Resistance to wheat stem rust in selected accessions of Iranian wheat landraces

WIT Award

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ABSTRACT

Stem rust is a potentially destructive fungal disease of wheat worldwide. In 1998 *Pgt* pathotype TTKSK virulent to *Sr31* was detected in Uganda. The same pathotype was confirmed in Lorestan and Hamedan provinces of Iran in 2007. We used a derivative of race TTKSK to phenotype 62 Iranian wheat landraces (resistant to stripe rust in a previous study) at the seedling stage to this new pathotype (TTSSK). Twenty eight resistant accessions were evaluated for the presence of resistance genes *Sr2*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, *Sr35*, *Sr36* and *Srweb* using SSR markers. None carried *Sr2*, *Sr24* or *Sr26*, but the presence of *Sr22*, *Sr25*, *Sr35* and *Sr36* was indicated. Some susceptible landraces were predicted to carry *Sr2* which may were pollinated by cultivars carrying this gene so it requires further investigation. To evaluate defense gene expression in compatible and incompatible stem rust interactions we sampled resistant and susceptible cultivars at 0, 12, 18, 24, 72 hours post-inoculation (hpi). β-1,3 glucanase expression was studied using qGLU-S and qGLU-AS primers and a real-time PCR step-one ABI machine, with *β-tubulin* and *EF1-α* genes used as internal controls. In incompatible interactions defense gene expression was increased at 24hpi, but in compatible interactions the highest level of expression occurred at 12hpi and was significantly decreased at 18hpi. The results revealed that expression of defense genes such as β-1,3 glucanase was earlier in compatible than in incompatible interactions but the quantity of expressed gene is less in incompatible interactions. On the other hand, in susceptible genotypes the expression of defense genes increased immediately after inoculation and reduced sharply after establishment of the pathogen. In contrast, defense gene expression in resistant genotypes began to increase after the establishment of the pathogen.

Introduction

Stem rust caused by *Puccinia graminis* Pers. f.sp. *tritici* is one of the most important fungal diseases of wheat (Singh *et al* 2008). In 1998, severe stem rust infections were occurred in Uganda which had virulence on *Sr31* (Pretorious *et al* 2000) and designated as TTKS using North American nomenclature system (Roelfs and Martens, 1988) and then designated as TTKSK (Jin *et al* 2008). Subsequently, Ug99 was detected in Kenya, Ethiopia, Sudan and Yemen. A new variant of Ug99 was detected in Kenya in 2006 which had virulence to *Sr24* (Jin *et al* 2007). Ug99 was confirmed in Iran in 2007 (FAO 2008).

Plants use different defense mechanisms to protect themselves against pathogen. β-1,3-glucanase is a kind of pathogenesis-related proteins which can help defend plants against fungal infection by weakening and decomposing fungal cell walls and releasing elicitors that can induce a chain of the consequent defense reactions (Kombrink *et al* 2001; Lawrence *et al* 2000). β-1,3-Glucanase activity has been associated with resistance to rust fungi. (Sock 1990).

The group of Ug99 races is widely recognized worldwide including Iran. The aim of this study is evaluation of Iranian wheat landraces against TTKS at seedling stage in the greenhouse to introduce new resistance resources and evaluate defense gene expression level in compatible and incompatible interactions.

Experiments

Sixty two Iranian wheat Landraces which were deposited in National Plant Gene Bank of Iran were evaluated in the greenhouse at seedling stage using isolate of *P. graminis* f.sp. *tritici* which collected in "Dasht Azadegan" during 2009-2010. Identification of pathotype was performed by differential lines which were received from ICARDA and CYMMIT using Jin *et al.,* 2007 method. Infection types were assessed 14 days after inoculation using Stackman *et al* (1962) method. These landraces were evaluated against stripe rust and local race of stem rust at adult stage in the field.

Genomic DNA was extracted from frozen leaves based on the method of Dellaporta *et al* (1983). Polymerase chain reaction (PCR) assays were performed according to reported protocols for *Sr2*, *Sr22, Sr24*, *Sr25*, *Sr26*, *Sr35*, *Sr36*, *SrWeb* and *Sr52*.

To evaluate defense gene expression in compatible and incompatible wheat-stem rust interactions, cv. Morocco (susceptible) and KC-440 genotype (resistant) were used. Inoculation with water (mock) and stem rust isolate were done on 7-day-old seedlings of both lines. Sampling was carried out 0, 12, 18, 24, 72 hours post inoculation (hpi). RNA extraction and cDNA synthesis were done using RNX-plus and Fermentas first strand cDNA synthesis kit, respectively. β- 1, 3 glucanase gene expression level was studied using qGLU-S and qGLU-AS primers (Liu *et al* 2010) and realtime PCR step one ABI machine. Also, β-tubulin and EF1-α genes were used as internal control. Three technical replications were used in each experiment.

RESULTS

Identification of pathotype was carried out by inoculation of differential lines received from ICARDA and CYMMIT with purified isolate. Pathotype used was virulent against *Sr31, Sr36, Sr38* and *Sr13* but was not virulent against *Sr24* and designated as TTSSK using North American nomenclature system (Roelfs and Martens 1988). According to result the landrace accessions divided into susceptible and resistant groups (Figure 1). The resistant group includes 28 landrace accessions. Based on the results, 13 accessions were resistant in field experiment as well.

The resistant landraces were used for marker analysis to detect some *Sr* genes including *Sr2, Sr22, Sr24, Sr25, Sr26, Sr35, Sr36, SrWeb* and *Sr52*. The results showed that *Sr22, Sr25, Sr35* and *Sr36* are present in some of the resistant landraces. On the other hand, 21 susceptible landraces which were resistant at adult stage were used for *Sr2* analysis. Results showed that some of these landraces carried adult plant resistance gene/genes other than *Sr2*.

Based on the normalized data, the β-1, 3 glucanase gene expression increased and reached to highest amount (6 folds) at 12hpi in compatible interaction (Figure 2A). It then decreased rapidly at 18 and 24hpi. Conversely, in incompatible interaction, gene expression compared with the mock treatment increased at 24hpi (12 folds) and decreased sharply at 72hpi (Figure 2B). Melt cure analysis showed that the peak of the curve occurred around 80˚C for all genes that stated the specific amplification of the Q-PCR products.

Based on the results, in compatible interactions, defense gene expressions, such as those induced by β-1, 3 glucanase, increased after inoculation. After 18 hours, due to host susceptibility and suppression of signal transduction pathways, defense gene expression reduced and led to a host susceptible reaction. In contrast, in incompatible interaction, the highest amount of gene expression were observed at 24hpi. This period is essential for the penetration and establishment of the pathogen. Therefore, at 12hpi, defense gene expression was induced and reached the highest level after pathogen establishment.

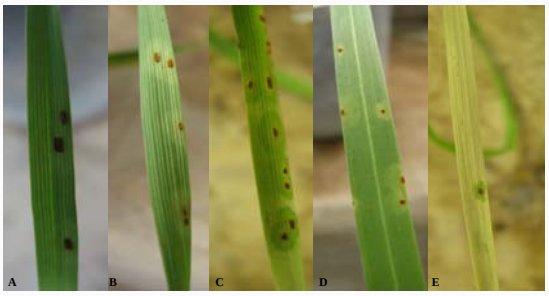

Figure 1- Different reaction of some landrace accessions (B-E) against *Pgt* (TTSSK race) compared to cv. Morocco as control (A).

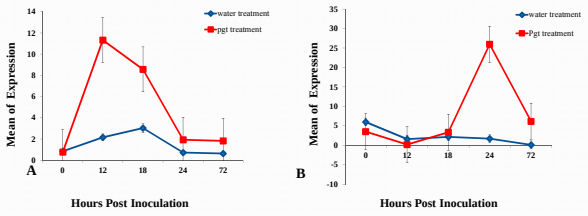

Figure 2- β- 1, 3 glucanase gene expression in compatible interaction (A) and incompatible interaction (B). β- tubulin is used as internal control to normalize the data.

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