

Tomato (*Solanum lycopersicum* L.) in the service of biotechnology

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Abstract Originating in the Andes, the tomato (*Solanum lycopersicum* L.) was imported to Europe in the 16th century. At present, it is an important crop plant cultivated all over the world, and its production and consumption continue to increase. This popular vegetable is known as a major source of important nutrients including lycopene, β -carotene, flavonoids and vitamin C as well as hydroxycinnamic acid derivatives. Since the discovery that lycopene has anti-oxidative, anti-cancer properties, interest in tomatoes has grown rapidly. The development of genetic engineering tools and plant biotechnology has opened great opportunities for engineering tomato plants. This review presents examples of successful tissue culture and genetically modified tomatoes which resistance to a range of environmental stresses improved, along with fruit quality. Additionally, a successful molecular farming model was established.

Keywords Tomato · Tissue culture · Transformation · Molecular farming

Introduction

Originating from the Andes, tomatoes (*Solanum lycopersicum* L.) were imported to Europe in the 16th century. At present, this plant is common around the world, and has become an economically important crop. Furthermore, this

plant is a model species for introducing agronomically important genes into dicotyledonous crop plants (Paduchuri et al. 2010). The tomato is considered a protective food because of its particular nutritive value, as it provides important nutrients such as lycopene, beta-carotene, flavonoids, vitamin C and hydroxycinnamic acid derivatives. Furthermore, this crop has achieved tremendous popularity especially in recent years with the discovery of lycopene's anti-oxidative activities and anti-cancer functions (Wu et al. 2011; Raiola et al. 2014). Thus, tomato production and consumption are constantly increasing. It is noteworthy that tomatoes are not only sold fresh, but also processed as soups, sauces, juices or powder concentrates. The tomato ranks 7th in worldwide production after maize, rice, wheat, potatoes, soybeans and cassava, reaching a worldwide production of around 160 million tons on a cultivated area of almost 4.8 million hectares in 2011 (FAOSTAT 2011).

From botanical point of view, the tomato is a fruit. Nevertheless, it contains a much lower sugar content compared to other fruits. It is a diploid plant with $2n = 24$ chromosomes. The tomato belongs to the *Solanaceae* family, which contains more than 3,000 species, including plants of economic importance such as potatoes, eggplants, tobacco, petunias and peppers (Bai and Lindhout 2007). In 1753, Linnaeus placed the tomato in the *Solanum* genus (alongside with potato) under the specific name *S. lycopersicum*. In 1754, Philip Miller moved it to its own genus, naming it *Lycopersicon esculentum* (Foolad 2007; Perlata and Spooner 2007). Nevertheless, the designation of the tomato was for a long time a subject of consideration and discussion by many scientists. The use of molecular data (genome mapping) and morphological information allowed for the verification of the *Solanaceae* classification when the genus *Lycopersicon* was re-introduced in the *Solanum*

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genus in the *Lycopersicon* section (Foolad 2007). Thus, after almost two centuries, the description of Linnaeus was confirmed. Due to the use numerous citations from recent references and in order to be consistent with much of the literature, the Linnaeus classification is followed in this review. Several reports indicate Peru as the centre of diversity for wild relatives of tomato. It seems justified to consider *S. lycopersicum cerasiforme* as an ancestor of the cultivated tomato because of its abundant existence in Central America (Bai and Lindhout 2007). Nevertheless, recent extensive genetic studies have revealed that the closest relative of the tomato is *Solanum pimpinellifolium* (The Tomato Genome Consortium 2012). It turned out that the genome sequences of both of the above-mentioned tomatoes as well as domesticated cultivars and *S. pimpinellifolium* showed only a 0.6 % nucleotide divergence. Domestication has triggered a range of traits (morphological and physiological) that distinguish domesticated crops from their wild ancestor. Studies on the domestication process, not only of tomatoes but also in the cases of maize and rice revealed that rapid phenotypic divergence is often controlled genetically by a relatively small number of loci (Koenig et al. 2013).

Among the members of family *Solanaceae*, many species of economic importance such as tomatoes, potatoes, tobacco, peppers and eggplants can be distinguished. In recent years, interest of scientists in the tomato as a model plant has significantly increased, also due to the fact that its genome has been sequenced (The Tomato Genome Consortium 2012). The tomato is an excellent model for both basic and applied research programs. This is due to it possessing a number of useful features, such as the possibility of growing under different cultivation conditions, its relatively short life cycle, seed production ability, relatively small genome (950 Mb), lack of gene duplication, high self-fertility and homozygosity, easy way of controlling pollination and hybridization, ability of asexual propagation by grafting and possibility to regenerate whole plants from different explants (Bai and Lindhout 2007; The Tomato Genome Consortium 2012). Among the existing tomato genotypes, cv. Micro Tom is considered to be a model system due to the aforementioned unique characteristics (Kobayashi et al. 2013). This dwarf tomato cultivar was created for ornamental purposes and originated by crossing two cultivars (Florida Basket and Ohio 4013-3), and shows small and ripened fruits as well as dark-green and rugose leaves. The phenotype of this cultivar is due to mutations in the *SELF-PRUNING* (SP), *DWARF* (D) and *MINATURE* (*mnt*) where the latter is likely associated with GA signaling (Martí et al. 2006). Additionally, in contrast to other model organisms such as *Arabidopsis* or rice, the tomato has many interesting features. For example, tomato plants produce fleshy fruits that are important for the

human diet. The tomato has sympodial shoots, and it is the only model plant with compound leaves. Furthermore, there exists a large pool of tomato mutants, which were either spontaneous or induced by chemicals or irradiation, that are available at the Tomato Genetic Resource Center (Lozano et al. 2009), LycoTill platforms and TOMA-TOMA base (Minoia et al. 2010; Saito et al. 2011). Over the years, the Botanical and Experimental Garden in the Netherlands has collected the germplasm of the *Solanaceae* species and preserved extensive ex situ plant collections of tomatoes (Bai and Lindhout 2007). This data is a crucial resource for breeders and scientists for improving the quality and yield of tomato cultivars. However, extensive knowledge concerning the molecular bases underlying these complex traits is necessary. This challenge requires comprehensive and genetically high-quality populations of mutants as well as the availability of these resources to the research community to promote functional analyses of tomatoes (isolation of key genes involved in development and growth regulation).

Tissue cultures of tomato

Traditional improvement methods are time-consuming and troublesome due to the time of breeding, and there is a problem with the choice of criteria appropriate for breeding purposes. Thus, the establishment of simple and efficient regeneration systems is a fundamental prerequisite of taking advantage of cell and tissue culture for genetic improvement (genetically transformed plants for commercial applications). The in vitro culture of the tomato has been successfully used in different biotechnological application including the clonal propagation of high-value commercial cultivars, virus-free plants, and genetic transformation (Namitha and Negi 2013; Hanus-Fajerska 2006; Li et al. 2011; Yarra et al. 2012).

The Flavr Savr tomato (also known as CGN-89564) was the first commercially grown, genetically engineered food to be granted a license for human consumption. The Food and Drug Administration approved the Flavr Savr tomato in 1994. Unfortunately, the tomatoes had a bland taste and they also were very delicate, proving difficult to transport. They were off the market by 1997. In China, the GM tomato Huafan No 1 (from Huzahong Agricultural University), which had long shelf life characteristics, was the first GM plant to be approved for commercialization in 1996. Other tomato varieties that have been authorized in some countries (USA, Japan, Mexico, Canada) include: 351N from Agritope Inc (Portland, USA), 8,338 and 5,345 from Monsanto (St. Louis, USA), 1345-4 from DNA Plant Technology Corp (Oakland, USA), B, Da, F from Zeneca Seeds. A detailed description is presented in Table 1.

Table 1 Transgenic tomato varieties approved for commercialization. Based on Yang et al. (2005) and Fukkuda-Parr (2012)

Company	Event	Trait	Year approved	Approved for	Country
Calgene	Flavr Savr CGN-89564	Delayed softening (developed by additional <i>PG</i> gene expressed)	1994	All uses in USA; Japan and Mexico for feed and for environment	USA
Calgene	Flavr Savr N 73 1436-11	Delayed ripening (developed by additional <i>PG</i> gene expressed)	1996	All uses in USA	USA
CAAS	About 10 events	Data not available	1998	Data not available	China
DNA plant technology	1345-4	Delayed ripening (developed by a truncated aminocyclopropane cyclase synthase (<i>ACC</i> gene))	1994	All uses in USA; food in Canada and Mexico	USA
Zeneca and Petoseed	B, Da, F	Delayed ripening (developed by additional <i>PG</i> gene expressed)	1994	All uses in USA; food in Canada and Mexico	USA
Monsanto	8338	Delayed ripening (developed by introduction of 1-aminocyclopropane-1-carboxylic acid deaminase (<i>accd</i>) gene)	1995	All uses in USA	USA
AgriTope	351N	Delayed ripening (developed by introduction the <i>S</i> -adenosylmethionine hydrolase (<i>SAM-K</i>) gene)	1995	All uses in USA	USA
Monsanto	5345	Insect resistant (developed by introduction of one <i>cryIAC</i> gene)	1997	All uses in USA; food in Canada	USA
Huazhong Agriculture University (HZAU)	Hufan no 1	Delayed ripening (developed by introduction anti-sense <i>EFE</i> gene)	1996	Data not available	China
Beijing University	PK-TM8805R (8805R)	Delayed ripening	1999	Food, feed, cultivation in China	China

It should be noted that elaborating a cost-effective and productive protocol for the mass propagation of high-quality tomato plants (via tissue culture) could significantly help reduce the market value of seedlings. An efficient regeneration system is also crucial for the success of such techniques as haploid regeneration, micropropagation, somatic hybridization, mutation selection and germplasm storage. As many independent studies on the tomato show, plant regeneration achieved through organogenesis is affected by several factors such as genotype, explant source, age of explants, media composition and environmental conditions (Mamidala and Nanna 2011; Namitha and Negi 2013; Sherkar and Chavan 2014; Wayase and Shitole 2014). There are many reports regarding tomato transformation and in vitro plant regeneration from different explants (including seed-cut cotyledons, hypocotyls, leaves, stem sections, pedicels, petioles and inflorescences) via organogenesis (Khoudi et al. 2009; Yasmeeen 2009; Goel et al. 2011; Koleva Gudeva and Dedejski 2012; Rai et al. 2013; Namitha and Negi 2013; Sherkar and Chavan 2014; Wayase and Shitole 2014). These reports also describe the recalcitrance of ‘non-competent’ tomato explants (partial or total inability to respond to in vitro culture) (Fuentes et al. 2008; Mamidala and Nanna 2011).

Thus, the improvement of the adventitious shoot regeneration system using tissue culture methods for tomato plants is still important due to the diverse morphogenic potential of the different genotypes. As mentioned above, scientists have used different types of explant, but it should be emphasized that the type of explants determines not only the frequency of the explants’ organogenesis but also determines the number of shoots produced per explant (Bahurpe et al. 2013; Jehan and Hassanein 2013). Namitha and Negi (2013) demonstrated that the efficiency of shoot regeneration ability followed the order hypocotyls > cotyledon > leaf. In earlier studies, Mamidala and Nanna (2011) reported that cotyledons explants showed organogenesis superiority over hypocotyls and leaf explants. It turned out that leaf explants showed effective regeneration only on one of the media tested (MS + 2 mg/L BAP, 6-Benzylaminopurine + 0.1 mg/L IAA, Indole-3-acetic acid), which suggests that using this type of explants can minimize genotype-dependent variations. In contrast to this report, Chaudry et al. (2010) observed the higher regeneration potential of hypocotyls than of leaf explants. On the other hand, Harish et al. (2010) reported that shoot formation efficiency was greatest in the order: hypocotyls > leaf > stem. Moreover, they noticed significant

differences in regeneration capacity between the six cultivars tested in terms of regeneration process duration as well as the number and size of the regenerated shoots. Ashakiran et al. (2011), who examined the effect of TDZ (Thidiazuron) on organogenesis induction from cotyledonary and leaf nodes, obtained similar results. On the other hand, Zhang et al. (2012) indicated that the location of the cutting wound in explants significantly affected callus induction and adventitious bud formation. They demonstrated that the highest frequency of bud induction occurred at the middle part of the cotyledon segment. They also proved that the way an explant was placed on a medium affected the differentiation rate of cotyledon buds, the back-up placing of the cotyledon onto the medium proved best.

It is well known that regeneration process depends on the age of explants. Moreover, it is reported that young explants have shown to give better morphogenic response than older ones (Harish et al. 2010). Dai et al. (1988) revealed that the regeneration capacity of tomato explants increased with their age. Depending on the type of explant, seedlings of different ages (7, 8, 10, or 14 days old) were used (Ishag et al. 2009; Kantor et al. 2010; Ali et al. 2012; Ajenifujah-Solebo et al. 2012; Bahurpe et al. 2013). Furthermore, much data suggests that the size of explants is essential for efficient plant regeneration in tomatoes. The optimal sizes for tomatoes are 0.7–2 cm long segments for hypocotyls and 5 mm × 5 mm for cotyledons (Ishag et al. 2009; Chaudry et al. 2010; Ajenifujah-Solebo et al. 2012).

There are two methods used to regenerate plantlets in vitro: somatic embryogenesis (direct or indirect) and organogenesis. From the point of view of conducting research on heredity or genetic engineering, the second pathway is more desirable as it allows the avoidance of genetic variation. Hence, most of the published procedures were based on direct organogenesis from intact explants (e.g. cotyledons, hypocotyl, leaf) or protoplast cultures or shoot development from meristematic cells (Ajenifujah-Solebo et al. 2012; Namitha and Negi 2013), while attempts to regenerate tomato via somatic embryogenesis are rather rare. However, Godishala et al. (2011) reported a simple and reproducible protocol for tomato cv S-22 regeneration via somatic embryogenesis. Additionally, Guan et al. (2012) demonstrated that shoot organogenesis and somatic embryogenesis occurred simultaneously during the in vitro regeneration of transgenic cherry tomato (*Solanum esculentum* var. *cerasi-forme*) mutant leaf explants treated by 6-BA combined with IAA. Interestingly, only the somatic embryogenesis pathway was observed during the regeneration of non-transformed cherry tomato plants under the same culture condition. Khuong et al. (2013) observed a similar effect on cv. Micro

Tom using trans-zeatin (TZ) (1 mg/L) combined with IAA (0.1 mg/L). They noticed very little callus formation, indicating direct shoot differentiation as described earlier for the Rio Grande cultivar and, on the other hand, they also noticed indirect embryogenesis via callus formation with the same kind hormonal regimen but that they had different concentrations (respectively 1 and 2 mg/L).

Exogenous fitohormones in a medium play an important role in regulating callus induction and organ differentiation or rooting. According to numerous reports IAA, NAA (α -Naphthaleneacetic acid), 2,4-D (2,4-Dichlorophenoxyacetic acid), ZT and 6-BAP are the hormones commonly used in in vitro cultures of tomato to ameliorate callus induction and plant regeneration (Kantor et al. 2010; Mamidala and Nanna 2011; Ashakiran et al. 2011; Zhang et al. 2012; Namitha and Negi 2013). KIN (Kinetin), 2iP (6-(γ,γ -dimethylallylamino) purine), TDZ, and IBA (Indole-3-butyric acid) are other plant growth regulators (PGR) that were tested (Ishag et al. 2009; Chaudry et al. 2010; Wu et al. 2011; Ashakiran et al. 2011). Tomato shoot induction from different types of explants was achieved in different cultivars through the modification of the media conditions. Moreover, it was shown that the type of basal medium used (e.g. MS or B5) (Murashige and Skoog 1962; Gamborg et al. 1968) may significantly affect the regeneration process rate (Ashakiran et al. 2011; Wu et al. 2011). Rashid and Bal (2010) demonstrated that MS fortified with kinetin 0.5 mg/L and BAP 0.5 mg/L was the optimal medium for inducing direct shoot regeneration. In contrast to these findings, Wu et al. (2011) emphasized the superiority of B5 basal medium to the MS medium. The regeneration rate for shoots from MicroTom explants on B5 was considerably higher than on MS (+12 %), and the best variant for regeneration (from cotyledons and hypocotyls) was MS supplemented with 1.5 mg/L 6-BA and 0.05 mg/L IBA, reaching 95.8 and 60 % respectively. Interestingly, the regeneration frequency of the MicroTom explants decreased with increasing IBA concentration. Kantor et al. (2010) discovered that MS supplemented with 1 mg/L zeatin and 0.05 mg/L IAA stimulated the highest number of regenerants. Zhang et al. (2012) obtained similar results for cotyledon explants: that MS supplemented with 2 mg/L zeatin and 0.01 mg/L IAA turned out to be most effective. On the other hand, MS with the combination of BAP (2 mg/L) and IAA (0.1 mg/L) was found to be the best for inducing shoot regeneration (74 %) and multiple shoot formation per explants from hypocotyls (Namitha and Negi 2013). In contrast to the media indicated by many authors, Ali et al. (2012) suggested that a MS medium supplemented with combination of 1.0 mg/L kinetin and 1.0 mg/L BA to be optimal for producing the highest number of shoots per explant from hypocotyls and cotyledons in tomatoes.

In plant tissue cultures, the growth and regeneration of plants can be improved by a small quantity of organic nutrients. In general, these adjuvants can be a potential source of vitamins, amino acids, fatty acids, peptides, carbohydrates or natural PGR at different concentrations (e.g. zeatin). One of them is coconut milk (CM), which contains a complex combination of several compounds. CM is predominantly used in orchid tissue culture. Afroz et al. (2010) used it to enhance the in vitro regeneration efficiency of five varieties of tomato. CM is known to induce plant cell proliferation and their rapid growth. Afroz et al. (2010) successfully replaced zeatin with CM and kinetin. They showed that coconut water alone was insufficient to promote satisfactory multiplication, but the combination of CW with IAA and kinetin allowed the achieving of faster regeneration (12–15 days from leaf explants and 20–25 days from hypocotyls) with a maximum number of shoot primordia. Bhatia and Ashwath (2008) used other adjuvants such as activated charcoal, ascorbic acid and casein hydrolysate to improve shoot regeneration response from cotyledon explants. The results showed that activated charcoal as well as ascorbic acid could improve the quality of the regenerated tomato shoots while casein hydrolysate can be effectively utilized to reduce callus response underneath the shoots, consequently decreasing the chance of somaclonal variation. While most scientists use the MS medium supplemented with a combination of auxins and cytokinins in different concentrations, Plana et al. (2006) reported an alternative procedure to regenerate tomato plants where there is a deficiency of exogenous fitohormones. The medium they proposed contained MS salts, 4 mg/L thiamine, 100 mg/L myo-inositol and 3 % sucrose. In practice, this procedure combines the pre-culture and seed cuttings to promote organogenesis without callus development. The main advantages of this method are simplicity, time efficiency and, most importantly, the proposed procedure allowed the obtaining of a shoot formation without developmental/morphological abnormalities (e.g. leaves and shoots without apical meristem and vitrified structures).

Environmental conditions such as light or temperature were found to be crucial for tomato regeneration. As it is well known, light (by the length of exposure or its quality) influences explant growth and differentiation processes. The response of tomato explants to tissue culture depends on the quality and quantity of light used during growth of a mother plant. Glowacka (2004) investigated the influence of red, yellow, green, blue and natural light on the micropropagation of tomatoes. The study showed a distinct influence of red and yellow light on shoot and internode elongation, and the plantlets were easy-to-cut. On the other hand, blue and

natural light inhibited shoot and internode elongation. Extending the regeneration period had no influence on the growth of the plantlets under red and yellow light. It turned out that red and yellow light had favorable influence on root formation. Since light is indispensable for the regeneration of tomato shoots, studies on tomato regeneration have exploited the 16 h photoperiod (Ali et al. 2012; Zhang et al. 2012; Namitha and Negi 2013). For example, Bhatia and Ashwath (2005) revealed that maximum shoot regeneration response (60 %) occurred in the explants exposed to 16 h of light and 8 h of darkness. The response decreased at 2 h dark (47 %) or 24 h (40 %) light. These results are in contrast to studies conducted by Tyburski and Tretyn (1999), who reported that tomatoes could be regenerated in the absence of light. The shoots that were regenerated under dark conditions were chlorotic, but they developed chlorophyll after exposure to a 16 h photoperiod. It was shown that the texture of the medium affected tomato regeneration. Velcheva et al. (2005) developed two distinct systems—solidified medium or liquid medium—for the regeneration of commercial tomato cultivars (Daniela 144, Brillante 179, Annan 3,017, Galina 3,019, and Bernadine 5,656) after a *Agrobacterium*-mediated transformation. In terms of the regeneration ability of different types of explants, their results are in agreement with the statement that hypocotyl explants are worse compared to the cotyledons when cultured on a solid medium and the liquid medium allowed the obtaining of similar regeneration efficiencies for both hypocotyls and cotyledons. Obviously, it cannot be ruled out that the physical parameters of a liquid culture (e.g. gas exchange, frequent passages or constant agitation of explants) played an essential role in efficient hypocotyl regeneration. Undoubtedly, the study by Velcheva et al. (2005) demonstrated some advantages of this procedure: regeneration is initiated from epidermal to subepidermal cells and the selection process in liquid media seems to be much more effective compared to similar selection performed on solid media.

Rooting is the final step of the regeneration protocol in plant tissue cultures. There are many factors affecting the rooting process (e.g. the physiological status of plantlets, medium composition, growth regulators). Mostly, MS or 1/2MS are used as a basal medium for rooting. Mensuali-Sodi et al. (1995), Rashid and Bal (2010) and Bahurpe et al. (2013) suggested that for root induction, the tomato does not require any exogenous plant growth regulators. However, in most cases, root formation would be achieved with auxins (IAA, NAA or IBA) alone with concentrations ranging from 0.1 to 1 mg/L (Chaudry et al. 2010; Ashakiran et al. 2011; Mamidala and Nanna 2011; Zhang et al. 2012; Namitha and Negi 2013; Sherkar and Chavan 2014; Wayase and Shitole 2014). Abundant rooting is usually observed after 2 weeks.

Genetic engineering of tomatoes

Methods of tomato transformation

The first *Agrobacterium*-mediated transformation of tomatoes was reported in 1986 (McCormick et al. 1986). Since then, several transformation protocols for different tomato cultivars have been developed using various explants (e.g. cotyledons, hypocotyls, leaves, fruits) (El-Siddig et al. 2011; Wu et al. 2011; Yarra et al. 2012; Garcia-Hurtado et al. 2012; Hasan et al. 2008; Orzaez et al. 2006, 2009; Orzaez and Granell 2009; Yasmeen et al. 2009). The process of plant genetic transformation is very complex, with many factors playing an important role including the application of nurse cells, the addition of acetosyringon to the culture or preculture media, bacterial factors (*Agrobacterium* strain, culture density) and tissue-specific factors (the genotype and the type of the explants) as well as the plasmid vector, the composition of the culture medium (concentration of fitohormones), the type and concentration of antibiotics, the cocultivation time, etc. (Fuentes et al. 2008; Jabeen et al. 2009; Sharma et al. 2009; El-Siddig et al. 2011; Wu et al. 2011; Guo et al. 2012; Chetty et al. 2013; Koul et al. 2014). Unfortunately *Agrobacterium*-mediated transformation is still not suitable for tomato varieties with low regeneration capacity. However, some attempts to establish an efficient transformation procedure for such cultivars (e.g. Cambell-28) have been made (Fuentes et al. 2008). The development of a system for stable genetic transformation of tomato plastids was a milestone in the transformation of the tomato (Ruf et al. 2001). This relatively new transformation technology allowed to investigate the possibility to elevate the provitamin A content in tomatoes (Apel and Bock 2009). Lycopene β -cyclase genes from an eubacterium *Erwinia herbicola* and from a higher plant, a daffodil (*Narcissus pseudonarcissus*), were introduced into the tomato plastid genome in order to enhance carotenoid biosynthesis and induce the conversion of lycopene to provitamin A. This research gave unexpected results, namely that the transplastomic tomatoes also showed a 50 % increase in total carotenoid accumulation in plants expressing the lycopene β -cyclase from daffodils. Another example of tomato chloroplast transformation was given by Zhou et al. (2008), who demonstrated that the HIV antigens p24 and Nef could be expressed in a plastid of tomato plants.

Recently, several procedures for the stable transformation of tomato plants have been reported (Hasan et al. 2008; Sharma et al. 2009; El-Siddig et al. 2011). Notwithstanding, there is still lack of an efficient, simple and reliable protocol, which significantly hinders the functional analysis of transgenes. To overcome this problem, scientists use the transient transformation methodology. This

alternative technology could provide a rapid tool for the functional analysis of the genes of interest (transgenes) (Wróblewski et al. 2005). An important breakthrough in the fast reverse genetics was achieved by using a powerful tool—virus induced gene silencing (VIGS) technology (Orzaez and Granell 2009; Fernandez-Moreno et al. 2013). Jaberolansar et al. (2010) and Romero et al. (2011) successfully demonstrated that the Tobacco Rattle Virus (TRV)-based VIGS vector could be used in tomato to silence genes. On the other hand, Zhou et al. (2012) applied Potato Virus X as a tool for virus-induced gene complementation for revealing a transcription factor network in the modulation of tomato fruit ripening. Furthermore, Orzaez et al. (2006), in order to shorten the time of the functional analysis of genes in fruit development, used an *Agrobacterium*-mediated transformation by infiltrating tomato fruit tissue. This new procedure, called “fruit agroinjection”, involves injecting *Agrobacterium* suspension into green fruits, resulting in complete fruit infiltration. The aforementioned method was found to be an invaluable tool for transient expression in fruits and also significantly facilitates functional research concerning that organ. Some of the data in the literature suggests fleshy fruits as an ideal target for genetic engineering (Spolaroe et al. 2001; Orzaez et al. 2006). Nevertheless, the identification and quantification of nonvisual phenotypes could be hindered by the irregular distribution of VIGS effects in fruit. For that reason, Orzaez et al. (2009) elaborated a simple visually traceable VIGS system for fruit. This methodology consists of two elements: (1) a tomato line expressing *Roseal* and *Delia* transcription factors under the control E8 promoter that show a purple-fruited (anthocyanin-rich) phenotype, and (2) the agroinjection of a modified TRV VIGS vector incorporating partial *Roseal* and *Delia* sequence into *Del/Ros1* plants, which was shown to be able to restore red fruit phenotype. Hasan et al. (2008), using the agroinjection procedure, obtained a stable transformed tomato fruit. The transformation efficiency ranged from 54 to 68 % in seedlings raised from seeds collected from the infiltrated fruits. Yasmeen et al. (2009) noticed that mature red fruit resulted in a higher frequency of transformation than immature green fruit, and the transformation efficiency was 40–42 %. Among the particularly popular methods there are those that avoid regeneration of the tissue culture as they allow the exclusion of complex, time consuming procedures, thus shortening the time of the entire process. The *in planta* transformation method is one of these. This method has been successfully used so far for various plant species (both monocotyledonous and dicotyledonous plants, including the tomato) to obtain transgenic plants. One of the versions of the *in planta* transformation method is the floral dip procedure. The floral dip method was used by Yasmeen et al. (2009) to obtain transgenic tomatoes.

For this purpose, they tested two approaches: the transformation of unopened flowers before pollination and the transformation open flowers after pollination. Yasmeen et al. (2009) showed that the floral stage as well as the gene construct had a significant impact on transformation efficiency. The results revealed that flowers treated before pollination gave higher percentages of transformation (12 % for *LFY* gene construct and 23 % for *GUS* gene construct) compared to those treated after pollination. Although the transformation efficiency was promising, some undesirable changes were observed. Compared to the control plants, the transgenic plants carrying transgenes (*API*) *Apethala* gene from *A. thaliana* or *LFY* (*LEAFY* gene from *A. thaliana*) were phenotypically different. They were shorter, their stems were not as erect and their leaves were curled. Additionally, these plants produced normal flowers earlier than the control plants, but they were infertile and failed to bear fruit. To our best knowledge, this kind of protocol is not widespread with regards to the tomato.

Applicable tomato transformation

At present, GM technology is widely acclaimed as being able to produce “upgraded crops”, including tomatoes, more rapidly and efficiently than selection breeding and therefore has the potential to reduce food shortages. Many plants important from economic point of view are genetically modified to resist a wider range of environmental conditions such as poor soil conditions (e.g. salinity and metal contamination) or drought, extreme temperatures (i.e. heat or cold). The genetic engineering allowed for increased productivity by enhancing efficiencies of metabolic or photosynthetic pathways.

Resistance to abiotic stresses

Transgenic approaches have been attempted to improve tolerance to abiotic stresses. A large number of genes either involved in signaling and regulatory pathways or encoding enzymes known to alleviate stress have been introduced to produce plants with increased stress resistance against salinity, high and low temperatures, oxidative stress, heavy metals or drought. Heavy metals that accumulate in soil are extremely harmful contaminants. Their toxic effects cause disturbances in cell membrane functioning, photosynthetic and mitochondrial electron transport, enzyme inactivation and basic cellular metabolism, thus leading to disturbances in the energy balance of a cell as well as hindering mineral management and growth suppression. For most, even a slight increase in the concentrations of metal ions in a cell is harmful, however in the course of evolution some

species have developed mechanisms to protect themselves from the harmful effects of high concentrations of heavy metals present in the environment. Barabasz et al. (2012) demonstrated that the expression of HMA4 (P_{1B} -ATPase) from *Arabidopsis halleri* in plants could be a useful approach to engineer altered metal distribution in tissues, which could be useful for biofortification or phytoremediation. It turned out that the expression of the *AhHMA4* gene facilitated Zn translocation from root to shoot and also induced Zn uptake in a Zn supply-dependent manner.

Drought is defined as water deficit in the environment and it is closely correlated with soil salinity. The presence of salt in the soil causes ionic and osmotic stresses, which lead to metabolic imbalances and nutritional deficiencies and may also cause oxidative stress. High soil salinity may damage plants during the vegetation period. It is believed that these two kinds of stresses are among the most devastating abiotic stresses that limit crop productivity worldwide. For example, droughts in Poland can occur in different seasons of the year, but they are most common in spring, occurring every few years. It is a very serious economic problem for any country because of large yield losses, and thus farmer income decreases and food prices increase. More recently, global warming may have been worsening this situation in most agricultural regions around the world. The ultimate aim is to develop crop plants with improved water use efficiency that can minimize drought-induced yield losses. Furthermore, drought stress tolerance may not only ameliorate productivity the land already in use but may also allow for the exploitation of cultivable land with limited water supplies. Over the last two decades, the number of publications concerning genetically modified plants for drought resistance has increased, indicating their scientific and applied importance. In this literature, many metabolic systems and candidate genes were targeted to achieve drought resistance. As is commonly known, resistance to abiotic or biotic stresses is a multifactor trait involving several genes. Therefore, genetic engineering for developing stress-tolerant crops based on the introgression of genes known to be involved in stress response and putative tolerance is being developed. Hence, numerous researchers focused on one or a few genetic changes to modify key metabolites (e.g. glycine betaine and proline) (Goel et al. 2011; Álvarez-Viveros et al. 2013) or proteins *Late Embryogenesis Abundance* (LEA) (Muñoz-Mayor et al. 2012) for drought resistance. Another strategy to increase the level of drought and salinity tolerance in plants consists of a transfer of genes encoding different types of proteins involved in molecular responses to abiotic stress such as osmoprotectants, chaperones, detoxifying enzymes, transcription factors, signal transduction proteins (kinases and phosphatases) and heat-shock proteins (HSPs) (Wang et al. 2011; Mishra et al. 2012; Li et al. 2013). It is known

that the mitogen-activated protein kinases are involved in tolerance-related signaling networks associated with various stressors, including drought stress. The results obtained by Li et al. (2013) using VIGS methodology confirmed this observation. It was found that *SpMPK1* (the mitogen-activated protein kinases from *S. pimpinellifolium*), *SpMPK2*, and *SpMPK3* genes played a crucial role in enhancing the drought tolerance of tomato plants by affecting the production and activity of H₂O₂ via the ABA-H₂O₂ pathway, and thus their inhibition reduced drought tolerance.

However, the modification of the expression of a single gene involved in resistance response such as listed above usually has a limited effect. A better solution seems to be the modification of the expression of transcription factors (TFs). This is an attractive target category for manipulation group, as it activates a cascade of genes that act together in enhancing tolerance towards different stresses. Most of them are classified into several transcription families such as AP2/ERF (APETALA2/Ethylene Responsive Factor), MYC, MYB, NAC, (Cys2His2 zinc finger), bZIP (basic leucine zipper) and WRKY (Shinozaki and Yamaguchi-Shinozaki 2007). Some of them are involved in plants' response to drought. Especially TFs from bZIP (e.g. ABA responsive element binding protein/ABRE binding factor (AREB/ABF)), AP2/EREB (e.g. DRE binding protein/CRT binding factor (DREB/CBF)), NAM (no apical meristem, ATAF1-2, CUC2 (cup shaped cotyledon) (NAC) (e.g. stress-responsive NAC (SNAC)), CCAAT-binding (e.g. C3H2 zinc finger protein ZFP) (Yang et al. 2010). AREB/ABF belong to the bZIP family plant TFs known to function in ABA signaling during dehydration and seed maturation. In response to ABA, an activated AREB/ABF binds to a *cis*-element known as an ABA-responsive element (ABRE) to trigger gene expression (Pandey et al. 2011). Up to now, participation of this kind of TFs in ABA-mediated stress signaling has been described for different plants such as *Arabidopsis thaliana*, rice, wheat and barley (Yang et al. 2010). Research by Yanez et al. (2009) revealed that the expression *SIAREB* in tobacco and tomato leaves was responsible for up-regulation of stress-responsive genes such as RD29B, the LEA genes ERD10B and TAS14 (dehydrin from tomato), the transcription factor PHI-2 and trehalose-6-phosphate phosphatizing gene. These results suggested that this class of bZIPs plays a role in abiotic stress response in the *Solanum* genus. In another study, Hsieh et al. (2010) observed that the overexpression of *SIAREB* is responsible for increasing tolerance to water and salinity in tomato plants. The overproduction of *SIAREB* in transgenic tomato plants regulated genes AtRD29A, AtCOR47, and SICI-like dehydrin under ABA and abiotic stress treatments. Mishra et al. (2012) inserted the transcription factor gene *ATHB-7* (*Arabidopsis*

thaliana homeodomain-leucine zipper class I genes) into the tomato genome. *ATHB-7* gene is induced in plants under drought stress via a mechanism that requires the production of ABA and acts as a negative growth regulator in *Arabidopsis* the expression of *A. thaliana* transcription factor gene *ATHB-7* in tomato plants significantly reduced the leaf stomatal density and stomatal pore size, which is probably crucial in preserving higher water potential. However, Mishra et al. (2012) observed in transgenic tomato line (DTL-20) a reduction in plant growth. This characteristic under-soil water deficit is common to many plant species.

Plants have developed several adaptation strategies that allow them to withstand saline stress. Among them we can distinguish sequestration of solutes, limitation of lipid peroxidation and the production of osmoprotectants. Numerous data indicates that there are potential benefits from obtaining transgenic plants overexpressing H⁺-pyrophosphatase and Na⁺/H⁺ antiporter, which increase tolerance to salinity. The data presented by Bhaskaran and Savithamma (2011) and Yarra et al. (2012) support the aforementioned hypothesis. Some research has indicated a significant role for vacuolar H⁺-ATPase (V-ATPase) under drought conditions. This multisubunit enzyme is necessary for plant growth because it is responsible for energizing secondary transport in the maintenance of ion homeostasis and in abiotic stress tolerance. Hu et al. (2012) demonstrated that the overexpression of *MdVHA-B* (subunit B of the V-ATPase from apple) in tomato plants resulted in high tolerance to drought stress as well as reduced malondialdehyde (MDA) contents and relative water loss, along with increased levels of free proline and H⁺ ATPase activity as compared to the control plants. It should be emphasized that MDA is a widely used marker of oxidative lipid injury whose concentration varies in response to abiotic or biotic stresses. Malondialdehyde accumulation takes place in plants due to membrane lipid peroxidation (Sharma et al. 2012).

As previously mentioned, the transfer of gene-coding transcription factors is one of the strategies to increase plant tolerance to drought and salinity. Rai et al. (2013) showed that the overexpression of *BcZAT12* in transgenic tomato plants caused a significant increase in their drought tolerance. Similarly, Mishra et al. (2012) demonstrated that transgenic tomato lines carrying the transcription factor of the *ATHB-7* gene from *A. thaliana*, were highly drought tolerant. On the other hand, Álvarez-Viveros et al. (2013) suggested that the overexpression of two genes, glyoxalase I gene (*GlyI*) and glyoxalase II genes (*GlyII*), might improve the salinity tolerance of tomatoes. It is known that methylglyoxal is produced during salt stress, and its detoxification is triggered by glycolases. Thus, transgenic plants subjected to a high concentration of NaCl (800 mM)

displayed both reduced lipid peroxidation and the production of H₂O₂. It is not only during drought stress, but also in cases of other stresses, that the scavenging of reactive oxygen species (ROS) is connected with the acting of a range of enzymatic and non-enzymatic antioxidants as well as of organic compounds as polyamines (PAs) (Gill and Tuteja 2010). Polyamines are considered as one of the oldest groups of substances, and include tetramine spermine (Spm), putrescine (Put) and cadaverine (Cad). In plants, polyamines not only play a role in abiotic and biotic stress, but also in many other physiological processes (organogenesis, embryogenesis, floral initiation and development, leaf senescence, fruit development and ripening) (cf. Alcazar et al. 2010). Recent studies have revealed that polyamine signaling is involved in direct interactions with different metabolic pathways and entangled hormonal cross-talks (e.g., abscisic acid involved in the regulation of abiotic stress responses) (Alcazar et al. 2010). Furthermore, many studies using transgenic overexpression or loss-function mutants confirmed protective role PAs in plant response to abiotic stress. As mentioned previously, the example of polyamines is putrescine (Put) in biosynthesis, in which arginine decarboxylase (ADC) is involved. Wang et al. (2011) showed that transgenic tomato lines with an overexpression of the *PtADC* gene isolated from *Poncirus trifoliata* performed better in plant dehydration and drought stress. As expected, under these stress conditions, ROS accumulation significantly decreased as compared to the control plants.

Numerous data have indicated that the heterologous overexpression of ornithine decarboxylase, ADC, S-adenosyl-L-methionine decarboxylase (SAMDC), spermidine synthase (SPDS) from different animal or plant sources in such plants as tomatoes, rice and tobacco has displayed tolerance traits against different stress conditions (including salt stress, osmotic stress, freezing, heat, drought, etc.) (cf. Alcazar et al. 2010).

In countries with cold climates, tomatoes are grown in greenhouses. Maintaining controlled temperature conditions raises the costs of breeding. Thus, a reasonable solution to manage this problem seems to be obtaining genetically-engineered tomato plants resistant to low temperatures. Generally, cold stress is responsible for, among others, the induction of osmotic disorders. Thus, tolerance to cold activates enzymes responsible for the synthesis of osmoprotectants and antioxidant defense. Osmotin and osmotin-like proteins have been demonstrated to accumulate in response to various biotic and abiotic stresses in plants. Patade et al. (2013) gave clear evidence that the accumulation of both osmotin and proline during cold stress in transgenic tomato lines imparted cold tolerance to them. Several studies on species more tolerant to cold allowed the determination of genes regulated by cold, known as *COR*

genes (cold-regulated). All genes belonging to the *COR* group have two characteristic sequences in promoter: the C-repeat (CRT) and the dehydration responsive element (DRE)-related motifs that interact with the CRT/DRE binding factor (CBF1). When the aforementioned gene from *A. thaliana* was introduced into tomatoes, the transgenic plants revealed higher chilling tolerance (Hsieh et al. 2002). However, transgenic tomato plants showed growth retardation with reduced fruit, seeds and fresh weight numbers. Moreover, transgenic tomato plants contained higher levels of proline than wild-type plants under normal or water-deficient conditions. Singh et al. (2011) obtained similar results by introducing into tomatoes the *AT-CBF1* gene, which is driven by the inducible promoter RD29A (which contained several *cis*-acting elements, including DRE, ABRE). However, Singh et al. (2011) did not observe any morphological disorders. The use of RD29A promoter instead of constitutive promoter in the tomatoes led to the development of cold-tolerant transgenic plants without any phenotypic abnormalities.

On the other hand, in terms of global warming, obtaining transgenic plants resistant to high temperature seems to be fully justified. It is commonly known that the accumulation of polyamines, including betaine, putrescine, spermidine or spermine, under abiotic stresses plays a crucial role in plant defense response to unfavorable conditions. SAMDC is one of the pivotal regulatory enzymes involved in biosynthesis of these compounds. Cheng et al. (2009) reported that transgenic tomatoes carrying the *SAMDC* gene from *Saccharomyces cerevisiae* produced 1.7–2.4 times more polyamines and therefore showed enhanced tolerance to high temperatures as compared to the control plants. Similarly to cold and heat stresses, ultraviolet B (UV-B, 280–320 nm) causes both the production and accumulation of toxic ROS. Furthermore, the interaction of high temperatures and UV-B could trigger sunscald phenomenon (tissue browning and desiccation) among the crop plants' fruit. Under such unfavorable conditions, the plants protect themselves by producing antioxidant enzymes including superoxide dismutase (SOD) and ascorbate peroxidase (APX). The data provided by Wang et al. (2006) clearly indicates that an overexpression of cAPX (cytosolic ascorbate peroxidase) in transgenic tomato plants significantly enhances resistance to high temperature (40 °C) compared to wild-type plants.

Examples of successful genetic engineering of tomatoes for enhanced resistance to abiotic stresses are presented in Table 2.

Resistance to biotic stresses

The enormous economic success of crop plants, including the tomato, is due to the application of pesticides and control of bacterial and viral diseases. Currently, pests and

Table 2 Examples of successful genetic engineering of tomato

Fruit trait	Targeted gene	References
<i>Fruit quality (organoleptic and nutritional)</i>		
Flavor and aroma	Thaumatococin, <i>GES</i> , <i>LeAADC1A</i> , <i>LeAADC2</i>	Bartoszewski et al. (2003); Davidovich-Rikanati et al. (2007); Mathieu et al. (2009); Tieman et al. (2006)
Size	<i>fw2.2</i>	Cong and Tanksley (2006); Liu et al. (2003)
Firmness	β -galactosidase, <i>EXP1A</i> (expansin)	Brummell et al. (1999); Smith et al. (2002)
Parthenocarpy	<i>Arf8</i> ; <i>IAA9</i> ; <i>SIARF7</i> , <i>SI-IAA27</i>	Bassa et al. (2012); de Jong et al. (2011); Goetz et al. (2007); Wang et al. (2005)
Soluble solids content	<i>Lin5</i> (invertase 5)	Zanor et al. (2009)
Carotenoid content	<i>Dxs</i> , <i>CrtB</i> , <i>CrtR-b2</i> (carotene beta hydroxylase), <i>CrtI</i> , <i>CrtY</i> , <i>PSY-1</i> , <i>Cyc-B</i> , <i>LCY-B</i> , <i>CHY-B</i> , <i>CRY-2</i> , <i>DET-1</i> , <i>COP1LIKE</i> , <i>CUL4</i> (Cullin4), <i>FIBRILLIN</i> , <i>spermidine synthase</i>	Apel and Bock (2009); D'Ambrosio et al. (2011); Davuluri et al. (2005); Dharmapuria et al. (2002); Enfissi et al. (2005); Fraser et al. (2002), (2007); Giliberto et al. (2005); Liu et al. (2004); Neily et al. (2011); Simkin et al. (2007); Wurbs et al. (2007); Wang et al. (2008)
Flavonoid content	<i>CHI</i> , <i>CHS</i> , <i>CHI</i> , <i>F3H</i> , <i>FLS</i> , <i>STS</i> , <i>CHR</i> , <i>FNSII</i> , <i>MYB12</i> , <i>SIMYB12</i> , <i>Del</i> , <i>Ros</i> , <i>ANTI</i> , <i>AN2</i>	Adato et al. (2009); Ballester et al. (2010); Bassolino et al. (2013); Butelli et al. (2008); Colliver et al. 2002; Maligeppagol et al. (2013); Muir et al. (2001); Schijlen et al. (2006); Schreiber et al. (2012)
Carboxylic acids	<i>SlAco3b</i>	Morgan et al. (2013)
Ascorbic acid content	<i>GalLDH</i> , <i>GME</i> , <i>GCHI</i> , <i>ADCS</i>	de la Garza et al. (2004), (2007); Garcia et al. (2009); Gilbert et al. (2009); Zhang et al. (2011); Waller et al. (2010)
<i>Abiotic stress</i>		
	<i>GlyI</i> , <i>GlyII</i> , <i>cAPX</i> , <i>SpMPK1</i> , <i>SpMPK2</i> , <i>SpMPK3</i> , <i>Osmotin</i> , <i>HMA4</i> (P1B-ATPase), <i>SAMDC</i> , <i>mtlD</i> , <i>codA</i> , <i>AVP1</i> , <i>PgNHX1</i> , <i>BcZAT12</i> , <i>TaNHX2</i> , <i>tas14</i> , <i>PtADC</i> , <i>MdVHA-B</i>	Álvarez-Viveros et al. (2013); Barabasz et al. (2012); Bhaskaran and Savithramma (2011); Chen et al. (2009a); Goel et al. (2011); Hu et al. (2012); Khare et al. (2010); Li et al. (2013); Mishra et al. (2012); Muñoz-Mayor et al. (2012); Park et al. (2005); Patade et al. (2013); Rai et al. 2013; Wang et al. (2006), (2011); Yarra et al. (2012)
<i>Biotic stress</i>		
	<i>AFP</i> , <i>amiR-AV1-3</i> , <i>hCAP18/LL-37</i> , <i>Bs2</i> , <i>CHI</i> , <i>alfAFP</i> , <i>ech42</i> , <i>Cry 2Ab</i> , <i>LF</i> , <i>CryIAC</i>	Chen et al. (2009b); El-Siddig et al. (2011); Herbet et al. (2011); Horvath et al. (2012); Jung (2013); Lee et al. (2002); Ma et al. (2011); Rashid and Bal (2011); Saker et al. (2008), (2011); Shah et al. (2010); Vu et al. (2013)
<i>Pharmaceuticals protein</i>		
	<i>PfCP-2.9</i> , <i>BACE1</i> , <i>IL-12</i> ; <i>F1-V</i> , <i>sDSP</i> , <i>hIgA_2A1</i> , <i>Tα1</i> , <i>miraculin</i> , <i>hFIX</i> , <i>AGAP</i> , <i>Hiv-1 Tat</i> , <i>HBsAg</i>	Alvarez et al. (2006); Alvarez and Cardineau (2010); Baesi et al. (2011); Biswas et al. (2012); Chen et al. (2009a); Cueno et al. (2010); Elías-López et al. (2008); Hirai et al. (2010); Juárez et al. (2012); Kantor et al. (2013); Kato et al. (2011); Kim et al. (2012); Kurokawa et al. (2013); Lai et al. (2009); Li et al. (2011); Lou et al. (2007); Ramirez et al. (2007); Soria-Guerra et al. (2007), (2011); Zhang et al. (2007); Youm et al. (2008)

diseases are controlled by pesticides, but plants' acquisition of resistance to pesticides as well as the appearance of new diseases may be side effects. Therefore, genetic engineering seems to be a reasonable solution to limit the usage of pesticides. Molecular studies on plant resistance mechanisms allowed the identification of genes whose manipulation could improve resistance to pathogens. Furthermore, an examination of plants susceptible to different pathogens allowed the identification of genes which are crucial for plant susceptibility to pathogens and could be potential

targets for RNA interference (RNAi) strategy. Below, we discuss some of the achievements in this field.

Diseases caused by geminiviruses strongly affect the infected crops' yield, leading to significant economic losses. Aerial spraying is traditional method of eliminating virus infections in crop plants. This approach is extremely effective, but unfavorable from environmental protection point of view. Many studies have shown that virus-encoded RNAi suppressors are responsible for pathogenesis in host plants. For this reason, they are very important targets for

antiviral strategies. Vu et al. (2013) generated tomato plants overproducing amiRs (artificial micro RNAs) to silence viral AV2/AV1 (coat proteins) transcripts. Since this study revealed that some of the transformants displayed tolerance/resistance against Tomato Leaf Curl New Delhi Virus, it is believed that the amiR strategy could be effectively employed to protect crop plants against viruses.

In the course of their evolution, plants have developed specific intracellular immune receptors encoded by disease resistance (R) genes. These genes recognize gene products originating from different pathogen species. For example AvrBs2 (an effector that is highly conserved in a number of *Xanthomonas* species) is recognized by Bs2 R protein in pepper (a close relative of tomato). Horvath et al. (2012) investigated whether the obtained Bs2 transgenic tomato lines are resistant to different bacterial strains. Their results showed that all tested genotypes from the Bs2 lines had developed high resistance to bacteria as compared to the control group. Another strategy to improve plant defense against different pathogens (including fungi and bacteria) consists of constructing genetically-engineered plants that express antimicrobial peptides. Jung (2013) obtained transgenic tomato lines by producing a human antimicrobial peptide (hCAP18/II-37)—cathelicidin; these lines show high expressions of PR protein (Pathogenesis-related), lipid transfer protein, and antifungal protein and exhibited significant resistance to bacterial soft rot and bacterial spot diseases. Mitochondrial alternative oxidases (AOXs) and important components of the alternative respiratory pathway in plants. This particular AOX pathway can be induced by the pathogens ROS, salicylic acid (SA) and high light intensity. It is known that the induction of SA is strictly linked to defense responses in plants, therefore it has been speculated that the alternative pathway might be connected with plant resistance to pathogens. This hypothesis was supported by the results obtained by Ma et al. (2011). They provided evidence that all plants with modified AOX expression levels could cope with Tomato Spotted Wilt Virus in contrast to non-transformed, wild-type plants.

Early defense responses include changes in the plant cell membrane. Potassium and chloride ions leak from a cell and are replaced by calcium ions. This leads to increased synthesis of hydrogen peroxide (H₂O₂), which is a toxic to plants as it inactivates enzymes strictly associated with ROS such as APX, SOD, catalase and glutathione peroxidases (GPx). Interesting results were obtained by Herbertte et al. (2011), who investigated the role of selenium-independent glutathione peroxidase in the response to abiotic (mechanical damage) and biotic (exposition on *Oidium neolyopersici* and *Botrytis cinerea*) stresses in transgenic tomato lines overexpressing GPx. They reported that in the case of mechanical damage, GPx overexpression alleviated

stress, while when plants were challenged by biotic stress GPx overexpression abolished plant defense response and increased formation of necrotic lesions. The authors concluded that GPx helped cells overcome elevated ROS generation in abiotic stress response, whereas in biotic stresses GPx activity clashed with ROS-mediated signaling events. In the light of the aforementioned data expression of δ -endotoxins in transgenic plants, this could prevent lepidopterus insect-caused damage. Such research was conducted by Saker et al. (2011), who evaluated resistance of transgenic tomato plants expressing the *Cry2Ab* (δ -endotoxins from *Bacillus thuringiensis*) gene to *Helicoverpa armigera* (Hübner) and *Phthorimaea operculella* (Zeller). This study showed that the mortality of larvae fed with transgenic plants was 100 %, as compared to only 8 % for the control plants.

Tomato plants are exposed to attack of a broad spectrum of pathogens. In the case of tomatoes, Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), Verticillium wilt (*Verticillium dahliae*), early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*) are major fungal diseases. Several attempts to generate transgenic tomato plants showing tolerance to fungal pathogens were made. For example, Shah et al. (2010) obtained transgenic tomato lines expressing endochitinase (*ech42*) gene from *Trichoderma virens*. These transgenic lines revealed enhanced resistance to fungal pathogens as compared to control plants. On the other hand, Chen et al. (2009b) generated tomato plants resistant to *B. cinerea* and the resistance levels were related to the expression levels of the transgene, displaying the gene-dosage effect. The highest resistance was noticed in the case of plants containing *CHI-AFP* (bivalent gene chitinase and alfalfa defensin).

Examples of successful genetic engineering for enhanced tolerance to biotic stresses and production of biopharmaceutical in tomato are presented in Table 2.

Improvement of fruit quality

Tomato fruits have two categories of intrinsic qualities, organoleptic properties and nutritional value. Organoleptic qualities include the texture of the fruit, their taste and aroma. With regards to nutritional values, tomato fruits are a low-fat, high-fiber, low-calorie source of many vitamins and minerals and many other substances such as sugars, flavonoids, ascorbic acids and folate, and carotenoids. Other very important qualities of tomato fruits are their color, shape, fruit firmness and shelf life. Examples of successful genetic engineering to enhance fruit quality traits have been presented in Table 2.

From the point of view of a potential consumer, the fruit's taste appears as a very important organoleptic trait. Until now, only two successful attempts to change tomato

flavor have been reported. Bartoszewski et al. (2003) generated transgenic lines of tomatoes expressing biologically active thaumatin, a sweet-tasting, flavour-enhancing protein produced by the fruits of the African plant *Thaumatococcus daniellii* Benth. Their results revealed that fruits from the T₂ plants were sweeter as compared to the control, wild-type fruits and possessed a specific aftertaste. This achievement demonstrated that it was possible to overcome poor fruit taste in breeding tomato lines such as those bearing a non-ripening mutation. Furthermore Davidovich-Rikanati et al. (2007) reported simultaneous modification of fruits' flavor and aroma. They obtained transgenic lines with modified both flavor and aroma by expressing *Ocimum basilicum* geraniol synthase (*GES*) under the control of the tomato ripening-specific polygalacturonase promoter (PG). Geraniol synthase belongs to the monoterpenes group, which are important contributors to many fruit scents and also intermediate in carotenoid biosynthesis. Generally, they are synthesized from geranyl diphosphate (GDP) where *GES* catalyzes the conversion of GDP to geraniol, which is a pivotal precursor of the isomeric monoterpenes aldehydes having different aroma (e.g. lemon, rose-like aroma). While the tomato ripens, carotenoid biosynthesis is highly active, but the ripened fruits contains small amounts of monoterpenes (Iijima et al. 2004). Davidovich-Rikanati et al. (2007) indicated that this modification could be applicable to other carotenoid-accumulating fruit species important from agricultural and horticultural point of view. Furthermore, because volatile terpenoids have some advantages in antimicrobial, antifungal or pesticidal activities, this modification can be very useful for improving the fruits' shelf life or reducing pesticide application.

The unique flavor of the tomato fruit is a combination of different components, including sugars, amino acids, lipids and carotenoids. It is well known that the flavor of commercially-produced tomatoes is unsatisfactory, so flavor improvement appears as one of major challenges for scientists. Unfortunately, until now very few genes involved in biosynthesis of volatile compounds have been identified (Mathieu et al. 2009). Tieman et al. (2006) showed that overexpression of *LeAADC1* (carotenoid cleavage dioxygenase gene) or *LeAADC2* in fruits brought about a nearly tenfold increased emission of pathway products involving 2-phenylacetaldehyde, 2-phenylethanol and 1-nitro-2-phenylethane. On the other, hand antisense silencing of tomato genes (*LeAADC2*) reduced the emission of these volatiles by 50 %. Interestingly, Mathieu et al. (2009) described new quantitative trait loci (QTLs) that affected the volatile emission of red-ripe tomato fruits. These results suggested that QTLs could be used as a tool to identify genes responsible for changes in volatile levels.

Genetic modification of tomato fruit firmness has been achieved by engineering genes involved in the regulation

of a single enzymatic step in the cell wall-formation pathways. Brummell et al. (1999) and Smith et al. (2002) provided evidence that expansin or β -galactosidase controlled fruit softening and firmness in the case of genetically modified tomatoes.

The flavor and firmness of tomato fruit not the only important factors for potential consumers, fruit size is also of importance. Therefore, some research has been conducted regarding to this fruit characteristic. Detailed studies on dosage series of the *fw2.2* gene encoding plant-specific protein regulating cell divisions, particularly in fruits, were performed on transgenic tomatoes, allowing for fruit size to be altered (Liu et al. 2003; Cong and Tanksley 2006). Since the detailed mechanism by which *fw2.2* participates in fruit development is unknown, deciphering this enigma is one of the keys to understanding the phenomenon of fruit development. Cong and Tanksley (2006) suggested that *fw2.2* might have mediated this process through gene co-option and recruitment of a cell cycle control pathway. As it turns out, *fw2.2* interacted with the highly conserved regulatory unit of CKII b ((beta) subunit of a CKII kinase) and thus may affect regulation of cell division via CKII mediated pathways.

Parthenocarpic fruits can be obtained in two ways: natural or artificial without ovule fertilization. This phenomenon is very desirable not only in the case of edible fruits, but also in the case of fruit crops that may be difficult to pollinate or fertilize such as tomatoes. It is noteworthy that parthenocarpy resulted in increasing the content of the soluble solid. In light of numerous publications, in order to induce and develop parthenocarpic tomato fruit, genetic transformation targeting a single TF (transcription factor) has been used. For example, Wang et al. (2005) reported that the downregulation of *Aux/IAA9* (the auxin/indole-3-acetic acid (*Aux/IAA*) and auxin response factor (*ARF*) familie) TF triggered parthenocarpic fruit development. On the other hand, Goetz et al. (2007) obtained a similar effect by the overexpression of auxin response factor 8 (*ARF8*) and Bassa et al. (2012) by the overexpression of *SI-IAA27*. Moreover, de Jong et al. (2011) revealed that transgenic tomato lines with decreased *SIARF7* mRNA levels promoted fruit parthenocarpic development, indicating that this TF might act as a negative regulator of the fruit set. Furthermore, it turned out that *SIARF7* played a pivotal role in the modulation of the GA response during the early stages of tomato fruit development.

Not only organoleptic attributes, but also the nutritional properties of tomato fruits have recently attracted attention of scientists. To prove involvement of cell wall invertase (*LIN5*) in controlling the content of soluble solid, Zanon et al. (2009) exploited the RNAi approach in transgenic tomato plants. As a result, the transgenic plants displayed several changes in morphology (including flower

architecture, reduced fruit size and reduced seed amount) and metabolic pathways (particularly sugar metabolism and hormones). Numerous attempts have been made to obtain transgenic lines with increased levels of lycopene, xanthophylls and β -carotene (Fraser et al. 2007). Neily et al. (2011) reported obtaining transgenic tomato lines that showed 1.5–2-fold increase in polyamine content by the overexpression of the *SPDS* gene, an enzyme crucial for polyamine biosynthesis. It has to be emphasized that the constitutive expression of the *SPDS* gene enhanced the accumulation not only of spermidine but also putrescine. Remarkably, the transgenic tomato fruits also revealed an increase in carotenoid accumulation, especially of lycopene (1.3- to 2.2-fold), and increased ethylene production (1.2- to 1.6-fold) as compared to wild-type fruits. Based on these results, it is believed that a high level of accumulation of polyamines in tomatoes regulates the steady-state level of transcription of the genes responsible for the lycopene metabolic pathway, resulting in a higher accumulation of lycopene in the fruit. The research conducted by D'Ambrosio et al. (2011) is another example of engineering high carotenoid content in transgenic tomato fruit. They found that plants of transgenic tomato lines carrying tomato carotene beta hydroxylase 2 transgene showed statistically higher content of total carotenoids (including β -xanthophylls, violaxanthin, neoxanthin) as compared to the control. Flavonoids, another group of compounds that occurring in tomato fruit, cause great interest among scientists because of their anti-inflammatory and antioxidant properties. Several strategies are used to achieve an enhanced level of flavonoids in tomato fruits. The first approach is strictly connected with engineering of single structural genes involved in crucial steps in the flavonoid biosynthesis pathway such as chalcone isomerase (*CHI*) or chalcone synthase (*CHS*) (Muir et al. 2001; Colliver et al. 2002). However, more spectacular results were obtained when multiple genes were targeted within the flavonoid pathway. For example, Colliver et al. (2002) reported that the ectopic expression of *CHS*, *F3H* (flavonole hydroxylase), and flavonole synthase (*FLS*) from the *Petunia hybrida* in tomato fruits resulted in enhanced levels of flavonoids and in peel tissue. Furthermore, they reported that *CHS* and *FLS* transgenes had a synergistic effect on flavonoid biosynthesis in tomato flesh tissues when they acted together. Another strategy demonstrated the possibility of targeting the flavonoid pathway in tomatoes towards synthesizing atypical flavonoids, e.g. grape stilbene synthase (*STS*), that normally are not present in tomato fruit. The results obtained by Schijlen et al. (2006) revealed that *STS* overexpressing tomatoes showed an increased accumulation of resveratrol aglycone in the tomato fruit peel. Another strategy consists of engineering transcription factors in order to increase the content of the

flavonoid compounds. Such attempts were undertaken by several research teams (Adato et al. 2009; Ballester et al. 2010; Schreiber et al. 2012; Bassolino et al. 2013; Maligeppagol et al. 2013). Maligeppagol et al. (2013), with fruit-specific expressions of two transcription factors *Delila* and *Rosea1*, isolated from *Antirrhinum majus* generated transgenic tomato plants accumulating a 70–100-fold higher amount of anthocyanin in the fruit. The transgenic tomato plants were identical to the control plants, except for the accumulation of high levels of anthocyanin pigments in the mature fruit. Insightful analysis confirmed the elevated expression of the downstream genes of the anthocyanin pathway due to the expression of the aforementioned TFs, and that the anthocyanin expression levels coincided with fruit ripening stages, with the highest expression occurring at the breaker stage. Similar studies were conducted by Butelli et al. (2008) and Orzaez et al. (2009) on tomato line (cv. MicroTom) expressing *Delia* and *Rosea1* TFs under the control E8 promoter (tomato fruit-specific E8 promote) where the fruit displayed the purple-fruited phenotype. In their study, Ballester et al. (2010) exploited VIGS to down-regulate *SIMYB12* (TFs family MYB form *S. lycopersicum*) gene expression in tomato fruit to demonstrate direct involvement of *SIMYB12* in the establishment of a pink phenotype. The research of Schreiber et al. (2012) confirmed the hypothesis that *ANTI* (gene encoding homologous R2R3) was the gene responsible for anthocyanin accumulation in tomato fruit peels.

In contrast to above-mentioned achievements in flavonoid pathway engineering, relatively less research has been conducted regarding the enhanced level of carboxylic or ascorbic acids in tomato fruit. It is believed that high organic acid content is a very important attribute of fresh tomato fruits. Notwithstanding, the complexity of their metabolism makes it difficult to choose the best way to influence carboxylic acid levels. Morgan et al. (2013) analyzed a tomato introgression line with increased levels of fruit citrate and malate at the breaker stage to identify a metabolic engineering target that was afterward investigated in transgenic plants. Morgan et al. (2013) analyzed transgenic lines of tomato fruit expressing an antisense construct against *SIaco3b* (one of the two tomato genes encoding aconitase). They indicated that in transgenic tomato lines, both the aconitase transcript level and aconitase activity were reduced. Increased levels of both citrate and malate were noticed in the ripe fruit, and as a result the total carboxylic acid content was raised by 50 % at maturity. It is known that ascorbic acid plays an essential role as an antioxidant in defense against various abiotic stresses, so several scientists have investigated the effect of increased ascorbate accumulation in transgenic plants on their tolerance of oxidation, cold and salt stresses. Zhang et al. (2011) revealed that the overexpression of GDP-D-

mannose-3',5'-epimerase genes (*SIGME1* and *SIGME2*) resulted in increased ascorbate accumulation in tomato fruits, improving their tolerance to abiotic stresses. Interactions between ascorbate levels and fruit metabolism were studied using RNAi technology. Garcia et al. (2009) generated transgenic tomato lines with silenced L-galactono-1,4-lactone dehydrogenase, producing fruits with improved ascorbic acid content. In contrast to the studies of Zhang et al. (2011), they silenced GDP-D-mannose-3',5'-epimerase, which resulted in a reduced level of ascorbic acid in the fruit (Gilbert et al. 2009). Recently, Garchery and Gest (2013), using the RNAi approach, obtained transgenic lines with decreased ascorbate oxidase activity and thus the plants showed increased levels of total ascorbic acid.

Folate (all forms of vitamin B) is essential for numerous bodily functions, such as regulating cell growth and functioning, and also has positive effect on the nervous system and brain. Moreover, it participates in maintaining the genetic material in the transmission of hereditary cell characteristics, regulating their distribution; it improves the digestive system, and is involved in the formation of gastric juice for the efficient operation of the liver, stomach and intestines; it stimulates hematopoiesis, i.e. the formation of red blood cells; and protects the body against cancer (particularly cancer of the uterus). Folate deficiency leads to neural tube defects in developing embryos and other human diseases including diarrhea, macrocytic anemia, neuropathy, mental confusion, pregnancy complications, different kind of cancers, etc. (Bailey 2010). Because the human body cannot synthesize folate *de novo*, it has to be supplied through diet. A potential source of folate are leafy vegetables (e.g. spinach, broccoli, cabbage), but in folate occurs slightly smaller quantities in tomatoes, lentils, beets, sunflowers, etc. In plants, folates are synthesized from pteridine p-aminobenzoate (PABA) and glutamate precursors (de la Garza et al. 2007). Because folate is very important for human health and plants are known as one of the major source of folate, some attempts to enhance plants' folate levels have been undertaken (Raiola et al. 2014).

As is the case with ascorbic acid, there is very little research related to modification of folate content in tomato fruit. de la Garza et al. (2004) reported a twofold increase in folate content in tomato fruits overexpressing GTP cyclohydrolase I. 3 years later, the same research team reported a 25-fold increase in folate accumulation in transgenic tomato fruits (de la Garza et al. 2007). On the other hand, Waller et al. (2010) showed that in engineered fruit overexpressing foreign GTP cyclohydrolase I and aminodeoxychorismate synthase genes, the expression of endogenous genes was not changed, but those of three downstream pathway genes, aminodeoxychorismate lyase, dihydroneopterin aldolase and mitochondrial folylpolyglutamate synthase, increased by up to 7.8-, 2.8-, and 1.7-

fold respectively, apparently in response to the build-up of specific folate pathway metabolites.

Molecular farming of tomatoes

Molecular farming is technology involving the use of plants, and potentially also animals, as the means of producing compounds that are of therapeutic value (safely and inexpensively). Until recently, the production of recombinant proteins was based on bacteria, mammalian or insect *in vitro* cultures and fungal cell cultures, which are insufficient to producing more complex polypeptides. Moreover, these technologies as applied now have some limitations resulting from differences in metabolic pathways and translation processes and refer mainly to expression systems based on bacterial, insect or fungal cell cultures. This may generate changes in the molecular structure of recombinant proteins, resulting in the limitation or total loss of their desirable activities. Plants offer many advantages over these systems in terms of safety, cost, time involved, protein complexity, storage and distribution issues. In this system, the desired foreign protein can be produced e.g. at 2–10 % of the cost of a microbial fermentation system and at 0.1 % of mammalian cell cultures, although it depends on the protein of interest, product field and the plant used. Additionally, plants have a higher eukaryote protein synthesis pathway very similar to animal cells with only minor differences in protein glycosylation. Therefore, plant biosynthesis pathway ensures correct structures even in the case of highly complex proteins. Furthermore, the use of plants avoids the risk of contamination with animal pathogens, such as viruses, that could be harmful to humans (e.g. HIV, hepatitis viruses, prions). It should be emphasized that no plant viruses have been found to be pathogenic to humans. Purification of the desired polypeptide from plants is often easier than from bacteria. Moreover, in some cases (e.g. edible vaccines), the purification process can be omitted. Whereas transgenic plants or virus-infected plants can be grown on field requiring only water, minerals and sunlight, mammalian cell cultivation is a very expensive process, requiring bioreactors that cost several hundred million dollars when production is scaled up to commercial levels. So far, more than 100 diagnostic and therapeutic recombinant proteins as well as vaccines have been produced in various plants including tobacco, potato, tomato, lettuce, carrot, cereals, legumes (Wiktorek-Smagur et al. 2012). Here, we present some examples of the production of recombinant pharmaceutical proteins in tomatoes.

Malaria is a potentially fatal tropical disease that is caused by protozoan parasites of the genus *Plasmodium*. Vaccines are a cost-effective way to overcome infectious diseases of this magnitude. Notwithstanding, the available

malaria vaccines produced by conventional methods are still unaffordable for most people due to their high price. Thus, Kantor et al. (2013) initiated the production of antigen gene *PfCP-2.9* of *Plasmodium falciparum* in tomato fruits. This research is the first report of a successful transformation with the expression of a malaria antigen (*PfCP-2.9*) in transgenic tomato plants of the T₀ and T₁ generations. Kantor et al. (2013) found that transgenic tomatoes produced 35 mg per gram of fresh weight of leaves of malaria antigen protein. The TSP (Total Soluble Protein) extracted from the tomato leaves was similar to that obtained by other researchers (Biswas et al. 2012).

Alzheimer's disease (AD) is the most common cause of dementia in older adults. The cause and progression of Alzheimer's disease are not well understood. AD is a neurological disorder in which the death of brain cells causes memory loss and cognitive decline. Research indicates that the disease is associated with plaques and tangles in the brain. In patients with Alzheimer's disease, the presence of insoluble protein deposits (A β amyloid) in the brain was revealed. It seems that inhibiting the formation of A β by using vaccines directed against A β would be one of the most promising approaches towards the treatment of AD. Such attempts were undertaken by Youm et al. (2008). They obtained tomato plants with a satisfactory level of A β protein expression to be used in an oral immunization assay on mice. Elías-López et al. (2008) explored the use of transgenic tomato (TT) expressing IL-12 (interleukin) (TT-IL-12, transgenic tomato interleukine) as a single polypeptide as a possible strategy to produce constant and therapeutic IL-12 levels when administered through the oral route in a well-characterized murine model of progressive pulmonary tuberculosis. They demonstrated that oral administration of TT-IL12 crude fruit extracts ameliorated protective immunity and reduced lung tissue damage during early and late drug-sensitive and drug-resistant mycobacterial infection in an albino, laboratory-bred strain of the house mouse (BALB/c).

On the other hand, Kim et al. (2012) initiated the production of human β -secretase (BACE1) in transgenic tomato fruits, which serves as a vaccine antigen that would promote immune response. Furthermore, the proteolytic activity of the tomato-derived rBACE1 was similar to that of a commercial sample of *Escherichia coli*-derived BACE1. In 2006, Alvarez et al. received transgenic tomato plants with the *Yersinia pestis fl-v* fusion gene encoding for F1-V, an antigen fusion protein. The immunogenicity of F1-V against a challenge with subcutaneous *Y. pestis* was confirmed in mice that had been vaccinated orally with freeze-dried fruits (Alvarez and Cardineau, 2010). In 2007, Soria-Guerra et al. obtained transgenic tomato plants (cv AC) expressing a synthetic gene encoding a novel synthetic recombinant polypeptide, sDTP (Diphtheria-

Pertussis-Tetanus) containing two adjuvant and six DPT immunoprotective exotoxin epitopes. In the course of subsequent studies, Soria-Guerra et al. (2011) examined whether the ingestion of tomato-derived sDTP protein induced specific antibodies in mice. The results showed that the sera of the immunized mice tested for IgG antibodies, the response to pertussis, tetanus and diphtheria toxin, and showed responses to the foreign antigens. Furthermore, the high response of IgA against tetanus toxin was apparent in the gut.

There are many causes of infectious diarrhea (viruses, bacteria and parasites). *Norovirus* is the most common cause of viral diarrhea in adults, but rotavirus is the most common cause in children under 5 years of age. Recombinant production of rotavirus antigens in plants has been proposed as an alternative to traditional production platforms. Juárez et al. (2012) obtained transgenic tomato plants expressing a recombinant human immunoglobulin A (hIgA_{2A1}) selected against the VP8* peptide of rotavirus SA11 strain. The amount of hIgA_{2A1} protein reached 3.6 ± 0.8 % of the TSP in the fruit of the transformed plants. Fruit-derived products suitable for oral intake showed anti-VP8* binding activity and strongly inhibited virus infection in an in vitro virus neutralization assay.

Thymosine (T α 1) plays a crucial role in the treatment of diseases induced by viral infections (e.g. hepatitis B and C) and also cancers as an immune booster. For clinical use, T α 1 is mainly derived from animal thymus extraction or chemical synthesis. However, Chen et al. (2009b) reported the possible production of the aforementioned protein in tomato plants. They revealed that T α 1 protein reached a maximum of 6.098 μ g/g fresh weight in mature tomato fruit. Moreover, the specific activity of T α 1 protein produced by tomato plants was higher than that from the synthetic *E. coli* system. Some research demonstrated that tomato plants can be exploited for the production of hepatitis B surface antigen (Lou et al. 2007; Baesi et al. 2011; Li et al. 2011). All of the above findings support the concept of using transgenic tomato plants as a model for edible vaccines or producing antibodies. However, there are some disadvantages of this technology such as the short shelf life of fresh tomato fruits. To overcome this problem, food-processing techniques such as freeze-drying could be applied. Plant material prepared in this way can be stored for a long period of time and directly consumed without cooking. Notwithstanding, this procedure allows the plant-made vaccine to equalize and concentrate. To date, several studies have demonstrated the use of this technique to produce vaccines in transgenic tomatoes (Alvarez et al. 2006; Elías-López et al. 2008; Alvarez and Cardineau 2010; Soria-Guerra et al. 2011).

Tomato plants are used not only for production of vaccines or antibodies, but also for the production of other

recombinant proteins such as miraculin. This protein is a taste-modifying glycoprotein extracted from a miracle fruit (*Richadella dulcifica*) and changes sour taste into sweet taste (Kato et al. 2011). In transgenic tomato plants, the recombinant miraculin content reached a concentration of up to 90 μg per g fresh weight (FW) of tomatoes (Hirai et al. 2010). Further studies by Kurokawa et al. (2013) revealed the miraculin accumulation levels in red fruits varied among the lines. Miraculin gene expression was driven by the E8 promoter and HSP terminator cassette (E8–MIR–HSP) in transgenic tomato plants, and the miraculin concentration was the highest in ripening fruits, 30–630 μg per gram of FW. The results achieved by Kurokawa et al. (2013) confirmed that combination of the appropriate promoter and terminator cassettes was important for significantly increasing the accumulation of recombinant proteins in a ripening fruit.

A number of studies over the past decades have proved that transgenic plants (including tomatoes) can be used as bioreactors for the production of recombinant therapeutic proteins (Wiktorek-Smagur et al. 2012). Zhang et al. (2007), using *Agrobacterium*-mediated transformation, demonstrated that the *hFIX* (human coagulation Factor IX) gene was expressed specifically in tomato fruits. The highest expression level was 15.84 ng/g FW (approx. 0.016 % of total soluble protein) and found in mature fruit. The analgesic–antitumor peptide (AGAP) from the venom of *Buthus martensii* Karsch is another therapeutic protein produced in transgenic tomato plants is (Lai et al. 2009). Earlier studies showed that AGAP would be useful in clinical therapy as an antitumor drug.

Examples of successful genetic engineering for biopharmaceutical in tomatoes are presented in Table 2.

Conclusion

Since the tomato (*S. lycopersicum* L.) was imported to Europe in the 16th century, it has become one of the most important vegetables around the world. Recently, interest in the tomato has significantly increased because of its nutritional values as well as its anti-cancer and anti-oxidative properties. In this review, we looked into new insights from recent developments in tomato biotechnology. Generally, it is known that traditional methods for improving tomatoes are time-consuming and troublesome due to breeding times. For this reason, it is necessary to develop efficient methods for the in vitro regeneration of different varieties of tomato. This would make a prerequisite step for further modification of tomato genome. Since more than 10,000 tomato varieties exist, it seems obvious that establishment of one universal protocol for regeneration is rather impossible since it would require

very extensive analytical research on the physiological and genetic background of tomatoes' regeneration capacity. At present, it seems more likely to establish a tissue culture protocol for select commercially important tomato cultivars preceded by wide screening of their regeneration potential. According to numerous data outlined in this review, the in vitro culture of tomatoes has been successfully used in different biotechnological applications. It should be pointed out that different genotypes of tomato are characterized by diverse morphogenic potential, and unfortunately there are some reports describing their partial recalcitrance or total inability to respond to in vitro cultures. Therefore, improvement of existing regeneration protocols is still required. Despite various difficulties, currently a procedure of successful stable *Agrobacterium*-mediated transformation of tomato plants has been achieved. In the light of the numerous data presented here, genetic engineering has opened amazing opportunities for tomato plant improvement. So far, transgenic tomato lines have been generated with enhanced resistance for wide range of stresses, including abiotic and biotic ones. This has become possible through the overexpression several genes or TFs. Additionally, understanding the underlying physiological process in response to different stresses could help in determining what promoter or TFs would be appropriate to use for transformation. It should be pointed out that constantly expending knowledge regarding the physiological and genetics basis of stress tolerance, along with genetic transformation technologies, could allow for essential progress in the development of tomato cultivars with improving stress tolerance. Moreover, using GM technology, researchers are able to obtain tomato fruits with improved nutritional and organoleptic values. Finally, the credibility of the use of tomatoes in molecular farming has been proven beyond all doubt. Although promising achievements in tomato engineering, the culture of GM tomato face serious problems in most leading producer countries. The cultivation of GM tomatoes was stopped in the USA in 2002, so only China remains a producer of GM tomatoes. The main reason for this seems to be a negative opinion of the public towards GM plants. There is a general belief that GM crops are harmful for human health as well as the environment. Therefore, one of the tasks of the scientific community is not only the production of GM crops, but also educate the public about the benefits they bring to us. It should be pointed out that broad research has provided no evidence that transgenic crops cause a greater risk to human or animal health than stereotyped crops. The Federal Office of Consumer Protection and Food Safety of Germany and partners published the BEETLE (Biological and Ecological Evaluation towards Long-term Effects) report to provide scientific data (reviewed over 100 publications) to the European Commission (FOCPFS 2009).

The BEETLE report gave clear evidence that, so far, no adverse effect to human health from eating GM plants have been found. Furthermore, although unexpected harmful effects are known, none have appeared in GM plants. Additionally, to convince consumers about GM plants, the use of marker-free transgenic plants (e.g. deprived of resistance of herbicides or antibiotic) could be a good argument. The continuously expanding knowledge of genomics of tomatoes' wild relative species, including knowledge about e.g. introgression of genetic information from related species into cultivated tomato, would significantly limit the risk of harmful effects on human or animal health or on the environment.

Although GM tomatoes are promising for improving the quality of human life, their potential has been seldom validated in field trials. Such trials as well as BEETLE report have to be expanded and their results have to be provided to society in order to raise awareness. It is only if the safety of GM crops and the benefits they bring to breeders and consumers, that biotechnology-derived plants will contribute to the success of their development.

Conflict of interest The authors declare that they have no conflict of interest.

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