Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

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Breeding approaches for abiotic stress management in vegetable crops

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Abstract
Vegetables 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. 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. 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 or genes that encode proteins conferring stress tolerance or enzymes present in pathways leading to the synthesis of functional and structural metabolites.

Keywords: Abiotic stress, management, breeding approaches and transgenic.

Introduction
Recent experimentation with transgenic plants has led to increased salinity tolerance, with emphasis on the areas of ion homeostasis, osmotic regulation and antioxidant protection. A case study of the major challenges and opportunities to improve stress tolerance in plants using salinity is presented [40]. As different abiotic stresses are inter-related (e.g. salinity and osmotic stress), our ability to improve crop performance may well be determined by combining different, apparently unrelated approaches for introducing several stress tolerance mechanisms into specific crop plants. 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 [39].

There has been considerably more experimentation directed at the engineering of glycinebetaine synthesis than for any other compatible solute. Unlike proline, glycinebetaine degradation is not significant in plants [24], but the problems of metabolic fluxes, compounded with the compartmentation of the substrate and product pools, has made the engineering of appreciable levels of glycinebetaine problematic. In plants that are naturally glycinebetaine accumulators (spinach and sugarbeet), synthesis of this compound occurs in the chloroplast, with two oxidation reactions from choline to glycinebetaine. The first oxidation to betaine aldehyde is catalyzed by choline monooxygenase (CMO), an iron-sulfur enzyme. Betaine aldehyde oxidation to glycinebetaine is catalyzed by betaine aldehyde dehydrogenase (BADH), a non-specific soluble aldehyde dehydrogenase [25]. In E. coli, these reactions are cytosolic; in this species the first reaction is catalyzed by the protein encoded by the bet A locus choline dehydrogenase (CDH), which is an NAD+ -dependent enzyme, and BADH in E. coli, is encoded by the betB locus. In *Arthrobacter globiformis,* the two oxidation steps are catalyzed by one enzyme choline oxidase (COD), which is encoded by the codA locus [18]. The cod A gene of *A. globiformis* offers an attractive alternative to the engineering of glycinebetaine synthesis as it necessitates only a single gene transformation event. This strategy was employed for engineering glycinebetaine synthesis in Arabidopsis [1]. The 35S promoter driven construct for transformation included the transit peptide for the small subunit

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of Rubisco so that the COD protein would be targeted to the chloroplast. Improved salinity tolerance was obtained in transgenic *Arabidopsis* that accumulated, as a result of the transformation, 1 μmol/g fresh weight glycinebetaine. The same construct was used by for transformation of *Brassica juncea* [23] and tolerance to salinity during germination and seedling establishment was improved markedly in the transgenic lines. COX from *Arthrobacter panescens*, which is homologous to the *A. globiformis* COD, was used to transform Arabidopsis, Brassica napus and tobacco [3]. This set of experiments differs from those above in that the COX protein was directed to the cytoplasm and not to the chloroplast [38]. Improvements in tolerance to salinity and drought and freezing were observed in some transgenics from all three species, but the tolerance was variable. The levels of glycinebetaine in the transgenic plants were not significantly higher than those of wild type plants, but increased significantly with the exogenous supply of choline to plants, suggesting that the supply of choline is a significant constraint on the synthesis of glycinebetaine [21]. Environmental stresses play crucial roles in the productivity, survival and reproductive biology of plants as well as crops [29]. Biotic and abiotic stresses, including drought, extreme temperature, scarcity of water, reducing quality of irrigation water and salinity in soil and water are problems which are becoming really acute [8]. These stresses can severely affect growth and development of both rootstocks and/ or scions, thus reducing both fruit production and fruit quality [37]. To overcome these stresses only few scientific approaches are available, one by making use of certain tolerant varieties/rootstocks or by the use of pesticides etc. But there are very few tolerant varieties is available to overcome abiotic stresses and use of pesticides is harmful for human as well as soil health[35]. So, in this situation, rootstock is only the viable option available before us which were used for propagating fruit trees for more than 2000 years [44].

Abiotic stresses
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.

2. 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 [4].

3. 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 [34].

4. 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 [43]. Soluble salts can cause harm to plant, if they are in high concentration in water or soils.

Breeding and Biotechnology in Management of Abiotic Stresses
1. Salt tolerance
Lyon (1941) reported that *Lycopersicon pimpinellifolium* was less sensitive than *L.esculentum* to Na_2SO_4 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 [41]. 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 cyclen [32]. 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 [33].

2. 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[21]. 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 [34]. 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 [26]. 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 [18, 19, 31].

3. 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 nonstress 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 [37].

4. Waterlogging tolerance

The 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 [22]. The additive gene action is the major determinant of inheritance of waterlogging tolerance [28].

5. Cold tolerance

Narrow sense heritability's were 28, 56, 45, and 74%, respectively for inhibition at 5 °C and at 16 °C, 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 16 °C 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 8-10 ^{0}C [7, 25]. 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 [8]. 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 [10].

Breeding Approaches to Overcome Abiotic Stress in Vegetable Crops
1. Synthesis of compatible solutes

The cellular response of salt tolerant organisms to both long- and shortterm salinity stress includes the synthesis and accumulation of a class of osmoprotective compounds known as compatible solutes. These relatively small, organic osmolytes include amino acids and derivatives, polyols and sugars, methylamines etc. The osmolytes are consider to stabilize proteins and cellular structures and can increase the osmotic pressure of the cell [4, 24]. This response is homeostatic for cell water status and protein integrity, which is perturbed in the face of soil solutions containing higher amounts of NaCl and the consequent loss of water from the cell. The accumulation of osmotically active compounds in the cytosol increases the osmotic potential to provide a balance between the apoplastic solution, which itself becomes more concentrated with Na + and Cl ions, and the vacuolar lumen, which in halophytes can accumulate up to 1 M Na +

(and Cl). For a short-term stress, this may provide the cells with the ability to prevent water loss. However, for continued growth under salinity stress, an osmotic gradient (towards the cytosol) must be kept in order to maintain turgor, water uptake and facilitate cell expansion.

2. Antioxidant protection

An important aspect of salinity stress in plants is the stress-induced production of reactive oxygen species (ROS) including superoxide radicals (O_2^-), hydrogen peroxide (H2O2) and hydroxyl radicals (OH). ROS are a product of altered chloroplast and mitochondria metabolism during stress. These ROS cause oxidative damage to different cellular components including membrane lipids, protein and nucleic acids [21, 25]. The alleviation of this oxidative damage could provide enhanced plant resistance to salt stress. Plants use low molecular mass antioxidants such as ascorbic acid and reduced glutathione and employ a diverse array of enzymes such as superoxide dismutases (SOD), catalases (CAT), ascorbate peroxidases (APX), glutathione S-transferases (GST) and glutathione peroxidases (GPX) to scavenge ROS. Transgenic rice over-expressing yeast mitochondrial Mn-dependent SOD displayed enhanced salt tolerance[31]. The overexpression of a cell wall peroxidase in tobacco plants improved germination under osmotic stress [1, 2, 3]. Transgenic tobacco plants over-expressing both GST and GPX displayed improved seed germination and seedling growth under stress [4]. Subsequent studies [5] demonstrated that in addition to increased GST/GPX activities, the transgenic seedlings contained higher levels of glutathione and ascorbate than wild-type seedlings, showed higher levels of monodehydro ascorbate reductase activity and the glutathione pools were more oxidized. These results would indicate that the increased glutathione-dependent peroxidase scavenging activity and the associated changes in glutathione and ascorbate metabolism led to reduced oxidative damage in the transgenic plants and contributed to their increased salt tolerance [22].

3. Salt tolerance in transgenic plants

Salt tolerance in transgenic experiments as described above has been mostly carried out using limited number of seedlings/ mature plants in laboratory experiments. However, the level of salt tolerance of crops ultimately needs to be evaluated as yield from a producer's fields. The evaluation of field performance under saline stress conditions is difficult because of the variability of salt levels in fieldconditions [9, 11] and the potentialfor interactions with other environmental factors, including soil fertility, temperature, light intensity and transpirational water loss. Some work also aims at the prediction of field performance carried out in trial plots, or using a solution based method where the salinity of the medium can be readily adjusted to required values [27]. This type of experiment often precludes measuring yield through lack of space and estimates of tolerance obtained from such experiments are often not borne out by the response of plants in the field [17, 18, 19, 21]. Evaluating tolerance is made more complex by variation in sensitivity to salt during the life cycle. For example, it is known that grain yield in rice is much more depressed by salt than is vegetative growth [20]: germination is relatively salt resistant. In tomato, tolerance at germination is not correlated with the ability to grow under salt stress: both are controlled by different mechanisms [21], although some genotypes display similar tolerance at germination and during vegetative growth [22]. It thus needs to

be recognized that the assessment of stress tolerance in the laboratory often has little correlation to tolerance in the field. Although there have been many successes in developing stress tolerant transgenic in model plants such as tobacco, Arabidopsis or rice [23], there is urgent need to test these successes in crops. Rice has the advantage that it is both the model monocot and an important crop. However, same is not the case when transgenes are tested with tobacco or *Arabidopsis* [27]. This brings a number of technical and financial challenges associated with transforming many of the crop plants, particularly the monocots. First, transformation of any monocot other than rice is still not routine and to develop a series of independent homozygous T2 lines is costly, both in money and time. Second, the stress tolerance screens will need to include a field component since many of the stress tolerance assays used by basic researchers involve using rich nutrient media which include sucrose. This type of screen is unlikely to have relationship to field performance. Finally, since saline soils are often complex and may include NaCl, $CaCl_2$, $CaSO_4$ and Na_2SO_4, plants that show particular promise will eventually have to be tested in all of these environments.

4. R-gene-mediated resistance defense pathways
R-gene mediated resistance is named systematic acquired resistance (SAR). SAR occurs in tissues that are distant from the initial stressed site and is characterized by an increase in the expression of several genes encoding pathogenesis-related (PR) proteins. The expression of PR proteins can be regulated by a variety of stresses including wounding, chemical elicitors, hormones, and UV-light [44]. PR proteins include at least 11 families, among them β-1, 3-glucanases, chitinases, and peroxidases. We have followed the activities of chitinase, β-1, 3-glucanase, and peroxidase in tomato leaves inoculated with viruliferous and nonviruliferous whiteflies, from 1 to 60 dpi slight increase with time in the activity of all three enzymes in leaves of noninoculated plants, which might be related to aging of tissues. Insect and virus treatments lead to a minor increase in chitinase activity [20]. In contrast, a pronounced increase in β-1, 3-glucanase activity was correlated with whitefly feeding, starting at 28 dpi and amplifying thereafter; yet viruliferous and nonviruliferous whiteflies had similar effects. S and R plants presented similar patters of chitinase and β-1, 3-glucanase activities upon treatments. The pattern of peroxidase activation was quite different: S leaves challenged with viruliferous whiteflies showed a level of peroxidase significantly higher than in uninfected or mock inoculated plants starting at 36 dpi and thereafter. Thus, TYLCV infection caused an increase of peroxidase activity in S but not in R tomato lines. The activation of the same PR proteins has been also assayed in response to some abiotic stresses (not shown) [6, 19, 29]. Salt applied in situ led to the activations of all three PRs after several days of treatment, followed by a pronounced decrease. No significant differences have been observed at the stage of activation of the PRs in S vs. R plants, but the intensity of decline of β-1, 3-glucanase and peroxidase activities was somehow less pronounced in R than in S tomatoes. Chitinase, β-1, 3-glucanase, and peroxidase activities in *in vitro*-treated tomato leaves showed similar patterns (data not shown). Under our experimental conditions, the downstream events in biotic and abiotic stress response, such as activation of HR/PCD and expressions of PRs (chitinase, β-1, 3-glucanase, and peroxidase), were similar in S and R tomatoes, except at

the very late (more than 7-8 weeks) of TYLCV infection [12, 3, 15, 27].

5. 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 [18, 41, 42]. Therefore, it is imperative to know about suitable selection criteria, which differ for each species and stress. Some of these are:

6. 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 [40]. It is caused by variation in perched water tables and by undulations in the landscape [7]. 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.

7. Selection
The natural selection in heterogeneous populations of crop plants should increase the frequency of genes for temperature extremes. The natural selection would not only be useful for temperature stress but for other stresses also, if population have been grown at locations where the probability of a stress near the critical level is high [8, 17]. The artificial selection involves sorting out and propagating individual plants/ genotypes from mixed/segregating populations. For selection to be effective there must be genetic variation which can be identified and distinguished from environmental variations [42].

8. 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* [44]. Tepary bean is most commonly suggested source of drought tolerance, although other *Phaseolus* species merit consideration also [9, 16]. From interspecific hybridization between *Pisum sativum and P. fulvum*, developed two promising lines with heat and drought tolerance.

9. 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 [35].

10. 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 DNA recombinant techniques [15, 36].

Conclusion

Plants have evolved strategies to perceive and to cope with deleterious conditions, whether biotic (attacks by pathogens, including fungi insects and viruses) or abiotic (including temperature, water, and salt stresses). In most cases the induced defense responses are regulated by a network of interconnecting signal transduction pathways, in which a variety of stress proteins play cross-reactive roles [10, 13, 14]. A rapid respond to these stresses determines the adaptive capacity and, therefore, the likelihood of survival of the threatened plants [18]. Recently, the downregulation of many expressed genes in Arabidopsis-phloem aphids interactions was shown[25], moreover, large fraction of the downregulated genes were involved in plant metabolism and not directly in plant protection (as PR proteins) [12].

Over the last 50 years, many researchers have argued for the development of salt-tolerant crops from true halophytes. Although halophytes are present in a wide diversity of plant forms, to date very few halophytic crops can compete effectively with glycophytic crops [2, 8, 13]. Moreover, research on the physiology of tolerance suggests that the overall trait is determined by a number ofsub-traits any of which might, in turn, be determined by any number of genes. We believe that by comparing different genes and genetic combinations, researchers will be able to advance the field more quickly, and develop the stress tolerant germplasm. Abiotic stresses, especially salinity, drought, temperature and oxidative stress, are the primary causes of plant loss worldwide [11]. Therefore, rootstock breeding aimed at overcoming abiotic stresses need to be quickly and fully implemented, with intensive molecular-assisted traditional breeding and genetic engineering. Identification of ideal rootstock in vegetable crops continues to remain a great challenge. Traditionally, indigenous wild species were used as rootstocks being propagated through seeds. The concept of scientific approach to identify efficient rootstocks for desired purpose started with focused efforts at different research centers. The potential to produce new rootstocks by either conventional means or in combination with modern technologies is substantial and demonstrated [7]. It is envisaged to choose right rootstocks so that the potential possibilities of the scion variety and rootstock and environment could gainfully be harnessed in sustainable fruit production.

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