



# Transgenic tomatoes for abiotic stress tolerance: status and way ahead

Ram Krishna<sup>1,2</sup> · Suhas G. Karkute<sup>2</sup> · Waquar A. Ansari<sup>2</sup> · Durgesh Kumar Jaiswal<sup>1</sup> · Jay Prakash Verma<sup>1,3</sup> · Major Singh<sup>4</sup>

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## Abstract

Tomato (*Solanum lycopersicum*) is one of the most important vegetable crops; its production, productivity and quality are adversely affected by abiotic stresses. Abiotic stresses such as drought, extreme temperature and high salinity affect almost every stage of tomato life cycle. Depending upon the plant stage and duration of the stress, abiotic stress causes about 70% yield loss. Several wild tomato species have the stress tolerance genes; however, it is very difficult to transfer them into cultivars due to high genetic distance and crossing barriers. Transgenic technology is an alternative potential tool for the improvement of tomato crop to cope with abiotic stress, as it allows gene transfer across species. In recent decades, many transgenic tomatoes have been developed, and many more are under progress against abiotic stress using transgenes such as *DREBs*, *Osmotin*, *ZAT12* and *BADH2*. The altered expression of these transgenes under abiotic stresses are involved in every step of stress responses, such as signaling, control of transcription, proteins and membrane protection, compatible solute (betaines, sugars, polyols, and amino acids) synthesis, and free-radical and toxic-compound scavenging. The stress-tolerant transgenic tomato development is based on introgression of a gene with known function in stress response and putative tolerance. Transgenic tomato plants have been developed against drought, heat and salt stress with the help of various transgenes, expression of which manages the stress at the cellular level by modulating the expression of downstream genes to ultimately improve growth and yield of tomato plants and help in sustainable agricultural production. The transgenic technology could be a faster way towards tomato improvement against abiotic stress. This review provides comprehensive information about transgenic tomato development against abiotic stress such as drought, heat and salinity for researcher attention and a better understanding of transgenic technology used in tomato improvement and sustainable agricultural production.

**Keywords** Tomato · *Solanum lycopersicum* · Genetic engineering · Abiotic stress · Sustainable agriculture

## Introduction

Plants are the key to life as they are an important source of almost 90% of calories and 80% of the protein intake for the human population. Besides, the animals also obtain their

food directly or indirectly from the plants. Approximately 3000 plant species were being used as food by humans; however, at present, the world mainly depends on only about 20 crop species for their major calorie requirements of which 50% contribution is by eight species of cereal crops (Popp 1951). Minerals and vitamins are provided by 30 species of fruits and vegetables. It is estimated that earth can support 15 billion strict vegetarian people or 5 billion on a mixed diet but the global population will increase up to 10 billion by 2050. The major challenge of agriculture is to provide food and nutritional security to the growing and urbanizing world population. The world food security status, i.e., the equilibrium between increasing food requirement of the world population and worldwide agricultural output, combined with discrepancies between supply and demand at the regional, national, and local scales is alarming (Ingram 2011). It has noticeably worsened during the recent decades,

✉ Jay Prakash Verma  
verma\_bhu@yahoo.co.in; jpv.iesd@bhu.ac.in

<sup>1</sup> Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi 221005, India

<sup>2</sup> Division of Vegetable Improvement, ICAR-Indian Institute of Vegetable Research, Varanasi 221305, India

<sup>3</sup> Hawkesbury Institute for the Environment, Western Sydney University, Hawkesbury Campus, Locked Bag 1797, Penrith, Sydney, NSW 2750, Australia

<sup>4</sup> ICAR-Directorate of Onion and Garlic Research, Rajgurunagar, Pune 410505, India

culminating recently in the 2008 food crisis. It is essential to note that in mid-2011, food prices were back to their heights of the middle of the 2008 crisis (FAO 2011). Severe climatic conditions further pose a significant challenge to the sustainable production of agriculture. Roughly, the biotic factors cause 20–40% direct loss of global agricultural productivity (Bommarco et al. 2013).

The twentieth century agricultural research was more focused on increasing the crop productivity to fulfill the food requirement of world's growing population by reducing the crop yield losses due to biotic and abiotic stress factors. In the present scenario, priorities of agricultural research have not been changed, but the extra complexity has been developed by the reduced room for maneuver available environmentally, economically, and socially (Brown 2012). This is a consequence of shrinkage of natural resources that are accessible to agriculture including water, agricultural land, arable soil, biodiversity, the availability of non-renewable energy, manpower, fertilizers (Parfitt et al. 2010) and the operation of some key inputs, such as high-quality varieties and planting material (Andersen 2005). Furthermore, losses in yield are caused due to diseases which also lead to post-harvest quality losses and the possible accumulation of toxins during and after the cropping period.

## Abiotic stress

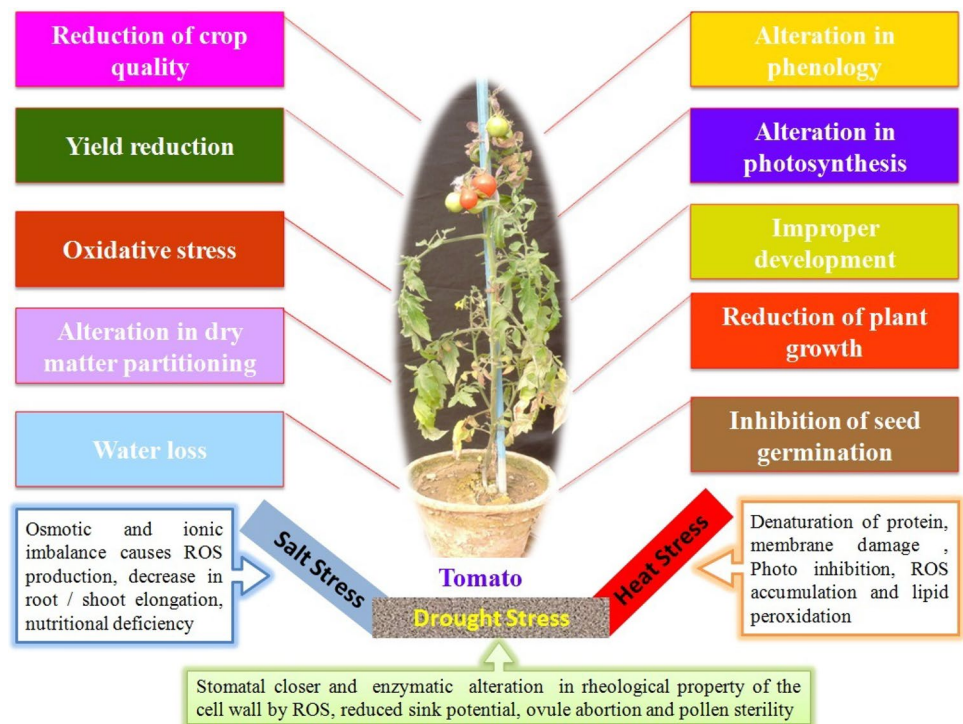
The term 'abiotic stress' consists of numerous stresses imposed by complex climatic circumstances, such as high-intensity light, UV radiation, low and high temperatures, freezing, drought, and salinity. Crop losses due to unfavorable environmental conditions have become frequent progressively over the previous decades (Boyer et al. 2013) and climate models also forecast an increased frequency of droughts, floods and extreme temperatures (Gourdji et al. 2013). The yields of major food crops such as rice, wheat and corn are decreasing according to the integrated climate change and crop production models with severe impact on global food production (Rosenzweig et al. 2014). Even with the progressive rise in major crop production by germplasm improvement and agricultural practices since the 1960s, an uncertainty of climate irregularity has increased owing to greater sowing densities, resulting in increased antagonism for nutrients and water (Lobell et al. 2014). Almost all kinds of abiotic stresses accompany oxidative stresses in plants (Demidchik 2015). Accumulation of reactive oxygen species (ROS) damages macromolecules (protein, DNA, lipid and carbohydrates) ultimately leading to cell death by membrane damage (Gill and Tuteja 2010). A group of abiotic stresses acting synergistically or additively can cause more serious oxidative stress. Therefore, controlling cellular ROS for

homeostasis is an important way to achieve enhanced multi-stress tolerance (Greco et al. 2012). The oxidative stress mitigation depends upon the reduced accumulation of ROS, the increased capability of the antioxidative system or direct ROS scavenging by the agents themselves (Turk et al. 2014). The potentiality of the antioxidant system depends on enzymatic (catalase, CAT; ascorbate peroxidase, APX; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; guaiacol peroxidase, GOPX and glutathione-S-transferase, GST superoxide dismutase, SOD) and non-enzymatic antioxidative components (ascorbic acid, ASH; glutathione, GSH) which act in concerted form to manage ROS (Gill and Tuteja 2010; Parmar et al. 2017). After exposure of plants to abiotic stresses, whether applied independently or in combined form, primed plants showed enhanced activity of both enzymatic and non-enzymatic systems compared to non-primed plants. Abiotic stresses cause a decrease in the overall performance of plants such as seed viability, germination, the weak establishment of seedling and growth (Amooaghaie and Nikzad 2013; Li et al. 2014). Abiotic stresses also lead to osmotic stress which can further cause loss of turgor pressure and ultimately restrict growth and development. Plants maintain their osmotic homeostasis partially via accumulation of osmoregulatory compounds such as free proline (Gujjar et al. 2018) and other amino acids, soluble proteins and soluble carbohydrates (Krasensky and Jonak 2012). Osmoregulation is necessary for normal plant physiology such as cell turgor pressure maintenance, stomatal movement, and hence improved photosynthesis and growth.

## Impact of abiotic stresses on growth, yield and nutritional quality of tomato

Abiotic stress is the primary cause for huge annual crop loss globally. It is estimated that about 70% of yield reduction is directly caused by abiotic stresses (Acquaah 2009). Different abiotic stresses are harmful to plant growth and development due to a series of changes in morphological, physiological, biochemical and molecular activities (Rai et al. 2013a, b). Drought, high temperatures and salinity are often interrelated and may trigger similar cellular damage including denaturation of structural and functional proteins (Fig. 1) (Gill and Tuteja 2010). For example, drought, salinity and heat are expressed primarily as osmotic stress, resulting in the disturbance in cellular homeostasis and ionic distribution (Kumar and Verma 2018). Even mild stress of these environmental factors may affect crop productivity significantly depending on the stage of the life cycle of tomato.

**Fig. 1** The overall impact of salt, drought and heat stress on a tomato plant growth and yields under various stress conditions



## Impact of drought stress

Drought is the most adverse and widespread environmental factor among the abiotic stresses (Rai et al. 2013a, b). Drought is a meteorological term, usually defined as a period of precipitation below normal which affects plant productivity in an agricultural or natural system. Water is the only base of all life forms on the earth, 70% surface of the earth is occupied by water (Chai et al. 2016), but freshwater is only about 2.5%. The whole global agriculture is fully based on freshwater and, consume about two-thirds of whole water withdrawals (Gan et al. 2013). The over-utilization of water for irrigation has been done in many parts of the world (Chai et al. 2014). Hence, the shortage of freshwater in arid and semiarid part of the globe is becoming a very serious problem. Tomato has a huge requirement of water (Battilani et al. 2012), particularly in the Mediterranean climatic parts (Saadi et al. 2010). Drought stress affects photosynthetic and defense machinery of plants. It inhibits the photochemical activities and decreases enzymatic activity of the Calvin cycle (Fig. 1). The drought stress causes a series of morphological, physiological, biochemical, and molecular changes in plants (Rai et al. 2013a). In drought stress, transpiration rate enhances than water uptake which leads to a decrease in relative water content (RWC) and thus cellular dehydration and osmotic stress. Stress-induced generation of ROS is a major aspect of drought stress (Rai et al. 2013b). ROS can be generated by many metabolic processes such as chloroplast electron transport, mitochondrial respiration and

photorespiratory metabolism (Laloi et al. 2004). At higher concentrations, ROS causes damage to almost all the cellular structures including nucleic acids, lipids and proteins by oxidation which may lead to programmed cell death (Laloi et al. 2004). Drought affects virtually every phase of plant physiology and also metabolism triggering many reactions (Verslues et al. 2006), and also negatively affects the cell division (Dixit 2008). This ultimately results in retarded plant growth rate (Sanchez-Rodríguez et al. 2010). Drought affects both plant height and leaf area in tomato. The most common adverse effect of drought on crop plants is the reduction in biomass production. Under drought stress, stomatal closure effectively saves moisture in plants; however, it also results in reduced photosynthesis. ROS generated in thylakoids during drought stress leads to a reduction in chlorophyll and carotenoid contents. Drought stress also results in a change in the ratio of chlorophyll 'a' and 'b', and carotenoids. Thus, overall growth and development of tomato plants are adversely affected by drought stress leading to drastic reduction in yield.

## Impact of heat stress

It is predicted that the frequency and harshness of extreme climatic conditions such as heat waves will increase due to global warming, posing a major constraint for sustainable agriculture, food and nutritional security. Along with slow temperature increase, the frequency and duration of heat waves are also expected to rise in the near future in several

parts of the globe. The gradual and constant changes in temperature can rigorously affect the probability of adaptation or yet survival of numerous crops of agricultural importance (Bita and Gerats 2013). A typical heat stress is caused due to 5–15 °C rise in temperature above the optimum temperature for plant growth and development (Fragkostefanakis et al. 2015). Depending on the duration and strength of the stress, plants can activate native response mechanisms to promote defense and revival from heat stress. The high temperatures cause a huge alteration in gene expression that brings about higher amounts of molecular chaperones such as heat shock proteins, scavenger proteins, ROS and other enzymes which lead to accumulation of plant metabolites functioning in the protection of biological systems and cellular structures (Shah et al. 2013; Fragkostefanakis et al. 2015). Exposure to extremely high temperatures or duration of heat stress can cause permanent disturbance of homeostasis that can damage the biological membranes and cytoskeleton, and also result in denaturation and aggregation of proteins.

Most vegetables including tomato are  $C_3$  crops which have the optimum temperature of 20–32 °C, for highest photosynthesis. Plant behavior against high temperature depends on species, growth stage and plant age, and can decrease 5–60% photosynthesis (Ruiz-Vera et al. 2015). Despite enhanced biomass production due to slight temperature increment, the yield of seed may reduce by 14%. However,  $C_3$  plants such as tomato can normally sustain 80% photosynthetic rates of its highest over large temperature duration. Majority of  $C_3$  plants can grow better when the temperature increases, but not beyond 25 °C. The study covering 127 publications (Lin et al. 2010) revealed that global warming considerably increased the biomass production by an average of 12.3% in whole terrestrial plants and an increase of 5.2% in herbaceous plants which are most suited for vegetables. The high temperature is beneficial to plants only when it is below the critical thresholds. With the increase in temperature, plants also enhance their transpiration rate due to water vapor gradient and need for cooling. In vegetables, transpiration losses are reduced by stomata closing with the penalty of decreased photosynthesis, particularly in combination with drought (Dixon and Aldous 2014). Above the optimum temperatures, the yield significantly decreases due to reduced pollen viability, germination, pollen tube growth and loss of stigma receptiveness or a reduced supply of nutrients to the pistil growing pollen tube (Karapanos et al. 2010). For example, when tomato plants were exposed to 43–45 °C temperature for 3–7 days before anthesis for 2 h, the pollen viability decreased by 20%, although the rate of viable pollen germination and the total pollen grains number were unaffected (Frank et al. 2009). High temperature negatively affects the growth, health, yield, and product quality, because of damages to cell membranes, proteins, nucleic acids, and negative effects on pigment synthesis and

degradation (Fig. 1) (Shah et al. 2013; Dixon and Aldous 2014). Various disorders such as sun scald, blossom end rot (BER), fruit cracking and unequal ripening of fruits and green shoulders are very common in tomatoes due to heat stress. High-temperature stress can reduce size, weight and appearance of the fruit (Abdelmageed and Gruda 2009), and also affects the components such as lycopene, sugars and acids causing an unfavorable impact on flavor and taste. One of the most important constituents of tomato fruit is lycopene which is responsible for fruit color, anti-cancerous and health-promoting properties. The synthesis of lycopene is repressed above 32 °C temperature. However, the increment in phytonutrients content such as anthocyanin, ascorbic acid (vitamin C), flavonoids and phenols (rutin, caffeic acid) is the positive impact of high temperature leading an elevated antioxidant capability. In contrast, reduction in the content of ascorbate and sugar accumulation in cherry tomato is reported (Wang and Frei 2011; Dixon and Aldous 2014).

### Impact of salt stress

Salt stress is considered as one of the major abiotic stresses which constrain the agricultural productivity of vegetable crops especially in semi-arid or coastal regions (Flowers 2004). Poor quality of irrigation water and poor drainage systems further enhance the problem of soil salinity. Salt stress leads to quick osmotic stress in root tissues affecting the water uptake of the plant which results in reduced plant growth (Fig. 1). Consequently, the salt stress results in disturbance of ionic balance and causes necrosis and premature death of old leaves (Julkowska and Testerink 2015). The main characteristic of the plant's response against salinity stress is the adaptation by morphological and physiological changes involving a large number of genes and pathways (Julkowska and Testerink 2015). The initial response of plant to salt stress is quite distinct, and among others involve the changes in free cytoplasmic  $Ca^{2+}$  level,  $Ca^{2+}$ /calmodulin-dependent kinase activation, production of secondary signaling molecules such as ROS and abscisic acid (ABA), and the initiation of salt overly sensitive (SOS) pathway to regulate ion homeostasis (Julkowska and Testerink 2015; Kumar and Verma 2018). Signaling mechanism involving hormones such as ABA, gibberellin (GA), brassinosteroid (BR) and jasmonic acid (JA) is also found to play a key role in regulating the plant's response at the later stages of stress exposures (Ryu and Cho 2015). Although mild salt stress is used to improve the nutritional quality of tomato fruit by enhancing amino acids, glucose, fructose, titratable acid, vitamin C and total soluble solid contents (Nebauer et al. 2013). Tomato is highly prone to high soil salinity conditions and cannot tolerate or tolerate with very low yields. The salt stress above the electrical conductivity (EC) (3–5.5 dS/m) reduces the leaf area index (LAI<sub>m</sub>)



and total chlorophyll content significantly, and reduces the tomato yield by 12–32% (Zhai et al. 2015). The blossom end rot (BER) of tomato is an important physiological disorder which causes up to 50% losses and the occurrence of BER is enhanced by salinity (Lu et al. 2012).

## Traditional breeding

Breeding in tomato is focused on several aspects depending on its use either fresh or for processing. Along with quality traits, more importance needs to be given to breeding for biotic and abiotic stresses as these are the major threats to tomato production in the present scenario. Conventional breeding involves the use of tomato genotype or its wild relatives having the desirable trait and transferring the trait into another cultivar with good genetic background. However, abiotic stress tolerance is a complex trait and is complicated to transfer it through traditional breeding. The stress tolerance at the phenotypic level is an outcome of several molecular, biochemical and physiological processes making it a complex trait. It has been successfully utilized in developing fruits with better quality and disease resistance. Hybrids with multiple resistances to several pathogens have also been developed in vegetable crops. Tomato varieties with resistance to at least 15 pathogens, although with varying stability and level of expression, are widely cultivated (Grube et al. 1999). Tomato varieties with some resistance to fungi or oomycetes, bacteria, virus, and nematodes are available (Silva Dias and Ryder 2011; Prasanna et al. 2015). The cultivated tomato genotypes are quite susceptible to different environmental stresses that they encounter throughout their life. Comparatively, many related wild species of tomato, viz. *Solanum chilense*, *Solanum pennellii*, *Solanum pimpinellifolium*, and *Solanum habrochaites*, are reported showing significant tolerance against such stresses due to their diverse genetic backgrounds harboring many genes and *QTLs* that are responsible for stress tolerance traits (Khan et al. 2017; Bondel et al. 2018; Liu et al. 2018). The biggest problem in transferring these traits from wild to cultivated species is the higher genetic distance between them resulting in crossing barriers. Therefore, an alternative, modern biotechnological approach such as transgenesis is frequently applied for overcoming such type of genetic barriers.

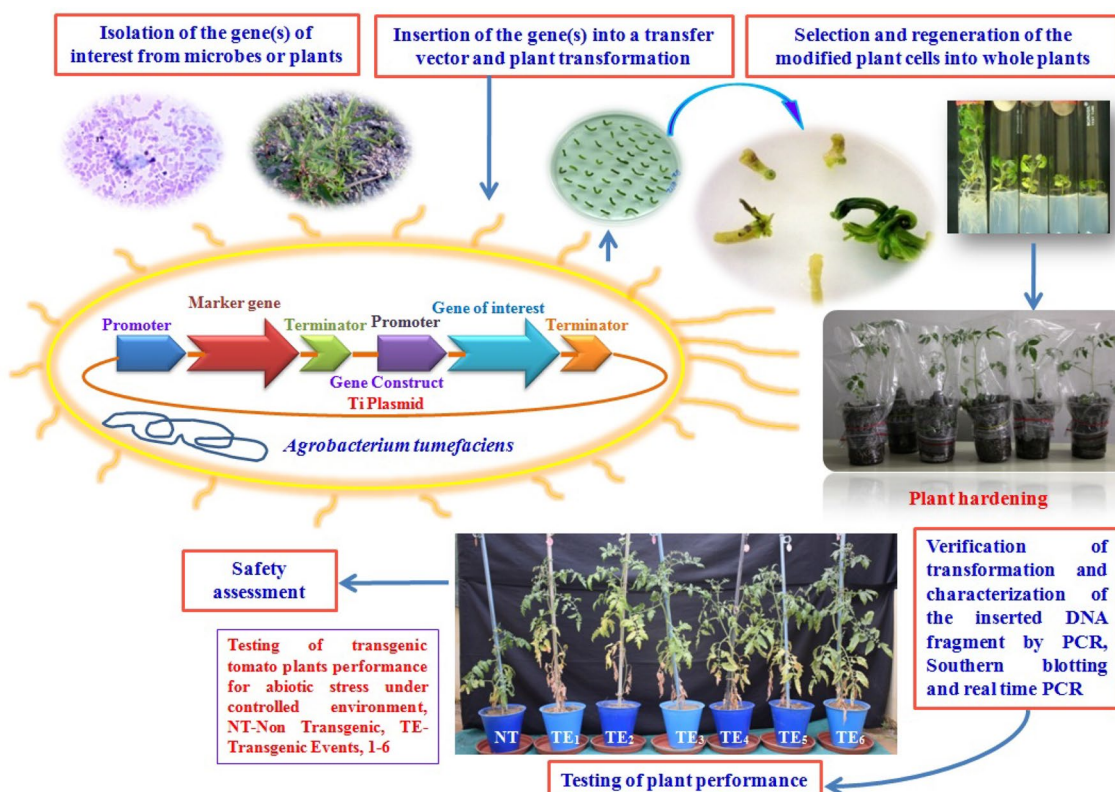
## The rationale for transgenic technology application

The traditional plant breeding has been enormously used in tomato crop improvement for fruit quality and disease resistance. However, it is limited by a number of factors such as time taking method of producing crosses and

backcrosses, saturation of the genetic pools that can be utilized for improvement and almost a decade of time required in releasing a new variety. Further, conventional breeding may not be helpful all the times to transfer the target trait from wild gene pool to developed varieties (Gould 1992). The alternative to traditional breeding that overcomes all these limitations is a recombinant-DNA technology or genetic engineering which allows transplanting of genetic distinctiveness across the species. Transgenic technology is based on identification and characterization of genes responsible for the desired trait. These genes can be then utilized to transform and manipulate any existing crop variety. It is, in fact, a continuum between plant breeding-based varietal development and manipulation of useful genes by recombinant DNA technology to develop transgenic crops (Fig. 2). Moreover, it overcomes the species barrier and thus, the cloned gene from one species can be introduced to other species of high agriculture importance (Peleman and Van Der Voort 2003). This approach reduces almost half of the time required to develop a new tomato variety compared to conventional plant breeding. An efficient plant regeneration system for the development of stable transgenic plant from explant tissue is the foremost requirement for a successful genetic engineering program. It is also important to optimize the protocol for transformation of the plant with a foreign or modified host gene. Plant genetic engineering requires a combination of these two approaches. *Agrobacterium tumefaciens* has been used in early experiments of gene transfer for introducing foreign genes in plants (Rai et al. 2012; Kumar et al. 2018). In case of solanaceous species, *Agrobacterium* is a very effective tool for plant transformation due to its reproducibility. Alternatively, the method of biolistic has emerged as an easy and potential method of transformation. It facilitates the successful genetic transformation of several plant tissues without any restriction. In gene gun or particle bombardment technique, DNA coated metal particles are rapidly bombarded into plant tissues and resulting transformants generally show their stable transformation. With the help of recombinant DNA technology, several genes have been characterized for their functions and that can be well utilized to develop new transgenic tomatoes with superior quality and resistance to biotic and abiotic stresses.

## Transgenic tomatoes resistant or tolerant to abiotic stresses

Sessile nature of plants causes their continuous exposure to vagaries of environmental conditions and, therefore, they have adopted a cellular mechanism to respond to extreme environmental conditions by altering their growth, productivity and quality. A number of studies have been carried out to understand this mechanism of plant's response to various



**Fig. 2** Various steps in tomato transgenic development through application of *Agrobacterium* mediated gene transformation in tomato plants under tissue culturing techniques

stresses such as drought, heat, salinity, cold, heavy metals, oxidative stress and hypoxia due to waterlogging (Karkute et al. 2019). To avoid these abiotic stresses, plants have evolved a convoluted perception and signaling network pathways which perceive signals and modulate the expression of particular gene/s (Zhang et al. 2004) that further leads to biochemical, physiological and morphological changes to make the plants adapt to the stress condition (Fig. 3). Although significant achievement has been made to understand these pathways in *Arabidopsis* as a model plant system, several studies have been reported that characterized these pathways in tomato as well. Nevertheless, enormous efforts have been made to develop tomato plants that can show significant tolerance or resistance to abiotic stresses.

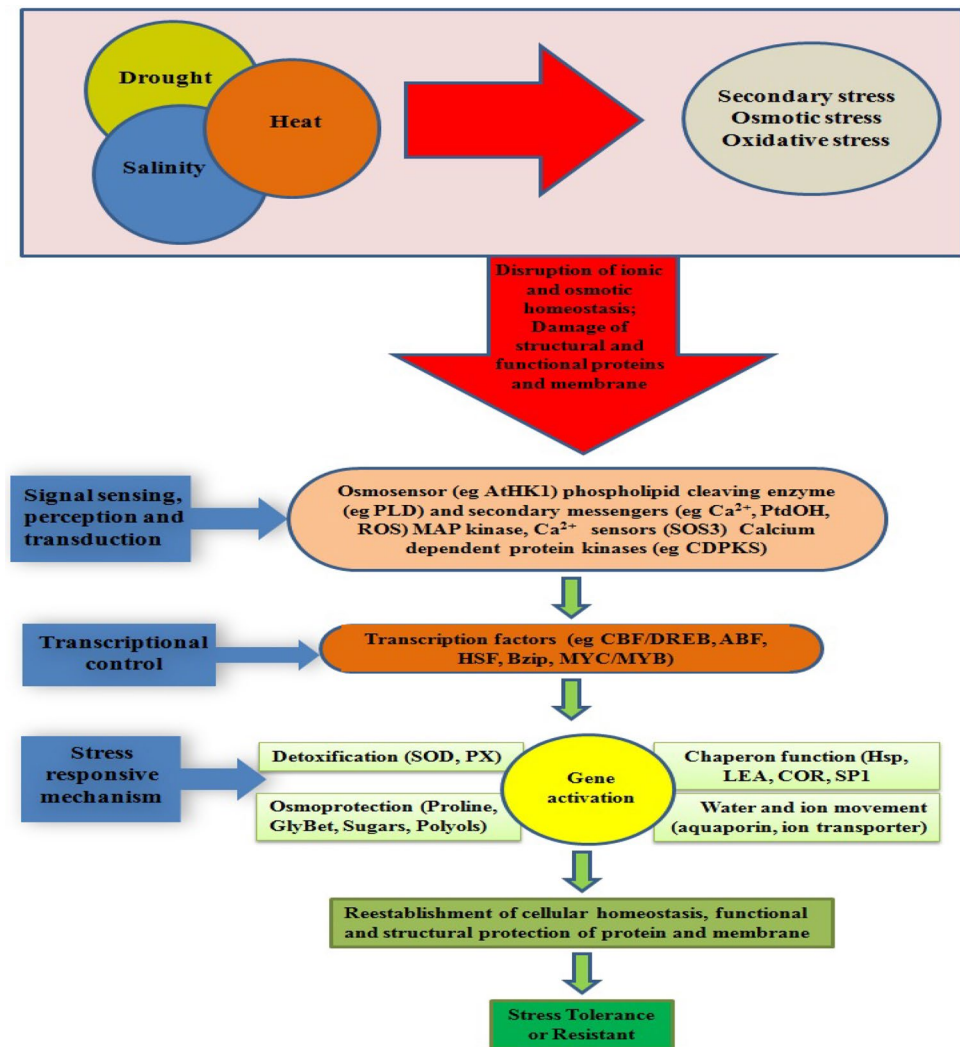
### Drought stress tolerance

The response of a plant to drought condition is uttered in a complex network of morphological, physiological, and molecular changes. Various biochemical and physiological screening methods are usually implemented to identify the best genotypes showing drought tolerance traits. Such genotypes have been exploited to mine the drought-responsive genes and to study the drought tolerance mechanism. A clear

understanding of the mechanism of drought adaptation in a plant will further help in developing crop varieties that can tolerate drought devoid of impacting crop quality and productivity. However, significant work is required before this knowledge can be translated into developing stress-tolerant tomato varieties. In *Arabidopsis*, a number of drought-responsive genes have been identified and these genes can also be overexpressed in tomato for achieving the drought stress tolerance in tomatoes.

Abiotic stress tolerance is a complex trait that involves a large number of genes. Plants exposed to stress condition respond through the changes in both RNA and protein expression profiles. Transcriptome analysis using microarray identified approximately 130 drought-responsive genes (Seki et al. 2001) involved in various functions such as transcription modulation, ion transport, transpiration control and carbohydrate metabolism (Fig. 3). Several transcription factors, *Dehydration-responsive element-binding protein 1A (DREB1A)*, *C-repeat/dehydration responsive element-binding factor (CBF)* and *Heat shock factor (HSF)* have been identified that specifically regulate stress-responsive genes (Sakuma 2006). Carbohydrate metabolism is one of the important parts of the general cellular response to acclimation to stress and contribute to osmotic adjustment under

**Fig. 3** Downstream signaling process and transcription controls which activate stress-responsive mechanisms to re-establish homeostasis repair damage



stress, and the cell wall invertase and sucrose synthase genes play a vital role in this process making them suitable target to manipulate for stress tolerance trait (McDowell 2011). Several other genes that can be manipulated are *ERECTA* gene regulating transpiration efficiency in *Arabidopsis* (Masle et al. 2005), and *Na<sup>+</sup>IH<sup>+</sup> exchanger (NHX)* and *Arabidopsis vacuolar H<sup>+</sup> pyrophosphatase (AVP1)* genes involved in ion transport (Zhang and Blumwald 2001). So far, several genes playing roles in the stress response have been reported in a number of species; however, identifying the best gene that can confer significant tolerance in crop plants is a major challenge. Nevertheless, there are several reports (Table 1) where the overexpression of drought-responsive genes in tomato showed significant tolerance to drought stress and some of them have been discussed here.

Transformation studies done to improve drought adaptation in plants have resulted in both tolerance and avoidance traits. One of the strategies of drought avoidance is leaf senescence which gets accelerated in drought-sensitive

plants to decrease canopy size. Thus, drought resistance can be developed in plants by suppressing the drought-induced leaf senescence. Cytokinin acts as an inhibitor of leaf senescence and, therefore, transgenic plants overexpressing isopentenyl transferase (*IPT*) under the control of senescence-associated receptor protein kinase promoter (*PSARK*) were developed (Rivero et al. 2007). The *IPT* is a key enzyme involved in cytokinin biosynthesis. These transgenic plants did not show senescence even after 15 days of drought stress treatment in a glasshouse and maintained a reduced photosynthetic capacity. Rewatered transgenic plants recovered full leaf turgor with two to three times higher water use efficiency and resumed growth and maximum photosynthetic capacity, whereas, control plants were unable to recover from wilting.

Heterologous constitutive expression of *Arabidopsis CBF1* (C-repeat/dehydration responsive element-binding factor 1) in tomato resulted in enhanced plant tolerance to drought as well as cold and salt stress (Hsieh et al. 2002).

**Table 1** Transgene used for the development of drought stress tolerance, their function and mechanism of action

S. no.	Gene	Function	Mechanism of action	References
1	<i>CBF1</i>	Stress-induced transcription factors	Expression of stress responsive gene	Lee et al. (2003)
2	<i>HI-S, drought-induced linker histone</i>	DNA packaging and organization of chromosomes in the nucleus	Modulation of mechanisms related to the stomatal function	Scippa et al. (2000)
3	<i>H<sup>+</sup>-pyrophosphatase</i>	Facilitate auxin fluxes	Enhance pyrophosphate-driven cation transport into root vacuolar fractions	Park et al. (2005)
4	<i>bspA</i>	Protein protection	Enhance desiccation tolerance by protecting proteins in membranes and cytosol	Roy et al. (2006)
5	<i>Coda</i>	Accumulation of glycine betaine	Osmolyte accumulation protects against oxidative damage	Park et al. (2007)
6	<i>LeNCED1</i>	Increases in abscisic acid (ABA) accumulation	Stomatal closure and increased water-use efficiency (WUE)	Tung et al. (2008)
7	<i>Osmotin</i>	Stress-responsive multifunctional protein	Osmotin provides protection via different mechanisms related with programmed cell death	Goel et al. (2010)
8	<i>PtADC</i>	Induce the stress-responsive gene	Improve dehydration and drought tolerance	Wang et al. (2011)
9	<i>DREBs/CBFs; ABF3</i>	Stress-induced transcription factors	Enhanced expression of downstream stress-related genes confers drought tolerance	Rai et al. (2012)
10	<i>ZAT12</i>	Stress-induced transcription factors	Enhanced expression of downstream stress-related genes confers drought	Rai et al. (2013a)
11	<i>AtGAMT1</i>	Suppress gibberellin	GAMT1 overexpression inhibited the expansion of leaf-epidermal cells	Nir et al. (2014)
12	<i>SINAC4</i>	Stress-responsive transcription factor	Modulation of ABA-independent signaling networks	Zhu et al. (2014)
13	<i>GalUR</i>	GalUR encodes Lgalactono-1,4-lactone as a precursor of ascorbic acid	Ascorbic acid detoxifies superoxide anion radical and hydroxyl radical, and also plays a crucial role in scavenging ROS	Lim et al. (2016)

However, this enhanced tolerance also caused a reduction in plant growth and yield. To avoid the yield and growth penalty, the same *CBF1* gene was expressed under the control of abscisic acid (ABA)/stress-inducible promoter from barley *HAV22* gene (Lee et al. 2003). These transgenic plants showed significant tolerance to drought, chilling and salt stress without any deleterious effects on plant growth and yield (Rai et al. 2013b). Overexpression of vacuolar H<sup>+</sup>-pyrophosphatase with constitutive promoter in tomato plants leads to efficient pyrophosphate-driven cation transport into root vacuoles along with enhanced root biomass (Park et al. 2005). In another study, transgenic tomato plants with a novel 66-kD *BSP* (boiling stable proteins) gene from *Populus tremula* showed desiccation tolerance against water stress. The environmental stress induces linker histone proteins in many plant species, but their exact role has not been identified yet. However, it has been proposed that a drought-induced linker histone of tomato, *HI-S*, may be involved in a structural role where it protects DNA from damage under drought stress and a functional role in gene regulation during stress (Scippa et al. 2000). Further, the transgenic tomato plants overexpressing antisense histone *HI-S* have been developed and found to have normal growth and development except shortening of internodes, which shows

that *HI-S* is not an important gene for the basal functions of tomato development.

### Heat stress

Heat stress induces reproductive development which results in shortening of the photosynthetic period and ultimately affects the fruit or seed production. The global distribution of crop species is determined by its capacity to acclimate to the environmental conditions. The acclimation of the plant species to high-temperature stress is the result of its highly conserved response that involves multiple pathways, regulatory networks and cellular compartments (Kotak et al. 2007). Heat stress in plants produces reactive oxygen species (ROS), and the accumulation of these ROS is harmful to cells. These enhanced ROS are neutralized in plants by an inducible protective system which includes antioxidants. They also help to restore and regulate the ROS at the cellular steady-state levels (Fig. 3). The other adaptive strategies in plants towards stress include programmed cell death or tissue degradation, loss of membrane semi-permeability, change in gene expression profiles and accumulation of stress-responsive transcripts and their synthesis (Shah et al. 2013).



Cells are protected from the adverse effects of heat stress by *heat shock proteins (HSPs)*. A small heat shock stimulant is sufficient to induce the expression and synthesis of small *HSPs*, which in turn develops thermo-tolerance in plants (Morrow and Tanguay 2012). In plants, at least 20 types of small *HSPs* have been reported and they are located in different cell organelles such as chloroplast, endoplasmic reticulum and mitochondria, cell membranes and cytosol (Waters 2013). *HSPs* are highly studied for their molecular properties because of their role in heat stress response. Interestingly, the *HSPs* are highly conserved proteins across different species. Since their discovery, *HSPs* from different species have been reported, cloned, and sequenced for their functional characterization.

The signaling pathways leading to activate the expression of *HSPs* involve a number of proteins and it begins with temperature sensing. Heat stress signal stimulates the heat shock factors which then binds to heat shock element present in the promoter region of *HSPs* (Larkindale 2005). Cytosolic *Hsp17.4* and chloroplastic *Hsp21* in tomato were shown to be induced under temperature stress and imparts acquired thermo-tolerance (Larkindale 2005). Similarly, chloroplastic *Hsp21* was found to be induced in leaves, flowers and fruits after exposure to heat stress. Overexpression of chloroplastic *Hsp21* in transgenic tomato protects photosystem II from temperature-dependent oxidative stress (Neta-Sharir 2005). Mitochondrial *Lehsp* gene of tomato overexpressed in transgenic tobacco conferred thermo-tolerance at 48 °C (Sanmiya et al. 2004).

Transcription factor *Zinc finger protein ZAT12 (ZAT12)* has been shown to play a role in multiple abiotic stress tolerance and its overexpression in transgenic plants induced the expression of ROS-scavenging genes leading to salt, drought or osmotic stress tolerance (Shah et al. 2013). Arabidopsis *DREB2A* transcription factor is heat stress-induced regulator of heat stress response and it induces *Heat stress transcription factor A-3 (HsfA3)* gene under heat stress (Schramm et al. 2008). Besides transcription factors and *HSPs*, the osmolytes such as amino acids, polyamines, quaternary ammonium compounds, sugars and sugar alcohols play a significant role in the adaptation of plants to osmotic stresses

and enhancing their biosynthesis by modulating the synthetic pathways can lead to the development of stress tolerance in plants (Table 2).

## Salt stress

Physiological response to particular stress is determined by a large number of component traits and is governed by a number of corresponding genes. Identification and characterization of such genes by the combination of genome-wide expression analysis using DNA arrays, RNA-Seq, etc. and *QTL* analysis along with modern biotechnological tools will provide the desired information to utilize further for developing stress-tolerant crops by breeding or transgenic methods. Several genes have been identified so far that have a potential for providing salinity stress tolerance in plants (Table 3). Cellular salt stress response starts with calcium sensor protein, *SOS3*, which is activated by sensing the  $\text{Ca}^{2+}$  ion concentration in cell and it binds to *SOS2* which is a Ser/Thr protein kinase. *SOS2* then regulates the activities of a tonoplast  $\text{Na}^+/\text{H}^+$  antiporter *NHX1* and plasma membrane  $\text{Na}^+/\text{H}^+$  antiporter *SOS1* (Sahi et al. 2006). Besides, a putative osmosensory histidine kinase of Arabidopsis (*AtHK1*)–*MAPK* cascade is one of the signaling pathways that may regulate genes involved in ROS scavenging and osmotic homeostasis (Fig. 3). Understanding the mechanism of ion transporters and their regulators, and *CBF* regulons will boost the development of salt stress-tolerant crops. Considerable work has been done in the field of transgenics to develop plants that can tolerate salinity stress by effectively managing cellular salt concentration levels. To manage the salt stress, it is essential to maintain a low cytosolic  $\text{Na}^+$  concentration which can be achieved by restricting the inflow, increasing outflow, or increasing vacuole sequestration of  $\text{Na}^+$  (Lata and Prasad 2011).  $\text{Na}^+/\text{H}^+$  antiporter genes are well characterized for their function in salt transport across the cellular membranes. Therefore, transgenic tomatoes overexpressing *A. thaliana* tonoplast membrane  $\text{Na}^+/\text{H}^+$  antiporter, *AtNHX1*, governed by a strong constitutive promoter, were developed in the anticipation of gaining stress tolerance (Deinlein et al. 2014). These transgenic tomatoes were able

**Table 2** Transgene used for development of heat stress tolerance, their function and mechanism of action

S. no.	Genes	Function	Mechanism of action	References
1	<i>Hsp101</i>	Heat shock protein	Acquired thermo-tolerance	Nieto-Sotelo (2002)
2	<i>sHSP (mitochondrial)</i>	Inducer of mitochondrial heat sock protein	Thermo-tolerance	Nautiyal et al. (2005)
3	<i>sHSPs (ER)</i>	Inducer of ER stress	Attenuated the lethal effect of tunicamycin	Zhao et al. (2007)
4	<i>SAMDC</i>	Polyamines	Accumulation of polyamines	Cheng et al. (2009)
5	<i>LeUCP</i>	Active oxygen species metabolism	Increase redox level and antioxidant capacity	Chen et al. (2013)
6	<i>LeCDJ1</i>	Protein	Overexpression facilitated heat tolerance	Kong et al. (2014)
7	<i>SiCDJ2</i>	Chloroplast-targeted DnaJ protein	Protects Rubisco activity under heat stress	Wang et al. (2015)

**Table 3** Transgene used for development of salt stress tolerance, their function and mechanism of action

S. no.	Genes	Function	Mechanism of action	References
1	<i>BADH-1</i>	Overexpression of betaine aldehyde dehydrogenase	BADH gene catalyzes the conversion of betaine aldehyde into glycine betaine	Moghaieb et al. (2000)
2	<i>NHX1</i>	Overexpression of the <i>NHX1</i>	Overexpressed <i>NHX1</i> vacuolar Na <sup>+</sup> /H <sup>+</sup> antiporter	Zhang and Blumwald (2001)
3	<i>cAPX</i>	Overexpression of <i>APX</i>	Minimize cellular damage by scavenging the super oxides	Wang et al. (2005)
4	<i>CaKRI</i>	Overexpression of <i>LeSOD2</i> , <i>LeAPX2</i> and <i>LeAPX3</i>	High transcript level of antioxidative enzyme machinery scavenges the ROS	Seong et al. (2007)
5	<i>ToOsmotin</i>	Osmotic adjustment	Overexpression leads to the accumulation or compartmentation of solutes and also protects the native structure of proteins	Goel et al. (2010)
6	<i>Ectoine (ectA, ectB and ectC)</i>	Compatible solute	Enhance peroxidase activity and decrease MDA contents by ectoine accumulation	Moghaieb et al. (2011)
7	<i>AtSISOS2 (AtSISOS2)</i>	Homeostasis of Na <sup>+</sup> and K <sup>+</sup>	Upregulation of the plasma membrane Na <sup>+</sup> /H <sup>+</sup> ( <i>SISOS1</i> ) and endosomal-vacuolar K <sup>+</sup> , Na <sup>+</sup> /H <sup>+</sup> ( <i>LeNHX2</i> and <i>LeNHX4</i> ) antiporters, responsible for Na <sup>+</sup> extrusion out of the root, active loading of Na <sup>+</sup> into the xylem, and Na <sup>+</sup> and K <sup>+</sup> compartmentalization	Huertas et al. (2012)
8	<i>TaNHX2</i>	Na <sup>+</sup> /H <sup>+</sup> antiporter	Na <sup>+</sup> /H <sup>+</sup> antiporters are involved in intracellular ion (Na <sup>+</sup> ), pH regulation and K <sup>+</sup> homeostasis in plants	Yarra et al. (2012)
9	<i>HAL5</i>	Maintaining Na <sup>+</sup> /K <sup>+</sup> homeostasis	Maintenance of Na <sup>+</sup> and K <sup>+</sup> transporter levels such as SIHKT1 and 2, and SIHAK5	García-Abellan et al. (2014)
10	<i>MdSOS2L1</i>	Codes for <i>MdSOS2L1</i> protein kinase	MdSOS2L1 protein kinase physically interacts with MdCBL1, MdCBL4 and MdCBL10 proteins to increase tolerance	Hu et al. (2016)
11	<i>coda</i>	Encode for glycine betaine	Glycine betaine enhanced NaCl-induced expression of genes encoding the K <sup>+</sup> transporter, Na <sup>+</sup> /H <sup>+</sup> antiporter, and H <sup>+</sup> -ATPase	Wei et al. (2017)

to flower and set fruit under the salt stress of 200 mM NaCl with very low concentrations of sodium in fruits. The *NHX1* system is highly conserved among different plant species and can be effectively manipulated to achieve improved salt tolerance in crop species.

One of the ways to improve salt tolerance is to increase the level of osmolytes in plants. It has been observed that the level of glycine betaine, which is a quaternary ammonium compound in many organisms, increases under salt or other osmotic stresses. Transgenic rice overexpressing *choline oxidase A (coda)* gene showed an increased synthesis of glycine betaine and thereby enhanced salinity tolerance (Mohanty et al. 2002). In sorghum, *Betaine-aldehyde dehydrogenase (BADH)* gene that codes for betaine aldehyde dehydrogenase catalyzes the conversion of betaine aldehyde into glycine

betaine. Overexpression of *BADH* gene in transgenic tomato improved salt stress tolerance by contributing to the maintenance of osmotic potential (Goel et al. 2010). Similar results have been reported (Wang et al. 2013) where the constitutive expression of *Suaeda liaotungensis BADH* gene in tomato significantly enhanced salt stress tolerance. In another report, *BADH-1* gene was transferred to tomato hairy root lines using root-inducing plasmid (pRi) and these transgenic tomatoes showed higher accumulation of betaine (Amudha and Balasubramani 2011).

## Conclusion and the way ahead

Transgenic technology has the potential to enormously contribute to sustainable agriculture, food and nutritional security. Considering the drastic change in temperature and erratic rainfall patterns, transgenic tomatoes developed for conferring abiotic stress tolerance are the need of the hour for fulfilling the continuous demand of tomato fruits. Modification of plant genome is a viable approach to engineer tomatoes with improved nutritional values, environmental benefits and inherent resistance against abiotic stresses in a friendly and sustainable environment. It has been reported that manipulation of single or multiple genes linked to abiotic stress response provides plants with tolerance to such stresses. In future, plants with tolerance to single abiotic stress will not be a solution for sustainable agriculture, because the abiotic stresses are interrelated. For example, salt stress often results in water deficit to plants or heat stress also ultimately results in drought stress. Therefore, plants with multiple stress tolerance are required that can not only survive in harsh environmental conditions but also give sufficient yields. This can be achieved by stacking or pyramiding of the existing GM traits in tomato for different abiotic stress in a quick, simple and efficient way.

The deeper understanding of the mechanism of stress tolerance in plants by metabolomics could further open the new approaches by manipulating metabolic pathways to develop stress-tolerant plants. Modification of metabolic pathways is quite complicated as the majority of the proteins of a pathway interact with several other proteins. Thus, efficient metabolic engineering will only be achieved by controlling multiple genes on the same or interlinked pathways. Multigene manipulation has also been advanced to a great level by utilizing both conventional and novel techniques which provides a framework for future improvements. Although the transgenic crops are being successfully cultivated since almost 2 decades in the USA and more than a decade in India without any known detrimental impact on humans or environment, the path to land a new transgenic crop in farmers' field is not smooth. Besides the in-depth understanding of the science behind the transgenes and their mode of action and all the possible studies to evaluate safety that shows no harmful effect of transgenics, the hurdle for its acceptance is a political and social affair. There is an utmost need for creating awareness among farmers and the public about the benefits of transgenic crops along with their scientific basis. It is important to make the public understand that the transgenic crops are very much safe to humans as well as the environment by facts and data of biosafety studies carried out on transgenic crops. The impressive

example of tackling these hurdles successfully is the case study of Bangladesh permitting commercial cultivation of transgenic brinjal. The agencies involved in the biosafety evaluation of transgenic crops should consider inviting the representative of the organizations opposing this technology at each and every stage of development and evaluation of a transgenic crop. This would make them aware of the process of safety evaluation of the transgenic crops. The proponents of transgenic technologies should bring the documents in public domain answering each and every doubt of opponents with supporting scientific data in a way that can be understood by farmers and public. Ultimately, the intensive efforts are required to harvest the fruits of such a wonderful technology in the forthcoming time in developing countries such as India.

## Compliance with ethical standards

**Conflict of interest** There is no conflict of interest among the authors; all authors contributed equally.

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