Accepted Manuscript

The auxin-regulated protein ZmAuxRP1 coordinates the balance between root growth and stalk-rot disease resistance in maize

Jian-rong Ye, Tao Zhong, Dong-feng Zhang, Chuan-yu Ma, Li-na Wang, Li-shan Yao, Qian-qian Zhang, Mang Zhu, Ming-liang Xu

PII: S1674-2052(18)30310-1
DOI: https://doi.org/10.1016/j.molp.2018.10.005
Reference: MOLP 675

To appear in: MOLECULAR PLANT
Accepted Date: 17 October 2018


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

All studies published in MOLECULAR PLANT are embargoed until 3PM ET of the day they are published as corrected proofs on-line. Studies cannot be publicized as accepted manuscripts or uncorrected proofs.
The auxin-regulated protein ZmAuxRP1 coordinates the balance between root growth and stalk-rot disease resistance in maize

Jian-rong Ye*, Tao Zhong*, Dong-feng Zhang*, Chuan-yu Ma, Li-na Wang, Li-shan Yao, Qian-qian Zhang, Mang Zhu, Ming-liang Xu*

Affiliations
State Key Laboratory of Plant Physiology and Biochemistry/National Maize Improvement Center/College of Agronomy and Biotechnology/Center for Crop Functional Genomics and Molecular Breeding, China Agricultural University, 2 West Yuanmingyuan Road, Beijing 100193, P. R. China

* These authors contribute equally to this work
* Corresponding Author: mxu@cau.edu.cn

Running title: ZmAuxRP1 balances growth and defense in maize

Short summary: The maize auxin-regulated gene ZmAuxRP1 responds to pathogen challenges by rapidly and transiently reducing its expression that led to retarded root growth and enhanced disease resistance. ZmAuxRP1 promotes the biosynthesis of indole-3-acetic acid (IAA), while suppresses the formation of defense compound benzoxazinoids. The concerted interplay between IAA and benzoxazinoids can regulate the growth–defense balance to optimize plant fitness.
ABSTRACT

To optimize fitness, plants must efficiently allocate their resources between growth and defense. Although phytohormone crosstalk has emerged as a major player in balancing growth and defense, the genetic basis by which plants manage this balance remains largely elusive. Previously, we identified a quantitative disease-resistance locus, \( qRfg2 \), in maize (\( Zea mays \) L.) that protects against the fungal disease \( Gibberella \) stalk rot. Here, through map-based cloning, we demonstrate that the causal gene at \( qRfg2 \) is \( ZmAuxRP1 \), which encodes a plastid stroma-localized auxin-regulated protein. \( ZmAuxRP1 \) responded quickly to pathogen challenges with a rapid, yet transient reduction in its expression that led to arrested root growth, but enhanced resistance to \( Gibberella \) stalk rot and \( Fusarium \) ear rot. \( ZmAuxRP1 \) was shown to promote the biosynthesis of indole-3-acetic acid (IAA), while suppress the formation of defense compound benzoxazinoids. \( ZmAuxRP1 \) presumably acts as a resource regulator to modulate indole-3-glycerol phosphate and/or indole flux at the branch point between IAA and benzoxazinoid biosynthetic pathways. The concerted interplay between IAA and benzoxazinoids can regulate the growth–defense balance in a timely and efficient manner to optimize plant fitness.

Key words: maize, quantitative disease resistance, \( Gibberella \) stalk rot, \( Fusarium \) ear rot, indole-3-acetic acid, benzoxazinoids

INTRODUCTION

Plants are naturally exposed to a wide range of biotic and abiotic stresses and hence must fine-tune their resource allocation between growth and defense to optimize fitness (Huot et al., 2014; Wang and Wang 2014). Thus, plants have evolved a defense to pest/pathogen threats that can be deployed at the expense of growth; however, they can resume growth once the threat is averted (Huot et al., 2014). Phytohormonal crosstalk appears to play a key role in the growth–defense tradeoff by
implementing resource reallocation to cope with the most life-threatening stress (Ning et al., 2017). As a growth-promoting phytohormone, auxin regulates a stunning array of plant growth and developmental processes (Kieffer et al., 2010). Additionally, auxin negatively regulates plant immunity due to its antagonistic effect on the salicylic acid (SA) signaling pathway and up-regulation of expansins/extensins that loosen plant cell walls for pathogen proliferation (Catalá et al., 2000; Wang et al., 2007). Reciprocally, SA can protect the auxin/indole-3-acetic acid (AUX/IAA) transcriptional repressors from degradation to suppress auxin signaling (Wang et al., 2007; Fu and Wang 2011; Wang and Wang 2014). Moreover, jasmonic acid (JA) alters the auxin distribution in plants to suppress normal growth by interfering with auxin transport (Huot et al., 2014).

IAA is the primary auxin in plants and is mainly synthesized from tryptophan (Trp) via indole-3-pyruvate (IPA) (Won et al., 2011), although other Trp-derived intermediates, such as tryptamine (TAM), indole-3-acetamide (IAM), indole-3-acetonitrile (IAN), and indole-3-acetaldoxime (IAOx) have also been proposed as IAA precursors (Brumos et al., 2014). In Arabidopsis, IAOx is also an intermediate for biosynthesis of two antimicrobial secondary metabolites, 3-thiazol-2'-yl-indole (camalexin) and indole glucosinolates, thus linking auxin-mediated plant growth to pathogen defense (Glawischnig et al., 2004; Truman et al., 2010). Suppression of auxin signaling by the microRNA miR393 redirects metabolic flow to increase indole glucosinolate levels and decrease camalexin levels (Robert-Seilaniantz, et al., 2011).

In Poaceae, benzoxazinoid 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and its C7-methoxy derivative (DIMBOA) are potent secondary metabolites that contribute to allelopathy and defense (Ahmad et al., 2011; Dutartre et al., 2012). DIMBOA reportedly functions as a defense compound in innate immunity against pests and diseases in maize (Gierl and Frey 2001; Meihls et al, 2013; Makowska et al., 2015). Ten enzymes (BX1–BX9 and IGL1) catalyze the formation of DIMBOA-glucopyranoside (DIMBOA-Glc) from indole-3-glycerol phosphate (IGP)
(Tzin et al., 2015). The first step is the conversion of IGP to indole, which occurs in the plastids and is catalyzed by Benoxazinless1 (BX1) and indole-3-glycerol phosphate lyases (IGLs). The reactions that convert indole to DIBOA take place in microsomes and are catalyzed by four cytochrome P450 monooxygenases (BX2–5). DIBOA is glucosylated by the UDP-glucosyltransferases BX8 and BX9 (Tzin et al., 2015). Conversion of DIBOA-Glc to DIMBOA-Glc is catalyzed by the cytosolic dioxygenase BX6 and methyltransferase BX7 (Jonczyk et al., 2008). Both DIBOA-Glc and DIMBOA-Glc are stored in vacuoles and can be hydrolyzed to release biocidal DIBOA and DIMBOA upon tissue disruption (Meihls et al., 2013). DIMBOA-Glc can be further O-methylated to form HDMBOA-Glc by three homologous O-methyltransferases, BX10, BX11, and BX12 (Meihls et al., 2013; Handrick et al., 2016). Additionally, DIMBOA-Glc can also be converted into HDM$_2$BOA-Glc via three connected steps: 1) DIMBOA-Glc is firstly converted into TRIMBOA-Glc catalyzed by BX13 (2-oxoglutarate-dependent dioxygenase); 2) TRIMBOA-Glc is then O-methylated by BX7 to form DIM$_2$BOA-Glc; 3) DIM$_2$BOA-Glc is further O-methylated by BX14 to generate HDM$_2$BOA-Glc (Wouters et al., 2016).

The α- and β-subunits of tryptophan synthase (TSA and TSB, respectively) localize to the plastids where they form an active αββα heterotetramer, in which TSA cleaves IGP to form indole and channels it directly to TSB for conversion to tryptophan (Dutartre et al., 2012). However, the TSA homologs BX1 and IGL are monomers in the plastids and catalyze the formation of indole that either acts as a DIMBOA-Glc precursor or a volatile signal (Gierl and Frey 2001; Makowska et al., 2015). Thus, BX1 and IGL divert IGP and/or indole from primary metabolism (Trp) towards secondary metabolism (DIMBOA-Glc). This may be a crucial step in the evolution of secondary metabolic pathways (Frey et al., 2009; Chu et al., 2011). TSBs are divided into type 1 and type 2, and different TSB isoforms are responsible for Trp biosynthesis under different growth or environmental conditions (Erb et al., 2015). BX1 is responsible for constitutive DIMBOA production in young seedlings and IGL
is induced in response to stress signals, such as wounding, herbivory, or JA (Meihls et al., 2013; Erb et al., 2015). The coexistence of BX1 and IGL in maize indicates that the chemical-defense capacity of the plant is augmented with multiple synthase isoforms.

Plants have evolved diverse mechanisms to regulate growth and defense tradeoffs to optimize fitness in response to their dynamic environment (Ning et al., 2017). In rice, the Pigm locus confers broad-spectrum resistance to the fungus Magnaporthe oryzae. PigmS competitively attenuates PigmR homodimerization to suppress resistance; however, upon pathogen attack the PigmR-mediated grain yield penalty can be counteracted by the PigmS-mediated increase in yield (Deng et al., 2017). The transcription factor TBF1 is involved in the switch from growth to defense that occurs upon immune induction. The TBF1 promoter and 5’-leader including two pathogen-responsive upstream open-reading frames (uORFs) form a TBF1 cassette. TBF1 controls translation of AtNPR1 resulting in broad-spectrum disease resistance without compromising plant fitness (Xu et al., 2017). In comparison, although crosstalk between phytohormone signaling pathways plays a key role in growth–defense tradeoffs, its genetic basis and molecular mechanism remain to be elucidated (Huot et al., 2014).

Maize stalk rot is one of the most devastating and prevalent diseases globally, causing yield loss and deterioration of grain quality. Maize resistance to stalk rot is a quantitatively inherited trait. The disease-resistance quantitative trait loci (QTL) have been reported to act in additive, epistatic, dominant, or partially dominant ways (Yang et al., 2010; Zhang et al., 2012; Zhang et al., 2013). Although maize ear rot is a harmful disease worldwide, no resistance QTL have been cloned (Yang et al., 2017). Therefore, the discovery of resistance QTL or genes is a crucial step for the development of marker-assisted selection to breed resistant varieties. Here, we describe the map-based cloning of the causal gene ZmAuxRP1 at the stalk rot resistance QTL qRfg2, which encodes an auxin-regulated protein to fine-tune growth and disease resistance in maize.
RESULTS

Map-based identification of a candidate gene ZmAuxRP1 at qRfg2

We previously mapped the Gibberella stalk rot resistance QTL qRfg2 to a ~300-kb region on chromosome 1 (Zhang et al., 2012). With two rounds of fine-mapping, we refined the qRfg2 locus to a ~2.6 kb region containing a single candidate gene GRMZM2G063298 (Figure 1A and 1B; Supplemental Figure 1A and Supplemental Table 1). qRfg2 acts in a dominant genetic mode to reduce the disease severity index (DSI) by ~10% (Supplemental Figures 1B and 1C). From a single BC₈F₁ plant, we developed a pair of near-isogenic lines (NILs) named Y331-R (with qRfg2) and Y331-S (without qRfg2). Compared with Y331-S, Y331-R plants displayed a significant reduction in the disease severity of not only Gibberella stalk rot but also Fusarium ear rot (Supplemental Figures 1D and 1E). BAC contigs were built to reconstruct the qRfg2 genomic sequences for the resistant parent line (1145) and susceptible line (HZ4) (Supplemental Figure 2).

Comparison of the cDNA and genomic sequences revealed that GRMZM2G063298 consists of five exons and encodes a 536-residue protein with a single domain of unknown function 966 (DUF966) (Figure 1B and Supplemental Figure 3A). The GRMZM2G063298 coding regions in the parental lines 1145 and Y331 differ from each other by nine SNPs and one indel, resulting in two amino acid changes and one amino acid deletion (Supplemental Figure 3B). The most salient feature is the presence of different numbers of auxin-response elements in the mapped regulatory region: Y331 has seven copies of the core ‘TGTC’ motif but 1145 has five (Supplemental Figures 3C and 4). Thus, GRMZM2G063298 may be an auxin-regulated gene, and has been accordingly named ZmAuxRP1. We collected 68 ZmAuxRP1 orthologues from 47 plant species in the gramene database (http://www.gramene.org/) and conducted phylogenetic analysis. Maize ZmAuxRP1 has the highest sequence similarity with the sorghum protein SORBI_3001G130700 (Supplemental Figure 5A). Moreover, we conducted phylogenetic analysis of 61 DUF966-domain-containing proteins retrieved from six gramineae plants, including
Zea mays, Sorghum bicolor, Oryza sativa, Triticum aestivum, Brachypodium distachyon and Setaria italica. These 61 DUF966 proteins were clustered into seven clades (Supplemental Figure 5B), and those in the ZmAuxRP1-containing clade I showed high amino-acid identity between any two of them (Supplemental Figure 5C).

Validation and characterization of ZmAuxRP1

The native resistance gene ZmAuxRP1 fused to the CaMV-35S promoter displayed 10–33-fold enhanced expression of transgenic plants (EE+) relative to their non-transgenic siblings (EE−) (Figures 1C and 1D). Unexpectedly, EE+ plants were more susceptible to Gibberella stalk rot than their EE− siblings (Figure 1E), and more importantly, similar results were obtained for Fusarium ear rot (Figure 1F). RNA interference (RNAi)-mediated silencing of the endogenous ZmAuxRP1 allele reduced its expression by 1.5–2-fold (Figures 1G and 1H). The transgenic plants (RNAi+) were more resistant to Gibberella stalk rot than their non-transgenic siblings (RNAi−) across T1, T3, and T5 families (Figure 1I). Thus, ZmAuxRP1 is the causal gene at the qRfg2 locus, and its mRNA abundance showed an inverse relationship with maize resistance to Gibberella stalk rot and Fusarium ear rot.

Publicly available RNA-seq data indicate that ZmAuxRP1 is predominantly expressed in the ovule, ear primordia, and immature seeds (Supplemental Figure 6). ZmAuxRP1-GFP fluorescence overlapped with the chlorophyll autofluorescence, indicating that ZmAuxRP1 localizes to the plastids (Figure 2A), which are the key organelles that host major biosynthetic pathways and have central roles in energy production, redox homeostasis, and retrograde signaling (Pogson and Albrecht 2011; Stael et al., 2015). Furthermore, ZmAuxRP1 was exclusively found in the plastid stroma fraction by immunoblotting analysis (Figure 2B).

ZmAuxRP1-dependent disease resistance and root growth

Under normal growth conditions, Y331-R did not differ from Y331-S in the root growth (Supplemental Figures 7A and 7B). Accordingly, ZmAuxRP1 expression was similar in 5–7-day-old seedlings at all survey points except for 16 and 32 h after the
5-day mark (Supplemental Figure 7C). However, upon pathogen challenge, Y331-R differed significantly from Y331-S in terms of resistance, root growth, and ZmAuxRP1 expression. At 48 h after F. graminearum inoculation (hai), Y331-R seedling roots generally exhibited mild disease symptoms such as a light-brown color and only a few hyphae were visible in the vascular tissue. However, most Y331-S seedling roots displayed severe disease symptoms such as dark-brown color, shrunken size, and massive hyphae colonization of the entire vascular tissue (Figures 3A and 3B). Y331-R displayed a significantly lower DSI than Y331-S (Figures 3A and 3C).

Interestingly, after inoculation, primary root growth was significantly arrested in Y331-R with an extension of only 5.17±1.10 cm, compared with 7.21±0.82 cm in Y331-S (Figures 3A and 3D). Accordingly, ZmAuxRP1 expression profiles differed between these two NILs. In Y331-R, ZmAuxRP1 expression decreased dramatically at 2 hai and only reverted to its initial level at 32 hai. However, in Y331-S, ZmAuxRP1 expression decreased at 2 hai but soon reverted to its initial level at 4 hai and remained at least at this level thereafter. Compared with Y331-S, Y331-R had significantly lower ZmAuxRP1 expression at all time points except at 48 hai (Figure 3E). Hence, a mutually antagonistic interaction exists between root growth and disease resistance, and the resistant ZmAuxRP1 allele is more responsive to F. graminearum infection than the susceptible allele.

We then used the transgenic plants to examine the genetic effect of ZmAuxRP1 on root growth. EE+ seedlings with elevated ZmAuxRP1 transcript levels had longer primary roots, more lateral roots, and enhanced root apical meristem (RAM) activity compared with EE− seedlings (Supplemental Figures 8A to 8D). By contrast, RNAi+ seedlings with reduced ZmAuxRP1 transcripts had a shorter primary root, fewer lateral roots, and reduced RAM activity compared with RNAi− seedlings (Supplemental Figures 8E to 8H). Thus, ZmAuxRP1 presumably potentiates RAM activity to sustain rapid root growth while increasing host vulnerability to pathogen invasion.

ZmAuxRP1 expression forms a positive feedback loop with IAA biosynthesis
Elevated ZmAuxRP1 transcript levels were accompanied by auxin-enriched phenotypes, such as strong RAM activity, supernumerary lateral roots, and vigorous primary roots. In the paired samples EE−/EE+, RNAi−/RNAi+, and Y331-S/Y331-R at 24 and 48 hai, ZmAuxRP1 expression, consistently represented as FPKM (fragments per kilobase per million reads) from RNA-seq data, was found to be closely associated with endogenous IAA content (Figure 4A). The DR5:GUS reporter is widely used to monitor endogenous auxin content (Ulmasov et al. 1997). Ectopic overexpression of ZmAuxRP1 in the DR5:GUS Arabidopsis led to a significant increase in IAA content, resulting in shorter hypocotyls under dark culture conditions and an increase in beta-glucuronidase (GUS) activity in the shoots, lateral root primordia, and root tips (Figures 4B to 4G). These findings demonstrate that ZmAuxRP1 positively regulates IAA biosynthesis.

Given that ZmAuxRP1 may be an auxin-regulated protein with multiple auxin-response elements in the promoter region, we want to know how auxin regulates ZmAuxRP1 expression, root growth, and stalk rot resistance. We treated maize seedlings with exogenous naphthalene acetic acid (NAA). Compared with H2O-treated controls, seedling roots grew faster when treated with 0.1 µM NAA but slower when treated with ≥1 µM NAA (Supplemental Figures 9A and 9B). Under 0.1 µM NAA treatment, the Y331-R primary roots were significantly elongated, reaching almost the same length as those of Y331-S. Synchronously, both Y331-R and Y331-S showed enhanced disease severities, yet the difference between them was similar to that during H2O treatment. In contrast, root growth was arrested after treatment with 5 µM NAA, which in turn significantly reduced the disease severity of both Y331-R and Y331-S (Supplemental Figures 9C and 9D). Treatment with 0.1 µM NAA stimulated ZmAuxRP1 expression, which peaked at 1 h in Y331-R and at 3 h in Y331-S; whereas, treatment with 5 µM NAA resulted in gradually increasing ZmAuxRP1 expression levels (Supplemental Figures 9E and 9F). To explore the intrinsic nature of NAA-induced ZmAuxRP1 expression, we performed a dual luciferase transient expression assay in maize protoplasts with the short/long
ZmAuxRP1 promoter fragments from both the resistant 1145 and susceptible Y331 lines (Figure 5A). Overall, the short ZmAuxRP1 promoter resulted in stronger normalized luciferase (LUC) activity than the long one. The LUC gene driven by the 1145 ZmAuxRP1 promoter could be rapidly yet transiently induced by 0.1 µM NAA; however, this change was not too intensive for the Y331 ZmAuxRP1 promoter (Figures 5B and 5C). It is thus speculated that auxin is capable of directly and transiently regulating the ZmAuxRP1 gene expression, and that the resistant allele is more responsive to 0.1 µM NAA than the susceptible one. Taken together, ZmAuxRP1 expression is inferred to form a positive feedback loop with IAA biosynthesis.

ZmAuxRP1-induced transcriptome reprogramming

Transcriptome reprogramming in maize seedling roots was examined by RNA-seq with multiple paired samples. The increase in ZmAuxRP1 transcript abundance led to fewer upregulated than downregulated differentially expressed genes (DEGs), as revealed in the paired EE+/EE− transcriptomes. However, the F. graminearum-inoculated Y331-R resulted in more upregulated than downregulated DEGs compared to the non-inoculated Y331-R. This is also true for the inoculated Y331-S, although the upregulated DEGs were less than half the numbers of the inoculated Y331-R (Supplemental Figure 10A). Gene ontology (GO) analysis revealed that most downregulated DEGs in the EE+ transcriptome were remarkably enriched in defense responses to abiotic/biotic stresses, JA/SA biosynthesis/signaling pathways, and reactive oxygen species (ROS)-related processes. Reciprocally, most upregulated DEGs in the inoculated Y331-R transcriptome were enriched in the defense response pathways. In the inoculated Y331-S, relatively few upregulated DEGs were enriched in these pathways (Supplemental Figure 10B). KEGG analysis indicated that the top enriched pathways of DEGs were those related to plant immunity, such as biosynthesis of secondary metabolites, phenylpropanoid biosynthesis, and plant-pathogen interactions (Supplemental Figure 10C). Transcript abundance of genes encoding pathogenesis-related (PR) proteins, cytochrome P450,
glutathione S-transferases, transcription factors (NAC, WRKY, and TIFY), or proteins involved in the JA/SA metabolism decreased remarkably in EE+ compared to EE−, yet increased in the inoculated Y331-R relative to the non-inoculated control. However, in the inoculated Y331-S, only some of these genes had increased transcript abundance (Supplemental Figure 10D). Taken together, ZmAuxRP1 suppressed defense-oriented reprogramming of transcriptome, and the extent of decrease in ZmAuxRP1 expression on F. graminearum infection presumably leads to the difference in transcriptome reprogramming between Y331-R and Y331-S.

ZmAuxRP1 reversely regulates the biosynthesis between IAA and benzoxazinoids

Furthermore, we investigated metabolome changes in maize seedling roots in response to changes in ZmAuxRP1 transcript abundance. In contrast to the elevated IAA and 4-Cl-IAA (also synthesized through the IPA pathway), two key defense-related phytohormones (SA and JA) and benzoxazinoids (HBOA-Glc, DIMBOA-Glc, DIMBOA) decreased significantly in EE+ relative to EE− (Figures 6A to 6D). Considering that IAA and benzoxazinoid biosynthetic pathways are partially overlapped and share common upstream precursors, we then examined the quantities of key metabolites and gene transcript abundance involved in these two pathways. When comparing EE+ to EE−, the amounts of two important upstream metabolites, namely chorismic acid and anthranilate, changed little (Figure 6A); whereas, of the seven genes encoding synthase isoforms in the conversion of IGP to indole, five were significantly downregulated, including TSA1, TSB1, TSB2, TSB2c, and IGL1. Intriguingly, no IGP was detected in either EE+ or EE−, indicating a rapid turnover of IGP by the synthases. In the next IAA biosynthetic pathway, the IAA precursor Trp and its derivatives (IAOx, IPA, IAM, and IAN) remained astonishingly unchanged in their quantities; while two TAR (tryptophan aminotransferase related)- and two YUC (flavin-containing monooxygenase)-encoding genes were significantly upregulated by >2-fold (Figure 6A and Supplemental Table 2). The conversion of IPA to IAA by YUC was regarded as a rate-limiting step in IAA biosynthesis (Fu and Wang 2011).
The increases in two TARs and two YUCs, coupled with constant Trp and its derivatives, would be expected to promote IAA biosynthesis, which in turn significantly upregulated downstream growth-promoting genes, like eight expansin and five extensin genes (Supplemental Table 2). Benzoxazinoid biosynthesis diverges from the primary Trp biosynthesis at IGP and/or indole (Frey et al., 2009; Chu et al., 2011). The *IGL1* gene, which is crucial for benzoxazinoid biosynthesis under environmental stimuli, was dramatically downregulated in EE* compared to EE−. This may have caused the observed reduction in benzoxazinoids, for instance HBOA-Glc and DIMBOA-Glc (Figure 6A). Moreover, some critical genes encoding *O*-methyltransferases, like *BX10*, *BX11* and *BX14*, were also significantly downregulated (Supplemental Table 2). Coincidentally, DIMBOA content correlated inversely with IAA content, as revealed by comparing Y331-R with Y331-S at 24 hai (Figures 4A and 6E).

**DISCUSSION**

Most maize diseases are caused by necrotrophic pathogens; in turn, maize disease resistance largely shows quantitative traits and controlled by multiple small-effect QTL (Yang et al., 2010; Yang et al., 2017). It proves to be a hard work to isolate naturally-occurring resistance QTL, especially for those very small-effect QTL (Yang et al., 2017). In the current study, *qRfg2* is a minor resistance QTL against stalk rot disease, which could only explain less than 10% of total phenotypic variation (Zhang et al., 2012). With the established fine-mapping strategy (Yang et al., 2012), we managed to narrow down the *qRfg2* into a 2.6-kb region harboring a single candidate gene *ZmAuxRP1* (Figure 1), which was later confirmed by transgenic functional validation (Figure 1). *ZmAuxRP1* encodes an auxin-regulated protein with a single DUF966 domain; so far, no detailed information has been reported in its function on auxin metabolism, let alone disease resistance. *ZmAuxRP1* seems to be a distinct quantitative resistance gene which could negatively regulate maize resistance to both *Gibberella* stalk rot and *Fusarium* ear rot diseases, while positively regulate root
growth. Moreover, ZmAuxRP1 is the first natural resistance gene cloned against ear rot. The pleiotropic effects on growth and multiple disease resistance suggest that ZmAuxRP1 may be implicated in certain metabolisms required for both growth and immune response.

As an auxin-regulated protein, ZmAuxRP1 was shown to promote IAA biosynthesis, while suppressing the biosynthesis of benzoxazinoids. Consequently, the elevated ZmAuxRP1 transcript level in EE+ resulted in high IAA and low benzoxazinoid contents (Figure 6). This shift in the balance between IAA and benzoxazinoids in turn has a profound influence on both growth and defense. High IAA directly boosted root growth to increase host vulnerability to pathogen infection. Indirectly, high IAA reduced SA, JA, and abundant defensive metabolites to suppress plant immunity. Furthermore, low benzoxazinoids intensified this crippled plant immunity. In the inoculated Y331-R, however, the reduced ZmAuxRP1 expression level just acts the opposite way to arrest root growth and enforce plant immune responses to diseases (Figures 3 and 6). Although both IAA and benzoxazinoids are involved in disease resistance, they are implemented apparently in different ways. IAA has more broad inverse impacts on plant immunity through both developmental process and phytohormone crosstalk; whereas, benzoxazinoids mainly devote themselves to enforce plant immunity (Wouters et al., 2016; Maag et al., 2016). The exogenous application of 0.1 µM NAA stimulated growth to achieve no difference in the root lengths between Y331-R and Y331-S; whereas, failed to remove the difference in disease resistance between them (Supplemental Figure 9). This probably resulted from the difference in the quantity of benzoxazinoids caused by differential ZmAuxRP1 expression levels.

Coincidently, IAA and benzoxazinoid biosynthetic pathways are overlapped in upstream steps and diverge at the conversion step of IGP to indole. In maize, seven synthase isoforms function to convert IGP to indole in the plastids, the same place where ZmAuxRP1 is localized (Figure 2). ZmAuxRP1 was found to inversely regulate most synthase-encoding genes, as observed in EE+ with the elevated ZmAuxRP1
expression as well as in the inoculated Y331-R with the reduced \textit{ZmAuxRP1} expression (Supplemental Table 2). Of the seven synthase-encoding genes, \textit{BX1} and \textit{IGL1} function in diverging the benzoxazinoid biosynthesis from the primary IAA biosynthesis (Figure 6). \textit{BX1} normally shows constitutive expression, while \textit{IGL1} could be induced by biotic/abiotic stresses (Meihls et al., 2013; Frey et al., 2009).

Compared to other synthase-encoding genes, \textit{IGL1} is more responsive to the varying \textit{ZmAuxRP1} expressions, which was downregulated by > 5 folds to the elevated \textit{ZmAuxRP1} expression in EE\textsuperscript{+}; while upregulated by >3 folds to the reduced \textit{ZmAuxRP1} expression in the inoculated Y331-R (Supplemental Table 2). The synthases involved in the conversion of IGP to indole may overall have very strong enzymatic activity, since no IGP was detected in either EE\textsuperscript{+} or EE\textsuperscript{-} seedlings. Yet, allocation of the substrate IGP between IAA and benzoxazinoid biosynthesis may depend on the relative enzymatic activities at the branch point of these two pathways. The relative low (or high) IGL1 probably utilizes less (or more) IGP and/or indole for benzoxazinoid biosynthesis. Thus, \textit{ZmAuxRP1} presumably influences on the synthase isoforms to mediate the IGP/Indole flux at the branch point between IAA and benzoxazinoid biosynthesis. Since no any physical interaction was found between \textit{ZmAuxRP1} and one of the seven synthase isoforms, it remains unclear how \textit{ZmAuxRP1} regulates synthase isoforms to reallocate IGP and/or indole.

Upon pathogen infection, plants suppress growth and allocate more resources towards activating the immune response to fend off the pathogens (Huot et al, 2014; Ning et al., 2017). \textit{ZmAuxRP1} is primed to respond quickly to pathogen infection with a rapid yet transient reduction in its gene expression, which temporarily reduces IAA biosynthesis and promotes benzoxazinoid biosynthesis to elicit the defense response. When the pathogen attack is averted, \textit{ZmAuxRP1} expression increases back to the level required for normal growth (Figures 3–6). Thus, \textit{ZmAuxRP1} controls the timing and duration of the plant immune response by regulating resource reallocation. The concerted interplay between IAA and benzoxazinoids could balance plant growth and defense in a timely and efficient manner to optimize plant fitness (Figure 7).
MATERIALS AND METHODS

Plant materials

The resistant line 1145 (donor parent) and susceptible line Y331 (recurrent parent) and their backcross mapping populations have been described (Zhang et al. 2012). Advanced BC₈F₁ to BC₁₀F₁ backcross populations were used for further fine-mapping of qRfg2. In the BC₈F₁ population, an individual with the shortest qRfg2 region was self-pollinated twice to generate a pair of near-isogenic lines (NIL), namely Y331-R (resistant allele) and Y331-S (susceptible allele) at the qRfg2 locus. The genetic backgrounds of Y331-R and Y331-S were found to be >99.9% identical based on a GoldenGate 3KSNP assay (Illumina, San Diego, CA, USA).

Artificial inoculation and symptom scoring in the field

Gibberella stalk rot: Maize kernels were sterilized and inoculated with *F. graminearum* (kindly provided by Prof. Xiaoming Wang, Chinese Academy of Agricultural Sciences), followed by incubation at 25°C under complete darkness for 15 days. Artificial inoculation was conducted at the silking stage at the experimental station of China Agricultural University (Shangzhuang, Beijing). Prior to inoculation, the infected maize kernels were pooled and thoroughly mixed to ensure uniformity of the inocula. Artificial inoculation was conducted by burying ~70 g infected kernels in a hole ~5–10 cm away from each plant stalk, followed by irrigation to increase soil moisture for fungal growth and infection. Disease severity was surveyed according to our previous protocols (Yang et al., 2010).

Fusarium ear rot: Immature maize kernels at 15 days after pollination were inoculated with *Fusarium moniliforme* (kindly provided by Prof. Jianyu Wu, Henan Agricultural University). Disease severity of each ear was scored at maturity stage by using a rating scale of 1–5, where grades 1, 2, 3, 4, and 5 correspond to 0–1%, 2–10%, 11–25%, 26–50%, and 51–100% of kernels exhibiting visual symptoms, respectively.

Disease severity index (DSI) (%) = \( \Sigma \) (grade × number of plants in grade) × 100/(5 ×...
total number of plants) (Robertson-Hoyt et al., 2006).

Artificial inoculation and symptom evaluation of maize seedlings

The cyan fluorescent protein-transgenic *F. graminearum* strain was used to artificially inoculate maize seedlings (Ye et al. 2013). The *F. graminearum* conidia were prepared with liquid mung bean broth (30 g mung beans per 1 L H₂O) and incubated at 25°C for 7 days at 150 rpm. The conidia were harvested and suspended in 3% liquid mung bean broth at 2 × 10⁷ spores/mL. Maize kernels were soaked in water for 12 h, then surface-sterilized with 10% (v/v) H₂O₂ for 30 min, washed with distilled water, incubated in saturated CaSO₄ for 12 h, and cultivated for 2 days in the dark at room temperature. Thereafter, germinated seeds were cultured for 2 days in a wet chamber under a 16-h light/8-h dark cycle at 26°C. The 5-day-old seedlings were artificially inoculated according to our previous study (Ye et al. 2013). The inoculated seedling roots were scored for symptoms in the diseased region at 48 h after inoculation (hai) with a rating scale of 1–5: grade 1, light-brown without shrinkage in the diseased region; grade 2, brown colour with a little shrinkage in the diseased region; grade 3, brown colour with obvious shrinkage in the diseased region; grade 4, dark-brown with moderate shrinkage in the diseased region; grade 5, dark-brown with severe shrinkage across a large area of the diseased region (Supplemental Figure 11).

DSI (%) = Σ(grade × number of plants in grade) × 100/(5 × total number of plants).

Fine-mapping of *qRfg2*

The minor QTL *qRfg2* was previously mapped on the long arm of chromosome 1 between markers SSRZ319 and CAPSZ459 (Zhang et al., 2012) (Supplemental Table 1), which confers resistance to *Gibberella* stalk rot. A total of 29 BC₃F₁ recombinants in the *qRfg2* region were screened and further backcrossed to Y331 to produce BC₉F₁ fine-mapping populations. From BC₉F₁ populations, eight recombinants in the newly mapped *qRfg2* region were identified and again backcrossed to Y331 to produce BC₁₀F₁ progeny for further fine-mapping. All progeny from a given recombinant were grown in duplicate in the field, and each
plant was investigated for its genotype at the \textit{qRfg2} locus and disease scale. DSI was calculated for each genotype, and the paired Student's \textit{t}-test was used to examine significant differences between homozygous and heterozygous genotypes. For Y331-R and Y331-S, each NIL was grown in three replicates with 20–40 individual plants per replicate. DSI was calculated for each replicate and used to test significant differences between the two NIL using the paired Student's \textit{t}-test.

BAC library screening, sequencing, and gene annotation

BAC libraries were constructed for both the resistant 1145 and susceptible HZ4 inbred lines (Yang et al., 2013). Six markers (STSZ499, STSZ511, STSZ514, STSZ519, STSZ525, and SSRZ319) in the \textit{qRfg2} locus (Supplemental Table 1) were used to screen both BAC libraries. The resultant positive BAC clones were digested with \textit{Bam}HI for fingerprint analysis to construct 1145 and HZ4 BAC contigs. The minimal tiling paths consisting of overlapping BAC clones from both 1145 and HZ4 contigs were subjected to sequencing and gene prediction. All sequences were assembled into a continuous sequence at the \textit{qRfg2} region for both 1145 and HZ4. Repeat sequences were masked with RepeatMasker, and non-repetitive sequences were annotated using FGENESH and BLAST searches in GenBank. Nucleotide sequences of the predicted genes were aligned for the \textit{qRfg1} region among 1145, HZ4, and the reference B73.

Construction of expression vectors for functional validation

\textbf{\textit{p35S}:ZmAuxRP1-EE} vector: The positive 1145 BAC clone #104A2-1 screened from 1145 BAC library (Yang et al., 2013) was digested with \textit{Bgl}II and \textit{Xba}I. The resultant DNA fragment containing the native \textit{ZmAuxRP1} with a 2288-bp promoter, 3191-bp coding sequence, and 2766-bp 3’-untranslated region was inserted into the \textit{Bam}HI-\textit{Xba}I double-digested \textit{pGreen0229} (http://www.pgreen.ac.uk/JIT/pG0229.htm), which yielded the enhanced \textit{ZmAuxRP1} expression vector, \textbf{\textit{p35S}:ZmAuxRP1-EE}, in which native \textit{ZmAuxRP1} was driven by the CaMV35S promoter.
**p35S:ZmAuxRP1-RNAi vector:** The XhoI-EcoRI sites and PstI-BamHI sites were introduced into the 5’ terminus of the two primers, RNAi-FP/RP (Supplemental Table 1), respectively, to amplify the 210-bp 1145 ZmAuxRP1 cDNA. The resultant PCR product was inserted into vector pEASY-T1 (TransGen Biotech, Beijing, China), which was named P1. P1 was digested with XhoI and EcoRI (New England Biolabs, Beverly, MA, USA), and the digested ZmAuxRP1 fragment was inserted into pGreen-HY104 (provided by Dr. S. Yang, China Agricultural University), named P2. Finally, P1 was inserted into P2 after PstI and BamHI digestion to produce the RNAi vector p35S:ZmAuxRP1-RNAi.

Both the p35S:ZmAuxRP1-EE and p35S:ZmAuxRP1-RNAi constructs were introduced into the maize hybrid HiIII (B73×A188) via Agrobacterium tumefaciens (strain EHA105). The T₀ positive plants from each transgenic event were self-crossed to produce T₁ plants. In the following generations, heterozygous plants were selected for self-pollination to produce generations T₂ to T₆, which were used to examine the genetic effects of the candidate gene ZmAuxRP1 on resistance against Gibberella stalk rot and Fusarium ear rot in field tests. For each transgenic event, plants in each generation were grown in three replicates, and each plant was genotyped with the construct-specific primers (Supplemental Table 1). DSI for both transgenic and non-transgenic plants was calculated and used to test for significant differences in resistance using the paired Student's t-test.

**Subcellular localization of ZmAuxRP1**

The 1611-bp full-length ZmAuxRP1 cDNA was amplified from 1145 by RT-PCR with primers SC-FP/RP and cloned in pEASY-T1 (Supplemental Table 1). The full-length ZmAuxRP1 cDNA was digested with KpnI and BamHI and inserted into the KpnI-BamHI double-digested vector pEZS-NL. This resulted in the fusion gene ZmAuxRP1-GFP, driven by the 35S promoter, namely p35S:ZmAuxRP1-GFP. Polyethylene glycol–mediated maize protoplast transformation was conducted as Yang et al (2013). GFP fluorescence was detected using an LSM510 laser-scanning confocal system (Zeiss).
Chloroplast preparation and Immunoblotting

For preparation of intact chloroplasts from maize seedlings, over 20 g of seedlings was collected and homogenized in 100 mL of HS buffer (330 mM sorbitol, 5 mM ascorbate, 0.05% (w/v) BSA, 2 mM EDTA, 1 mM MgCl₂, 50 mM Hepes-KOH, pH 7.6) on ice. Chloroplast isolation and lysis, together with preparation of soluble and membrane fractions, were done according to Aronsson and Jarvis (2011). About 20 µg soluble or membrane proteins were separated by SDS-PAGE, the proteins were transferred to polyvinylidene difluoride membranes by semidry blotting. The ZmAuxRP1 antibody used here was prepared with artificial synthesis of the short peptide (32-HLEHPHFVEVPLASPEGLYLRDVINHL-58) as antigen. The peptide was coupled to the carrier protein for the preparation of immunogen. Later a single epitope specific antibody was obtained with the antiseraum immunity affinity purification.

Resistance performance and root apical meristem (RAM) activity of maize seedlings

The 5-day-old Y331-S and Y331-R seedlings were divided into two groups. One group was continuously grown for 2 days, whereas the other group was inoculated with F. graminearum and incubated for 2 days. Elongated primary root length was recorded for both NIL with or without pathogen inoculation during a period of 48 hours from 0 hai (the time of inoculation) to 48 hai. The inoculated maize seedlings were also scored for their resistance performance at 48 hai. Three biological replicates were carried out for each genotype with ~30 seedlings per replicate, and average root extension and DSI were calculated for both NILs for testing significant differences with the paired Student’s t-test.

For the ZmAuxRP1 enhanced or RNAi transgenic plants, T₄ seeds were germinated for 3 days and then continuously cultured for 4 days to measure primary root length and lateral root number. Each seedling was genotyped with PCR using primers bar-F/R for p35S:ZmAuxRP1-EE or RNAi-F/R and AuxRP-F/R for


p35S:ZmAuxRP1-RNAi (Supplemental Table 1). Three biological replicates were carried out for each T₄ transgenic event. The average primary root length and lateral root number were calculated for both transgenic and non-transgenic plants and used to test for significant differences between these plants using the paired Student's t-test.

For in vivo detection of root apical meristem (RAM) activity, the EdU (5-ethynyl-2′-deoxyuridine) was used with EdU detection cocktail (Invitrogen). Briefly, after ~6 days of culture, maize seedlings were treated with 1 μM EdU for 45 min and fixed in 4% (w/v) formaldehyde in PBS (with 0.1% (w/v) Triton X-100) for 30 min. Fixer was washed away with PBS (3 × 10 min). Maize seedlings were then incubated in EdU detection cocktail for 30 min in the dark, followed by PBS washes (3 × 10 min). The RAM was observed under an Olympus SZX16 microscope, and images were acquired using a GFP-specific filter (EdU and GFP).

Ectopic overexpression of ZmAuxRP1 in Arabidopsis, and GUS histological analysis

The 1611-bp full-length ZmAuxRP1 cDNA was amplified with primers OX-FP/RP (Supplemental Table 1) and cloned into pEASY-T1. The ZmAuxRP1 cDNA fragment was obtained by double-digestion with BamHI-KpnI and cloned into i-p1205 to construct the overexpression vector p35S:ZmAuxRP1-OX. p35S:ZmAuxRP1-OX was transformed into DR5:GUS Arabidopsis using the floral dip method, followed by screening of transgenic Arabidopsis plants with hygromycin B. Arabidopsis seeds were surface-sterilized with 70% ethanol and plated on half-strength Murashige and Skoog medium (pH 5.8) containing 1% sucrose and 0.8% agar and stored at 4°C for 2 days; then, they were grown vertically at 22°C in darkness for 3 days and imaged. For GUS staining, Arabidopsis seedlings at 10 days after germination (DAG) were selected and transferred to GUS staining solution [1 mM X-Gluc dissolved in 0.5% (v/v) DMSO, 0.5% (v/v) Triton X-100, 1 mM EDTA, 0.5 mM K₃Fe(CN)₆, 0.5 mM K₄Fe(CN)₆, 0.5 M sodium phosphate buffer, pH 7] and incubated for 10 min under vacuum. After vacuum infiltration, samples were incubated at 37°C for 12 h. The staining reaction was stopped by three rounds of 70% ethanol washes at 37°C and...
visualized under a differential interference contrasts (DIC) microscope (Olympus).

**Exogenous application of NAA**

The 5-day-old seedlings from both Y331-S and Y331-R were used to measure the effects of exogenous NAA on seedling root growth and resistance performance. Different NAA concentrations were applied to 5-day-old seedlings by direct addition to the culture solution. To determine the effect of NAA on resistance performance, exogenous NAA (0.1 or 5 µM) was directly added into the macroconidia solution. The primary roots at 48 h after treatment were sampled for resistance performance and seedling root measurement. The experiments were done with three biological replicates, and the paired Student's t-test was used to test the significance of differences between the two NILs.

**Promoter activity assay**

The *cis*-elements were identified by searching the PLACE database (http://www.dna.affrc.go.jp/PLACE). Different *ZmAuxRP1* promoters were cloned with various lengths, the L-promoter is 1703 bp (for 1145) or 1727 bp (for Y331) upstream of the *ZmAuxRP1* start codon; the S-promoter is 426 bp (for 1145) or 418 bp (for Y331) upstream of the *ZmAuxRP1* start codon (Supplemental Table 1). These promoter segments were modified by introducing a *Hind* III site at the 5’ end and an *Nco* I site at the 3’ end of the sequences, allowing these promoters to be cloned as a transcriptional fusion with the *LUC* gene in the pGreenII 0800-LUC vector. The *Renilla* luciferase (REN) gene driven by the cauliflower mosaic virus (CaMV) 35S minimal promoter was used as an internal reference. The transformed protoplasts were incubated for 18 h at 28°C. Prior to activity analysis, the protoplasts were treated with 0.1 µM NAA for 1, 3, or 6 h. Sampled protoplasts were lysed in 100 µl lysis buffer (Promega, E1910) and assayed using the Dual-Luciferase Reporter Assay System (Promega, E1910), following the manufacturer’s instructions. Ratios of LUC to REN activities were used to define normalized promoter activity. Three biological replicates, each with four technical replicates, were set for each construct.
RNA-seq analysis

The primary roots of 7-day-old seedlings were harvested from the differentially treated samples, including T4 transgenic and non-transgenic plants or inoculated and non-inoculated NIL, followed by immediate freezing in liquid nitrogen and storage at –80°C until RNA extraction. Total RNA extraction and poly(A) RNA isolation were performed according to the manufacturer's protocol (Invitrogen). A fraction of 100 ng poly(A) RNA from each sample was fragmented for RNA-seq library construction according the manufacturer's recommendations (Illumina) and sequenced with the Illumina Hiseq3000. Approximately 40 million clean reads were used for mapping, calculation, and normalization of gene expression. Reads were aligned to the masked maize genome database Ensemble Zea_mays. AGPv3.26 (http://plants.ensembl.org/Zea_mays/Info/Index). Overall, >88% of reads were mapped to maize protein-coding genes. Calculation and normalization of gene expression were based on the FPKM (fragments per kilobase of exon model per million mapped reads) using Cufflinks, version 2.1.1. Differentially expressed genes (DEGs) were defined using a fold change (FC) >2.0 or <0.5 with \( P < 0.05 \) and a false discovery rate (FDR) of \( \leq 0.05 \) compared with expression level measured in the control transcriptome, according to Ye et al (2013). Gene ontology enrichment and KEGG enrichment (KEGG enrichment/pathway graph) were performed using the identified DEGs.

Real-time RT-PCR

Total RNA was isolated from primary seedling roots or other maize tissues using TRIzol reagent (Invitrogen). First-strand cDNA was synthesized from 1 \( \mu \)g total RNA with M-MLV (Moloney murine leukemia virus) reverse transcriptase (Promega). Quantitative RT-PCR was performed using a CFX96 real-time PCR detection system with iQ SYBR green supermix (Bio-Rad, Hercules, CA, USA). Primers were designed with Primer Express Software according to standard parameters for real-time RT-PCR assays (Bio-Rad). Primers GAPDH-FP/RP were used for the GAPDH2 internal control, and primers Aux-FP1/RP1 were used for ZmAuxRP1. Expression
analysis in transgenic *Arabidopsis* used primers Aux-FP2/RP2 for *ZmAuxRP1* and primers ACT-FP/RP for the internal control *ACT2* (*At3g18780*) (Supplemental Table 1). Each expression analysis was carried out with more than three biological replicates, with three technical replicates for each biological replicate.

**Quantification of phytohormones and DIMBOA**

The maize seedling roots from differentially treated samples were used to quantify phytohormones and DIMBOA. These samples included the T4 transgenic and non-transgenic maize seedlings at 7 DAG, inoculated and non-inoculated NIL seedlings at 24 and 48 hai, and *Arabidopsis* transgenic and non-transgenic seedlings at 10 DAG. These samples were collected, immediately frozen in liquid nitrogen, and stored at −80°C. The content of each of IAA, JA, SA, and DIMBOA was quantified using ultra-high-pressure liquid chromatography-coupled tandem mass spectrometry (UHPLC-MS/MS). Refer to Liu et al. (2012) for experimental details. Samples from three biological replicates were quantified individually.

**Metabolite profiling with LC-MS**

Primary roots were collected from 7-day-old maize seedlings and dipped in liquid nitrogen. After freeze-drying and homogenization, the dried root samples (~100 mg) were extracted via ultrasonic treatment on ice for 30 min in 1 mL each of 100% methanol, 70% methanol, and 100% isopropanol, followed by centrifugation for 10 min at 11,000 rpm at 4°C. The supernatant was collected for subsequent analyses. Chromatographic analysis of the maize root extracts was performed using HPLC (DIONEX UltiMate 3000, USA) whereas secondary metabolites were profiled and identified using LC-MS (Thermo Scientific Orbitrap Fusion Mass Spectrometer, FSN10185). All manipulations were performed as described (Walker et al., 2011). Metabolite profiling was recorded in both negative and positive modes, and compounds were identified according to standardized guidelines using the mass spectrum and retention-time index matched to the reference collection of authenticated standard substances and of frequently observed but not yet identified
mass spectral tags from the Golm Metabolome database, GMD (http://gmd.mpimp-golm.mpg.de) and to the collection of mass spectra of the NIST08 database (http://www.nist.gov/srd/mslist.htm). Numerical analyses were based on the peak height values of the recorded mass feature, i.e. the response values. These values were corrected for the weight of each sample and for the response of the internal standard from each respective LC-MS chromatogram to obtain normalized responses. Samples from six biological replicates were prepared and quantified separately.

**Statistical analysis**

Datasets were analyzed using Prism 6 software (GraphPad Software). Statistical analysis was performed with the paired Student's *t*-test. All values represent the mean ± SD. *P* < 0.05; **P** < 0.01; ***P** < 0.001.

**SUPPLEMENTAL INFORMATION**

**Supplemental Table 1.** Primers and markers used in these experiments.

**Supplemental Table 2.** Expression changes of the genes that related to IAA and benzoazinoids biosynthesis pathway.

**Supplemental Figure 1.** Fine-mapping of qRfg2 and its genetic effect on Gibberella stalk rot and Fusarium ear rot.

**Supplemental Figure 2.** Screening and assembly of positive BACs and sequence characterization of the qRfg2 region among the resistant 1145, susceptible HZ4, and reference B73 lines.

**Supplemental Figure 3.** Sequence characteristics of ZmAuxRP1 between the two parental lines 1145 and Y331.

**Supplemental Figure 4.** Sequence characterization of ZmAuxRP1 between the parent line 1145 and Y331.

**Supplemental Figure 5.** Phylogenetic and alignment analysis of ZmAuxRP1 proteins from various plant species.
Supplemental Figure 6. Expression of ZmAuxRP1 in various tissues from different developmental stages.

Supplemental Figure 7. Root growth and ZmAuxRP1 expression in the uninfected NIL Y331-R and Y331-S.

Supplemental Figure 8. Primary root length, lateral root number, and RAM activity in T4 EE+ and EE− seedlings.

Supplemental Figure 9. Effect of exogenous NAA on root growth and disease development in maize seedlings.

Supplemental Figure 10. ZmAuxRP1-induced transcriptome reprogramming in maize seedling roots.

Supplemental Figure 11. Disease severity grades for inoculated maize seedling roots.

ACKNOWLEDGEMENTS

We thank Prof. Xaoming Wang for providing Fusarium graminearum. We thank Dr. Shijuan Yan (Guangdong Academy of Agricultural Sciences) for help with phytohormone and metabolome analyses. This work was funded by the Ministry of Agriculture of China (grant numbers: 2018ZX0800917B) and the National Natural Science Foundation of China (31671704).

AUTHOR CONTRIBUTIONS

M. Xu and J. Ye designed the project. T. Zhong, J. Ye, and D. Zhang conducted experiments. L. Wang, Q. Zhang, C. Ma, and M. Zhu participated in some experiments. T. Zhong and J. Ye analyzed the data. J. Ye and M. Xu wrote the manuscript, and M. Xu supervised the project. All authors read and approved the manuscript.


FIGURES AND LEGENDS

Figure 1. Map-based cloning of the resistance QTL $qRfg2$ against *Gibberella* stalk rot and *Fusarium* ear rot.

(A) Phenotypes of stalk, transverse, and longitudinal sections from resistant (left) and susceptible (right) plants.

(B) Fine-mapping of the resistance QTL $qRfg2$ to an interval of ~2.6 kb. After BAC library screening, sequencing, and gene prediction, a single candidate gene (GRMZM2G063298) was identified and named ZmAuxRP1.

(C) Schematic diagram of the $p35S:ZmAuxRP1$-EE construct. The native ZmAuxRP1 gene from the resistant line 1145 was cloned into pGreen0229. ZmAuxRP1 was driven by the CaMV 35S promoter (35S) and terminated with the CaMV terminator (CaMV Ter).

(D) ZmAuxRP1 expression in two transgenic events (3-1 and 4-3) was 33-fold and 10-fold higher in the transgene-positive (EE+) plants than in the transgene-negative (EE-) plants, respectively, as detected in 10 plants for each group.

(E–F) The disease severity index (DSI) was greater for EE+ plants than EE- plants in field inoculation tests for *Gibberella* stalk rot in both the 3-1 and 4-3 transgenic events in 2014 (T1 family) and 2015 (T3) (E), and also for *Fusarium* ear rot in 3-1 plants in 2015 and 2016 (F).

(G) The $p35S:ZmAuxRP1$-RNAi construct containing the 209-bp sense and antisense ZmAuxRP1 cDNAs, 1023-bp GUS linker, and flanking 35S and CaMV Ter sequences.

(H) ZmAuxRP1 expression was ~33% lower in the transgene-positive (RNAi+) plants compared with transgene-negative (RNAi-) plants for the 12-5 and 13-9 transgenic events.

(I) The DSI was lower in RNAi+ plants than RNAi- plants for the 12-5 and 13-9 transgenic events in field inoculation tests in 2013 (T1 family), 2014 (T3), and 2015 (T5). Values are the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 (according to a paired Student’s t-test); NS, not significant.
Figure 2. Subcellular localization of ZmAuxRP1.

(A) The \textit{p35S:ZmAuxRP1-GFP} construct containing the \textit{ZmAuxRP1-GFP} fusion driven by the CaMV 35S promoter was transformed into maize protoplasts and visualized via confocal laser-scanning microscopy at 24 h after transformation. The GFP signal from the control pEZS-NL vector was evenly distributed throughout the maize cell cytoplasm (bottom), whereas the signal from \textit{p35S:ZmAuxRP1-GFP} exclusively co-localized with the red autofluorescence of chloroplasts. Thus, ZmAuxRP1 specifically localized to chloroplasts/plastids. Bar = 20 µm. DIC, differential interference-contrast. (B) ZmAuxRP1 specifically localized to chloroplast stroma, but not associate with chloroplast membrane by western blot analysis with a ZmAuxRP1-specific antibody.
Figure 3. Characterization of the resistance performance, root growth, and ZmAuxRP1 expression in NIL Y331-R and Y331-S after inoculation with *F. graminearum*.

(A) Maize seedlings at 48 h after inoculation (hai) with *F. graminearum*. Images in the dashed boxes are the magnified diseased root regions that are indicated with arrows.

(B) Microscopy images of transverse sections of infected seedling roots. *F. graminearum* hyphae (green signals) were detected in several epidermal cell layers and partial vascular tissue in Y331-R seedling roots, whereas massive *F. graminearum* hyphae were observed in seedling roots, through the epidermal and cortical cell layers up to the vascular tissue in Y331-S roots. Scale bar = 20 µm. Ep, epidermal cells; Cc, cortical cells; Va, vascular cylinder; DIC, differential interference-contrast.

(C-D) Disease severity index (DSI) and primary root extension at 48 hai for Y331-R and Y331-S plants. Y331-R seedlings had a lower DSI (C) and less root extension (D) compared with Y331-S seedlings at 48 hai. Values are the mean ± SD.

(E) Dynamic ZmAuxRP1 expression in seedling roots after inoculation with *F. graminearum*. Prior to inoculation with *F. graminearum* (0 hai), ZmAuxRP1 transcript abundance did not differ significantly between Y331-S and Y331-R, whereas ZmAuxRP1 transcript abundance decreased significantly in Y331-R after inoculation compared with Y331-S until 48 hai. Values are the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 (according to a paired Student's *t*-test); NS, not significant.
Figure 4. *ZmAuxRP1* expression correlates strongly with endogenous IAA content.

(A) Relationship between *ZmAuxRP1* expression and endogenous IAA content in paired samples of *T*$_4$ EE$^{-}$/EE$^+$, *T*$_4$ RNAi$^{-}$/RNAi$^+$, and Y331-S/Y331-R (at 24 or 48 hai). Increased *ZmAuxRP1* expression in *T*$_4$ EE$^+$ seedling roots associated with an increase in IAA content compared with *T*$_4$ EE$^-$ seedling roots. In contrast, reduced *ZmAuxRP1* expression in inoculated Y331-R seedling roots at 24 hai was associated with a reduction in IAA content compared with Y331-S seedling roots. Similar results were observed for *T*$_4$ RNAi$^+$/RNAi$^-$ and Y331-R/Y331-S at 48 hai, although the differences did not reach statistical significance. FPKM, fragments per kilobase per million reads; FW, fresh weight.

(B–G) Ectopic overexpression of *ZmAuxRP1* in DR5:GUS Arabidopsis. Comparison of the *ZmAuxRP1*-specific RT-PCR products between the transgenic (OX) and DR5:GUS (CK) Arabidopsis, with *ACT2* (*At3g18780*) as an internal control (B). *ZmAuxRP1* overexpression in Arabidopsis (OX) significantly increased IAA content compared with CK Arabidopsis (C). When grown in darkness, the 3-day-old OX seedlings had shorter hypocotyls than CK seedlings (D). The 10-day-old OX seedlings displayed more intense GUS staining in shoots (E), lateral root primordia (F), and root tips (G) compared with CK seedlings. Red arrows (F) indicate lateral root primordia. Bar = 1 mm. Values are the mean ± SD. *P* < 0.05, **P** < 0.01, ***P*** < 0.001 (paired Student's *t*-test); NS, not significant.
Figure 5. The effect of exogenous auxin on ZmAuxRP1 expression and its promoter activity.

The activity of the ZmAuxRP1 promoter from parent lines 1145 and Y331 responds differently to exogenous auxin. (A) The location of AuxREs and length of the long (L) or short (S) ZmAuxRP1 promoters from 1145 and Y331, the L-promoter is 1703 bp (for 1145) or 1727 bp (for Y331) upstream of the ZmAuxRP1 start codon; the S-promoter is 426 bp (for 1145) or 418 bp (for Y331) upstream of the ZmAuxRP1 start codon. (B) Schematic diagrams of the different ZmAuxRP1 promoters fused with the LUC gene. The Renilla luciferase (REN) gene driven by the cauliflower mosaic virus (CaMV) 35S minimal promoter was used as a control. (C) The activity of different-length ZmAuxRP1 promoters from 1145 and Y331. The two S-promoters were associated with stronger normalized luciferase (LUC) activity than the two L-promoters. Both the S-promoter and L-promoter from 1145 could be rapidly yet transiently induced by 0.1 µM NAA; however, the S-promoter and L-promoter from Y331 showed little induction by auxin.
Figure 6. ZmAuxRP1-induced changes in the abundance of key metabolites and the expression of genes involved in IAA and DIMBOA biosynthetic pathways.

(A) Metabolite content and expression of genes involved in IAA and DIMBOA biosynthesis in EE+ and EE− seedling roots. The relative content of intermediate metabolites and difference in expression of relevant genes was calculated by comparing EE+ with EE− seedling roots. In contrast to the increased IAA/4-Cl-IAA levels in seedling roots from T4 EE+ plants compared to EE− plants, DIMBOA-Glc and its precursor HBOA-Glc contents were significantly reduced. Expression of YUCs and TAR4s was greater in seedling roots from T4 EE+ plants relative to those from EE− plants, whereas the expression levels of IGL1, TSAαs and TSBβs were lower, and that of BX1 was unchanged.

(B–D) HPLC analysis revealed that the elevated ZmAuxRP1 expression in EE+ seedling roots resulted in significantly reduced DIMBOA (B), SA (C), and JA (D) contents compared with EE− seedling roots.

(E) DIMBOA content was significantly higher in Y331-R seedling roots than in Y331-S seedling roots at 24 hai, but the difference was not significant at 48 hai. FW, fresh weight. Values are the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 (paired Student’s t-test); NS, not significant. ANT, anthranilate; BX1, Benzoxazinless1; CA, chorismic acid; DIMBOA-Glc: 2-(2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one)-β-D-glucopyranose; HBOA-Glc: 2-(2-hydroxy-2H-1,4-benzoxazin-3(4H)-one)-β-D-glucopyranose. IAA, indole-3-acetic acid; IAM, indole-3-acetamide; IAN, indole-3-acetonitrile; IAOx, indole-3-acetaldoxime; IGL, indole-3-glycerol phosphate lyase; IGP, indole-3-glycerol phosphate; IPA, indole-3-pyruvic acid; TSA, tryptophan synthase α; TSB, tryptophan synthase β; Trp, tryptophan; YUCCA, flavin-containing monooxygenase; TAR4, Tryptophan aminotransferase-related protein 4.
Figure 7. A model for ZmAuxRP1-mediated balancing of the biosynthesis of IAA and DIMBOA-Glc in maize.

ZmAuxRP1, a plastid-localized auxin-regulated protein, promotes IAA biosynthesis while suppressing the formation of benzoxazinoids, which are potent defense-related metabolites in maize. Under normal growth conditions, both resistant and susceptible ZmAuxRP1 alleles are expressed in rapidly growing young tissues/organs so as to allocate more IGP/indole for IAA biosynthesis. Upon pathogen infection, the resistant ZmAuxRP1 allele responds to pathogen invasion with a rapid yet transient reduction of gene expression. Plants with reduced ZmAuxRP1 expression thus temporally allocate more IGP/indole for benzoxazinoid biosynthesis and less for IAA biosynthesis to reinforce defense capabilities. However, the susceptible ZmAuxRP1 allele responds with only a slight reduction of gene expression, and thus plants continue to grow and are vulnerable to pathogen invasion. ZmAuxRP1 presumably acts as an integral regulator responsible for resource reallocation between IAA and benzoxazinoid biosynthesis. Eventually, the concerted interplay between IAA and benzoxazinoids may rapidly and efficiently modulate the balance between growth and defense to optimize plant fitness. Solid arrows refer to pathways with identified enzymes, and dashed arrows refer to undefined pathways. ANT, anthranilate; BX1, Benzoxacinless1; CHA, chorismic acid; IGL, indole-3-glycerol phosphate lyase; IGP, indole-3-glycerol phosphate; Trp, tryptophan; TSA, tryptophan synthase α; TSB, tryptophan synthase β; YUC, flavin-containing monooxygenase (YUCCA).