

Recent insights into physiological and molecular regulation of salt stress in fruit crops

Abstract

Food and nutritional security of a burgeoning global population in face of unabated land degradation, depletion of fresh water and prime croplands, and climate change impacts remains a challenge. Salinization of agricultural lands caused by the natural and anthropogenic factors a severe obstacles to realizing sustainable agricultural production. Majority of the fruit crops are highly susceptible to salinity and related problems like water logging. Use of salt tolerant scion and rootstock cultivars is seen as a viable means of lessening the salt induced damages. Nonetheless, development and commercial release of salt tolerant cultivars in fruit crops has progressed rather slowly. Moreover, salt tolerance of most of the currently available cultivars breaks down when root zone salinity exceeds the critical threshold. This has led to a departure from conventional screening trials to molecular and genomics tools to broaden the understanding of salt stress regulation at the gene, protein and metabolite levels. The ultimate aim is to introgress such genes into established cultivars in a manner that is cost efficient and safe from human and environmental health perspectives.

Keywords: Fruit crops, Functional genomics, Ion toxicity, Omics approaches, Osmotic stress, Salinity; Sequencing technologies

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Anshuman Singh, PC Sharma

ICAR-Central Soil Salinity Research Institute, India

Correspondence: Anshuman Singh, ICAR-Central Soil Salinity Research Institute, Karnal-132001, India, Email anshumanari@gmail.com

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Introduction

Global population, likely to cross 9 billion by 2050, has necessitated substantial improvements in food production so that all the people have safe access to sufficient nutritious food.¹ Projected increases in global food demand can be met either by agricultural expansion into new areas or by raising the productivity of existing agricultural lands. In so far as further agricultural expansion is concerned, it seems to be virtually impossible due to heightening inter-sectoral competition for land use. A recent study predicts that relentless urbanization could usurp 1.8-2.4% of the highly productive global croplands by 2030.² Pervasive land use inevitably leads to the biodiversity loss, land degradation, water pollution and higher greenhouse gas emissions.³ Efforts towards the productivity enhancements on existing croplands, though appealing, may not lead to expected gains especially in the areas suffering from land degradation and fresh water shortages; problems that are likely to be accentuated by the climatic warming. Currently an estimated 2.5 billion global population suffers from land degradation and its adverse consequences to varying degrees.⁴ In the recent decades, the problem has attained alarming levels severely diminishing soil's ability to provide food and vital ecosystem services in several parts of the world. Continued accumulation of salts in soil and water, a process referred to as salinization, is a major cause of land degradation. Around 20% of the irrigated lands in arid and semi-arid regions have become less productive or, in extreme cases, uncultivable due to twin problems of water logging and salinity. In many situations, presence of salty water in the root zone renders the land virtually barren. Several rain fed areas also suffer from compounded impacts of soil erosion, salinity and water scarcity. Salt-affected soils (SAS) contain either soluble salts or exchangeable sodium or both in amounts harmful to soil properties and plant growth. Different countries adopt different criteria to classify the SAS. In India, for example, depending

on the values of soil saturation paste extract electrical conductivity (EC_e), pH (pH_s) and exchangeable sodium percentage (ESP), SAS are commonly grouped into saline ($EC_e \geq 4 \text{ dS m}^{-1}$, $pH_s < 8.2$ and high $ESP > 15$) and sodic ($EC_e < 4 \text{ dS m}^{-1}$, $pH_s > 8.2$ and $ESP > 15$) categories.⁵ Salts released from the weathering of rocks and minerals, and, to a lesser extent, wind- and seawater-borne salts keep accumulating over a geological time scale and give rise to natural saline soils (*i.e.*, primary salinity). Adverse effects of primary salinity on soil health and crop yields have been reported from about 100 countries transcending the continental boundaries. In most of the cases, naturally formed salt-affected lands have relatively deeper water tables ($> 2 \text{ m}$) and can be productively utilized by simple interventions such as leaching with fresh water and the use of salt tolerant cultivars. In contrast, secondary salinity is a man-made problem and describes the formation of waterlogged saline lands due to excessive irrigation and land clearing in irrigated and rain fed areas, respectively. Sodic soils, on the other hand, require the application of amendments (*e.g.*, gypsum) followed by water ponding for overcoming the structural problems caused by the excess Na^+ ions. Both of these practices are, however, increasingly becoming unsustainable due to growing fresh water shortages and the rising amendment costs. Furthermore, evidence is mounting that such measures have little utility in the areas affected by secondary salinity and that specialized drainage interventions (*e.g.*, sub-surface drainage) are needed to restore the productivity of waterlogged saline lands. In either case, availability of high yielding salt tolerant cultivars can considerably reduce the dependence on costly amelioration practices that often also have a high environmental footprint.⁶

Plant-based solutions are increasingly becoming important for reviving the productivity of salinized lands. Salt tolerant crops have nearly half the leaching requirement compared to salt sensitive crops implying that development of high yielding salinity tolerant cultivars

can greatly minimize the need for irrigation and drainage interventions under such situations. In rain fed areas, integration of woody perennials and pasture crops into existing farming systems is considered one of the effective measures for preventing the salinity onslaught. The role that tree plantations play in lowering the saline water table in irrigated lands, a process called bio-drainage, is well documented. In sodic soils, deep penetrating tree roots hasten the dissolution of calcite to release sufficient Ca^{2+} for counteracting the toxic effects of the excess Na^+ ions. There is evidence that cultivation of forest and fruit trees leads to the substantial improvements in soil properties of even highly degraded sodic lands. Moreover, tree plantations on salt-affected soils can act as strong carbon sinks to lessen the climate change impacts. In partially reclaimed or non-reclaimed lands, salt tolerant cultivars can give significantly higher yields in comparison to sensitive cultivars even with no or reduced use of amendments.⁷

Despite these benefits, slow progress in the development of salt tolerant cultivars is worrisome. In this backdrop, this article aims to underscore the importance of developing salt tolerant cultivars in fruit crops adjudged to be highly sensitive to salinity and the associated problems of drought or water logging. Effects of salinity on physiological processes and plant growth are delineated followed by a brief description of diverse plant responses under saline conditions. Consistent with the fact that conventional and marker-assisted breeding have not led to significant breakthroughs, a case has been presented to harness the potential of functional genomics, omics and genetic transformation tools to accelerate the development of salt tolerant genotypes.

Effects of salinity on plant growth

Plants growing on saline substrates suffer from three distinct, but interrelated, stresses: 'osmotic', 'salt-specific' and 'oxidative' that, depending on species, differentially contribute to the extent of salt induced injury. Besides osmotic and ionic stresses in saline soils, plants also suffer from high root zone pH, structural problems, nutritional imbalances, oxygen deficiency and diminished root respiration in the sodic soils. Salinity affects both annual and perennial plants in a similar manner; initially by perturbing the osmotic balance and subsequently by causing the salt-specific effects. However, annual and perennial species differ with each other in the rate of salt accumulation in the leaves and shoots. In salt stressed annual crops, salt concentrations in leaves may attain toxic levels even within few days or weeks. By comparison, salt injury symptoms usually appear after months or years of salt exposure in perennials.⁸ Depending on species, the plants may either equally tolerate both osmotic and salt-specific stresses or, alternatively, may show high susceptibility to both of these perturbations. For example, salt-induced (50mM NaCl) reductions in growth and net gas exchange in Carrizo citrange seedlings were not caused by the loss of leaf turgor but likely resulted from ion toxicity in leaves as salinity reduced both leaf water potential and osmotic potential such that leaf turgor was increased.⁹ Salinity increased leaf and root Na^+ and Cl^- contents in seedlings of *Citrus limonia* and rooted cuttings of olive cv. Arbequina with a concomitant reductions in total plant dry mass and net photosynthesis. Because leaf pressure potential was higher in salinized plants than in control, decrease in growth and gas exchange were apparently due to ionic toxicity.¹⁰ These observations suggest that a particular genotype may respond differentially to osmotic and salt-specific components of salinity and implies that use of salt excluder rootstocks can greatly alleviate the salt injury by delaying salt transport to shoots. Salt

exclusion by the roots may also minimize the energy expenditure for osmotic adjustment. It seems that in contrast to annual crops and forage species that usually become relatively salt tolerant with age, salt tolerance of fruit crops breaks down after a few years of growth. It is possible that during initial few years of salinity exposure, salt ions are retained in the basal stem and root tissues. With increase in the duration of salinity exposure, salts stored in the stems and roots gradually move to the leaves.¹¹ Salinity affects both vegetative and reproductive stages in plants. A crop or genotype tolerating excess salts during the vegetative growth may be adversely affected at the reproductive stage and vice-versa. Furthermore, QTL (quantitative trait loci) mapping studies have shown an intra-phase variation in salt tolerance; species displaying salt tolerance at the germination stage usually become salt sensitive during the subsequent growth stages.¹² In some crops, salt effects can be more detrimental at the reproductive stage suggesting that crops having fruit or grain as the harvestable product will suffer more damage at a given salinity compared to those crops where stem and/or root constitute the economic part.¹³

Osmotic stress

Elevated salt levels initially cause soil water deficit diminishing the capacity of plant roots to extract sufficient water for metabolic purposes. Water deficit stress may further exacerbate due to progressive water loss from the leaves. In citrus, deleterious effect of osmotic stress are essentially dependent on the pattern of salt accumulation. Sudden increase in salinity increases leaf abscisic acid (ABA) and ethylene levels hastening the leaf fall while the abscised leaves may still be deficient in Na^+ and Cl^- . In contrast, progressive salinization allows efficient osmotic adjustment by the plants to avoid the osmotic stress.¹⁴ Water stressed plants tend to enhance the endogenous ABA levels for preventing leaf dehydration through stomatal closure. Nonetheless, ABA regulated stomatal closure eventually causes the oxidative stress. Reduced stomatal conductance leads to CO_2 deficiency in chloroplasts causing disruptions in electron transport chain. Under these conditions, molecular oxygen (O_2) acts as a strong electron receptor leading to the formation of strongly oxidizing reactive oxygen species (ROS) such as singlet oxygen ($^1\text{O}_2$), hydroxyl radical (OH^\cdot), superoxide radical (O_2^\cdot) and hydrogen peroxide (H_2O_2) in the chloroplast.¹⁵ In saline soils, available water remains trapped by the salt ions compelling the plants to accumulate organic (compatible) and inorganic osmolytes to decrease the leaf water potential for creating a negative pump effect to maintain the water flux through xylem sap.¹⁶ Excess salts present in the cytoplasm are sequestered into cell vacuole by ion compartmentation to prevent ionic damage to the metabolic enzymes. At 100 mM and above concentrations, Na^+ and Cl^- hamper the enzymatic functions. Subsequent to their partitioning in the vacuole, different kinds of solutes accumulate in the cytoplasm (*i.e.*, osmotic adjustment) to balance the osmotic pressure of the ions in the vacuole. Nonetheless, this process involves greater energy expenditure compared to salt exclusion and ion compartmentation processes and may lead to ionic injury in the salt sensitive species. Furthermore, accumulation of metabolically benign organic solutes is rather energy expensive. For example, plants need to spend roughly 10 times more energy to accumulate proline than that needed for accumulating an equivalent amount of Na^+ .¹⁷

Salt-specific effects

While osmotic or water-deficit effect of salinity occurs when salt ions are still outside the plant, salt-specific or ionic stress commences

with ion entry inside the plants. In salt sensitive genotypes inefficient in salt exclusion, salt build-up in shoots and leaves is often rapid resulting in diverse physiological abnormalities. Among fruit crops, citrus, stone fruits, grapevines and avocado are highly sensitive to Cl^- injury. It does not mean that Cl^- is more toxic than Na^+ . Rather, these crops seem to be relatively better Na^+ excluders resulting in reduced Na^+ translocation to the leaf blades. Cl^- accumulation can be nearly twofold higher than sum of cations in citrus treated with NaCl and KCl salts.¹⁸ Besides Na^+ and Cl^- toxicities, decreased availability of essential cations (NH_4^+ , K^+ and Ca^{2+}) and anions (NO_3^-) is another cause of plant growth reduction in SAS. High levels of Na^+ cause the depolarization of plasma membrane hampering the passive uptake of K^+ . Moreover, Na^+ competes with K^+ for major binding sites in the key metabolic processes and also hastens K^+ efflux from cytoplasm. Reduced uptake of nitrate nitrogen (NO_3^-) in saline soils may be due to the fact that Cl^- and NO_3^- have nearly similar size and identical charge, and may compete for the transport sites. At higher salt levels, Cl^- may adversely affect transport of NO_3^- across membranes. Measurement of $^{15}\text{NO}_3^-$ net flux under high salinity conditions revealed that grape rootstock K 51-40 had lower $\text{NO}_3^-/\text{Cl}^-$ root selectivity with increasing salinity compared to 1103 Paulsen showing higher $\text{NO}_3^-/\text{Cl}^-$ root selectivity over the whole salinity range (0-45 mM).¹⁹ High affinity saturable system (HATS) and low affinity linear system (LATS) functional at low and high NO_3^- concentrations, respectively, modulate NO_3^- uptake in plants. In citrus plants, some of the salt induced symptoms such as reduced growth and low fruit yield resemble with those of nitrogen deficiency indicating that salt stress suppresses N uptake. NO_3^- absorption was severely affected by Cl^- ions in both Troyer citrange and Cleopatra seedlings; albeit to a greater extent in the former which showed higher tissue Cl^- concentrations.²⁰ In Troyer citrange seedlings, NO_3^- uptake rate linearly increased at 1-50 mM external $^{15}\text{NO}_3^-$ concentrations which seemed to be mediated by LATS. Nitrate pre-treatments had a repressive effect on NO_3^- uptake rate implying that LATS is under feedback control by the N status of plants.²¹ Addition of NO_3^- to irrigation water reduced Cl^- accumulation in salt stressed citrus and avocado rootstocks suggesting that Cl^- laden irrigation water may be used if NO_3^- is supplied at a molar concentration equivalent to half that of Cl^- .²² Na^+ and Ca^{2+} interactions at the surface of the plasma membrane and subsequent Ca^{2+} signaling events affect plant growth, photosynthesis, water absorption and ion transport in salt stressed plants. Under saline conditions, plants need 5-10 mM Ca in the external solution for growth. Surprisingly, plant growth may be suppressed when Ca concentration exceeds 10 mM. The optimal Na: Ca ratio is in range of 10-20 for majority of the crop plants.²³ Reduced activity of Ca^{2+} in the leaf cells is attributed to Na^+ induced decrease in the binding of Ca^{2+} to the plasma membrane, inhibition of influx and a higher efflux of Ca^{2+} , and loss of the Ca^{2+} from endomembranes.²⁴ Supplemental Ca applications may partly overcome the adverse effects of Na^+ in some crops. Apoplastic effects of Ca^{2+} on the transport of Na^+ and K^+ across the root plasma membrane seem to be the major process lessening Na^+ toxicity. It has been shown that root and shoot Ca^{2+} concentrations may not increase following Ca addition and that Ca applications do not reduce osmotic stress in salinized plants.²⁵ Supplementary Ca (5 mM Ca as CaCl_2) ameliorated the negative effects of NaCl (35 mM) on growth and fruit yield in strawberry (*Fragaria × ananassa* Duch) cvs. 'Oso Grande' and 'Camarosa' by decreasing the membrane permeability, correcting leaf Ca^{2+} deficiency and improving the plant water use.²⁶

Plant response to salinity

Fruit plants employ different strategies to endure the salt stress. These include accumulation of hormones such as ABA, osmotic adjustment, preferential accumulation of K^+ , maintenance of photosynthesis, activation of antioxidant defense system and salt exclusion. These responses are, by and large, crop- and genotype-specific, and are influenced by the factors such as growth stage, substrate, and agro-climatic conditions.

Hormonal regulation

Salinity depresses the plant growth and yield by reducing the growth of sink (assimilate consuming) organs as well as by suppressing photosynthesis in the source organs. Hormonal signals such as cytokinins, ABA, 1-aminocyclopropane-1-carboxylic acid (ACC) and auxin indole-3-acetic acid modulate assimilate synthesis, partitioning and use in the salt stressed plants. Hormonal regulation of source-sink relations during osmotic stress may enhance the energy availability for growth, root function and ion homeostasis, and may also delay Na^+ and Cl^- accumulation to the toxic levels.²⁷ In citrus plants, Cl^- accumulation triggers the synthesis of ethylene precursor ACC. Prolonged imposition of salinity may thus accelerate ethylene induced leaf abscission. Salinized plants accumulate ABA to counteract ethylene upsurge. Pre-treatment with ABA reduces ethylene release and leaf abscission in citrus probably by preventing Cl^- accumulation in leaves.²⁸ Some ABA analogs (8'-methylene ABA and 8'-acetylene ABA) also delay the deleterious effects of salinity on citrus plants.²⁹ The rate and extent of leaf fall in salt stressed plants is also reduced by ethylene inhibitors like CoCl_2 or silver thiosulphate.³⁰ At 250 mM NaCl, grape rootstocks Dogridge, 1613, St. George and Salt Creek showed nearly threefold increase in root ABA levels compared to control. Higher ABA levels were negatively correlated with Na^+/K^+ ratio indicating that ABA accumulation inhibits Na^+ uptake.³¹ Little is known about ethylene-responsive element binding factor (ERF) genes and their functions in fruit plants. ERF citrus gene *Cit ERF* is expressed in plants under polyethylene glycol, low temperature and NaCl treatments.³²

Osmotic adjustment

Halophytes (salt tolerant plants) endure exceptionally high salinity levels by accumulating Na^+ and Cl^- ions to create a gradient for water uptake. Glycophytes (salt sensitive plants), on the other hand, achieve osmotic adjustment (OA) mainly by the synthesis of compatible solutes such as proline, sugars and organic acids—a process that involves heavy energy use. It is due to this reason that growth and yield are severely affected in glycophytes even at moderate salinity.³³ Organic solutes are classified into three major groups: amino acids (e.g., *proline*), onium compounds (e.g., *glycine betaine*), and polyols/sugars (e.g., *mannitol*). Accumulation of non-toxic compatible solutes in the cytoplasm is a part of an overall mechanism to raise the osmotic pressure and thereby maintain both turgor and the driving gradient for water uptake. In addition, some of them can also protect enzymes and cell membranes and may prevent ROS induced injury.³⁴ In fruit crops, the predominant OA strategy depends on the relative salt tolerance of genotypes, growth conditions and management practices. Proline is a major compatible osmolytes in salt stressed plants. In addition to well defined role in OA, proline may also act as a metal chelator, an antioxidant and a signaling molecule. Proline is synthesized by the plants in response to a wide

range of abiotic stresses including drought and salinity, and thus proline content is often used as a biochemical marker to distinguish the salt tolerant and sensitive genotypes. Observations in date palm (*Phoenix dactylifera* L.) suggest that free proline over-accumulates in the roots and leaves under high salinity, drought, high temperature, ABA treatment and other stresses. Differential accumulation of proline under different abiotic stresses implies that proline cannot be used as a unique marker in breeding programs aimed at improving drought or salt tolerance.³⁵ Proline is synthesized from ornithine and glutamate under normal and saline conditions, respectively. As chlorophyll is also synthesized from glutamate, its use by the salt stressed plants in proline biosynthesis may lead to chlorophyll loss as noted in Yaghooti and 1103P grapevines that showed the highest total chlorophyll but the lowest proline levels at 50–100 mM NaCl. Glycine betaine (GB) accumulation in salt stressed plants is known to maintain protein stability and photosynthetic machinery. Salt tolerant ‘Yaghooti’ and ‘1103P’ grapevines accumulated more GB than proline. By avoiding proline biosynthesis, these cultivars maintained optimum chlorophyll levels for normal photosynthesis.³⁶ Application of exogenous proline (5 mM) considerably minimized the salt damage in salt-sensitive *Citrus sinensis* ‘Valencia late’ cell line exposed to 100 mM NaCl.³⁷ Mannitol seems to be a potential osmoregulator in leaf mesophyll in salt stressed olive plants where increase in leaf mannitol concentration shows a positive correlation with the increasing root zone salinity.³⁸ Salt treated (0–75 mM NaCl) Mazzetto almond scions grafted on Garnem rootstock had higher proline, soluble sugar, K⁺ and Ca²⁺ concentrations but lower Na⁺ compared to Mazzetto grafted on GF677.³⁹ In general, plants facing drought, salt, low temperature and flooding tend to have relatively higher soluble sugar concentrations, whereas those suffering from high light intensity, heavy metals and nutrient deficiencies exhibit lower soluble sugar levels. Nonetheless, plant sugar levels may vary with the genotype and the magnitude of stress. Among soluble sugars, sucrose and glucose may act either as substrates for cellular respiration or as osmolytes to maintain cell homeostasis. In contrast, fructose plays a major role in the synthesis of secondary metabolites in stressed plants.⁴⁰ Total soluble sugars in leaves increased with increasing salinity (up to 80 mM NaCl) but decreased with the further increase in salinity in olive cultivars Zard and Roghani.⁴¹ Sucrose, glucose and fructose concentrations declined in the leaves of Cleopatra mandarin, and in both leaves and roots of Troyer citrange with increase in salinity (0–80 mM NaCl) implying that sugar levels tend to be higher in salt sensitive than in tolerant genotypes.⁴²

Preferential accumulation of potassium

It is seen that some fruit genotypes preferentially accumulate K⁺ in leaf and stem tissues to withstand the adverse effects of salt ions. Regardless of rootstock, grafted lemon trees (cv. Fino 49) had lower leaf Cl⁻ but higher K⁺ when 10 mM potassium nitrate was added to 50 mM NaCl.⁴³ Trifoliate orange seedlings inoculated with two arbuscular mycorrhizal fungi (*Glomus mosseae* and *G. versiforme*) showed significantly lower leaf Na⁺ and Ca²⁺ levels but higher K⁺, glucose, fructose and proline concentrations under 100 mM NaCl suggesting the major role of K⁺, proline and monosaccharides in OA.⁴⁴ Mango rootstock *M. zeylanica* maintained higher photosynthetic assimilation at 60 mM NaCl than *M. indica* 13-1 due to a higher root K⁺/Na⁺ ratio and lower leaf/root Na⁺ in.⁴⁵ Pistachio rootstock *P. atlantica* outperformed *P. vera* under salt stress by restricting Na⁺ and Cl⁻ uptake and maintaining higher K⁺/Na⁺ and Ca²⁺/Na⁺ selectivity.⁴⁶

Salt stress (EC_e 6.5 and 10.7 dS m⁻¹) significantly enhanced Na⁺ accumulation in bael (*Aegle marmelos* Correa) cultivars NB-5, NB-9, CB-1 and CB-2. However, cultivar NB-5 exhibited relatively similar Na⁺ concentrations in leaf, stem and root tissues as well as maintained higher K⁺ concentrations in aerial parts. Restricted Na⁺ uptake and preferential K⁺ accumulation enhanced the salt tolerance in NB-5 cultivar.⁴⁷ K⁺ and Ca²⁺ levels in leaves and stems of tamarind (*Tamarindus indica* L.) plants either remain unchanged or increased up to 40 mM NaCl compared to control.⁴⁸ Salt stress significantly increased K⁺ concentration in leaves and shoots of salt sensitive fig (*Ficus carica* L.) genotypes suggesting that high cytosolic K⁺ levels may lead to better sequestration of Na⁺ ions in vacuoles and may therefore enable poor Na⁺ excluders to sustain large amounts of Na⁺ in leaves.⁴⁹

Maintenance of photosynthesis

Decrease in stomatal conductance is one of the earliest responses in salt stressed plants to arrest the water loss from leaves. Stomatal closure also reduces CO₂ diffusion into the leaves resulting in reduced internal CO₂ partial pressure and consequently low photosynthetic rates. In many species, non-stomatal biochemical limitations also impair the photosynthesis. In Valencia orange, Taylor lemon and Ellendale tangor scions grafted on Cleopatra mandarin rootstock, salt induced (75 mM NaCl) reduction in photosynthesis was attributed to stomatal closure, Na⁺ toxicity and chlorophyll degradation. Na⁺ ions had a greater limiting effect on photosynthesis and transpiration than Cl⁻.⁵⁰ In ‘Séjène’ grapevines irrigated with 100 mM NaCl water, salinity tolerance was linked to vine vigor, maintenance of photosynthetic capacity and retention of Na⁺ in the shoots and the older basal leaves. Cl⁻ exclusion, osmotic adjustment through K⁺ and Ca²⁺ accumulation in leaves, appeared to play minor roles in salt tolerance.⁵¹ At 10.7 dS m⁻¹ salinity, the minimum and the maximum reductions in photosynthesis occurred in salt tolerant NB-5 and salt sensitive CB-2 bael cultivars, respectively.⁴⁷ Reduced photosynthetic efficiency under salt stress is ascribed to stomatal (e.g., diffusional resistance to CO₂ transport) and non-stomatal (physical and biochemical factors) limitations. While there is a better understanding of stomatal factors, non-stomatal limitations to photosynthesis remain somewhat obscure. Accordingly, it is suggested that instead of analyzing stomatal and non-stomatal limitations investigations on diffusional and non-diffusional limitations of photosynthesis may provide a better idea of photosynthetic depression in salt treated plants.⁵²

Anti-oxidant enzymes

Continued accumulation of reactive oxygen species (ROS) leads to the oxidative stress in salt stressed plants. Decrease in stomatal conductance due to oxidative stress and excessive Na⁺ accumulation in the cytosol together impair the photosynthetic machinery resulting in only partial utilization of light by the photosynthetic pigments. This accelerates ROS production in leaves and eventually leads to lipid peroxidation, DNA and protein denaturation, and ion (e.g., K⁺) efflux.⁵³ Salt stressed plants activate different enzymatic [superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) and peroxidase (POD)] and non-enzymatic [ascorbic acid (AsA), glutathione (GSH) and tocopherols] antioxidant systems for ROS scavenging. These antioxidants alter the gene expression, act as redox buffers and as a metabolic interface to regulate the optimum induction of acclimation responses.⁵⁴ Salt tolerant and salt sensitive

genotypes differ in their antioxidant that also varies with the crop growth stage and management practices. Simultaneous occurrence of two or more abiotic stresses (e.g., salinity, drought and high light intensity) alters the expression of transcripts regulating antioxidant activity compared to a single stress (e.g., salinity). Salt tolerant apple rootstocks (Dongbeihuanghaitang, Daguhongsanyehaitang and Qiuzi) showed higher activities of ROS scavenging enzymes than salt sensitive (Yingyehaitang and Lushihongguo) ones.⁵⁵ Exogenous application of AsA minimized the hypoxia-induced oxidative damage in both tolerant (*M. hupehensis*) and sensitive (*Malus sieversii*) apple rootstocks by up regulating the activities of antioxidants like APX, GR, SOD, POD and CAT; albeit to a greater extent in *M. sieversii*.⁵⁶ Citrus rootstock *Jatti khatti* showed significantly higher activities of SOD and CAT than Attani-1 and Attani-2 rootstocks at 50 mM NaCl salinity. POD levels in different rootstocks increased only up to 25 mM salinity.⁵⁷ Salinity also triggers the synthesis of phenolic compounds in plants. Oleuropein is identified as the main phenolic compounds protecting the olive trees from salt shock probably by acting as a glucose-reservoir for osmoregulation as well as a component of the antioxidant defense system.⁵⁸ Both enzymatic and non-enzymatic antioxidant levels increased in salt stressed Carrizo citrange seedlings while malondialdehyde content (a measure of lipid peroxidation) remained at a moderate level indicating that deleterious effects of salinity in Carrizo citrange are mainly due to toxic effects of Cl⁻ ions and not due to oxidative stress.¹⁶

Salt exclusion

Salt exclusion describes the ability of plants to filter the salts at the root level. While water passage is allowed, the dissolved salts are not absorbed. In a strict sense, complete salt exclusion ability can be seen only in salt resistant halophytes. In fruit crops, salt exclusion in most of the cases describes the ability of a rootstock to retain salt ions in the roots and/or basal stem tissues such that insignificant amounts of salts are translocated to the photosynthetically active leaves. Several salt excluder rootstocks having better Na⁺ and Cl⁻ exclusion properties have been reported. Genetic features such as ploidy level may influence the relative salt tolerance of different rootstocks. For example, tetraploid citrus seedlings often show greater salt tolerant than diploid genotypes. Genome duplication enhances the salt exclusion capacity of citrus presumably by altering the physiological and anatomical traits. At 40 and 80 mM NaCl salinities, tetraploid seedlings of *Citrus macrophylla* accumulated considerably lower Na⁺ and Cl⁻ in leaves than diploid seedlings. Furthermore, leaf K⁺ concentrations dropped only in salinized diploid seedlings reflecting the inherent differences in mineral uptake.⁵⁹ Fine root turnover, a unique root trait found in citrus relative *Poncirus trifoliata*, ensures that salt stressed plants continuously produce the fine roots to remove the excess salts. This trait, instrumental in delaying ion translocation to leaves, can be introgressed in related citrus species through intergeneric hybridization.⁶⁰ In citrus, Na⁺ and Cl⁻ exclusion mechanisms are independent traits suggesting that a good Na⁺ excluder may not be an effective Cl⁻ excluder and vice versa.⁶¹ Polyembryonic mango rootstocks often outperform the monoembryonic cultivars under saline conditions.⁶² Salt exclusion capacity may break down when salt levels exceed the critical threshold. Own-rooted grape rootstock Dog Ridge, initially considered to be an efficient excluder up to 6.5 dS m⁻¹ NaCl induced salinity, does not sustain prolonged saline irrigation when grafted with Thompson Seedless scion.⁶³ In fruit crops, salt exclusion appears to be a genotype-specific trait and higher exclusion capacity of a particular cultivar does not necessarily represent the salt

tolerance of the species. While salt stressed Cleopatra and Shekwasha showed negligible chlorosis symptoms and leaf drop, Fuzhu, Willow leaf, Beauty, King of Siam and Nasnaran were severely affected due to excessive Cl⁻ translocation from roots to leaves (leaf/root Cl⁻ ratio > 1). Despite heavy leaf abscission, some of the leaves on salinized plants were still green indicating an escape mechanism whereby leaves containing excessive toxic ions are dropped while younger leaves are retained to sustain the photosynthesis.⁶⁴ At 50 mM Cl⁻, xylem Cl⁻ concentration in Cl⁻ excluder 140 Ruggeri grapevines was nearly sevenfold lower than that in Cl⁻ includer K51-40. Higher Cl⁻ exclusion capacity of 140 Ruggeri seems to be due to restricted loading of Cl⁻ in xylem and subsequent lower translocation from roots to shoots.⁶⁵ Among nine olive cultivars, 'Arvanitolia Serron' and 'Lefkolia Serron' were found to be the most salt tolerant cultivars under NaCl stress. These cultivars accumulated less Na⁺ and Cl⁻ in leaves by retaining them in roots. 'Arvanitolia Serron' also maintained high K⁺ levels in younger leaves under salinity.⁶⁶ Salinity induced growth suppression was much greater in olive cultivar 'Barnea' than in 'Arbequina'. While the former had a high leaf Cl⁻ concentration, the latter skipped salt damage by excluding Cl⁻ at the root level.⁶⁷ Japanese pear 'Akibae' grafted on *P. betulaeifolia* rootstock showed lower Na⁺ and Cl⁻ levels and higher photosynthetic rate when treated with 25 or 100 mM NaCl than scions grafted on *P. calleryana* and *P. pyrifolia* rootstocks suggesting that salt exclusion capacity of *P. betulaeifolia* remains functional even after grafting.⁶⁸ Salt stress decreased plant growth in fig genotypes. 'S×P' and 'S×K' were the most sensitive and the most tolerant genotypes, respectively. Cl⁻ concentrations in different plant parts increased but were higher in roots than in leaves indicating salinity tolerance in fig depends on the degree of Na⁺ and Cl⁻ exclusion from shoots.⁴⁹

Role of ion channels

Sodium and potassium channels

While plants in normal soils maintain a high cytosolic K⁺/Na⁺ ratio, salt stress disrupts the ionic balance as excess Na⁺ promotes the passive transport of Na⁺ over K⁺. Na⁺ and K⁺ channels often fail to discriminate between these two ions as they have strongly similar hydrated ionic radii. Na⁺ ions can be transported into the cell through low- and high-affinity K⁺ transporters. Among three different low affinity K⁺ channels identified in the plants, voltage dependent inward rectifying channels (KIRCs) and outward rectifying channels (KORCs) exhibit a high K⁺/Na⁺ selectivity ratio at physiological K⁺ and Na⁺ concentrations. In contrast, voltage-independent cation channels (VICs) have a relatively high Na⁺/K⁺ selectivity and seem to modulate Na⁺ uptake at high salinity levels.⁶⁹ Low-affinity K⁺ channels have low K⁺/Na⁺ selectivity and are relatively more affected by Na⁺. Thus, salinized plants need to maintain high-affinity K⁺ channels for K⁺ uptake.⁷⁰ Integrated membrane proteins (IMPs) also regulate solute movements. High-affinity K⁺ transporters (HKTs), a class of IMPs found only in plants, vary in Na⁺/K⁺ selectivity with some being highly selective for Na⁺ and others for K⁺. Na⁺/K⁺ selectivity of these HKTs also varies with the ionic environment.⁷¹ In *Arabidopsis*, several families of Na⁺ transporters have been reported. Among them, Class I HKT-type transporters are involved in Na⁺ uptake and translocation, *SOS1* excludes Na⁺ from the cytosol and *NHX* regulates Na⁺ sequestration in vacuole.⁷² A grapevine inward K⁺ channel 'VvK1.1' shares many functional similarities with *Arabidopsis* *AKT1* channel like inward rectification and regulation by calcineurin B-like (CBL)-interacting protein kinase (*CIPK*) and Ca²⁺-sensing CBL partners.

Application of 50 mM NaCl or KCl did not cause any significant change in '*VvK1.1*' transcript accumulation in roots and leaves.⁷³ Chawga grapevines accumulated K⁺ in roots and shoots even at high salinity. Salinity enhanced the expression of *VvKUP1* and *VvKUP2* transporters and *VvK1.1* channel in roots and leaves but expression was higher in roots than in leaves.⁷⁴ Transcriptome analysis in salt stressed olive cv. Kalamon led to the identification of 387 unique ion transporter transcripts including 9 Na⁺ transporters, 30 K⁺ transporters and several other transcripts related to proton transport mechanism.⁷⁵

Chloride channels

In plants, Cl⁻ catalyzes certain enzymatic activities, serves as a co-factor in photosynthesis, stabilizes membrane potential and regulates the cell turgor and pH. However, excess Cl⁻ causes toxicity symptoms, i.e., at 4–7 mg g⁻¹ concentration in salt sensitive and at 15–50 mg g⁻¹ in salt tolerant species. Exclusion by the roots and slower transport to the vegetative parts can reduce or prevent Cl⁻ induced injury symptoms in sensitive fruit crops such as citrus and grapes.⁷⁶ Despite the fact that excess Cl⁻ is more harmful than Na⁺ in many plants, little is known about Cl⁻ absorption and transport mechanisms. Similar to Na⁺, reduced loading into xylem, intracellular compartmentation and root efflux are some of the major processes controlling Cl⁻ entry and translocation in plants. While slower loading into xylem prevents excess Cl⁻ accumulation in shoots, preferential accumulation of Cl⁻ in leaf epidermis lowers the likely damage to mesophyll cells performing photosynthesis. Reduced Cl⁻ loading into shoots (Cl⁻ exclusion), previously thought to be governed by a single dominant gene, is regulated by several genes. Aquaporins (regulating the water flow in roots) and ATP binding cassette (ABC) transporters are some of the well known genes directly or indirectly involved in Cl⁻ flux in plants. Transcriptome analysis of two citrus genotypes differing in Cl⁻ exclusion revealed the possible involvement of several anion transporter families.⁷⁷ Different pathways for the water and ion movement in roots include apoplastic, symplastic and transmembrane pathways. Under high salinity conditions, direct apoplastic transport without biological selectivity for ion transport may become predominant. In grape rootstocks 1103 Paulsen (salt tolerant) and K 51-40 (salt sensitive), there was no correlation between transport of Cl⁻ and the apoplastic tracer (3-hydroxy-5,8,10-pyrenylsulphonic acid) indicating similar bypass flow of salts to the xylem in both the rootstocks and that differences in membrane transport explain the differences in Cl⁻ transport to the shoot.¹⁹ According to a model proposed for the symplastic regulation of Cl⁻ homeostasis in salt includer Carrizo citrange and excluder Cleopatra mandarin rootstocks, high affinity Cl⁻/H⁺ symporters govern Cl⁻ uptake at low external Cl⁻ concentrations. When Cl⁻ is added in millimolar concentrations, a high influx develops and the plants tend to modify the kinetic properties of influx transporters and activation of plasma membrane anion efflux channels from epidermal cells. Symplastic regulation differs in the includer and excluder rootstocks in the rate of Cl⁻ translocation to the xylem which is much lower in excluder Cleopatra than in includer Carrizo.¹⁸ In *Arabidopsis*, *CCC*, *SLAC/SLAH*, *ALMT* and *CLC* gene families encode different Cl⁻ channels and transporters. However, only *At CCC* and *At CLCc* play an important role in Cl⁻ homeostasis under high salinity.⁷² Plant cation-chloride co transporters (*CCCs*) can improve shoot salt exclusion by catalyzing the retrieval of Na⁺ and Cl⁻ ions from the root xylem. A *CCC* gene from grape cultivar Cabernet Sauvignon (*VviCCC*) shares a high degree of similarity with other *CCCs* and probably belongs to the Na⁺-K⁺-2Cl⁻ co transporter

class. Expression of *VviCCC* in an *Arabidopsis* *ccc* knockout mutant decreased shoot Cl⁻ and Na⁺ contents to wild-type levels when exposed to 50 mM NaCl.⁷⁸ Membrane transporter genes like *NRT1-2* are differentially expressed in salt stressed Cleopatra and Carrizo plants suggesting their potential roles in Cl⁻ homeostasis.⁷⁹ Chloride channels (*CLCs*) also seem to be involved in plant adaptation to salt stress by regulating Cl⁻ homeostasis. *CLC*-encoding gene *CsCLCc* expressed in the leaves and roots of trifoliate orange was up regulated by ABA, low temperature and NaCl.⁸⁰

Genetic approaches for improving salt tolerance

Genetic improvement for salt tolerance in crop plants can be achieved either by identifying the natural variations through direct selection or QTL mapping. As previously mentioned, limited success with direct selection under field conditions is ascribed to the influence of environmental factors on whole plant response to salt tolerance that seems to be a developmentally regulated trait; plants differentially respond to salt stress at different developmental stages.¹² Furthermore, conclusive evidence is not yet available regarding the relative contributions of different physiological adaptations to the overall performance of a particular genotype at a particular growth stage under salt stress even in extensively studied crops like rice.⁸¹ Limiting the scope for selection based on physiological criteria. This has enhanced the interest in identifying the genes and gene products that can be transferred to established cultivars through marker-assisted breeding and genetic transformation.

Salt stress signalling

Situations where a single abiotic stress affects plant productivity seldom exist. In fact, plants often experience two or more stresses at a given time. Simultaneous occurrence of abiotic stresses such as drought, heat and excess salts implies that stress sensing and signaling are intricate processes. Furthermore, as underground root portion and aboveground foliage may be differentially affected by a single stress or different yet simultaneously occurring stresses (e.g., water deficit, salinity and high temperature),⁸² understanding how plants sense and respond to stress condition is absolutely essential; especially in grafted plants consisting of two distinct genotypes. It is increasingly becoming evident that plant signaling is not an isolated process but essentially involves the interaction between several transduction events in a complex manner. Besides the complicated transduction chain among different abiotic stresses, interactions between biotic and abiotic stress signaling mechanisms also occur.⁸³

Genetic basis of salt tolerance

Plants exposed to stress conditions express specific stress-related genes which can broadly be grouped into three major categories:^{84,85}

- genes encoding proteins with known enzymatic functions in metabolic processes such as detoxification and osmolyte biosynthesis,
- genes encoding proteins with unknown functions, and
- regulatory class genes involved in signaling cascades and in transcriptional control.

Interest in identifying and cloning the genes conferring tolerance to one or more abiotic stresses has steadily increased as conventional breeding methods have not led to concrete outcomes. Moreover,

marker-assisted breeding based on the identification of the genomic regions (quantitative trait loci; QTLs) tightly linked to a trait of interest and subsequent introgression into established cultivars continues to suffer from the problem of linkage drag as insertion of such QTLs often brings along undesirable traits from the donor parents.

Salt tolerance is a complex trait involving responses at the cellular, organ and whole plant levels. Polygenic nature of salt tolerance is an important limitation to the development of salt tolerant varieties through conventional breeding. Physiological and genetic bases of salt tolerance remain obscure in most of the crop plants. Precise delineation of even morphological and physiological traits imparting salt tolerance, and not the much complex gene networks, continues to be a challenge in the woody perennials. In citrus fruits, for example, over five dozen such traits seem to be directly or indirectly involved in determining the whole plant response under saline conditions.⁸⁶ In fruit crops, only a few studies have been conducted to understand the genetic basis of salt tolerance. Even in some of the most studied crops like grapevine, it is still unclear whether salt tolerance is a monogenic or polygenic trait.⁸⁷ Extensive field and laboratory trials for salt stress screening are expensive and tedious. In addition to the cost and resource constraints, accurate determination of absolute salt tolerance under field (*ex vitro*) conditions is not possible due to complex interactions occurring between the plants and environmental variables. This has led to interest in relatively controlled pot studies. Nonetheless, as plants employ a range of physiological adaptations to withstand salt stress, data from such studies often poorly match with those of field trials. Of late, *in vitro* culture studies under controlled conditions requiring far lesser time and space are being advocated as an ideal system for identifying the salt tolerant lines. However, as with pot experiments, poor correlations between *in vitro* and *in vivo* responses and the difficulties in regenerating plants from the selected salt tolerant lines are the major barriers to the use of *in vitro* screening techniques.⁸⁸

QTL mapping

QTLs are the genomic stretches linked with the variation of a quantitative trait of interest (e.g., salt tolerance). In plants, such quantitative variation results from the combined action of multiple segregating genes and environmental factors. QTL analysis involves crossing two parents differing in one or more quantitative traits. The resulting hybrid population is analyzed to link the QTL to the known DNA marker for the indirect selection of the trait of interest. While most of the QTL mapping studies are conducted with the goal of marker-assisted selection (MAS), germplasm characterization and gene cloning are some other areas where QTL mapping can play a significant role.⁸⁹ In hybrid tomato rootstocks (*Solanum lycopersicum* var. *cerasiforme* x *S. pimpinellifolium*, *S. lycopersicum* var. *cerasiforme* x *S. cheesmaniae*), salt tolerance is a heritable trait governed by at least eight medium sized QTLs inherited from the wild parents.⁹⁰ In the same hybrid populations, it was observed that chromosome 7 is the only genomic position where the QTLs governing both the cations exist together.⁹¹ A total of 98 QTLs putatively linked to salt tolerance were detected in a hybrid citrus population [Cleopatra mandarin (salt tolerant) x Trifoliolate orange (salt sensitive)]. A cluster of QTLs governing plant vigor and leaf boron concentration pointed a genomic region in linkage group 3 as the most relevant one to improve salt tolerance using the Cleopatra parent as donor.⁸⁶ QTL mapping unravelled 70 potential QTLs in a

BC₁ population [*Citrus grandis* x (*Citrus grandis* x *Poncirus trifoliata* x)] of which 69% were associated with salinity. Further analysis of 16 regions of the citrus genome indicated that 6 of them were involved in both growth and dry mass production under salt stress.⁹² A subsequent study in the same population revealed a probable overlap between the genomic regions controlling salt and cold tolerance.⁹³ Randomly amplified polymorphic DNA (RAPD) analysis revealed that certain DNA fragments may characterize genes coding for salt tolerance in date palm. For example, RAPD Primer OPC-02 amplified a 1400 bp fragment in salt tolerant cv. Bugal White.⁹⁴

Association mapping

Biparental QTL mapping used for dissecting genomic regions linked to salt tolerance provides little knowledge as how allelic variations present in the whole gene pool interact and affect the salt tolerance. Furthermore, such QTLs may contain hundreds or sometimes thousands of the putative genes.⁹⁵ This has necessitated the development of more efficient approaches for understanding the genetic control of quantitative traits. Recent advances in genotyping, genome sequencing and statistical analysis have made association mapping (AM) an appealing proposition for exploiting the vast genetic diversity in crops. Also called linkage disequilibrium (LD) mapping, AM seeks to resolve complex trait variations by exploiting historical and evolutionary recombination events at the population level. While QTL mapping considers only variations between two individuals, AM explores the phenotypic and genetic differences across a natural population.^{96,97} Of late, whole genome sequences of strawberry, apple, peach, papaya and grape are available and sequencing is underway in other fruit crops. Availability of low cost high-throughput sequencing techniques may further lower the sequencing and genotyping costs and may also help refine such draft genome sequences. These developments could eventually lead to the use of genome sequences as references to identify single nucleotide polymorphisms and copy number variations. It is worth mentioning, however, that unique growth and genetic characteristics of fruit crops like long juvenile phase, large tree size and high heterozygosity need to be considered when collecting genotyping and phenotyping data to ensure the selection of an appropriate AM strategy.⁹⁸

Functional genomics approaches

Plant genomes often possess more genes compared to other living organisms; a fact explained by the sessile and autotrophic nature of plants compelling them to synthesize an array of compounds needed for protection against adverse conditions. Availability of DNA sequence information in crops has spurred the interest in the analysis of gene function. Availability of complete genome sequences has allowed the determination of the number and structure of genes in a particular genome and their organization on the chromosome.⁹⁹ Currently, 'quantitative' genome data available in different species are being converted into 'qualitative' information through a process of value addition to the nucleotide sequence information by assigning new functions to the unknown genes.¹⁰⁰ It has been shown that nearly 54% of the higher plant genes can be assigned some degree of function by comparing them with the sequences of genes of known function. Unfortunately, knowing the general function frequently does not provide an insight into the specific role in the organism necessitating large-scale functional genomics to assign well defined roles to the genes.¹⁰¹ Based on the sequencing information, the function of a specific gene can be predicted either by 'forward' or

'reverse' genetics approaches. In forward genetics, genetic basis of a trait is elucidated by examining the altered (mutated) phenotype (*i.e.*, phenotype to genotype). In contrast, in reverse genetics, a particular gene is altered and the resultant phenotype is studied (*i.e.*, genotype to phenotype). Classical forward genetics studies are conducted by phenotypic screening of mutant populations obtained either by chemical or physical mutagenesis. In the mutant phenotype, time-consuming map based cloning (MBC) is carried out to identify the sequence change underlying the mutant phenotype. However, such forward genetics techniques are largely unsuited for high-throughput functional analyses even if saturated genetic maps are available. In reverse genetics, sequence information (*i.e.*, a specific nucleotide sequence or a set of sequences) is selected to explore the links between nucleotide sequence and the underlying function(s) by selecting the mutations that disrupt the sequence. Tagging by either transposable elements or T-DNA constructs can identify the function of a specific gene by uncovering a specific phenotype. Moreover, when a particular function is encoded by more than one gene, reverse approaches are the only way to perform a step-by-step analysis of such redundant functions. In some cases, it has been found that *Arabidopsis* insertional knockout mutants do not show an informative phenotype probably due to functional redundancy among genes or the fact that mutations are conditional and do not readily change plant morphology. In such cases biological function has to be inferred from measurements of gene expression and activity.^{100,102}

High-throughput omics approaches

Preceding observations suggest that the success of functional genomics would largely depend on more efficient high-throughput tools such as transcriptomics, proteomics and metabolomics collectively referred to as the 'omics approaches'. Omics technologies can be defined as the global (genome-wide or system-wide) experimental approaches to assess the gene function.¹⁰³ Functional genomics is strikingly similar to 'systems biology' in that it heavily relies on comprehensive profiling of virtually each gene expression product to simultaneously monitor all the biological processes operating as an integrated system.¹⁰⁴

Transcriptomics

A 'transcriptome' can be defined as the complete set of cell- or tissue-specific messenger RNA (mRNA) molecules expressed by an organism. While plant genome remains stable, transcriptome expression changes with the plant growth stage and environmental stimuli. Different techniques can be used to estimate the alterations in transcriptome in response to stress conditions. Over the years, rapid developments in inexpensive high-throughput next-generation sequencing have revolutionized transcriptome sequencing for studying the global transcriptional networks. Polyamines (PAs) like putrescence (Put), spermidine (Spd) and spermine (Spm) play a protective role under salinity stress. Exogenous application of such PAs partly alleviates salt induced damages in plants. Expression profiling of the genes involved in PA biosynthesis (*ADC*, *ODC*, *SAMDC*, *SPDS*, *SPMS*) and catabolism (*PAO* and *DAO*) showed that biosynthetic genes except *ODC* were induced by NaCl treatment in sour orange (*Citrus aurantium* L.) plants.¹⁰⁵ Nearly 1900 genes were found to be differentially expressed in the roots of mock-treated and salt-treated plants of date palm cv. Deglet Beida. As several such genes belonged to DNA/RNA and protein biosynthesis and signaling pathway categories, they are believed to play functional roles in salt

tolerance.¹⁰⁶ In date palm cv. Khalas, 2630 and 4687 genes were differentially expressed in leaves and roots, respectively, under salt stress. Of these, 194 genes were commonly expressed in both the organs. Gene ontology analysis showed the higher expression of transcripts involved in photosynthesis, carbohydrate metabolism and oxidative phosphorylation in leaves and those involved in membrane transport, phenyl propanoid biosynthesis, amino acid metabolism and caesarian strip development in roots.¹⁰⁷ An integrated analysis of miRNA and mRNA expression and their regulatory networks in *Citrus junos* Siebold cv. 'Ziyang' roots showed that differentially expressed mRNA and miRNA were involved in ABA-activated signaling pathway and ROS metabolism under dehydration and/or salt stress.¹⁰⁸ There was higher abundance of transcripts linked to flavonoids biosynthesis, sugar metabolism and transport, and aquaporin genes in Grenache grapevines recovering from water stress. Transcripts involved in ABA metabolism and signaling (ABA-8'-hydroxylase, serine-threonine kinases, RD22 proteins) were only up regulated in plants recovering under high transpiration.¹⁰⁹ Increase in the transcript abundance of RuBisCo activate was detected on day 4 and day 12 in water- and salt-stressed Cabernet Sauvignon vines, respectively. While water stress induced higher number of transcripts involved in metabolism, transport and the biosynthesis of cellular components, salinity stress elicited a higher number of transcripts linked to transcription, protein synthesis and metabolism.¹¹⁰ Salt tolerant grape genotypes (H6 and Gharashani) differed with salt sensitive (Shirazi and Ghezel Uzum) in gene expression under salinity. Expression profile of *VvNHL1* in leaves of all genotypes and in roots of tolerant genotypes was similar to that of *VvEDS1*. In contrast, *VvChS* and *VvPAL* transcripts significantly increased only in the leaves of tolerant genotypes.¹¹¹ A total of 209 and 36 differentially expressed transcripts were identified in salt tolerant Kalamon and salt sensitive Chondrolia Chalkidikis olive cultivars in response to NaCl stress. In Kalamon, all the transcripts were significantly down regulated in the post-stress period. Two cultivars shared 21 differentially expressed transcripts in response to NaCl-stress. Comparison of transcriptional regulatory networks in olive with that of *Arabidopsis* suggested similarity in TF homologues regulating salt stress.¹¹²

Proteomics

Acclimation of plants to abiotic factors occurs through alterations in gene expression leading to the changes in plant transcriptome, proteome and metabolome. Nonetheless, changes in gene expression may not always correspond to the changes at the protein level necessitating the study of proteomic changes so that proteins directly eliciting plant stress response may be identified. Proteome analysis techniques decipher the complete set of proteins present in a biological sample and thus allow a detailed understanding as how diverse proteins contribute to stress regulation in plants. Advances in proteomics have led to the identification of different salt stress responsive proteins involved in signal transduction, photosynthesis, osmotic adjustment, ion homeostasis and activation of anti-oxidant defense system. Genes encoding such proteins have been cloned and engineering in several glycophytes.¹¹³ Hydrogen peroxide (H₂O₂) and nitric oxide (NO) play key roles in signal transduction pathways activating plant defense under stresses. Proteomic analysis showed that 85 leaf proteins underwent significant quantitative variations in salinized *Citrus aurantium* L. plants. Most of these protein variations were not detected in plants pre-treated with either H₂O₂ or sodium nitroprusside (SNP, a NO releasing chemical). Both

H₂O₂ and SNP pre-treatments before salt treatment alleviated salinity-induced protein carbonylation and shifted the accumulation levels of leaf *S*-nitrosylated proteins to those of unstressed plants. Results indicated an overlap between H₂O₂ and NO signaling pathways in acclimation to salinity and that the oxidation and *S*-nitrosylation patterns of leaf proteins are specific molecular signatures of citrus plant vigour under stressful conditions.¹¹⁴ In salt stressed sour orange plants, protein carbonylation and tyrosine nitration were depressed by some PAs whereas protein *S*-nitrosylation was elicited by all PAs. A total of 271 *S*-nitrosylated proteins commonly or individually affected by salinity and PAs were identified.¹⁰⁵ Proteome analysis of roots and leaves revealed a synergetic responsive network under drought and salt stresses in date palm. While 55 protein spots were common under both the stresses, salinized plants showed 35 spots (15 and 20 with higher and lower protein abundance, respectively). In comparison, polyethylene glycol induced drought stress resulted in only 3 and 6 spots with higher and lower protein abundance, respectively.¹¹⁵ Proteins involved in photosynthetic assimilation and protein synthesis declined in Chardonnay and Cabernet Sauvignon grapevines under water deficit and salinity stresses. Several proteins up regulated in tolerant cultivar Chardonnay were of an unknown function compared to Cabernet Sauvignon where proteins involved in protein metabolism were upregulated.¹¹⁶ A survey of 1047 proteins in the grape berry pericarp (skin and pulp) revealed that 90 such proteins had differential expression in the skin and pulp. Of 695 proteins surveyed from seed tissue, 163 were identified and indicated that seed and pericarp proteomes were nearly completely distinct from one another. Water-deficit stress altered the abundance of approximately 7% of pericarp proteins, but had little effect on seed protein expression.¹¹⁷

Metabolomics

Despite significant breakthroughs in transcriptome and proteome analyses, the understanding of gene function remains perplexing. Currently, about 30–40% of the open reading frames have unknown function(s) highlighting the need for determining the biological function(s) of these so-called orphan genes.¹¹⁸ Metabolomics refers to comprehensive high-throughput analyses of complex metabolites produced by the plants. Nonetheless, plant metabolic composition is often very complex and may entail very high expenditure and technical requirements.¹¹⁹ Interest in Metabolomics is driven by the fact that biochemical response of an organism to a conditional perturbation can be characterized by its effect on the differential accumulation of individual metabolites. Phenotype of an organism is largely the result genotype and environment interactions. However, phenotypic expression is also regulated by different sub-cellular physiological processes suggesting that precise delineation of metabolic fluxes involved in biochemical pathways is absolutely essential to develop a clear understanding.¹²⁰ In fruit crops, metabolites such as proline, mannitol, glycine betain, anti-oxidant molecules, chlorophyll pigments, etc. are routinely assessed to evaluate plant response to salinity. However, understanding of plant adaptation to salinity as a function of the complete metabolite network remains poor. Metabolite profiling revealed that there were higher concentrations of glucose, malate, and proline in water-stressed than salinized Cabernet Sauvignon vines. Metabolite differences were linked to differences in transcript abundance of many genes involved in energy metabolism and nitrogen assimilation processes like photosynthesis and photorespiration.¹²¹ In grapes, about half of the 32 metabolites surveyed showed tissue-specific differences in abundance with

water-deficit stress affecting the accumulation of seven of these compounds.¹¹⁷

Genetic transformation

Genetic improvement of perennial fruit trees through conventional approaches is cumbersome. Prolonged juvenile phase, large tree size and the need to screen large number of seedlings are some of the factors hindering the success in tree breeding. For example, development of an improved apple cultivar takes nearly 20 yr and entails a cost of approximately €400 000 (at 2002 prices). Furthermore, these time and cost requirements hold true when an established cultivar is used as the parent in hybridization. More money and time may be needed to transfer desirable traits from a distant or wild relative. Add to this the time required for removing the undesirable alleles introduced from the wild parent (5–6 generation cycles each of 4–10 yr). In a nutshell, nearly five decades will be required to obtain a new cultivar and one more decade to commercialize the cultivar and to gain the consumer acceptance.¹²² In comparison, introgression of genes using *Agrobacterium* mediated or other direct transfer methods like electroporation and microinjection may substantially lower the time and costs of traditional selection and hybridization programmes. The fact that genetic transformation has been successfully attempted in over 100 species until now and that some genetically modified edible crops are available on the market has proved catalytic to the development of transgenic cultivars in fruit crops. Nonetheless, different biological, regulatory and public opinion constraints need to be adequately addressed before actual field applications of such transgenic products.¹²³ Stable transformation of transgenes involved in regulatory networks improves the salt tolerance of transgenic plants. Over expression of chemically synthesized grape gene *VvbHLH1* significantly enhanced salt and drought tolerance in transgenic *Arabidopsis thaliana* plants by up regulating the genes involved in flavonoid biosynthesis, ABA signaling pathway, proline biosynthesis and ROS scavenging.¹²⁴ Transgenic kiwifruit plants containing *Arabidopsis* vacuolar Na⁺/H⁺ anti porter gene (*AtNHX1*) tolerated up to 200 m mol l⁻¹ NaCl attributed to better osmotic adjustment and higher antioxidant activities.¹²⁵ Introgression of *Arabidopsis* gene *CBF3/DREB1A* into *Citrus macrophylla* genome significantly improved salt tolerance compared to wild-type plants.¹²⁶ Over expression of *Musa DHN1* gene improved the salt tolerance of transgenic banana lines by enhancing proline accumulation and decreasing the lipid peroxidation in leaves.¹²⁷ Recently, grafting is also being advocated as a potentially novel technique to allay some of the fears related to transgenic cultivars. In grafting, either genetically engineered scion or rootstock having potential to confer tolerance to stress conditions is used as one of the graft components. In so far as the movement of transgenic product(s) across a graft union is concerned, transmission of genomic or organelles DNA over such a long distance is highly unlikely. However, some of the heritable changes induced by epigenetic modifications of genomic DNA may be transmissible.¹²⁸

Conclusion and future perspective

In the last few decades, there has been a paradigm shift in the ways and means employed to unravel the complex physiological and genetic bases of salt tolerance in crop plants. Over the years, marker-based and genomics techniques are increasingly being utilized to identify the genes linked to salt stress adaptation in plants. Advent of next generation sequencing tools and omics approaches has marked

the beginning of a new phase in the area of functional genomics. Fruit physiologists and breeders are increasingly tapping these genomic resources to identify and characterize the transcripts, genes, proteins and metabolites linked to key salt tolerance traits so that they can be transferred to the high yielding but salt sensitive cultivars. Most of the current knowledge using these high-end techniques comes from crops like citrus and grapes reflecting their huge commercial value. It is expected that continual refinements would further lower the costs and enhance the efficiency of currently available genomics tools leading to their large-scale use in other fruit crops for the development of commercial products.

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Conflict of interest

None.

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