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Applied methodology of transgenics in vegetables for salinity stress tolerance: A comprehensive review

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Abstract

Vegetables play a vital role in human life as they are rich sources of essential vitamins, minerals, dietary fiber, and antioxidants, contributing to balanced nutrition, disease prevention, and overall health. They are also keys to ensuring food and nutritional security, especially in developing countries where vegetables form a major part of daily diets. However, vegetable production is increasingly threatened by salinity stress. In India, where large areas of arable land are affected by soil salinization due to irrigation practices, poor drainage, and climate change, vegetable cultivation faces severe challenges. Globally, rising salinity in agricultural soils exacerbated by seawater intrusion, overuse of chemical fertilizers and water scarcity is degrading vegetable productivity. Plants defend themselves against salinity stress through a combination of physiological, biochemical and molecular strategies. Different transgenic methods such as *Agrobacterium*-mediated transformation, gene gun (biolistic) delivery and CRISPR/Cas-based genome editing enable the precise introduction or modification of genes that improve salinity tolerance in vegetables. These approaches allow incorporation of traits like ion homeostasis, osmolyte biosynthesis, and antioxidant defense, which are difficult to achieve through conventional breeding due to genetic limitations and long breeding cycles. Thus, transgenic breeding provides a faster, targeted, and reliable strategy to develop salt-tolerant vegetable varieties, ensuring stable yields and quality under saline condition.

Keywords: Salinity tolerance, transgenic approaches, CRISPR/Cas9, *Agrobacterium*-mediated transformation, gene-gun

Introduction

Although humans use around three thousand plant species for sustenance, the majority of the world's population currently relies on 20 crop species to meet their primary calorie demands, with eight cereal crop species accounting for 50% of this total (Krishna *et al.*, 2019) [40]. Abiotic (drought, salt, heat, water logging, etc.) and biotic (virus, viroids, bacteria, fungi, nematodes and insects) stresses, as well as population growth are the primary causes of the limited availability of vegetables. These stresses may also lower the crops' yield and quality (Ingram, 2011; Prasanna *et al.*, 2015; Karkute *et al.*, 2019; Singh *et al.*, 2021; Soumia *et al.*, 2021) [30, 57, 33, 65, 72]. The primary obstacle that contemporary agriculture is facing now includes fulfilling the nutritional and food security of the world's expanding population.

Due to its persistence throughout the crop life cycle, salt stress is the second most damaging abiotic stressor. One of the most significant environmental factors limiting the economic production of vegetable crops is salinity stress (Hong *et al.*, 2021) [29]. According to Tiwari *et al.*, large-scale irrigation-associated intensive farming, excessive groundwater consumption, irrigation of crops with low-quality water and regular changes in the climate all have an impact on soil salinity. Furthermore, evaporation from irrigation dams, a lack of leaching rain and elevated bore water salinity might all contribute to extended water stress conditions in the soil, which would raise the salinity of the soil profile. According to Giordano *et al.* (2021) [21], vegetable crops are more vulnerable to climate change than other horticultural crops and salinity stress in particular affects the growth and development of these plants throughout their ontogeny.

The physiological result of too much salt in plant cells is salinity stress, which negatively impacts the metabolism of the plant. When the ECe (Electrical Conductivity of a Saturated Soil Extract) is ≥ 4 dS/m, or around 40 mM NaCl, and produces an osmotic pressure of 0.2 megapascal (MPa), the soil is classified as saline (USDA). Due to the significant impact that salinity stress has on agricultural production, an estimated 12 billion USD are lost globally each year on average (Zahedi *et al.*, 2019) [93]. As a result, high salinity affects roughly 10% of the world's total land surface (950 Mha), 20% of its cultivated land (300 Mha), and roughly 50% of its irrigated land (230 Mha) (Abiala *et al.*, 2018) [1].

Although, the development of stress-tolerant vegetable cultivars has benefited greatly from traditional breeding techniques. However, we still lack the best ways to stop the financial losses caused by salt stress on crops, especially in regions with heavy irrigation (Machado and Serralheiro, 2017) [46]. One of the most important tools for creating stress-tolerant vegetable crops is transgenic technology for salt stress tolerance (Kumar *et al.*, 2017) [41]. Vegetables respond differently to salt stress. While some crops, including beans (*Phaseolus vulgaris*), carrots (*Daucus carota*), and onions (*Allium cepa*) are vulnerable to salinity stress, other vegetables, like asparagus (*Asparagus officinalis*), may withstand it. Therefore, understanding the genetic underpinnings of the ability of vegetables to tolerate salinity is a prerequisite for plant breeders looking to create superior genotypes through the use of transgenic breeding methods.

For the purpose of improving plant genetics, transgenic breeding provides a viable substitute for traditional breeding. Techniques for genetic engineering and transgenic breeding have advanced significantly over the past 20 years in manipulating genes to induce desirable traits in transgenic plants. An integrated understanding of the molecular and physiological mechanisms controlling plant tolerance and production is made possible by transgenic techniques, which give us the ability to identify the candidate genes, miRNAs and transcription factors (TFs) involved in particular plant processes. This phenomenon's accuracy and precision guarantee future plant enhancements will be very successful. Transgenic breeding has therefore shown promise as a means of improving crops under abiotic stress. We have provided up-to-date information on biotechnology solutions for salt stress in vegetable crops in this review.

Impacts of Salinity Stress on the Plants

Oxidative stress in vegetables brought on by salinity may have an impact on the quality and quantity of the same since it may cause a variety of physiological and biochemical alterations in the plants (Kashyap *et al.*, 2020, 2021) [36]. Among these, membrane damage, substance leakage that results in plasmolysis and water imbalance, disruption of the ROS detoxification system, modifications to nutrient flux and dynamics and photosynthetic characteristics are the most frequent. Physiological processes including respiration, photosynthesis, transpiration, hormone control, water use efficiency, germination, antioxidant generation and plasma membrane permeability are ultimately impacted by these alterations.

The first physical characteristic response to salt stress is a decrease in leaf extension and stomatal closure. The excessive build up of Na^+ and Cl^- in plant lower leaves, along with its detrimental effect on ion concentration, can be found there. Thus, a drop in the leaf area index results from the build up of salts and an imbalance in nutrient intake (Shah *et al.*, 2021) [67].

Because of hyperosmotic pressure, cells in sodic soils lose more water than they absorb. The physical signaling, gene expression, metabolic and physiological processes and activities that result from water deficits generate a cascade of events that lowers the leaf area index, photosynthesis, biomass accumulation and yield (Machado *et al.*, 2017; Wei *et al.*, 2021) [46, 83]. The primary cause of rising Na^+ and Cl^- ion concentrations and plant toxicity in sodic soils is that 50-80% of soluble salts are NaCl. (Azizi *et al.*, 2021; Dong *et al.*, 2021) [7, 12]. These ions have a major effect on the signaling route of photosynthetic activity or the biochemical process of enzyme activity. Moreover, the Na^+ in saline soil solutions competes with K^+ for absorption because of physical, chemical, and transport system interactions between the two, which may result in a K^+ deficit in plant tissues (Rubio *et al.*, 2020; Fang *et al.*, 2021) [60, 15]. A K^+ concentration deficit in tissues, particularly in leaves, reduces growth because it is crucial for photosynthetic characteristics such stomatal conductance and function and cell turgor regulation.

Salt stress has a significant impact on the intricate plant physiological and biochemical process of photosynthesis in vegetables (Shams *et al.*, 2021) [69]. In vegetables that are susceptible to salt, stomata closure may result in a reduction in carboxylation and photosynthetic efficiency. By causing chlorophyll breakdown and over expressing pheophorbide *a* oxygenase (CaPAO), salinity stress also affects photosynthesis (Shams *et al.*, 2021) [69]. The maximum quantum yield of photosystem II (PSII) and the substomatal concentration of CO2 (Ci) are all altered by high salinity. In the end, it causes cell death by destabilizing the structure and permeability of the cell membrane as well as the structure and function of the proteins (Kanojia *et al.*, 2018; Muthu *et al.*, 2020) [32, 51]. Concurrently, it was shown that salinity stress decreased stomatal conductance and transpiration rates, degraded pigments and light-harvesting complexes and consequently decreased the quantum yield of photosynthetic energy and energy dissipation through non-photochemical means in lettuce, onion, tomato and sweet potato (Yang *et al.*, 2022) [89].

Under several abiotic stresses, a notable decrease in plant height has been observed. Salinity exposes plants to severe water shortage circumstances, which decrease leaf growth and leaf areas in a number of species, including cowpea (Manivannan *et al.*, 2007) [47], poplar (Wullschleger *et al.*, 2005) [86] and wheat (Sacks *et al.*, 1997) [64]. The dropping of older plant leaves is one illustration of the physiological changes brought on by salt (Shao *et al.*, 2008) [68]. Sharp and LeNoble (2002) [71] established a correlation between the ABA content of plants and the increase in the root to shoot ratio brought on by salt conditions. The distribution of biomass is closely linked to plant productivity in salinity.

Regardless of the main effects of salt stress, the build up of reactive oxygen species (ROS), which are harmful and alter gene expression and DNA methylation, is the secondary consequence of salinity on plant cells (Ranjan *et al.*, 2021) [62]. Additionally, ROS can result in lipid peroxidation, which increases the permeability and fluidity of the membrane.

Table 1: Salt Tolerance of some Vegetables

Sensitive	Moderately Sensitive	Moderately Tolerant	Tolerant
Daucus carota	*Brassica oleracea*	*Solanum lycopersicum*	*Asparagus officinalis*
Raphanus sativum	*Ipomoea batatas*	*Ocimum basilicum L.*	
Solanum melongena	*Curcuma longa*	*Beta vulgaris subsp. Vulgaris*	
Brassica rapa	*Allium sativum*	*Cucurbita pepo*	
Allium cepa	*Colocassia esculenta*	*Yellow scallop*	
Abelmoschus esculentus	*Solanum tuberosum*		
Manihot esculentua	*Curcubita moschata*		
Phaseolus vulgaris	*Citrullus lanatus*		
	Capsicum annum		

(Source: Shams and Khadivi, 2023) [70]

Mechanism of Salt Tolerance

A variety of morphological, biochemical, molecular and physiological characteristics that control plant development and productivity are linked to salinity tolerance (Alexieva *et al.*, 2001) [4]. Reducing water loss from the cuticle and stomata and optimizing root water intake to sustain the osmotic adjustment also aid in morphological and physiological adaptation towards resistance to the salt-induced osmotic stress (Rai *et al.*, 2021) [61]. A cascade of molecular networks controls tolerance and adaptation to salt stress, triggering response mechanisms such as the synthesis of stress proteins, the up regulation of antioxidants and the accumulation of compatible solutes to restore cell homeostasis, to repair and shield damaged proteins and membranes (Nahakpam and Shah, 2011) [52].

Because of the highest leaf relative water content (LRWC), leaf area index, biomass accumulation and photosynthesis, some vegetable crops, like *Beta vulgaris*, have the natural capacity to withstand the negative effects of high NaCl in the root zone or on the leaves without negatively affecting their yield. Certain crops can lessen the negative effects of NaCl by reducing osmotic adjustment and the absorption of Na^+ and Cl^- ions into the shoots. Through active or passive transport pathways, ions can flow through the root's membranes. However, a few organic acids and amino acids, including proline, glycine betaine, oxalate and malate, have been found to constitute putative osmotic processes in higher plants. Additionally, in response to salinity and drought stress, carbohydrates can agglomerate selectively (Liang *et al.*, 2018; Fang *et al.*,2021) [44, 15]. Plants developed ROS-scavenging mechanisms as part of their antioxidant defense systems. Under salinity stress, antioxidant enzymes like as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) are used to reduce ROS and preserve redox equilibrium (Shah *et al.*, 2021) [67].

Plants build up compatible, innocuous biomolecules that are essential to plant processes in order to withstand these different abiotic stressors. These consist of hormones including ABA, salicylic acid, and brassinosteroids, as well as polyamines, heat shock proteins (HSPs) and nitric oxide (NO). The protein known as late embryogenesis abundant (LEA) has been shown to be effective against a number of stresses including salinity, drought and cold (Xu *et al.*, 1996) [87]. These accumulating solutes play a part in the solubilization of proteins like glycine, ectoine and betaine as well as in uncharged solutes with ROS scavenging properties like mannitol and pinitol (Breś *et al.*, 2022) [10]. When Sodium Nitroprusside (SNP) is applied to soybeans (*Glycine max* L.), the physiological and morphological characteristics are improved in saline environments. By mimicking plant hormone and signal molecules, SNP as a Nitric Oxide (NO) donor has been shown to significantly improve the capacity for regeneration and tolerance to salinity (Jabeen *et al.*, 2020). NO is a vaporous molecule that plays an important part in signal transduction, plant growth and the regulation of several developmental processes, including cell death, seed germination, stomata closure, root growth, blooming and respiration (Pi *et al.*, 2016) [56].

Additionally, the phytohormone abscisic acid (ABA), which regulates plant physiological responses to many abiotic stressors, is synthesized in response to salinity-induced osmotic stress. Through the activation of genes involved in the biosynthesis pathway, increased salinity can promote the synthesis of ABA in plants. By changing the expression of stress-responsive genes, ABA not only communicates salinity to cells but also shields them from salt stress (Kaya *et al.*, 2020) [37]. One of the anatomical characteristics of salt-tolerant genotypes generally is an increase in the thickness of the leaf cuticle and leaf epidermis. Furthermore, physical characteristics those are well-known physiological mechanisms for plants to withstand salinity stress include reduced photosynthesis, closed stomata, high leaf temperature and a decrease in leaf turgor and chlorophyll concentration. Additionally, in monocotyledon vegetables like sweet corn, asparagus, yams, leeks and onions, intact bulliform cells, bulliform cell area, chloroplast content and chloroplast ultra-structure specifically, the length, width and width/length of chloroplasts can serve as effective markers for salt-unsusceptible genotypes (Fang *et al.*, 2021) [15].

Plants use two strategies to keep the salt from getting to the leaf surface. Salt ions can either collocate in the apoplast or enter the vacuole. There shouldn't be more salt ions present than what has accumulated in the vacuole (Volkmar *et al.*, 1998). Proline and total sugar content were both markedly elevated in melon (*Cucumis melo*) cultivars subjected to salinity stress (Akrami *et al.*, 2019). When exposed to salt stress, *Phaseolus vulgaris*, *Zygophyllum xanthoxylum* and *Lycopersicum esculentum* showed reductions in photosynthetic rate, stomatal/non-stomatal factors, deterioration in chlorophyll and carotenoid pigments and chloroplast destruction (Van *et al.*, 2020) [78]. Numerous techniques, such as transgenic development, transcription factor (TF) modulation and grafting, have been developed to assure vegetable yield during salt stress.

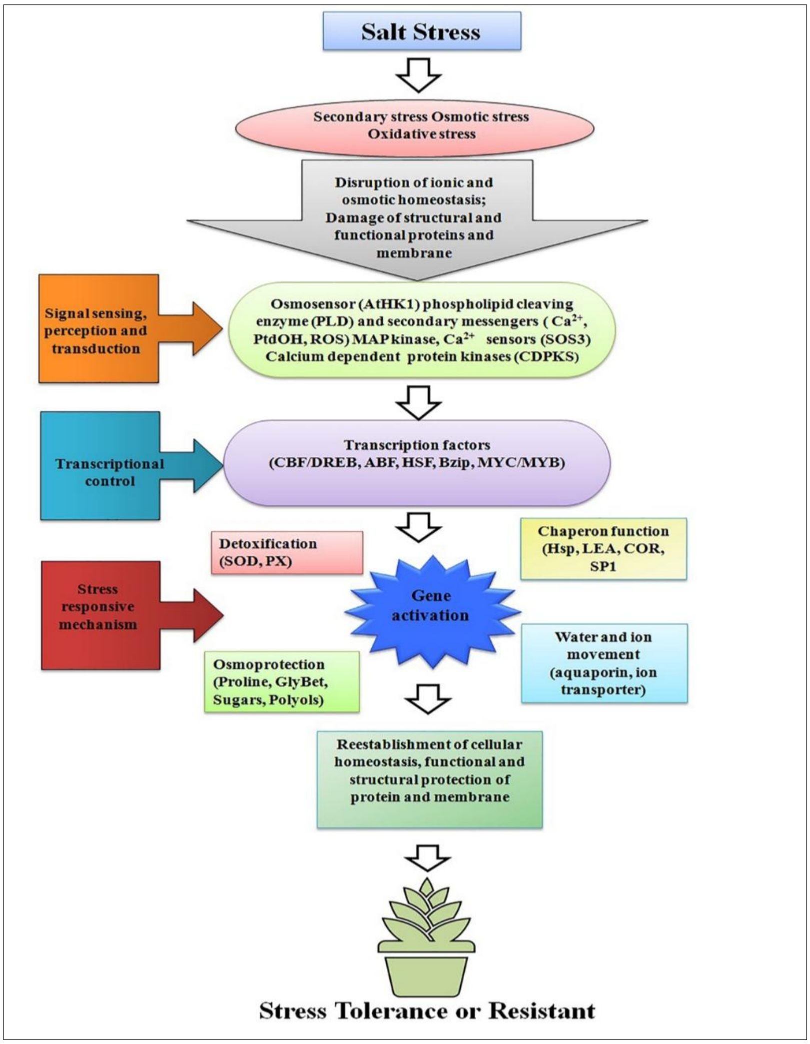
Transgenic Vegetables for Salt Stress Tolerance

Vegetables are consumed raw, semi-cooked, cooked and/or processed all over the world and are the least expensive source of nutrients, vitamins and antioxidants. While salt stress has no effect on vegetable productivity, it does have an impact on the crops' nutritional value. Since many vegetable crop gene pools lack unique salt tolerance, transgenes have been transplanted from non-parent sources such as bacteria, fungi, plants and animals. In order to create transgenic vegetable crops, the desired vegetables' genes for salt stress must first be found, described and then transferred. Salt stress induces the transgene and transgene overexpression or downregulation starts a chain reaction of stress-regulating phenomena that leads to salt tolerance (Figure 1).

Among vegetable crops, Solanaceous crops like potato, tomato, capsicum and chilli constitute major group of vegetables consumed all over the world and out of these, potato is the most important and ranks third in the world in terms of economic importance and a key agriculture crop for food and nutritional security. Potato is cultivated globally and very sensitive to salt stress and more than 60% crop loss is caused abiotic stresses including salt (Upadhyaya *et al.*, 2011; Xu *et al.*, 2014; Shafi *et al.*, 2017) [76, 88, 66]. To improve potato yield and quality under salt stress condition, many transgenic potato plants have been developed using different genes with different modes of action (Shafi *et al.*, 2017; Wang *et al.*, 2019; Ali *et al.*, 2020) [76, 81, 3]. The transfer of numerous osmoprotectant genes, such as P5CS, mtlD and AtBADH to potatoes greatly increases their ability to withstand salt stress (Karthikeyan *et al.*, 2011; Rahnama *et al.*, 2011; Zhang *et al.*, 2011) [35, 59, 95].

Similar to potatoes, tomatoes are the most processed product worldwide and the second most important vegetable fruit crop in the Solanaceae family. The tomato is a great source of vitamins, particularly vitamin C, minerals, proteins and carbs. There are numerous phytochemicals found in tomatoes, such as lycopenes and carotenes, which have anticancer effects and other health advantages (Rai *et al.*, 2021) [61]. Osmoprotectant genes such as BADH-1, ToOsmotin, Ectoine (ectA, ectB, and ectC), and coda gene have been altered in tomato farmed for salt stress tolerance. This transformation lessens the effects of salt stress by encoding osmoprotectant solutes (Moghaieb *et al.*, 2000, 2011; Goel *et al.*, 2010) [50, 22]. Numerous Na+/H+ antiporter genes, including NHX1, TaNHX2 and LeNHX4, which control the Na+/H+ to preserve cellular homeostasis, have also been altered to preserve cellular acidity under salt stress (Yarra *et al.*, 2012) [91]. Additionally, transgenes like as cAPX, MdSOS2L1, AnnSp2, LeNHX2 and SlSOS2, At FeSOD, and BcZAT12 have undergone transformation and function in various ways.

AVP1, which encodes vacuolar H^+-pyrophosphatase, which controls the proton pump and ultimately maintains the cellular acidity to prevent salt stress, was introduced into bottle gourds through the transfer of HAL1, which encodes for 32 kDa water soluble proteins that protect against salt-induced osmotic stress in watermelon (Park *et al.*, 2014; Han *et al.*, 2015) [55, 24]. Different genes, including CodA, PgNHX1, OsNASI, BnSIP1-1, APX, SOD and LEA4-1, have been transplanted to cole crops to tolerate salt stress because they are extremely sensitive to it (Park *et al.*, 2005) [54].



(Source: Behera *et al.*, 2022) [8]

Fig 1: Mechanisms of transgene action in transgenic plants; downstream signaling process and transcription controls that stimulates stress-responsive mechanisms to re-establish cellular homeostasis and damage repair

Transgenic Vegetables for Salt Tolerance

Plant biotechnological techniques, such genetic engineering and molecular breeding, provide the potential for quickly producing enhanced and genome-edited crops. Genetic engineering helps to overcome reproductive barriers in different plant species (Noman *et al.*, 2017) [53]. Through genetic mutation and enhancements, transgenic breeding effectively increased crop yields in a brief breeding period (Gantait & Mondal, 2018) [16]. These days, genome sequences are available for a variety of plant species, including those with vast and complex genomes, such as rice, tomatoes, cucumbers and *Arabidopsis* (Hameed *et al.*, 2018) [23]. To create transgenic crops with improved resistance to salt stress, it is crucial to identify the candidate genes that give plants resistance to various abiotic stresses. Using genetic engineering and hybridization with marker-assisted selection, the genes governing salt tolerance can be integrated into the genetic background of any desired cultivar after they have been identified using QTL mapping. Over the past 20 years, a lot of research has been done on plant engineering techniques for abiotic stress resistance. Researchers looked into the expression of genes that might be involved in signalling pathways for plant defence that encode proteins that provide resistance to abiotic stress. Although there have been some significant advancements in the present attempts to increase plant stress tolerance through genetic transformation, the endeavour is quite challenging due to the genetically intricate mechanisms of abiotic stress tolerance.

The potential for genetic improvement for a range of biotic and abiotic challenges is limited by the difficulty of tissue culture and genetic transformation in chilli plants (Kothari *et al.*, 2010) [39]. Subramanyam *et al.*, (2011) [73] used an *Agrobacterium tumefaciens*-mediated gene transfer technique to ectopically express the tobacco osmotin gene, effectively increasing the tolerance of chilli pepper (*Capsicum annum* L. cv. Aiswarya 2103) plants. In biochemical analysis, the T2 generation of transgenic pepper plants showed increased levels of proline, glycine betaine, ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD), chlorophyll, and relative water content (RWC). They also survived at salinity levels up to 300 mM NaCl.

Because of their high commercial worth, tomatoes are regarded as one of the most significant vegetable crops in the world. Wang *et al.* (2005) [80] used the cytosolic ascorbate peroxidase (cAPX) gene, which was obtained from peas (*Pisum sativum* L.), to create transgenic tomato plants that expressed resistance to chilling and salt stress. Ascorbate peroxidase is essential for quenching hydrogen peroxide (H_2O_2) in plant cells, protecting them from oxidative damage brought on by salt stress and cold. After five weeks at 9°C, the seed germination rate of transgenic plants was higher (26-37%) than that of the natural type (3%). In transgenic plants exposed to salt stress (200-250 mM), APX activity was shown to be 10-25 times higher, resulting in relatively less leaf damage. Lim *et al.* (2016) [45] inserted a strawberry D-galacturonic acid reductase (GalUR) gene into cherry tomato (*Solanum lycopersicum*) lines to boost the ascorbic acid content and improve tomato plants' resistance to salinity stress. When compared to wild-type plants, transgenic tomato plants were found with high fruit ascorbic acid concentrations were found to be more resilient to abiotic stress caused by viologen, NaCl, and mannitol. In addition to demonstrating increased expression levels of antioxidant genes like APX and CAT, which provide transgenic plants extra salt tolerance capabilities, the transgenic plants were able to survive under salt stress levels of up to 200 mM.

Numerous abiotic stressors, such as salinity, cold and oxidative stress, are the main elements restricting the production and cultivation of the root vegetable crop known as sweet potatoes (*Ipomoea batatas*). It has been noted that tolerance level of plant cells to various stressors is enhanced by increased glycine betaine synthesis. A chloroplastic betaine aldehyde dehydrogenase (SoBADH) gene from *Spinacia oleracea*, which is involved in the production of glycine betaine, was introduced into sweet potato cv. Sushu-2 by Fan *et al.* (2012) [13]. By protecting against cell damage by preserving cell membrane integrity, increasing photosynthetic activity, lowering ROS production, and activating ROS scavenging mechanisms, the overexpression of the SoBADH gene in transgenic sweet potatoes enhanced tolerance to salinity, oxidative stress and low temperatures.

In order to improve tolerance to salinity stress, Bulle *et al.* (2016) [11] created transgenic chilli pepper (*Capsicum annuum*) plants that express the wheat Na^+/H^+ antiporter gene (TaNHX2). PCR, Southern hybridization and RT-PCR were used to verify transgene incorporation and expression in T1 generation. When compared to non-transformed plants under salt stress, transgenic lines had lower levels of H_2O_2 and malondi-aldehyde and higher levels of proline, chlorophyll, superoxide dismutase, ascorbate peroxidase and relative water content in biochemical tests. To counteract salt stress, tomatoes have already been shown to overexpress the TaNHX2 gene (Yarra *et al.*, 2012) [91]. Han *et al.* (2015) [24] sought to increase bottle gourds' resistance to salt.

Table 2: Vegetables with Transgenic Breeding Methods for Salinity Tolerance

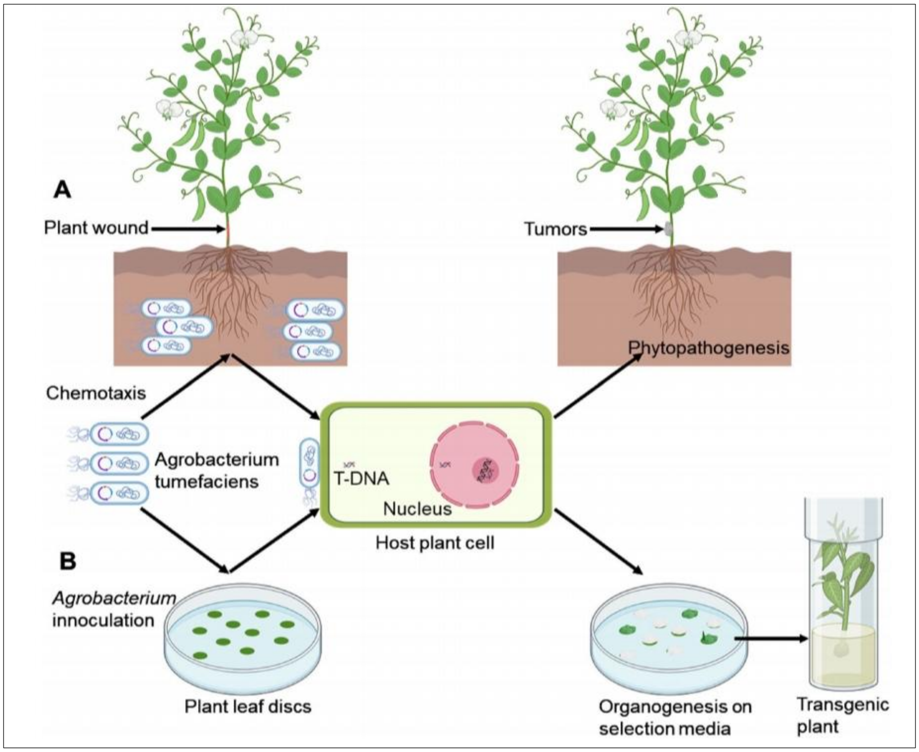
Vegetable	Gene(s) Used	Transgenic Method Used	Function	Reference
Tomato (*Solanum lycopersicum*)	codA (from *Arthrobacter globiformis*)	*Agrobacterium*-mediated transformation (overexpression)	Glycine betaine synthesis (osmoprotection)	Goel *et al.*, 2011 [22]
Tomato	AHK1 (from *Arabidopsis*)	*Agrobacterium*-mediated transformation	Na^+/H^+ antiporter; vacuolar ion sequestration	Zhang & Blumwald, 2001 [94]
Tomato	AVP1 (from *Arabidopsis*)	Overexpression via *Agrobacterium*	Vacuolar H^+-pump for ion homeostasis	Gaxiola *et al.*, 2001 [18]
Potato (*Solanum tuberosum*)	BADH (from spinach)	*Agrobacterium*-mediated transformation	Glycine betaine pathway	Holmström *et al.*, 2000 [28]
Potato	PR10 (from potato)	Native gene overexpression using 35S promoter	ROS scavenging, antioxidant defense	Hanafy *et al.*, 2024 [25]
Potato	SlCYS1 (cystatin)	*Agrobacterium*	Protease inhibitor; ROS and salt stress	FAO AGRIS,

			protection	2024 [14]
Potato	DREB1A (TF from Arabidopsis)	*Agrobacterium*, rd29A promoter	Induces stress-responsive genes	Bouaziz *et al.*, 2005
Carrot (*Daucus carota*)	AHK1 (from *Arabidopsis*)	*Agrobacterium*-mediated transformation	Ion sequestration in vacuoles	Wu *et al.*, 2005 [86]
Carrot	BADH (Betaine Aldehyde Dehydrogenase) from spinach	Chloroplast transformation via particle bombardment	Increases glycine betaine → better osmotic balance, salt tolerance	Kumar & Daniell, 2004
Carrot	P5CS (from mothbean)	*Agrobacterium*-mediated transformation	Increases proline → enhances osmotic adjustment under salt stress	Goel *et al.*, 2003 [22]
Cabbage (*Brassica oleracea*)	P5CS (from mothbean)	*Agrobacterium*-mediated transformation	Proline biosynthesis for osmotic balance	Siripornadulsil *et al.*, 2002
Eggplant (*Solanum melongena*)	AHK1 (from rice)	*Agrobacterium*-mediated transformation	Ion homeostasis (Na^+/H^+ antiporter)	Singh *et al.*, 2013
Chili Pepper (*Capsicum annuum*)	CaAl2B1	Overexpression using *Agrobacterium*	Oxidative stress resistance	Kong *et al.*, 2012
Chili pepper	TaNHX7 (from wheat)	*Agrobacterium*	Vacuolar Na^+/H^+ antiporter	FAO AGRIS, 2024 [14]
Onion (*Allium cepa*)	GsH and GhH (from rice)	Biolistic transformation (particle bombardment)	Methylglyoxal detoxification	Rahman *et al.*, 2016
Onion	OSISAP1 (stress-associated protein)	Microparticle bombardment (gene gun) into embryogenic onion tissue	Transgenics survived at 400 mM NaCl vs. non-transgenics dying at 200 mM; improved tolerance to salt & alkaline stress likely via enhanced cellular protection	Yi Chuan Xue Bao (1999)
Onion	PDH45 (Pea DNA helicase)	*Agrobacterium*-mediated transformation with PDH45 + GUS + hptH	Better salt tolerance via reduced ROS, higher antioxidant activity (SOD, CAT), increased proline, and protein content	Bhosale, 2016 [9]
Okra (*Abelmoschus esculentus*)	AdNAC53 (a NAC transcription factor)	Overexpression in *Arabidopsis* for functional assays; also VIGS (Virus Induced Gene Silencing) in okra for knock-down / regulatory confirmation	Regulation of root architecture, salt response	He *et al.*, 2024 [26]
Vegetable soybean	SP5CS (from *Solanum torvum*)	*Agrobacterium*, CaMV35S promoter	Proline biosynthesis (osmolyte)	Lee *et al.*, 2015
Cucumber (*Cucumis sativus*)	CmHKT1;1	*Agrobacterium*-mediated transformation	Enhanced Na^+ exclusion and K^+ uptake, improved K^+/Na^+ balance	Gao *et al.*, 2020 [17]
Watermelon (*Citrullus lanatus*)	CmHKT1;1	*Agrobacterium*-mediated transformation	Selective Na^+ transport from rootstock to scion, reduced Na^+ accumulation in shoots	Sun *et al.*, 2018 [75]
Citrullus lanatus (Watermelon)	HAL1 (S. cerevisiae)	*Agrobacterium*-mediated transformation; overexpression (CaMV 35S + RNA4 leader)	Reduces Na^+ toxicity; improves ionic balance; better root/leaf growth under NaCl	Xu *et al.*, 2003
Cucurbita moschata (Pumpkin) → Cucumber	CmHKT1;1 (Na^+ transporter)	Overexpression in cucumber; also used via grafted pumpkin rootstock	Restricts Na^+ transport to shoots; enhances K^+/Na^+ ratio	Sun *et al.*, 2018 [75]
Cucurbita moschata (Pumpkin)	CmCNIH1 (cargo receptor)	CRISPR/Cas9 knockout in pumpkin roots	Regulates localization of CmHKT1;1; loss leads to salt sensitivity	Wei *et al.*, 2023 [84]
Bottle gourd (as rootstock)	AVP1 (*Arabidopsis* H^+-pyrophosphatase)	Transgenic bottle gourd lines expressing AVP1; homozygous T2; also used as rootstock for grafting watermelon; compared with wild type	Improved relative water content; better recovery after desalinization; in grafted watermelon, higher plant biomass, better photosystem II quantum yield under salt stress	Han *et al.*, 2015 [24]

Transgenic Breeding Strategies

Agrobacterium-mediated transformation is a widely used method for transferring genetic material into plant cells, in which *Agrobacterium tumefaciens* is exploited as a natural plant pathogen to transfer T-DNA into host plant genomes (Wang *et al.*, 2023) [82]. This approach is effective and suitable for both monocot and dicot plant species. It has been effectively used for crops including cotton, soybean and maize as well as model plants like tobacco (Karlson *et al.*, 2022) [34] and *Arabidopsis thaliana* (Gelvin, 2003) [19]. Potential disruption of endogenous genes or regulatory elements during T-DNA integration is one of the challenges in *Agrobacterium*-mediated transformation, which may result in unexpected physiological effects in plants (Anjanappa and Gruissem, 2021) [5]. The different transformation efficiencies that exist among plant species and genotypes add to the complexity and call for specialized optimization techniques. Furthermore, there are worries regarding the possible spread of antibiotic resistance genes from GM plants into the environment when using antibiotic selection markers to identify altered cells (Phillips, 2008).

In tomato (*Solanum lycopersicum*)**,** genes such as *AtNHX1* (a vacuolar Na^+/H^+ antiporter from *Arabidopsis*) have been introduced to improve salt tolerance by enhancing sodium compartmentalization within vacuoles. Similarly, overexpression of the *BADH* (betaine aldehyde dehydrogenase) gene in tomato has led to increased glycine betaine production, improving osmoprotection under saline conditions. In brinjal (eggplant)**,** transformation with genes like *OsTPS1* (trehalose-6-phosphate synthase from rice) has shown enhanced salt tolerance by stabilizing proteins and membranes. Carrot (*Daucus carota*) has also been genetically modified using *Agrobacterium* to express stress-responsive genes such as *MnSOD* (manganese superoxide dismutase), which improves oxidative stress management under saline environments. In lettuce (*Lactuca sativa*)**,** *Agrobacterium*-mediated expression of *P5CS* (Δ^1-pyrroline-5-carboxylate synthetase) has resulted in higher proline accumulation, contributing to improved salt tolerance. These examples demonstrate the potential of *Agrobacterium*-mediated transformation in developing salinity-tolerant vegetable cultivars through targeted genetic interventions.



(Source: Rahman *et al.*, 2024) [58]

Fig 2: *Agrobacterium*-mediated transformation for genetic improvement of plants. A: Natural infection of plants producing crown gall disease, B: Engineering *Agrobacterium* for plant genetic transformation

Gene gun-mediated transformation, also known as biolistic particle bombardment, is a flexible technique that introduces foreign DNA into plant cells without requiring particular interactions with plant microbes. A shock wave or electrical discharge is used to propel high-velocity microprojectiles coated with the desired genetic material into plant tissue. The capacity of this method to change a variety of plant species, including resistant ones, makes it useful (Kikkert *et al.*, 2005) [36]. Nevertheless, these techniques have drawbacks that may reduce their levels of transformation efficiency, including random DNA integration and possible cell/tissue damage (Su *et al.*, 2023) [74]. In vegetables such as tomato, carrot and lettuce, genes involved in ion homeostasis, osmoprotectant synthesis, and antioxidative defence—like *NHX1* (vacuolar Na^+/H^+ antiporter), *P5CS* (proline biosynthesis), and *SOD* (superoxide dismutase)—have been introduced using biolistics to enhance salt stress tolerance. This method facilitates rapid gene delivery and expression, helping plants maintain cellular homeostasis and reduce oxidative damage under saline conditions.

Plant cells undergo protoplast fusion, which produces protoplasts by removing their cell walls. It is possible to fuse these protoplasts to produce hybrid cells that combine genetic material from many sources (Yue *et al.*, 2021) [92]. The ability to transfer genetic material between distantly related plants and the ability to circumvent species boundaries are just two benefits of protoplast fusion (Reed and Bargmann, 2021) [63]. Numerous plant species have successfully used this strategy, allowing for the creation of important crop variants and the manufacture of unique features. Among its drawbacks are its poor fusion efficiency and the possibility of unstable or chromosomal rearrangements in the resultant hybrid cells (Yue *et al.*, 2021) [92]. In vegetables such as tomato**,** carrot**,** and cabbage, protoplast fusion has been used to introduce traits like disease resistance, abiotic stress tolerance (including salinity) and improved nutritional quality. For example, somatic hybrid plants generated through protoplast fusion have combined salt tolerance from wild relatives with desirable agronomic traits of cultivated varieties. Additionally, protoplast fusion facilitates genetic recombination without sexual reproduction, thus speeding up the development of novel hybrids with enhanced traits. Coupled with modern genome editing techniques, protoplast fusion remains a promising tool for precise and efficient gene transfer in vegetable breeding programs.

Both monocots and dicotyledons are among the many plant species that can be electroporated. Electric pulses are used to create temporary holes in the cell membrane that allow foreign chemicals to enter the cell efficiently. High delivery efficiency is provided by electroporation, but it can potentially cause tissue injury and lead to nonspecific genetic material absorption (Gehl, 2003) [20]. In order to achieve targeted and desired genetic alterations while reducing any negative consequences, it is crucial to optimize the pulse settings and conditions while using this procedure.

According to Yang *et al.* (2023) [90], PEG is frequently utilized as a delivery system to introduce genetic material into plant protoplasts. PEG helps molecules including DNA, siRNA, miRNA and others enter protoplasts. PEG and the necessary genetic material are both present in the solution that is used to incubate the protoplasts. PEG allows genetic material to enter the protoplasts by causing cell membrane permeability and fusion. Osmotic pressure caused by PEG's large molecular weight and hydrophilia causes the protoplast membrane to momentarily rupture. Genetic material can enter the cell more easily as a result of this disturbance. However, the overall effectiveness of PEG-mediated transformation may be limited due to the possibility of cytotoxic effects on cells and low regeneration rates (Mathur and Koncz, 1998) [48].

Polyethylene glycol (PEG)-mediated gene transfer has been widely utilized in vegetable crops to enhance and study salinity tolerance. This method involves introducing salt stress-responsive genes into protoplasts, allowing researchers to rapidly analyze gene function without the need for stable transformation. For example, in tomato (*Solanum lycopersicum*), genes such as *SlSOS1* and *SlNHX1*, which regulate ion transport and homeostasis under salt stress, have been transiently expressed via PEG-mediated transformation to evaluate their role in salinity tolerance. Similarly, in chickpea (*Cicer arietinum*), PEG-mediated gene transfer has enabled the introduction of *CaCBL* genes, involved in calcium signaling, to assess their contribution to salt stress responses. In eggplant (*Solanum melongena*), antioxidant enzyme genes have been transiently expressed using PEG to study their effect on mitigating salinity-induced oxidative damage. Overall, PEG-mediated gene transfer provides a rapid and efficient tool for functional genomics in vegetables, facilitating the development of salinity-tolerant varieties by enabling detailed gene characterization and genome editing in protoplast systems.

The pollen tube pathway serves as a technique to deliver foreign DNA into plant cells across both monocot and dicot species. This method involves injecting pollen grains with exogenous DNA, which is then transported by the elongating pollen tubes toward the ovary. Nevertheless, the efficiency of this approach is hindered by obstacles encountered by the pollen tubes during growth, competition between the plant's native DNA and the introduced DNA, as well as the vulnerability of the foreign DNA to degradation (Laforest and Nadakuduti, 2022) [42]. Current research is focused on enhancing this method by refining the DNA delivery process, improving the stability of the DNA during pollen tube extension, and mitigating competition with endogenous DNA. Ongoing research aims to optimize this delivery system by enhancing DNA stability and improving the uptake process to achieve higher transformation rates in vegetable crops.

Originating from the adaptive immune system of bacteria and archaea, CRISPR/Cas has quickly developed to use the Cas9 protein and single guide RNA (sgRNA) to pinpoint and alter specific DNA regions in the target genome (Wright *et al.*, 2016) [85]. Since the system is programmable, scientists may create a sgRNA molecule that will attach to the desired DNA sequence precisely, guiding the Cas9 protein to make precise changes such gene insertions, deletions, or replacements (Li *et al.*, 2023; Wang *et al.*, 2023) [43, 82]. CRISPR technology allows for efficient and versatile native genome editing. Because of its accuracy, genetic sequences can be added, removed, or altered at precise locations, and organism characteristics can be tailored without introducing extraneous DNA. Compared to conventional techniques, this flexibility in altering the native DNA reduces off-target effects and unintentional changes more.

CRISPR technology has been increasingly applied to enhance salinity tolerance in various vegetable crops by precisely editing genes involved in salt stress responses. For instance, in tomato plants, CRISPR-Cas9 has been used to modify the SlSOS1 gene, which regulates sodium ion transport, thereby reducing toxic ion build up and improving salt tolerance. Similarly, in cucumber, the CsHKT1 gene, responsible for sodium uptake, has been targeted to limit sodium accumulation under saline conditions. In chilli peppers, genes related to osmotic stress signaling, such as CaERF1, have been edited to strengthen the plant’s ability to cope with salt stress. These examples demonstrate the potential of CRISPR to tailor specific genetic pathways, enabling vegetable crops to better withstand saline environments and maintain productivity.

Table 3: Summary of delivery methods used in plant transformation

Transformation techniques	Targeted tissues	Species	Delivery methods	Limitations
Agrobacterium	Root, shoot apical meristem, leaf, flower, hypocotyl, cotyledon	Monocots, dicotyledons	DNA	Host limitation
Biolistic/gene gun	Callus, shoot apical meristem, embryo, leaf	Monocots, dicotyledons	DNA, siRNA, miRNA, ribonucleo proteins	Cell damage, low integration efficiency, low expression levels
Protoplast fusion	Callus, shoot apical meristem, protoplast	Monocots, dicotyledons	DNA, siRNA, miRNA	Variable efficiency, requirement of cell fusion
Electroporation	Leaf, protoplast, meristem, pollen grain	Green algae, monocots, dicotyledons	DNA, siRNA, miRNA, proteins	Damage tissue, non-specific
Polyethylene glycol (PEG)	Protoplast	Monocots, dicotyledons	DNA, siRNA, miRNA	Cytotoxicity, low regeneration
Pollen tube pathway	Pollen tube	Monocots, dicotyledons	DNA	Low efficiency
Nanoparticles	Leaf, protoplast	Monocots, dicotyledons	DNA, siRNA, miRNA	Low efficiency

Conclusive Remarks

Plants react differently to salt stress, which can induce both osmotic and ionic stress. Determining the proline, ABA, and Na^+:K^+ ratio is crucial and these could be used to boost agricultural plant tolerance mechanisms. Under salt stress, a large number of genes that control plant tolerance pathways are activated. Enhancing and releasing novel salt-tolerant crop plant cultivars into the environment requires an understanding of the molecular mechanisms underlying salt stress. In order to adjust to salt stress, physiological, biochemical and molecular variables must also be taken into account. Moreover, integrated transgenic techniques are essential for genetic engineering to address the issue and provide extensive knowledge about tolerance mechanisms. Transgenic breeding approaches have emerged as a promising strategy to enhance salinity stress tolerance in vegetable crops, offering targeted and efficient solutions where conventional breeding often falls short. By introducing genes responsible for ion homeostasis, osmolyte accumulation, antioxidant defence and stress signaling, transgenic methods enable the development of cultivars with improved growth, yield and quality under saline conditions. While challenges such as biosafety concerns, regulatory barriers and public acceptance remain, the integration of transgenic breeding with advanced tools like genome editing and marker-assisted selection holds immense potential. Ultimately, harnessing these technologies after addressing a few challenges can contribute to sustainable vegetable production in saline-affected regions, ensuring food and nutritional security in the face of climate change and soil degradation.

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