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Transgenic Approaches Towards Controlling Fusarium Head Blight in Wheat

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Abstract
Fungal diseases cause huge losses in terms of yield and quality of wheat grains which ultimately affect food security of a country. Previously, a number of techniques including agrochemical, breeding and chromosome hybridization adopted were time consuming as well as failed in host resistance ability. In wheat, a recalcitrant crop, genetic improvement remained behind than other cereals. Molecular mapping, marker assisted selection (MAS), functional genomics and cloning have enabled the breeders to develop reliable resistance against fungal diseases. First transformation in wheat was done in 90s and afterwards several antifungal genes have been transformed as an alternative for fungicides. Among the fungal diseases, Fusarium head blight (FHB), producing mycotoxins, not only affects plant but human as well as mongastric animals. FHB antifungal genes such as Fhb1, NPR1 (Arabidopsis thaliana nonexpressor of pathogenesis-related gene), FsTRI101 (Trichothecene 3-O-acetyltransferase from Fusarium sporotrichioides), transcription factors TaWRKY45 (WRKY from Triticum aetivum) and HvUGT1324 (UDP-glucosyltransferase from Hordeum vulgare) have been introduced either to reduce toxin severity or confer fungal resistance. HIGS (host induced gene silencing) has also recently contributed in imparting disease resistance against FHB. There is still much more to be discovered that will further confer more reliable resistance against FHB in future.

Key words: Genetic engineering, wheat, HIGS, FHB, disease resistance

Introduction

Among the cereal, wheat is a unique gift of nature (Goutam et al. 2013) fulfilling the feeding requirement of about one third of world population (Inamullah et al. 2006), covering 17% of the global cropping area and contributing a total of 18% of the global human calories intake (Peng et al. 2011; FAOSTAT 2018). In the developing world increasing potential of wheat yield is one of the important strategies for solving food security issues (Duveiller et al. 2007). As several biotic and abiotic constraints pose serious threats towards this major cereal crop, causing a significant decrease in its grain quality and quantity (Limbalkar et al. 2018; Ghimire et al. 2020). Increasing grain yield along with grain quality of crops is the current challenge for

the breeders (Goutam et al. 2013). For developing resistance against different stresses (Todorovska et al. 2009; Kamal et al. 2010), the approaches need to be employed along with the increased capability to adapt to the different climatic changes (Olmstead and Rhode 2011). Plant pathogens signify real threat to world agriculture. More than 200 different diseases have been characterized in wheat, of which about 50 cause serious economic losses (Wiese et al. 2000). Savary et al. (2019) reported 31 pests of wheat causing approximately 21% yield losses. Among all pathogens, fungi are the leading agents responsible for major diseases (Zhou 2011). Fusarium head blight (FHB), caused by Fusarium graminearum, F. avenaceum and F. culmorum, is a destructive disease of barley and wheat in warm and tropical regions globally (Bai and Shaner 2004; Buerstmayr et al. 2009). Since the early 1990s, FHB has risen back in wheat and barley crops globally, probably because of conservation tillage practices, increased maize production, and climate change. Generally, wheat has been found vulnerable to Fusarium infection in different stages like from anthesis to the soft dough stage of grain development. During wet seasons, the disease results in huge economic losses leading to epidemics and mycotoxin accumulation at higher and unacceptable level in grains (McMullen et al. 2012). The fungal pathogen interferes with seed development by infecting wheat heads in flowering stage, resulting in shriveled grains which are of light weight that can easily flown away with chaff during harvesting. The pathogen damages cell walls, starch granules and storage proteins during infection of grains. The affected crop results in lower grain quality and quantity (Xu et al. 2005; Schmidt et al. 2016). The FHB may also lead to production of secondary metabolites {such as deoxynivalenol (DON), moniliformin (MON), zearalenone (ZEN) and nivalenol (NIV)}, of the fungal pathogens and their derivatives and contaminate the grains (Tralamazza et al. 2016; Chilaka et al. 2017) all of which can have toxic effects on human health and animals (Häggblom and Nordkvist 2015) and could be a significant danger in the food chain as reported (Magan and Aldred 2007). Wheat cultivars vary in their response to FHB; ranging from high resistance to high susceptibility but no genotype has been found immune (Bai and Shaner 2004). Husbandry techniques and agrochemicals have been used for reducing fungal infections and spread but failed to improve host resistance ability. Along with these, fungicides are also not promising applications to get rid of these pathogens which sometimes in turn can increase the concentration of mycotoxins (Magan et al. 2002).

Genetically disease resistance as well as plant breeding techniques are appropriate only within closely related species and taking long time (15 to 20 years) as reported (Rommens and Kishore 2000). In addition, there is also lack of wild plants with resistances against all conceivable pathogens (Bergelson and Purrington 1996). The cytogenetics tools significantly eased chromosome mediated gene transfer as well as hybridization into crops from wild crops (Fedak 1999; Jauhar 2003). Much attraction has been made towards molecular breeding, plant genetic engineering and integrating disease resistant genes (Xing et al. 2008). Introduction of the target genes using genetic engineering is offering a suitable and swift approach for the enhancement of tolerance against the pathogens (Miroshnichenko et al. 2014). The target genes include the ones encoding the enzymes which can detoxify DON, and the genes involved in biosynthesis of the antifungal proteins having fungicidal, fungistatic, or inhibitory activity to the FHB pathogens (Ferrari et al. 2012; Hou et al. 2015).

Transgenic wheat overexpressing genes against FHB

The wheat breeding programs on large scale have not developed varieties with higher resistance to FHB, apparently, because of the partial and quantitative resistance in wheat. Despite the extensive search of wheat germplasm resources single genes for higher resistance to FHB have not been found (Leonard and Bushnell 2003). In s response to Fusarium infection in wheat, several pathogenesis-related (PR) genes like β-1,3-glucanase, thionin and thaumatin like proteins (tlp-1) are activated (Li et al. 2001; Kang and Buchenauer 2002; Han et al. 2005; Zhou et al. 2005; Bernardo et al. 2006). These PR genes collectively confer basal resistance to pathogens during infection by encoding proteins with different mechanism of action against

the fungal pathogens. During FHB infection in wheat, particularly, expression of PR1, PR-2 (β-1,3-glucanase), PR-3 (chitinase), PR-4 (antifungal), and PR-5 (tlp-1) proteins was found increased in spikes (Pritsch et al. 2000, 2001). Although plant breeders have achieved improved resistance to Fusarium spp. in wheat, the lack of durable resistance to FHB could be a problem for the growers. These reports show that defense response to FHB infection is induced involving several defense response genes. Due to somewhat recalcitrant to transformation and in vitro regeneration, the improvement in wheat transformation remained behind the other cereals (Gao et al. 2011). First transformation of wheat was carried out by gene gun or particle bombardment and the first transgenic wheat conferring resistance to the herbicide Basta® (Bayer Crop Science, Leverkusen, Germany) was achieved by targeting embryogenic immature callus culture. The extended culture time having 0.2% transformation frequency produced sterile transgenic line and resulted in low callus response rate with somatic variation in regenerated plants (Vasil et al. 1992). Later the transformation frequency obtained was upto 2% and also shortened the time to 8-9 weeks for transgenic plants by bombarding osmotically treated immature embryos that have been cultured for 5-7 days (Altpeter et al. 1996). Additional research gave rise to the first successful Agrobacterium-mediated transformation in wheat cv. Bobwhite (Cheng et al. 1997) with stable transgenic plants with transformation frequency of 1.1-1.6% obtained in 3 months. Similarly, the gene, FsTRI101, from F. sporotrichioides (encoding acetyltransferase), previously known as TriR, was introduced into Bobwhite cultivars which resulted in the fractional protection against the spread of FHB in inoculated wheat spikes (Okubara et al. 2002). The transgenic wheat transformed with a rice tlp, and the wheat line co-transformed with wheat chitinase and β-1,3-glucanase genes, developed symptoms of FHB slower than the non-transformed control under greenhouse condition (Anand et al. 2003). Several types of FHB resistance have been reported, but the type I and type II are widely accepted. The type I is referred to the resistance to initial infection and the type II is the resistance to spread of the pathogen in the spike (Mesterhazy 1995). Other types of the resistance to FHB, as reported, are the resistance to grain infection (type III), to FHB and DON (type IV) and to DON accumulation (type V) (Boutigny et al. 2012). Previous studies of Lunn et al. (2001), Mares & Mrva (2008) and Perlikowski et al. (2014) revealed that alpha-amylase activity can enable the kernels more vulnerable to FHB after infection. Similarly, a gene NPR1 from Arabidopsis thaliana (AtNPR1) was engineered to develop FHB resistant wheat lines (Makandar et al. 2006). Mackintosh et al. (2007) generated transgenic wheat lines, by introducing the α-1-purothionin, tlp-1, and β-1,3-glucanase genes, that exhibited enhanced resistance to FHB infection under greenhouse condition. The transgenic lines were also evaluated under field condition for FHB severity (%), accumulation of DON, and scabby kernels (%) and concluded that overexpression of the defense-responsive genes in the transgenic wheat enhanced resistance to FHB under greenhouse and field condition. Expression of fusion proteins, Fusarium spp. specific antibody fused to an antifungal peptide exhibited a significantly improved resistance FHB pathogen and also reduced the pathogen in the field, representing the first antibody-based crop protection (Hu et al. 2008). A number of expressed sequence tags (ESTs) corresponding to WRKY genes were found up-regulated in response on exposure to the abiotic stress conditions (Houde et al. 2006, Gregersen and Holm 2007) as well as to F. graminearum infection (Golkari et al. 2007). The transcription factors, TaWRKY45 involved in the defensive response to Fusarium attack also appears to show a positive part in resistance to FHB in wheat (Bahrini et al. 2011). Other reports also have described transgenic-induced resistance conferred by different genes against several fungal phytopathogens which emphasize the need of evaluating the resistance of the transgenic wheat against the FHB (Mackintosh et al. 2007; Shin et al. 2008). The transgenic wheat expressing yeast ribosomal protein L3 exhibited enhanced resistance to FHB (Di et al. 2010). Overexpression of a defensin, isolated from radish, in transgenic wheat conferred enhanced resistance to F. graminearum and Rhizoctonia cerealis (Li et al. 2011). Ferrari et al. (2012)

reported that transgenic Arabidopsis and wheat overexpressing polygalacturonase-inhibiting proteins increased resistance to the F. graminearum (Table 1).

Table. List of genes and transcription factors isolated from different sources and used against Fusarium head blight (FHB).

Gene	Description of gene/transcription factor	Pathogen/Source
	Reference	
Tlp	thaumatin-like protein	Oryza sativa
	Chen et al. (1999)	
FsTRI101	Trichothecene 3-O-acetyltransferase	Fusarium
sporotrichioides	Okubara et al. (2002)	
Tlp, β-1,3-glucanase, cht	thaumatin-like protein, chitinase	O. sativa,
Triticum aestivum	Anand et al. (2003)	
AtNPR1	nonexpressor of pathogenesis-related	Arabidopsis
thaliana	Makandar et al. (2006)	
α-1-purothionin, tlp-1, β-1,3-glucanase	thaumatin-like protein	Hordeum
vulgare, T. aestivum	Mackintosh et al. (2007)	
b-32	ribosome-inactivating protein	Zea mays
	Balconi et al. (2007)	
Tri101	trichothecene 3-O-acetyltransferase	F.
sporotrichioides	Alexander, (2008)	
Cht II	barley class II chitinase gene	Hordeum
vulgare	Shin et al. (2008)	
TaWRKY45	Triticum aestivum transcription factor	Triticum
aestivum	Bahrini et al. (2011)	
RsAFP2	Raphanus sativus antifungal protein	Raphanus
sativus	Li et al. (2011)	
PvPGIP2	Polygalacturonase-inhibiting proteins	Phaseolus
vulgaris	Ferrari et al (2012)	
Rs-CWP2, (Chi) gene,	anti-fungal peptides (AFPs)	Hordeum
vulgare	Liu et al. (2012)	
MsrA1 & Pep3 (synthetic genes)		
HvUGT13248	barley UDP-glucosyltransferase	H. vulgare T.
aestivum	Li et al. (2015; 2017)	
TaFROG	T. aestivum Fusarium Resistance Orphan Gene	Triticum
aestivum	Perochon et al. (2015)	
ScNPR1	Secale cereal NPR1	Secale cereale
	Yu et al. (2017)	
TaWRKY70	Pathogen-inducible transcription factor	Triticum
aestivum	Kage et al. (2017)	
Bradi5g03300 UGT gene	UDP-glycosyltransferases	Brachypodium
distachyon	Gatti et al. (2018)	
Ta-UGT3	UDP-glucuronosyltransferases	Triticum
aestivum	Xing et al. (2018)	
HvUGT13248	H. vulgare UDP-glycosyltransferases	H. vulgare
Mandalà et al. (2019)		
AtLTP4.4	A. thaliana lipid transfer protein	Pichia pastoris
McLaughlin et al. (2020)		
wfhb1-1	Triticum aestivum Fusarium head blight gene	Triticum
aestivum	Paudel et al. (2020)	

TaUGT6	UDP-glucosyltransferase gene	Triticum aestivum
	He at al. (2020)	

In addition, over-expression of the transcription factors and the signaling molecules with FHB attack have been reported to accelerate FHB resistance in wheat (Bahrini et al. 2011). Over-expression of the resistant genes in response to the pathogen invasion and also the genes encoding stress hormones like ethylene, methyl jasmonate and salicylic acid has also improved resistance to FHB (Makandar et al. 2012). Co transformation of alien genes could be an appealing strategy for improving resistance to FHB. To check this, Liu et al. (2012) carried out co-transformation of various anti-fungal peptides (AFPs) in elite wheat (cv. Yangmai11). Among different lines, identified through qRT-PCR and Southern blot, the two lines 451 and 513 expressing two AFPs showed a stable and considerably enhanced resistance to FSB (Fusarium seedling blight) and FHB, whereas all the other lines were found resistant to only FHB. Therefore, it is necessary to properly link and express AFPs in transgenic wheat to accomplish an improved overall resistance to the Fusarium pathogens (Liu et al. 2012).

Mediating the metabolic pathways of disease resistance by inserting the regulatory genes, transgenic plants can exhibit higher resistance against FHB. Such as Yu et al. (2017) successfully expressed ScNPR1 (Secale cereale-NPR1), a regulatory gene responsible for systemic acquired resistance (SAR), in transgenic wheat plants. The expression was found higher after Fusarium graminearum inoculation, thus indicating the potential of transgenic wheat lines in imparting improved resistance against FHB. A wheat gene WFhb1-1, consisting of an exon and encoding 127 amino acids inhibited growth of both the F. graminearum and P. pastoris in the culture. Overexpression of the WFhb1-1 in non-Qfhb1-carrier wheat caused a significant decrease ($p < 0.01$) in Fusarium-damaged rachis and kernel and the DON contents in harvested kernels.

Detoxification of DON and resistance to FHB

One of the several mycotoxins that Fusarium spp. produce and mainly associated with epidemics of FHB is deoxynivalenol (DON). DON, a member of mycotoxigenic sesquiterpene ep-oxides, and called the trichothecenes, is commonly found in FHB-affected grains. It enhances disease severity and sets a health hazard to humans and monogastric animals (Okubara et al. 2002). Like FHB, DON can also lead to premature chlorosis or bleaching of plant tissue, as was demonstrated in both wheat heads and barley leaves (Lemmens et al. 2005; Bushnell et al. 2003, 2010; Ansari et al. 2007; Diamond et al. 2013). Therefore, resistance to the DON has been innate component of FHB resistance (Gunupuru et al. 2017).

DON, as reported, is produced 24 h post-inoculation (Chen et al. 1995), and a significant rise in level has been observed by 96 h post-inoculation as reported by Savard et al. (2000). Vascular transport of DON, upwards and downwards, and to the neighboring healthy heads has been reported (Kang and Buchenauer 1999), and so as a virulence factor of the pathogen (Bai et al. 2001) which facilitates colonization of some of the plant hosts by certain trichothecene-producing Fusarium spp. Genetic modification of crops for DON detoxification may result in reduction of the mycotoxin concentration in grains and increased plant resistance against infection as well. The role of DON in infection was demonstrated when host plants were challenged with the fungal strains with no trichothecene synthesis ability and could not cause infection (Proctor et al. 1995; Maier et al. 2006). A number of efforts have been made to detoxify or biotransform DON to non-toxic form. Proctor et al. (1995) made a fungal mutant (trichothecene-deficient mutant) that was unable to produce trichodiene synthase, a trichothecene biosynthesis catalyzing enzyme. When wheat was inoculated with the TRI5- strain, head blight developed more slowly than the wheat lines inoculated with wild-type strains, and was concluded that the trichothecene may lead to virulence. This was further confirmed by evaluating the role of toxin in FHB. F. graminearum wild strains developed

significantly more disease symptoms and reduced the yield compared to the TRI5¯ mutant (Desjardins et al. 1996; Bai et al. 2001). Lemmens et al. (2005) found that the resistant Fhb1 allele was able to conjugate DON to glucose to produce DON-3-O-glucoside (D3G), the less-toxic form. Similarly, transgenic wheat expressing a toxin-modification gene, Tri101, exhibited reduction in DON accumulation and subsequent reduction in FHB severity (Alexander 2007). A modest reduction in disease severity of the transgenic wheat overexpressing defense genes and DON-resistant genes has been reported (Mackintosh et al. 2007; Shin et al. 2008; Di et al. 2010). Karlovsky, (2011) suggested biological detoxification of the DON and its use in genetically modified crops and feed additives. Fungal acetyltransferases and plant glucosyltransferases targeting carbon 3 of trichothecenes, glycosylation of trichothecenes, were suggested to be promising candidates for engineering resistance to FHB. The transgenic wheat overexpressing UDP-glucosyltransferase (HvUGT13248) from barley restricted type II resistance (disease spread in the spike) compared to the non-transformed control, and also efficient conjugation of the DON with D3G, a less toxic form of the mycotoxin was noted (Li et al. 2015). In their later experiments, Li et al. (2017) demonstrated that the HvUGT13248 gene was also capable of converting nivalenol (NIV), a trichothecene mycotoxin, into nivalenol-3-O-β-D-glucoside (a non-toxic). Genetically engineered E. coli expressing the HvUGT13248 glycosylated NIV more efficiently than the DON. In their subsequent research, overexpression of the HvUGT13248 in Arabidopsis, wheat and yeast resulted in increased resistance to nivalenol, and it was demonstrated that the HvUGT13248 has the potential to detoxify DON and NIV and confer resistance to DON- and NIV-generating Fusarium spp.

Perochon et al. (2015) characterized a wheat orphan gene that increased resistance to FHB, and was named as TaFROG (T.aestivum Fusarium Resistance Orphan Gene).. The temporal induction of the TaFROG by F. graminearum in wheat was found linked to activation of the TaPR1 (T. aestivum PR-1 gene. Unlike the TaPR1, induction of the TaFROG by F. graminearum was found toxin dependent. Overexpression in transgenic wheat lines indicated that TaFROG has contribution in host resistance to both the DON and F. graminearum (Perochon et al. 2015). In their next set of experiments, Perochon et al. (2019) characterized TaNACL-D1 (a T. aestivum NAC–like transcription factor) that interacts with the TaFROG and evaluated its role in the FHB. The wheat lines overexpressing TaNACL-D1 were found more tolerant to FHB than the control plants. Thus, it was concluded that the protein TaFROG interacts with the TaNACL-D1, and develops part of the disease response in wheat. Gatti et al. (2018) functionally characterized transgenic wheat over-expressing the Bradi5g03300 UGT gene. The transgenic lines showed a high level of type II resistance (spike colonization) and a significant reduction in the mycotoxin contents. The transgenic lines over-expressing Ta-UGT3 encoding UDP-glucuronosyltransferases in wheat displayed significantly increased resistance (type II) to FHB and reduced DON contents in the grains compared to the wild type control. Over-expression of the Ta-UGT3 also resulted in alteration of the endogenous hormones like SA and JA in the spikes. These results suggested that the Ta-UGT3 can positively regulate the defence responses to F.g, probably by regulating the defence and DON-induced genes (Xing et al. (2018). Mandalà et al. (2019) evaluated the UGT-mediated response to FHB and FCR (Fusarium crown rot) in transgenic durum wheat over-expressing the Hv-UGT13248 . The two transgenic lines inoculated with F. graminearum at anthesis stage significantly decreased FHB severity (up to 30%) as compared to with the non-transformed plants. Although seeds of all the tested lines were affected by the infection, the DON contents were significantly decreased in both the transgenic lines. These results indicated that both the FHB and FCR infections are positively affected by the UGT-mediated DON detoxification mechanism. He at al. (2020) also characterized and expressed a novel UDP-glucosyltransferase gene, TaUGT6 in wheat and found enhanced resistance to FHB and DON accumulation in wheat.

Mandalà et al. (2021) stacked genes in wheat and produced two types of transgenic lines i) Durum wheat double-transgenic (UGT+PMEI) line expressing the HvUGT13248 and AcPMEI genes, encoding a barley UGT and a kiwi pectin

methylesterase inhibitor, respectively and ii) a bread wheat transgenic (UGT+PGIP) line, expressing the HvUGT13248 gene and PvPGIP2 gene, encoding a bean polygalacturonase inhibiting protein, respectively. It was observed that both types of the transgenic plants showed enhanced resistance against F. graminearum by reducing the FHB symptoms (by 10–20%) compared to the lines with the singletransgenes, and by 50% when compared to the control plants.

Antimicrobial proteins (AMPs) against FHB

The AMPs have a diverse action against bacterial, fungal and viral pathogens, which make them attractive for enhancing plant defense against the pathogens. AMPs have been overexpressed in different plant species for enhanced disease resistance (Marcos et al. 2008). Milk, saliva, tears and mucous secretions of most of the mammals contain lactoferrin, a cationic glycoprotein (80 kDa) which plays an important role in the immune system of newborns (Valenti et al. 2006). Lactoferrin has strong activity against bacterial, fungal and viral pathogens (Farnaud & Evans 2003). Makandar et al. (2006) reported that the AtNPR1regulating the activation of systemic acquired resistance, confered a heritable, type II resistance to F. graminearum when expressed in Bobwhite Transgenic wheat 'Bobwhite' lines expressing a bovine lactoferrin were tested for resistance to FHB infection under in vitro and greenhouse conditions for several generations. The transgenic lines exhibited consistent disease resistance to FHB infection compared to non-transformed control plants (Han et al. 2012). Badea et al. (2009) tested 5 synthetic peptides (10R, 11R, BMAP-18, MsrA2 (methionine sulfoxide reductase) and MsrA3) against FHB under in vitro conditions and concluded that 10R was found the most effective in restricting conidial germination, whereas the MsrA2 restricted mycelial growth. As single peptide was not found effective against all the Fusarium isolates tested, two or more peptides with different mode of action can be more effective in controlling FHB in wheat. Later the two peptides, 10R and MsrA2 co-expressed in leaves and spikes of transgenic wheat exhibited 50% reduction in FHB infection (Badea et al. 2013). Koch et al. (2012) evaluated antifungal activity of thanatin (21 amino acids) isolated from soldier bug (Podisus maculiventris), against the F. graminearum and concluded that conidial germination and mycelia growth of the pathogen was inhibited. Moreover, detached leaves assay of transgenic A. thaliana overexpressing thanatin exhibited enhanced resistance against colonization with F. graminearum. TAD1 (T. aestivum defensin 1), encoding a plant defensing, is induced with cold acclimation in winter wheat. Sasaki et al. (2016) demonstrated that overexpression of the TAD1 in transgenic wheat plants conferred resistance to Typhula ishikariensis (snow mold fungus) and F. graminearum causing FHB. Bolouri et al. (2017) tested antimicrobial activity of metchnikowin, an insect-derived AMP against F. graminearum and concluded that the metchnikowin targeted the fungal enzyme β (1,3)-glucanosyltransferase Gel1 (FgBGT), responsible for fungal cell wall integrity. A recombinant lipid transfer protein, isolated from Pichia pastoris, displayed strong antifungal potential against F. graminearum. Over expression of the LTP gene, AtLTP4.4, in transgenic wheat substantially decreased F. graminearum growth in 'Bobwhite' and 'RB07' lines under greenhouse condition, and lead to reduction in fungal lesion size in the detached leaf assays and suppressed the DON contents in the field (McLaughlin et al. 2020).

HIGS against FHB in wheat

RNA interference (RNAi), a post-transcriptional gene silencing, is a regulatory mechanism of gene expression in many eukaryotes (Baulcombe 2004; Fire 2007). RNAi, a powerful tool in silencing a target gene (Baulcombe 2004; Waterhouse and Fusaro 2006), is carried out by expression of dsRNA homologous to a target sequence to silence expression of the target sequence. Host-induced gene silencing (HIGS) is an emerging system of conferring resistance in plants against pathogens. In this system, an important gene of the pest is targeted by generating a DNA construct wih an inverted repeat of that important gene. When the

susceptible host is transformed with the construct having inverted repeat, the transcription in the plant generates dsRNA. The plant recognizes the dsRNA as invasive molecules and breaks the dsRNA into siRNAs. When the pathogen attacks the transgenic plant, transfer of siRNAs from the plant cells into the pest induces silencing of the target genes required for development or virulence. HIGS has been reported as a promising strategy to increase plant resistance against fungal pathogens such as Blumeria graminis (Steven and Nelson 2009), Puccinica triticina (Nowara et al. 2010) and Fusarium spp. (Attia et al. 2022). The HIGS was first demonstrated by Koch et al. (2013) to control F. graminearum wherein both the transgenic Arabidopsis and barley expressing dsRNA from cytochrome P450 lanosterol C-14α-demethylase genes exhibited higher resistance to the F. graminearum as reported (Koch et al. 2013). Chitin, the major part of fungal cell wall, is catalyzed by chitin synthase. Cheng et al. (2015) reported that HIGS of a fungal Chs (Chitin synthase) gene in wheat elevated resistance to Fusarium pathogens and mycotoxins. This represents HIGS as a powerful tool against Fusarium infection under field tests conditions, therefore developing an environment-friendly, durable and novel resistance against toxins contamination in wheat seedlings and spikes. He et al. (2019) developed transgenic Brachypodium distachyon plants carrying RNAi construct to downregulate the expression of two protein kinase genes (Fg08731 and Fg00677), and cytochrome P450 lanosterol C14-α-demethylase (CYP51) encoding genes (CYP51A, CYP51B, and CYP51C) of F. graminearum, respectively. All the transgenic T2 lines harboring Fg00677-RNAi, Fg08731-RNAi, and CYP51-RNAi cassettes showed strong resistance to F. graminearum, depicting that silencing molecules produced by the transgenic plants altered the corresponding gene function leading to its reduced pathogenicity. Therefore, the two kinase genes (Fg00677 and Fg08731) can be used as effective targets for HIGS to enhance FHB resistance in wheat and other cereal crops. Wang et al. (2020) evaluated the HIGS for generating transgenic wheat against the FHB and DON contamination by silencing, simultaneously, three genes (FgSGE1, FgSTE12 and FgPP1) of F. graminearum. The RNAi inserts were found highly specific to the target genes. The FgSGE1-STE12-PP1 RNAi constructs, generated siRNAs in the transgenic wheatwhich were transferred to the pathogen during infection. The siRNAs in the pathogen caused decrease in transcript levels of the target genes in the hyphae of F. graminearum. These findings suggested that HIGS targeting the genes playing role in metabolism of the fungal pathogen could be effective enough to be employed as an alternative strategy for development of FHB and DON resistant crops.

Conclusions and future prospects

FHB has been a devastating fungal disease that results in losses in yield and quality of wheat grain. In addition, accumulation of mycotoxin, trichothecene in the diseased grain may also affect animal and human health. Breeding efforts have produced varieties with partial resistance to FHB because of deficiency of effective resistant genes and complexity of the resistance as well. Advances in biotechnology research can hold promise of understanding mechanism of resistance to FHB. Marker-free transformation and HIGS technology could be able to contribute to imparting resistance to FHB. Genetic engineering approach to identify the best candidate genes and pyramid more than one gene (transgene stacking) in wheat could be the better option for researchers to enhance level of resistance to FHB. In addition, CRISPR/Cas9, a genome-editing tool enabling researchers to alter genomic sequences in a precise way, can be used to modify the genes (such as Nuclear Transcription Factor X box-binding-Like 1 (TaNFXL1) and ABC transporter (TaABCC6) in wheat associated with susceptibility of FHB.