Genetically Modified Foods: Engineered tomato with extra advantages

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Abstract

Currently, genetically modified crops are among the most important constituents in all aspect of our life. Recent controversies about genetically modified crops in United Kingdom and other European countries indicate the apparent differences in public opinion on this subject across the Atlantic, where people do seem untroubled with GMO as compared to other countries. Among plants after potato, tomato is the model plant for genetic changes. A number of genetic modifications of tomato are available especially against different insects, pests and fungus. Gene silencing techniques against viruses have been applied for silencing the expression of damage causing gene. Genetic modifications inducing significant events regarding fruits ripening and growth in tomato and vaccines are of the particular importance. In this review article, we have covered genetic modifications which cause insect, virus and fungus resistance; confer fast growth, chilling, salinity and drought tolerance, increased and improved nutrients, yield, fruit ripening, quality and color of the tomato. Factors affecting photosynthesis and metabolic rate are also discussed in this review.

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Introduction

During the process of crop development, tomato (Solanum lycopersicum) evolved from Solanum pimpinellifolium [1,2]. Tomato is among the most important vegetables and extensively used as raw or cooked [3]. Beside a source of antioxidants, minerals, fibers and vitamins it is also an excellent model system for genetic studies, fruit development and ripening process [4,5]. Insect pest and other different many kind of microbial pathogens outbreak tomato. Microbial pathogens include lepidopteron, Helicoverpa armigera and common fruit borers which mainly attack on fruit while Spodoptera litura destroy leaves [6,7]. Incorporation of Bt genes in tomato, cotton, and sugarcane has showed considerable resistance against lepidotermans [8-10]. Tomato has served as an excellent model for fleshy fruit development and ripening [11,12]. Well characterized ripening mutants, high density genetic maps, small genome size, short life cycle, efficient and stable transformation made tomato an excellent model for studying viruses, development and fruit ripening process through genetic modification [13-16]. Cold, heat and soil salinity are the major environmental factors that significantly affect the productivity and quality of tomato and other crops [16-18]. Chilling, drought and salinity stress have been found interconnected in effecting water relations at cellular, tissue, organ and whole plant level leading to physiological, morphological biochemical and molecular changes [19-22]. A lot of genetic modifications in tomato have been done. The aim of this review is not to explain all of them but to mention the important events related to tomato fruit development and ripening; insects and viruses resistance; chilling and soil salinity resistance.

Methods

Search Strategy and Selection Criteria

The following key terms were provided in Google Scholar, PubMed and PubMed Central for mining the scholarly literature relevant. Research papers with specific focus on tomato pathogens (insects, fungus and viruses), tomato growth, nutrition and fruit ripening were included. Most of the included articles were published during 2013-2014. But exceptions were made for few articles published back in 1990s based upon strong relevancy to topic. A total of 51 research articles were consulted to construct this review.

Discussion

Against Insects:

Transformation of Cry1Ac gene against lepidopteron pest in Tomato

Tomato is the part and parcel of our daily food. Importance of tomato in food industry can also not be undermined because it is involved in production of other food products. Presently, there are many yield limiting factors for tomato crop but lepidopteron insects pose a serious threat in devastating this precious crop. In order to check the attack of these insects, modified BT genes (Cry1Ab) were transformed in high yielding commercial tomato variety by Agrobacterium mediated transformation method. This transformation played a pivotal role in reducing the attack of lepidopteron insects. The results of this
study indicate the effective control of this problem [23].

Transformation of Tea Hydro peroxide Lyase Gene, CsiHPL1 in tomato
Tomato crop is an attractive crop for many insects and other pest organisms causing several problems in it. Defense system of tomato plant is highly sensitive and vulnerable to insect attack ultimately triggers the occurrence of dangerous diseases in tomato. Chloroplast localized tea gene, CsiHPL1 is found to be highly effective in strengthening the defense system of tomato crop. Moreover, transformation of CsiHPL1 not only protects tomato crop against all fungal attacks but also create herbivore resistance in tomato [24].

The transcription factor SISHINE3 modulates defense responses in tomato plants
Cuticle is an important part of plant leaf. Several transcription factors are involved in cuticle production, like SISHINE3, an ortholog of the Arabidopsis WIN/SHN3. Interaction of pathogens with their surroundings and acting as a barrier against physical stresses and pathogens are all the functions of leaf cuticle. Moreover, it also acts as chemical deterrent in enhancing and regulating the plant defense system. Many transcription factors are said to be involved in enhancing the production of cuticle in leaves making tomato plant resistant against all types of stresses [25].

The N gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato
Tobacco mosaic virus has become a major threat for the production of tomato plant. Studies show that resistance can be created in tomatoes by genetic transformation of virus resistant genes. In a recent study, viral resistant N gene has been isolated from transgenic tobacco plant, cloned and transferred in commercial tomato variety ultimately created resistance against tobacco mosaic virus in tomato [26].

Against Viruses:

Transformation of Coat protein gene from Tobacco Mosaic Virus in tomato
Viruses are the natural enemies of living organisms. Similarly plants are also among the easy victims of viral infection. Tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV) have been causing losses in tomato crop. Yield losses about 25-30% have been reported in tomato crop due to this viral attack. Coat protein genes (CP genes) are found producing more than 90% resistance in tomato plants against TMV and ToMV. Integration of CP genes in tomato genetic makeup was done through Agrobacterium mediated transformation to create viral resistance. These CP gene has no negative impact on fruit yield [27].

Genetic transformation of Tomato Yellow Leaf Curl Virus against virus
Like other viral diseases, Tomato Yellow Leaf curl Virus (TYLCuV) also cause huge fruit losses in tomato crop. This viral disease was controlled by the genetic transformation of TYLCuV genes which encode Capsid proteins (VI). This strategy has against this virus has reduced the risk of leaf curling in esculentum spp [28].
Transformation of glycoprotein producing gene
Preparation of vaccine for protection against any disease is an uphill task now a day. Biotechnologists and molecular biologists utilize different means and mediums for manufacturing valuable products. Most interestingly, transgenic tomato plants have been used for this purpose. Glycoprotein (which coats the outer surface of rabies virus) producing gene is transferred in tomato plants through Agrobacterium mediated transformation procedure. Analysis through electron microscopy and other techniques show cell organelles like Golgi bodies, plasma cells, vesicles and cell walls of parenchyma tissues contain Glycoprotein proteins. In this way, transgenic tomato plants can be used as a tool for producing oral vaccines against rabies [29].

Against Fungus:

Wasabi defensing gene (WD) expression in tomato
Pathogens are potential threat for the survival of tomato species causing diseases and ultimately affecting fruit yield of this crop. Fusarium oxysporum is the most problematic fungus for tomatoes. Studies show that cysteine rich peptides have ability to inhibit the growth of fungi. Wasabi defensing (WD) genes, rich in cysteine peptides offer resistance to tomato crop against fungal attack. The wasabi defense (WD) gene was transferred in tomato plants through Agrobacterium mediated transformation procedure. Root specific promoters LjNRT2 was used expressing Wasabi defensen (WD) genes in roots. Hence Fungus can be controlled effectively in tomato plants by WD genes [30].

Growth and Nutrition:

Transformation of LeMYC2 gene in tomato
From commercial point of view, tomatoes having shorter stature are more acceptable as compared to taller ones because reproductive growth is more important than vegetative growth. Many genetic promoters are reported which enhance reproductive growth while discourage vegetative development. LeMYC2 and MYC2 are the promoters responsible for root growth and repressing vegetative length. They are also reported to enhance fruit development ultimately increasing the yield of tomato crop. Hence their transformation is blessing for tomato yield [31].

Expression of bacterial enzyme ACC Deaminase for plant growth
Plant growth depends directly upon light because plants need it for accomplishing photosynthesis. Root growth in tomato plants has been affected directly from the source of light. It has been reported in a research that UV-B light has more impact on root length as compared to other sources. In the presence of UV-B, root grew to a greater length than light and dark conditions. Moreover, root length is reduced by only ACC enzyme, a precursor of ethylene while hypocotyl growth is not much sensitive towards ACC enzyme [32].

Boron-deficient tolerant tomato with overexpression of AtBOR1 gene
Boron is the most important constituent of tomato growth and development. Boron
deficient conditions effects tomato growth. In Arabidopsis thaliana, AtBOR1 (major borate transporter gene) or boric acid channel gene improves plant growth under boron deficiency conditions. To study the expression of these genes in tomato, three lines were developed in one study. First two lines showed normal growth under B-deficient conditions while non-transgene and weekly expressed third line were having restricted stems and failed to develop new leaves. Shoot biomass was high under Bo-conditions in the strongly expressing ATBOR1 transgenes and Bo-deficiency phenotype symptoms were not seen in newly developing leaves [33].

**Frost Tolerance:**

**Chilling tolerance in transgenic tomato through Carrot antifreeze protein**

All plants are sensitive to cold and major damage is done by frost. Tomato is the most important food crop. Along with other production factors chilling temperature is also the major factor in the destruction of tomato crop. During chilling temperature, crystals are formed in extracellular matrix which ultimately causes dehydration in cytoplasm and result in shrinking of cell membrane. Tomato has been made resistant to frost. This tolerance was developed by the transformation of antifreeze protein gene from carrot. Transgene were most resistant against frost damage and were having very less electrolyte occurrence due to chilling temperature as compared to non-transgenic plants [20].

**Candidate gene expression profiling in two contrasting tomato cultivars under chilling stress**

Tomato, at all development stages, has sensitivity to chilling temperature. Genetic variation for chilling tolerance exists between cultivars, but interspecific variation was not studied. In one study, seedling of different cultivars were analyzed and two were found most resistance against chilling tolerance. Then the gene candidate profile of these cultivars was studied through RT-PCR. Those candidate genes were chosen which were having affiliation to be induced with chilling or having putative role in CBF/DREB and ROS mediated pathways. Other than CBF regulon, ROS and C2H2 type Zinc finger protein mediated signaling pathways were also found involved in chilling tolerance. Transgenic plants were having up-regulation of these transcription factors as compared to wild types [34].

**Fatty acid desaturease 7 and Lipoxygenase gene expression by Glycine betaine**

Cold stress is the environmental stress factor which greatly effects the plant growth, yield and quality. Cold stress mainly due to the accumulation saturated lipids. Glycine betaine accumulation helps in destaturating the lipids by enhancing the expression of naturally destaturating genes. Its role is well defined but its mode of action is not well understood. In one study, its mode of action was analyzed in two different cultivars of tomato; one sensitive and other moderate resistant against chilling. Differences in lipoxxygenase (TomLOXF) and fatty acid desaturaase 7 (FAD7) gene expression profiles and physiological parameters, like growth rates, relative water contents, osmotic potential, membrane leakage and lipid peroxidation level were studied.
Results showed that cultivar with enhanced accumulation of Glycine betaine were having more expression of TomLOXF and FAD7 genes and were more resistant to chilling temperature without disturbing other parameters [35].

**Photosynthesis and Metabolic Rate:**

**Glycine Betaine biosynthesis reduces heat-enhanced photo-inhibition in tomato**

Heat enhanced photo inhibition is the big hurdle for continuous photosynthesis. Under high heat plant fails to work properly. Glycine betaine is a solute that accumulates rapidly and creates heat tolerance in plants. To enhance the expression of GB, betaine aldehyde dehydrogenase (BADH) gene from spinach was transformed in tomato. Higher photosynthetic activities and increased tolerance against heat was observed in tomato plants after the insertion of this gene. This increased tolerance accelerated the repair of photosystem II (PSII) followed by the reduction in heat enhanced photo inhibition. Significant reduction in hydrogen peroxide and super oxide radical as compared to non-transgene was observed. Moreover, it was concluded that exogenous GB cannot directly reduce heat tolerance in tomato plants and lesser concentrations of anti-oxidant should be maintained in order to decrease the ROS (reactive oxygen species) to protect membrane from mechanical injuries [36].

**ABA hormone for tomato fruit ripening**

Tomato fruit ripening is mainly controlled by the action of hormones and tight genetic controls. Recent studies have shown that abscisic acid signaling have also effect on different aspect of fruit maturation and ripening. Previously, it was demonstrated that ABA-regulated transcription factor are involved in stress responses in tomato and expressed mainly in seeds/fruit tissues, but recently it is proven that it has role in the expression of genes which are involved in primary metabolic process. Analysis of transgenic pericarp tissue from fruit, harvested at three stages, showed increased contents of organic acid, hexose, hexose-phosphates and amino acid in immature, mature green and red ripe fruit. These changes are directly correlated with enzymes coding genes involved in primary metabolites and partially with secondary as well [37].

**Salinity and Drought Tolerance:**

**S. adenosyl-L-methionine synthase transformation against salinity in Tomato**

Salinity is the major problem in those soils which are having heavy salt concentration of alkali especially sodium. Almost whole crop can be destroyed through this problem but gene are present which confer resistance to high concentration of salts. S. adenosyl –L-methionine synthases can create tolerance in tomato against alkali through polyamine metabolism. SAM tolerance can be generated by the introduction of SAM synthase cDNA (SISAMS1) into tomato genome. Transgenic plants showed great resistance against alkali without disturbing other nutrients. Fruit setting and yield was enhanced significantly. Tomato tolerance against alkali have important role in modulating polyamine metabolism, resulting in maintaibility of nutrients [38].
**Gibberellin methyl transference transformation against transpiration in tomato**

Plant transpiration rate has very important role regarding drought tolerance. If transpiration rate is reduced then plant can reserve and use water during drought stress. Tomato plant has also drought stress problem. This problem was overcome by the transformation of Arabidopsis thaliana Gibberellin methyl transfrase1 (AtGMT1) in tomato. Actually AtGMT1 encodes an enzyme that catalyzes the methylation of active GAs to generate inactive methyl esters. Transgene having deficiency of active GAs have less leaf transpiration rate due to reduced stomatal conductance. GAMT1 overexpression inhibit the expansion of leaf epidermal cells leading to the formation of smaller stomata with reduced stomatal pores. Thus reduced stomatal pores are of beneficial for reserving water contents and creating strength to afford drought stress in tomato [39].

**Yield, Fruit Ripening, Quality and Color**:

**Tomato carrying a gene StAPX for creating water stress influencing yield and nutrition quality of tomato plant**

Nutrition and quality of tomato fruit is very important. StAPX gene was transformed to create water stress for analyzing its effects on tomato fruit yield and quality, hydrogen peroxide contents and net photosynthetic rate under 50-70% soil moisture with full field capacity in comparison with wild type tomatoes. Results revealed that transgenic harboring StAPX gene were having more yields than wild type tomatoes and quality was same like wild type. So under water stress tolerance tomato can yield more production by the introgression of stAPX gene in cultivated tomatoes without any abnormalities [40].

**Better chloroplast functioning and tomato quality**

Chloroplast is the most important constituents of photosynthesis. As this process is heavily dependent on sugars transported from leaves to carry out the demanding process like fruit development and ripening and finally for organoleptic properties of fruit. Transcriptional factors which enhance chloroplast development in fruit can result in higher contents of tomato fruit and also sugars. It is the indication that photosynthesis process plays vital role in fruit development and quality in tomato [41].

**Tomato ovary and fruit development by microRNA 156-Targated SPL/SBL box transcription factor**

Potato (Solanum lycopersicum) is well understood at molecular level, but genetic pathways associated with tomato ovary and early fruit development was still unknown till study discussed here on [42]. Possible role of microRNA156/SQUAMOSA promotor binding protein like (SPL or SBP box) module (miR156) is studied in this study which revealed that SBP genes expressed in the ovary and early fruit development while in meristematic tissues and including placenta and ovules miR156 over expressed. Transgenic plants over expressing AtMIR156b have normal flower and fruit development morphology with fruit characterized by growth of extra carpels and ectopic structures as compared to non-
transgenic plants. Meristem maintenance genes are expressed in the developing ovaries with extra carpels, while expression of MADS box genes was repressed in miR156 over expressers. Collectively describing, miR156 node is involved in meristematic maintenance phase of ovaries tissues controlling initial step of fruit development and determinacy [43].

**NAC and SINCA4 a new transcription factor for tomato fruit ripening**

Tomato fruits ripening are well understood by NOR transcription factors like a NAC domain family but its molecular genetic relating fruit ripening is of poor knowledge. As tomato fruit ripening is a complicated process affected by endogenous hormonal and genetic regulators and external signals. SINAC4, a new NAC domain protein, was studied to understand the phenomenon of fruit ripening in tomato. SINAC4 showed high accumulation in sepal and at the onset of fruit ripening. Under various stress treatments like wounding, NaCl, dehydration and low temperature SINCA4 expression boosted. SINCA4 reduced expression by RNAi resulted in delayed fruit ripening is the indication that ethylene synthesis genes are directly related with the expression of SICA4 factor. Transgene showed significant down regulation of ripening genes through reduced expression of SINCA4. So to overcome the fruit ripening process SINCA4 is the most important transcription factor which control this phenomenon by up regulating ethylene synthesis gene in the fruit ripening regulatory network [21].

**Tomato MADS box family play vital role in fruit ripening**

MADS RIN tomato protein have very important role in fruit ripening. Their role was confirmed through two homologous tomato proteins named FUL1 and FUL2. These proteins are related in ethylene biosynthesis and consequently enhancing the expression of fruit ripening genes. This statement was verified when the expression of these proteins was knocked out. Knocking out the expression of this protein resulted in blocking ethylene synthesis and decreased expression level of ACC SYNTHASE2 (ACS2) gene which is responsible for fruit ripening. Actually these proteins are the MIKC domains of MADS box family proteins. A pointed tip at the blossom end of fruit was observed with over expression of FUL1 protein but, FUL2 has not any role in phenotype. In short, FUL proteins are vital for the fruit ripening in tomato [44].

**Inheritance and effect on ripening of antisense polygalacturonase genes (PG) in transgenic tomatoes**

The polygalacturonase (PG) cell wall hydrolase have part in tomato fruit ripening. In subjected study, PG was put in analysis for its role in fruit ripening. Tomato lines were deprived from PG through antisense RNA. For this purpose, chimeric genes were designed to express anti-PG RNA constitutively. All lines were having single copy of PG antisense gene, which led to a reduction in PG enzyme activity. The reduction in fruit ripening was observed 5-50% in lines except one line in which reduction in fruit ripening was eve of PG enzyme activity. PG was segregated after
two generation and plants homozygous for PG antisense gene showed a reduction of 99% in expression of PG enzyme and ripening process. *In-vivo* PG is involved in depolymerisation of Pectin but other ripening parameters like ethylene production, lycopene accumulation were not affected [45].

**Tomato Goldent2-like (GLK1 and GLK2) transcription factors enhance fruit ripening**

Plastid and chlorophyll are the two most important constituents for fruit ripening. But they are affected by numerous environmental and genetic factors, and are positively correlated with photosynthesis and Photosynthate accumulation. Actually, fruit ripening is the summation of changes rendering fleshy fruit tissues and palatable to seed dispersing organisms. Sugar contents are greatly influenced by plastid numbers and photosynthetic activity in unripe fruits and in later stages by starch and sugar catabolism. GOLDEN2-LIKE (GLK) transcription factor control plastid and chlorophyll levels. Like other plants tomato has also two most important GLK, GLK1 and GLK2. They have similarly genetic peptides, but GLK1 is important in leaves and GLK2 predominantly in fruit ripening [46].

**CRTB gene from Bacterium (Erwinia uredovora) enhances tomato fruit ripening**

Tomato plastid have significant role in fruit ripening as mentioned in previous heading; if its expression is enhanced then ripening process can be elevated. Phytoene synthase enzyme enhances the expression of plastid and ultimately increase in ripening process occurs. This statement was proved by the transformation and overexpression of CRTB gene of bacterium *Erwinia uredovora* in tomato. Transgenic analysis of tomato revealed that by the overexpression of CRTB gene plastid expression was increased without disturbing other constituents like isoprenoids, tocopherols, plastoquinone and ubiquinone [47].

**Escherichia coli heat-labile enterotoxin B subunit in transgenic tomato fruit**

Cheap, safe and effective vaccines are the need of time produced through audible plants. Keeping this most important concern in view, an effort was made to transfer Heat-labile Enterotoxin B subunit (LTB) in tomato plants. Integration and transgenic protein expression was confirmed through southern Blot and ELISA. Transgenic produced pentameric LTB protein bound specifically to GM1gangloside. So, it is the indication that LTB protein produced in tomato are the potential candidates for the production of inexpensive and safe plant based vaccines [48].

**SIMYB12 transcription factor leads to pink tomato fruit color**

Carotenoids and flavonoids are the most important items for the tomato color. Homozygous *Solanum chemielewskii* introgression on the short arm of chromosome 1 is consistent with the position of Y mutation which results in colorless epidermis and pink colored fruit combined with red flesh. Pink fruits neither lack the naringenin chalcone in fruit peel while carotenoids are not affected by this mutation. The expression of all the genes encoding biosynthetic enzymes are down
regulated in pink color which is the indication that pink phenotype gene produce proteins as transcription factor rather than biosynthetic enzyme. MyB and basic –loop helix transcription factors are putatively involved in the regulating transcription genes in the flavonoid pathway. MyB12 gene expression is correlated well with the decrease in the expression of structural flavonoid gene in peels. Moreover, MYB12 is located on chromosome 1 and segregate perfectly into pink fruit color. This was proved by virus induced gene silencing of SIMYB12 that this silencing resulted in decrease of naringenin chalcone production which is involved with pink color fruit. It is of conclusion that SIMYB12 have an important role in regulation of flavonoid pathway in tomato fruit and being a candidate for Y mutation [49].

Polyamine buildup in tomato enhances phytonutrient content, juice quality
In many organisms, polyamines have been implicated in numerous physiological and developmental process. But lacks the determination of in-vivo function yet. Yeast S-adenosyl-methionine decarboxylase gene (ySAMdcv; Spe2) transferred with inducible promoter E8 in tomato to enhance polyamine and spermine in tomato fruit during ripening. Transgenic plants along with their five successive segregations results revealed that increased conversion of putrescine into higher polyamines, consequently ripening specific products (spermidine and spermine) was observed. Non-transformed plants were having low lycopene as compared to transgene. Conclusively, it can be said that along with ethylene polyamine in fruit ripening specific manners are also vital [50].

Floral developments and Female sterility:
Auxin response factors like ARF6 and ARF8 of Arabidopsis thaliana have very important role in florescence, stem elongation and lateral stages of petal, stamen and gynoecium developments. All seeds plants have these factors as well as microRNA167, which inhibit the expression of these Auxin factors. Micro RNA transformed into tomato plant resulted in reduction in leaf size, reduced internode length and shortened petals, stamens and styles. Basically these factors were repressed by microRNA overexpression and resulted in overall defective vegetations. These factors bind to those genes which are involved in floral development as promoter and augment their expressions. Moreover, the inactivation of these factors also induced female sterility as wild type pollens were unable to germinate on the stigma surface. It can be concluded that vegetative growth and floral development is heavily based on conserved regions of these factors [51].

Conclusion
Tomato is an excellent model for genetic transformation of the required genes. Agrobacterium mediated transformation support this cause well. Transcription factor are the excellent source for augmenting the expression for fruit ripening and growth development in tomato. Female sterility and insect problem along with chilling stress problems can be countered effectively.
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