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Abiotic stress management in herbaceous crops using breeding and biotechnology approaches

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Abstract
In the face of a global scarcity of resources, abiotic stresses like water stress, temperature stress, mineral stress, oxidation stress are major limiting factors in plant growth and development. Vegetable crops are generally sensitive to environmental extremes, and thus high temperatures and limited soil moisture are the major causes of low yields in the tropics and will be further magnified by climate change. The response of plants to environmental stresses depends on the plant developmental stage and the length and severity of the stress. Plants may respond similarly to avoid one or more stresses through morphological or biochemical mechanisms (Capiati *et al.,* 2006). Adapting agri-horti system to future conditions is essential to meet the need of the growing population and increasing demand for vegetables, and other horticultural products. This enormous and difficult task requires tremendous research efforts from multiple disciplines. Stress physiology studies identify mechanisms of stress tolerance and provide an approach, method, and traits for screening stress-resistant genotypes. Molecular biology and genomic investigations lead to a better understanding of the structural organization and functional properties of genetic variation for stress-related traits, allow gene-based selection through identification of molecular markers and high-throughput genotyping techniques, and increase the gene pool available, including new sources of stress-tolerant traits or transgenes. The genetically complex responses to abiotic stresses are multigenic and thus more difficult to control and engineer. Plant engineering strategies for abiotic stress tolerance rely on the expression of genes that are involved in signaling and regulatory pathways (Seki *et al*., 2003) or genes that encode proteins conferring stress tolerance or enzymes present in pathways leading to the synthesis of functional and structural metabolites (Wang *et al.,* 2004). Plant breeders need to translate these findings into stress-tolerant crop varieties by using all tools available that include germplasm screening, marker-assisted selection, plant transformation, and conventional breeding methods.

Keywords: abiotic stress, breeding, molecular biology, tolerance and mechanism

Introduction

Phenotypic performance of a plant/ line/ population is determined by genotype, environment and genotype x environment interaction. The environment of an individual may be simply defined as the sum total of all the factors other than the individual concerned. The various factors of environment are called biotic and abiotic depending upon their biological/ nonbiological nature. In an optimal environment there is no interference by any environmental factor with the complete expression of genotypic potential of a plant/ line; such an environment is therefore termed as stress free environment. When some factors of the environment interferes with the complete expression of genotypic potential or significant deviation from the ideal conditions, preventing plants from expressing their full genetic potential for growth, development, and reproduction is termed as stress (Rehman *et al.,* 2005) [19]. Stresses are also classified as biotic (pathogens, pests, weeds) and abiotic (environmental factors). Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment. The non-living variable must influence the environment beyond its normal range of variation to adversely affect the population performance or individual physiology in a significant way (Vinebrooke *et al*., 2004) [27].

Characteristics of abiotic stresses

The relative importance of different abiotic stresses is mainly region/ location specific. The occurrence and the degree of some of the stresses are unpredictable e.g. drought. A given abiotic stress may increase/ decrease the level of another abiotic stress e.g. in a saline soil, moisture stress would enhance salinity stress. Different crop varieties show large differences in their abilities to tolerate abiotic stress. Different growth stages of crops may show marked differences in their tolerance to an abiotic stress. Stress during the reproductive phase of a crop causes far more economic loss than comparable stress during the earlier phases or vegetative phases. The effects generated by one abiotic stress may overlap some of those generated by other e.g. salinity stress generates some features produced by drought stress.

Plant responses to stress

Abiotic stress may trigger a series of responses in plants that include changes in gene expression and cellular metabolism. The duration, severity and frequency with which a stress is imposed, and the affected organs and tissues, developmental stage, and genotype also influence plant responses to stress. Consequently a combination of different conditions can cause different plants responses to the same type of stress. There are three types of stress resistance mechanisms occurs in plants viz. Avoidance mechanisms: These prevent exposure to stress. Tolerance mechanisms: These permit the plant to withstand stress. Acclimation: Plants alter their physiology in response stress.

1. Water Stress

Among the environmental variables affecting plant growth and development water stress is one of the most important. A water stress may arise either from an insufficient water or drought stress or from excessive water activity or water logging.

2. Temperature Stress

Temperature is basic to life processes, which increase with temperature within a limited range. This effect is expressed as Q_{10}, which is the ratio of the rate of a biochemical process at one temperature to that at a temperature 10^0 C lower. When temperature rises beyond the upper limit of the range i.e., it goes above the optimal temperature, the relation between life processes and temperature is disturbed. Similarly, when the temperature goes below a threshold, which is often close to zero, life processes are disturbed enough to cause injury and death in sensitive genotypes. Each plant species, more particularly each genotype, has an optimal range of temperature for its normal growth and development; the specific temperatures would depend not only on the genotype but also on the stage of growth and development of the given genotype. When temperature moves beyond this optimal range, it generates temperature stress, i.e., temperature interferes with the performance. Temperature stress may be grouped into the following three categories heat stress, chilling stress and freezing stress.

3. Salinity Stress

Salt stress has become an ever increasing threat to food production. It is a major factor limiting the crop productivity. Increased salinization of arable lands is expected to have a devastating global effect, resulting in 30% land losses within the next 25 years and up to 50% by the year 2050 (Wang *et al*., 2003) [28]. Soluble salts can cause harm to plant, if they are in high concentration in water or soils. Generally an array of stresses interplay in saline soils and reduces productivity. Salt affected soils are mainly of two types: saline and alkaline.

Genetics of Tolerance

- **Drought tolerance:** The performance of two bean populations, consisting of 78 and 95 recombinant inbred lines from the crosses Sierra/ AC-1028 and Sierra/lef-2RB, was examined under moisture stress and non-stress condition regimes. Two genotypes from each population yielded in the top 10% under both stress and non-stress conditions. Heritability estimates for yield in Sierra/ AC-1028 population, based on five years of data, averaged 0.55 and 0.59, respectively for Sierra/Lef-2RB population the corresponding values were 0.20 and 0.19. Heritability for plant biomass was 0.52 for stress and 0.55 for non-stress in the Sierra/Lef-2RB population. Hundred seed weight was the highly heritable trait in both the populations, with heritability estimates of 0.80 for the Sierra/AC-1028 population and 0.65 for the Sierra/Lef-2RB population. Generally leaf characters like waxy bloom, glossiness, glaucousness, and glabrous leaves are under oligogenic control. Some other traits like ABA accumulation in wheat, constitutive proline accumulation in *Brassica* and resistant to flower abscission and ability to support pod formation in rajma seems to be determined by oligogenes (Singh, 2003) [25].
- **Waterlogging tolerance:** Boru (1996) proposed that different genes could relate to different mechanisms of tolerance to waterlogging, therefore waterlogging tolerance could be substantially improved by combining all tolerance genes into one genotype. Cao *et al*. (1994) [2] also indicate that additive gene action is the major determinant of inheritance of waterlogging tolerance.
- **Heat tolerance:** The heat tolerance trait in the interspecific somatic and sexual hybrids between cabbage (*B. oleraceae var. capitata)* x Chinese cabbage *(B.campestris. var peknensis),* and between Chinese kale (*B. oleracae var alboglabra)* x Chinese cabbage was intermediate between of the parents (Hossian *et al.*, 1995). Leaf conductivity was used in common bean to study the inheritance of heat tolerance in the three crosses involving two heat tolerant parents; P.I, 271998 and 5BP-7 (Marsh *et al.*, 1985) [15]. In one cross, both additive and dominance effects were significant. In a second cross, only additive effects were important, and in third cross, additive, dominance and epistatic effects were important. The quantification of high temperature tolerance and the characterization of its genetic control are necessary for germplasm enhancement efforts. Saulter *et al.* (1990) [22-23] found that the ethylene evaluation rate is genetically controlled, with additive-dominance and epistatic effects present or ethylene evaluation rate. Ethylene production of bean plant increases under heat environment. The heat tolerance in cowpea was conferred by a recessive gene during floral bud development and by a dominant gene during pollen and anther development (Hall, 1990) [10].
- **Cold tolerance:** Narrow sense heritability's were 28, 56, 45, and 74 %, respectively for inhibition at 5oC and at 16oC, for seedling vigour, plant vigour, and days to bloom in a cross of NY 590 x BBL 92. Cold tolerance at these stages was inherited independently. Pod set at 16oC behaved as a recessive compared to setting at warmer temperatures. Bean lines with good pod and plant type have been bred with the ability to germinate at 8-100 ^{0}C (Dickson, 1971) [4]. Heritability for germination at low temperature was about 35%. No specific segregation pattern was detectable. Additive dominance and reciprocal effects were observed, with the predominance of the additive effects (Melo *et al.*, 1997) [17]. Inheritance

of several traits involved in chilling tolerance has been studied in some crops like tomato, rice etc. Electrolyte leakage following chilling, and germination, seedling vigour, floret sterility and plant growth under chilling stress are all under polygenic control; only leaf discoloration under chilling are governed oligogenically in case of rice. Electrolyte leakage after chilling stress was reported to reflect the growth under low temperature of different genotypes. Heritability estimates for germination were high in tomato and cucumber. In, case of broccoli, Brassica oleraceae, the genetic evidence suggested that two dominant, epistatic genes conditioned freezing tolerance (Bouwkamp and Honma, 1969).

- **Salt tolerance:** Lyon (1941) [14] reported that *Lycopersicon pimpinellifolium* was less sensitive than L.*esculentum* to Na2SO4 and F1 hybrids between these two species were identical in salt tolerance to the sensitive parent. Single major gene controlled the presence or absence of tolerance. Rush and Epstein (1981) made the crosses between a salt tolerant wild tomato (*L.cheesmani*) and a domestic cultivar (*L. esculentum*). Selections were made from resulting progenies for salinity tolerance at germination, seedling establishment and reproductive stage of their cycle. The selected progenies were tested for survival and fruit production in salinized solution culture experiments and in field greenhouse trails where they were irrigated with various dilutions of sea water applied to sand. Salt tolerance was shown to be heritable trait. Plants selected from the F2 and successive backcrosses to cultivar Walter survived and produced fruit when irrigated with up to 70% sea water in the sandy soil culture trails, whereas cultivar Walter did not survive. The choice of breeding approaches and methods may depend on the following considerations:
- The method of reproduction or mode of pollination (self-pollinated vs. cross pollinated, vegetative or sexual, tolerance to inbreeding, seed increase method etc.
- The mode of gene action (multigenic vs. monogenic, dominant vs. recessive, heterosis, epistasis etc.)
- What is the effect of the abiotic stress on the crop to be improved?
- What germplasm is available that contains the necessary genetic variation to initiate improvement?
- What breeding scheme will be used to facilitate improvement?
- What will be the specific goals of the breeding effort?
- Priority assigned to goal in relation to the other agronomic traits (biotic stress, quality factors etc.).

Breeding Approaches

i. Direct (empirical or agronomic approach): It is the selection for absolute performance (growth rate or yield), under actual stress or selection for only a small reduction in performance under stress. A superior variety at the potential level will also yield relatively well under sub potential levels e.g., tolerance to drought may be present in such a variety which is expressed as an unidentified component of stability in performance over various locations. In the process of breeding, yield and stability are tackled as one complex. The accumulation of environmentally stable yield genes equates with better performance under stress situations (Fischer *et al*., 1982) [9]. Testing over a large number of sites with varying moisture availabilities, although experience, should enable the elimination of those genotypes which may have yield negative traits under stress.

ii. Indirect (the physiological or analytical) approach: This approach to breeding for yield performance in a stress environment maintains that potential yield is irrelevant. Varieties must be selected, developed and tested under the relevant conditions. It implies screening and selection for morphological or physiological characteristics that may be correlated with or that contribute to stress resistance. Specific traits that enable the plant to better withstand stress are identified and introduced into superior genetic background.

Methods of Breeding

1. Classical methods: The classical approach uses the genetic variation already available and also uses sexual cycle to recombine DNA through independent assortment of chromosomes and through crossing-over. Induced mutations are also now a part of classical breeding approaches. In any of these approaches emphasis is on the selection of individual plants and their progenies. Therefore, it is imperative to know about suitable selection criteria, which differ for each species and stress. Some of these are:

i. Development of suitable selection criteria: Selection for abiotic stress resistance is difficult because of unpredictability of climatic conditions. The salt affected regions are typically very patchy in their salinity and areas with no salinity as well as areas with high salinity are present in the same field. This patchiness may occur over distances of less than a meter horizontally or over much large distances. It is caused by variation in perched water tables and by undulations in the landscape. As a result of this patchiness and because of ease with which plants can be grown in uniform salinized nutrient solution, many selections have been done in nutrient solution. Selections in nutrient solution may not perform well in saline soils because of the patchy field environment is altogether different from that of salinized solution. The stresses such as drought, waterlogging, freezing etc. are sporadic and rare events, therefore it is difficult to make a direct selection for stress tolerance in the field. Though the root characteristics have been suggested as criteria no convenient methods are available to evaluate for root characteristics for breeding purposes. The possibility of evaluating for root and height characters at seedling may be explored in the crops which are transplanted. An alternative to selection in stressed environments is to adopt indices based on morphological, biochemical and physiological traits associated with tolerance (Blum, 1988) [1].

Table 1: Selection of plants according their genotype and phenotypic for various stress.

Characteristic	Measurement	Selection criteria
Yield potential	Screening in artificially created environments.	Genotypes performing well in such environments can be selected.
Leaf rolling	Visually scored.	High scored genotype is selected. Mainly done in drought stress.
Water retention	Measured as dehydration rate.	Genotypes with high water retention can be selected
Membrane stability	Conductometer	Plants with solute leakage should not be selected.

Leaf necrosis and death.	Visual evaluation	Mineral stress causes necrosis and death.
Pollen tube growth, pollen fertility.	Pollen grain screening.	Pollen tube growth rate is a selection criteria used in salt stress. Meiotic stage of pollen mother cell is very sensitive to chilling therefore if genotype shows tolerance it can be selected.
Chlorophyll content	SPAD.	Used especially in cases of water logging, cold stress e.g. Tomato, Cucumber.
Sensitivity of reproductive phase	Flower/ pod/ fruit/ seed production.	Plants tolerant during reproductive phase should be selected.
Root colour and shape	Root decolourization and deformation.	Aluminum toxicity sensitive plants produce deformed and decolorized roots.
Root length	Relative root length	Deep root system is a identified as a target for selection especially in case of drought stress, mineral stress.
Germination percentage	Germination test	Seedling that emerge and establish first escape stress.
Photosynthetic rate	Respirometer infrared gas analyzer.	Plants with high photosynthetic rate can be selected.

The selection criteria primarily depends on morphological characters which would be used by plant breeders for the selection of parents as well as desirable segregates followed by hybridization are desirable. During selection characters with high heritability's and high correlation with yield under stress across the locations/ environments may be preferred. Monte *et al*. (1993) [18] working on peas, used laboratory screening for freezing stress tolerance and at the same time the selection in the field for quality and yield and other agronomic traits related to production. This dual selection approach would risk that selection in the laboratory may have effect on other positive agronomic traits.

ii. Hybridization

a. Interspecific hybridization: If the level of tolerance in the agronomically improved cultivars is not adequate, the collections of the crop species may be screened for sources of desired variability. This variability may be used in the hybridization of the on-going programme. After hybridization the segregating populations can be handled using one of the following methods:

Pedigree and bulk-pedigree selection: In this method one parent is chosen for genetic properties that are useful while other is chosen for its complementing and desirable agronomic characteristics. Selection for plants with desired combination of characters is started in the F2 generation and the progenies of the selected F2 plants are reselected in succeeding generations until genetic purity is reached. It is more useful for simply inherited traits with high penetrance and heritability. A disadvantage of the system is that early generations can be expected to be heterogeneous and quite unstable in their response to environmental interactions. In bulk method, selection is delayed until a later generation, commonly the F6-F8. This is to reduce environmental effects because at this time segregation will have virtually ceased. The increased homozygosity of these generations will increase the correspondence between genotype and phenotype. The progenies may be evaluated against check which are planted after few or alternatively after each row in the segregating generation. When the lines/ progenies are partially fixed, these may be evaluated either through the use of moving average method or augmented design.

Backcross method: The method is a form of recurrent hybridization by which a gene for superior characteristics may be added to an otherwise desirable variety. One parent is adapted variety which lacks a gene for superior characteristics that is present in a second variety. Beginning in the F1 the hybrid is successively backcrossed to the adapted parent variety for several generations. The purpose of this method is to recover the genotype of the recurrent parent, except for the addition of a gene for the superior characteristic which comes from non-recurrent parent. The tolerant and sensitive parents may be grown along with in each generation. The growth of inbred lines derived from cross of the phosphorous efficient donor parent and the recurrent parent in common bean was measured in low phosphorous nutrient solution culture and in afield nursery on a soil moderately deficit in phosphorous Several inbred backcross lines that resembled recurrent parent in general morphology were identified as phosphorous efficient in the nutrient solution culture. In general these lines accumulated 30-50% more shoots dry weight and more phosphorous in the shoot tissue at first flower than recurrent parent in the low phosphorous field but did not differ from it in a field plot amended with phosphorous fertilizer. Transfer of quantitative trait through inbred backcross method proved to be useful for developing bean germplasm tolerant to soils low in available phosphorous.

Mass selection/recurrent selection: The mass/ recurrent selections are important in accumulating genes for tolerance/ resistance in the populations/ genotypes against abiotic stress. A mass selection involving relatively controlled sib crosses between identify elite populations from test crosses. The stress tests could be applied at a level that will eliminate 50% individuals. The survivors can be sib-crossed in as feasible. Seed from resulting F1 hybrids can be bulked to form the F2 bulk population for the next cycle of selection. The remnant seed from each cycle could be increased for use in breeding programme and to assess genetic gain for stress tolerance.

b. Interspecific hybridization: If the genes for stress tolerance are not available in cultivated types, these can be transferred from the wild relatives/species which are the rich source of stress tolerant genes. The commercial cultivars could be improved by the transfer of genes for abiotic stresses from their wild relatives and their co-specific subspecies e.g., salt tolerance from *Lycopersicon cheesmani* to *L. esculentum* (Epstein *et al.*, 1980) [7]. Tepary bean is most commonly suggested source of drought tolerance, although other *Phaseolus* species merit consideration also. From interspecific hybridization between *Pisum sativum and P. fulvum*, Drozd (1981) [6] developed two promising lines with heat and drought tolerance.

2. Mutation breeding: Mutagenesis may be employed in an effect to produce the desired variability in cases where such variation is not readily available. Large number of mutant varieties have been developed from crosses using mutants as one of the parents and also several cultivars have been developed from the parent variety using induced mutation e.g. in *Solanum melongena* variety PKM-1 by mutagen gamma rays for resistance to drought is reported.

3. Using models to develop stress resistant varieties: The need for development of genotypes for target areas can be done through use of models. Loomis *et al. (*1971) [13] defined a model as a summary of a coherent body of experimental data in a logical structure that may be a causal or an associative nature. The data used are obtained from interrelated phenomena and consequently are coherent, i.e., connected by common principles. A simple descriptive model from a few controlled environments was developed and this model was used for prediction of response in other natural environments. The response of six flowering genotypes to a wide range of photo thermal conditions was studied in the controlled environment by Summerfield *et al.* (1985) [26]. The flowering response was found to be governed by both the temperature and the photoperiod is described below: 1/f = a + selected genotypes with in a genetically diverse population may be effective. It may be possible to b T + CP, where f is the time, T is the mean temperature, P is the mean photoperiod during that period and a,b,c are the genotypic constants.

Constraints of coventional breeding

- Long time trails
- High cost
- Transfer of undesirable genes.
- Distant Hybridization barriers
- Inbreeding depression in cross pollinated plants
- Ineffective phenotypic selection
- Difficult to produce viral resistant plants in case of clones.
- Not possible to transfer a gene from micro organisms
- Ineffective diagnosis of diseases

Biotechnological approaches or non-conventional approaches

Reiger *et al.* (1976) [20] defined genetic engineering as genetic manipulations by which an individual having a new combination of inherited properties is established. Two major approaches to genetic engineering were specified cellular approach and molecular approach.

a. The cell/tissue culture approach: The tissue culture is a cyclic procedure whereby types of hormones are incorporated into a medium and their concentrations can be modified to force either differentiated or undifferentiated growth. Differentiated growth involves the production of either shoots or roots, while undifferentiated growth is the production of a timorous mass of cells called callus. *In vitro* screening for drought tolerance in sweet potato cultivated Lizixiang was subjected by Wang *et al.* (2003) [28]. Embrogenic suspension cultures were subjected to stress medium (MS) containing 0-35 % PEG6000. Their result indicated that 30% PEG6000 can be used for the potential selection for drought stress. Dobranski *et al.* (2003) [5] studied the effects of manitol on different genotypes of potato in callus and plantlet culture. In callus-test, the relative increase of callus mass was a useful parameter for the determination of osmotic tolerance of genotypes at cellular level. Tissue culture techniques for screening germplasm for heat tolerance have been used by many workers.

b. Molecular approach or genetic marker based approach: Traditionally morphological traits controlled by a single locus have been used as genetic markers by the plant breeders. Different selection criteria developed through use of co-dominant morphological markers and used for the improvement of abiotic stress resistance e.g., in Chinese cabbage thick leaves, high levels of electrolytes, high chlorophyll content, few stomata, and vigorous root growth are related to heat tolerance. Although morphological markers have been used as predictors of genetic response to selection, they are not useful for selection based on root traits which are crucial in drought and heat tolerance. For the improvement of these, biochemical markers or DNA markers may be of greater use. Isozymes are differently charged protein molecules that can be separated using electrophoretic procedures. Since enzymes catalyze specific reactions, it is possible to visualize the location of a particular enzyme on a gel by supplying the appropriate substrate and cofactors, and involving the product of the enzymatic reaction in a colour-producing reaction. Bands visualized from specific enzymes represent protein products, have a genetic basis and can provide genetic information as co dominant markers. In case of DNA markers superior genes can be selected if molecular marker is closely linked to the gene of interest. The selection based on molecular markers is known as marker assisted selection. Since most of the morphological and physiological traits associated with abiotic stress resistance such as root development or osmotic adjustment are difficult to manipulate using conventional screening methods. The recombinant DNA technology can be successfully applied to crop improvement for stress tolerance only when biochemical events which help the plant with the stress conditions have been identified.

c. Marker assisted selection: The selection based on molecular markers is known as marker assisted selection. The marker assisted selection helps in overcoming the difficulties associated with low heritability, recessiveness and difficult screening assays during the gene transfer and selection. Understanding of genetic bases of heat tolerance to high temperature is important for improving the productivity of crop plants in regions of heat stress. Molecular markers help in locating position of gene on chromosomes which can be used in improvement of the crop for desirable characters. Recombinant inbred lines of Chinese cabbage were analyzed using Isozyme, RAPD, and AFLP techniques to find molecular markers that are linked to heat tolerance QTL (Zheng *et al.*, 2002, 2004) [31-32]. They located nine molecular markers closely linked with heat tolerance QTL including 5 AFLP markers, 3RAPD markers and 1 PGM Isozyme markers. The 9 heat tolerance linked markers distributed in 5 independent occasions in the genome of Chinese cabbage. In tomato, genetic resources from salt tolerance have been identified largely within the related wild species, and considerable efforts have been made to characterize the genetic control of tolerance at various developmental stages. The inheritance of several tolerance related characters have been determined and QTLs associated with tolerance at individual developmental stages have been identified and characterized. Zhang *et al.* (2003) [30] identified 9 QTL's for salt tolerance during seed germination and 8 QTLs during vegetative stage. Mostly different QTL's were identified for salt tolerance during those stages suggesting different genetic and physiological mechanism contributing to the salt tolerance.

d. Transgenic: The genetic engineering is one of the components of biotechnology which allows genes that carry instructions for a particular feature to be isolated and moved from one organism to another, resulting in a genetically modified organism or a transgenic plant which can be defined as the organisms with a gene or gene construct of interest that has been introduced by molecular or recombinant DNA techniques.

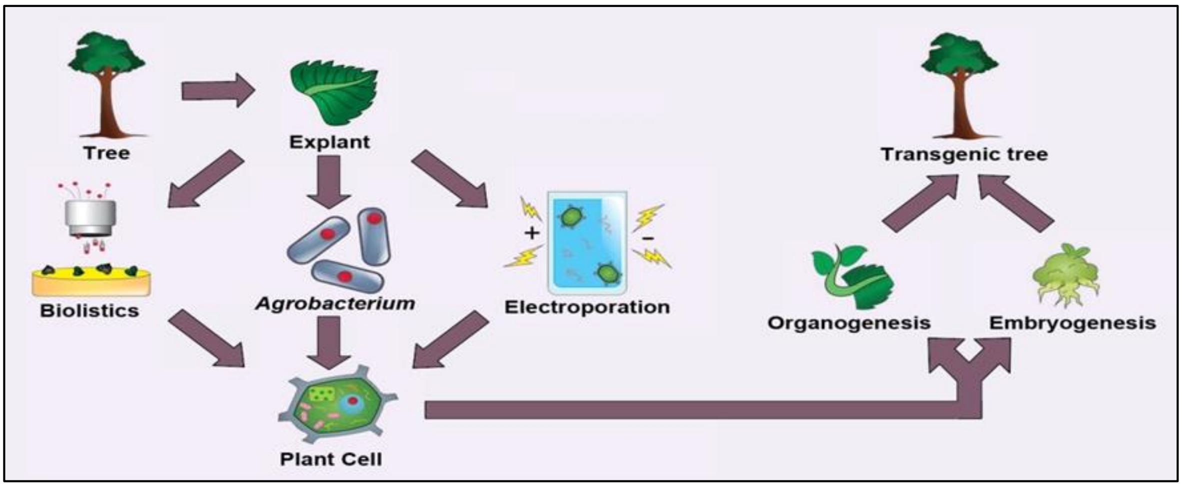

Fig 1: Steps involved in transfer of gene from one plant to another plants

Other tools in managing abiotic stresses

i. Use of anti- transpirants: Anti transpirants are chemical compounds and their role is to practice plants for hardening to stresses, as a method of reducing the impact of drought created by many abiotic conditions, such as salinity, heat or high temperature, reduced precipitation, reduced soil moisture etc., it is gradually reduced by applying very dilute solutions of chemicals that close stomata or by emulsions of wax or latex to form a thin film covers over the stomates or by reflect back a portion of the incident radiation. Thus, based on mode of action, antitranspirants have been classified into film forming type, reflecting type and metabolic inhibitors. Use of antitranspirants reduces the rate of transpiration in plants by penetrating the luxurious loss of water to atmosphere via stomata. These also improve water use efficiency by reducing transpiration rates.

ii. Grafting: Grafting vegetables originated in East Asia during the 20th century and is currently common practice in Japan, Korea and some European countries. Grafting, in this context, involves uniting of two living plant parts (rootstock and scion) to produce a single growing plant. , it can provide tolerance to soil-related environmental stresses such as drought, salinity, low soil temperature and flooding if appropriate tolerant rootstocks are used. Grafting of eggplants was started in the 1950s, followed by grafting of cucumbers and tomatoes in the 1960s and 1970s. Romero *et al.* (1997) [21] reported that melons grafted onto hybrid squash rootstocks were more salt tolerant than the non-grafted melons. However, tolerance to salt by rootstocks varies greatly among species, such that rootstocks from *Cucurbita* spp. are more tolerant of salt than rootstocks from *Lagenaria siceraria* (Matsubara, 1989) [16]. Grafted plants were also more able to tolerate low soil temperatures. *Solanum lycopersicum* x *S. habrochaites* rootstocks provide tolerance of low soil temperatures (10^{o}C to 13^{o}C) for their grafted tomato scions, while eggplants grafted onto *S. integrifolium x S. melongena* rootstocks grew better at lower temperatures (18^{o}C to 21^{o}C) than non-grafted plants. Vegetables generally are unable to tolerate excessive soil moisture. Tomatoes in particular are considered to be one of the vegetable crops most sensitive to excess water. In the tropics, heavy rainfall with poor drainage induces water-logged conditions that reduce oxygen availability in the soil thereby causing wilting, chlorosis, leaf epinasty, and ultimately death of the tomato plants. Genetic variability for tolerance of excess soil moisture is limited or inadequate to prevent losses. Research at AVRDC - The World Vegetable Center has shown that many accessions of eggplant are highly tolerant of flooding. Thus, the Center developed grafting techniques to improve the flood tolerance of tomato using eggplant rootstocks which were identified with good grafting compatibility with tomato and high tolerance to excess soil moisture. Tomato scions grafted onto eggplant rootstock grow well and produce acceptable yields during the rainy season. In addition to protection against flooding, some eggplant genotypes are drought tolerant and eggplant rootstocks can therefore provide protection against limited soil moisture stress.

iii. Use of micro-organisms: Several soil microorganisms may have mechanisms for alleviation of abiotic stresses in plants such as water and temperature stress, salinity, heavy metals etc. Some of these include tolerance to salinity, drought (*Azospirillum* sp., *Pseudomonas syringae*, *P. fluorescens*, *Bacillus* sp.) and nutrient deficiency (*Bacillus polymyxa*, *Pseudomonas alacaligenes*). Other than bacteria, salinity- and drought-tolerant isolates of *Trichoderma harzianum* and the effect of other strains of *Trichoderma* in amelioration of such abiotic stresses have also been reported. Arbuscular mycorrhizal fungi (*Glomus mosseae*, *G. etunicatum*, *G. intraradices*, *G. fasciculatum*, *G. macrocarpum*, *G. coronatum* etc.) help in alleviating abiotic stresses in different crops by enhancing nutrient uptake (phosphorus, nitrogen, magnesium and calcium), biochemical (accumulation of proline, betaines, polyamines, carbohydrates and antioxidants), physiological, molecular and ultra-structural changes. *Bacillus subtilis, Bacillus cereus, serratia spp*, has been reported to impart drought tolerance in cucumber.

Conclusion

Environmental stress is the primary cause of crop losses worldwide, reducing average yields for most major crops by more than 50%. Latitudinal and altitudinal shifts in ecological and agro-economic zones, land degradation, extreme geophysical events, reduced water availability, and rise in sea level and salinization are postulated (FAO 2004) [8]. Unless measures are undertaken to mitigate the effects of climate change, food security in developing countries will be under threat. Climate change may affect agriculture more through water availability than temperature. Scientists predict that the altered hydrologic cycles will change the pattern of precipitation and cause wet areas becoming wetter while dry areas become drier. There is an urgent need to mitigate these abiotic stresses through development of new tolerant crop varieties that will thrive in future conditions Drought-tolerant cultivars are needed to improve water use efficiency, enhance water conservation, reduce irrigation costs, and maintain or increase the acreage of horticultural crops. For certain areas or specific seasons that are prone to flooding, new varieties should be developed to adapt to the wet soil. Global warming is not uniform and may lead to some weather extremes. Although the general trend of temperatures is going up, we are seeing some regions or certain seasons with very cold weather, even breaking low temperature records. Warmer springs might make crop seedlings emerge faster, but they could be vulnerable to damage from late spring frosts or freezes. So cold tolerance is still an important trait for many crops. We need cold tolerant cultivars to extend growing areas or seasons, especially in inland areas with limited frost-free days. In addition, 20% of the world's irrigated lands are affected by salinity (Zhu, 2001) [33], a situation worsened by climate changes. Global warming increases the rates of evapo transpiration and crop requirement of irrigation water, which may bring more salt into the soil in arid and semiarid regions. The migration of salt water into groundwater is being exacerbated by increasing freshwater demands that are depleting coastal aquifers and the projected sea level rise caused by global warming. Acreage impacted by salinity problems is expected to increase. Growers in these regions require cultivars that can resist salt stress to sustain crop production. Growers often face multiple abiotic stresses. For example, heat and drought conditions often come together. Abiotic stresses frequently are intertwined with biotic stresses. High temperatures and elevated CO_2 levels may favor weed growth in competition with crops, making weed control a challenge for growers. Heavy rainfall may increase root diseases. Climate change may promote infestation/epidemics of plant pests/diseases because higher winter minimum temperatures enable insects, pathogens, or disease vectors to overwinter more effectively. The need to produce stress tolerant crops was evident even in ancient times (Jacobsen and Adams, 1958) [12]. We should breed cultivars with multiple resistances or tolerances against abiotic and biotic stresses, including herbicide tolerant traits. However, efforts to improve crop performance under environmental stresses have not been much fruitful because the fundamental mechanisms of stress tolerance in plants remain to be completely understood. Conventional knowledge has almost saturated in finding the solutions for the sprawling abiotic stress' resulting due to climatic change and other causes and plant breeding is a long-term process, often taking more than 10 years to develop a new variety. Genetic engineering has proved its worth in tweaking the plants' ability to cope with the various abiotic stresses. The main advantage of Genetic engineering is that it can transcend across the species barrier. Transgenic plants have shown great promise in tolerance to abiotic stresses such as heat, cold, drought, and salt but much is need to be done to realize the full potentiality of latest technologies.

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