecific and partial, general or field resistance. Horizontal resistance is generally controlled by polygenes i.e. many genes with small effects and

it is pathotype nonspecific. In this case, the reproduction rate is not zero but it is less than one. Poly genes, govern horizontal resistance.

Methods of Breeding for Disease Resistance

The methods of breeding for disease resistance are essentially same as those used for other agronomic traits. They are :

1. Introduction
2. Selection
3. Hybridization
4. Budding & Grafting
5. Mutation Breeding
6. Biotechnological methods.

1. **Introduction :** Resistant varieties may be introduced for cultivation in a new area.

Eg.

□□Early varieties of groundnut introduced from USA have been resistant to leaf spot (Tikka)

- Kalyanasona and Sonalika wheat varieties originated from segregating material introduced from CIMMYT, Mexico, were rust resistant.
- African bajra introductions have been used in developing downy mildew resistant cms lines.

2. **Selection :** Selection of resistant plants from commercial varieties is easiest method.
Eg.
- Kufri Red potato is selection from Darjeeling Red round
- Pusa Sawani behind (yellow mosaic) selection from a collection obtained from Bihar
- MCU I was selection from CO4 for black arm resistance in cotton

3. **Hybridization :** Transfering disease resistance from one variety or species to the other.
a.Pedigree method is quite suitable for horizontal resistance. Artificial disease epiphytolics are produced to help in selection for disease resistance.
Eg. In wheat Kalyana Sona, Sonalaka, Malvika 12, Malvika 37, Malavika 206, Malavika 234, Laxmi in Cotton (Gadag 1 x CO2) for leaf blight resistance
b. Backcross method is used to transfer resistance genes from an undersirable agronomic variety to a susceptible, widely adoptable and is agronomically highly desirable variety. If the resistant parent is a wholly unadapted variety, backcross method is a logical choice. If resistant variety also possess some good qualities then chose pedigree method of handling segregating material.
4. **Budding & Grafting :** The disease resistance in vegetatively propogated material is transferred by adopting either by budding or grafting. By grafting or budding the resistant material, the resistance can be transferred.
5. **Mutation Breeding :** When adequate resistance is not available in the germplasm ; Mutation breeding is resorted to induce resistance. This is also us ed to break the linkages between desirable resistant genes and other desirable genes.

Precautions

1. The donor parent must possess the required amount of resistance
2. It must be simply inherited without any linkage
3. The recovery in the recipient parent should be more
4. Proper condition for full expression of the resistant genes has to be provided

Advantages with breeding for disease resistance

1. Helps in reducing the losses caused by patogens
2. Reduces the high cost of disease control by chemical treatment
3. Helps to avoid the use of poisonous fungicides
4. Only method available to some specific diseases like viruses, wilt etc.

Limitations

1. Linkage of resistant genes with genes of inferior quality
2. Occurrence of physiological races of varying capacities
3. Self sterility in host plants

Utilization and achievements

1. Rice ADT 10 x Co4 (resistant to blast)
2. Potato *Solanum* tuberosum x *Solanum* demissum (susceptible to late blight) (wild resistant to late blight) F1 backcrossed with *Sol. Tuberosum* Resistant variety

Varieties resistant to different diseases

Rice : Blast Co25, Co26,
Wheat : all three rusts : NP 809
Yellow rust : NP 785, NM86
Black rust : NP 789
Brown rust : NP 783, NP 784
Sugarcane : Red rot Co 419, Co 421, Co 527
Cotton : Wilt Vijay, Kalyan, Suyog
Groundnut : Tikka leafspot Ah 45
Chilli : Mosaic resistant Pusa red, Pusa orange

INSECT RESISTANCE

Global average loss due to insect pests is 14%. Estimated losses in individual crops vary from 5% in wheat to 26.7% in rice and still more in crops like cotton & sugarcane.

Insect Resistance :

1. The ability of a plant to withstand, oppose or overcome the attack of an insect in known as insect resistance.
2. It is the property of a variety or a host crop due to whic h it is attacked by an insect pest to a significantly lower degree than are other varieties of the same host.

Biotypes : Strains of a species of an insect pest, differing in their ability to attack different varieties of the same host species (syn: Physiological races)

Host Habitation :

1. Polyphagy 3. Seasonal Oligophagy
2. Oligophagy 4. Monophagy

1. Polyphagy : Insects feed on a vide range of hosts avoiding few plant species. Eg. Scales & moths.
2. Oligophagy : Live on one taxonomic unit only. Eg. Hessianfly on wheat
3. Seasonal oligophagy : Insects may live on many species in one part of the year and on few in another part of the year. Eg : Aphids.
4. Monophagy : Avoid all hosts except one particular species or variety Eg. Boll weevil on cotton.

Mechanism of Insect Resistance :

Insect resistance is grouped into four categories :

1. Non preference
2. Antibiosis
3. Tolerance
4. Avoidance

1. **Non preference** : Host Varieties exhibiting this type of resistance are unattractive or unsuitable for colonization, oviposition or both by an insect pest. This type of resistance in also termed as non-acceptance and anti-xenosis. Non preference involves various morphological and biochemical features of host plants such as – color, hairness, leaf angle, taste etc.

2. **Antibiosis** : Antibiosis refers to an adverse effect of feeding on a resistant host plant on the development and/or reproduction of the insect pest. In severe cases, it may even lead to the death of the insect pest. Antibiosis may involve morphological, physiological or biochemical features of the host plant; some cases of insect resistance involve a combination of features. Eg. Resistance to BPT is due to antibiosis & non preference.

3. **Tolerance** : An insect tolerant variety is attacked by the insect pest to the same degree as a susceptible variety. But at the same level of infestation, a tolerant variety produces a higher yield than a susceptible variety. Ability of the host plant to withstand the insect

population to a certain extent which might have damaged a more susceptible host. Tolerance is mainly a host character and it may be because of greater recovery from pest damage. Eg. Rice varieties tolerant to stem borer/gall midge produce additional tillers to compensate yield losses (as in stem borer in sorghum) or due to the ability of host to suffer less damage by the pest eg. aphid tolerance in Sugarbeet & Brassica sps. And green bugs tolerance in cereals. Inheritance of tolerance is complex in many cases and is supposed to be governed by polygenes.

4. **Avoidance** : Pest avoidance is the same as disease escape , and as such it is not a case of true resistance Mostly insect avoidance result from the host plants being at a much less susceptible developmental stage when the pest population is at its peak. Eg. 1. Early maturing cotton varieties escape pinkboll worm infestation, which occurs late in the season.

Nature of Insect Resistance / Factors for insect -resistance

Insect resistance may involve :

1. Morphological
2. Physiological (or)
3. Biochemical features of the host plant

1. **Morphological features** : Morphological factors like, hairiness, colour, thickness and toughness of tissues etc. are known to confer insect resistance.

a) Hairiness of leaves is associated with resistance to many insect pests leaf beetle in cereals, in cotton to Jassids , in turnip to turnip aphid.

b) Colour of plant : Color may contribute to non preference in some cases. For example : Red cabbage, Red leaved brussel's sprouts are less favored than green varieties by butterflies and certain Lepidoptera for oviposit ion. Boll worms prefer green cotton plants to red ones.

c) Thickness and Toughness of plant – Tissues prevent mechanical obstruction to feeding and oviposition and thereby lead to non-preference as well as antibiosis.

Eg.

1. Thick leaf lamina in cotton contributes to Jassid resistance
2. Solid stem in wheat confers resistance to wheat stem sawfly
3. Thick and tough rind of cotton bolls makes it difficult for the boll worm larve to bore holes and enter the bolls.

Other characters : also contribute to insect resistance.

Eg. 1. *Gossypium arboretum* varieties with narrow lobed and leathery leaves are more resistant to Jassids than are those with broad lobed and succulent leaves.

2. Cotton varieties with longer pedicels are more resistant to boll worms.

2. **Physiological Factors :** Osmotic concentration of cell sap, various exudates etc; may be associated with insect resistance.

Eg.

1) Leaf hairs of some *solanum sps*. secrete gummy exudates. Aphids and coloradobeetles get trapped in these exudates.
2) Exudates from secondary trichomes of *Medicago disciformis* leaves have antibiotic effects on alfalfa weevil.
3) Cotton- High osmotic concentration of cell sap is associated with Jassid resistance.

3. **Biochemical Factors**: Several biochemical factors are associated with insect resistance in many crops. It is believed that biochemical factors are more important than morphological and physiological factors in conferring non-preference and antibiosis.
Eg.
1) High concentrations of gossypol is associated with resistance in several insect pests in cotton.
2) In rice – high silica content in shoots gives resistance to shoot borer

Genetics of Insect Resistance

Insect resistance is governed by -
1. Oligogenes
2. Polygenes
3. Cytoplasmic genes

1. **Oligogenic Resistance** : Insect resistance is governed by one or few major genes or oligogenes, each gene having a large and identifiable individual effect on resistance. Oligogenic resistance may be conditioned by the dominant or the recessive allele of the concerned gene. The differences between resistant and susceptible plants are generally large and clear-cut. In several cases, resistance is governed by a single gene (monogenic resistance)
Eg. In wheat to green bugs In cotton to Jassids In apple to woolly aphis In rice to plant & leaf hopper.
2. **Polygenic Resistance** : It is governed by several genes, each gene producing a small and usually cumulative effect. Such cases of resistance.
1) Involve more than one feature of the host plant
2) Are much more durable than the cases of oligogenic resistance.
3) Difference between resistance & susceptible plants are not clear cut
4) Transfer of resistance is much more difficult

Examples for polygenic resistance
1) In wheat to cereal leaf beetle
2) In alfalfa to spotted aphid
3) In rice to stem borer
4) In maize to ear worm and leaf aphid Evolution of resistance breaking biotypes is almost rare.

3. **Cytoplasmic Resistance :** governed by plasmagenes
Eg. 1. Resistance to European corn borer in maize

2. Resistance to root aphid in lettuce

Sources of Insect Resistance

1. A cultivated variety
2. Germplasm collections.
3. A related wild species
4. An unrelated organisms

1. Cultivated variety : Resistance to many insect pests may be found among the cultivated varieties of the concerned crop. Varieties SRT 1, Khand waz ; DNJ 286 and B 1007 of *G. hirsuturn* are good sources of resistance to Jassids.

2. Germplasm collection :

Eg.

1) In apples for rosy apple aphid, green apple, apple maker and apple saw-fly.

2) In cotton, several strains resistant to Jassids.

3. Related wild species :

Eg.

1) Resistance to both the species of potato nematodes has been transferred fro *Solanum vernei* to potato

2) Jassid resistances is known in wild relatives of cotton *G. tomentosum; G.anomalum* and *G.armourianum*

4. An unrelated organism : It is done through recombinant DNA technology

a) T he 'Cry' gene of *Bacillus thuringiensis* is the most successful example.

Other genes of importance are the

b) Protease inhibitor encoding genes found in many plants eg. the cowpea pea, trypsin inhibitor (cp TI) gene.

Breeding Methods for Insect Resistance

1. Introduction
2. Selection
3. Hybridization
4. Genetic Engineering

1. Introduction :

Eg. *Phylloxera vertifoliae* resistance grape root-stocks from U.S.A. into france.

2. Selection :

Eg.

1) Resistance to potato leaf hopper

2) Resistance to spotted alfalfa aphid

3. Hybridization : Pedigree oligogenic characters Back cross Polygenic characters

4. Genetic Engineering : *B.theningiensis* (cry gene) resistance in maize, soybean, cotton etc.

Screening Techniques for determining resistance

The most crucial and, perhaps , the most difficult task in breeding for insect resistance is the identification of insect resistant plant during segregation generations. There are two types of screenings.

1. Field Screening
2. Glass house screening

Field Screening :
The techniques designed to promote uniform infestation by an insect pest in the field are
1. Inter planting a row of known susceptible variety between two rows of testing material.
2. Screening in highly prone areas
3. in case Soil insect pests to be tested in sick plots only
4. Testing in a particular season when the infestation is very high. Eg. Rice stem borer in off season.
5. Transferring manually equal number of eggs or larvae to each test plant.

Glass house screening
Result from glass house tests are much more reliable than those from field tests since both the environment and the initial level of infestations are more or less uniform for all the plants being tested.
Problems in Breeding for Insect Resistance :
1. Breeding for resistance to are insect pest may leads to the susceptibility to another pest. Eg. Glabrous strains of cotton are resistant to bollworms but susceptible to Jassids.
2. Reduction is quality or make unfit for consumption.
3. Linkage between desirable & undesirable genes. Inter specific varieties are generally low yielding and their produce is often of inferior quality.
4. Screening for resistance is the most critical and difficult step is a breeding programme it necessitates a close co-ordination among scientists belonging to different disciplines.
5. It is a long term programme.

Achievements
INDIA
1. India – cotton varieties – G 27, MCU 7, LRK 516 – resistant to boll worms.
2. Rice – variety vijaya – resistant to leaf hopper
Rice – TKM 6, Ratna – Stemborer
Rice –Vajram, chaitanya, Pratibha – BPH

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.

1. **Drought Escapes** : It is due to ability of a genotype to mature early, before occurrence of drought. Drought escape is most common is plants grown is desert region.
Eg. Early maturing varieties of sorghum, maize, bajra, wheat, rice etc; give more yield than late maturing under drought.

2. **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 is their tissues and lose less water. This is possible either because of :
i) Increased water uptake (due to increase in root development) plants are called water spenders.(or)
ii) 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, osmatic adjustment of stomata, cuticular wax, increased water uptake ; Reduced Transpiration : Inc rease is concentration of Abscisic Acid (ABA), closure of stomata, ABA plays role in reduction of leaf expansion, Promotion of root growth etc.

3. **Drought Tolerance** (Dehydration tolerance) : Ability of plants to produce higher yield even under 'low water potential'. In cereals drought tolerance generally occur 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

4. **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

a. Morphological
1. Earliness
2. Reduced tillering
3. Leaf characters : Leaf rolling , Leaf folding, Leaf shedding, Leaf reflectance
4. Reduced leaf area : Narrow leaf, Change in leaf angle
5. Hairiness (presence of hairs on leaf and other parts, lowers leaf temperature and reduce transpiration)
6. Colour of leaves
7. Wax content
8. Awns (eg. wheat and barley)
9. Root system (rooting depth and intensity)

b. Physiological
1. Photosynthesis (efficient system like C4) under stress, photosynthetic efficiency is reduced due to chloroplast damage.
2. Reduced Transpiration and reduced respiration losses
3. Stomatal behavior (closure of stomata, also change in size and number of stomata)
4. Osmotic adjustment
5. Leaf enlargement (increase in thickness)
6. Leaf cuticle wax (increases)

c. Biochemical
1. Accumulation of proline and betaine
2. Increase in Abscisic acid (barley) and Ethylene (maize & wheat)
3. Protein synthesis (increases under stress)
4. Nitrate – reductase activity

Sources of drought resistance
1. Cultivated varieties
2. Land (old or desi primitive varieties)
3. Wild relatives (reported in several crops)

For example :
S.No. Crop Wild sps Resistant to
i Wheat *Aegilops. variabilis* drought
Aegilops speltoides "
Aegilops umbellulata "
Aegilops squarrosa "
ii Sugarcane *Sacharum. spontaneam* Drought & salinity
4. Transgenes :

Eg. ‘Rab’ (Responsive to abscisic acid) in rice

Screening / Evaluation

1. Field Env. Highly desirable
2. Green house Env. More precisely controlled than field

Breeding Methods and Approaches

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

1. Yield and yield components are best evaluated under non stress / optimal environments, while
2. 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

1. Introduction
2. Selection
3. Hybridization
4. Mutation
5. Biotechnology

Limitations :

1. Generally resistant varieties have low yield
2. Do not have much wider adaptability (as abiotic resistant is location specific)
3. Drought resistant genes may have linkage with undesirable genes.
4. Transfer of resistant genes from wild types may post problem.
5. Drought resistance is a consequence of a combination of characters and single character can be used for selection.
6. 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)
7. Creation of controlled moisture stress Environments
8. 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:

1. 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.
2. 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).
3. Root systems are suddenly plunged into an anaerobic condition. This switching from aerobic to anaerobic respiration disrupts root metabolism.
4. Carbohydrates level get depleted it is due to

a. Dissipation of metabolism
b. High water temperature
c. Low light

Characteristics of plants in response to water logging stress :

1. Reduced growth / elongation.
2. Chlorosis, senescence and abscission of lower leaves
3. Wilting & leaf curling
4. Hypertrophy (increase in size of organ due to increase in cell size)
5. Epinasty (downward growth of petioles)

Mechanisms of tolerance:

1. Adventitious root formation on lower part of stem (close to surface so that O2 tension is quickly restored after transient water logging) eg. Tomato
2. Lenticel (i.e. raised pores in the stem of plants) formation
3. Aerenchyma formation (soft plant tissue continues air spaces found in acquatic plants) in the cortex that provide canal paralled to the axis of the root through which gases can diffuse longitudinally (eg. rice)
4. 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 characterstics.

1. Capacity to carry out functional activity at low O2 concentration (i.e. High cytochrome activity)
2. Ability for photosynthesis under low light intensity
3. Capacity to synthesis food rapidly
4. Regeneration capacity of shoots when damaged by flood

5. Ability to withstand drought at later growth stage
6. Deep root system
7. 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 fond 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:

1. Soil reclaimation : costly, time consuming and short lived
2. Resistant varieties : less costly, more effective, long lasting require longer period to develop.

Behavior / characteristics of plants to salt :

1. Land races more tolerant than High yielding varieties. Tolerant plants varieties are found is salt affected areas
2. Salt tolerance capacity differs from species to species. Also genetic differences exist among cultivars for their salt tolerance capacity.
3. Different crop plants show differential response to salinity

Salinity Crops

a. Highly tolerant crops Sugarbeat, sunflower, barley (grain), cotton, datepalm, asparagus

b. Moderately Tolerant crops Barley (Forage), rye, soghum, 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, rajmabean, carrot, okra, onion (orange)

4. 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
5. In rice tall, coarse grained, late maturing varieties- more tolerant
6. In sugarcane different strains have differential tolerance Barley more tolerant than wheat.

Symptoms of plants to salt stress :

1. Retardation / cessation of growth
2. Necrosis
3. Leaf abscession
4. Loss of turgor
5. Ultimate death of plant

Mechanisms of salt tolerance :

2 types of mechanisms

1. **Salt Tolerance** : Plants respond to salinity stress by accumulating salt, generally in their cells or glands and roots etc.
2. **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.
Hallophytes (plants that grew in salty or alkaline soils) show tolerance by ion accumulation mechanism.

Breeding methods

Breeding methods are same but breeding strategies are

1. 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.).
2. Selection should be done is 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 nuturient 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

1. Germination (%) is saline medium
2. Dry matter accumulation (seeding / plant dry wt.) / Early vigour
3. Leaf senescence or death – Estimated by total dead leaf area or No. of dead leaves
4. Leaf necrosis
5. Leaf ion content
6. Osmoregulation (Determined as maintenance of turgor under stress) Measured as proline or CHo accumulation or accumulation of glycine, betaine etc.
7. Yield – Economic yield

Problems

1. Creation of reliable controlled salinity Env.
2. Scoring for salinity resistance
3. Genetic control – it is complex & polygenic
4. Mechanisms of resistance poorly understood. Salinity may have interaction with other stresses.

COLD TOLERANCE

When temperatures remain above-freezing *i.e.* >00C to <100-150C it is called chilling
When temperature. remain below freezing *i.e.*<00C 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 :

1. Reduced germination
2. Poor seedling establishment
3. Stunted growth
4. Wilting
5. Chlorosis
6. Necrosis
7. Pollen sterility
8. Poor fruit set / seed formation
9. Reduced root growth
10. Locked open stomato
11. ABA accumulation

At subcellular level

12. Reduces membrane stability
13. Poor chlorophyll synthesis (affected)
14. Reduced photosynthesis & respiration
15. 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 specifie d period as a result of which chilling tolerance of the concerned plants increases.

Mechanisms of chilling tolerance:

1. Membrane lipid un-saturation
2. Reduced sensitivity of photosynthesis
3. Increased chlorophyll accumulation
4. Improved germination
5. Improved fruit / seed set
6. Pollen fertility

Sources of chilling Tolerance :

1. Late adopted breeding populations eg. maize
2. Germplasm (eg. That collected from high altitude , low temperature geographic regions)
3. Induced mutants for cold tolerance
4. Cold tolerant somaclonal variants
5. Related wild species eg. Tomato

Selection criteria

Based on -

1. Germination test
2. Growth under stress (measured as plant dry matter accumulation)
3. Chlorophyll Loss under chilling stress eg. rice, cucumber, tomato (measured as seedling colour)
4. Membrane stability : (Assayed in terms of solute leakage from tissues)
5. Photosynthesis : Chilling injury to photosynthesis is assayed as variable chlorophyll fluorescence at 685 nm
6. Seedling mortality
7. Seed / Fruit set
8. Pollen fertility (apply during injury at PMC)

B. 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 00C, 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 is most plant species.

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

Extracellular ice formation in a cases the conc entrations of extracellular solutes, there by water is withdrawn from the cells during extracellular ice formation. This creates waterstress in the frozen tissue / plant.

2. 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 thawaing which is often called as frost plasmolysis

□□Cells may become highly turiel due to uptake of excess water.

3. Suspercooling :

Cooling of water below 00C without ice crystal formation is called supercooling

In plants water may cooldown to -1 to-150C is herbeceour sps and to -40 to -450C in hardy trees.
This becomes possible apparently because internal ice-nucleators are absence in such cases.
This is regarded as an important. Mechanism of freezing avoidance 4. Stress due to external factors : Consequent to freezing
1) Ice sheet formation below and above the ground causes reserve depletion anoxia etc. in plants.
2) Tissues killed during freeze-thaw are highly prone to pathogen attacks
3) Auto toxicity mary 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.
1.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

2. **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:
1) Osmotic adjustment
2) Amount of bound water
3) Plasma membrane stability
4) Cell wall components properties
5) Cold-responsive proteins Eg. ABA
Sources of freezing tolerance
1. Cultivated varieties
2. Germplasm lines
3. Induced Mutations
4. Related wild species Eg. Wheat *Agropyron* sps; rye
Barley – *H. jubatum, H.brachyantherum x H.bogdanii, H.jubatum x H.compressum*
Oats – Avena sterilis

5. Transgenes : Eg. chemical Synthe sized antifreeze protein gene, ala 3, in tobacco

Selection criteria:

Based on

1. Field survival
2. Freezing test in laboratory
3. Cryo freezing
4. Osmoregulation

Problems in breeding for freezing tolerance

1. Freezing Tolerance is a complex trait & involves several components. So, it is not ready measurable under field conditions
2. Breeding work under field conditions is highly influenced by other environ factors and biotic stresses
3. Due to large G X E for the trait field survival shows poor heritability
4. Freezing tolerance also shows a large GXE interaction which limits progress under selection
5. Laboratory tests are yet to be developed to screen large breeding population