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Research review paper

Recent advances in genes involved in secondary metabolite synthesis, hyphal development, energy metabolism and pathogenicity in *Fusarium graminearum* (teleomorph *Gibberella zeae*)

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ABSTRACT

The ascomycete fungus, *Fusarium graminearum* (teleomorph *Gibberella zeae*), is the most common causal agent of *Fusarium* head blight (FHB), a devastating disease for cereal crops worldwide. *F. graminearum* produces ascospores (sexual spores) and conidia (asexual spores), which can serve as disease inocula of FHB. Meanwhile, *Fusarium*-infected grains are often contaminated with mycotoxins such as trichothecenes (TRIs), fumonisins, and zearalenones, among which TRIs are related to the pathogenicity of *F. graminearum*, and these toxins are hazardous to humans and livestock. In recent years, with the complete genome sequencing of *F. graminearum*, an increasing number of functional genes involved in the production of secondary metabolites, hyphal differentiation, sexual and asexual reproduction, virulence and pathogenicity have been identified from *F. graminearum*. In this review, the secondary metabolite synthesis, hyphal development and pathogenicity related genes in *F. graminearum* were thoroughly summarized, and the genes associated with secondary metabolites, sexual reproduction, energy metabolism, and pathogenicity were highlighted.

Contents

1. Introduction

Fusarium graminearum (teleomorph *Gibberella zeae*) is an ascomyceteous fungus that causes *Fusarium* head blight (FHB) in cereal crops, including wheat, barley, rice, and oats, as well as ear rot and stalk rot in maize (Fernando et al., 1997; Goswami and Kistler, 2004; Munkvold, 2003; Parry et al., 1995; Sutton, 1982). As a major global pathogen of cereals, the threat caused by this fungus is multifaceted. It leads not only to yield and quality losses but also contaminate grains by producing mycotoxins that are hazardous to livestock and humans (Glenn, 2007; Hussein and Brasel, 2001; Placinta et al., 1999). The losses can happen at two stages. In the first, research has shown that the

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Table 1
Gene involved in the secondary metabolites production in *F. graminearum*. AUR, aurofusarin; DON, deoxynivalenol; NRPS, nonribosomal peptide synthetase; PKS, polyketide synthase; TRI, trichothecene; WT, wild-type strain; ZEA, zearalenone; β-ZOL, β-zearalenonol; ROS, reactive oxygen species.

Genes	Proteins	Phenotype of mutants	Functions	References
Clm1	Longiborneol synthase	*Clm1* gene disruptants produced no culmorin but were able to convert exogenously added longiborneol to culmorin	Clm1 encodes a longiborneol synthase and is required for culmorin biosynthesis in *F. graminearum*	Gardiner et al. (2009a); McCormick et al. (2010)
Fgl1	A secreted lipase	Δ*Fgl1* mutants showed reduced extracellular lipolytic activity and to reduced virulence to both wheat and maize, and it exhibited up-regulated DON production during wheat head infection and revealed a dramatically enhanced ZEA production on kernels	Fgl1 may be involved in hyphal growth during infection of the spikelet and activation and expression of other enzymes responsible for fast growth of fungal hyphae. Fgl1 may also involve in regulation of eight PKS genes and ZEA production	Voigt et al. (2005, 2007)
FgLaeA	Global regulator	Deletion of *FgLaeA* led to earlier induction of perithecia formation as well as drastically reduced disease symptoms in wheat. Overexpression of *FgLaeA* caused the increased production of TRIs and additional metabolites	FgLaeA may be a member of putative FgVeA complex and controls secondary metabolism, sexual development, and virulence	Kim et al. (2013)
Fgos1	Osmosensor histidine kinase	Δ*Fgos1* mutants produced a reduced amount of AUR. The transcript levels of *Pks12* and *Gip2* were reduced in the Δ*Fgos1* mutants	FgOs1 is a putative component of the osmotic stress signal transduction pathway. FgOs1 plays role in AUR biosynthesis and regulates Pks12 and Gip2	Ochiai et al. (2007)
Fgos4, *Fgos5* and *Fgos2*	MAPK kinase pathway	Mutants of *Fgos4*, *Fgos5*, and *Fgos2* showed markedly enhanced AUR production and failed to produce TRIs in aerial hyphae. Also, the transcript levels of *Pks12* and *Gip2* were enhanced. Expression of *Tri4* and *Tri6* were markedly reduced.	This osmoregulatory MAPK pathway regulates secondary metabolism associated with AUR and TRIs. It's very likely that this MAPK pathway affects AUR by regulating Pks12 and Gip2	Ochiai et al. (2007)
Fgp1	Wor1-like Protein	Deletion of the *Fgp1* results in greatly reduced pathogenicity and loss of TRI toxin accumulation in infected wheat plants and in vitro. The Δ*fgp1* mutants show defects in asexual and sexual spore development	Fgp1 is essential for TRI production. It affects asexual and sexual reproduction. Fgp1 may also regulates expression of gene clusters and other genes encoding PKS or NRPS proteins	Jonkers et al. (2012)
FgVe1	Velvet	Disruption of *FgVe1* caused phenotypes include hyperbranching of the mycelium, suppression of aerial hyphae formation, reduced hydrophobicity of the mycelium and highly reduced sporulation	FgVe1 modulates the production of the AUR pigment and is essential for the expression of Tri genes and the production of TRIs. It is a positive regulator of virulence. It may also affect hyphal development and reproduction	Merhej et al. (2012)
FgVelB	Velvet	Δ*FgVelB* strains produced fewer aerial mycelia with less pigmentation;Production of TRI and ZEA was dramatically reduced compared with the WT strain. The Δ*FgVelB* strains were incapable of colonizing host plant tissues;The Δ*FgVelB* strains produced no fruiting bodies but retained male fertility under sexual development conditions	FgVelB regulates mycotoxin production, sexual reproduction and pathogenicity, probably by acting as a member of a possible velvet protein complex	Lee et al. (2012)
Gip1	A putative laccase	Δ*Gip1* mutants produced no AUR on PDA and showed yellowish color	Gip1 are required for AUR production in *F. graminearum*, and it is downstream of Pks12 in the AUR biosynthetic pathway	Y.T. Kim et al. (2005)
Gip2	A putative transcription factor	Δ*Gip2* mutants could not produce AUR on PDA. Overexpression of *Gip2* increases AUR production and reduces mycelial growth	Gip2 is required for AUR biosynthesis, and it was required for transcription of the genes in the AUR biosynthetic cluster	Kim et al. (2006)
GzGpa1	Gα subunit	Deletion of *GzGpa1* resulted in female sterility and enhanced DON and ZEA production	GzGpa1 is required for normal sexual reproduction and repression of toxin biosynthesis	Yu et al. (2008)
GzGpb1	Gβ subunit	Production of DON and ZEA was enhanced in the Δ*GzGpb1* mutants. Deletion of *GzGpb1* resulted in 75% of the hyphal growth and mutants were much less virulent than the WT	GzGpb1 negatively control mycotoxin production like *GzGpa1*. *GzGpb1* are essential for the virulence of *F. graminearum*	Yu et al. (2008)
Hep1	Heterochromatin protein	AUR genes are highly up-regulated and AUR production is greatly enhanced, while gene expression and metabolites are lower for the TRI cluster in the *Hep1* deleted strains	Hep1 has a repressive role on AUR gene cluster and a positive function for DON biosynthesis	Reyes-Dominguez et al. (2012)
Lh1 (*Tri1*)	P450 oxygenase	Δ*Lh1* mutants no longer produced 15-acetyl DON, but rather accumulated calonectrin and 3-deacetylcalonectrin	*Lh1* gene encodes a P450 responsible for oxygenation at one or both of these positions (C-7 and C-8) in the TRIs biosythesis pathway	McCormick et al. (2004)
Map1	MAPK	DON and 3-acetyl DON production were reduced in Δ*Map1* mutants. Δ*Map1* mutants lost pathogenicity, and also lost their ability to form perithecia in vitro	The Map1 signaling protein controls multiple events in disease establishment and propagation, including root colonization, wheat ear colonization, DON synthesis and perithecia formation	Urban et al. (2003)
Mgv1	MAP kinase	DON production and virulence were reduced in mutants. Mutants had weak cell walls and were hypersensitive to cell wall degrading enzymes. They were self-incompatible when tested for heterokaryon formation and were female-sterile	Mgv1 in *F. graminearum* is involved in multiple developmental processes related to sexual reproduction (essential for female fertility), plant infection, and cell wall integrity	Hou et al. (2002)
Nrps2	NRPS	Δ*Nrps2* mutants did not produce ferricrocin, which differed from the WT strain	Nrps2 is responsible for the biosynthesis of ferricrocin that is an intracellular siderophore	Tobiasen et al. (2007)
Nrps6	A putative NRPS	Deletion of *Nrps6* resulted in reduced virulence and hypersensitivity to H_2O_2 as well as increased sensitivity to iron depletion	Nrps6 may be responsible for the biosynthesis of siderophores, whose role is to supply an essential nutrient, iron, to the pathogenic fungi in planta	Oide et al. (2006)

(continued on next page)

Table 1 (*continued*)

Genes	Proteins	Phenotype of mutants	Functions	References
Pac1	pH regulatory factor	Δ*FgPac1* mutant showed a reduced development under neutral and alkaline pH, increased sensitivity to H_2O_2 and an earlier Tri gene induction and toxin accumulation at acidic pH	Pac1 negatively regulates Tri gene expression and toxin production in *F. graminearum*	Merhej et al. (2011b)
Pks4 (also named *Zea2*)	PKS	Δ*Pks*4 mutants could not produce ZEA and β-ZOL	Pks4 is required for ZEA production. And PKS4-encoded protein or its product stimulates expression of PKS13	J.E. Kim et al. (2005); Lysøe et al. (2006)
Pks12	PKS	Δ*Pks12* mutants produced no AUR on PDA and showed yellowish color. And it has higher growth rate and a 10-fold increase in conidia production compared to the WT	The product of *Pks12* is the originator of AUR. Pks12 is upstream of Gip1 in the AUR biosynthetic pathway. So *Pks12* is responsible for the biosynthesis of AUR and is involved in ZEA production	Malz et al. (2005); Y.T. Kim et al. (2005)
Pks13 (also named *Zea1*)	Non-reducing PKS	Δ*Pks13* mutants could not produce ZEA and β-ZOL	Pks13 may catalyze iterative condensation steps for the synthesis of the unreduced moiety of ZEA	J.E. Kim et al. (2005)
Tri5	Trichodiene synthase (TRIase)	Δ*Tri5* mutants could not produce TRIs, and it exhibited reduced virulence of *F. graminearum* on some hosts	*Tri* encodes a TRIase which catalyzes the first step in TRIs biosynthesis	Proctor et al. (1995)
Tri6 and *Tri10*	Transcription factor	Both mutants had greatly reduced pathogenicity and toxin production	Tri6 and Tri10 are responsible for regulation of TRIs biosynthetic and related genes	Seong et al. (2009)
Tri7	4-O-Acetyltransferase	Δ*Tri7* mutants of 88–1 (88–1 produced NIV and 4-ANIV) produced NIV but no 4-ANIV	Tri7 protein is involved in acetylation of the oxygen at C-4 of NIV to produce 4-ANIV	Lee et al. (2002)
Tri8	TRI C-3 deacetylase	Δ*Tri8* mutants were altered in their ability to biosynthesize 15-acetyl DON and instead accumulated 3,15-diacetyl DON, 7,8-dihydroxycalonectrin, and calonectrin	*Tri8* gene encodes an esterase responsible for deacetylation at C-3	McCormick and Alexander (2002)
Tri13	A putative P450	Δ*Tri13* mutants of 88–1 produced DON instead of NIV and 4-ANIV	*Tri13* gene is the determinant for the DON-NIV switching in *F. graminearum*	Lee et al. (2002)
Tri14		Δ*Tri14* mutants showed reduced virulence, and do not produce a detectable quantity of DON on plants	1) Tri14 acts as a positive regulator of DON synthesis; 2) Tri14 may play is in the export of DON outside of the mycelia; 3) Tri14 involved in the synthesis of another pathogenicity factor	Dyer et al. (2005)
Zra1	ABC transporter	Deletion of *Zra1* resulted in reduced ZEA production	Zra1 may functions as a transporter in ZEA synthesis	Lee et al. (2011a)
Zeb1	Isoamyl alcohol oxidase	Δ*Zeb1* mutants produced β-ZOL rather than ZEA in the liquid medium	Zeb1 is responsible for the chemical conversion of β-ZOL to ZEA in the biosynthetic pathway	J.E. Kim et al. (2005)
Zeb2	A putative transcriptional activator	Δ*Zeb2* mutants could not produce ZEA and β-ZOL	Zeb2 may play an important role in the regulation of the PKS gene cluster for ZEA production	J.E. Kim et al. (2005)
Zif1	b-ZIP transcriptionfactor	Δ*Zif1* mutants had significantly reduced DON production and virulence, and it formed smaller and fewer perithecia than the WT and was defective in sexual reproduction. It was also hypersensitive to ROS	Zif1 may regulate some subsets of genes and so is essential for female fertility and involved in DON production	Wang et al. (2011)

disease reduces crop yield and lowers the market grade of cereal crops. This directly results in significant losses for farmers. Secondly, *F. graminearum* can produce a variety of mycotoxins (Desjardins et al., 1993; Kimura et al., 2007) that are easily transferred to compound feeds, leading to rejection or downgrading of grain at marketing, resulting in indirect loss (Goswami and Kistler, 2004; Kazan et al., 2012; McMullen et al., 1997). Therefore, the surveillance of grain and animal feed for the *Fusarium* mycotoxins continues to attract worldwide attention.

F. graminearum survives and over-winter on or within plant tissue residues including small grain stems and roots as well as maize stalks and ear pieces (Kazan et al., 2012; Sutton, 1982). It produces both sexual spores (ascospores) within perithecia and asexual spores (conidia). These spores are resistant to environmental stress conditions and are well suited for dispersal into susceptible host tissues (Trail et al., 2005). Infected crop debris is the main source of inoculum in the form of ascospores and conidia for *F. graminearum* (Dill-Macky and Jones, 2000; Osborne and Stein, 2007; Tschanz et al., 1976; Xu, 2003). Ascospores can be forcibly discharged into the air from perithecia and are estimated to be more important than conidia in FHB epidemics because FHB inoculum requires aerial dispersal to the cereal heads (Sutton, 1982; Trail et al., 2002). However, macroconidia might be spread by splash dispersal during rain and alternatively by insect vectors like wheat midges, which also contributes to the FHB dispersal (Beyer et al., 2004; Mongrain et al., 2000). Besides, both ascospores and conidia can be found at nearly any time during the mature stages of the infected cereal crops (Beyer et al., 2005; Trail et al., 2002; Tschanz et al., 1976). Overall, both ascospores and conidia play important roles in the development and propagation of *F. graminearum*.

Diseases caused by various phytopathogens result in a serious threat to global food security and yield losses. The most widely studied plant pathogenic fungus may be the rice pathogen *Magnaporthe oryzae*, and its major signaling pathways involved in plant infection have been summarized recently (Li et al., 2012). Similarly, *F. graminearum* is also an important plant pathogenic fungus. With the completion of *F. graminearum* genome sequencing (Cuomo et al., 2007), an increasing number of functional genes involved in secondary metabolite synthesis, hyphal differentiation, sexual and asexual reproduction, virulence and pathogenicity have been identified recently. Previously reviews have mainly focused on genes involved in trichothecenes biosynthesis, pathogenicity and signal transduction of the *Fusarium* genus (e.g., Brown et al., 2004; Idnurm and Howlett, 2001; Kimura et al., 2007; Merhej et al., 2011a; Xu, 2000). Recently, Kazan et al. (2012) reviewed new advances on pathogenesis, toxin biosynthesis and host resistance mechanisms. They focused on the molecular aspects of the host–pathogen interaction, including *F. graminearum* colonization mechanisms, toxin biosynthesis inducers, host genes expressed during pathogenesis, host resistance, and plant protection. However, there has been no recent review that systematically analyzed the secondary metabolite synthesis, hyphal development

and pathogenicity related genes of *F. graminearum*. Here, we summarize the genes controlling various aspects of secondary metabolite synthesis, hyphal development and pathogenicity of *F. graminearum*. We aim to provide an updated summary of the regulation of these mycotoxins, hyphal development and the pathogenicity of this fungus, in the hopes of identifying better targets to fight against this undesirable "cereal killer".

2. Genes involved in the secondary metabolites production in *F. graminearum*

Fungi have the potential to produce a wide range of secondary metabolites, including mycotoxins, antibiotics, and pigments (J.E. Kim et al., 2005; Y.T. Kim et al., 2005). *F. graminearum* can produce several mycotoxins, including trichothecene derivatives, polyketides zearalenone (ZEA) and fusarin C (Desjardins et al., 1993; Kimura et al., 2007), among which trichothecenes (TRIs) are related to the pathogenicity of *F. graminearum*. Several enzyme families are commonly involved in the synthesis of these secondary metabolites in fungi, such as polyketide synthetases (PKSs), nonribosomal peptide synthetases (NRPSs), and cytochrome P450 family (P450) (Table 1) (Idnurm and Howlett, 2001). Fungal PKSs are large multidomain enzymes (belongs to type I PKSs) with an iterative function (Lysøe et al., 2006). PKSs catalyze the biosynthesis of polyketides, which are a structurally diverse class of natural products including antibiotics, toxins and pigments (Y.T. Kim et al., 2005; Zhou et al., 2008). 16 PKS genes have been identified in the genome of *F. graminearum*, including six non-reducing PKSs (Gaffoor et al., 2005; Kroken et al., 2003).

2.1. Polyketides zearalenone (ZEA)

ZEA is an estrogenic polyketide (Malz et al., 2005), synthesized by the head-to-tail condensation of acetate units via the acetate–malonyl–coenzyme enzyme system. In order to identify the enzymes involved in this biosynthetic pathway, J.E. Kim et al. (2005) deleted five non-reducing PKSs (*Pks3*, *Pks13* [also named *Zea1*], *Pks14*, *Pks15* and *Pks16*) and identified *Pks13* as the ZEA PKS gene. Subsequently, targeted deletions of additional ORFs closely linked to *Pks13* revealed that three more genes (*Pks4* [also named *Zea2*], *Zeb1* and *Zeb2*) also participated in ZEA production by *F. graminearum* (Fig. 1). The Zeb1 protein catalyzes an oxidation step for the conversion of β-zearalenonol to ZEA, and Zeb2 may be a transcriptional activator of the cluster members (J.E. Kim et al., 2005). Subsequently, Gaffoor and Trail (2006) furtherly characterized the functions of Pks4 and Pks13, Pks4 was predicted to have a domain order of KS-AT-DH-ER-KR-ACP and Pks13 of KS-AT-ACP, disruption of either gene resulted in the loss of ZEA production under inducing conditions. It was proposed that Pks4 and Pks13 make up the core biosynthetic unit for ZEA, with the first ten carbon additions catalyzed by Pks4 and the remaining three rounds of C_2 additions by Pks13 (Gaffoor and Trail, 2006). The hypothesis was supported by data from Lysøe et al. (2006) in which the expression of *Pks13*, located in the same cluster as *Pks4*, decreased dramatically in the Δ*Pks4* mutants. Thus, *Pks4*, *Pks13*, *Zeb1* and *Zeb2* form a ZEA biosynthesis gene cluster in *F. graminearum*.

Except the PKSs, several other genes have also shown to be involved in ZEA production (Table 1). Fgl1, a secreted lipase, has been proven to regulate eight PKS genes and ZEA production. In

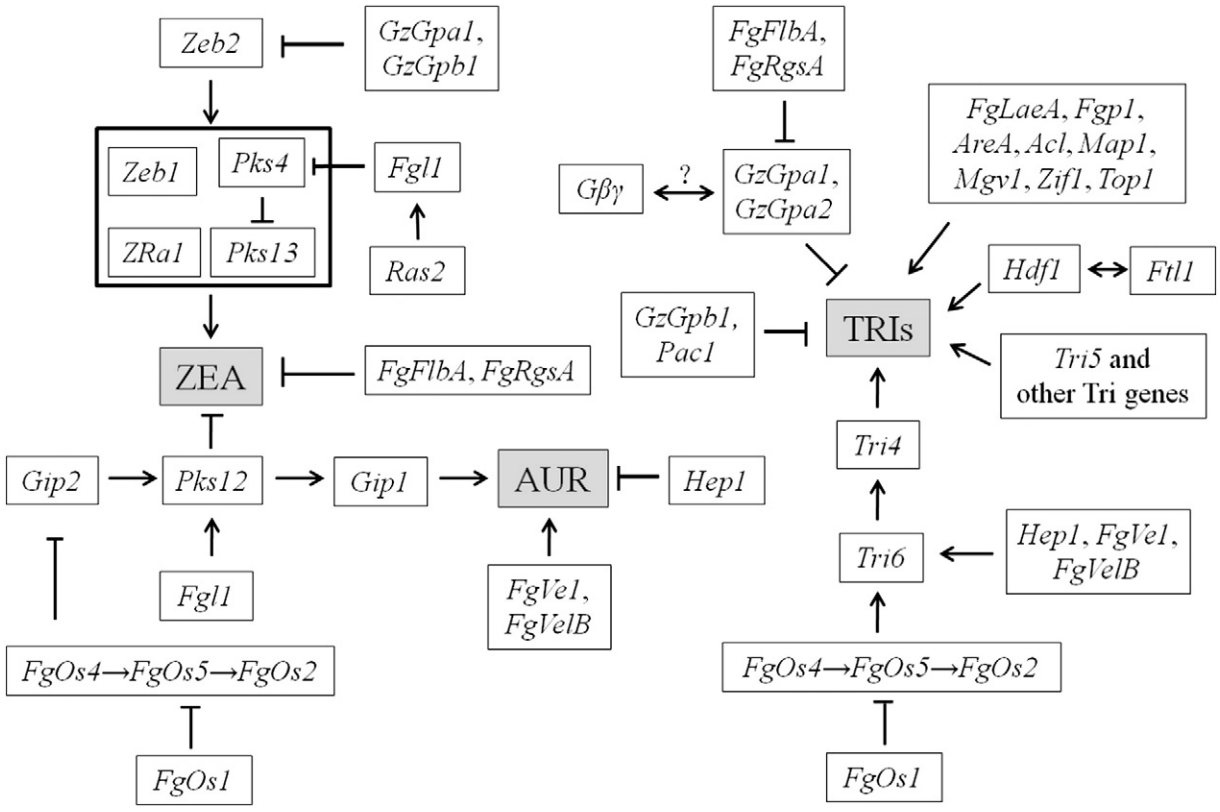

Fig. 1. Partial genes involved in the secondary metabolites production in *F. graminearum*. The relationship of these genes involved in the secondary metabolites synthesis are complicated, several genes can alter the expression of other genes, positive regulation is indicated by an arrow, negative regulation by a line ending by a transversal bar, and double-headed arrow shows two genes can be regulated by each other, while "?" represents the interaction of two genes need to be further confirmed. The gray boxes show these secondary metabolites produced by *F. graminearum*, and double boxes show these genes can regulate other genes by the same mechanism (positive or negative regulation). ZEA, zearalenone; TRIs, trichothecenes; AUR, aurofusarin.

Table 2
Genes involved in sexual and asexual reproduction in *F. graminearum*. DON, deoxynivalenol; TRI, trichothecene; WT, wild-type strain.

Genes	Proteins	Phenotype of mutants	Functions	References
Cch1	Voltage-gated calcium ion channel	Δ*Cch1* mutants were found to have asci which did not forcibly discharge spore. Mycelial growth was significantly slower, and sexual development was slightly delayed in the mutants; mutant mycelia showed a distinctive fluffy morphology, and no cirrhi were produced	Cch1 may have a role in forcible spore discharge in *F. graminearum* and this channel has subtle effects on growth and development	Hallen and Trail (2008)
Chs5, *Chs7*	Chitin synthase	Neither mutants did not produce perithecia or caused disease on barley heads. Neither mutants formed balloon-shaped hyphae and intrahyphal hyphae and that cell wall rigidity of the mutants was weaker than that of the WT	GzChs5 and GzChs7 are indispensable for perithecia formation and pathogenicity as well as normal septa formation and hyphal growth in *F. graminearum*	Kim et al. (2009)
Fbp1	F-box proteins	Δ*Fbp1* mutants showed reduced growth rate, changed colony morphology and pigmentation as well as reduced virulence. Δ*Fbp1* mutants produced asci that contain incomplete octads of abnormal spores	Fbp1 participates in the formation of a SCF^{FBP1} complex controlling many regulatory processes for major traits of *F. graminearum* including sexual development, vegetative growth, pigmentation and fungal virulence	Han et al. (2007)
Fgssk2, *Fgpbs2* and *Fghog1*	FgHog1 MAPK pathway	Mutants all had reduced hyphal growth, they were not only hypersensitive to osmotic stress but also had increased sensitivity to oxidative, cytoplasm membrane, and cell wall stresses. They were female sterile but retained male fertility	FgHog1pathway is involved in hyphal growth, branching, plant infection, and stress responses in *F. graminearum*	Zheng et al. (2012)
Fsr1	A putative striatin	Δ*Fsr1* mutants showed reduced virulence on barley and resulted in loss of fertility and perithecia formation	Fsr1 regulates virulence and sexual reproduction likely by acting as a scaffold for a signal transduction pathway	Shim et al. (2006)
Ftl1	Transducin	Δ*Ftl1* mutants were female sterile and had reduced conidiation. It also was defective in spreading from infected anthers to ovaries and was more sensitive than the WT to plant defensins MsDef1 and osmotin, and it also had significantly reduced HDAC activity	Ftl1 appears to be a component of HDAC protein complex that has a role in repression of other transcription factors and thus plays a critical role in the penetration and colonization of wheat tissues as well as conidiation	Ding et al. (2009)
Gea1		*Gea1* deletion mutants produced normal-shaped perithecia and ascospores, yet ascospores were observed to precociously germinate inside the perithecium. Moreover, *Gea1* deletions resulted in abnormal ascus walls that collapsed prior to ascospore discharge	Gea1 is required for ascus wall development	H. Son et al. (2013)
Gpmk1	MAPK	Δ*Gpmk1* mutants had reduced conidial production, were sexually sterile and non-pathogenic. Endoglucanase, xylanolytic and preteolytic activities in Δ*Gpmk1* mutants are lower than WT	Gpmk1 is responsible for signal transduction processes. Gpmk1 regulates the early induction of extracellular enzymes that may participate in the infection process.	Jenczmionka et al. (2003); Jenczmionka and Schäfer (2005)
GzSnf1	SNF1 protein kinase	Δ*GzSnf1* mutants had reduced mycelial growth and virulence. They produced 30% fewer perithecia, and the germination and nucleation of both ascospores and conidia were delayed	Snf1 affects fungal virulence; developmental processes; spore maturation and germination; and the utilization of certain carbon sources	Lee et al. (2009a)
GzSyn2	Syntaxin-like SNARE protein	Δ*GzSyn2* mutants completely lost both self and female fertility, and virulence on barley was reduced by 75%	GzSyn2 is essential for self and female fertility, but not for male fertility	Hong et al. (2010)
Hdf1	Histone deacetylase	Mutants had reduced virulence and DON production, and failed to spread from the inoculation site to other parts of wheat heads or corn stalks. It was defective in sexual reproduction and reduced in conidiation	Hdf1 may interact with Ftl1 and function as a component in a well-conserved HDAC complex in the regulation of conidiation, DON production, and pathogenesis	Li et al. (2011)
Hex1	Hexagonal peroxisome protein	Both *Hex1* gene deletion and overexpression reduced the production of asexual spores and reduced virulence on wheat spikelets	Hex1 gene plays a direct role in the asexual reproduction and virulence of *F. graminearum*	M. Son et al. (2013)
Mid1	Stretch-activated ion channel	Δ*Mid1* mutants exhibited a >12-fold reduction in ascospore discharge activity and produced predominately abnormal two-celled ascospores with constricted and fragile septae. The vegetative growth rate of the mutants was 50% of the WT, and production of macroconidia was >10-fold lower than in the WT	Mid1 plays role in ascospore development and forcible discharge, vegetative growth and conidiation	Cavinder et al. (2011)
ppg1	Pheromone precursor	Δ*ppg1* mutants showed reduced fertility in self-fertilization tests, and reduced male fertility in outcrossing tests	A putative pheromone-receptor pairs (ppg1/pre2) enhances, but is not essential for, selfing and outcrossing	Lee et al. (2008)
ppg2	Pheromone precursor	Δ*ppg2* mutants had no discernible effects on sexual function	-	Lee et al. (2008)
pre1	Pheromone receptor	Δ*pre1* mutants had no discernible effects on sexual function	-	Lee et al. (2008)
pre2	Pheromone receptor	Δ*pre2* mutants had reduced fertility in self-fertilization tests, and reduced female fertility in outcrossing tests	A putative pheromone-receptor pairs (ppg1/pre2) enhances, but is not essential for, selfing and outcrossing	Lee et al. (2008)
Ras2	Ras GTPase	Disruption of *Ras2* caused slower growth on solid media, delayed spore germination, female sterility and significant reductions in virulence	Ras2 may regulate growth and virulence in *F. graminearum* by regulating the Gpmk1 MAP kinase pathway and Fgl1	Bluhm et al. (2007)
Roa		Mutants showed an abnormal size and shape of asci and ascospores but did not affect vegetative growth. The asci of mutants discharged fewer ascospores from the perithecia but achieved a greater dispersal distance	Roa has a specific role in ascospore morphology and discharge (sexual development) in *F. graminearum* via affecting turgor pressure	Min et al. (2010)
Top1	Topoisomerase I	Δ*Top1* mutants had reduced virulence and DON production. Asexual sporulation was reduced and Δ*Top1* mutants did not develop sexual spores when subjected to an in vitro perithecia production assay	Top1 is involved in both asexual and sexual development, as well as the pathogenicity of *F. graminearum*	Baldwin et al. (2010)

Δ*Fgl1* mutants, four Pks genes (*Pks2*, *Pks11*, *Pks12* and *Pks14*) exhibited decreased expression, whereas four others (*Pks4*, *Pks7*, *Pks9* and *Pks15*) showed an elevated expression (Voigt et al., 2007). Recently, three putative ABC transporters (Zral, Zra2 and Zra3) were found to be significantly down-regulated in Δ*Zeb2* mutants. However, only one (*Zra1*) of the three was found to be significantly up-regulated

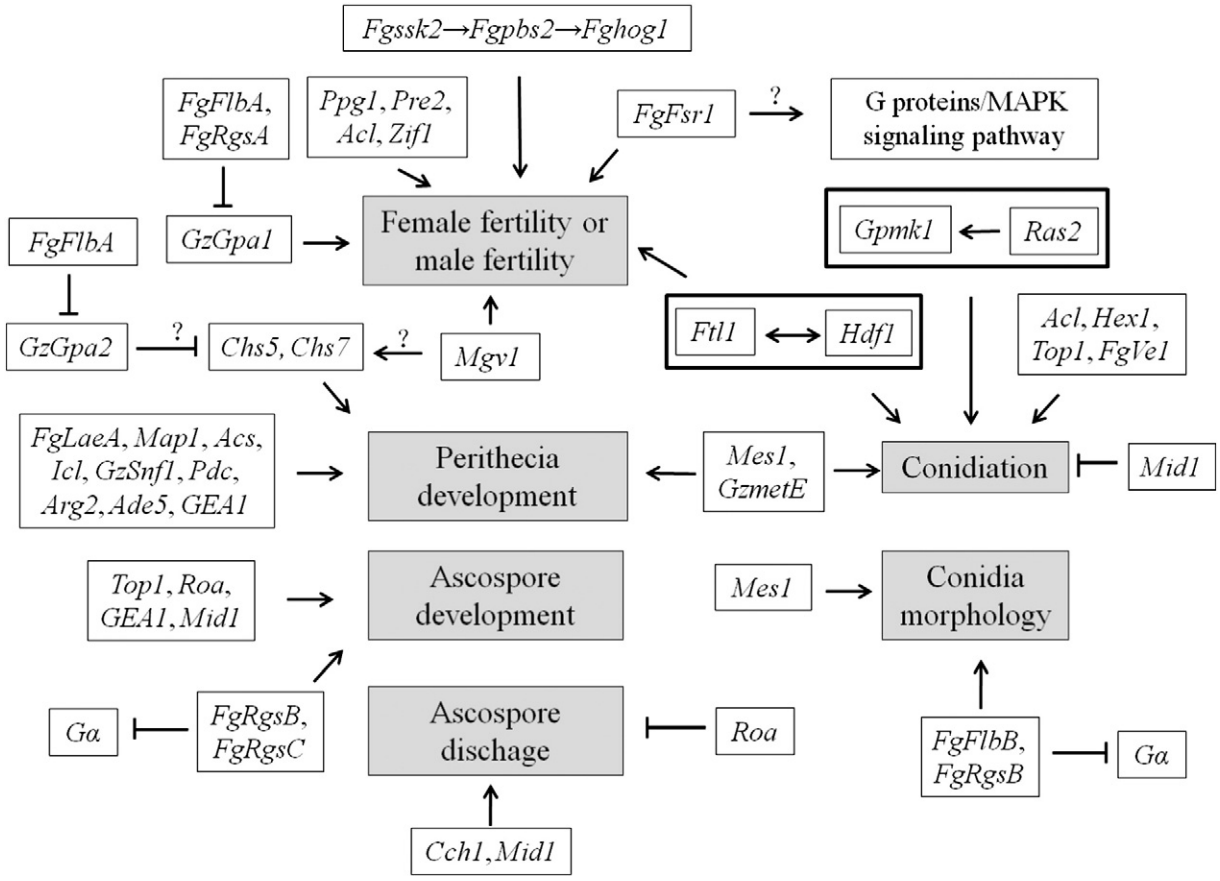

Fig. 2. Partial genes involved in sexual and asexual reproduction, mycelial differentiation and conidiation in *F. graminearum*. The relationship of these genes involved in the hyphal development and conidiation are also complicated. Similarly, several genes can alter the expression of other genes, positive regulation is indicated by an arrow, negative regulation by a line ending by a transversal bar, and double-headed arrow shows two genes can be regulated by each other, while "?" represents the interaction of two genes need to be further confirmed. The gray boxes show biological processes, such as sexual and asexual reproduction, and conidiation, and double boxes show these genes can regulate other genes by the same mechanism (positive or negative regulation).

(by 20-fold) in the wild-type strains supplemented with ZEA, and the deletion of *Zra1* resulted in reduced ZEA production (Lee et al., 2011a). These results suggest *Zra1* positively regulate ZEA production.

2.2. Trichothecenes (TRIs)

In addition to ZEA, TRIs and their derivatives are also important toxins produced by *F. graminearum*. Several species of the genus *Fusarium* and related fungal genera can produce TRIs and contaminate agricultural crops and commodities. TRIs are a broad class of sesquiterpene epoxides that inhibit eukaryotic protein synthesis and thereby can impair human and animal health (Desjardins et al., 1993). There are four types of TRIs (A–D). Type A TRIs include mainly the T-2 toxin, HT-2 toxin, anguidin (diacetoxyscirpenol) and their derivatives, and type B TRIs include mainly vomitoxin (deoxynivalenol, DON), nivalenol (NIV), and their acetylated derivatives. While type C and type D TRIs are groups of non-*Fusarium* TRIs (Kimura et al., 2007).

Several genes involved in the TRIs biosynthetic pathway have been cloned and characterized (Table 1, Fig. 1) (Merhej et al., 2011a). For example, *Tri5* (previously designated *Tox5*) encodes a trichodiene synthase which catalyzes the first step, changing farnesyl pyrophosphate to trichodiene, in TRIs biosynthesis (Proctor et al., 1995). Lee et al. (2002) clarified the roles of Tri13 and Tri7 using two chemotypes of *F. graminearum*, 88-1 and H-11. According to the TRIs type they produce, strain 88-1 belongs to the NIV chemotype and strain H-11 belongs to the DON chemotype. Recent results suggested that the Tri13 protein was responsible for the oxygenation at C-4 during the synthesis of NIV while Tri7 protein was involved in acetylation of the oxygen at C-4 of NIV to produce 4-acetyl-NIV (also known as fusarenon-X). Moreover, the *Tri13* gene is the determinant for the DON-NIV switching and *Tri7* gene is responsible for further modification of NIV in *F. graminearum*. *Tri8* encodes an esterase and is responsible for deacetylation at C-3 to produce 15-acetyldeoxynivalenol (McCormick and Alexander, 2002). In *Fusarium sporotrichioides*, *Tri1* (*FsTri1*) encodes a P450 oxygenase and is responsible for adding the isovalerate group to the C-8 position. Gene disruptant of *F. graminearum Lh1* (homologue of *FsTri1*) no longer produced 15-acetyldeoxynivalenol, which is oxygenated at C-7 and C-8, indicating that *GzTri1* plays a role in oxygenation at one or both of these positions (C-7 and C-8) (McCormick et al., 2004). Interestingly, *F. graminearum Tri14* does not share sequence similarity with any previously described genes in the databases. The Δ*Tri14* mutants could synthesize DON on cracked maize kernel medium but caused 50–80% less disease than wild type strains and they do not produce a detectable quantity of DON on plants. So *Tri14* may be required for DON production on wheat but not in vitro (Dyer et al., 2005). Moreover, some fungal genes for TRIs biosynthesis (*Tri* genes) are known to be under the control of transcription factors encoded by *Tri6* and *Tri10*. *Tri6* and *Tri10* regulate overlapping sets of genes that include a common group of multiple genes for both primary and secondary metabolism (Peplow et al., 2003). Both *Tri6* and *Tri10* deletion mutants showed greatly reduced pathogenicity and toxin production. In addition, transcript levels for over 200 genes were altered by over two folds in Δ*Tri6* or Δ*Tri10* mutants, including nearly all known *Tri* genes.

The results suggest that *Tri6* and *Tri10* genes act as regulators of TRIs biosynthetic and related genes in *F. graminearum* and other fungi (Gardiner et al., 2009a; Seong et al., 2009). Other Tri genes include *Tri4* (oxygenase), *Tri101* (3-O-acetyltransferase), *Tri3* (3-acetyltrichothecene 15-O-acetyltransferase) and *Tri11* (ITD C-15 hydroxylase). These results led to a proposed biosynthetic network for TRIs in *F. graminearum* (Kimura et al., 2007).

In addition to the above genes, DON production were substantially reduced in Δ*Mgv1* mutants and Δ*Map1* mutants, suggesting that Mgv1 and Map1 (two MAPKs) positively regulated DON production (Hou et al., 2002; Urban et al., 2003). Conversely, *Fgl1* negatively regulates DON synthesis (Voigt et al., 2007). Moreover, pH has a great effect on expression of the Tri5 gene and DON production and it seems that low pH could significantly increase DON production (Gardiner et al., 2009b). The pH regulatory factor Pac1 regulates Tri gene expression and TRIs production in *F. graminearum* (Merhej et al., 2011b). In addition to those genes, some other genes also participate in DON production (Fig. 1), such as *Zif1* (Wang et al., 2011), *FgLaeA* (Kim et al., 2013), *Fgp1* (Jonkers et al., 2012), and *Hdf1* (a histone deacetylase encoding gene, which interacted with *Ftl1*) (Li et al., 2011).

2.3. Polyketide pigments

Aside from the toxins, *F. graminearum* can produce polyketide pigments such as aurofusarin (AUR) and rubrofusarin. All of the known PKSs required for the production of fungal pigments have the same domain structure and belong to the same enzyme class, the non-reducing PKSs (Kroken et al., 2003). AUR is a golden yellow polyketide pigment. So far, relatively few genes involved in the AUR production have been reported (Fig. 1). Pks12, a type I PKS, is one such gene and its disruption caused no AUR production and a lighter colony color than wild type. Another gene *Gip1* functions downstream of Pks12 in the AUR biosynthetic pathway and may be responsible for changing a precursor to AUR (Y.T. Kim et al., 2005). Other AUR-related genes are regulated by *Gip2*. Deletion of *Gip2* leads to loss of AUR production and overexpression of *Gip2* increases AUR production, as well as reduces mycelial growth (Kim et al., 2006). Targeted mutagenesis of *Pks12* in *F. graminearum* caused not only the absence of AUR but also an increase in the level of the mycotoxin ZEA (Malz et al., 2005). Since both AUR and ZEA are polyketides, it is possible that Pks12 is also involved in ZEA production. Recently, two velvet genes, *FgVe1* and *FgVelB*, were confirmed to play critical roles in AUR production (Lee et al., 2012; Merhej et al., 2012). Specifically, *FgVe1* is essential for the expression of *Tri* genes and the production of TRIs and *FgVelB* positively regulates TRIs and ZEA production. Moreover, *Hep1* is also involved in AUR synthesis in *F. graminearum* (Reyes-Dominguez et al., 2012).

2.4. Small peptides

F. graminearum can also produce small peptides synthesized by NRPSs (Table 1). NRPSs are multifunctional proteins involved in synthesizing small peptides independently of the ribosomal protein synthesis machinery. The products of certain fungal NRPSs play critical roles in plant–microbe interactions (Lee et al., 2005; Tobiasen et al., 2007). For example, deletion of *Nrps6* in the maize pathogen *Cochliobolus heterostrophus* causes concomitantly a reduction in virulence and an

Table 3
Genes involved in energy metabolism in *F. graminearum*. MM, minimal medium; TRI, trichothecene; WT, wild-type strain.

Genes	Proteins	Phenotype of mutants	Functions	References
Acl (*Acl1* and *Acl2*)	Adenosine triphosphate (ATP) citrate lyase	Mutants did not produce any initial structures for fruiting bodies and showed severe reduction in vegetative growth and conidiation. They showed a complete loss of self and female fertility as well as a reduction in asexual reproduction, virulence, and TRIs production	Acl is a key enzyme in the generation of cytosolic acetyl-CoA. Reduction of Acl-mediated histone acetylation caused defects in sexual reproduction. Both sub-units (Acl1 and Acl2) of Acl are required for both fungal development and virulence in *F. graminearum*	Son et al. (2011a)
Acs (*Acs1* and *Acs2*)	Acetyl-CoA synthetase	Deletion of *Acs* resulted in a defect in sexual development that was mainly due to a reduction in 1-palmitoyl-2-oleoyl-3-linoleoyl-rac-glycerol production, which is required for perithecium development and maturation	Acs1 is required for perithecium maturation as well as cytosolic and peroxisomal acetyl-CoA production in *F. graminearum*. Acs2 has accessorial functions for Acs1 and has compensatory functions for Acl as a nuclear acetyl-CoA producer	Lee et al. (2011b)
Arg2	Acetylglutamate synthase	Radial growth of the Δ*Arg2* mutants was reduced, and could not grow on MM. Δ*Arg2* mutants did not produce perithecia and showed severely reduced virulence on barley heads	*Arg2* is involved in arginine biosynthetic pathway and is responsible for the arginine auxotrophy in Δ*Arg2* mutants	Kim et al. (2007)
AreA	A transcription factor	The *AreA* deletion resulted in an inability to use nitrate as a sole nitrogen source, markedly reduced virulence, loss of TRIs biosynthesis, and mutants showed immaturity of asci and did not produce mature ascospores (urea restored normal sexual development)	AreA-dependent regulation of nitrogen metabolism is required for vegetative growth, sexual development, TRIs biosynthesis, and virulence in *F. graminearum*	Min et al. (2012)
Cbl1	Cystathionine β-lyase	The Δ*Cbl1* mutants had much less aerial hyphae than the WT, and it was methionine auxotrophic and was significantly reduced in plant infection	Cbl1 catalyzes the conversion of cystathionine to homocysteine, which is a precursor for methionine synthesis	Seong et al. (2005)
GzMcl1	Methylisocitrate lyase	Δ*GzMcl1* mutants failed to grow on propionate, and double deletion of both *GzIcl1* and *GzMcl1* caused reduced virulence on host plants	GzMcl1 is required for the methylcitrate cycle in *F. graminearum*	Lee et al. (2009b)
GzIcl1	Isocitrate lyase	Δ*GzIcl1* mutants showed defects in growth on acetate and in perithecium formation but not in virulence on barley and wheat	GzIcl1 is the key enzyme of the glyoxylate cycle and is essential for self-fertility in *F. graminearum*	Lee et al. (2009b)
GzmetE	A putative homoserine O-acetyltrasferase (HOA)	Δ*GzmetE* showed pleiotropic phenotypes, including reduced virulence on host plants, lack of sexual development and different mycelial pigmentation. Mutants produced only a few or no conidia on solid medium	HOA, the first enzyme of the methionine biosynthetic pathway, is responsible for the methionine auxotrophy, which results in such pleiotropic effects on *F. graminearum*	Han et al. (2004)
Lip1	Triglyceride lipase	Δ*Lip1* mutants were reduced lipolytic activities on MM supplemented with either saturated or unsaturated lipid. They also exhibited growth deficiency on MM supplemented with the saturated triglyceride tristearin	*Lip1* encodes a secreted lipase for exogenous lipid hydrolysis and is required for utilization of triglyceride tristearin	Feng et al. (2005)
Msy1	Methionine synthase	Mutants had much less aerial hyphae than the WT, and it was defective in wheat head infection and methionine auxotrophic	Msy1 may catalyze the conversion of homocysteine to methionine	Seong et al. (2005)
Pdc (*Pdc1*, *Pdc2* and *Pdc3*)	Pyruvate decarboxylase	Δ*Pdc1* mutants produced highly wettable mycelia, and had reduced lipid accumulation in the aerial but not the embedded mycelia. They produced many immature perithecia compared with the WT and most of the immature perithecia were barren. Embedded mycelia of the *Pdc1* deletion mutants grow much slower	Pdc1 functions upstream of Acs1 in the PAA pathway. It may function as a key metabolic enzyme crucial for lipid production and is involved in vegetative growth of embedded mycelia in *F. graminearum*. *Pdc2* and *Pdc3* have no discernible effects on *F. graminearum*	Son et al. (2012)

Table 4
Other genes involved in virulence, resistance and growth in *F. graminearum*. DON, deoxynivalenol; RGS, regulator of G protein signaling; WT, wild-type strain; ZEA, zearalenone; MBC, methyl benzimidazol-2-ylcarbamate.

Genes	Proteins	Phenotype of mutants	Functions	References
Ade5	Phosphoribosylamine-glycine ligase	Δ*Ade5* mutants had reduced radial growth, and could not grow on MM, and it did not produce perithecia and showed severely reduced virulence	Ade5 may take part in purine synthetic pathway, which is important for a wide spectrum of biological processes	Kim et al. (2007)
Cps1	Adenylate-forming enzyme	Δ*Cps1* mutants showed reduced virulence, and they grew slower than the WT on nitrate-containing medium	The Cps1-controlledproduct may be a general virulence factor	Lu et al. (2003)
FgAtg15	Autophagy-like lipase	Deletion of *FgAtg15* leads to defects in conidiogenesis, conidial shapes, germination, growth rate, and aerial hyphae formation. *FgAtg15* disruptants showed severely attenuated infection towards wheat and dramatically reduced DON levels	FgAtg15 is involved in numerous developmental processes such as hyphal growth, conidial development, DON production and pathogenicity	Nguyen et al. (2011)
FgFlbA	RGS	Deletion of *FgFlbA* caused reduction in conidia production, precocious germination of conidia, higher levels of DON and ZEA production and reduced virulence. They did not develop perithecia by self-fertilization and lost its capacity for female fertility	Involved in conidia production, germination rate of spores, sexual development, mycotoxin production and virulence	Park et al. (2012)
FgFlbB	RGS	Δ*FgFlbB* mutants produced shorter and thinner conidia with fewer septa	Involved in conidia morphology	Park et al. (2012)
FgRgsA	RGS	Δ*FgRgsA* mutants showed significantly reduced vegetative growth, decrease in germination rate, higher levels of DON and ZEA and reduced virulence	Involved in vegetative growth, conidia gemination, mycotoxin production and virulence	Park et al. (2012)
FgRgsB	RGS	Δ*FgRgsB* mutants grow much slower, produced longer and wider conidia, discharged much fewer ascospores per perithecium and exhibited reduced virulence	Involved in vegetative growth, conidia morphology, sexual development and virulence	Park et al. (2012)
FgRgsC	RGS	Δ*FgRgsC* mutants discharged much fewer ascospores per perithecium	Mainly involved in sexual development	Park et al. (2012)
FgStuA	Transcription factor	The deletion mutant was greatly reduced in pathogenicity on wheat heads and in production of secondary metabolites. Spore production was significantly impaired in Δ*FgStuA*, which did not develop perithecia and sexual ascospores, and lacked conidiophores and phialides, leading to delayed production of aberrant macroconidia	FgStuA is a global transcription factor that regulates pathogenicity, spore development, and secondary metabolism in of *F. graminearum*	Lysøe et al. (2011)
GzGpa2	Gα subunits	Mutants had severely reduced pathogenicity and increased chitin accumulation in the cell wall	GzGpa2 have multiple functions in pathogenicity and chitin synthesis	Yu et al. (2008)
GzSyn1	Syntaxin-like SNARE protein	The Δ*GzSyn1* had 71% reduced hyphal growth, but produced perithecia with normal ascospores. The Δ*GzSyn1* virulence on barley was reduced by 67%	GzSyn1 is required for normal vegetative growth and virulence	Hong et al. (2010)
Mes1		Deletion of *Mes1* reduces sexual and asexual reproduction, severely perturbs the shape of macroconidia and hyphae, alters the pattern of cell wall deposition and the organization of sterol-rich rafts, and attenuates virulence on wheat heads	Mes1 contributes to virulence by facilitating the formation of a stable polarity axis during both hyphal growth and the development of reproductive structures	Rittenour and Harris (2008)
Tub1	β1-tubulin	Deletion of *Tub1* in the HR isolate GJ33 of *F. graminearum* resulted in increased resistance to carbendazim	Tub1 plays a role in the sensitivity of *F. graminearum* to carbendazim	Liu et al. (2010)
Tub2	β2-tubulin	The Δ*β2tub* mutants grew normally on MBC-free PDA medium and were supersensitive to carbendazim	It conferred *F. graminearum* resistance to benzimidazole fungicides and this gene can be used as a genetic marker	Chen et al. (2009); Liu et al. (2010)

increase in sensitivity to H_2O_2. Except for increased sensitivity to iron depletion, deletion of *Nrps6* in *F. graminearum* resulted in the same phenotypic changes as in *C. heterostrophus*, a result suggesting that *Nrps6* is conserved among diverse species of filamentous ascomycetes (Oide et al., 2006). Moreover, *Nrps6* of *F. graminearum* may be responsible for the biosynthesis of extracellular siderophores, whose role is to supply an essential nutrient, iron, to the pathogenic fungi in planta and not to act as phytotoxins, depriving their hosts of iron. Deletion of another *Nrps*, *Nrps2*, caused no marked change in *F. graminearum* but it was proven responsible for the biosynthesis of ferricrocin that is an intracellular siderophore (Tobiasen et al., 2007). However, Nrps2 affects sexual development in teleomorph of *F. graminearum*, which may be partly due to their iron deficiency (Oide et al., 2007). To date, 15 NRPS genes have been identified from *F. graminearum* (Tobiasen et al., 2007). However, little is known about their functions.

2.5. Other genes involved in secondary metabolites synthesis

In addition to the genes directly involved in the biosynthesis pathway of secondary metabolites, other genes with broad spectrum effects also play important roles in synthesis of these metabolites. These include G protein subunits, cAMP/PKA, and mitogen activated protein (MAP) kinase signal cascades that are all well-conserved pathways. For example, deletion of both *GzGpa1* (Gα subunit) and *GzGpb1* (Gβ subunit) enhanced ZEA and DON production in *F. graminearum* (Yu et al., 2008), suggesting that both genes negatively control mycotoxin production (Fig. 1). Moreover, an osmosensor histidine kinase (FgOs1) and an osmoregulatory MAPK pathway (consists of FgOs4, FgOs5 and FgOs2) have been shown to regulate secondary metabolism associated with AUR and TRIs in *F. graminearum* (Fig. 1) and this MAPK pathway was confirmed to operate similarly to their homologues of *N. crassa*, os-4, os-5, and os-2. All these three genes are responsible for the regulation of *Pks12* and *Gip2* and this osmoregulatory MAPK pathway has additional function in TRIs biosynthesis (Ochiai et al., 2007).

In summary, *F. graminearum* can produce diverse secondary metabolites, such as polyketides, TRI derivatives and small peptides, and the corresponding synthetases are PKSs, TRIs synthases and NRPSs. At present, these genes have been identified in the genomes of *F. graminearum* and other plant pathogenic fungi (Table 1) (Cuomo et al., 2007; Merhej et al., 2011a; Tobiasen et al., 2007; Zhou et al., 2008). In fact, a large portion of them are multifunctional and take part in more than one metabolite synthesis. However, only a few are functionally characterized and further studies are needed to clarify the functions of those genes and their interactions.

3. Genes involved in sexual and asexual reproduction in *F. graminearum*

As ascospores and conidia can serve as disease inocula of the FHB, the genes related to reproduction are likely involved in pathogenicity of

F. graminearum. There are diverse genes controlling the sexual and asexual developments of *F. graminearum* (Table 2, Fig. 2). In *Saccharomyces cerevisiae*, the α and a factors determine the mating type in karyogamy. Similarly, there are four pheromone precursor genes, *ppg1*, *ppg2*, *pre1* and *pre2* in *F. graminearum* (Kim et al., 2008; Lee et al., 2003). However, *ppg2* and *pre1* had no discernible effects on sexual function. While *ppg1* or *pre2* deletion mutations showed reduced fertility in self-fertilization tests by approximately 50%, with Δ*ppg1* reduced male fertility and Δ*pre2* reduced female fertility in outcrossing tests (Fig. 2). Even though the pheromone-receptor pairs (*ppg1*/*pre2*) enhance selfing and outcrossing in *F. graminearum*, they are not essential for sexual reproduction (Lee et al., 2008). Map1 in *F. graminearum* is the homologue of *M. oryzae* Pmk1 (a homologue of yeast Fus3/Kss1), which is responsible for pheromone response. So *ppg1*/*pre2* may function upstream of Map1. *Map1* deletion mutants were unable to form perithecia in vitro and were non-pathogenic (Urban et al., 2003). These results suggest that sexual development in fungi are intrinsically related to virulence.

Mgv1 is a multifunctional MAPK in *F. graminearum* and is a homologue of *M. oryzae* Mps1 (function in penetration and sporulation), essential for female fertility (Hou et al., 2002). Similarly, another MAPK (Gpmk1) in *F. graminearum* is also involved in both sexual and asexual reproduction (Fig. 2), as Δ*Gpmk1* mutants showed reduced conidial production and were sexually sterile (Jenczmionka and Schäfer, 2005; Jenczmionka et al., 2003). Ras2 is a GTPase that functions upstream of the Gpmk1 MAP kinase pathway and Fgl1. It is required for female fertility and spore germination (Bluhm et al., 2007). Recently, the FgSsk2–FgPbs2–FgHog1 MAPK cascade was identified as required for female fertility (Fig. 2), and is also involved in hyphal growth, stress responses, and plant infection (Zheng et al., 2012). It seems that most of the MAPK pathways and related regulatory genes contribute to *F. graminearum* reproduction.

Moreover, disruption of *FgFsr1* resulted in reduced virulence and loss of fertility and perithecial development in *F. graminearum*. And Fsr1 contains multiple protein-binding domains and has been hypothesized to regulate signaling, acting as a scaffold for signal transduction (Shim et al., 2006). But whether G proteins and MAP kinase signaling pathways interact with the Fsr1 pathway is unknown at present. Furthermore, *Ftl1* was a newly identified transducin beta-like gene that positively regulates female fertility and conidiation in *F. graminearum*. Its homolog in the budding yeast, Sif2, is a component of the Set3 complex (a well-conserved HDAC protein complex) that regulates sporulation. It is possible that Ftl1 regulates conidiation and plant infection by novel mechanisms (Ding et al., 2009). This is because ascospore discharge results from the buildup of turgor pressure generated by ion fluxes (K^+ and Ca^{2+}) and mannitol accumulation (Trail et al., 2002). This conclusion was verified by two calcium ion channels' (*Cch1* and *Mid1*) critical role for forcible discharge of ascospores (Cavinder et al., 2011; Hallen and Trail, 2008).

In addition to these signal pathways, there are several other genes playing important roles in sexual and asexual reproduction in *F. graminearum* (Fig. 2). For example, *Fbp1* encodes a F-box protein that presumably participates in the formation of the $SCFF^{BP1}$ complex required for ubiquitin-mediated degradation of regulatory and signaling proteins. Its role on sexual reproduction was confirmed by the abnormal ascospores produced by *Fbp1* REMI and deletion mutants (Han et al., 2007). Chs5 and Chs7 are two chitin synthases and are indispensable for perithecia formation and pathogenicity as well as for normal septa formation and hyphal growth in *F. graminearum* (Kim et al., 2009). GzSnf1 (sucrose nonfermenting 1 protein kinase) is critical for normal sexual and asexual reproduction, spore maturation and germination in *F. graminearum* (Lee et al., 2009a). Recently, another gene *Zif1*, which is involved in DON production, was shown essential in female fertility in *F. graminearum*. Deletion of the *Zif1* ortholog *MoZif1* in the rice blast fungus also caused reductions in virulence and in invasive growth (Wang et al., 2011). Other genes involved in *F. graminearum* reproduction include *Arg2* (acetylglutamate synthase), *Ade5* (phosphoribosylamine-glycine ligase) (Kim et al., 2007), *Mes1* (Rittenour and Harris, 2008), *Top1* (a topoisomerase) (Baldwin et al., 2010), *GzSyn2* (Hong et al., 2010), *Hdf1* (Li et al., 2011), *Roa* (a novel gene) (Min et al., 2010), *Gea1* (H. Son et al., 2013), and *Hex1* (M. Son et al., 2013) (Table 2).

Indeed, the relationships among sexual and asexual development related genes and pathogenicity-related genes (Table 2, Fig. 2) are complex. For example, *Hdf1* and *Zif1* were previously described as important for DON production and virulence. And *Hdf1* interacts with *Ftl1* as components of the HDAC complex. Both Δ*ftl1* and Δ*hdf1* mutants had reduced HDAC activity. While *Hdf1* affects DON production, conidiation and sexual reproduction, *Ftl1* is only involved in female fertility and conidiation. Interestingly, ZEA could enhance or inhibit perithecial formation depending on the concentration applied (Wolf and Mirocha, 1973). Therefore, it's likely that the ZEA-related genes may also have regulatory effect on sexual reproduction. Those results indicate that different genes have both common and divergent functions, which form a complicated network that regulate reproduction of *F. graminearum* as well as other cellular functions.

4. Genes involved in energy metabolism in *F. graminearum*

In fungi, both hyphal growth and pathogenic process require energy. It has been reported that the glyoxylate cycle is associated with fungal and bacterial virulence (Lorenz and Fink, 2001). Tricarboxylic acid (TCA) cycle and glyoxylate cycle play important roles in energy metabolism of fungi. In addition, fungi have a methylcitrate cycle. Fungal pathogens employ glyoxylate bypass for glycometabolism, fatty acid and amino acid catabolism as well as to use acetyl coenzyme A (CoA) that can be generated from pyruvate. Propionyl-CoA is generated along with acetyl-CoA and is toxic to fungi. Pathogenic fungi can oxidize propionyl-CoA to pyruvate via the methylcitrate cycle during infection process (Lee et al., 2009b). The glyoxylate cycle is an anaplerotic pathway of the TCA cycle that allows fungal growth on C_2 compounds by bypassing the CO_2-generating steps of the TCA cycle. The unique enzymes of this route are isocitrate lyase (Icl) and malate synthase (Mls) (Dunn et al., 2009). In the glyoxylate cycle, propionyl-CoA is oxidized to pyruvate in four steps via the methylcitrate cycle, and *GzMcl1* encodes a methylisocitrate lyase that catalyzes the last step in the methylcitrate cycle (i.e., the cleavage of methylisocitrate into pyruvate and succinate). Its role in the methylcitrate cycle was confirmed by Δ*GzMcl1* mutants failing to grow on propionate (Table 3). Meanwhile, the deletion of *GzIcl1* caused defects in growth on acetate and in perithecium formation, indicating that *GzIcl1* is essential for self-fertility in *F. graminearum*. Though the two mutants exhibited no major changes in other traits, double deletion of both *GzIcl1* and *GzMcl1* caused significantly reduced virulence on host plants, indicating that both genes have redundant functions for plant infection in *F. graminearum* by participating in different metabolic pathways for the use of fatty acids (Lee et al., 2009b).

Citrate also could pass through the tricarboxylate carriers and then cleaved by the cytosolic adenosine triphosphate (ATP) citrate lyase (Acl) into oxaloacetate and cytosolic acetyl-CoA. Once cytosolic acetyl-CoA enters the nucleus, it becomes nucleocytosolic acetyl-CoA that is particularly important in the acetylation of histones. In mammalian cells, Acl regulates the glycolytic process through histone acetylation (Son et al., 2011a). In plants, there are two sub-units of Acl responsible for *de novo* biosynthesis of lipids and in the cytosol for the mevalonate pathway or fatty acid elongation (Rangasamy and Ratledge, 2000). The two sub-units of Acl in *F. graminearum* are responsible for histone acetylation (related to sexual reproduction) and involved in asexual reproduction, virulence, and TRIs production (Son et al., 2011a).

In fungi, there exists a special pyruvate–acetaldehyde–acetate (PAA) pathway to produce acetyl-CoA. The PAA pathway converts pyruvate produced from glycolysis into acetate which is eventually transformed into acetyl-CoA (Son et al., 2012). In *F. graminearum*, three pyruvate

decarboxylases (Pdc1, Pdc2 and Pdc3) act upstream of Acs1 in the PAA pathway. Deletion of *Pdc1* reduces lipid accumulation in the aerial mycelia and reduces growth of embedded mycelia. The deletion mutants produced many immature perithecia, most of which were barren. However, Pdc2 and Pdc3 have no discernible phenotypic effects on *F. graminearum* (Son et al., 2012). *Acs1*, which encodes an acetyl-CoA synthetase, is required for perithecium maturation. Deletion of *Acs1* caused reduction in 1-palmitoyl-2-oleoyl-3-linoleoyl-rac-glycerol production, which is required for perithecium development and maturation (Lee et al., 2011b). So it's probably that Acs1 is responsible for the synthesis of this compound in *F. graminearum*. In short, Pdc catalyzes pyruvate into acetaldehyde and then acetaldehyde is oxidized to form acetate. Subsequently, acetate is converted into acetyl-CoA by Acs1 and other enzymes.

Amino acid metabolism is also related to the glyoxylate cycle and is important for a wide spectrum of biological processes in all living organisms. Methionine is an important amino acid which is a component of proteins. Methionine auxotrophy leading to loss of virulence has been reported in a human pathogenic fungus, *Cryptococcus neoformans*. Hoa (homoserine O-acetyltrasferase) is the first enzyme of the methionine biosynthetic pathway. Deletion of a Hoa gene in *F. graminearum*, *GzmetE*, led to pleiotropic phenotype changes such as virulence on host plants, conidiation, sexual development and mycelial pigmentation (Han et al., 2004). Restoration of hyphal growth on minimal medium by the addition of two intermediates (cystathionine and homocysteine) but not by homoserine or cysteine suggests that *GzmetE* functions before cystathionine and homocysteine and after homoserine or cysteine. Meanwhile, *Cbl1* and *Msy1* encode cystathionine β-lyase and methionine synthase respectively in *F. graminearum*. And it has been confirmed that Cbl1 catalyzes the conversion of cystathionine to homocysteine, which is a precursor for methionine synthesis. It was followed by the conversion of homocysteine to methionine that was catalyzed by Msy1 (Seong et al., 2005). So GzmetE catalyzes O-succinylhomoserine (comes from homoserine) and cysteine to form cystathionine, Cbl1 changes cystathionine into homocysteine and homocysteine is consequently converted to methionine by Msy1 in *F. graminearum*. In addition, Arg2 is an acetylglutamate synthase, the first enzyme in the biosynthesis of the arginine precursor ornithine. Arg2 is required for perithecia formation and high virulence of *F. graminearum* (Kim et al., 2007).

In addition to the amino acid metabolism, carbon and nitrogen sources play irreplaceable role in the growth and development of *F. graminearum* (Table 3). *Lip1* encodes a triglyceride lipase, and Δ*Lip1* mutants showed greatly reduced lipolytic activity and growth deficiency on minimal medium supplemented with specific lipids as substrate. And expression of *Lip1* was activated in planta during the fungal infection process, which suggested that *Lip1* is required for utilization of triglyceride tristearin associated with growth during infection of *F. graminearum* (Feng et al., 2005). Meanwhile, AreA is a global nitrogen metabolism regulator in filamentous fungi. AreA in *A. nidulans* and Nit2 in *N. crassa* activate the expression of metabolic enzymes and permeases required for utilization of secondary nitrogen sources when the favored nitrogen sources are limited. AreA functions similarly in *F. graminearum* and is involved in vegetative growth, sexual development, TRIs biosynthesis, and virulence (Min et al., 2012). In summary, changes in the energy metabolism will affect the many physiological activities and lead to phenotype alterations such as changes in growth rate, reproduction and virulence.

5. Other genes involved in mycelial development, resistance, and pathogenicity in *F. graminearum*

In addition to the above genes, there are many other genes also involved in hyphal development and pathogenicity in *F. graminearum* (Table 4). For example, StuA is a key developmental regulators in fungi, its homolog in *F. graminearum* (FgStuA) was shown to regulate pathogenicity, spore development, and secondary metabolism (Lysøe et al., 2011). Previous studies have shown that disruption of *F. graminearum Cps1* (encoding an adenylate-forming enzyme) resulted in reduced virulence. However, none of the Cps1 homologs previously deposited in GenBank has a known function, suggesting that Cps1-controlled product may be a general virulence factor (Lu et al., 2003). Ade5 is a phosphoribosylamine-glycine ligase that is responsible for purine biosynthesis and is required for perithecia formation and high virulence of *F. graminearum* (Kim et al., 2007). Moreover, three other genes *Zif1* (bZIP transcription factor), *Nos1* (ubiquinone oxidoreductase) and *Tbl1* (transducing beta-subunit-like gene and later was assigned *Ftl1*) are also involved in plant infection by *F. graminearum* (Seong et al., 2005), with *Ftl1* participating in the penetration and colonization of wheat tissues (Ding et al., 2009). Four other genes *Mes1* (Rittenour and Harris, 2008), *Cch1* (Hallen and Trail, 2008), *GzSyn1* (Hong et al., 2010) and *FgAtg15* (Nguyen et al., 2011) contribute to pathogen growth and virulence. Besides, regulators of G protein signaling (RGS) proteins make up a highly diverse and multifunctional protein family that plays a critical role in controlling heterotrimeric G protein signaling (Wang et al., 2013). RGS proteins have been characterized in *S. cerevisiae*, *M. oryzae* and *F. graminearum* (Dohlman et al., 1996; Park et al., 2012; Zhang et al., 2011). Recently, eight RGS and RGS-like proteins were also functionally analyzed in *M. oryzae*, and they were found to be involved in a complex process governing asexual/sexual development, appressorium formation, and pathogenicity (Zhang et al., 2011). Meanwhile, seven versatile RGS genes (*FgFlbA*, *FgFlbB*, *FgRgsA*, *FgRgsB*, *FgRgsB2*, *FgRgsC* and *FgGprK*) were reported in *F. graminearum* (Park et al., 2012). Some of the RGS mutants showed similar phenotypes as those of their homologous genes in *A. nidulans* and *M. oryzae*, suggesting RGS proteins are conserved among ascomycete fungi. Among the RGS genes, *FgFlbA* deletion in *F. graminearum* manifested a similar conidiation phenotype as *A. nidulans*. The phenotypic defects are related to cell wall integrity and *FgFlbA* is confirmed to interact with *GzGpa1* and *GzGpa2* (Park et al., 2012). GzGpa2 is a Gα subunit which has multiple functions in pathogenicity and chitin synthesis (Yu et al., 2008). So it can be hypothesized that *FgFlbA* regulate GzGpa2 to further control Chs5 and Chs7, affecting cell wall integrity and finally impairs both the sexual and asexual reproduction.

In addition to those genes, the drug-resistance genes have received increasing attentions. Repeated use of fungicides often selects for resistance, which is a major practical problem in the use of fungicides. For example, resistance to benzimidazole fungicides has been detected in many fungal species. In most cases, resistance was correlated with single-point mutations in the β-tubulin gene that encodes the target at which the fungicide binds. The tubulin gene family in *F. graminearum* has five members, including α2-tubulin, α-tubulin, β1-tubulin, β2-tubulin and the γ-tubulin genes. However, it seems that α2-tubulin, α-tubulin, β1-tubulin, and the γ-tubulin do not confer benzimidazole resistance in *F. graminearum*. Even though Tub1 (β1-tubulin) was later demonstrated to be associated with resistance to carbendazim, deletion of *Tub1* in the HR (high level of resistance to carbendazim) isolate in *F. graminearum* resulted in increased resistance to carbendazim (Liu et al., 2010). It was demonstrated that specific point mutation of Tub2 (β2-tubulin) could confer benzimidazole resistance in *F. graminearum* (Chen et al., 2009; Liu et al., 2010). Interestingly, the expression levels of *Tub2* in Δ*Tub1* mutants showed different degrees of increase, which could partially explain the increased carbendazim resistance of Δ*Tub1* mutants (Liu et al., 2010).

6. Conclusion and perspectives

Over the years, scientists have made efforts to develop predictive models and biocontrol methods to deal with the disastrous FHB (Yuen and Schoneweis, 2007). For example, De Wolf et al. (2003) tested several risk assessment models and found that only narrow time periods around crop anthesis can be used to predict FHB epidemics. Palazzini

et al. (2007) isolated 22 bacterial strains from wheat anthers that can reduce the growth of *F. graminearum* and the production of DON. Recently, Prandini et al. (2009) introduced different predictive models for FHB and related mycotoxin contamination from five countries and discussed how those predictive models could be optimized. Meanwhile, in order to develop more practical and efficient strategies to control FHB, functional analyses of *F. graminearum* genes and better understanding of the regulatory relations are needed. Although *F. graminearum* is one of the best studied plant pathogens, the genetic basis of its life cycle and pathogenicity are poorly defined. Genomics and postgenomic studies are being conducted to understand the genetic basis of this "cereal killer" (Jiang et al., 2013; Trail, 2009). Indeed, the genome sequencing of the fungus *F. graminearum* is helping to analyze the connection between different genes involved in secondary metabolites synthesis, hyphal development, and pathogenicity. Recently, Son et al. (2011b) established a database of over 11,000 phenotypes (phenome), which would help to better understand how *F. graminearum* regulates traits important for growth, development, stress response, pathogenesis, and toxin production and how transcriptional regulations of these traits are interconnected. In this review, we systematically categorized the functional genes involved in secondary metabolite synthesis, sexual reproduction, energy metabolism, and pathogenicity. We tried to identify the relationships among them in order to provide further insight into the genetic basis of interactions between *F. graminearum* and its plant hosts.

Although all the reported genes involved in secondary metabolite synthesis, hyphal development and pathogenicity have been classified roughly by their main functions (Tables 1–4), it is difficult to determine which specific gene is involved in which specific biological process. This was mainly due to the multifunction nature of many genes and the overlapping effects of different genes (Figs. 1–2). Indeed, the core components of key signaling or regulatory pathways have shown broad-spectrum effects depending on the upstream signals and downstream sensors. So the versatile trait is particularly marked in those regulatory genes. For instance, G proteins, cAMP/PKA, and MAP kinase pathways, always work closely together to regulate sexual fertility, development, and virulence in fungi (Shim et al., 2006; Yang et al., 2011). Meanwhile, complicated relationships exist among different developmental and infection processes. First, all the biochemical reactions require energy as the foundation. Therefore, if the energy metabolism was blocked or repressed, many other traits such as growth, secondary metabolite synthesis, sexual reproduction, conidiation and virulence will be all impaired. On the other hand, only under appropriate growth conditions could other functions proceed normally. Hence, growth-related genes will affect other traits such as plant infection. In conclusion, different mechanisms and pathways involved in various morphogenesis are inter-related and inter-dependent through most of the development stages. Further characterization of the network is needed to better understand the molecular mechanisms and interaction relationships of secondary metabolite synthesis, hyphal development and pathogenicity in *F. graminearum* in order to control the disastrous FHB. The expanded research efforts will likely lead to the development of effective strategies for managing FHB and other diseases in the near future.

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