Research on Biotic and Abiotic Stress Related Genes Exploration and Prediction in *Brassica rapa* and *B. oleracea*: A Review

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ABSTRACT Global population is increasing day-by-day, simultaneously, crop production need to increase proportionately. Whereas, increase crop production being restricted due to abiotic and biotic stresses. Abiotic stresses are adversely affected crop growth and development, leading to crop loss globally and thereby causing huge amount of economic loss as well. Contrary, pathogens are attacked the plants imposing biotic stress and severely hampers the yield. Therefore, it is prime need to understand the molecular mechanism and genes involved to minimize the biotic and abiotic stresses for mitigating the *Brassica* vegetable crop losses. The stress responsive, pathogens related genes are involved in tolerance or resistance to stress in plants that are cross-talk with different types of stress components in signal transduction pathways. The plants have their own mechanism to overcome biotic and abiotic stresses to follow the abscisic acid (ABA)-dependent and ABA-independent pathways. Several transcription factors such as WRKY, Alfin-like, MYB, NAC, DREB, CBF are integrating to various stress signals and controlling the gene expression through networking with their related cis-elements. To develop stress tolerance and/or resistant crops plants, there is need to realize both of the plant and pathogenic disease development mechanisms. Therefore, this article is focused on (i) major and devastating stresses on vegetable crops, (ii) role of genes to overcome the stresses, and (iii) differential genes expressed under biotic and abiotic stresses in *Brassica oleracea* and *B. rapa* for getting insight of the mechanisms of development of resistance lines.

Keywords Biotic and abiotic stress, Gene expression, Transcription factor, *Brassica*

INTRODUCTION

The concept of stresses on plant is “unfavorable and environmental constraints in plants” or “all agents can act as stressors, producing both stress and specific action,” and “there exist stressor-specific responses and non-specific general responses” (Haag 2013). *Brassica* crops have experience many different types of stresses. Those are biotic stresses like fungal, bacterial, viral diseases and insect pests, while abiotic stresses are salinity, extremes temperature (cold, heat), water shortage (drought or dehydration), water logging, etc. The plant can respond to abiotic stresses not only by fast acclimations but also by particular long term adaptations such as leaf shape, size and thickness, stomata density and distribution, chloroplast structure and function by increasing the levels of photo-

protecting enzymes and stress metabolites (Obidiegwu *et al.* 2015). These types of adaptation may take place within few days or in a week depending on plant nature. Biotic stress is the result of damage of plants occurs by other living organisms, such as bacteria, viruses, fungi, parasites, insects, weeds, and cultivated or native plants. Abiotic stress is the lifeless factors on the living organisms in a specific environment. The lifeless variable must control the environment beyond its normal range of variation to unfavorably affect the population performance or individual physiology of the organism in a significant way. The biotic and abiotic stresses are very importance for crop production worldwide; it's significantly reducing the yield of crops. These stresses are major constraints for crop growth, which consequently hampers the productivity of crop plants. Crops yield are reduced about 25% worldwide

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due to diseases and insects infestation (Savary *et al.* 2012). Globally, abiotic stress is the key sources of crop loss, reducing more than 50% average yields for most major crop plants (Acquaah 2007). Particularly, drought and salinity are becoming devastating in many regions of the world, and may cause serious salinization of more than 50% of all arable lands by the year 2050" (Wang *et al.* 2003).

However, the biotic stress is one of the important features that have significant effect on crop growth and development and ultimately responsible for huge losses of crop yield. Moreover, abiotic stress is another confronts inducing a strong pressure on crop production. Now-a-days, research has generally centralize on understanding plant responses to abiotic or biotic stresses (Qin *et al.* 2011; Todaka *et al.* 2012; Thakur and Sohal 2013; Stotz *et al.* 2014). In the field condition, plants have to face more than one stress simultaneously, and the response cannot be predicted based on the plant's response to the individual single stresses (Atkinson and Urwin 2012). Plants can demonstrate different levels of sensitivity depending on the natural condition and the developmental stage of the plant (Mittler and Blumwald 2010). Different type of interactions can take place between the defenses mechanism after sensitivity of the stresses. It is confusing, whether biotic and abiotic stresses are rather antagonistic, synergistic or additive, inducing more or less susceptibility to a specific kind of stress. In most of the times, exposed on different type of stresses can also lead to antagonistic responses in the plants (Yasuda *et al.* 2008; Ton *et al.* 2009), for examples, under drought stress condition common beans show more symptoms when infected by *Macrophomina phaseolina* (Suleman *et al.* 2001) and applied ABA on tomato leaves increases the susceptibility to *Botrytis cinerea* (Audenaert *et al.* 2002).

Brassica is a very important vegetable group worldwide which comprises some 100 species, including rapeseed (*Brassica napus* L.), mustard (*Brassica juncea* L.), cabbage (*Brassica oleracea* L.), and turnip rape (*Brassica rapa* L.) that are mainly grown for oil, condiments, vegetables or fodder (Ashraf and McNeilly 2004). These crops are sternly challenged by biotic stresses (fungal and bacterial pathogens, viral diseases) and abiotic stresses including drought, heat, cold or salinity are the major factors that

cause enormous losses of yield. *Fusarium oxysporum* is the plant pathogen that infects Brassica crops causing the wilt disease leading to great loss in quantity and quality of oilseed Brassica (Gaetan 2005). The blackleg or stem cankers causing pathogen *Leptosphaeria maculans* is the most devastating fungal diseases for oilseed Brassica, particularly canola (*B. napus* and *B. rapa*) (Howlett *et al.* 2001; Fitt *et al.* 2006). Brassica vegetables are highly susceptible to bacterial soft rot disease which is the most severe and destructive disease that causes serious damage and economic losses across the members of Brassica. Many abiotic stresses directly or indirectly affect the Brassica crops, due to the fact that they are mainly grown in arid and semiarid areas. However, its growth and yield have greatly decreased owing to abiotic stresses.

Most of the Brassica species were grown normally at 15°C-22°C temperature. The above 35°C temperature and below 10°C temperature was injurious to reproductive organs at different developmental stages of Brassica crops (Angadi *et al.* 2000). *B. rapa* was more sensitive to heat stress than *B. napus* and *B. juncea*. However, generally the Brassica spp. of tropical and subtropical origins is sensitive to chilling (0°C-15°C) and freezing (< 0°C) stress and lacks this mechanism of cold acclimation (Sanghera *et al.* 2011).

Stresses could be managed in two ways in vegetable crops I. Crop management II. Biotechnological approaches. Abiotic stress effects can be managed by agronomic practices such as irrigation by quality water, soil amendment, altering sowing time, etc. This strategy is not sustainable and economic for vegetable production (Farooq *et al.* 2009). Therefore, we need the permanent solution for vegetable production against stresses. Plants have their own defense mechanism that regulate the expression of stress related genes will be necessary for the stress tolerance crop variety development (Dita *et al.* 2006). Exposure of plants to biotic and abiotic stress leads to the adjustment of stresses using their genetical mechanism and produced high yield during stress condition. On the other hand, when plants face up to adverse conditions, few genes are active against such stresses, researcher are used this active genes to developed stress tolerance crop varieties. Therefore, this review has focused to explore the probable genes for specific stresses.

MAJOR AND DEVASTATING STRESSES ON VEGETABLE CROPS

The global warming trend is expected to continue and changes in temperature, precipitation and carbon dioxide that will affect plant growth and development, spread of pests and diseases and influence the environmental factors on vegetable crops production. Globally, vegetables crops yield are reduced more than 50% due to abiotic stresses (Bray *et al.* 2000). Climatic changes will influence the severity of abiotic stress imposed on vegetables crops. The genera *Brassica* are an important and diverse vegetable crops grown worldwide, include a number of economically important crops such as *B. rapa*, *B. oleracea*, *B. napus*, etc. (Cardoza and Stewart 2004) and these crop plants are affected by biotic and abiotic stresses. The *Brassica* vegetables are soft and succulent and generally consist of more than 85% water. Therefore, water scarcity as well as excessive water significantly influences the yield and quality of these crops; abiotic stress particularly cold, salinity and drought stresses dramatically reduce productivity of these crops. Different types of stresses are faced by *Brassica* vegetables during their growth cycle and

try to overcome those stresses by adapting or producing different mechanisms (Table 1).

Cold is one of the devastating stresses for *Brassica* vegetable crops. Under cold stress condition, water in plant body become ice and rupture the plant cell, ultimately significant yield losses with great risks for future global food security. Cold stress is even more destructive stress when it imposed in seedling and reproductive stages. Drought stress causes an increase of solute concentration in the soil, leading to an osmotic flow of water out of plant cells. Extreme drought stress conditions will negatively impact on crop yield. Due to soft and succulent in nature of the cabbage family, majority numbers of the vegetable crops are sensitive to drought stress, mainly during flowering and seed development stages.

Salinity is also the similar trends of problem like drought for vegetables crops. It is projected that about 17% and 30% of cultivated lands and irrigated agricultural land of the worldwide are affected by high salinity, respectively (Vijayvargiya and Kumar 2011). In tropical areas, high evapo-transpiration results in substantial water loss due to hot and dry environments, thus leaving salt around the plant roots which interferes with the plants ability to uptake

Table 1. Enlist different types of stresses normally induced threat to vegetable cultivation and their possible causes and responses for combat against such stresses.

Types of stress	Agent	Probable response
Biotic	Bacteria	Plant produce callogen and tyloses which is responsible against Bacterial pathogens.
	Fungi	Formation of ROS which activated against fungi.
Abiotic	Low temperature (chilling)	Activated DREB1/CBF genes and produce detoxification of ROS and altering glyoxalate pathway that developed low temperature tolerance plants.
	High salinity	WRKY, NAC, MYB TFs activated salt resistant genes that altering glyoxalate pathway and waxy layer formation; finally produce salinity resistant plants.
	Drought/water shortage	Stomatal closure reduces transpiration that manages the drought stress.
	Heat	Efficient protein repair systems and general protein stability support survival, temperature can lead to acclimation.
	Natural mineral deficiency (e.g., nitrogen shortage)	
	Water logging	Development of cavities mostly in the roots that facilitate the exchange of oxygen and ethylene between shoot and root (aerenchyma).
Hormonal factors	SA	SA regulated the stomatal closure that directly involved in drought stress.
	JA and ABA	JA uses as stress signaling

ROS: reactive oxygen species, SA: salicylic acid, JA: Jasmonic acid, ABA: abscisic acid.

water. Under salt stress, plant is reflected in loss of turgor, growth reduction, wilting, leaf curling, leaf abscission, decreased photosynthesis, respiratory changes, loss of cellular integrity, tissue necrosis and potentially death of the plant (Cheeseman 1988). Most of the vegetable crops especially *Brassica* crops are sensitive to salinity stress during seedling and early growing stages. Therefore, it is well characterized that the response of plants to abiotic stresses depends on the plant growing stage and the duration and severity of the stress (Bray 2002).

Like abiotic stresses, *Brassica* are also highly susceptible to several biotic stresses. *Fusarium* wilt, Club root, soft rot, Black rot are the importance diseases (Ahmed *et al.* 2012a; Kayum *et al.* 2015a). Two pathogens *Pectobacterium carotovorum* subsp. *carotovorum* and *F. oxysporum* f.sp. *conglutinans* are specifically attacked *Brassica* crops and cause soft rot and *Fusarium* wilt diseases. Bacterial soft rot and *Fusarium* wilt are the most severe and destructive disease, which causes huge damage and enormous losses

across the members of *Brassica*. Control of this pathogen is difficult due to its wide range of host's selectivity and to survive on plant debris. These two diseases break out devastatingly when attack at seedling stage (Ahmed *et al.* 2012a). Many plant defense or defense-related genes contain W-box sequences in their promoters region, including pathogenesis-related (PR) genes and the regulatory *NPR1* gene (Yu *et al.* 2001). The W-box sequences are necessary for the inducible expression of these defense genes.

ROLE OF GENES TO OVERCOME THE ABIOTIC STRESSES

Plants are developed different defense mechanism through activation of complex signaling cascade in varying stress conditions (Abou Qamar *et al.* 2009). Plant exposed to abiotic and biotic stresses, promote to activate protein kinase cascades and specific ion channels keep

Fig. 1. Simple model of different signaling pathway involved in plants to overcome the abiotic stresses.

ABA: abscisic acid, ROS: reactive oxygen species.

turn on, or producing reactive oxygen species (ROS), phytohormones such as salicylic acid, jasmonic acid, abscisic acid (ABA), etc. (Fujita *et al.* 2006). A simple model has been proposed in Fig. 1, where different mechanisms of responses to abiotic stresses by plants along with their corresponding are presented for better understanding.

According to our present knowledge on stress signaling pathways, the transcriptional regulatory networks of abiotic stress signals and gene expression have been studied (Chaves *et al.* 2003; Yamaguchi-Shinozaki and Shinozaki 2006; Pérez-Clemente *et al.* 2013). The major findings of those reports can be divided into the following major steps: signal perception, signal transduction, transcription factors activation, activation of cis-acting elements, stress responsive gene expression. Plant cell sensors or receptors located in the cell wall or membrane which perceive stress stimuli. Abiotic stress signal transduction is taken place through (i) ABA-dependent and (ii) ABA-independent pathways. In the ABA-dependent pathway, ABRE is the main ABA responsive elements that activate the stress responsive genes. On the other hand, in the ABA-independent pathway dehydration responsive elements (DRE) is involved in drought, cold and salt stress responsive genes regulation (Fig. 1). These signals are received by cell membrane sensors that pass from plants when they faced the abiotic stresses, accordingly cells try to response the signals through producing ROS, calcium ions, inositol phosphate etc. inside the cells. However, high level of ROS in intracellular space may cause damage the lipid, protein and finally DNA. Moreover, phosphorylation mediated by protein kinase help to activated or suppressed different numbers of transcription factors (TFs) and bind specifically to cis-acting elements in the stress-responsive genes promoters and control their transcriptional level for combat against abiotic stresses (Danquah *et al.* 2014).

In the meantime, other upstream components are control TFs at the transcription level (Hirayama and Shinozaki 2010) and subjected to different levels modifications at the post-transcription stage, such as ubiquitination and sumoylation, thus forming a complex regulatory network that control the stress responsive genes expression level. These genes are involved in detoxification of ROS, altering

glyoxalate pathway, stomatal closure, waxy layer formation (Dong *et al.* 2006; Miura *et al.* 2007; Mizoi *et al.* 2013). Therefore, breeder should keep in consideration of the above mentioned components in the Fig. 1, from signal receptors to the downstream stress responsive genes that constitute the generic pathway for plant abiotic stress signal transduction (Fig. 1) for developing abiotic stress tolerance cultivars.

DIFFERENTIAL GENES EXPRESSED UNDER ABIOTIC STRESSES IN *Brassica*

The abiotic stresses are becoming alarming situation for *Brassica* crop production worldwide due to quickly changes of global environment. Previous studies have showed that different genes are differentially expressed under such type of stresses in *B. rapa* and *B. oleracea*. WRKY and Alfin-like transcription factors have been well documented in response to many abiotic stresses in *B. rapa* (Kayum *et al.* 2015a; 2015b). Among the WRKY genes, *BrWRKY22*, *BrWRKY44*, *BrWRKY70*, and *BrWRKY72* in Chiifu showed about 175-, 32-, 54-, and 42-fold higher expressions, respectively during cold stress. Therefore, *BrWRKY22*, *BrWRKY44* genes could be introgressed or over-expressed in *B. rapa* vegetables for getting cold tolerant lines. Additionally, under salt stress condition, *BrWRKY17*, 57, and 58 showing about 5-, 2-, and 3-fold higher expression, respectively and *BrWRKY51*, 65, 98, and 104 showed their highest expression after drought stress treatment and were about 85-, 15-, 18-, and 38-fold higher than the control, respectively (Kayum *et al.* 2015a). On the other hand, *BrAL2*, 3, 7, 9, 12, 13, 14, and 15 showed higher expression in response to all abiotic stresses (cold, salt, and drought) (Kayum *et al.* 2015b). Lee *et al.* (2012) found that all eight CBF/DREB1 genes responses against cold stress and some of them also responsive to salt, drought and ABA treatment. Several numbers of NAC TFs are involved in various abiotic stress of Chinese cabbage (Liu *et al.* 2014). Several numbers of BZR transcription factor and longevity assurance gene of *B. rapa* showed responsiveness against cold, drought and ABA stresses (Ahmed *et al.* 2012c; Saha *et al.* 2015). Alfin-like transcription factors in *B. oleracea*

(BoAL1, 4, 5, 6, 7, 8, 9, 10, and 12) have been reported in response to abiotic stresses like cold, salinity, drought and ABA (Kayum *et al.* 2016) and Wang *et al.* (2015) showed that *BrMYB210*, *BrMYB137*, *BrMYB88*, *BrMYB154*, and *BrMYB222* significantly responses against cold and osmotic stress and *BrMYB261* (an ortholog of *AtMYB28*) had no response to cold treatment, but was expressed dramatically by osmotic stress. Among the MYB genes, six genes (*BrMYB140*, *BrMYB172*, *BrMYB229*, *BrMYB208*, *BrMYB137*, and *BrMYB210*) were significantly up-regulated by ABA treatment but down-regulated by auxin treatment. However, *BrMYB210* (an ortholog of *AtMYB96*) showed down-regulation against auxin treatment in leaves (Wang *et al.* 2015). Ahmed *et al.* (2015) showed that 12 BoCRGs were expressed differentially after cold stress treatment in two contrasting cabbage lines, and *BoCRG54*, 56, 59, 62, 70, 72, and 99 were predicted to be involved in cold regulatory pathways.

ROLE OF GENES TO OVERCOME THE BIOTIC STRESSES

Plants have two levels of immune system that protects them against different types of pathogens. At the first, a pattern recognition receptor (PRR) recognizes pathogens attacks on the surface of the plant cell. The PRR produces signaling and pass to nucleus that activate defense related genes. Thereby, the cells are tried to stop further invasions of the pathogens through producing huge amount of callose, tyloses and ROS. Plant cells are deposited callose and tyloses just inside cell wall, resulting in cell walls thicker which protect the microorganisms (Fig. 2). Secondly, the plant cells are started secretion of high amount of ROS and activation of pathogenesis related (PRs) damaging threatening pathogens (Kumar 2013). At the same time, pathogens also are injecting sets of affecter molecule inside the cell that try to damage or defeat the plant immunity system. The affecter molecules damaging transduction signaling and response that disrupt plant defense system and ultimately plants are infected by

Fig. 2. Schematic model of plant-pathogen interaction and molecular mechanisms involved in resistance and susceptible reactions to pathogens attacks.

PRR: pattern recognition receptor, PRs: pathogenesis related, ROS: reactive oxygen species.

pathogens in susceptible plants (Fig. 2; Oppel *et al.* 2009).

DIFFERENTIAL GENES EXPRESSED UNDER BIOTIC STRESSES IN *Brassica*

Biotic stress is very devastating stress for plants as well as vegetables crops. Several numbers of genes have well documented that they are differentially expressed against different biotic stresses. However, when infection with *F. oxysporum* f.sp. *conglutinans*, six *BrWRKY* genes (*BrWRKY4*, 65, 72, 97, 133, and 141) showed significantly higher expression and were about 8-, 6-, 6-, 3-, and 5-fold higher, respectively (Kayum *et al.* 2015a). *BrWRKY141* showed about 180-fold higher expression after *P. carotovorum* subsp. *carotovorum* infection (Kayum *et al.* 2015a). After infection with *F. oxysporum* f. sp. *conglutinans*, *BrAL2*, 3, 4, 7, 9, 10, 13, 14, and 15 showed several fold higher expression, and *BrAL2*, 3, 7, 9, 13, 14, and 15 were expressed in response to both biotic and abiotic stresses (Kayum *et al.* 2015b). Ahmed *et al.* (2012a) reported that three chitinase genes (*BrCLP1*, *BrCLP2*, and *BrCLP3*) responses against *P. carotovorum* subsp. *carotovorum* infection. The defensin-like family protein (*BrDLFP*) expression significantly increased after infection with *P. carotovorum* subsp. *carotovorum* in Chinese cabbage (Ahmed *et al.* 2012b). On the other hand, Alfin-like two genes (*BoAL8* and *BoAL12*) were induced after infection with *P. carotovorum* subsp. *carotovorum* in *B. oleracea* (Kayum *et al.* 2016). Among the 12 thaumatin-like proteins, three of which expressed differentially after *P. carotovorum* subsp. *carotovorum* infection in cabbage plants (Ahmed *et al.* 2013).

CONCLUSIONS

Crop growth, development and yields are severely affected by environmental cues and different types of pathogens. Most of the vegetables crops are vulnerable to those stresses. The cellular metabolic components calcium, ROS, protein kinase, protein phosphatase are activated and promotes signal transduction pathways

inside cells during stress and convey the abiotic stress signal that are necessary to regulate TFs. The TFs are controlled the downstream genes that regulate the abiotic stress through activate their corresponding cis-acting elements. On the other hand, biotic stress signals are responded by the plants through initiation of callogen, ROS, tyloses, etc. in the signal transduction pathways. Plant cells are received the external signal of each stress and consequently turn on the responsive to produce particular molecules to over-come such stress. Some molecules are involved in the defense response to the specific stress that contributes to protect the plant and express its resistance/tolerance to that stress. The molecular engineering is a new approach which is used to modify a gene coding and introduction specific genes in crop plants leading to increase tolerances against stresses. We need to understand the functions or mechanisms of the stress responsive candidate genes. Thereafter, introgression of those candidate genes through conventional breeding or marker assisted back crossing or even in urgent situation genetic transformation will lead to develop resistance or tolerance cultivar of *B. oleracea* and *B. rapa*.

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