

P. B. Kavi Kishor  
Manchikatla Venkat Rajam  
T. Pullaiah *Editors*

# Genetically Modified Crops

Current Status, Prospects and  
Challenges Volume 2

 Springer

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Challenges Volume 2

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*Editors*

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## Foreword

To get easy access to food and to improve the productivity of crop plants, humans have used methods of domestication and improvement through selective breeding, based on useful phenotypic traits. It was through the work of Gregor Mendel that we learnt about the genetic basis of plant traits. The first hybrid corn was developed in 1922 by an intelligent breeding strategy. Following the discovery of DNA as the genetic material, work of a number of groups led to the concept of gene as the unit of DNA that controls a phenotypic character of an organism. And it was in 1973 that Herbert Boyer and Stanley Cohen developed genetic engineering by inserting DNA from one bacterium to another. Around the same time Jeff Schell and Marc Van Montagu discovered that it is due to the transfer of the plasmid DNA of *Agrobacterium tumefaciens* that results in tumor formation in plants. This research was a by-product of curiosity-driven science and based on fundamental scientific discovery. Using this information, and developing plant transformation technology, group of Mary-Dell Chilton and R. Fraley and scientists from the Monsanto Company created the first transgenic plant. During the mid-1990s, with the creation of GM tomato, the initial wave of GM plants was set in motion. However, due to certain issues of public acceptability and stringent regulatory laws that were put in place in different countries, the growth of this technology was slowed down. Van Montagu, whom I have had the pleasure of meeting and knowing for a long time, wrote an insightful article in the *Annual Review of Plant Biology* in 2011 titled “It is long way to GM Agriculture.” Even then this technology has been used in many crops and the global biotech crop area is steadily increasing within many countries which have adopted this technology for crop improvement in their agriculture systems. Unfortunately, due to various social and political issues the adoption of this technology has received resistance. This trend needs to be reversed. In the meanwhile, one has seen the emergence of new technologies like RNAi to silence the expression of genes to understand their role as also to develop novel transgenic plants with useful traits. And since 2015, gene editing technologies have evolved which have become useful and efficient tools to manipulate DNA in plant cells. And now we are moving onwards to the precision genome engineering though prime genome editing which does not involve double-strand breaks and donor DNA templates. Hopefully, these interventions will not be subjected to as much stringent regulatory procedures and will also find better acceptability in the society.

An article was published in *EMBO Reports* by Fagerstrom et al. in 2013, entitled “Stop Worrying Start Growing” with the subtitle, “Risk research on GM crops is a dead parrot, it is time to start reaping the benefits of GM.” This is even more true today. The present volumes by Professors Kavi Kishor, Rajam, and Pullaiah have been compiled to convey the same message by presenting achievements and opportunity of employing different technological tools for genetic improvement of plants. I have known the editors of this volume for a long time. They have themselves made significant contributions in the area of plant biotechnology and are well acquainted with GMOs, in all its perspectives. They are also aware of the views of opponents of this technology. Accordingly, taking these into considerations too, they have broadly outlined the status, prospects, and challenges of different genetic interventions in various plants of economic importance for improving traits like developing resistance to viral, insect, and other diseases and for conferring tolerance to abiotic stresses. With rapid advancements in genome sequencing methodologies and functional genomics tools, it has now been possible to identify the genes which can be deployed in a very precise manner using efficient transformation techniques.

These volumes cover, among cereals, a chapter on rice that deals with the use of GM technology to address the problem of food and nutrition security and a chapter each on wheat and finger millet. Legumes, which remained recalcitrant for a long time and an efficient transformation system was not available, have now been tamed. This family of plants have received special attention, and a chapter each on pigeonpea, chickpea, cowpea, and peanut have found a place in this volume. Among vegetables there is a detailed account on the present status on brinjal, tomato, cucurbits, and one chapter each on redpepper and capsicum. Other plants of importance which have been included are sugarcane, cassava, banana, papaya, citrus, mulberry, and jatropha. Work on two oil plants, sunflower and safflower, has been presented in two independent chapters. This approach of illustrating the use of the technology for each species separately, rather than group them on specific trait, I find, provides better perspective to evaluate the importance of GM technology with respect to each plant species.

These volumes, I am very sure, will be useful to all students and practitioners of biotechnology, be in colleges, universities, and private organizations, as well as for policy makers and regulators in the government agencies. I look forward to the deployment of the safe use of new tools and techniques of genetic manipulation for the improvement of important plants on a large scale in our agriculture and horticulture system. This will help, along with other breeding methodologies, including marker-assisted breeding, to sustain productivity with limited inputs. We hope to see hunger-free world in the years to come.

International Centre for Genetic Engineering  
and Biotechnology  
New Delhi, India  
June 06, 2020

Sudhir K. Sopory

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## Preface

Plants provide us many essential things in life, including food, feed, cloth, wood, paper, medicinal compounds, industrial products, and most importantly the life sustaining molecule oxygen to breath. Plants are also crucial to clean lifesaving water. There are only six crop plants, viz., rice, wheat, corn, potato, sweet potato, and cassava, which provide about 80% calories to humans. There are other important crops like sugarcane, barley, sorghum, bean, soybean, coconut, and banana, which are also being consumed by humans. But crop plants are vulnerable for various biotic factors (pathogens and pests) and extreme environmental conditions or abiotic stresses (e.g., high salinity, drought, heat and cold, heavy metal and submergence) because of their sessile nature. These stresses cause a colossal loss of crop yields and impair nutritional quality. Otherwise, one can realize the potential and harvest 100% agricultural productivity from all crops. In addition, global warming, shrinking water resources, arable land, and population growth are aggravating the problem of food security. In fact, these are key scientific issues in agriculture besides post-harvest losses and impairment in nutritional quality. Then the critical question that arises in our minds is how to harness the full yield potentials of crops without compromising the quality component. The answer lies evidently in the exploitation of diverse technologies, particularly plant breeding and genetic engineering. Between plant breeding and genetic engineering, the former has contributed significantly for more than seven decades to crop improvement and in fact almost all the new and improved varieties were virtually derived through breeding strategies. However, breeding methods suffer from certain limitations like incompatibility barriers or narrow mobilization of useful genes between closely related species. This leads to the problem of using only limited gene pool and there is no way to transfer a single beneficial gene since we generally transfer a cluster of genes/chromosomes during the crosses, thus subject  $F_1$  hybrids for 4–5 back-crosses to chuck away the unacceptable. It takes nearly 10–12 years to develop a new variety with desirable traits and may not be cost-effective. In contrast, genetic transformation by *Agrobacterium* or other gene delivery systems or transgenic technology offers several advantages such as precise gene transfer from any source to crop plants. This means a huge gene pool exists for transfer of desirable traits across species and takes relatively 7–9 years to develop a transgenic line of interest. Consequently, genetic engineering holds great promise for crop improvement and is essential since huge gap exists between

food production and rate of population growth. Today's world population is about 7.7 billion and expected to reach 9.7 billion by 2050, and further to an estimated 11 billion by 2100. Human hunger and malnutrition are the major problems, especially in Asian countries due to accelerating birth rate. So, it is a challenge for plant biologists and biotechnologists to resolve the problem of human hunger and malnutrition through crop improvement programs. In reality, about 70% increase in food production is required by 2050 to feed the growing masses; otherwise we may face great famines in the near future. Indeed, this suggests that a second green revolution is the need of the hour to bring food security to the world population, and this can only happen if we couple the conventional breeding strategies with genetic engineering technologies.

Transgenic technology has already proven to be novel and a potential alternative for crop improvement, and a handful of transgenic varieties like cotton, corn, soybean, and canola have been commercialized globally. This has led to a substantial increase in crop yield and quality and reduced use of harmful pesticides, reduction in CO<sub>2</sub> emissions, and a decrease in the cost of crop production, besides improving the economy of marginal farmers. The first transgenic variety, *flavr savr*—the slow ripening tomato, was commercialized in 1994 in the USA, and since then there is a steady increase in the adoption of the first generation of genetically modified (GM) crops such as corn, cotton, and soybean for insect resistance, herbicide tolerance, and improvement of oil quality. In 2018, about 475 million acres (191.7 million hectares) of land were under the cultivation of various GM crops in 26 countries (21 developing and five developed countries), including five top countries—USA, Argentina, Brazil, Canada, and India (with the adoption of only Bt cotton) with the largest area of GM crops grown, and an additional 44 countries imported these GM crops. To date, about 525 different transgenic events in 32 crops have been approved for cultivation in different parts of the world. Currently, the next generation of transgenic plants displayed potential for the production of bio-ethanol, bio-plastics, and many pharmaceutically important recombinant proteins and compounds. Interestingly, the recent genome engineering or editing technology is quickly gaining importance for maneuvering genes in crop plants using the gene editing tool, the CRISPR-Cas system. This technology is aiding us in the improvement of many agronomically important traits such as yield, stress tolerance, and nutritional quality. Soon, the gene-edited crop plants with new traits, but not having an alien gene, will be commercialized. Such an endeavor will assist us in meeting the increasing food demands and global food security. This technology can be safely exploited since it has minimum or no regulatory issues. GM crops have the most rapid adoption rate in the history in spite of public concerns as compared to the traditional hybrids like corn, which took more than seven decades for global penetration. Transgenic varieties were released only after passing the tests against environmental aggressiveness, toxicity, allergenicity, after fulfilling the stringent regulatory guidelines laid down by the respective countries, and after exhibiting their superiority for field performance vis-à-vis the untransformed or wild-type plants.

The present book brought in two volumes has updated information about the current status of GM crops. While the first volume covers genetic modification



studies in cereals, pulses, and oil-yielding crops, the second one includes information on important vegetable, fruit-yielding, and commercial crops. These volumes on GM crops will be handy to students of life science stream of both undergraduate and postgraduate studies, research scholars, postdocs and researchers working in plant and agricultural biotechnology organizations, faculty members, biotech companies and professionals alike.

Lastly, we would like to express our heartfelt gratitude to Springer Nature for kindly consenting to bring out this book in two volumes and for extending support through various phases and for the timely completion of publishing. Our heartfelt thanks are also due to Prof. Sudhir K. Sopory, ICGEB, New Delhi, for writing the foreword. We would like to thank all the authors/coauthors who have contributed the review articles and also for their cooperation and erudition.

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Anantapur, Andhra Pradesh, India

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He was awarded the Panchanan Maheshwari Gold Medal, the Dr. G. Panigrahi Memorial Lecture award of the Indian Botanical Society, and the Prof. Y.D. Tyagi Gold Medal of the Indian Association for Angiosperm Taxonomy. He has authored 51 books, edited 19 books, and published over 330 research papers. He was a member of the Species Survival Commission of the International Union for Conservation of Nature (IUCN).



# Transgenic Tomatoes for Abiotic Stress Tolerance and Fruit Traits: A Review of Progress and a Preview of Potential

P. Hima Kumari, S. Anil Kumar, G. Rajasheker, D. Madhavi, N. Jalaja, K. Kavya Shridhar, K. P. Scinthia, D. Divya, M. Swathi Sri, Ch. Akhila, E. Sujatha, P. Rathnagiri, and P. B. Kavi Kishor

## Abstract

Tomato (*Lycopersicon esculentum* Mill.) is the second most important vegetable crop of the world. It is rich in nutrition with zero cholesterol, but highly sensitive to abiotic stresses, especially salt, drought, and high temperatures. Development of transgenic tomatoes that are climate resilient coupled with high nutritional value and improved shelf-life of fruit is the need of the hour. Utilization of conventional plant breeding methods and genetic engineering technologies must therefore be vital to achieve these goals. Tomatoes overexpressing transgenes and transcription factors conferred tolerance against different abiotic stresses with increased fruit production in comparison with wild-type (WT) plants. Similarly, delayed fruit ripening and nutritional quality of the fruit have been achieved in tomato. The present review describes the current status of the development of transgenic tomatoes that are tolerant to diverse abiotic stresses alongside delayed fruit ripening and other quality attributes and projects the potential areas for future research.

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**Keywords**

*Lycopersicon esculentum* · Fruit quality · Transgenic tomato · Abiotic stresses · Polyamines

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## 1 Introduction

Plants always encounter various biotic and abiotic stresses which can trigger a series of biological, morphological, and physiological changes leading to metabolic derailment of cellular activities, resulting in reduced growth as well as yield (Rodríguez et al. 2005; Zhou et al. 2011). Biotic and abiotic stresses bring severe metabolic alterations and affect up to 50% of crop productivity every year (Boyer 1982; Wang et al. 2003; Oerke 2006). Plants respond to these stresses by a cascade of interactions which are complex and integrative (Atkinson and Urwin 2012). It is crucial to produce 50% more food by 2050 in order to meet the demands of the growing population (Godfray et al. 2010). This is further compounded, because of the limitations in the availability of water, land, and other natural resources. Abiotic stresses, especially salinity, drought, and high temperature, are responsible for reduced crop growth and cause economic losses in agricultural production. Further, demands are also raising worldwide for more nutritious fruits.

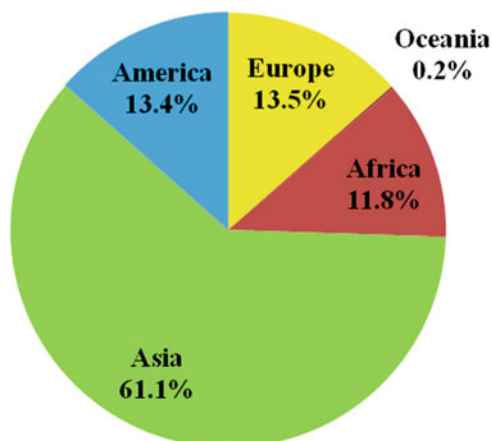
Tomato (*Lycopersicon esculentum* Mill.) is the second most produced and consumed vegetable crop originated from South America and spread throughout the world after Spanish colonization. It is a diploid ( $2n = 24$ ) crop that belongs to the genus *Lycopersicon* of Solanaceae family (Weese and Bohs 2007). The word “tomato” is derived from Spanish “tomate”. Tomato was renamed from *Solanum lycopersicum* L. to *Lycopersicon esculentum* based on molecular and phylogenetic data (Foolad 2007; Peralta and Spooner 2007). Botanically tomato is a fruit berry, but due to its vast usage for culinary purposes, it is treated as vegetable. Due to the presence of 16 elements and important nutrients, it is often regarded as “poor man’s orange”. Tomato has become a model organism due to its smaller genome, short life span, absence of gene duplication, ability to grow in a wide variety of climatic conditions, and ease of controlled pollination and hybridization (Ranjan et al. 2012; Bergounoux 2014; Schwarz et al. 2014). Currently, total world production of tomatoes is about 182 million tonnes. India ranks second with 20 million tonnes after China with 60 million tonnes in its production (FAOSTAT 2017). Asia dominates the global tomato production (Fig. 1).

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## 2 Morphological and Geographical Diversity of Tomato

Tomato shows remarkable diversity in morphological/phenotypic characters and geographical distribution. It typically grows up to 1–3 m (3–10 ft) in height and has a weak stem that often sprawls over the ground and vines over other plants.

**Fig. 1** Schematic representation of production share of tomatoes during 2017 by different regions of the world. (Source: FAOSTAT 2017)



Branches are usually sub-opposite with basic cyme inflorescence. It grows as perennial in its native habitat and as an annual in temperate climates. Cultivated tomatoes are generally self-pollinated, though controlled crosses can be made. Tomatoes vary in size, for example, tom berries measure 5 mm, cherry tomatoes 1–2 cm, and wild beefsteak 10 cm or more in diameter. The most widely grown commercial tomatoes vary in the diameter range of 5–6 cm. It also shows variation in fruit weight ranging from 20 g (cherry tomato) to 500 g (beef tomato), and on average, common tomato weighs approximately up to 100 g. Most cultivars produce red fruits, but a number of cultivars produce yellow, orange, pink, purple, green, black, and white also. Multicoloured and striped fruits are quite striking. Different fruit shapes are observed in tomato including round, oblate, pear, torpedo, or bell-shaped. More than ten quantitative trait loci (QTLs) are associated with size and shape of cultivated tomatoes (Tanksley 2004). Today, cultivars are seen all over the world, but the wild species are restricted to certain areas (Table 1).

### 3 Nutritional Value of Tomatoes

Tomato (100 g) is one of the low-calorie (18 kcal) vegetables with zero cholesterol levels. The water content of tomato is around 95% and carbohydrates and fibre constitutes the other 5%. It has important nutrients like flavonoids, folic acid, carotenoids, lycopene, and ascorbic acid (AsA), which act as antioxidants. Tomato suppresses cancer cell proliferation, protects from prostate cancers, digestive tract, and cardiovascular disorders (Franceschi et al. 1994; Giovannucci et al. 1995; Levy et al. 1995; Willcox et al. 2003). The consumption of tomato significantly increases the lycopenes, total skin carotenoids, phytofluene, and phytoene levels in human serum which protects the skin against UV-light-induced erythema (Aust et al. 2005). The tomato epicarp contains naringin, which reduces the inflammation, atherosclerosis, cardiovascular disorders, diabetes mellitus and acts as an antioxidant (Bharti

**Table 1** List of cultivated and wild tomatoes and their geographical distribution

S. no.	Cultivated/wild	Geographical distribution
1.	<i>Lycopersicon esculentum</i> (Syn.: <i>Solanum lycopersicum</i> ) (cultivar)	All over the world
2.	<i>Solanum galapagense</i> (wild)	Galapagos islands
3.	<i>Solanum cheesmaniae</i> (wild)	Galapagos islands and Ecuador
4.	<i>Solanum pimpinellifolium</i> (wild)	Ecuador and Chile
5.	<i>Solanum chilense</i> (wild)	Peru and Chile
6.	<i>Solanum chmielewskii</i> (wild)	Peru and Bolivia
7.	<i>Solanum habrochaites</i> (wild)	Ecuador and Chile
8.	<i>Solanum pennellii</i> (wild)	Peru and Chile
9.	<i>Solanum eorickii</i> (wild)	Ecuador, Peru, and Andean valley
10.	<i>Solanum arcanum</i> (wild)	Peru and Andean valley
11.	<i>Solanum huaylasense</i> (wild)	Peru
12.	<i>Solanum peruvianum</i> (wild)	Peru and Chile
13.	<i>Solanum corneliomuelleri</i> (wild)	Peru

et al. 2014). Tomatoes also play a vital role in bone health with significant increase in the growth of femur and tibia (Choudhary et al. 2016).

#### 4 Need for Genetic Engineering of Tomato with Altered Traits

Genetic engineering is as an alternative to conventional plant breeding methods for the development of transgenic crops by introduction of alien genes with better agronomic characters. Conventional methods of breeding takes 5–7 years for the development of new tomato cultivars (Vinocur and Altman 2005; Causse et al. 2007). But through genetic engineering techniques, one can introduce the genes of interest within a short span of time and develop a line with traits of interest. Genetically modified crops for biotic and abiotic stresses are gaining importance all over the world. McCormick et al. (1986) produced the first transformed tomato. The *flavr savr* (also known as CGN-89564-2 and pronounced as “flavour saver”) is the first commercial genetically modified tomato used for human consumption (Kramer and Redenbaugh 1994). Tomatoes have a short shelf life, but need to be transported for long distances within the country and also to other countries. This necessitates to develop fruits with improved shelf life. Hence, *flavr savr* tomatoes were marketed initially, but withdrawn subsequently. Salt, drought, and other abiotic stress factors show adverse effects on tomato production and fruit quality. We do not have tomato cultivars that can withstand high-salt, severe water-deficit conditions or low and high temperatures. So, to overcome these stresses, one way is to grow tomato plants in saline irrigated lands, and to adopt crop rotation methods. But, this is an ineffective method as most of the soils are saline and availability of fresh water



is meagre. Another way to overcome these problems is to genetically engineer elite varieties with genes that confer tolerance to salt, drought, cold, and high temperature stresses (Table 2). Therefore, development of tomato and other crops that are tolerant to abiotic stresses is crucial especially in the wake of climate change. Hiwasa-Tanase et al. (2012) reviewed the role of tomato fruits as biofactories for the production of recombinant products and artificial sweeteners. Klee and Giovannoni (2011) elaborately reviewed the process of tomato fruit ripening. In the present study, we reviewed the development of tomato transgenics for abiotic stress tolerance and fruit quality attributes including ripening that have not been covered earlier.

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## 5 Development of Transgenic Tomato Plants for Salt Stress Tolerance

Salinity results in the loss of water content and turgor from leaf cells which limits the ability of plant growth and decreases the final productivity. Salinity also affects the closure of stomatal apertures and reduces the photosynthetic rate with an increase in the formation of reactive oxygen species (ROS) resulting in an oxidative stress. The strategies and mechanisms for sodium ( $\text{Na}^+$ ) transport include  $\text{Na}^+$  exclusion from the cell, or its inclusion into vacuole and intracellular compartmentation and acquisition of potassium ( $\text{K}^+$ ) to cope with osmotic stress during different developmental stages of plant growth.  $\text{K}^+$  maintains ion homeostasis of the cell, membrane potential, photosynthesis, and enzyme activation. In many crops, salt tolerance is achieved by  $\text{Na}^+$  exclusion. This minimizes the damage caused by the accumulation of  $\text{Na}^+$  ions (Munns 2002; Tester and Davenport 2003). Tomato production has been limited by a high level of salinity in the soil or irrigation water. It is sensitive to moderate levels of salinity like most other crop plants. Seed germination, vegetative growth, and reproductive stages of tomato show high sensitivity to salt stress, and economic yield is drastically reduced under these conditions (Maas 1986; Bolarín et al. 1996). Salinity stress results in the  $\text{Na}^+$  toxicity which impairs electroneutrality of the cell by altering the metabolic process. Regulations of  $\text{Na}^+$  transport rate from root to the shoot and tissue tolerance are the critical factors for salinity tolerance (Shabala 2013; Maathuis 2014). Elevated salinity levels for longer period compounds the problem of drought stress also (Munns and Tester 2008). A number of candidate genes associated with salt stress tolerance have been identified and overexpressed for salt stress tolerance in tomatoes which are briefly described below.

Transgenic tomato plants overexpressing a vacuolar  $\text{Na}^+/\text{H}^+$  antiporter (*NHX*) accumulated high amount of  $\text{Na}^+$  in leaves but not in fruits when grown in the presence of 200 mM NaCl (Zhang and Blumwald 2001). Tomatoes expressing  $\text{Na}^+/\text{H}^+$  antiporter-like protein (*NHXL*P) conferred tolerance to salt stress by low accumulation of  $\text{Na}^+$ . Transgenics displayed higher proline,  $\text{K}^+$ , improved cambial conductivity, and fruit yield in comparison with WT plants (Kumari et al. 2017). Transgenic tomato co-expressing *Arabidopsis thaliana* vacuolar  $\text{H}^+$ -pyrophosphatase (*AVP*I) and *Pennisetum glaucum* vacuolar  $\text{Na}^+/\text{H}^+$  antiporter

**Table 2** List of tomato transgenics developed for abiotic stress tolerance

Genes and their source	Improved tolerance to	References
<i>GRF9</i> from <i>Arabidopsis</i>	Improved phosphate deficiency	Zhang et al. (2018a)
<i>SbNHXLP</i> from <i>Sorghum</i>	Salt stress	Kumari et al. (2017)
Choline oxidase gene ( <i>codA</i> ) from <i>Arthrobacter globiformis</i>	Salt stress	Wei et al. (2017)
<i>EgCBF3</i> from <i>Elaeis guineensis</i>	Abiotic stress	Ebrahimi et al. (2016)
Osmotin-like protein (OLP) and Chitinase ( <i>Chi1</i> ) from <i>Solanum</i> and rice	Salt and drought stresses	Kumar et al. (2016)
<i>LeFAD7</i> from tomato	High temperature	Nakamura et al. (2016)
<i>FaGalUR</i> from strawberry	Salt and cold conditions	Cai et al. (2015)
miR399 from rice	Salt and cold conditions	Gao et al. (2015)
<i>ICE1</i> from <i>Arabidopsis</i>	Cold stress	Juan et al. (2015)
<i>AtHMA4</i> from <i>Arabidopsis</i>	Enhanced Zn translocation	Kendziorrek et al. (2014)
<i>BADH</i> from spinach	Reduction in heat-induced photoinhibition	Li et al. (2014)
<i>StAPX</i> from tomato	Drought stress	Sun et al. (2014)
<i>MdoMYB121</i> from apple	Salt, drought, and cold stresses	Cao et al. (2013)
<i>ZAT12</i> from <i>Brassica carinata</i>	Drought and oxidative stresses	Rai et al. (2012, 2013a)
<i>AtDREB1A</i> from <i>Arabidopsis</i>	Drought stress	Rai et al. (2013b)
<i>BADH</i> from <i>Suaeda liaotungensis</i>	Salt stress	Wang et al. (2013)
<i>HMA4 (PIB-ATPase)</i> from <i>Arabidopsis halleri</i>	Increased uptake of zinc	Barabasz et al. (2012)
<i>MDHAR</i> from tomato	Increased tolerance to salt and osmotic stresses	Li et al. (2012)
<i>SpUSP</i> from tomato	Drought stress	Loukehaich et al. (2012)
Dehydrin ( <i>TAS1A</i> ) from tomato	Salt and drought stresses	Muñoz-Mayor et al. (2012)
<i>CaRma1H1</i> from <i>Capsicum annum</i>	Drought stress	Seo et al. (2012)
<i>MhGLB1</i> from <i>Malus hupehensis</i>	Hypoxia stress	Shi et al. (2012)
<i>mhgai2</i> from apple	Salt and drought stresses	Wang et al. (2012a)
<i>MdCIPK6L</i> and <i>MdCIPK6LTI75D</i> from apple	Salt, drought, and chilling stress	Wang et al. (2012b)
Co-expression of <i>AVP1</i> and <i>PgNHX1</i> (vacuolar H <sup>+</sup> pyrophosphatase from <i>Arabidopsis thaliana</i> and <i>PgNHX1</i> from <i>Pennisetum glaucum</i> )	Salt stress	Bhaskaran and Savithramma (2011)
<i>CaXTH3</i> xyloglucan endotransglucosylase/hydrolase from <i>Capsicum annum</i>	Salt and drought stresses	Choi et al. (2011)

(continued)

**Table 2** (continued)

Genes and their source	Improved tolerance to	References
<i>MdVHP1</i> from apple	Salt stress	Dong et al. (2011)
<i>codA</i> from <i>Arthrobacter globiformis</i>	Salt and drought stresses	Goel et al. (2011)
<i>MdSPDS1</i> (spermidine synthase 1) from apple	Salt stress	Neily et al. 2011
<i>SIXTH1</i> (xyloglucan endotransglucosylase/hydrolase) from tomato	Altered fruit characteristics	Ohba et al. (2011)
<i>HALI</i> from yeast	Salt stress	Safdar et al. (2011)
<i>At-CBF1</i> (C-repeat binding factor 1) from <i>Arabidopsis</i>	Cold stress	Singh et al. (2011)
<i>CAX1</i> from <i>Arabidopsis</i>	Ca <sup>2+</sup> accumulation and fruit shelf-life improvement	Park et al. (2005)
<i>AtNHX1</i> from <i>Arabidopsis</i>	Salt stress	Zhang and Blumwald (2001)

(*PgNHX1*) genes showed higher degree of salt tolerance when compared to plants where the genes were expressed individually. With the overexpression of *PgNHX1* gene, higher salt stress tolerance was noticed. The tolerance mechanism has been found to be due to the exclusion of Na<sup>+</sup> at the root level and also due to sequestration of cytosolic Na<sup>+</sup> into the vacuoles. Transgenics also displayed higher proline and chlorophyll content compared to WT plants (Bhaskaran and Savithramma 2011). Genes associated with osmolyte biosynthesis have been deployed for salt stress tolerance with great success. Osmolytes act as osmotic balancing agents and have the ability to quench/scavenge the ROS. Transgenic tomatoes expressing betaine aldehyde dehydrogenase (*BADH*) gene, driven by *CaMV35S* promoter, displayed salt stress tolerance. Similarly, overexpression of *BADH* driven by stress-inducible promoter *P5* showed higher tolerance to salt stress than *CaMV35S* promoter (Wang et al. 2013). Tomato transformed with the choline oxidase gene (*codA*) from *Arthrobacter globiformis* accumulated glycine betaine (GB), which was not reported in WT plants. Upon exposure to salt stress, the *codA* transgenics showed higher photosynthetic rates, antioxidant enzyme activities, and lower accumulation of reactive oxygen species (ROS). The quantitative real-time PCR (qRT-PCR) experiments in *codA* transgenics revealed higher GB, enhanced expression of K<sup>+</sup> transporter, Na<sup>+</sup>/H<sup>+</sup> antiporter, and H<sup>+</sup>-ATPase genes under salt stress conditions. The *codA* gene also regulated the ion channel and transporters as evident by high K<sup>+</sup> to Na<sup>+</sup> ratio in transgenics treated with salt (Wei et al. 2017). Genes associated with antioxidative metabolism have also been tested with over all positive effect. Monodehydroascorbate reductase (MDHAR) catalyses the reduction of monodehydroascorbate (MDHA) to ascorbate (AsA) and the increased AsA alleviates the photoinhibition of PSII. Tomatoes expressing sense *MDHAR* showed higher mass, height, and increased tolerance to salt and osmotic stress than the

antisense and WT. The AsA levels were high in sense plants followed by WT and antisense (Li et al. 2012). Tomato containing the gene *mhgai2* exhibited more resistance to drought and salt stresses than the WT plants at the seedling stage (Wang et al. 2012a). In addition to transgenes, an array of transcription factors were also employed in genetic transformation of tomato for tolerance to salt, drought, and cold (Liu et al. 1998; Lindemose et al. 2013; Nakashima et al. 2014). Of the various transcription factors, members of the MYB family were widely studied. *MdoMYB121* was induced under multiple stress conditions. Compared to WT plants, transgenic tomato expressing *MdoMYB121* showed better tolerance to salt stress (Cao et al. 2013). Tomato overexpressing strawberry *FaGalUR* gene exhibited elevated tolerance to salt stress with twofold increase in AsA content in tomato fruit (Cai et al. 2015). Transgenic tomato overexpressing *athmiR399d* under the control of *rd29A* promoter displayed tolerance to salt stress. It also increased the biomass of tomato under low-temperature and phosphate (P) deficiency conditions (Gao et al. 2015). Overexpression of *Capsicum annuum* gene (*CaRma1H1*), an endoplasmic reticulum-localized protein, displayed enhanced tolerance to both salt and drought stresses compared to WT tomato plants (Seo et al. 2012). Interestingly, tomato overexpressing osmotin-like protein (*OLP*) and chitinase (*Chi11*) double construct exhibited tolerance to salt as well as drought. These plants showed higher proline,  $K^+$ , total biomass, and relative water content, than the WT under salt and drought stress conditions in the pots (Kumar et al. 2016). Thus, an array of genes isolated from diverse pathways and different species exhibited tolerance to salt stress in tomato upon overexpression, but field level trials were not conducted in majority of the cases mentioned above. However, potential exists for further validating a great deal of transcription factors that have been isolated which can modulate downstream genes and impart salt stress tolerance.

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## 6 Transgenic Tomatoes for Water-Deficit Conditions or Drought-Affected Areas

Drought or water deficit is an important abiotic stress which results in reduced growth and final productivity (Jacob 2008). Plants have developed efficient cellular and molecular mechanisms to cope with water-deficit conditions (Kramer and Boyer 1995). Drought stress results in ion imbalance as well as production of ROS (Mittler 2002). To maintain the osmotic potential of the cells, plants accumulate inorganic solutes in the vacuoles (like  $Na^+$  and  $K^+$ ) and compatible solutes like proline, GB, and sugars in the cytoplasm and thus favour the uptake of water from the soil. Antioxidative compounds and ascorbate peroxidases (APX) quench the ROS and degrade  $H_2O_2$  to water. Transgenic tomatoes overexpressing  $GA_3$  Della protein (*mhgai2*) are insensitive to exogenous gibberellins and are more resistant to drought than WT plants, but produced smaller flowers and seeds (Wang et al. 2012a). Similarly, tomatoes overexpressing *Solanum lycopersicum* thylakoid-bound ascorbate peroxidase gene (*SlAPX*) displayed higher APX activity, with subsequent tolerance to drought (Sun et al. 2014). Transgenics recorded higher yields compared

to WT plants (Sun et al. 2014). Transcription factors also play crucial roles during water-deficit conditions. Cao et al. (2013) developed transgenics by introducing *MdoMYB121* gene, which showed tolerance to water-deprived conditions. Similarly, tomatoes overexpressing *AtDREB1A/CBF3* driven by *rd29A* promoter exhibited drought tolerance with lower levels of superoxide and  $H_2O_2$  compared to WT plants. A significant increase in the antioxidant activity was noticed in transgenic plants (Rai et al. 2013b). Tomato overexpressing *ZAT12*, a  $C_2H_2$  zinc finger, confirmed tolerance against drought and oxidative stresses by accumulating higher proline in comparison with WT plants (Rai et al. 2012, 2013a). The universal stress protein (SpUSP) is induced by salt, drought, abscisic acid (ABA), and oxidative stresses. Loukehaich et al. (2012) demonstrated that transgenic tomato plants expressing *SpUSP* exhibited increased tolerance to water deficit conditions. *SpUSP* expression enhanced the accumulation of ABA which in turn regulated water loss by closing the stomata. Besides drought tolerance, *SpUSP* tomato plants performed well under oxidative stress. It appears that *SpUSP* gene interacts with annexin and modulates ABA-induced stomatal closure, which prevents water loss, and hence imparts drought tolerance (Loukehaich et al. 2012). Late embryogenesis abundant (LEA) proteins or dehydrins play a prime role during drought stress tolerance. Transgenic tomato expressing dehydrin *TAS14* driven by *CaMV35S* exhibited tolerance to both salt and drought stresses with increased accumulation of  $K^+$  and sugars in comparison with WT plants (Muñoz-Mayor et al. 2012).

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## 7 High-Temperature Stress-Tolerant Transgenic Tomato

Moderate heat stress inhibits the repair mechanism of photosystem II (PSII) without causing photo-damage directly (Chen and Murata 2011). On the other hand, high temperature induces PSII inhibition and hence affects plant growth and metabolism. Osmolyte GB accumulates rapidly under heat stress and provides tolerance to plants by acting as a compatible solute. Also, it enhances repair of PSII induced by heat stress by lowering the levels of ROS (Allakhverdiev et al. 2007; Yang et al. 2007). Transgenic tomato overexpressing *BADH* exhibited higher GB accumulation with increase in chlorophyll fluorescence compared to WT plants upon exposure to 42 °C. Transgenics revealed an increase in D1 protein content, associated with PSII repair indicating that the osmoprotectant GB plays a pivotal role in temperature stress tolerance. Arguably, exogenous application of GB do not reduce the ROS content directly (Li et al. 2014). Tomato plants transformed with fatty acid desaturase gene (*LeFAD7*) evinced high-temperature tolerance along with lower amounts of unsaturated fatty acids when compared to WT plants. Nakamura et al. (2016) developed transgenics where the fatty acid desaturase gene (*LeFAD7*) was RNA-silenced. Transgenic lines grew under high-temperature conditions. Further, such a high temperature tolerance was conferred in the nontransgenic tomato scions after grafting onto the silenced root stocks. Such a novel technique may be critical for developing many crop plants with high-temperature tolerance. This can avoid the spread of engineered genes into wild species. However, a comprehensive

understanding of high-temperature tolerance mechanisms, identification of candidate genes such as heat shock proteins (HSPs) and heat shock factors (HSFs), RNA-binding proteins (chaperones), and their modulation is highly crucial to develop tomato transgenics that can withstand the climate changes in future.

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## 8 Low-Temperature or Cold Stress-Tolerant Transgenic Tomatoes

Tomato is highly sensitive to low temperatures or cold stress conditions. Both growth and yield decrease, and plants become non-productive if the temperature drops to 5 °C (Lin et al. 2000). For protecting the crop plants from low temperatures, and for generating cold stress-tolerant plants, several antifreeze proteins (AFPs), cold-induced candidate genes, and transcription factors have been identified and isolated (Thomashow 1999). Calcineurin B-like protein (CBL) kinase (*CIPK*) responds to different abiotic stresses. Tomato overexpressing both *MdCIPK6L* and *MdCIPK6LT175D* exhibited enhanced tolerance to multiple stresses like drought, chilling, and salt (Wang et al. 2012b). Experiments conducted by Cao et al. (2013) with *MdoMYB121* gene overexpression in tomato revealed tolerance to cold stress conditions. The *ICE1* gene [C-repeat/DRE-binding factor, (CBF)], an inducer of *CBF* expression 1, when overexpressed in tomato displayed tolerance to cold stress with an increase in proline content and catalase activities compared to WT plants. Malondialdehyde (MDA) content remained lower in transgenics than the WT plants (Juan et al. 2015). This indicates that *CBF* has the ability to regulate antioxidative genes such as *catalase* and also improve the accumulation of the osmolyte proline. Likewise, transgenic tomatoes expressing strawberry *FaGalUR* gene displayed tolerance to low temperatures alongside twofold increase in AsA level in tomato fruits (Cai et al. 2015). When compared to the WT plants, enhanced expressions of ethylene biosynthesis-related genes and antifreeze proteins (AFPs) like *SICH13*, *SIPR1*, *SIPR-P2*, and *SILAP2* were recorded in transgenic tomatoes with the introduction of oil palm transcription factor *EgCBF3*. *EgCBF3* expression resulted in the delayed leaf senescence and enhanced chlorophyll content suggesting the role of this gene in ethylene biosynthesis-related as well as in AFP genes and, hence, could protect crops from low-temperature stress tolerance (Ebrahimi et al. 2016). However, the mechanistic explanation for the upregulation of ethylene biosynthetic pathway and chlorophyll biosynthetic pathway genes is largely obscure. The nonsymbiotic hemoglobins-1s (*nsHb-1s*) have very high affinity for oxygen and involved in oxygen transport. Tomato overexpressing the transgene *MhGLB1* (*nsHb-1s*) improved the plant tolerance to hypoxia by the decrease in photosynthetic transpiration and stomatal conductance rates compared to WT plants (Shi et al. 2012).

## 9 Transgenic Tomatoes with Heavy Metal and Mineral Stress Tolerance

Among heavy metals, cadmium (Cd) and nickel (Ni) are the most hazardous that cause considerable damage to plant productivity. Exposure of plants to these toxic metals triggers various physiological and metabolic alterations (Villiers et al. 2011). The widespread effects of these metals on plant growth (Sharma and Dubey 2007) include leaf chlorosis, necrosis, root activity, altered photosynthetic, and reduced biosynthetic activities leading to the death of plants eventually (DalCorso et al. 2010). However, plants have developed several tolerance mechanisms which include modulation in physiological and biochemical processes and changes in global gene expressions (DalCorso et al. 2010; Urano et al. 2010). Inhibition of metal uptake or avoidance involves restriction of metal entry to the cell by extracellular precipitation, biosorption to cell walls, reduced uptake, or increased efflux. Further, plants adapt to heavy metals by intracellular metal chelation through the synthesis of organic acids (e.g., malate), glutathione (GSH), or metal binding ligands such as phytochelatin (PCs), and metallothioneins (MTs). Vacuolar compartmentation of metals is a strategy adapted by many plants to avoid the toxic effects of metal stress in the cytoplasm. Further, induction of the antioxidant defence system is crucial to counter the toxic effects caused by metal-induced ROS. Cadmium (Cd) content was quantified in six tomato cultivars, and its effects on the expression of *LeNRAMP3*, *LeFER*, *LeIRT1*, and *LeNRAMP1* were evaluated. The six tomato cultivars accumulated high Cd concentrations and were able to transport it to fruits. Among the evaluated genes, the Cd-induced level of *LeFER* expression appeared to provide evidence regarding the capacity of foliar Cd accumulation in tomato (Hartke et al. 2013). Transgenic tomato was generated by heterologous expression of *AhHMA4p1::AhHMA4* from *Arabidopsis halleri*. This is a Zn export protein implicated in loading of Zn into xylem. *AhHMA4* induces uptake of Zn in a Zn-dependent manner and also the activation of Fe-uptake in roots if the Zn ions are taken up in excess. Expression of *AhHMA4* gene may also cause cell wall remodelling due to overload of Zn into the apoplast, and thus help in metal homeostasis network in tomato (Barabasz et al. 2012). Though Fe-Zn homeostasis has been studied to some extent in transgenic tomato, heavy metal-tolerant transgenics have not yet been generated in tomato using genetic engineering technologies.

## 10 Biofortification in Transgenic Tomato

Several biofortification strategies have been pursued to improve mineral quality like zinc (Zn) and iron (Fe) in tomato. Zn plays an important role in vegetative growth by regulating root-to-shoot metal translocation through the xylem (Palmgren et al. 2008). Deficiency of Zn results in the reduced crop yields and also Zn malnutrition in humans. Transgenic tomatoes expressing *AtHMA4* showed enhanced Zn translocation to shoots, which helps in Zn biofortification. But, strangely, overexpression of



*AtHMA4* resulted in decreased Fe in transgenics compared to WT plants by upregulation of Fe-deficiency marker genes (*LeFER*, *LeFRO1*, *LeIRT1*). Tomatoes transformed with *AhHMA4p1::AhHMA4* displayed improved Zn uptake by facilitating root-to-shoot Zn translocation. It also induced the uptake of Fe in the roots. Thus, it appears that *AtHMA4* overexpression in tomato alters the cross-homeostasis (Kendziorek et al. 2014). But, no attempts were made till date to identify the number of genes associated with Fe and Zn transport and their tissue-specific expressions under Fe- and Zn-deficient and -sufficient conditions, and also translocation of these metals to the fruits. Such a comprehensive identification and understanding their modulation would help us greatly to generate tomatoes with better accumulation of Fe and Zn contents. Phosphorus (P) plays a pivotal role in plant growth and development, and its deficiency results in decreased productivity and quality of crops. *miR399* is essential for phosphate homeostasis, and its overexpression resulted in P toxicity and retarded growth. The *athmiR399d* showed increased biomass under low temperature and P deficiency conditions (Gao et al. 2015). Thus, it is possible to manipulate P content in tomato. Further work on the efficient uptake of P is necessary by genetic engineering or genome-editing technologies. Overexpression of *Arabidopsis* General Regulatory Factor 9 (*GRF9*) improved the tolerance of plants to low phosphate (P) with improvement in root biomass and enhanced P content under hydroponic conditions compared to WT plants. Transgenic tomato also showed higher uptake of P content and transcript levels of *LePT1* and *LePT2* in both normal and low-P hydroponic solutions. *GRF9* overexpression resulted in the exclusion of protons from the roots under low P conditions in transgenic tomato and promotion of fruit production (Zhang et al. 2018a) indicating the importance of the gene *GRF9* in fruit yield/final productivity. Calcium ( $\text{Ca}^{2+}$ ) maintains membrane stability and cell wall structure, and its deficiency results in low plant productivity. Exogenous supply of  $\text{Ca}^{2+}$  before harvest of fleshy tomato fruits showed improvement in shelf life by maintaining plasma membrane integrity and cell wall firmness (Gerasopoulos et al. 1996). Transgenic tomato expressing *Arabidopsis* H<sup>+</sup>/cation exchangers (*CAX*) displayed increased accumulation of  $\text{Ca}^{2+}$  and prolonged shelf life in comparison with the WT plants (Park et al. 2005), indicating that  $\text{Ca}^{2+}$  is associated with tomato fruit shelf life. Methylselenocysteine (MeSeCys), a derivative of the amino acid, has anticancer activity in animals. Selenium (Se) hyperaccumulating plants convert Se to MeSeCys by the enzyme selenocysteine methyltransferase (SMT). Overexpression of a cDNA encoding SMT in tomato resulted in high accumulation of MeSeCys in the fruit, but not in the leaves when roots were fed with selenite or selenate (Brummeli et al. 2011). More interestingly, MeSeCys was found heat stable and not destroyed by the processing of the fruit to tomato juice (Brummeli et al. 2011). These results indicate that biofortification of tomato fruit with an anticancer compound methylselenocysteine is possible and can be utilized for controlling cancer. However, more efforts are needed to make such transgenics affordable and acceptable by the consumers.



## 11 Modulation of Tomato Fruit Traits Like Shelf Life and Pigments/Carotenoids

Tomato is a fleshy fruit, and several flavour compounds and pigments accumulate during the process of ripening. Such compounds/pigments attract animals/birds that devour them and disseminate the indigestible seeds of tomato at a distant place. This helps them in proper seed dispersal and ensue successful establishment of progeny. Pesaresi et al. (2014) discussed the role of plastid modifications in the tomato fruit maturation and ripening. A large body of information indicates the possible involvement of crosstalk via plastid to nucleus (retrograde) and nucleus to plastid (anterograde) signalling. Nearly 3000 proteins observed in the chloroplast are encoded by nuclear genome, but translated in the cytoplasm. All these proteins are then transported into the cell organelles (Richly and Leister 2004; Li and Chiu 2010). This implies that chloroplast transition to chromoplast involves sizeable exchange of information between the plastids and nucleus. Such an exchange of information between the two organelles is essential to meet the needs of the changing energy and metabolic demands (Chi et al. 2013). Therefore, maturation and ripening of tomato fruit are dynamic and highly complex. A comprehensive understanding of the events are essential to regulate fruit maturation and subsequently improve fruit quality traits including pigments that have antioxidative properties. Carotenoids protect the photosynthetic apparatus from excess light and lycopene  $\beta$ -cyclase (*lyc-b*) is an essential enzyme for the synthesis of  $\beta$ -carotene via methylerythritol phosphate (MEP) pathway of isoprenoid biosynthesis. Transgenic tomato fruits expressing *Lycb-1* under the control of CaMV35S showed 4.1-fold increases in the production of  $\beta$ -carotene and 30% of total carotenoid content in comparison with WT plants. Expression of *Lycb-1* altered the other pathways including fatty acids and flavonoid biosynthesis (Guo et al. 2012). miRNAs also involve in regulating carotenoid content in tomato by targeting the biosynthetic pathways (Koul et al. 2016). Transgenic tomato overexpressing oat arginine decarboxylase displayed improved fruit harvesting attributes (Gupta et al. 2019). Tomato fruit ripening is a complex and genetically regulated process which completes with seed formation. It is a climacteric fruit, where ripening is associated with increased production of ethylene. Ripening of the fruit is regulated by thousands of genes that control fruit softening, accumulation of sugars, volatile compounds, and pigments which increase the palatability. Palma et al. (2019) reviewed the role of ethylene receptors, anthocyanin and carotenoid biosynthesis, auxin signalling, effect of light and vitamin C, and modification of organic acids, and cell wall degrading enzymes involved in the process of fruit ripening. Several studies report the maturation of tomato involving ripening-inhibitor (*rin*), non-ripening (*nor*), and colourless non-ripening (*cnr*) mutants. Different transgenes and transcription factors (TFs) were employed for slowing fruit ripening and improving palatability. The list of such transgenics is shown in Table 3. NON-RIPENING (NOR) NAC transcription factors (TFs) play important roles in fruit ripening, and controls leaf senescence (Ma et al. 2019). In addition to these TFs, it is known that ethylene TFs also play an indispensable role in fruit ripening and shelf life of fruits (Klee and Giovannoni 2011). Miraculin, a glycoprotein, is an

**Table 3** List of tomato transgenics and mutants developed for fruit traits

Genes and their source	Fruit improvement	References
<i>ACS1</i> from grapes	Decreased ethylene production for enhanced shelf life	Ye et al. (2018)
<i>GRF9</i> from <i>Arabidopsis</i>	Phosphate deficiency	Zhang et al. (2018a)
<i>XTH2</i> and <i>XTH10</i> from apple	Increased ethylene production	Zhang et al. (2017a)
<i>CmLOX18</i> from <i>Cucumis melo</i>	Increased production of C6 volatiles	Zhang et al. (2017b)
<i>MYB12</i> from <i>Arabidopsis</i>	Increased flavonoid content	Choudhary et al. (2016)
<i>FaGalUR</i> from strawberry	Increased ascorbate accumulation	Cai et al. (2015)
<i>Ornithine decarboxylase</i> from mouse	Increased fruit quality	Pandey et al. (2015)
<i>SISAMS1</i> from tomato	Increased fruit set and yield and tolerance to alkali stress	Gong et al. (2014)
<i>BZR1-1D</i> from <i>Arabidopsis</i>	Increased accumulation of carotenoids	Liu et al. (2014)
<i>S-adenosylmethionine decarboxylase</i> from human	Delayed ripening	Madhulatha et al. (2014)
<i>GAD RNAi</i> from tomato	Reduced glutamate accumulation	Chew and Seymour (2013)
<i>RNAi ACO1</i>	Delayed ripening and increased shelf life	Eglous et al. (2013)
<i>DAHPS</i> from bacteria	Increased aroma	Tzin et al. (2013)
<i>hpRNAi-ACO1</i>	Reduced ethylene production and increased shelf life	Behboodian et al. (2012)
<i>GGP</i> from <i>Actinidia chinensis</i>	Increased ascorbate accumulation	Bulley et al. (2012)
<i>Lycb-1</i> from tomato	Increased $\beta$ -carotene and total carotenoid accumulation	Guo et al. (2012)
<i>Prosystemin</i>	Increased lycopene content	Liu et al. (2012)
Selenocysteine methyltransferase ( <i>SMT</i> )	Increased selenium accumulation	Brummell et al. (2011)
<i>ODO1</i> from <i>Petunia</i>	Increased phenylpropanoid compound levels	Cin et al. (2011)
<i>DHAR</i> from <i>MDHAR</i>	Increased ascorbate accumulation in fruit but not in leaves	Haroldsen et al. (2011)
<i>Miraculin</i> from <i>Richadella dulcifica</i>	Increased miraculin accumulation (a taste-modifying protein)	Hirai et al. (2011)
<i>Miraculin</i> from <i>Richadella dulcifica</i>	Conversion of sour to sweet taste	Hiwasa-Tanase et al. (2011), Kurokawa et al. (2013)
<i>Stilbene synthase</i> from grapes	Induce parthenocarp	Ingrosso et al. (2011)
<i>AP2a</i> mutant	Regulates carotenoid and chlorophyll metabolism	Karlova et al. (2011), Chung et al. (2010)
<i>AFSK</i> (an NAK-type protein kinase) from apple	Higher floral abscission and affection of pollen development	Kim et al. (2011)
<i>gr</i> (green ripe) mutant	Delayed ripening	Barry and Giovannoni (2007)

(continued)

**Table 3** (continued)

Genes and their source	Fruit improvement	References
<i>CNR</i> (colourless non-ripe) mutant	Delayed ripening	Manning et al. (2006)
<i>rin</i> (ripening inhibitor) mutant	Delayed ripening	Vrebalov et al. (2002)
<i>Nr</i> (never ripe) mutant	Delayed ripening	Yen et al. (1995), Lanahan et al. (1994)
<i>rin</i> mutant	Degradation of polyuronide but not fruit softening	Giovannoni et al. (1989)

alternative to more traditional sweeteners. It was discovered in red berries of the miracle fruit of West Africa, which converts sour into sweet tastes (Kurihara and Beidler 1968; Theerasilp and Kurihara 1988). Transgenic tomatoes expressing miraculin driven by E8 tissue-specific promoter and heat shock protein (Hsp) terminator accumulated 30–630 µg miraculin per gram fresh weight of tissue which was four times higher than transgenic tomatoes expressing miraculin driven by the constitutive 35S promoter (Hiwasa-Tanase et al. 2011; Kurokawa et al. 2013). Yet, such fruits have not been marketed, and tomatoes with sweet taste are still a dream to come true to the consumers.

In tomato, fruit ripening involves a cascade of physiological and biochemical events like softening, change of fruit pigment, development of flavour components, and more importantly biosynthesis of ethylene. Levels of ethylene content increase which can subsequently trigger multiple physiological changes brought out simultaneously by the expression of several genes. It is known that the MADS-box genes help by way of non-hormonal ripening. Tomato *rin* mutant has large sepals and loss of inflorescence determinacy. Cloning of the *rin* locus revealed the presence of two tandem MADS-box genes, namely *LeMADS-RIN* and *LeMADS-MC*. While *RIN* is associated with fruit ripening, *MC* plays a role in sepal development. The *rin* mutation alters the expression of *LeMADS-RIN* and *LeMADS-MC* genes (Vrebalov et al. 2002). It appears that MADS-box transcription factor *RIN* is an essential regulator of ripening gene expression network. It interacts with many genes and controls the changes in fruit colour, flavour, texture, and taste during ripening. *RIN* interacts with the promoters of genes responsible for the overall ripening including the transcriptional regulation, cell wall metabolism, and ethylene and carotenoid biosynthesis. Contrary to this, macrocalyx (*MC*) has very low expression in tomato fruit, but the function of the fusion gene *RIN-MC* is not completely clear. It is *RIN* which acts as a rate-limiting factor in ethylene and carotenoid biosynthesis by interacting with the promoters of several genes. While overexpression of *RIN-MC* in tomato impaired the process of fruit ripening, downregulation of *RIN-MC* in the *rin* mutant stimulated the normal yellow colour of the fruit (Li et al. 2018). The experiments conducted by Li et al. (2018) infer a negative role for *RIN-MC* fusion gene in fruit ripening, and it encodes a chimeric transcription factor which can regulate many genes associated with ripening. Further, *RIN* function depends on the normal functioning of *ncr* gene (Martel et al. 2011). Martel et al. (2011)

demonstrated that RIN recruitment to target loci depends on a functioning allele at the ripening-specific transcription factor COLOURLESS NONRIPENING gene locus. Thus, it appears an interaction between the ripening regulators is highly crucial.

Biosynthesis of ethylene is regulated by two key enzymes, 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) which transform *S*-adenosyl-L-Met (SAM) into ACC and convert it further into ethylene (Kende 1993). Both ACS and ACO genes show different spatial and temporal expression patterns (Barry et al. 2000; Jiang et al. 2011; Liu et al. 2015; Ye et al. 2017). A total of seven ACO genes have been identified, and *LeACO1* and *LeACO4* are highly expressed in tomato leaf and flower tissues (Seymour et al. 2013; Chersicola et al. 2017). Overexpression of *VvACS1* is the only rachis-specific ACC synthase (ACS) gene in tomato which showed increased activity in rachis without any increase in fruit. Ectopic expression of *VvACS1* resulted in decreased ethylene production in flowers, fruits, and leaves of tomato. Its expression does not downregulate the expression of endogenous tomato *ACS1* and *ACS6* genes. *VvACS1* (from grapes) expression in tomato resulted in decreased auxin and increased zeatin contents, suggesting the role of ethylene in auxin transport and distribution during fruit ripening (Ye et al. 2018). Thus, the phytohormone ethylene is at the centre stage of fruit ripening and its regulation is critical for improving shelf life of tomato fruits. Several fruit ripening, single recessive gene mutants such as *ripening inhibition (rin)* as described above, *non-ripening (nor)*, *alcobaca (alc)*, *never ripe (nr)*, and *green ripe (gr)* have been known that prolong the shelf life of tomatoes (Chialva et al. 2016; Osei et al. 2017). These gene mutants modify several of the ethylene's downstream effects, thus inferring a complex fruit ripening gene/protein network. Therefore, production of ethylene in these gene mutants is limited and hence fruits fail to ripe, thus they contribute to postharvest fruit quality. Further, the discovery of such gene mutants improved our understanding of the molecular mechanisms that help to control fruit ripening. However, in a heterozygotic condition, such mutants exhibit natural fruit colour that is acceptable by consumers, and fruits ripe naturally with enhanced shelf life. Thus, potential exists for the utilization and exploitation of these genes for genetic engineering and for improving the shelf life of tomato fruit with consumer acceptance.

Brassinosteroids (BRs) also regulate fruit ripening besides ethylene. Transgenics expressing *Arabidopsis* BR response transcription factor Brassinazole resistant 1 (*BZR1-1D*) showed enhanced accumulation of carotenoids, soluble sugars, and ASA during fruit ripening. Transgenics also showed upregulation of *SIGLK2* gene involved in chloroplast development. The 2,4-epibrassinolide (EBR)-treated ethylene-insensitive mutant pericarps have never ripen with the accumulation of carotenoids. Thus, EBR and *BZR1-1D* play vital roles in the accumulation of carotenoids which is attributed to the fruit quality (Liu et al. 2014). Transgenic lines overexpressing *BZR1-1D* showed 411 differentially expressed proteins with enhanced light reaction pathway during ripening. The increase in 2-oxoglutarate-dependent dioxygenase (2-ODD2), a protein involved in gibberellin biosynthesis, was noticed during all the four developmental and ripening stages (Liu et al. 2016).

Jasmonic acid (JA) is also involved in carotenoid biosynthetic pathway; thereby it can control fruit quality. Both lycopene and ethylene contents decreased significantly in the fruits of JA-deficient mutants (*spr2* and *defl*). This indicates that JA is a crucial player in improving lycopene content in tomatoes. On the other hand, transgenics overexpressing *35S::prosystemin* (*35S::prosys*) displayed increased levels of JA and ethylene. Exogenous application of methyl jasmonate (MeJA) to the mutant fruits *spr2* and *defl* increased the levels of fruit lycopene. Similarly, exogenous application of MeJA to Never ripe (Nr) and the ET-insensitive mutants increased the lycopene accumulation significantly. Thus, JA appears to promote lycopene biosynthesis independent of ethylene (Liu et al. 2012). But the mechanistic explanations for the roles of these hormones during fruit ripening and accumulation of lycopene are largely not explained properly.

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## 12 Transgenic Tomato with Improved Fruit Aroma

Fruits have characteristic aroma volatiles that impart a fruity flavour contributed by ester compounds (Song and Forney 2008; Deflippi et al. 2009). More than 400 different types of volatile compounds have been recorded in tomato, but few of them have an impact on its organoleptic properties (Sitrit et al. 2008). This implies that metabolic engineering of tomato fruits for better taste and flavour qualities is possible. Sitrit et al. (2008) have chosen a terpenoid pathway gene that encodes geraniol synthase (GES) for this purpose. GES produces geraniol, an acyclic monoterpene alcohol, which has a good odour and also acts as a precursor to other scented volatiles like geranial, citronellol, and geranyl acetate. Overexpression of *GES* gene in tomato under the influence of polygalacturonase promoter (*PG*) resulted not only in enhanced levels of geraniol, but also aroma and overall flavour of the transgenic fruits (Sitrit et al. 2008). These results indicate that it is feasible to alter aroma and other quality traits through genetic engineering in tomato and other fruit crops. 3-Deoxy-d-arabino-heptulosonate-7-phosphate synthase (DAHPS) is the first enzyme of the shikimate pathway which produces the aromatic volatiles. Transgenic tomato expressing bacterial feedback-insensitive *AroG* gene encoding a 3-deoxy-d-arabino-heptulosonate-7-phosphate synthase (DAHPS) under the influence of a fruit ripening-specific promoter E8 enhanced the levels of metabolites and aroma (Tzin et al. 2013). The synthesis of 2,4,6-carbon chain esters occurs from the degradation of linoleic and linolenic acids. LOX enzymes contribute for the synthesis of ester compounds from alcohols (Baldwin et al. 2000; Contreras and Beaudry 2013) and help improve the flavour quality. When *CmLOX18* gene isolated from melon was overexpressed in tomato, it enhanced the biosynthesis of C6 volatiles like hexanal, (Z)-3-hexanal, and (Z)-3-hexen-1-ol, during fruit ripening (Zhang et al. 2017b).

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### 13 Transgenic Tomato with Enhanced Vitamin C or Ascorbic Acid

Ascorbic acid (AsA) is an essential component for collagen biosynthesis. Very rich in tomato, it acts as an antioxidant and protects DNA damage from oxidative stress (Raiola et al. 2014). Synthesis of AsA does not occur in humans due to the mutation of L-gulonono-1,4-lactone oxidase enzyme; hence, humans obtain it from plant sources (Chatterjee 1973). AsA protects the plants from ROS, but its deficiency activates cell death via redox mechanisms which are independent of natural senescence of the plants (Conklin et al. 1996; Pavet et al. 2005). Biochemical and transcriptomic analyses revealed the relation of AsA content with pectin methylesterase (PME) activity and the degree of pectin methylesterification (DME) in *Solanum pennellii* introgression line (IL12-4-SL). SolyPME, SolyPG, and UGlcAE have been found as candidate genes responsible for an increase in AsA production by affecting the alternative D-galacturonate pathway (Rigano et al. 2018). Overexpression of strawberry FaGalUR gene resulted in twofold increase in AsA levels in tomato fruit with enhanced oxidative and salt stress tolerance in comparison with WT plants. These results suggest that tomato has an alternative D-galacturonate pathway for ascorbate biosynthesis as pointed out by Cai et al. (2015).

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### 14 Increased Flavonoid Content

Transgenic tomato fruits expressing *AtMYB12* transcription factor displayed significant increase in flavonoid content. Mice fed with such transgenic tomato fruit extracts containing *AtMYB12* transcription factor recorded significant increase in femur and tibia bone growth in comparison with WT fruits (Choudhary et al. 2016). This implies that exciting prospects lie to generate transgenic tomato fruits with improved flavonoid content for better bone growth in humans.

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### 15 Transgenic Tomato for Expression of Malarial Antigens

It is important to develop transgenics that express animal proteins in high quantities. Kantor et al. (2013) developed transgenic tomatoes expressing the PfCO-2.9 protein which is a chimera of the antigens *MSP1* and *AMA1* of *Plasmodium falciparum*. Thus, successful transformation of tomato was reported with the expression of malarial antigen (*PfCP-2.9*). This study holds great promise to express other antigens of commercial importance in tomato.