

# Mechanisms of increasing salt resistance of vegetables by grafting

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## Abstract

Soil salinity is a serious threat to horticultural production. Improving salt tolerance of vegetables by breeding is a difficult task as salt tolerance is a quantitative trait, regulated by a large number of genes. As a traditional agronomic method, grafting, which is widely practiced in vegetables, especially among members of Cucurbitaceae and Solanaceae, is a useful tool for reducing yield loss caused by salinity. However, the underlying mechanisms of this phenomenon remain largely unknown. Numerous studies have been conducted to uncover these mechanisms by which grafting improves salt tolerance in vegetables. This review summarizes the studies that have been conducted on this topic. In this review, the effects of salt stress on vegetable crops were discussed, and the four main mechanisms by which grafting increases the salt resistance of vegetables, namely, restricting the transport of toxic ions, enhancing the antioxidant system, enhancing the stability of the photosynthetic system, and sending root signals, were discussed. This review concludes by identifying several prospects for future research on increasing the adoption of grafting in vegetables under salinity stress.

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## Introduction

Salinity is one of the major factors that limit crop production. Over 1,500 million hectares of land suffer from increased salinity<sup>[1]</sup>. Soil salinity has two distinct types: primary salinity and secondary salinity<sup>[2]</sup>. The former is the result of natural processes, such as rock disintegration. During these processes, soluble salts, such as sodium, calcium, magnesium and sulfates, permeate into the water environment where they are accumulated in the soil by rain and wind<sup>[3]</sup>. Sodium chloride is one of the most easily transported salts in these processes<sup>[1]</sup>. By contrast, secondary salinity is the result of human activities, such as overfertilization under a protected horticultural condition, replacement of perennial crops with annual crops, overuse of chemical fertilizers, all of which increases soil salinity, especially in vegetable production<sup>[4]</sup>. It is predicted that by 2050 the high salinity of arable land will make half of it unusable for agriculture<sup>[5]</sup>. High salinity negatively impacts the yield and quality of horticultural crops<sup>[6]</sup>. Most vegetable crops are glycophytes; thus, they are susceptible to soil salinity even at mild or moderate salt levels<sup>[7]</sup>. About 50–70 mM of NaCl concentration in nutrient solution could lead to irreversible injury to cucumber<sup>[8–10]</sup> and tomato<sup>[11–13]</sup>. In contrast, the salinity threshold level of pepper is 20 mM of NaCl<sup>[14]</sup>.

To deal with the adverse effects of salinity on vegetable production, researchers have attempted to enhance the salt tolerance of vegetables by breeding strategies. However, owing to the complexity of salt tolerance, which is a quantitative trait, few salt-tolerant species have been developed for commercial use. Recently, vegetable grafting has become widely adapted as a commercial measure<sup>[15–19]</sup>. Vegetable grafting is an ancient practice. Grafting of Cucurbitaceae was

mentioned in the ancient book, 'Si-Sheng-Zhi-Shu', which was published in China in the first century<sup>[16]</sup>. The first commercial use of grafting of vegetables was in Asia in the 20<sup>th</sup> century. Grafting of eggplants (*Solanum melongena*) started in the 1950s, followed by grafting of cucumber (*Cucumis sativus*) and tomato (*Solanum lycopersicum*) in the 1960s and 1970s, respectively<sup>[4]</sup>. Grafting was initially used to overcome soil diseases<sup>[20]</sup>. However, grafting has been gradually recognized to significantly improve plant resistance to abiotic stress, including salinity, drought, and high temperature and chilling<sup>[21]</sup>. The spread of grafting in recent years and its impact on horticultural production have been the main driving force for hundreds of studies and comprehensive reviews on vegetable grafting and salinity<sup>[1,4,22–30]</sup>. However, the mechanisms by which grafting enhances the salt tolerance of vegetables remain unclear, and some novel findings on this topic have been neglected.

This review aims to present the latest knowledge of the effects of salinity on vegetable crops and the role grafting plays in increasing salt tolerance. In this review, two main families of vegetables, namely, Cucurbitaceae and Solanaceae, were selected as case studies to present the current scientific consensus on the mechanisms by which grafting reduces the effects of stress on these important vegetables.

## Adverse effects of salt stress on vegetables

### Effects on plant growth

Stress imposed by saline conditions includes two components: (1) osmotic effect, which is caused by the increase in osmotic potential of water that decreases the ability of water uptake; and (2) toxicity of salt ions, especially Na<sup>+</sup> and Cl<sup>-</sup>,

which is harmful to the physiological processes of various plants. In the first stage, saline conditions do not substantially alter plant growth because most toxic ions are collected in the vacuoles. This process is the reason why halophytes are more tolerant to stress than glycophytes<sup>[31–32]</sup>. In this phase, both leaves and roots are inhibited, but leaves do not present salt injury symptoms. Another remarkable change is stomatal conductance. The stomata close very quickly when they detect changes in osmotic pressure (within 3 h) and rebound during a prolonged stress period (after 6–12 h)<sup>[10,33]</sup>. In the second stage, the cells are unable to store the toxic ions in vacuoles. As a result, their concentration in the cytoplasm increases and the activity of many enzymes is severely inhibited<sup>[1]</sup>.

### Effects on photosynthesis

Vegetables subjected to excessive salt stress are usually associated with reduced photosynthetic capacity<sup>[34]</sup>. In particular,  $\text{Na}^+$  and  $\text{Cl}^-$  interfere with chlorophyll production<sup>[9]</sup>. Salinity can also reduce the content of carotenoids and xanthophylls and the fluorescence intensity of chlorophyll<sup>[35]</sup>. Stomatal closure limits the fixed rate of carbon dioxide. Therefore, the rate of light energy absorption by photosynthetic pigments exceeds the rate of its consumption in chloroplasts<sup>[36]</sup>. This condition can lead to photosynthetic damage of PSII through the formation of reactive oxygen species (ROS), thus inducing oxidative stress and damage of the photosystem<sup>[37,38]</sup>.

### Effects on nutritional balance

Salt has an antagonistic effect on the absorption and transport of nutrients in plants, which may damage the nutritional balance of vegetables<sup>[39]</sup>. In fact, salinity can reduce the solubility of micronutrients by altering the pH of the soil solution<sup>[40]</sup>. However, this effect on micronutrients depends on the species and salinity levels<sup>[41]</sup>. Salinity also reduce nitrogen absorption via the interactions between  $\text{Na}^+$  and  $\text{NH}_4^+$  or  $\text{Cl}^-$  and  $\text{NO}_3^-$ . High  $\text{Na}^+$  levels reduce  $\text{Ca}^{2+}$  levels but stabilizes  $\text{K}^+$  levels in roots and reduces  $\text{K}^+$  levels in leaves<sup>[41]</sup>. Moreover, high  $\text{Na}^+$  levels reduce  $\text{Mg}^{2+}$  levels in leaves but not in roots. Furthermore, the presence of soluble salts ( $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{SO}_4^{2-}$ ) in soil reduces phosphate absorption<sup>[7,42]</sup>. In addition, high concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  in soil or water may depress nutrient-ion activities and produce extreme ratios of  $\text{Na}^+/\text{Ca}^{2+}$ ,  $\text{Na}^+/\text{K}^+$ ,  $\text{Ca}^{2+}/\text{Mg}^{2+}$ , and  $\text{Cl}^-/\text{NO}_3^-$  in leaves<sup>[39]</sup>. Therefore, the plant becomes susceptible to nutritional disorders as well as osmotic stress induced by  $\text{Na}^+$  or  $\text{Cl}^-$ .

## Mechanisms by which grafting increases the salt resistance of vegetables

### Grafting restricts the transport of toxic ions and maintains nutrient absorption

Sodium is the most commonly encountered salt ion that is toxic to crops<sup>[43]</sup>. Regulation of  $\text{Na}^+$  influx, efflux, and compartmentation are important for plants to cope with  $\text{Na}^+$  accumulation in shoots. Grafted cucumber has a higher crop performance with  $\text{Na}_2\text{SO}_4$  than with  $\text{NaCl}$  because of the inability of rootstock to restrict transfer of  $\text{Cl}^-$  from roots to shoots<sup>[44]</sup>. Thus, restriction of  $\text{Na}^+$  is the key mechanism to improve the salt tolerance of vegetables via grafting.

Given that only a small proportion of  $\text{Na}^+$  can be retrieved from shoots and returned to roots via the phloem in plants<sup>[45]</sup>,

the key factor that determines  $\text{Na}^+$  accumulation in scions is the restrictive ability of rootstock to load  $\text{Na}^+$ , both in Cucurbitaceae<sup>[8,46,47]</sup> and Solanaceae<sup>[12,48–51]</sup>. Sodium restriction by rootstocks is based on two main functions concerning  $\text{Na}^+$  regulation. The first one is  $\text{Na}^+$  exclusion in the root maturation zone, and the other one is  $\text{Na}^+$  loading in vascular bundle sheath cells. Pumpkin (*Cucurbita moschata*)-grafted cucumbers show a higher rate in effluxing  $\text{Na}^+$  from roots than self-grafted cucumber plants. This observation is consistent with the findings from non-grafted cucumber and pumpkin that indicated higher  $\text{Na}^+$  exclusion capacity in pumpkin roots than in other plant parts<sup>[45]</sup>. Sodium extrusion is mediated by the  $\text{Na}^+/\text{H}^+$  antiporter located on the plasma membrane (PM). Under  $\text{NaCl}$  stress, an increase in  $\text{Na}^+$  extrusion is concurrent with a higher  $\text{H}^+$  influx in pumpkin (*Cucurbita moschata*) than in cucumber. The strong  $\text{Na}^+$  exclusion in pumpkin (*Cucurbita moschata*)-grafted cucumber is due to the active  $\text{Na}^+/\text{H}^+$  antiporter across the PM that is powered by PM  $\text{H}^+-\text{ATPase}$ . High-affinity  $\text{K}^+$  transporter-1 (HKT1) encodes a  $\text{Na}^+$  preferential transporter that controls  $\text{Na}^+$  delivery from root to shoot via  $\text{Na}^+$  withdrawal from xylem sap<sup>[52,53]</sup>. Limiting  $\text{Na}^+$  radial transport to the stele is the key way to reduce the content of  $\text{Na}^+$  in the scion in pumpkin grafted cucumber<sup>[54]</sup>. Rootstock HKT1 genotype affected fruit  $\text{Na}^+$  and non-commercial tomato fruit yield<sup>[55]</sup>. These findings may play a key role in salt tolerance breeding of vegetables.

Numerous studies (Table 1) have suggested that salt tolerance can be improved by rootstocks, which limits the uptake of  $\text{Na}^+$  but not of  $\text{Cl}^-$ . However, some studies still focused on the function of rootstocks in the regulation of  $\text{Cl}^-$  distribution<sup>[56]</sup>. Grafting the cucumber variety 'Aramon' onto pumpkin (*Cucurbita moschata*) rootstocks reduces  $\text{Na}^+$  uptake, enhances  $\text{K}^+$  transport toward young leaves, and induces  $\text{Cl}^-$  recirculation to old leaves. Thus, cucumber can avoid overaccumulation of  $\text{Cl}^-$ , and the pumpkin (*Cucurbita moschata*) rootstocks regulate the recirculation of  $\text{K}^+$  and  $\text{Cl}^-$ <sup>[57]</sup>. Similar results were obtained in *Solanaceae*. Various pepper species grafted onto salt-tolerant rootstocks (e.g., *Capsicum chinense* or *Capsicum baccatum*) can achieve high plant productivity by limiting  $\text{Cl}^-$  transport to leaves, thereby allowing the uptake of other cations (i.e.,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$ ) for low osmotic potential at low energy cost<sup>[58]</sup>. Moreover, the increase in root length density and root surface area of the salt-tolerant rootstock may be an adaptation to salinity. Salinity may also reduce nitrogen absorption via the interactions between  $\text{Na}^+$  and  $\text{NH}_4^+$ .

Yang et al.<sup>[41]</sup> evaluated the multiple effects of salt and rootstock on nitrogen metabolism of grafted watermelon. The increase in activity of glutamate dehydrogenase (GDH) may have participated in ammonium assimilation in gourd (*Lagenaria siceraria*)-grafted seedlings when the glutamine synthetase (GS) or glutamate synthase (GOGAT) pathway was inhibited by salinity stress ( $\text{NaCl}$ ), and thus increased the salt tolerance of watermelon<sup>[41]</sup>. Grafting also increases the uptake of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in grafted plants by decreasing  $\text{Na}^+$  uptake, suggesting that the mechanism by which grafting reduces  $\text{Na}^+$  uptake is also related to  $\text{Ca}^{2+}/\text{Na}^+$  or  $\text{Mg}^{2+}/\text{Na}^+$  selectivity<sup>[49]</sup>.

### Grafted plants have higher antioxidant activity under salt stress

The relationship between an antioxidant system and salt tolerance has been found in many plant species. It has been

## How grafting increases vegetable salt tolerance

**Table 1.** Studies on the mechanism by which grafting limits salt ion transport.

ID	Scion species	Rootstock species	Salt treatments	Growth conditions	Tested traits	Reference
1	'Jinchun No. 2' ( <i>Cucumis sativus</i> )	'Chaojiqianwang' ( <i>Cucurbita moschata</i> )	90 mM NaCl	Hoagland's solution	Ca <sup>2+</sup> , K <sup>+</sup> , and Na <sup>+</sup> content; plasma membrane (PM) H <sup>+</sup> -ATPase activity; expression levels of <i>SOS1</i> and <i>PMA</i>	[45]
2	'Brennus' and 'London' ( <i>Cucumis melo</i> )	'RS841 improved' ( <i>Cucurbita maxima</i> Duch. × <i>Cucurbita moschata</i> )	40 and 80 mM NaCl	Hoagland's solution	WUE; Cl <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , SO <sub>4</sub> <sup>2-</sup> , PO <sub>4</sub> <sup>-</sup> , Na <sup>+</sup> , K <sup>+</sup> , Ca <sup>2+</sup> , Mg <sup>2+</sup> , and NH <sub>4</sub> <sup>+</sup>	[59]
3	'Jinchun No. 2' ( <i>Cucumis sativus</i> )	'Chaojiqianwang' ( <i>Cucurbita moschata</i> )	90 mM NaCl	Hoagland's solution	FW and DW; Na <sup>+</sup> , K <sup>+</sup> , and Cl <sup>-</sup> concentrations; Na <sup>+</sup> concentration in xylem; gas exchange parameters	[8]
4	'Ekron' ( <i>Cucumis sativus</i> )	'P360' ( <i>Cucurbita maxima</i> × <i>Cucurbita moschata</i> )	20 mM CaCl <sub>2</sub> , 30 mM NaCl or 10 mM CaCl <sub>2</sub> + 15 mM NaCl	Quartziferous sand	Fruit dry matter; total soluble solid content; Fv/Fm; GPX activity; K <sup>+</sup> , Ca <sup>2+</sup> , Na <sup>+</sup> , Mg <sup>2+</sup> , and Zn <sup>2+</sup> contents	[44]
5	'Cyrano' and 'Akito' ( <i>Cucumis sativus</i> )	'P360' and 'PS1313' ( <i>Cucurbita maxima</i> × <i>Cucurbita moschata</i> )	40 mM NaCl	Quartziferous sand	Yield; shoot and root biomass; net photosynthetic rate; K <sup>+</sup> , Na <sup>+</sup> , and Cl <sup>-</sup> concentrations	[46]
6	'Deer No. 99' ( <i>Cucumis sativus</i> )	<i>Cucurbita ficifolia</i>	100 mM NaCl	50% Yamazaki solution	Enzyme activities; salt damage score; Na <sup>+</sup> , Ca <sup>2+</sup> , and K <sup>+</sup> distributions	[60]
7	'Aramon' ( <i>Cucumis sativus</i> )	'Becada' ( <i>Cucurbita moschata</i> )	50 mM NaCl	Nutrient solution	Gas exchange, plant growth, and ion concentrations in xylem sap	[57]
8	'Baimi' ( <i>Cucumis melo</i> )	'Jingxin 3' ( <i>Cucurbita maxima</i> × <i>Cucurbita moschata</i> )	150 mM NaCl	Hoagland's solution	Growth parameters; photosynthetic measurement; antioxidant system; enzymes for sugar metabolism; Na <sup>+</sup> , K <sup>+</sup> , Ca <sup>2+</sup> , and Mg <sup>2+</sup> concentrations	[47]
9	'Xintaimici' ( <i>Cucumis sativus</i> )	'Chaojiqianwang' ( <i>Cucurbita moschata</i> )	75 mM NaCl	Hoagland's solution	<i>CmHKT1;1</i> expression; cation uptake experiments in yeast cells; cucumber transformation with <i>CmHKT1;1</i>	[54]
10	'Ikram' ( <i>Solanum lycopersicum</i> )	'Charlotte' ( <i>Solanum tuberosum</i> )	5.0 dS m <sup>-1</sup> NaCl	Sandy loam soil	Stomatal conductance; fluorescence parameters; Na <sup>+</sup> , K <sup>+</sup> , and Ca <sup>2+</sup> concentrations in roots, stems, leaves, and fruits	[12]
11	'Tom 121' and 'Tom 174' ( <i>Solanum lycopersicum</i> )	'Tom 121' and 'Tom 174' ( <i>Solanum lycopersicum</i> )	50 mM NaCl	Greenhouse soilless culture conditions	Stomatal conductance (gS); osmotic potential; Na <sup>+</sup> and Cl <sup>-</sup> concentrations	[50]
12	'Adige' ( <i>Capsicum annuum</i> )	'ECU-973' ( <i>Capsicum chinense</i> ) and 'BOL-58' ( <i>Capsicum baccatum</i> )	32 mM Na <sup>+</sup> and 41 mM Cl <sup>-</sup> (sandy soil); 40 mM NaCl (hydroponic)	Sandy soil and hydroponics	K <sup>+</sup> , Ca <sup>2+</sup> , Mg <sup>2+</sup> , Na <sup>+</sup> , and Cl <sup>-</sup> concentrations; proline determination; photosynthetic activity; chlorophyll fluorescence	[61]
13	'Red stone' ( <i>Solanum lycopersicum</i> )	'Long purple' ( <i>Solanum melongena</i> ), 'datura' ( <i>Datura patula</i> ), and 'orange nightshade' ( <i>Solanum luteum</i> )	5 and 10 mM NaHCO <sub>3</sub>	1/4 concentration of Hoagland's solution	Biomass; Mg <sup>2+</sup> , Ca <sup>2+</sup> , Mn <sup>2+</sup> , Zn <sup>2+</sup> , and Cu <sup>2+</sup> concentrations; proline content	[62]
14	'Cuore di Bue' ( <i>Solanum lycopersicum</i> )	'Maxifort' and 'Arnold' ( <i>Solanum lycopersicum</i> × <i>Solanum habrochaites</i> )	20 and 40 mM NaCl	Perlite–peat substrate	Yield, fruit number, fruit weight, TSS, and Na <sup>+</sup> content	[48]
15	'Chaoyan 298' ( <i>Solanum lycopersicum</i> )	'Yanqi' ( <i>Lycium chinense</i> )	7.42 g kg <sup>-1</sup> (total salt include Na <sup>+</sup> , K <sup>+</sup> , Ca <sup>2+</sup> , Mg <sup>2+</sup> , Cl <sup>-</sup> , SO <sub>4</sub> <sup>2-</sup> , HCO <sub>3</sub> <sup>-</sup> )	Coastal saline soil	SPAD, fruit production, shoot biomass, leaf ion	[63]
16	'Angela' ( <i>Solanum melongena</i> )	'Maxifort' ( <i>Solanum lycopersicum</i> )	55.5 mM Na <sup>+</sup> and 148 mM Cl <sup>-</sup>	Sand tanks	Yield and ion uptake in leaves and fruits	[49]

reported that grafting with salt-tolerant rootstocks can improve the antioxidant capacity of scions under salt stress (Table 2). To protect the plants themselves from reactive oxygen species (ROS) induced oxidative damage, plants up-regulate antioxidant system to maintain the dynamic balance of ROS production and extinction. Plants have evolved nonenzymatic and enzymatic antioxidation mechanisms. Enzymatic antioxidants include catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR). The most common nonenzymatic antioxidants are glutathione (GSH in its reduced form), ascorbate (AsA in its reduced form), carotenoids, and tocopherols<sup>[7]</sup>.

Grafting can improve the activities of antioxidant enzymes. This phenomenon has been confirmed in different vegetable grafting complexes. In *Cucurbitaceae*, cucumber grown under Ca(NO<sub>3</sub>)<sub>2</sub> stress has higher activities of antioxidant enzymes if

they are grafted with selected bottle gourd rootstocks and suffers less oxidative damage. Using two genotypes as the scions, Yang et al. & Shu et al. reported that the enhancement of salt tolerance in rootstock-grafted watermelons is related to the activation of antioxidant systems activated by using rootstock grafting, including the SOD, POD, and APX<sup>[64,65]</sup>. The AsA-GSH cycle is the key mechanism for scavenging ROS in plant chloroplasts. Rootstock-grafted watermelons have higher APX, GR, and DHAR activities in their chloroplasts under 100 mM NaCl and higher MDHAR activity under 50 mM NaCl than self-grafted plants<sup>[41]</sup>. Zhen et al. suggested that the alleviation of growth inhibition of grafted rootstocks under salt stress may be related to the improvement of photosynthesis and the enhancement of antioxidant enzyme activity<sup>[37]</sup>. In Solanaceae, eggplant seedlings, using salinity tolerant eggplant cultivar as rootstock, were more tolerant to stress by excess of Ca(NO<sub>3</sub>)<sub>2</sub> than non-grafted seedlings, owing to the effectively

**Table 2.** Studies on the mechanisms by which grafting enhances the antioxidant system.

ID	Scion species	Rootstock species	Salt treatments	Growth conditions	Tested traits	Reference
1	'Jinyou No. 3' ( <i>Cucumis sativus</i> )	<i>Cucurbita maxima</i> × <i>Cucurbita moschata</i>	80 mM Ca(NO <sub>3</sub> ) <sub>2</sub>	1/2 Hoagland's solution	H <sub>2</sub> O <sub>2</sub> , Fv/Fm, and antioxidant enzymes	[70]
2	'Xiuli' ( <i>Citrullus lanatus</i> )	'Chaofeng Kangshengwang' ( <i>Lagenaria siceraria</i> )	100 mM NaCl	1/2 Hoagland's solution	NR, NiR, GS, GOGAT, and NADH-GDH activities	[41]
3	'Hybrid 1010' ( <i>Cucumis sativus</i> )	<i>Cucurbita moschata</i> , <i>Lagenaria siceraria</i> , <i>Citrullus lanatus</i> , and <i>Cucurbita maxima</i>	50 and 100 mM	Vermiculite and compost	Chlorophyll contents, net assimilation rate, stomatal conductance (gs), electrolyte leakage (EL), antioxidant enzyme activities, and endogenous phytohormones	[71]
4	'Naomi' and 'Artvin' ( <i>Solanum melongena</i> )	'AGR703' ( <i>Solanum aethiopicum</i> ), 'Yula', and 'Vista' ( <i>Solanum incanum</i> × <i>Solanum melongena</i> )	6–7 dSm <sup>-1</sup> NaCl	3:1 perlite: vermiculite	Leaf ion concentrations, antioxidant enzymes, and lipid peroxide	[67]
5	'Bark' ( <i>Solanum lycopersicum</i> )	'LA1995', 'LA2711', 'LA2485', and 'LA3845' ( <i>Solanum lycopersicum</i> )	100 and 200 mM NaCl	Peat moss, vermiculite, and perlite	Plant growth and yield, antioxidant enzymes, and UPGMA-based phylogenetic tree	[72]
6	'Jingxin No.2' ( <i>Citrullus lanatus</i> )	'Quanneng Tiejia' and 'Kaijia No.1' ( <i>Cucurbita moschata</i> )	200 mM NaCl	Hoagland's solution	Photosynthetic analysis, chlorophyll a fluorescence, K <sup>+</sup> and Na <sup>+</sup> contents, antioxidative enzyme activities, H <sub>2</sub> O <sub>2</sub> generation, and lipid peroxidation	[73]

scavenging system of free radicals and the protective mechanisms of antioxidant enzymes and polyamines<sup>[66]</sup>.

Leaf MDA and antioxidant enzyme activity are used as indicators of the level of salt tolerance of grafted eggplants. Rootstock-grafted eggplants have higher SOD, CAT, and APX activity levels than self- and nongrafted plants, and thus they have a better salt tolerance and growth rate<sup>[67]</sup>. Wei et al. observed that grafted eggplants maintain lower H<sub>2</sub>O<sub>2</sub> content and higher antioxidant enzyme activity under Ca(NO<sub>3</sub>)<sub>2</sub> stress than nongrafted plants<sup>[68]</sup>. Penella et al. reported that the antioxidant system of ungrafted pepper did not function as an ROS scavenger under salt stress although the ungrafted plant also showed an increased activity of the primary antioxidant enzymes, indicating that the rootstock may promote the antioxidant system to play a role in response to salinity<sup>[58]</sup>.

Polyphenols are phytochemicals with antioxidant activity. Kacjan Maršić et al. reported that grafting of pepper leads to a substantial increase in total phenolic content under 40 mM

NaCl conditions compared with nongrafted plants<sup>[14]</sup>. Similarly, López-Serrano et al. observed that salinity treatment considerably increases the total phenolic content of grafted pepper, which indicated that phenolic compounds are thought to help prevent the formation of reactive oxygen species and protect photosynthetic organs<sup>[51]</sup>.

H<sub>2</sub>O<sub>2</sub> induced by NADPH oxidase may act as a cascade reaction to trigger the antioxidant system in plants, thereby alleviating the oxidative damage induced by salt stress<sup>[69]</sup>. This finding confirms that ROS plays an important role in the salt tolerance of grafting complexes as an early signal (See Section 'Root signals play an important role in improving the salt tolerance of grafted vegetables').

### Grafting enhances the stability of the photosynthetic system

Grafting alleviates the inhibition of photosynthesis induced by salt stress (Table 3). Stomatal closure is the primary reason

**Table 3.** Studies on the mechanism by which grafting enhances the stability of the photosynthetic system.

ID	Scion species	Rootstock species	Salt treatments	Growth conditions	Tested traits	Reference
1	'Xiuli' ( <i>Citrullus lanatus</i> )	'Chaofeng Kangshengwang' ( <i>Lagenaria siceraria</i> )	100 mM NaCl	1/2 Hoagland's solution	Chlorophyll content, gas exchange parameters, chlorophyll fluorescence parameters, and Rubisco activity	[79]
2	'Jinchun No. 2' ( <i>Cucumis sativus</i> )	'Chaojiqianwang' ( <i>Cucurbita moschata</i> ) and 'Bouche' ( <i>Cucurbita ficifolia</i> )	90 mM NaCl	1/2 Hoagland's solution	Biomass, gas-exchange parameters (Pn), and synthetase (GS)	[9]
3	'Xiuli' ( <i>Citrullus lanatus</i> )	'Chaofeng Kangshengwang' ( <i>Lagenaria siceraria</i> )	100 mM NaCl	1/2 Hoagland's solution	Biomass, net photosynthetic rate, and proteomic analysis	[64]
4	'Jinchun No. 2' ( <i>Cucumis sativus</i> )	'Chaojiqianwang' ( <i>Cucurbita moschata</i> )	90 mM NaCl	1/2 Hoagland's solution	Growth parameters, gas-exchange parameter measurements, chlorophyll fluorescence, xanthophyll pigment analysis, and ultrastructure of chloroplasts	[78]
5	'Adige' ( <i>Capsicum annuum</i> )	<i>Capsicum chinense</i> , <i>Capsicum baccatum</i> , and <i>Capsicum annuum</i>	7.5 dS m <sup>-1</sup> with 57.5 mM of Na <sup>+</sup> and 71.2 mM of Cl <sup>-</sup>	Sandy loam soil	Biomass and ion determination, leaf water potential, and gas-exchange parameters	[11]
6	'Adige' ( <i>Capsicum annuum</i> )	A25 ( <i>Capsicum annuum</i> )	7.5 dS m <sup>-1</sup> with 57.5 mM of Na <sup>+</sup> and 71.2 mM of Cl <sup>-</sup>	Hydroponic greenhouse	Biomass and ion determination, gas exchange and PSII, and antioxidant enzymes	[58]
7	'Vedrana' ( <i>Capsicum annuum</i> )	'Rocal F1' ( <i>Capsicum annuum</i> )	20 and 40 mM NaCl	Perlite and rock wool flocks	Growth conditions, proline, photosynthetic measurements, sugars, and organic acids	[14]

## How grafting increases vegetable salt tolerance

that limits photosynthesis under salt stress. Stomatal closure under salt stress results in rapid reduction of carbon dioxide involved in photosynthesis. Rapid stomatal closure might be an alternative strategy to cope with salt stress. A recent study found that cucumbers grafted onto pumpkins (*Cucurbita maxima* × *Cucurbita moschata*) showed a more rapid stomatal closure than self-grafted cucumber. This reason was believed to be the enhanced adaptability of plants to early osmotic stress under saline conditions<sup>[65,74]</sup>. Photosynthesis is initially reduced by stomatal limitations, and then non-stomatal effects were not observed for hours or days after salt treatment until Na<sup>+</sup> or Cl<sup>-</sup> accumulated at higher levels in the leaves<sup>[75]</sup>. An early response of vegetables to salt stress is a reduction of leaf growth, which is caused by a drop in stomatal conductance in tomatoes<sup>[76]</sup>.

After the 'early osmotic stress', plants reopen their stomata and stomatal conductance recovers to a relatively normal level but still substantially lower than that under non-saline conditions<sup>[74,77]</sup>. In this stage, stomatal conductance is less affected in salt-tolerant rootstock-grafted combinations because the accumulation of large amounts of Na<sup>+</sup> in the leaves of scions forces the plant to close its stomata to reduce the amount of Na<sup>+</sup> delivered from roots to shoots via transpiration flow. Moreover, salt-tolerant rootstocks usually have a considerably higher ability to restrict Na<sup>+</sup> transport from roots to shoots<sup>[10]</sup>. A similar situation has been observed in Solanaceae. The stomatal conductance of tomato and grafted plants responds to saline water irrigation through stomatal closure<sup>[12]</sup>.

In plants under long-term salt stress, the decrease in photosynthesis is not only caused by stomatal closure, but also by non-stomatal factors. Grafting with salt-tolerant rootstocks can enhance photosynthetic rate by protecting chloroplast structure. For example, the reduction in PSII efficiency caused by salt stress is associated with the PSII complex, and pigment-protein complexes of the thylakoid membranes of chloroplasts. By investigating PSII efficiency, xanthophyll cycle, and chloroplast ultrastructure of nongrafted and pumpkin (*Cucurbita moschata*)-grafted cucumber plants, Liu found that chloroplasts are remarkably affected in the leaves of NaCl-treated cucumbers<sup>[78]</sup>. The starch grains of non-grafted plants and self-grafted plants were larger than those of rootstock grafted plants, reflecting that the salt stress affected cells to mobilize starch. A similar result has also been obtained in watermelon seedlings. In self-grafted watermelon, NaCl stress markedly reduces its chlorophyll content, inhibits its photochemical activity and CO<sub>2</sub> assimilation, and damages the structure of its photosynthetic apparatus. By contrast, grafting watermelon with bottle gourd rootstock increases its chlorophyll content, especially chlorophyll b, and minimizes the harmful effects of NaCl stress on PSII reaction center and thylakoid structures<sup>[79]</sup>.

Kacjan Maršič et al. analyzed the photosynthetic pigments in pepper leaves in both grafted and nongrafted plants. They reported that using the salt-tolerant rootstock 'Rocal F1' remarkably increases the level of photosynthetic pigments compared with 'Vedrana' nongrafted bell pepper<sup>[14]</sup>.

**Root signals play an important role in improving the salt tolerance of grafted vegetables**

The root is the first organ to be exposed to salinity. Early root signaling is crucial for plant adaptation to abiotic stress<sup>[80]</sup>. The early signals provided by rootstocks under salt stress play a decisive role in activating the salt tolerance of scions (Table 4). Most rootstock-borne signals are hormones and some are chemicals. Among these signals, ABA and ROS are the most widely studied root-borne signals for improving salt tolerance.

ABA is usually associated with stress response under abiotic stress. ABA can improve the salt tolerance of grafted vegetables by triggering rapid stomatal closure at the early stage of salt stress to avoid water loss. This process may be related to the ability of grafting to improve the sensitivity of scions to ABA. ABA sensitivity has been demonstrated to improve the water status of grafted tomato<sup>[81]</sup>, pepper<sup>[51]</sup>, and cucumber<sup>[74]</sup> under salinity. A study reported that grafting with pumpkin (*Cucurbita moschata*) rootstocks evidently improves the osmotic stress tolerance of cucumber scions under saline conditions<sup>[37]</sup>. During this process, ABA in the root acts as a signaling substance to mediate stomatal closure of aboveground cucumber scions, thereby preventing wilting due to excessive water loss under salt stress. The ABA signaling in roots may be part of the reason for the better performance of the pumpkin (*Cucurbita maxima* × *Cucurbita moschata*)-grafted cucumber than that of self-grafted plants under Ca(NO<sub>3</sub>)<sub>2</sub> stress<sup>[65]</sup>.

However, with the extension of salt stress, ABA concentration in shoots is negatively correlated with the salt tolerance of the grafted plants. Galvez et al. evaluated three pepper rootstocks with different growth and yield characteristics. They associated the invigorating effects of the rootstocks with the improvement in photosynthesis and K<sup>+</sup> homeostasis coordinately controlled by ABA<sup>[82]</sup>. Similarly, López-Serrano et al. reported that the reduction in leaf ABA content in plants grafted onto 'Niber' (a commercial pepper rootstock) under salinity keeps the stomata open, striking an appropriate photosynthesis balance and leading to NR activation<sup>[51]</sup>. Their results have provided clues for screening salt-tolerant rootstocks.

ROS signaling is critical to the acclimation of plants to salt stress conditions<sup>[83]</sup>. It is well known that hydrogen peroxide is one of the most common ROS signaling molecules with the ability to travel long distances. Root RBOH-dependent H<sub>2</sub>O<sub>2</sub> operates as a primordial signal to regulate the salt tolerance in

**Table 4.** Studies on the mechanism by which root signals improve the salt tolerance of grafted vegetables.

ID	Scion species	Rootstock species	Salt treatments	Growth conditions	Tested traits	Reference
1	'Jinchun No. 2' ( <i>Cucumis sativus</i> )	'Chaojiqianwang' ( <i>Cucurbita moschata</i> )	75 mM NaCl	Hoagland's solution	Photosynthetic rate, ABA, and ABA biosynthesis-related genes	[74]
2	'Jinchun No. 2' ( <i>Cucumis sativus</i> )	'Chaojiqianwang' ( <i>Cucurbita moschata</i> )	75 mM NaCl	Hoagland's solution	Biomass; MDA; Na <sup>+</sup> , H <sub>2</sub> O <sub>2</sub> , Na <sup>+</sup> , and H <sup>+</sup> fluxes along the stem; NADPH oxidase activity	[84]
3	'Adige' ( <i>Capsicum annuum</i> )	'NIBER®' ( <i>Capsicum annuum</i> × <i>Capsicum annuum</i> )	70 mM NaCl	Standard nutrient solution for pepper	Na <sup>+</sup> and K <sup>+</sup> concentrations; gas exchange; ABA, NR, and proline content; H <sub>2</sub> O <sub>2</sub>	[51]
4	'Gacela' ( <i>Capsicum annuum</i> )	'Creonte', 'Atlante', and 'Terrano' (commercial rootstocks)	35 mM NaCl	50% sand, 30% peat, and 20% vermiculite	Plant growth and fruit yield, gas exchange, and hormones (IAA, ZA, GA, SA, JA, ABA, ACC)	[82]

grafted cucumber by Na<sup>+</sup> exclusion<sup>[84]</sup> and stomatal closure<sup>[74]</sup>. However, NADPH oxidase-sourced H<sub>2</sub>O<sub>2</sub> is essential to maintain the transport capacity of H<sup>+</sup>/Na<sup>+</sup>. Diphenylene iodonium (an NADPH oxidase inhibitor) blocks salinity-induced H<sub>2</sub>O<sub>2</sub> production and reduces the salinity tolerance of grafted cucumber<sup>[84]</sup>. The mechanism by which NADPH oxidase-mediated H<sub>2</sub>O<sub>2</sub> signaling positively affects the SOS pathway has remained elusive until now. Previous studies have suggested that NADPH oxidase may operate as a sensor in plants. The NADPH works in tandem with Ca<sup>2+</sup>-permeable channels<sup>[80,83]</sup>.

The increase of H<sub>2</sub>O<sub>2</sub> in pepper-grafted plants has also been associated with high total antioxidant capacity and low lipid peroxidation. These results suggested that H<sub>2</sub>O<sub>2</sub> can be positively used to activate the antioxidant capacity of grafted plants and help them endure salt stress by acting as a signal substance rather than a damage to plant<sup>[51]</sup>.

### miRNAs mediating graft-dependent salt tolerance

In addition to regulating plant growth, miRNAs also respond to various abiotic stresses by negatively regulating the expression of target genes at the post-transcriptional level<sup>[26]</sup>. Many miRNAs, such as miR169, miR395, miR398, and miR399, have been identified in networks that respond to high salinity in *Arabidopsis thaliana*<sup>[85]</sup>, *Glycine max*<sup>[86]</sup>, and *Populus euphratica*<sup>[87]</sup>. A previous study found that grafting can alter the expression of miRNAs and their target genes; over 40 miRNAs were observed to change in the leaves of watermelon (*Citrullus lanatus*) after it was grafted onto bottle gourd (*Lagenaria siceraria*) or pumpkin (*Cucurbita maxima* × *Cucurbita moschata*)<sup>[88,89]</sup>. Under saline conditions, the post-transcriptional effects of the miRNA target network may be important in graft-dependent responses to salt stress. A study reported that the expression levels of most miRNAs in the leaves of pumpkin (*Cucurbita moschata*)-grafted cucumber seedlings increase after 6 and 24 h of salt stress compared with self-grafted cucumbers<sup>[90]</sup>. Moreover, the expression levels of most miRNAs in the roots of pumpkin (*Cucurbita moschata*)-grafted cucumber seedlings increase under normal and salt stress conditions<sup>[91]</sup>. The different responses to salinity of miRNAs might be decided by the genotype of rootstock. Xie et al. subjected two *Cucurbita* rootstocks, 'N12' (*Cucurbita maxima*) and 'N15' (*Cucurbita moschata*), with significant differences in Na<sup>+</sup> accumulation, to determine small RNA populations in root tissues after 4 h of salt treatment and controls<sup>[91]</sup>. Results indicated that the miRNAs between the two *Cucurbita* germplasms have differential expression under salt stress conditions. Owing to their vigorous vascular system, *Cucurbita* plants have been used as a model for understanding long-distance signal elements, including miRNAs transport. Tolstyko et al. demonstrated that the phloem transcriptome contains a subset of *Cucurbita maxima* pri-miRNAs that differs from a subset of pri-miRNA sequences abundant in a leaf transcriptome<sup>[92]</sup>. They validated this conclusion via interspecies grafting experiments, which showed that *Cucurbita maxima* pri-miR319a can be transported across the graft union, verifying the presence of pri-miR319a in sieve elements and indicating that phloem miRNA precursors may play a role in long-distance signaling in plants.

### Conclusions and challenges ahead

Grafting has been successfully practiced in many countries. This old technology can reduce the negative effects of salinity

on vegetables and provide an alternative way to cope with salt stress and maintain yield and quality.

The physiological mechanism of grafting to improve salt tolerance of vegetable crops has been discussed in detail in this review. It can be found that most studies focus on the effects of grafting on plant biomass accumulation, photosynthesis, salt ion accumulation and other aspects. However, there are few studies on the effect of grafting on improving the commodity quality of vegetables under salt stress. Recently, vegetable yield is no longer the only factor for production. How to improve the quality of vegetable crops under abiotic stress is the key question for future consideration<sup>[93]</sup>. Some rootstocks can improve the survival rate of vegetables under serious salt stress but cause deterioration of vegetable quality<sup>[94]</sup>. On the other hand, different genetic backgrounds and cultivation modes (hydroponics or substrate culture) significantly affect the salt tolerance of vegetables, and there was previously no standard for the evaluation of salt-tolerant rootstocks<sup>[95]</sup>.

There are few studies on the molecular mechanism of grafting to improve salt tolerance of vegetable crops. Considering that the key of grafting to improve salt tolerance lies in limiting salt ion transport, it is very important to reveal the function of related ion transporters located in the plasma membrane and vacuole membrane. Related genes and regulatory mechanisms need to be elucidated through a large number of studies. More importantly, hormone signaling plays an important role in rootstock – scion communication. Long distance transfer of genome – level mRNA is also widespread during grafting<sup>[96]</sup>. Mechanism of sensing salt signals in rootstock root, and long-distance signals between rootstock and scion under salinity need to be elucidated further in the future.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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