Review

Current trends in genetic manipulations to enhance abiotic and biotic stresses in tobacco

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Environmental stresses, both abiotic and biotic, are the main factors reducing crops productivity. Biotic stresses caused by fungi, viruses, bacteria and insects are the major threats. However, abiotic stresses have more adverse effects on crop yield and crop survival. Hitherto, tolerant plants were mainly produced by classical breeding techniques. Success in breeding for better adapted varieties to abiotic and biotic stresses depends on the concerted efforts of various research domains including plant and cell physiology, molecular biology, genetics and breeding. However, such process is time consuming. The production of transgenic plants by genetic engineering allows a much faster progress. This review focuses on stress tolerance on tobacco. Tolerance to salt, drought, water stress, low and high temperature, heavy metals, pathogens and pests were enhanced via overexpression of specific stress related genes from different sources.

Key words: Overexpression, genetic engineering, abiotic factors, biotic factors.

INTRODUCTION

Out of a US$1.3 trillion annual food production capacity worldwide, the biotic stresses (insects, diseases and weeds) cause 31 to 42% loss (US$500 billion), with an additional 6 to 20% (US$120 billion) post harvest loss due to insects, fungal rots and bacterial rots (FAO, 2005). FAO (2005) also investigated 6 to 20% (US$120 billion) loss by abiotic stresses (drought, flood, frosts, nutrient deficiencies, various soil and air toxicities). Goel and Madan (2014) reported that abiotic stresses lead to more than 50% of yield reduction. These huge losses exert high pressure in world food security program.

Plants are sessile organisms which are constantly exposed to a variety of biotic and abiotic stresses in their external environment. Abiotic stress causes cellular dehydration and accumulation of reactive oxygen species (ROS) such as hydrogen peroxide, which may function as signal to counteract these stresses, for instance by increasing endogenous osmotic pressure to prevent further water loss from the cell (Doltchinkova et al., 2013). In general, ROS are one of the major damaging factors that arise when plants are exposed to various abiotic stresses, including nutrient excess/depletion, flooding drought, desiccation, extreme temperatures, light/dark cycles, high salinity, ozone, anoxia and herbicides (Choet et al., 2013). Accordingly, an unfortunate consequence of salinity stress in plants is the excessive generation of ROS intermediates, such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and

Table 1. Gene manipulation to enhance abiotic stress tolerance in transgenic tobacco.

Gene/its product	Source	Role	Reference
GmERF7	Soybean	Enhanced salt tolerance	Zhai et al. (2013)
MDHAR	Acerola	Antioxidative	Eltelib et al. (2012)
ALDRXV4	*Xerophyta viscose*	Better salinity stress tolerance	Kumar et al. (2013)
AtNHX1	*Arabidopsis*	Increased salt tolerance	Zhou et al. (2011)
DgNAC1	chrysanthemum	Improved salt tolerance	Liu et al. (2011)
AVP1	*Arabidopsis*	Tolerated to shortage of water	Arif et al. (2013)
ALDRXV4	*Xerophyta viscosa*	Survived longer period of water deficiency	Kumar et al. (2013)
TaEXPB23	Wheat	Losing water more slowly	Li et al. (2011)
PtADC	*Poncirus trifoliata*	Reduce ROS	Wang et al. (2011)
LOS5/ABA3	*Arabidopsis*	Maintaining high water content	Yue et al. (2011)
TaEXPB23	*Arabidopsis*	Improved the water-stress tolerance	Li et al. (2013)
BcBCP1	*Boea crassifolia*	Tolerance to osmotic stress	Wu et al. (2011)
CbCOR15b	Shepherd's purse	Cold tolerance	Wu et al. (2012)
GbCBF1	Cotton	Enhanced chilling tolerance	Hui-Ming et al. (2011)
ZmMPK4	Maize	Low temperature tolerance	Zhou et al. (2012)
CfCBF3	Sweet pepper	Low temperature tolerance	Yang et al. (2011)
ZFP177	Rice	High temperature tolerance	Grover et al. (2013)
WRKY40	Pepper	High temperature tolerance	Dang et al. (2013)
MxCS1	*Malus xiaojinensis*	Improved Fe stress	Han et al. (2013)
TaVP1	Wheat	Enhanced Cd tolerance	Khoudi et al. (2012)

hydroxyl radicals (OH^-). ROS overproduction affects membrane potential and other essential macromolecules, such as photosynthetic pigments, carbohydrates, proteins, DNA and lipids inhibiting photosynthetic capacity.

Plants developed an array of complex mechanisms to rapidly sense environmental changes. Plants also developed a variety of mechanisms to adapt themselves to ever changing environments and these mechanisms are mediated through multiple signal transduction pathways acting in a global signal network. Typically, mitogen-activated protein kinase (MAPK) cascades are universal signaling pathways employed in such responses (Zhou et al., 2012). Plants adapt to unfavorable environmental conditions by accumulating highly soluble organic compounds of low molecular weight like sugars, sugar alcohols, amino acids and amino acid derivatives (Doltchinkova et al., 2013). In addition, plants detoxify ROS through a combination of antioxidants (ascorbate (AsA) and glutathione (GSH), and antioxidative enzymes [superoxide dismutase (SOD); ascorbate peroxidase (APX); monodehydroascorbate reductase (MDHAR); dehydroascorbate reductase, (DHAR); and catalase (CAT)] (Choe et al., 2013; Peshev et al., 2013). Antioxidative enzymes are involved in chloroplasts, cytosol and mitochondria that are known sources of ROS generation. In addition, transgenic plants with increased contents of compatible solutes show a strong potential for improving their stress tolerance (Doltchinkova et al., 2013). This review summarizes the gene sources and the effects on stress tolerance after transforming these genes into tobacco.

ABIOTIC FACTORS

Abiotic factors are yield limiting factors that potentially reduce crop productivity. Abiotic factors include salinity, drought, osmotic stress, heavy metals and natural and artificial toxic chemicals. Before the era of modern biotechnology, these problems were overcome by exposing plants for long periods to allow the plants to adapt, finally creating tolerant organisms. Plant biotechnology allows a quick introduction of a tolerance-related gene from a certain organism into susceptible plants (Table 1). Such genes are typically overexpressed by using promoters to enhance their functions. For instance, transgenic tobacco plants overexpressing *OsCBSX4* [a gene that produce a component of cystathionine β-synthase domain containing proteins (CDCPs)], obtained from rice (*Oryza sativa* L.) exhibited improved tolerance against salinity, heavy metals and oxidative stress (Singh et al., 2012). The higher accumulation of OsCBSX4 protein in *OsCBSX4* overexpressing transgenic plants and the exhibition of higher abiotic stress tolerance than wild type (WT) plants suggest a role in abiotic stress tolerance in plants.

Salt tolerance

Salinity deteriorates agricultural land and reduces crop

yield. The land affected by salinity is estimated to be about one-third of the world's cultivated land (Eltelib et al., 2012). Accordingly, plants have evolved a variety of mechanisms to deal with high salinity. For example, a low Na^+ content in the cytoplasm is maintained by compartmentation of Na^+ into the vacuole (Zhou et al., 2011). Vacuolar Na^+ compartmentation has two basic roles in salt tolerance physiology. First, it keeps Na^+ away from the sites of metabolism, and second, it increases the osmolarity of the cell to regulate cytoplasmic pH. These are attained using Na^+/H^+ antiporter that pumps excess vacuolar Na^+ into vacuoles, and membrane potential is created due to a charge difference. The membrane potential and H^+-inorganic pyrophosphatase provides energy for Na^+/H^+ antiporter (Zhou et al., 2011) and the energy establishes electrochemical H^+ gradient between the cytoplasm and the vacuole.

Ethylene-response factors (ERFs) play an important role in regulating gene expression in plant responses to biotic and abiotic stresses. Zhai et al. (2013) isolated a new ERF transcription factor, *GmERF7*, from soybean. Overexpression of *GmERF7* in tobacco plants led to higher levels of chlorophyll and soluble carbohydrates and a lower level of malondialdehyde compared to wild-type tobacco plants under salt stress conditions, indicating that *GmERF7* enhanced salt tolerance in transgenic plants.

Eltelib et al. (2012) introduced a monodehydro-ascorbate reductase (MDHAR) cDNA from acerola (*Malpighia glabra*) into tobacco plants via an *Agrobacterium*-mediated gene delivery system. Transgenic plants accumulated higher level of ascorbate (AsA) and showed higher MDHAR activities as compared with the control plants. These effects were also associated with a greater tolerance to salt stress in transgenic tobacco plants. Transgenic tobacco plants over-expressing aldose reductase (*ALDRXV4*) cDNA, obtained from *Xerophyta viscose*, showed better salinity stress tolerance than WT plants (Kumar et al., 2013).

Zhou et al. (2011) introduced *AtNHX1* (a vacuolar Na^+/H^+ antiporter gene) from *Arabidopsis thaliana* in tobacco. Salt tolerance was better in the *AtNHX1* over expression plants than WT plants, with higher germination rates and successful seedling establishment in the presence of toxic concentrations of NaCl. The authors also proposed that simultaneous overexpression of V-ATPase and vacuolar Na^+/H^+ antiporter might be appropriate to produce plants with a higher salt tolerance ability.

Furthermore, overexpression of a novel Chrysanthemum (*Dendronthema grandiform*) NAC transcription factor gene in tobacco was studied by Liu et al. (2011). A full-length cDNA designated *DgNAC1* has been isolated from Chrysanthemum and it was overexpressed in tobacco to assess its ability to improve stress tolerance. Indeed, it was found that 35S:DgNAC1 transgenic tobacco plants exhibited a markedly increased tolerance to salt stress.

Drought tolerance

Desiccation tolerance has been defined as the ability of an organism to equilibrate its internal water potential with that of moderately dry air, and then resume normal function after rehydration (Arif et al., 2013). Desiccation tolerant flowering plants require a slow drying time to activate mechanisms that protect membranes and organelles during desiccation. Thick cell walls, few vacuoles and deep and closed stomata were reported in tobacco plants overexpressing *Arabidopsis* vacuolar pyrophosphatase (AVP1), whereas WT tobacco showed larger vacuoles and kept their stomata open (Arif et al., 2013). As a result, the transgenic tobacco tolerated water shortage (dessication). In addition, AVP1 transformed tobacco showed better growth (increase in size and weight of shoots and capsules) and they produced more seeds than WT plants. Overexpression of a levansucrase gene from *Bacillus subtilis* in tobacco led to increased drought tolerance (Pilon-Smits et al., 1995), and similar stress tolerance effects were obtained by using other fructan synthesizing genes in a diverse array of plant species (Keunen et al., 2013).

In another study, Kumar et al. (2013) reported the efficacy of an aldose reductase (ALDRXV4) enzyme from *Xerophyta viscosa* Baker in enhancing the prospects of tobacco's survival under abiotic stress. Transgenic tobacco plants overexpressing *ALDRXV4* cDNA showed an alleviation of NaCl and mannitol induced abiotic stress. The transgenic plants survived longer periods of water deficiency than the WT plants. The increased synthesis of aldose reductase in transgenic plants correlated with reduced methylglyoxal and malondialdehyde accumulation and an elevated level of sorbitol under stress conditions. In addition, the transgenic lines showed better photosynthetic efficiency, less electrolyte damage, greater water retention, higher proline accumulation and a more favorable ionic balance under stress conditions.

Plants growth is the result of cell division and cell enlargement. Plants growth is regulated by cell extensions. Drought stress restrains plant growth by retarding both cell division and extension (Gao et al., 2007). Expansins are proteins that are the key regulators of wall extension during plant growth and they mediate pH dependent wall loosening, probably by disrupting hydrogen bonds between cellulose and matrix glycans (Li et al., 2011). *TaEXPB23* is a wheat expansin gene that was transformed into tobacco. The results indicate that the transgenic tobacco lines lost water more slowly than the wild-type plants under drought stress; their cells could sustain a more integrated structure under water stress than that of WT plants (Li et al., 2011).

In a study of Wang et al. (2011), *PtADC* (an arginine decarboxylase gene), isolated from *Poncirus trifoliata*, was introduced into tobacco to investigate its function in drought tolerance. It was demonstrated that the transgenic plants showed an improvement in dehydration

and drought tolerance. Under dehydration stress conditions, ROS accumulation was much lower in the transgenic tobacco plants as compared with WT plants. Similarly, Yue et al. (2011) transformed *Arabidopsis LOS5/ABA3* into tobacco. They found that transgenic plants showed less wilting, maintained higher water content and showed better cellular membrane integrity as compared with control plants. Moreover, the transgenic plants accumulated higher quantities of ABA and proline, and they exhibited higher activities of antioxidant enzymes.

Water stress tolerances

Water stress affects plant growth and significantly decreases crop productivity. Plants respond to water stress with physiological and developmental changes. Therefore, studying the molecular and physiological mechanisms by which plants adapt to water stress is important for improving agricultural practices.

As stated before, expansins are the key regulators of cell wall extension during plant growth. Li et al. (2011) produced transgenic tobacco plants with increased tolerance to water stress by overexpressing the wheat expansin gene *TaEXPB23* driven by the constitutive 35S cauliflower mosaic virus (CaMV) promoter. However, the growth and development of *35S::TaEXPB23* transgenic tobacco plants were altered under normal growth conditions, with a faster growth rate at the seedling stage, earlier flowering and maturation, and a shorter plant height compared to WT plants. Next, Li et al. (2013) altered cellular characteristics and carbohydrate metabolism in *35S::TaEXPB23* transgenic tobacco plants. During carbohydrate metabolism, lower starch accumulation was obtained due to higher amylase activity in the leaves of *35S::TaEXPB23* compared with WT plants. They also generated transgenic *Arabidopsis* plants that showed the same phenotype as the transgenic tobacco plants, which may have resulted from the altered expression of several flowering-related genes; after which, they produced TaEXPB23 transgenic tobacco plants using the stress-inducible *RD29A* promoter. The use of this promoter reduced the negative effects of *TaEXPB23* on plant growth and development. The *RD29A::TaEXPB23* transgenic tobacco plants had greater tolerance to water stress than WT plants, as determined by examining physiological and biochemical parameters. Therefore, the use of stress-inducible promoters, such as *RD29A*, may minimize the negative effects of constitutive transgene expression and improve the water-stress tolerance of plants.

Overexpression of *BcBCP1*, a phytocyanin-related early nodulin-like gene from *Boea crassifolia*, in tobacco under the control of CaMV 35S promoter enhanced tolerance to osmotic stress, as indicated by the less impaired growth, less damaged membrane integrity and lower lipid peroxidation levels after osmotic stress (Wu et al., 2011). Transgenic tobacco lines overexpressing *BcBCP1* showed higher photosynthetic rates, higher antioxidant enzyme activities and higher cytosyl ascorbic peroxidase transcription levels than non-transgenic tobacco plants, both under normal conditions and under osmotic stress.

Low temperature tolerance

Low temperature is one of the most important abiotic factors limiting growth and productivity of crop plants. Cold regulated (COR) genes are low temperature-responsive genes regulated by the C-repeat binding-factor (CBF) signaling pathway (Hui-Ming et al., 2011; Yang et al., 2011; Wu et al., 2012). When *CbCOR15b* from shepherd's purse (*Capsella bursa-pastoris*), was expressed in transgenic tobacco plants under chilling and freezing temperatures, transformants were found more tolerant under cold (Wu et al., 2012), judged on electrolyte leakage experiments, relative water content, glucose content and phenotypic observations. Similarly, a CBF gene named *GbCBF1* was isolated from cotton and introduced into tobacco (Hui-Ming et al., 2011). They found that under low temperature stress, the electrolytic leakage rate of transgenic tobacco was lower than that of the WT tobacco. However, free proline and soluble sugar contents of transgenic tobacco were higher than those of the WT tobacco. These results indicate that *GbCBF1* enhances cold tolerance in transgenic tobacco.

Various studies confirmed that the CBF family of transcription factors plays key roles in regulating cold stress responses. Yang et al. (2011) isolated CBF3 from sweet pepper (*Capsicum frutescens*) and transformed it into tobacco. Overexpression of *CfCBF3* under the control of the CaMV35S promoter in tobacco induced expression of orthologs of CBF3-targeted genes and increased chilling tolerance without a dwarf phenotype. Multiple biochemical and physiological changes, such as increased levels of proline and soluble sugars and lower contents of ROS, were observed in transgenic plants associated under chilling condition. Sucrose specific signaling pathways may be involved in such stress responses (Van den Ende and El-Esawe, 2014). In addition, overexpression of *CfCBF3* resulted in higher level of total unsaturated fatty acids, especially in phosphatidylglycerol (PG). During exposure to chilling stress, the transgenic lines were less susceptible to chilling-induced photoinhibition than WT plants. These results suggest that overexpression of *CfCBF3* led to modification of the fatty acid unsaturation and alleviated the injuries under chilling stress.

Another universal signaling pathway involved in responses to low temperature is the mitogen-activated protein kinase (MAPK) cascade. *ZmMPK4* is a mitogen-activated protein kinase gene from maize. Transgenic

tobacco overexpressing *ZmMPK4*, accumulated less ROS, more proline and soluble sugars, higher peroxidase and catalase activities and increased expression of stress-responsive genes expression, leading to enhanced low temperature stress tolerance compared to control plants (Zhou et al., 2012). Hence, overexpression of *ZmMPK4* in transgenic tobacco results in increased tolerance to low temperature stress.

High temperature tolerance

Production of plants tolerant to high temperature stress is of immense significance in the light of global warming and climate change. Plant cells respond to high temperature stress by re-programming their genetic machinery for survival and reproduction (Grover et al., 2013). High temperature tolerance in transgenic plants has largely been achieved either by overexpressing heat shock protein genes or by altering levels of heat shock factors that regulate expression of heat shock and non-heat shock genes (Horváth et al., 2012).

When a new gene encoding an aldo-keto reductases (*ARKs*), which catalyses the conversion of glucose to sorbitol, from rice is expressed in tobacco plants, these transgenic plants show a better tolerance to high temperature stress (Grover et al., 2013). Turóczy et al. (2011) investigated two mechanisms how overexpression of ARKs in tobacco enhances high temperature tolerance. First, many AKRs are effective in the synthesis of osmolytes, thus having important role in the osmoregulation, which is an important process in plants for the acquisition of desiccation tolerance due to high temperature. Second, AKRs have also been shown to be effective in the detoxification of lipid peroxidation and/or glycolysis derived reactive carbonyls such as malondialdehyde 4-hydoxy-nonenal, methylglyoxal.

Over-expression of rice Zn-finger protein gene *ZFP177* in transgenic tobacco plants resulted in enhanced tolerance following high temperature stress (Grover et al., 2013). Huang et al. (2008) proposed that a possible role for ZFP177 in temperature stress tolerance is to down-regulate accumulations of some proteins that are not benefit to resistance to temperature stress by ubiquitinylating and targeting them for degradation Likewise, overexpression of pepper *WRKY40* in tobacco enhanced high temperature tolerance (Dang et al., 2013). Dang et al. (2013) investigated the mechanism how the overexpression of *WRKY40* enhanced tolerance to high temperature and they found that the overexpression of *WRKY40* increase transcript levels of hypersensitive response asccociated genes (*NtHSR20*, *NtHSR515*), ethylene biosynthesis related genes (*NtACS6*, *NtEFE26*), ROS detoxification associated genes (*NtSOD1, NtGST1* and *NtAPX*), as well as the heat-shock genes (*NtHSP18*, *NtsmallHSP* and *NtHSF2)*. These genes interact in different ways and improve tobacco tolerance to high temperature.

Heavy metals tolerances

Metal ions (Fe, Mn, Zn and Cu) are essential elements for plant growth and development (Han et al., 2013; Yadav, 2010). Heavy metal pollution is increasing rapidly due to excessive emission of industrial waste, wastewater irrigation, unreasonable pesticides and plastic sheeting (Wu et al., 2013). Though metal concentrations are increasing in soil and water, still there is a shortage of essential metals since they are found in unusable form for plants. Excess heavy metals in plant cells result lipid peroxidation (membrane deterioration), altered ion homeostasis and generation of ROS (Choe et al., 2013; Yadav, 2010) and adversely affected gene expression and slow photosynthesis (Wu et al., 2013). Heavy metal accumulation in plants is especially dangerous besides crop productivity reduction since it allows heavy metal contamination to food chains (Ovecka and Takac, 2014; Wu et al, 2013).

Iron

Iron is one of the essential micronutrients required by all plant and it is chelated by citric acid. However, in calcareous soil solution where the concentration of free Fe is far below normally required (10^{-15} M), the solubility of these ions is very poor (Han et al., 2013). Therefore, Fe-deficiency is a worldwide problem for crop production. Han et al. (2013) introduced *MxCS1* gene (isolated from *Malus xiaojinensis*) into tobacco, promoting the synthesis of citrate synthase and increasing citric acid levels. Overexpression of *MxCS1* improved Fe stress tolerance in transgenic tobacco and flowers presented morphological abnormalities. Higher concentrations of Fe, Mn, Cu and Zn in young leaves and flowers were found in transgenic plants as compared with WT plants. In addition to its role in long-distance metal transport, citric acid may be involved in the regulation of metal transfer within cells as well (Han et al., 2013).

Aluminum

Aluminum (Al) stress represses mitochondrial respiration and leads to increased ROS levels in plants (Panda et al., 2013). Mitochondrial alternative oxidase (AOX) uncouples respiration from mitochondrial ATP production. In order to study the function of tobacco AOX gene under Al stress, Panda et al. (2013) overexpressed *NtAOX1* under the control of the cauliflower mosaic virus (CaMV) 35 S promoter in sensitive *Nicotiana tabacum* L. cell lines. Endogenous *AOX1* gene expression and AOX protein levels were higher in transformed tobacco cell lines than in WT cell lines. They explained that a decreased respiratory inhibition and reduced ROS production with a better growth capability were the significant features that characterized AOX1 transformed

Table 2. Genes used to enhance tolerance to biotic stresses in tobacco.

Gene	Source	Role	Reference
WRKY40	Pepper	Enhanced pathogen tolerance	Dang et al. (2013)
BrERF11	Chinese cabbage	Resistance to disease by *Ralstonia solanacearum*	Lai et al., (2013)
ech42	*Trichoderma virens*	Resistance to fungal pathogens	Shah et al. (2011)
cry1Ac and *cry1Ab*	Artificially constructed	Resistance to *Spodoptera exigua* and *Helicoverpa armigera* larvae	Sohail et al. (2012)
Cadherin gene silencing	*Bacillus thuringiensis*	Alleviated *Manduca sexta* larvae resistance to cry toxin	Porta et al. (2011)

cell lines under Al stress. These results demonstrate that AOX plays a critical role in Al stress tolerance with an enhanced respiratory capacity, reducing mitochondrial oxidative stress burden and improving the growth capability in tobacco cells.

Cadmium

Cadmium (Cd) is considered an extremely significant pollutant due to its high toxicity to many organisms. Cadmium generates oxidative stress by interfering with antioxidant defense responses, which leads to the indirect production of oxygen-free radicals. These highly reactive species lead to multiple effects including membrane peroxidation, loss of ions, protein cleavage, enzyme inactivation and DNA strand damage (Khoudi et al., 2012). Plants have evolved several mechanisms to cope with Cd, the most important of which is vacuolar sequestration. Cadmium can be directly transported into vacuoles by cations/H+ exchangers, such as CAXs (calcium exchangers), which are energized by the pH gradient established by proton pumps. Khoudi et al. (2012) overexpressed a cDNA encoding wheat vacuolar H+-pyrophosphatase (TaVP1, a V-H-PPase) to invest-tigate whether this proton pump would enhance Cd tolerance. *TaVP1*-expressing plants were found to be more tolerant to Cd compared to WT plants when exposed to various concentrations of Cd. Despite the fact that *TaVP1*-transgenic plants had higher Cd content in their shoots and roots, *TaVP1*-transgenic plants performed much better than WT plants. Taken together, Khoudi et al. (2012) suggested that higher expression of a vacuolar proton pump contributed to Cd tolerance.

BIOTIC FACTORS

The biotic factors that greatly affect productivity of tobacco include viruses, fungi and bacteria, weeds and insects. As a result, the potential crop losses are estimated to be 30, 18 and 15% caused by weeds, pests and microorganisms (Sutherst et al., 2011). The classical method to protect the crop losses is extensive usage of chemical treatments but this is costly and bad for the environment. For instance, according to FAO (2005)

report, the world market for fungicides was estimated to be US\$6 billion at the end-user level in 1999. However, overexpressing "resistant" genes has lead to promising results. Examples of such genes engineered into transgenic tobacco to enhance its defense under biotic stresses are shown in Table 2.

Microbial pathogens and viruses

Major losses of yield and deterioration in quality are the results of attack by an array of microbial pathogens especially fungi (FAO, 2005), and plants develop different mechanisms to counteract pathogens. Plants protection against infection by pathogens is achieved through the intervention of viral development or replication, called pathogen-derived resistance (PDR) (Koh et al., 2014). PDR of viruses can be divided into two categories: (1) RNA-mediated resistance that involves the transformation of a partial sequence of the virus genome into the plant, and (2) protein-mediated resistance that involves the transformation of the viral full-length protein-encoding gene into the plant. Besides PDR, Ding et al (2010) showed that RNA silencing serve as antiviral defense mechanisms.

Plant defense reactions are regulated by complicated signaling networks, which generally include ion fluxes across the plasma membrane, oxidative burst, changes in phytohormone and sugar levels, MAP kinase cascades and defense associated genes regulation by transcription factors (Lai et al., 2013; Bolouri and Van den Ende, 2013). For instance, overexpression of pepper *WRKY40* in tobacco strengthened *Ralstonia solanacearum* (a bacterial pathogen) tolerance (Dang et al., 2013). Similarly, overexpression of a Chinese cabbage *BrERF11* transcription factor enhanced disease resistance to *R. solanacearum* in tobacco (Lai et al., 2013). In addition, development of transgenic tobacco expressing an endochitinase (*ech42*) gene from the biocontrol fungus *Trichoderma virens* showed enhanced resistance to fungal pathogens (Shah et al., 2011).

Insects

Another major threat to crop production worldwide that

limit agricultural productivity are pests. The worldwide economic damage caused by insect pests to agricultural and horticultural crops and to orchards stands at a hundred billion dollars annually (FAO, 2005). Chemical insecticides are the most effective and still the predominant type of pest control strategies employed today. However, non-judicious and continuous use of these insecticides results in the development of resistance in insect populations against these insect-cides. Moreover, these have harmful effects on non-target/beneficial insects, potentially damaging natural environments. Insect resistant transgenic plants have emerged as potential alternatives to the synthetic insecticides. Genes encoding the insecticidal crystal protein were isolated from *Bacillus thuringiensis* (*Bt*) and successfully transformed into plants to produce insect-resistant plants (Porta et al., 2011; Sohail et al., 2012). *Bt* toxins are safe to non-target insects due to their specific activity towards target insects. Moreover, these toxins are not persistent in the environment unlike synthetic insecticides and can be used on large scales without any direct/indirect hazardous effects on humans.

However, transgenic plants with a narrow spectrum of insecticidal activity may not be protected against a wider array of insects. This problem was overcome by expressing more than one toxin simultaneously. Sohail et al. (2012) investigated a combination of toxins such as *cry1Ac* and *cry1Ab*, artificially constructed genes, to broaden the action spectrum in tobacco. They found 12% and 62% resistance to *Spodoptera exigua* and *Helicoverpa armigera* larvae. Both toxins showed synergistic effect in tobacco and broadened the spectrum of plant activity against insects.

Resistance to Cry toxins, produced by *B. thuringiensis,* has been linked with mutations in the *cadherin* gene (Porta et al., 2011; Sohail et al., 2012). One strategy effective to overcome larval resistance to Cry1A toxins is the production of Cry1AMod toxins that lack helix α-1since helix α-1is responsible for resistance (Porta et al., 2011). Cry1AMod are able to form oligomeric structures without binding to cadherin receptor and were shown to be toxic to cadherin-silenced *Manduca sexta* larvae and *Pectinophora gossypiella* strain with resistance linked to mutations in a cadherin gene (Porta et al., 2011). They found that Cry1AbMod protein produced in tobacco plants retains its functional toxic activity against susceptible and tolerant *M. sexta* larvae due to the silencing of *cadherin* receptor by RNAi.

CONCLUSION

The use of transgenes to improve the tolerance of tobacco to abiotic and biotic stresses remains an attractive option. Different genes contribute to specific traits for stress tolerance can be successfully engineered into tobacco and they improve tolerance dramatically. However, this review focuses on single gene transfers. In the future, multiple gene transfer might provide better enhancements to both abiotic and biotic stresses.

Conflict of Interests

The author(s) have not declared any conflict of interests

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