THE ROLE OF SOME MYB GENES IN DEFENSE RESPONSE OF WHEAT AGAINST STRIPE RUST PATHOGEN

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Wheat is one of the most important dietary crops and is a staple food in many countries. Several pathogens attack wheat in different growth stages, stripe rust is considered one of an important disease appears and causes damage by growing in the leaves and producing spores. In this study, the expression levels of four genes from myeloblastosis (MYB) Transcription factor family under compatible (CYR31) and incompatible (CYR23) strains of stripe rust were tested. The results showed *TaMYB1* transcript levels with CYR31 were induced at 12 hpi and 48 hpi compared with the control which were about 1.5-fold and 3.8-fold, respectively. While, the transcript levels with CYR23 were reduced with all incubated time, except 12 hpi and 48 hpi (about 2.3-fold - 1.7-fold, respectively). In addition, the transcript levels were significantly reduced at 72 hpi with both strains. Moreover, the same results were observed with *TaMYB7* under the same conditions. The expression levels were significantly induced at 12 hpi and 48 hpi, which were about 1.78-fold and 4-fold, respectively. But, at 24 hpi, the expression pattern of *TaMYB7* was induced with CYR23. *TaMYB2* transcript levels were reduced at all time points with both strains, except 12 hpi with CYR23. Furthermore, the *TaMYB30* transcript level was induced (3.4-fold) at 12 hpi with CYR23 compared with control. On another hand, after 12 hpi, the expression levels of *TaMYB30* were reduced. Altogether, it can be concluded that *TaMYB1*, *TaMYB2*, *TaMYB7* and *TaMYB30* may be played roles during the interaction between wheat and stripe rust pathogen in

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different stages.

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Domestication of agricultural crops, estimated at 2500 species globally (Meyer et al., 2012), has involved artificial selection of desirable traits that enhance yield and quality of the harvested product. While breeding for agronomic targets in high input environments has successfully increased global crop productivity (Lynch, 2007). Biotic stresses are challenges which face the plants during different growth stages. The plant depends on some of mechanisms for response and defense against different biotic stresses such as control opening/close the stomata, induce programmed cell death, induce some biochemical in the cell e.g., H_2O_2, O_2^-. All of these mechanisms are controlling by different genes, these genes have different roles in plant response against biotic and abiotic stresses. Among these genes, transcription factors, such as AP2/ERF, MYB, NAC, WRKY, bZIP, and bHLH, have been demonstrated to play an important regulatory role in plant response to abiotic stresses (Butt et al., 2017; Mao et al., 2017; Tang et al., 2017; Wu et al., 2017; Sun et al., 2018 and Zhu et al., 2018).

The MYB transcription factor contains a MYB domain that is highly conserved across all eukaryotes and is located at the N terminus, whereas the C terminus is variable, acting as a transacting domain, involves in the regulation of a wide range of functions in the MYB protein (Butt et al., 2017). In addition, MYB proteins contain one, two or three imperfect repeats containing 52 amino acid residues in their MYB domain (Stracke et al., 2001 and Butt et al., 2017). Based on the number of repeats in their MYB domain, the members from MYB family are divided into four groups, R2R3-MYB, MYB1-R, 4R-MYB, and R1R2R3-MYB in *Arabidopsis* (Stracke et al., 2001).

Since the first plant protein containing the MYB domain, named c1-encoded protein, is isolated in maize (Pazares et al., 1987), numerous MYB proteins have been identified in various plant species e.g., rice, soybean, *Arabidopsis* and sweet orange through genome-wide analysis method (Stracke et al., 2001; Du et al. 2012 and Liu et al., 2014). Subsequent researches showed that MYB transcription factor family plays a significant role in regulatory networks that involve in the whole process of plant growth and development. The R2R3-type MYB subfamily is divided into 22 subgroups.

However, 36 members of the R2R3-type MYB subfamily do not belong to any of these subgroups. The R2R3-MYB genes are playing different important roles in plants. For example, *AtMYB52*, *AtMYB54*, *AtMYB69* and *AtMYB52* are positive regulators dedicated to cell wall thickening in fiber cells. *AtMYB52*, *AtMYB54* and *AtMYB69* are hypothesized for regulating of the lignin, xylem, and cellulose biosynthesis, and *AtMYB103* is involved in cellulose biosynthesis (Dubos et al., 2010; Stracke et al., 2001 and Zhong et al., 2008). Some of genes in the R2R3-MYB subfamily regulate the cell fate and identity in *Arabidopsis* (Kirik et al., 2005; Lepiniec et al., 2006 and Li et al., 2009). In response to pathogens attack, *AtMYB44* has been shown to exhibit stronger antioxidative activities compared with other plant genes. The reactive oxygen species (ROS) burst is most likely associated with the *AtMYB44*-mediated signaling induced in response to biotic stresses (Shi et al., 2011 and Baohong et al., 2012). *AtMYB72* is a key regulator required in the roots during the early signaling steps of induced systemic resistance and is mediated by beneficial fungus and bacteria (Van et al., 2008 and Segarra et al., 2009). Moreover, TaMYB4 have role to plant defense response against strip rust disease (Al-Attala et al., 2014). Also, *BjMYB1* is potentially involved in host defense against fungal attack (*Botrytis cinerea*) through activating the expression of *BjCHI1* by binding to the Wbl-4 element in the BjC-P promoter (Gao et al., 2016). *MYB2* has been reported to regulate the interplay between JA and other hormones (Atkinson and Urwin, 2012). Moreover, *MYB2* have role to regulate abscisic acid (ABA) signaling (Abe et al., 2003). Lignification is a mechanism for disease resistant in plants. During defense responses, lignin or lignin-like phenolic compound accumulation was shown to occur in a variety of plant-microbe interactions (Bhuiyan et al., 2009). *AtMYB30* encodes an activator of the hypersensitive cell death program through the regulation of the synthesis of very-long-chin fatty acids. Additionally, *AtMYB30* positively regulates hypersensitive cell death and is involved in an amplification loop or signaling cascade that modulates salicylic acid synthesis (Vailleau et al., 2002; Raffaele et al., 2006, 2008 and Li et al., 2009). *TaMYB30* was shown to improve drought stress tolerance during the germination and seedling stages. Furthermore, the overexpression of *TaMYB30* alters the transcript levels of some drought stress-responsive genes and changes several physiological indices to allow plants to overcome adverse

conditions. Additionally, *TaMYB32* enhances the tolerance to salt stress in plants (Zhang et al., 2012a and b). Wheat stripe rust fungus (or yellow rust) caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is one of the most common and destructive diseases of wheat worldwide. Using resistant wheat cultivars is one of the most effective ways to control of wheat stripe rust fungus. A few genes have different functions in the wheat response to biotic stress.

This study investigated the role of *TaMYB1, TaMYB2, TaMYB7* and *TaMYB30* under biotic stress (compatible and incompatible strains) via qRT-PCR. Also, study the interaction between the proteins of these genes and the other proteins using STRING database.

MATERIALS AND METHODS

1. Plant Materials, Inoculation, and Treatments

Suwon 11 (Su11), which is a Chinese cultivar of wheat (*Triticum aestivum* L.), was used to study the expression and isolation of *TaMYB1, 2, 7* and *30* in response to biotic stress. Suwon 11 has been reported to carry the YrSu resistance gene and is highly resistant to *Pst* pathotype CYR23 (incompatible) and highly susceptible to CYR31 (compatible). Suwon11 seeds, *Pst* pathotypes (CYR23 and CYR31) were supported from State Key Laboratory of Stress Biology for Arid Areas, Northwest A and F University (NWAFU). The wheat seedlings were maintained and inoculated with CYR23 or CYR31 under controlled conditions, based on the protocols described by Stakman (1915), Kang and Li (1984) and Liu et al. (2006) and. The control treatment was inoculated with sterile distilled water. At the one-leaf stage, the seedlings were inoculated with stripe rust fungus (CYR23 or CYR31) on the surface of the wheat leaves. After inoculation, all of the plants were directly maintained in the growth chamber for 24 h in the dark with 100% relative humidity and a temperature of 15°C. Leaves were harvested at 0, 12, 24, 48, 72 and 120 hpi. These time points were chosen based on microscopic studies of the interactions between Suwon 11 and CYR23 or CYR31, as reported by Wang et al. (2007).

2. RNA Extraction and Reversion to cDNA

The RNA was extracted with Biozol™ Reagent (BioFlux, Tokyo, Japan). The quality and integrity of the total RNA were determined by running 1 µl of RNA with 9 µl of sterile distilled water in a formamide denaturing gel.

Additionally, the RNA quantity was estimated using a NanoDrop™1000 spectrophotometer (Thermo Fisher Scientific, USA). To synthesize cDNA from RNA, a Revert Aid First-strand cDNA synthesis kit from Fermentas (www.thermosscientific.com/fermentas) was used.

3. Quantitative RT-PCR Analyses Under Biotic Stress

Quantitative real-time PCR analysis was used to determine the expression profiles of *TaMYB1, 2, 7* and *30* in response to different treatments. For the qRT-PCR analyses, four pairs of primers were designed (Table 1) to amplify different fragments under the following conditions: 95°C for 20 s, 40 cycles of 95°C for 10 s, 55°C for 20 s and 72°C for 40 s. The total volume of the PCR reaction was 25 µl. A 7500 Real-Time PCR System was used to quantify the gene expression. A dissociation curve was generated for each reaction to ensure specific amplification. The threshold values (CT) were generated from the ABI PRISM 7500 software (Applied Biosystems, Foster City, CA, USA). The relative expression of *TaMYB*s were quantified using the comparative $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). The wheat elongation factor (GenBank accession number: Q03033) was used as the internal reference for the qRT-PCR analyses. Three independent replicates were used for each time point and control.

Table (1). Oligonucleotide primers used in the study.

Gene name	Primer sequence
TaMYB1	**F5'** AGAGCACGGGTTCCACCACGACA **3'**
	R5' GTTTCAGAGCAGGCCGAAGAATAAACAGAC **3'**
TaMYB2	**F5'** GCAGGAGGAGGACCACATC **3'**
	R5' ATCCGAACTGGCCGTACA **3'**
TaMYB7	**F5'** CGAGGGCAAGGTCAAGATGA **3'**
	R5' GACAGCGTCTCCGACCAGAA **3'**
TaMYB30	**F5'** GCTTCGTGCTCAGATTGC **3'**
	R5' GGTTGCCGAGAATCCTGT **3'**

4. Structural and Functional Analysis of *TaMYBs* Gene

The tool NetPhos 2.0 was used for the identification of major phosphorylation site. Protein-protein interaction study was performed using STRING database.

5. Data Analysis

SPSS software was used to calculate the standard deviations and to perform Tukey's test for the statistical analyses.

RESULTS

1. Cloning and Phylogenetic Sequence Analyses of cDNA of TaMYBs Genes cDNA

The sequences of *TaMYB 1, 2, 7* and *30* were download from NCBI database and the qRT-PCR primers were designed according to these sequences. All information about these genes such as open read farms (ORF), total number of amino acids were mentioned in table (2).

Table (2). Information of *TaMYBs* gene.

Gene name	ORF* total length (bp)	Amino acid	Isoelectric point	Molecular weight (Da)**	Accession no. in NCBI website
TaMYB1	897	299aa	8.50	31914	DQ353858
TaMYB2	882	294aa	5.74	31873	AB252145
TaMYB7	840	280aa	6.49	30888	AB252147
TaMYB30	2199	733aa	4.75	79145	JF951913

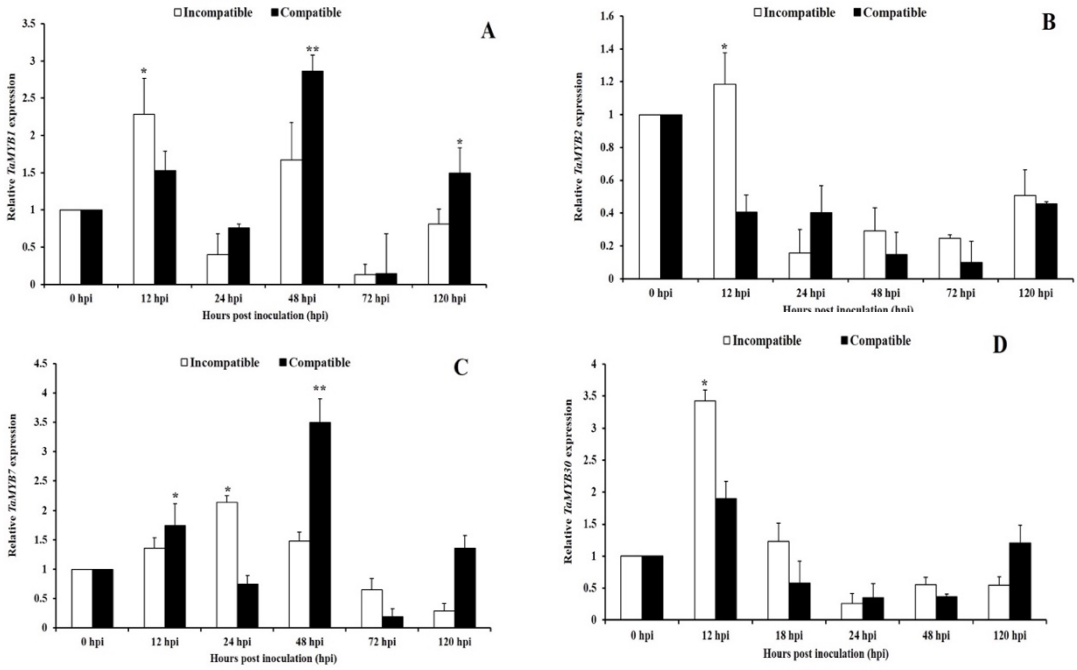
*ORF; Open Reading Frame.

**Da; Daltons (measuring unit of molecular weight).

2. *TaMYBs* Transcriptional Responses to the Fungal Pathogen (*Puccinia striiformis* f. sp. *tritici*)

The transcript abundance of *TaMYB1, 2, 7* and *30* in the inoculated leaves were evaluated using qRT-PCR method. With *TaMYB1*, the transcript level in the inoculated plant with CYR23 (incompatible) showed direct upregulation at 12 hpi and 48 hpi with infected leaves which were about 2.3-fold and 1.7-fold, respectively. Also, at 48 hpi with CYP31, the expression pattern was up-regulated to 3.9-fold compared with control and incompatible race. The transcript levels at 24 hpi and 48 hpi were down-regulated with both strains compared with control. In addition, the expression patterns of *TaMYB2* were investigated under the same condition. The results showed that the transcript levels of *TaMYB2* were downregulated in all time points with compatible and incompatible stains except at 12 hpi with CYR23. Moreover, under the interaction with compatible race (CYR31), the expression patterns

of *TaMYB7* were induced at 12 hpi and 48 hpi (1.7-fold and 3.9-fold, respectively). On other hand, at 24 hpi and 48 hpi, the transcript levels of *TaMYB7* were reduced compared with control. But, at 24 hpi, the expression pattern of *TaMYB7* was induced with CYR23. With *TaMYB30*, at 12 hpi, the transcript pattern of *TaMYB30* was upregulated compared with all-time points. These results demonstrate that TaMYB1, 7 and 30 may have difference roles in the response of wheat during *Puccinia striiformis* f. sp. *tritici* infection with compatible race (Fig. 1).



The relative gene expression was quantified using the comparative threshold ($2^{-\Delta\Delta CT}$) method. The mean and standard deviation were calculated using data from three independent biological replicates. *significantly and **high significantly.

Fig. (1). The expression pattern of *TaMYB* genes in wheat leaves inoculated with CYR23 (incompatible) and CYR31 (compatible). The samples were collected at 0, 12, 24, 36, 48 and 120 hpi. **A**, **B**, **C** and **D** show the expression patterns under *TaMYB1*, *TaMYB2*, *TaMYB7* and *TaMYB30*.

3. Protein-Protein Interaction of *TaMYB1*, *TaMYB2*, *TaMYB7* and *TaMYB30*

Protein-Protein interaction of *TaMYBs* network generated by the STRING database based on spring model is shown in table (3) and fig. (2). The averages local clustering coefficient of *TaMYB1*, *TaMYB2*, *TaMYB7* and *TaMYB30* were 0.558, 0.909, 0.429 and 0.558, respectively.

Table (3). The analysis of network stats of *TaMYB 1, 2, 7* and *30* proteins.

Gene name	No. of nodes	No. of edges	Average node degree	Average local clustering coefficient	Ppi enrichment p-value
TaMYB1	11	26	4.73	0.558	0.0961
TaMYB2	11	10	1.82	0.909	0.9880
TaMYB7	11	31	5.64	0.429	0.0147
TaMYB30	11	26	4.73	0.558	0.0943

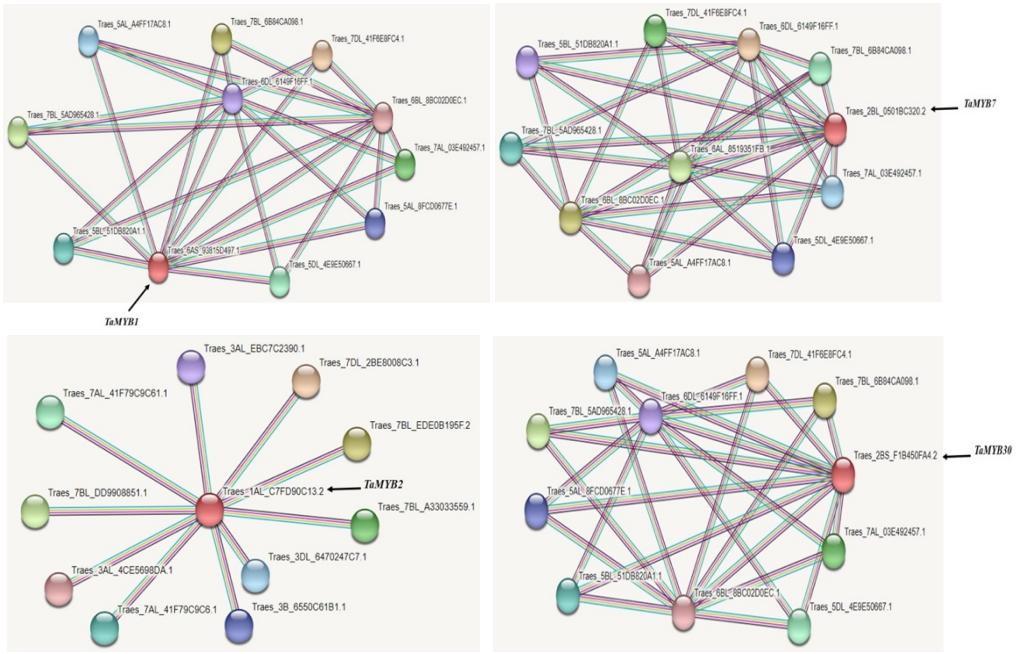


Fig. (2). Protein-protein interaction network (*TaMYB1*, *2*, *3* and *30*) generated by the STRING database based on spring model.

DISCUSSION

Stripe rust (yellow rust) is caused by *Puccinia striiformis* f. sp. *tritici* Eriks & E. Henn. (syn. *P. glumarum* Eriks and Henn.) and is widely regarded as one of the most destructive diseases that affect wheat (*Triticum aestivum* L.) worldwide. The flexibility and adaptability of this pathogen makes it very serious and it is present on all continents, where it can cause wheat production losses of 30–100% by destroying leaf tissue, as well as reducing the crop weight and quality (Chen, 2005 and Kolmer, 2005)

For more a decade ago, the concept of plant innate immunity as a two-layer defense system comprising broad-spectrum pattern triggered immunity (PTI) and isolate-specific effector-triggered immunity (ETI) was first proposed. PTI is thought to be controlled by conserved cell-surface pattern-recognition receptors (PRRs), such as receptor-like proteins and receptor-like kinases (RLKs) including wall-associated kinase genes (WAKs), after perception of conserved pathogen associated or plant-derived damage-associated molecular patterns, such as chitin or pectic oligogalacturonide derivatives of fungal or plant cell walls, respectively. After this first level of defense is overcome, plants may deploy ETI with highly variable, often dispensable resistance proteins (cytoplasmic NLRs or extracellular-receptor-like proteins) that detect matching avirulence (Avr) effectors, which are also highly variable. ETI frequently culminates in a hypersensitive response and is often described as faster and stronger than PTI. The above concepts are being challenged by the accumulation of new data suggesting that plant immunity is likely to be a continuous surveillance system that evolves to detect invading microbes. In particular, there may be no strict dichotomy between PTI and ETI, or between PRRs and resistance proteins (Postel and Kemmerling, 2009 and Dardick et al., 2012)

An R2R3-MYB protein contains two MYB domains and the majority of R2R3-MYB proteins regulate plant-specific functions including immunity against microbial pathogens (Stracke et al., 2001 and Dubos et al., 2010). For example, overexpression of the R2R3-MYB gene *HbMyb1* from *Hevea brasiliensis* enhances resistance to *B. cinereal* in transgenic tobacco *Nicotiana tabacum* cultivar (Ambawat et al., 2013). The R2R3-MYB gene *OsJaMyb* in rice (*O. sativa* spp. *japonica*) is responsive to infection by the blast fungus *Magnaporthe oryzae* (Lee et al., 2001). The *Botrytis cinerea*-induced mRNA

expression pattern of *BjMYB1* from *Brassica juncea* was similar to that of *BjCHI1* in the native host plant *B. juncea*. Overexpression of *BjMYB1* in stable, transgenic *Arabidopsis thaliana* plants enhanced host plant resistance to *B. cinerea*. These results suggest that *BjMYB1* might be involved in plant defense against fungal infection by interacting with the Wbl-4 element and regulating the expression of *BjCHI1* for host plant defense (Gao et al., 2016). Moreover, the expression levels of *TaMYB2* were reduced with all-time points in compatible and incompatible strains Previous studies have been reported that Some MYB genes are expressed in response to GA treatment in Petunia petals. Another plant hormone, Abscisic acid (ABA), induces expression of *AtMYB2* in *Arabidopsis*, a MYB gene that is also induced in response to dehydration or salt stress (Shinozaki et al., 1992). *AtMYB2*, *AtMYB74* and *AtMYB102* were up-regulated by drought stress (Urao et al., 1993; Abe et al., 2003 and Denekamp and Smeekens, 2003). *AtMYB2* was induced by dehydration and salt stress but not by cold and heat stress and thus *AtMYB2* is responsive to dehydration at the transcriptional level. Moreover, *OsMYB2*, was involved in salt, cold, and dehydration tolerance in rice (Yang et al., 2012). So, it can be concluded that *TaMYB2* have more function in plant response under abiotic stress.

Based on the obtained results, the expression patterns of *TaMYB7* were induced at 12 hpi and 48 hpi with compatible race of *Pst.* While, transcript level was induced at 24 hpi with incompatible race of *Pst.* The previous studies reported that carbohydrates are important components in the adjustment process, and many studies have shown that when plants were stressed by drought, low temperature, high temperature and certain biological stresses, the plant tissues will accumulate substantial levels of fructose and sorbitol to maintain cell turgor (Couee et al., 2006 and Lee et al., 2016). *MYB10, MYB4* and *MYB7* have upregulated with accumulated of carbohydrates in stressed plants. The previous study suggested that carbohydrates, which are components of osmotic regulation during pathogen infection, may contribute to the accumulation of flavonoids as defense factors against infection and disease expansion because of their antioxidant properties (Das et al., 2012; Di et al., 2017 and Zhu et al. 2017). So, the results suggested *TaMYB7* may be plays role under biotic stresses. With all studied TaMYBs, the expression levels were induced at 120 hpi, it could be related to help the

plant cell to regain balance after response to stress.

Activation of immunity is achieved by multiple transcriptional regulators that reprogram cell transcription to favour defense over routine cellular requirements (Moore et al., 2011). The arsenal of transcriptional regulators consists not only of DNA-binding transcription factors (TFs), but also of proteins that interact with and regulate these TFs. *MYB30*, an *Arabidopsis* R2R3-type MYB TF, as a positive regulator of plant defense and HR responses. *MYB30* activates genes related to the lipid biosynthesis pathway that leads to the production of very-long-chain fatty acids (VLCFAs) (Vailleau et al., 2002 and Raffaele et al., 2008). Previous study has been reported that *MYB30* is targeted by the *Xanthomonas campestris* pv. *Campestris* (*Xcc*) effector XopD, suppressing *MYB30* transcriptional activity and thereby plant resistance (Canonne et al., 2011). Here, the results showed that the transcript levels were induced at 12 hpi with incompatible race of *Pst* and then the expression patterns were reduced with all of time points. Altogether, it can be concluded that *TaMYB1*, *TaMYB2*, *TaMYB7* and *TaMYB30* may play important roles plant response against strip rust. Additionally, these genes may have different roles during wheat defense stages.

CONCLUSIONS

Form this study, It can be concluded that *TaMYB1*, *TaMYB2*, *TaMYB7* and *TaMYB30* may play difference roles during wheat response against strip rust pathogen. Moreover, these genes have responsibility to control several genes which are have roles in wheat defense response.

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دور بعض جينات MYB في الاستجابة الدفاعية للقمح ضد مرض الصدأ الأصفر

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يعتبر القمح واحد من أهم المحاصيل الغذائية في العالم وهو الغذاء الأساسي في العديد من البلدان. تهاجم العديد من المسببات المرضية القمح في مراحل النمو المختلفة، ويعتبر الصدأ الأصفر واحد من أهم الأمراض التي تسبب ضررًا كبيرًا خلال النمو في الأوراق وإنتاج الحبوب. في هذه الدراسة تم دراسة مستويات التعبير لأربع جينات من عائلة Myeloblastosis (MYB) تحت ظروف العدوى الصناعية من سلالتين؛ الأولى متوافقة (CYR31) والثانية غير متوافقة (CYR23) من الصدأ الأصفر مع صنف القمح المستخدم في الدراسة. وقد أظهرت النتائج أن مستويات التعبير ل *TaMYB1* مع CYR31 كانت مستحثة بعد ١٢ ساعة من العدوى و٤٨ ساعة من العدوى مقارنة مع الكنترول التي كانت حوالي ١.٥ ضعف و٣.٨ ضعف، على التوالي. في حين، ظهر انخفاض في مستويات التعبير الجيني مع CYR23 مع جميع الأوقات باستثناء بعد ١٢ و٤٨ ساعة من العدوى (حوالي ٢.٣ أضعاف - ١.٧ أضعاف، على التوالي). بالإضافة إلى ذلك، انخفض مستويات التعبير الجيني بشكل كبير عند ٧٢ ساعة من العدوى. وعلاوة على ذلك، لوحظت نفس النتائج مع *TaMYB7* في ظل نفس الظروف. حيث لوحظ ارتفاع مستويات التعبير بشكل كبير عند ١٢ و٤٨ ساعة من العدوى والتي كانت حوالي ١.٧٨ ضعف و٤ أضعاف، على التوالي. ولكن عند ٢٤ ساعة من العدوى، زاد التعبير الجيني من *TaMYB7* مع CYR23. على الجانب الآخر، انخفض مستويات نسخة *TaMYB2* عند جميع نقاط القياس مع كلا السلالتين باستثناء عند ١٢ ساعة من العدوى مع السلالة الغير متوافقة والتي لم ترتفع بصورة كبيرة. وبالإضافة إلى ذلك، زاد مستوى التعبير الجيني ل *TaMYB30* (٢.٣ أضعاف) عند ١٢ ساعة من العدوى مع السلالة الغير متوافقة مقارنة مع الكنترول. ولكن بعد ١٢ ساعة من العدوى، انخفضت مستويات التعبير في شتلات القمح المعاملة بعزلة غير ضارية أو متوافقة. بالإضافة إلى ذلك، كان مستوي التعبير الجيني *TaMYB30* مع سلالة متوافقة عند ١٢ ساعة من العدوى (١.٨ ضعف) مقارنة مع الكنترول. بالإجمال، يمكن استنتاج أن *TaMYB1*، *TaMYB2*، *TaMYB7* و*TaMYB30* قد تلعب أدوار مختلفة في الاستجابة لمقاومة القمح لمرض الصدأ الأصفر.