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Araştırma Makalesi - Research Article

Involvement of *ZmMPK14* in Plant Defense Revealed by Comparative Expression Analysis

ZmMPK14'ün Bitki Savunmasında Görev Aldığının Mukayeseli Ekspresyon Analiziyle Belirlenmesi

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ABSTRACT

Mitogen-Activated Protein Kinases (MAPKs) function in signaling pathways as modules cascading between stimulus activated sensors and response mechanisms.*ZmMAPK14*, a group C final MAPK of this cascade was identified as a differentially expressed message in cDNA-AFLP studies of both susceptible and resistant genotypes, where a gradual induction was displayed in the resistant genotype while a clear repression occurred in the susceptible genotype in response to *Puccinia sorghi* (*Ps*) inoculation. RT-qPCR verification studies, however, did not reveal the same pattern of expression in that both displayed inductions at different levels. *Ps* inoculation induced a limited expression increase fluctuating between 1.5 and 2.5-fold in the susceptible genotype while a dramatic upregulation starting at 12 h with a 149-fold and gradually increasing to a maximum level of 477 folds at 72 h in resistant genotype was observed. To obtain further evidence about its role in plant defense, *ZmMapk14* expression in response to applications of chitin, Salicylic Acid (SA) and H_2O_2 at six time points covering a 0-24 h interval were studied in both genotypes. All treatments induced *ZmMapk14* expression in the resistant genotype significantly at different levels while the expressional changes in the susceptible were more complex and limited similar to the *Ps* inoculation levels in the susceptible. Overall, the results show that *ZmMAPK14* display differential expression in resistant and susceptible genotypes in response to *Ps* inoculation and applied defense compounds, and it presumably functions in plant defense to *Ps*.

Keywords- ZmMAPK14, Plant Defense, SA, Chitin, H_2O_2

ÖZ

Mitojenle-Aktive olan Protein Kinazlar (MAPKs) stimulusla aktive olan sensörlerle yanıt mekanizmaları arasında sinyal iletiminde kaskadlar halinde fonksiyonel olan moleküllerdir. Bu kaskadların son basamağında bir grup C MAPK olan *ZmMAPK14* duyarlı ve dirençli genotipi cDNA-AFLP çalışmalarında diferansiyel ekspresyon gösteren bir mesaj olarak tanımlanmıştır: *Puccinia sorghi* (*Ps*) inokülasyonuyla dirençli genotipte göreceli bir indüksiyon görülürken, duyarlı genotipte belirgin bir represyon tespit edilmiştir. RT-qPCR çalışmaları diğer taraftan *Ps* inokülasyonuyla her iki genotipte farklı düzeylerde indüksiyon olduğunu göstermiştir: Duyarlı genotipte kontrole göre 1.5- 2.5 kat arasında değişim gösteren bir ekspresyon artışı görülürken dirençli genotipte 12. h'de 149 kat ile başlayan ve 72. h'de 477 kat tepe değerine ulaşılan dramatik göreceli bir artış gözlenmiştir. Söz konusu MAP Kinazın bitki savunmasında rolüyle ilgili daha somut bulgular elde etmek için, kitin, Salisilik Asit (SA) ve H_2O_2 uygulamalarıyla *ZmMapk14* ekspresyonunda değişim her iki genotipte 0-24 aralığını kapsayan altı örnekleme noktasında çalışılmıştır. Tüm uygulamalar dirençli genotipte istatistiki önemli indüksiyonlar ortaya çıkarırken, duyarlı genoptipte ekspresyon değişimleri *Ps* uygulamasındakine benzer düzede kompleks ve sınırlı

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olarak tespit edilmiştir. Sonuçlar, *ZmMAPK14* ekspresyonunun gerçekleştirilen uygulamalarla değişim gösterdiğini ve *Ps*'e karşı bitki savunmasında fonksiyonel olduğunu göstermektedir.

Anahtar Kelimeler-ZmMAPK14, Bitki Savunması, SA, Kitin, H_2O_2

I. INTRODUCTION

MAPKs, as constituents of the many signal transduction pathways, function between stimulus activated sensors and target molecules, which appear to regulate many aspects of the plant life from growth and development to biotic and abiotic stress responses [1,2]. Plant MAPK cascades formed by the members of three or more protein kinase sub-families (MAP4K, MAP3K, MAP2K and MAPK) are linearly organized and sequentially activated via phosphorylation in relaying signal to downstream target molecules [3,4].

A number of studies have been conducted in maize to identify MAP Kinase genes in genome-wide: Kong et al. [5,6] concluded that there are 74 MAP3Ks and 9 MAPKKs in the genome. Wei et al. [7], on the other hand, detected 9 MAP4Ks, 84 MAP3Ks, 18 MAPKKs and 19 MAPKs. Based on sequence and structural homology to the *Arabidopsis* and rice MAPKs, 19 maize MAPKs have been classified into four major clades corresponding to the groups, A, B, C and D in *Arabidopsis* [7]. According to the activation loop sequence motif, MAPKs can be either of two subtypes, as TEY or TDY, both of which are phosphorylated at threonine and tyrosine residues by MAP2Ks. Maize group A, B, C MAPKs are TEY type containing 4, 2 and 2 identified members respectively while the group D comprises 11 TDY type MAPKs [8,9]. In identification and naming maize MAPKs, different similarities and designations have been reported: Liu et. al. [8] designated the members of group C MAPKs as *ZmMAPK1* and *ZmMAKP2*, whereas Wei [7] named them as *ZmMAPK8* and *ZmMAPK9* while Sun et. al. [10] identified them as *ZmMAPK2* and *ZmMAPK8*. In a more comprehensive study, Mohanta et. al. [9] found them closer to the *AtMAPK7* and *AtMAPK14* and designated as *ZmMAPK7* and *ZmMAPK14* (used in this report as well) respectively. *ZmMAPK14* encodes a 370 aa polypeptide with the size of 42.46 kDa.

MAPK cascades, regarded as central signaling modules in plant immunity and regulation, have been shown to participate in both Pattern-Triggered Immunity (PTI) and Effector-Triggered Immunity (ETI) [4,11-14]. One of the early events was demonstrated to be the MAPKs activation occurring transiently in PTI after a pathogen/microbe-associated pattern (P/MAMPs) recognition and during ETI in a sustained manner [14,15]. Identified MAPK cascades have been shown to function in multiple defense responses ranging from synthesis of reactive oxygen species (ROS), plant stress/defense hormones and phytoalexins to cell wall strengthening, defense gene activation and hypersensitive reaction (HR) induction [4,15]. To counteract these defense responses and promote virulence, pathogens are shown to employ effectors to block MAPKs and preceding signaling components [13,15-18]. *Arabidopsis* MAPK3/6 (Group A) and MAPK4 (Group B) and their identified cascades are regarded as the hallmark of plant immunity [2,13]. Similarly, the group C orthologues i.e., *OsMAPK3* in rice [19], *GhMAPK2* in cotton [20,21] and a number of other MAPKs have been reported to participate in plant defense signaling [2]. Two well-characterized MAPK cascades are known to be activated upon perception of PAMPs in *Arabidopsis*: One is formed by MAPKKK3/5, MAPKK4/5 and MAPK3/6 and functions downstream of Pattern Recognition Receptors (PRRs) of FLS2 and EFR [22]. The other one consists of MAPKKK1, MAPKK1/2 and MAPK4 [23]. Several *Pseudomonas* effectors are known to target the components of both cascades in suppressing PTI and regulating plant immunity: HopAI1 suppresses PAMP induced gene expression and callose deposition by directly interacting with MAPK3 and MAPK6 while HopF2 appears to target MAPKKs to inhibit flg22 induced MAPK activation. As an effector, HopAI1 activates SUMM2 R protein mediated ETI by inhibiting kinase activity of MAPK4 in phosphorylation of CRCK3 [13].

Although plant MAPK cascades are regulated via posttranslational modifications, transcriptional modulations of MAP kinase genes are also frequently observed [24-26], indicating that initial production and turn-over compensation of signaling components along with the expressional dynamics are presumably important in regulation and signal transduction. Expressional modulations of MAPKs in response to pathogen infections and applications of defense signaling molecules are frequently reported in many publications [7,19,20,27,28]. One of the well-studied MAPK genes, *AtMAPK3* (orthologues in other plant species) is rapidly induced in *Arabidopsis* seedlings upon treatment with chitin and pathogens [4,29]. Similarly, *OsMAPK3* [19] and *GhMAPK2* [20] are also induced in response to pathogen/pest challenges and applications of defense hormones (SA and JA) and defense-related compounds such as ethylene and H_2O_2. A maize orthologue of these MAPKs, *ZmMAPK14* was identified as expressional modulation showing cDNA-AFLP tag in a previous study in that it displayed a marked repression

in samples of susceptible genotype and gradual induction in samples of the resistant genotype [30]. Present study was conducted to verify a previous cDNA-AFLP study observations and to obtain further evidence about its role in plant defense. Results of the RT-qPCR studies carried out to examine the expressional modulations induced in both susceptible and resistant genotypes by *P. sorghi* inoculations and the individual applications of defense inducing compounds, chitin, H_2O_2 and SA are reported here.

II. MATERIALS AND METHODS

A. Biological materials, growth conditions and treatments

P. sorghi isolate T09 and two maize lines, A188 inbred and *Rp1-G*, which are susceptible and resistant to T09 respectively, were used as biological materials in the study. Both A188 and *Rp1-G* seedlings were grown and maintained at 24-27 ºC with a 16/8h photoperiod in rust free clean chambers before the rust challenge and chemical treatments. Healthy fully-grown seven-day old seedlings were arranged in parallel as control and treatment groups. In rust treatment experiments, control group of both resistant and susceptible genotypes were rubbed with ddH_2O while treatment groups of both genotypes were inoculated with freshly collected urediniospores by rubbing ddH_2O-wetted fingers as in control groups. In chemical treatment experiments, plant materials were also prepared in the same way in that applications were carried out by spraying onto the leaves. Treatments were carried out by spraying individually 10 mM solutions of either H_2O_2, SA or 5 mg/ml sonicated shrimp shell chitin to their respective seedling set while sterile dH_2O (treatment solvent) was sprayed to the control plants of each treatment. Following rust inoculation, both control and treatment plants were incubated overnight (in dark) at 24 ºC in mist chambers with 95%< humidity and subsequently transferred to a room with a 16/8 h (day/night) photoperiod at 24-27°C. Each experiment was conducted as three biological replicates along with their controls. Control and treatment leaf tissue samples were collected in parallel from both control and treatment plants at each time point as 0 (control), 6, 12, 24, 48, 72 and 96 post-application hours in rust experiments while chemical treatment leaf tissues were sampled in the same way at 0 (control), 1, 2, 4, 6 and 24 h of post-application. In each sampling event of all experimental groups, ~7 cm segment (extending from tip to base) of the second leaf (which is ~0.1 g) was taken. Samples were immediately placed into sterile 1.5 ml eppendorf tubes and frozen in $N_{2(L)}$, stored in $N_{2(L)}$ until grinding and addition of RNA extraction buffer.

B. RNA isolation and RT-qPCR

Total RNAs were isolated from the frozen seedling leaf samples as two biological replicates using RNAzol Reagent (MRC, U.S.A.) as described in Südüpak [30].Nucleic acid quantity and quality measurements were made spectrophotometrically using the ACTGene nanodrop and average yield was computed as ~40-60 µg/100 mg leaf tissue with A_{260}/A_{280} ratios between 1.9-2 and A_{260}/A_{230} ratios between 2-2.3. From each sample, 2 µg total RNA was first treated with RNase free DNase I (Thermo-Fermentas) by incubating at 37 ºC for 30 min to eliminate gDNA contamination. Following treatment, 1 µl 25 mM EDTA was la added to each reaction to prevent Mg^{2+} catalyzed RNA hydrolysis during the DNase inactivation incubation, which was carried out at 65 ºC for 10 min. Subsequently, prepared RNAs were reverse transcribed into cDNAs using RevertAid™ H^-reverse transcriptase (Thermo-Fermentas) and an oligo-dT_{11} primer together with the gene specific primer pair for 18S rRNA in 30 µl reaction volumes. Following reverse transcription, mixtures were subjected to 94 ºC for 5 min to inactivate enzymes and 5x diluted aliquots were stored at -20 ºC.

Primers used in the study were designed using Primer3plus (sourceforge.net/projects/primer3). Output primers pair list were, then, examined in the analysis utilities of Primer Premier 6 program demo (Premier Biosoft International) and NCBI Primer Blast routine (ncbi.nlm.nih.gov/tools/primer-blast) to select the most appropriate primer pair to be ordered. Primer synthesis was carried out by thermofisher-invitrogen as desalted. Primer sequences and related information are given in Table 1.

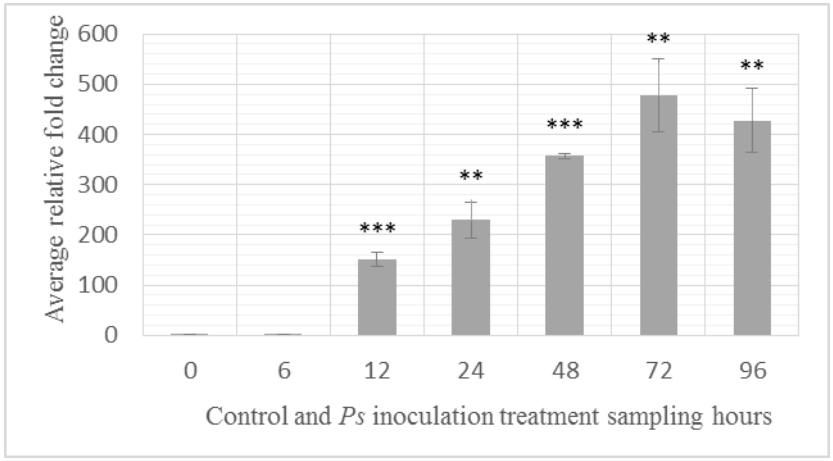
Table 1. Primers used in RT-qPCR studies

Primer pair	Primer sequences (F/R, 5'→3')	Amplified gene, product length (bp)	Used annealing temperature (ºC)	Primer Efficiency
HKG1 124	TTTGACTCAACACGGGGAAA CAGACAAATCGCTCCACCAA	*Zm* 18S rRNA (AF168884.1), 124	59	1.02
HKG2-2 120	TTTAAGGCTGCTGTACTGCTGTAGA CACTTTCTGCTCATGGTTTAAGG	*ZmAktin1* (J01238.1), 120	59	1.02
TC/TA-A4 105	CTCTCATGACATGCTTATTG GACATAGACGAAAACATCAG	*ZmMPK14* (GRMZM2G062914), 105	59	1.08

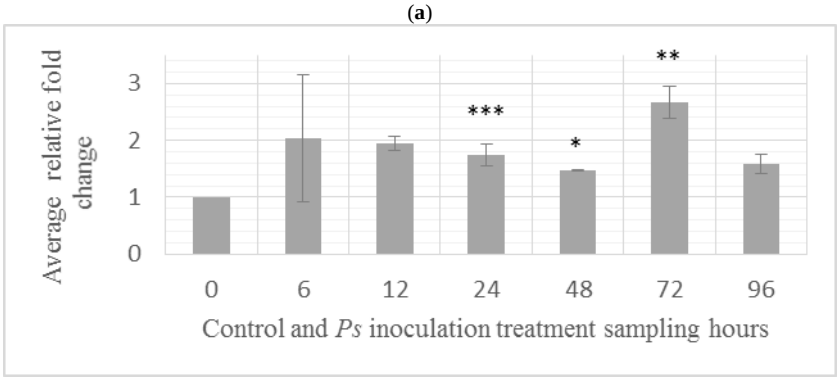
RT-qPCRs were carried out with Real Q-Plus 2x Green mix without ROX (Amplicon). Reactions were prepared as 20-µl mixtures, containing 10 µl Real Q-Plus, 10 picomoles (1µL) of the forward and reverse primers and 3 uL of the 5X diluted cDNA template which are constituted in 6 µL ddH_2O.RT–qPCRs were carried out in Rotor-Gene Q (Qiagen) Thermal cycler, which was programmed for a HotStart *Taq* DNA polymerase (TEMPase) activation period of 15 min at 95ºC, followed by 40-cycle qPCR with a denaturation of 20 s at 94 ºC, annealing at 59 ºC for 20 s and extension at 72 ºC for 30 s. Amplification kinetics were monitored at the end of the each extension step via (SYBR) Green fluorescence readings. Maize *Actin1*was used as the internal control (reference gene) and for normalization in *Ps* treatment studies whileZm18S rRNA was used as reference gene in chemical treatment experiments. In each run, a reference gene dilution array was employed as standards in Rotor-gene software Cq computations. Relative expression levels were computed manually according to delta-deltaCt method of Livak et. al. [31] using average Cq values obtained from two technical replications of biological duplicates. All standard statistical computations were carried out with Cq, deltaCq, delta-deltaCq values since they are known to show normal distribution. Comparisons between individual sampling points and control were carried out using *t-test* with delta-deltaCq values. As a last step, mean fold changes and their respective fold change ranges were obtained as 2^-(delta-deltaCq) and 2^-(delta-deltaCq±Standard Error) respectively.

III. RESULTS

ZmMAPK14 similar sequences were identified as the differential expression showing cDNA-AFLP Transcript Derived Fragments (TDFs) in the experiments involving the compatible and incompatible interactions of maize genotypes, A188 and *Rp1-G* respectively with *P. sorghi* race T09. Both TDFs were expressed at similar levels in controls of resistant and susceptible genotypes, whereas a gradual induction in the resistant genotype and a clear repression in the susceptible genotype samples were displayed in response to rust inoculations. This pattern of expression was also confirmed partially in sqRT-PCR studies of both genotypes. RT-qPCR studies, on the other hand, revealed a marked induction in response to *Ps* infection in the resistant genotype starting at 6 h with about 2-fold, rapidly increasing to 149 folds at 12 h, gradually reaching a expression maxima of 477-fold at 72 h (Figure 1a). *ZmMAPK14* expression in the *Ps* infected susceptible genotype with respect to control had an overall two-fold induction starting at 6 h and continuing up to 96 h of post-inoculation with slight fluctuations, contrary to the initial observation of marked repression upon *Ps* inoculation in cDNA-AFLP studies and partial repression in RT-sqPCRs (Figure 1b).



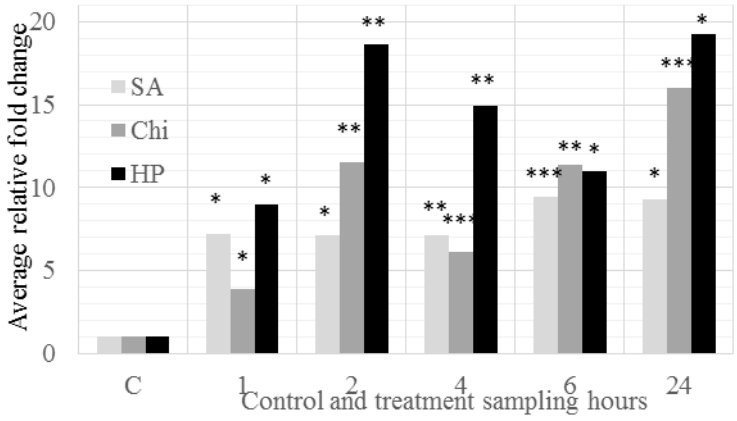
(**a**)



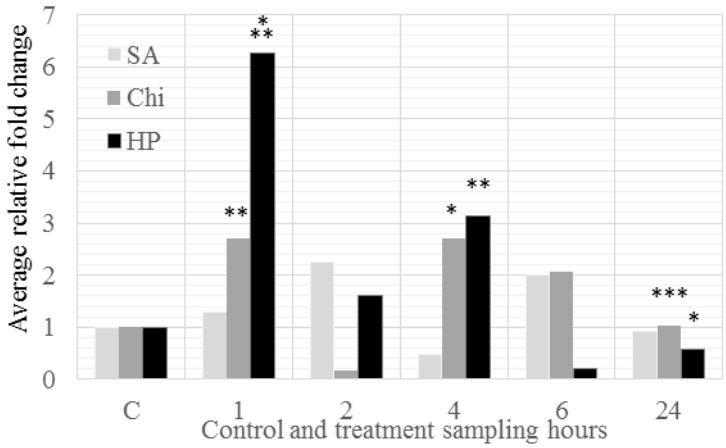
(**b**)

Figure 1. RT-qPCR assessments of *ZmMAPK14* expression change in response to *Ps* inoculation in *Rp1-G* (a) and A188 (b) leaves. Samples were collected from the control and infected plant leaves as biological duplicates at 0 (control), 6, 12, 24, 48, 72 and 96 h after *Ps* inoculation. Data were normalized to the expression level of the reference gene and represent (averaged) fold changes with respect to control at each time point. Error bars represent fold change ranges computed from ΔΔCq ± Standard Error. "*" indicates *t*-test *p*-value significance as * = $0.05<p<0.1$, ** = $0.01<p<0.05$, *** = $p<0.01$.

To examine the role of *ZmMAPK14* in plant defense, effects of SA, H_2O_2 and chitin applications on *ZmMAPK14* expression in both susceptible and resistant genotypes were studied. In the study, applied all treatments induced expressional changes in the resistant genotype samples at different levels and with different profiles. In the resistant genotype, chitin induced expression were significant at 4 h, followed by an over 11-fold at 6 h and a peak induction of 16 folds at 24 h. SA induced change started at 1 h with a 7-fold increase and maintained up to 6 h sampling point where increase reached over 9-fold and remained at this level. In response to H_2O_2 treatment, *ZmMAPK14* expression displayed a sharp induction at 1 h with a 9-fold increase, made a peak at 2 h with an over 18-fold induction, followed by a gradual decrease to 11-fold at 6 h and subsequently making a second peak at 24 h with a 19-fold induction (Figure 2a). In the susceptible genotype, H_2O_2 treatment induced an over 6-fold expression increase at 1 h similar to resistant genotype followed by relatively lower inductions, 1.5 fold at 2 h and 3 fold at 4 h (Figure 2b). Contrary to resistant genotype, SA treatment caused an overall 2-fold induction at 2 h and 4 h time points. Chitin treatment produced a 3-fold induction at both 1 h and 4 h time points (Figure 2b). Lower than control level expressions at 1 h chitin, 4 h SA and 6 h and 24 h HP treatments were also apparent in the susceptible.



(**a**)



(**b**)

Figure 2. RT-qPCR assessments of *ZmMAPK14* expression changes induced by the applications of H_2O_2 (HP), chitin (Chi) and SA treatments in resistant (a) and susceptible (b) genotypes. Samples were collected from the leaves of the control and treatment plants as biological duplicates at C (control), 1, 2, 4, 6 and 24 h. Graphs were prepared from the normalized expression levels as the relative fold changes with respect to control at each time point. "*" indicates *t*-test *p*-value significance as *=0.05<*p*<0.1, **=0.01<*p*<0.05, ***=*p*<0.01.

IV.DISCUSSION

Plant MAPKs appear to be regulated by not only reversible protein phosphorylation/dephosphorylation cycles, but also transcriptional control [24]. Transcriptional regulation of group C plant MAPK genes in response to biotic and abiotic stresses appears to be common. *ZmMAPK7*, a paralog of *ZmMAPK14*, is induced in response to exogenous applications of H_2O_2 [26]. Reported expression patterns of two *ZmMAPK14* orthologues, *OsMAPK3*in rice and *GhMAPK2* in cotton are also examples of this regulation mode [19,20]. Both were reported to display expressional modulations in response to the phytopathogen/pest infestations as well. *OsMAPK3* having 96% sequence similarity to *ZmMAPK14*, is expressed marginally in control plants and displayed an upregulation starting at 6 h continuing with a gradual increase up to 3 days following *Nilaparvata lugens* infestation. Similarly, *Magnaporthe grisea* avirulent race inoculation also resulted in a gradual expression increase after inoculation while the virulent race did not promote a noticeable expression change except a slight induction in the 8 h inoculation sample [19]. Similarly, *GhMAPK2*, showing 92% sequence similarity to *ZmMAPK14*, was reported to be transcriptionally induced in response to *Fusarium oxysporum* fs *vasinfectum* inoculation [20]. Initial detection and subsequent RT-qPCR expression validation experiments in the present study showed that *ZmMAPK14* mRNA was rapidly induced within an hour of *P. sorghi* inoculation and reached a maximum 477-fold induction at 72 h in the incompatible interaction. Expression changes observed in RT-qPCR experiments of the compatible interaction were slight and did not corroborate the repression detected in cDNA-AFLP studies. This differential expression observed between compatible and incompatible interactions closely resembles the expression changes reported for

OsMAPK3 [19] and *OsMAPK4* (another rice group-C MAPK) [32] displayed against the virulent and avirulent races of *M. grisea*. *OsMAPK3* was identified as *OmMAPK* homolog with a 99% amino acid identity and suggested to be a disease resistance factor functioning against *N. lugens* and *M. grisea* [19]. With these similarities and observed inductions, *ZmMAPK14* suggest itself as a factor, which plays a role in conditioning resistance to phytopathogens. Consistent with this assumption, transgenic tobacco lines overexpressing *GhMAPK2*, displayed heightened resistance to the tested viruses (TMV, CMV) and fungi (*F. oxysporum*, *P. infestans*) infections [21].

Reactive oxygen species (ROS), such as H_2O_2, are produced in plants in response to various biotic and abiotic stresses. As a biotic stressor, pathogen challenges induce the synthesis of H_2O_2 both transiently in PTI and in a sustained manner in ETI [11,33]. H_2O_2 are known to cross biological membranes efficiently and function as both a signaling molecule (seconder messenger) and a defense compound, thereby activating local and systemic defense responses and providing direct protection against invading pathogens to some extent respectively [34]. The role of MAPK signaling in ROS production downstream to MAPK cascades in both PTI and ETI are well known and characterized to some extent [4]. Numerous studies have revealed that H_2O_2 activates plant MAPKs [35] and induce expressional changes in MAPK genes, [10,26,27,36-38], however, the mechanism of this transcriptional regulation in plants remains elusive. H_2O_2 signal is presumably sensed either by receptors, ROS sensitive transcription factors like heat shock factors, NPR1 or ROS mediated inhibition of phosphatase, which subsequently turns on a signal transduction pathway, resulting in the orchestration of differential gene expression [39]. H_2O_2 induced *ZmMAPK14* expression change is evident in both susceptible and resistant genotypes as displayed in Figure 2. Amplitude and profile of induction exhibit obvious differences between them as described before. Promoter identification and a motif search conducted in this study revealed that the putative *ZmMAPK14* promoter contains a number of probable ROS-responsive cis-acting elements (ROSE), specifically similar to ROSE7/GCC box reported by Wang et al. [37]. ROSE7/GCC box is the binding site for the Ethylene Responsive element binding Factor 6 (ERF6), which acts as a transcriptional activator of ROS-responsive genes during oxidative stress [37,40]. Accumulating evidence also reveals that ERF6 is an important player and a common component of the ETI triggered by both CNLs (RPS2 and RPM1) and TNLs (RPS4 and RPP4) in conditioning resistance to biotrophic bacterial and oomycete pathogens. ERF6 has been shown to bind and control the expression of several well-known immune regulators, such as MPK3 and WRKY33 [4,41]. A STF1/HY5 binding sequence was also found in the *ZmMAPK14* promoter. HY5 transcription factor binds this sequence motif to promote ROS responsive gene expression [42]. Observed *ZmMAPK14* expression increase in response to rust inoculation may be due to the endogenous H_2O_2 increase triggered by the defense reaction. An experimental *ZmMAPK14* promoter analysis should reveal insight about its nature of ROS responsiveness.

Salicylic acid (SA), regarded as a plant defense hormone, is an important endogenous and exogenous signaling molecule, which promotes several local and systemic defense responses especially effective against biotrophs. SA is required for a robust Systemic Acquired Resistance (SAR) induction, and plants defective in SA synthesis and accumulation display increased disease susceptibility [43]. In the present study, spraying 10 mM SA onto both susceptible and resistant genotype seedling leaves resulted in a marked induction started at 1 h and maintained with an increase to 9-fold at 6 h and 24 h in the resistant genotype whereas expression increase was limited and complex in the susceptible genotype (Figure 2). SA signal is transmitted via NPR1, a master regulator of SA-dependent defense pathways, by conversion of cytoplasmic NPR1 oligomers to monomers, which are subsequently localized to nucleus where they are phosphorylated and interact with NPR3 and NPR4 in the induction of defense gene expression (e.g. PR genes) in a concentration dependent manner by interacting with transcription factors such as TGAs (basic leucine zipper TFs). NPR1 defective plants are also defective in both SA-induced gene expression and SAR activation [43]. Sun et. al. [10] reported the presence of a SA-responsive TCA-element at *ZmMAPK14* promoter, which may be an explanation for the observed SA caused expression change.

Chitin, a polymer of N-acetyl glucosamine, is not found in plants, but a major component of fungal cell walls, which are fragmented during infection to chito-oligosaccharides functioning as elicitor/PAMP. Chitin elicitor binding proteins, CEBiPs and receptor-like kinase, LysM RLK1/CERK1 in Arabidopsis and orthologous in rice and several other species have been identified as membrane proteins functioning in chitin perception and signaling [44]. Plant MAPKs function in chitin defense signaling via either direct activation of MAPKs or expressional modulation [4,45]. Chitin induces expressional modulations in a large number of defense-related genes including *AtMAPK3* [44]. Regulatory regions of pathogen responsive plant genes contain W-box elements, which are shown to be elicitor responsive [46]. Both Sun et. al. [10] and current study promoter bioinformatics revealed the presence of a fungal elicitor response element, Box-W1 (W-box) in the *ZmMAPK14* promoter. Box-

W1 elements, containing TTGAC(C/T) sequence motifs, are recognized by WRKY transcription factors, which are known to modulate the expressions of plant defense and defense related genes either directly or indirectly [11,47,48]. Consistent with the accumulating knowledge, chitin induced expressional modulation of *ZmMAPK14* observed in this study provides clues about its chitin responsiveness and defensive role.

V. CONCLUSIONS

Although no direct role, other than expressional change, for its involvement in plant defense has been obtained, the marked expressional induction in the resistant genotype in response to *Ps* inoculation and applications of defense inducing compounds suggest that it plays a direct or indirect role in defense signaling. Expression of *GhMAPK2* in transgenics revealed that genotypes display enhanced resistance to the tested fungi and viruses. *GhMAPK2* overexpressing transgenics also displayed an increased ability scavenge ROS and tolerate oxidative stress. Based on these findings, a defense signaling role in response to both pathogen infection and oxidative stress has been proposed [21,49]. Further studies similar to that of *GhMAPK2* should reveal more insight about the role of *ZmMAPK14* in plant defense and related functions.

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