Biotechnological Tools to Develop Abiotic Stress-Tolerant Plants

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Biotechnological tools include several methods used for plants to develop tolerance to abiotic stress. The genetic transformation of tomato relies highly on the tissue culture technique. Advances in the field of plant genetic transformation have enabled the identification of genes that are responsible for tolerance to different environmental stresses. Various biotechnological tools can be used to alter the tomato genes so that this species can more rapidly or better adapt to abiotic stress. Further advancement in understanding the genomics of wild relatives of tomatoes and other Solanaceae has facilitated their exploitation in various breeding programs aiming to introgress genes responsible for abiotic stress resistance in cultivars.

Solanum lycopersicum biotechnology drought salinity

1. Genetic Transformation Methods in Tomato

Multiple transformation techniques have been utilized to deliver foreign DNA sequences into an ample range of plant species [1]. The combination of recombinant DNA technologies, genetic transformation, and plant tissue culture are at the core of the production of transgenic plants in a variety of crops [2][3][4][5][6][7][8][9][10][11][12]. In tomato, the first genetic transformation protocol was developed in the 1980s [13], and still today, *Agrobacterium*-mediated techniques are widely employed for many tomato cultivars [14]. Transformation mediated by the *Agrobacterium* is a complex process. Briefly, the efficiency of gene delivery into tomato plants depends on various factors, such as the pre-culture of the explants, culture media, culture density, virulence and strain of *Agrobacterium*, phytohormones, type of explants, vectors, size of DNA insert, and genotype of the recipient plant [15][16]. **Table 1** presents a short selection of research efforts devoted to improving the process of tomato genetic transformation. Genetic transformation can also be obtained using *A. rizogenes* [17]. However, some detrimental phenotypes can be observed in tomato plants, such as shortened internodes, reduced seed setting, and wrinkled leaves. *A. rizogenes*–mediated transformation can be utilized for the in vitro production of compounds in tomato with biopharmaceutical properties. Besides indirect genetic transformations, direct methods like particle bombardment have also been reported for tomato [18]. This method was optimized by altering factors such as the quality and quantity of DNA, concentration of osmoticum in the tissue culture media, firing separation, and period of particle bombardment to which tomato explants are exposed [19].

Table 1. Investigations focused on improving the efficiency of tomato genetic transformation.

S. lycopersicum Cultivar	Transformation Method	Type of Explant	Transformation Frequency (TF)	References
Micro-Tom	Indirect	Embryonic part of the seedling	11%	[17]
NA	Indirect	Fruits	54 to 68.0%	[20]
Micro-Tom	Indirect	Cotyledons (embryonic part)	5.1%	[21]
Hezuo 908	Indirect	Hypocotyls and embryonic part	40%	[16]
Roma and Rio Grande	Indirect	Hypocotyls and leaf disks	24% and 8%, respectively	[22]
Momotaro, UC-97, and Edkawi	Indirect	Hypocotyls	54 to 67%	[23]
Castle Rock	Direct	Hypocotyls and part of cotyledons	26.5%	[24]
Cambell-28	Indirect	Cotyledons	21.5%	[25]
Pusa Ruby, Sioux, and Arka Vikas	Indirect	Cotyledons	41.4%, 22%, and 41%, respectively	[26]
Hezuo 908	Indirect	Embryonic part and Hypocotyl	40%	[16]
Shalimar	Indirect	Shoot and Leaf	NA	[27]
MicroTom	Indirect	Leaf	19.1%	[28]
NA	Indirect	Hypocotyls	33 to 59%	[17]
Pusa Ruby and DT-93	Indirect	Cotyledons	higher than 37%	[29]
Summer	Indirect	Hypocotyls and cotyledons	7%	[30]

Footnote: NA: not available.

Besides the addition of a new DNA sequence or (untargeted) mutation of the tomato genome, recent advances in recombinant DNA technology and reverse genetic approaches, such as antisense technology, RNA interference (RNAi), and genome editing by CRISPR-CAS9, have revolutionized functional genomics in plants. These approaches have been utilized in tomato cultivars to delay their ripening during abiotic stress, such as extreme temperature, by silencing the gene *vis 1* [31][32].

Overall, the genetic transformation of tomato is a mature and well-established technique that is employed by numerous laboratories around the world. Although improvements in regeneration and transformation efficiency are always welcome, the production of genetically modified tomatoes should not be considered a limiting factor for

biotechnological approaches since efficient and repeatable transformation and regeneration protocols are widely available.

2. Transformation Approaches Using rDNA Technologies (Genetic Engineering)

Climate change is predicted to increase the occurrence of abiotic stress, further hampering the ability of plants to yield [33]. Traditional plant breeding has limitations for creating a substantial level of tolerance against abiotic stress because it is time-consuming and often requires a complex breeding scheme to insert multiple sources of variability from wild relatives to a cultivated variety. Recombinant DNA (rDNA) technology–based tools have been traditionally considered alternatives to change the genetic constitution of plants. Different rDNA technologies have been employed to modify the tomato genome so that it can adapt to abiotic stress. These modifications include the exploitation of regulatory genes highly expressed during stress and coding for enzymes whose biochemical or enzymatic activity is useful to counteract abiotic stress [34].

2.1. Mannitol

Mannitol is an important polyol (sugar alcohol) produced from fructose metabolism and serves as a scavenger of free radicals and osmoregulation. The enzyme involved in fructose metabolism to obtain mannitol is mannitol-1-phosphate dehydrogenase, and the corresponding gene encoding this enzyme is *mt1D* [35][36]. In tomato, the constitutive expression of a bacterial *mt1D* gene driven by the CaMV 35S promoter provides improved tolerance against chilling, drought, and saline stress [37].

2.2. Glycine Betaine

It is an organic compound derived from the amino acid glycine, whose accumulation in plants may occur following abiotic stress. In plants, this compound is considered an organic osmolyte, ensuring, for instance, the regulation and preservation of the thylakoid membrane and, thus, sustaining the photosynthetic efficiency under stress [38]. Various studies have used biotechnological tools, such as the overexpressing of this compound, to facilitate an increased response of plants to abiotic stress tolerance. For example, the expression of the bacterial choline oxidase A (coda) in tomato targeted to the chloroplasts with a transit peptide resulted in an accumulation of glycine betaine in a relatively low (0.09 to 0.30 µmol·g^{-1} FW) but significant (up to 86% in chloroplasts compared to unstressed control plants) amount, sufficient to enhance tolerance to chilling at various phenological stages, as indicated by an increased yield in stress conditions of the transgenic plants [39].

2.3. Glutathione

Glutathione, an important antioxidant performing multiple functions in plants, is synthesized from amino acids (i.e., L-glutamate, cysteine, and glycine). The whole process requires two ATP molecules and is catalyzed by two glutamate enzymes—cysteine ligase (GCL) and glutathione synthetase (GSS). This tripeptide provides protection

at cellular and tissue levels in response to various reactive oxygen species (ROS), such as peroxides, superoxides, and hydroxyl radicals [40]. Glutathione has an important role during induced stress. The constitutive expression in tomato of a Se-independent glutathione peroxidase (GPx5) from *Mus musculus* resulted in an increased tolerance to mechanical stress [41]. Similarly, the concurrent constitutive expression of two glyoxalase (GlyI and GlyII) from *Brassica juncea* in tomato showed a reduced growth depression and membrane damage (as indicated by the level of lipid peroxidation and hydrogen peroxide production in leaves) following long-term exposure (3 months) to salinity (up to 800 mM NaCl) [42].

2.4. Osmotin

Osmotin is a 26 kDa protein, a member of the PR-5 family, which also includes zeamatin and thaumatin. It accumulates in plants as a defense mechanism against abiotic stress because of its prominent role in osmoregulation [43]. It has been reported that tomatoes constitutively expressing an osmotin gene from *Nicotiana tabacum* have higher levels of proline, increased chlorophyll contents, and higher water contents (under stress). These features were considered crucial in helping tomato withstand salt stress (150 mM NaCl for 10 days) [44]. The osmotin from *N. tabacum* was also used to increase pathogen resistance in transgenic barley, while it did not have a significant impact on insect-borne virus infections (by aphids and leafhoppers) [45]

2.5. Polyamines

Polyamine (PA) is a term used to indicate the wide class of organic molecules having multiple (more than two) amino acid groups. In plants, naturally occurring, low molecular–weight polyamines are associated with embryogenesis, organogenesis, anthesis, fruit development, ripening, and leaf senescence, but there is also evidence of their role in stress response [43]. The most common and abundant PAs in plants are putrescine (a diamine) and its derivatives spermidine and spermine [46]. Tomato transformed to constitutively express the arginine decarboxylase gene from *Poncirus trifoliata* (PtADC), indirectly involved in the biosynthesis of putrescine, showed increased levels of free PAs and improved tolerance to leaf dehydration and drought stress [47]. Tomato genetically transformed to constitutively overexpress the tomato *SlSAMS*$_1$ gene accumulated PAs and hydrogen peroxide and had an improved alkali stress tolerance. This gene is a member of the S-adenosylmethionine synthetase (SAMS) family, and it is stress-inducible. These genes catalyze the formation of SAM, which is also a precursor to PAs [48].

2.6. Trehalose

Trehalose is a highly soluble disaccharide made of glucose subunits, and it is present in a wide range of organisms, including prokaryotes, algae, mosses, fungi, protozoa, and mammals. Trehalose appears to be able to play a special function as a stress metabolite protecting the integrity of the cell against environmental stress and nutrient limitations [49]. Traditionally, trehalose has been of little importance for angisoperms, where another non-reducing saccharide, sucrose, has a predominant role in carbon storage and transport. Nonetheless, the discovery of gene families encoding trehalose phosphate synthases (TPSs) and trehalose phosphatases, along with their subsequent functional characterization, indicated that trehalose acts mainly as an osmoprotectant and as a signal

molecule involved in stress response. Recombinant DNA technologies have made it possible to modify genes governing trehalose metabolism in tomato. For example, the constitutive expression of *Saccaromyces cerevisiae ScTPS1* improved tolerance against drought or salt. Nonetheless, transgenic plants had phenotypic abnormalities and alterations in carbohydrate biosynthesis [50].

2.7. Biosynthesis of Ethylene

Ethylene is a well-known plant hormone whose commercial derivatives are also used to induce post-harvest tomato ripening. Because of its applied importance, there are several studies on genetically modified tomato cultivars with altered ethylene pathways in relation to fruit maturation. Ethylene production is typically increased in stressful environmental conditions. Several works have demonstrated that one of the positive effects of plant growth–promoting rhizobacteria (PGPR) is lowering the ethylene level under stress by cleaving and deaminating aminocyclopropane-1-carboxylic acid (ACC), the precursor of ethylene, by ACC-deaminases [51]. Tomato cultivars expressing a bacterial ACC deaminase under constitutive and inducible promoters were more tolerant to flooding [52].

2.8. Aquaporins

Aquaporins (AQPs) are trans-membrane proteins that allow the movement of water and small solutes between and within cells. Numerous studies have reported the potential roles of aquaporins in relation to abiotic stress in plants, water use efficiency (WUE), and solute transport in plants [53][54]. In tomato, over forty members of the AQP gene family have been linked to abiotic stress and plant development, mainly because of their expression pattern [55]. The overexpression of genes regulating the formation and functioning of aquaporins, such as *SlTIP;2*, in tomato increased the tolerance to abiotic stress. Interestingly, transgenic plants were more productive and had higher biomass than untransformed controls in normal and drought conditions [56]. An AQP from apple (MdPIP1;3) was also used to increase fruit growth rate and size mainly thanks to bigger cells, also increasing tolerance to drought stress [57]. Similarly, the overexpression of *SlPIP2;1* conferred to tomato's higher hydraulic conductivity and tolerance against drought stress [58][59].

2.9. Heat Shock Proteins

A set of relatively conserved, ubiquitous proteins, referred to as heat shock proteins, are synthesized by virtually all organisms, including plants, in response to various environmental stresses. These proteins often serve as intracellular chaperones and, for the establishment of protein-protein interaction, are involved in protein folding, assembly, translocation, degradation, and transport [60]. Different genes encoding HSPs (e.g., *HsfA1*, *HsfA2*, *HsfB1*, *LeHSP 17.6*) have been identified and delivered to tomato to facilitate the production of HSPs, with the common aim of helping plants better adapt to stress [61][62]. Moreover, the overexpression in tomato of *LeHSP21.5* diminished tunicamycin-induced ER stress [63]. Tunicamycin is an antibiotic that inhibits protein N-glycosylation, hence, inducing misfolded glycoproteins, and it is experimentally used to induce the unfolded protein response in living organisms. Furthermore, other plant chaperonins have been employed to increase the stress resistance in tomato [64]. For example, the overexpression of the tomato *SlDnaJ20* relieved ROS accumulation by ensuring high

levels of SOD and APX activities and was associated with higher fresh weights of six-week-old plants under heat stress [65].

2.10. Antioxidants

Many antioxidants have been reported in plants that act as buffers to regulate the redox potential of cells. Among antioxidant enzymes, the most exploited in plant biotechnology are probably glutaredoxins, catalases, ascorbate peroxidases (APX), and superoxide dismutases (SOD). Just to give a few examples of applications, a catalase gene (*katE*) from *E. coli*, introduced in the chloroplast genome of tomato under the RBCS promoter, increased catalase activity and better protected plants from oxidative stress induced by high light intensity, drought, or low temperature, compared to the untransformed control [66]. An ascorbate peroxidase from tomato (*LetAPX*) was expressed in Arabidopsis and conferred resistance to cold (4 °C for up to 24 h) [67]. Genetically modified tomato cultivars expressing the *A. thaliana Fe-SOD* gene promoted the increased performance and stability of the photosynthetic apparatus under UV stress [68].

2.11. Ion Transport Proteins

Cation and anion transporters comprise a large class of transmembrane proteins vital for any organism, serving the purpose of moving ions and other small molecules within and between cells. These proteins are fundamental for ion homeostasis and are involved in salt stress resistance because they participate in sodium and chloride uptake, translocation, and cellular compartmentalization [69]. Numerous investigations have reported that genes like *HAL1* and *HAL5*, encoding ion transport proteins in *S. cerevisiae*, when delivered to tomato using recombinant DNA technology, increased the tolerance of toward salinity [70]. Similarly, the *A. thaliana AtNHX1* gene inserted in the tomato genome resulted in improved salinity tolerance [71]. In both cases, a positive effect was associated with an improved K/Na ratio under saline conditions. The importance of K homeostasis in the tolerance to NaCl stress was also demonstrated by overexpressing the endosomal LeNHX2 ion transporter [64][72].

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