

Screening and biochemical characterization of wheat cultivars resistance to *Magnaporthe oryzae* pv *Triticum* (MoT)

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ABSTRACT

Global food security is seriously threatened due to increased frequency and occurrence of fungal diseases. One example is wheat blast caused by *Magnaporthe oryzae* is a fungal diseases of rice, wheat, and other grasses, that can destroy the whole food production to sustain millions of people. Wheat blast was first detected in february 2016 with a serious outbreak in Asia. Assessment of the available germplasm to stress tolerant/resistant is one of the best options for developing stress tolerant crop varieties. In this study, a total of sixteen wheat cultivars were collected and test their disease severity to blast pathogen *Magnaporthe oryzae* pv. *Triticum* (MoT). Among the varieties, BARI Gom 33 exhibited partially resistance against blast pathogen, whereas all other genotypes become susceptible to MoT. Different yield and yield contributing characters of both resistant and susceptible cultivars were also evaluated and found no significant differences among them. To understand the underlying mechanism of resistance in BARI Gom 33, antioxidant enzyme activity, concentration of reactive oxygen species and cellular damage after fungal infection were also evaluated and found that activities of ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) were higher in BARI Gom 33 than BARI Gom 25 and BARI Gom 31. The hydrogen peroxide (H₂O₂) and malonaldehyde (MDA) content in BARI Gom 33 was low compare to BARI Gom 25 and BARI Gom 31, which may due to greater increase of the APX, CAT and POD in resistant genotypes. Thus, it may suggest that a more efficient antioxidative defense system in BARI Gom 33 during the infection process of *M. oryzae* restricts the cell damage caused by the fungus. The identified genotypes can either be used directly in the blast prone area or as a source of resistance to further development of blast resistance high yielding wheat variety.

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INTRODUCTION

Wheat, the second major staple food source after rice has a crucial role to play for ensuring global food security. It is one of the first tamed crops by human among the grains. In developing countries, wheat is the second source of calories and first sources of protein in the diets of consumers [1]. Around 37.2 million hectares area of southern Asia are under wheat cultivation producing 98.7 million tons of wheat [2]. At present, hexaploid bread wheat accounted for 95% of the wheat grown throughout the world, following tetraploid durum wheat of about remaining 5% [3]. Global climate change along with worldwide financial crisis and depletion of natural resources cause serious threat to the living standard of millions of poor people. It is projected that wheat demand increased to 60 % by 2050 in the developing world [4]. At the same time, several

biotic and abiotic stresses constraints the widespread cultivation of wheat. Wheat blast caused by the ascomycetous fungus *Magnaporthe oryzae* (*Triticum* pathotype - MoT) [5] is currently the most devastating wheat disease limiting wheat production. Wheat blast is new to Asia, known to cause significant crop losses in some South American countries with warm and humid condition. The disease was first observed in the state of Paraná, Brazil, in 1985 [6]. *Wheat* blast emerged for the first time in 2016 in several southwestern and southern districts of Bangladesh viz., Pabna, Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal, and Bhola. Almost 3.5% of total wheat coverage (15000 ha) in Bangladesh was affected by the wheat blast that dwindled total wheat production by 15% [5,7]. Comparative genome analyses revealed that the fungal isolates appeared in Bangladesh was clonal and closely related to highly aggressive MoT isolates from South America [5,7,8].

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Wheat blast is mainly a disease of spike, however, can occur on all aerial plant parts. Severity of the disease greatly depends upon weather conditions, cultivars, and plant organs infected [9,10]. Although exact weather conditions required for a field epidemic are not clear, most severe blast outbreaks have coincided with wet years; warm temperatures and high humidity [10]. Whereas, disease management become challenging due to limited knowledge on the factors responsible for wheat blast epidemics. However, wheat blast fungus has physiologically and genetically complex character, with a range of aggressiveness and pathotypes. Most of the wheat cultivars are susceptible to wheat blast in Bangladesh [5]. Some studies showed the presence of partially tolerance but the mechanisms is totally unknown [11,12]. Upon any kind of environmental stress condition, wheat plants are being accounted by oxidative damage through accumulation of reactive oxygen species (ROS) inside the cells inhibiting both growth and grain yield [13]. Over-accumulation of ROS affects cellular redox homeostasis and causes oxidative stress [14,15] and plants tend to protect the damage of cell via activating antioxidant mechanism [13]. The antioxidant enzymes comprise of several enzymes such as ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (POD), superoxide dismutase (SOD) etc. In wheat, oxidative stress induced by different biotic and abiotic stresses, cause an alteration in the activity of SOD, APX, CAT, POD and in the ROS concentration are reported both in field and laboratory conditions [16, 17, 18, 19, 20]. Tolerant genotypes generally consist of higher antioxidant activity causing less damage to the cell, which is solely depends on genetic potentiality of cultivars [13, 21]. Although, involvement of antioxidant systems in pathogen infection have been well documented [22, 23]. Only few studies investigated the relationship between change in antioxidant mechanism to host resistance. There is no report/work in terms of the tolerance of commercially cultivated wheat genotypes and their tolerance mechanism in Bangladesh. Therefore, this study was aimed to find out blast resistant wheat varieties/cultivars among the old and newly released varieties in Bangladesh and also to measure antioxidant enzymatic activity, ROS accumulation and cell membrane damage (MDA), to know the genetic potential of tolerant and susceptible cultivars.

MATERIALS AND METHODS

Plant Materials and Growth Condition

Sixteen varieties/cultivars collected from Bangladesh Agricultural Research Institute (BARI) namely Kheri, Kanchan, Sourav, Gourav, Shatabdi, Sufi, BARI Gom 25, BARI Gom 26, BARI Gom 27, BARI Gom 28, BARI Gom 29, BARI Gom 30, BARI Gom 31, BARI Gom 32, BARI Gom 33 and BROUCK 100 were used for this study. Seeds were surface sterilized and sown on small pots containing soil. Twenty one days old seedlings were used for fungal inoculation. At the same time seeds were also sown in the experimental field laboratory under natural condition to evaluate different yield and yield contributing characters.

Inocula Preparation, Inoculation and Evaluation

Wheat blast isolates were cultured on readymade oatmeal agar at 26°C for 17 days. The surface of culture media was

then gently rubbed with a sterile paint brush to remove aerial mycelia and then exposed for 3 days to fluorescent light at 26°C to induce sporulation. Conidia from sporulated culture media were scraped and suspended in water containing Tween 20 (0.01%), then filtered and adjusted the concentration of conidia to $1 \times 10^5 \mu\text{L}$ water. The conidial suspension was sprayed on 21 days old wheat seedlings, incubated at 25°C temperature in a humid chamber for 24 hrs. After incubation, seedlings tray were transferred to a temperature controlled plant growth room at $28 \pm 1^\circ\text{C}$. Disease score were measured by following Standard Evaluation System (SES, IRRI) [24] after seven days of inoculation.

Antioxidant Enzyme Activity Assay

Activities of catalase (CAT) (EC 1.11.1.6) [25], Guaiacol peroxidase (POD) (EC 1.11.1.6) [26], and Ascorbate peroxidase (APX) (EC 1.10.3.3) [26] were measured in inoculated plants. Fifty milligrams of fresh plant sample were collected and homogenized with 3 ml of 50 mM potassium phosphate buffer (pH 8.0) in a mortar and pestle. The homogenate was centrifuged at 12,000 rpm for 10 min. In all stages, 4°C temperature was maintained. The clear supernatant was used for assaying CAT, POD, APX activity. To measure CAT activity 0.1 mL of enzyme extract was added to a 0.7 mL of 50 mM potassium phosphate buffer (pH 8.0), 0.1 ml of EDTA and 0.1 ml of H_2O_2 mixture and changes in absorbance were recorded immediately at 240 nm at 30 seconds interval for two minutes. POD activity was measured by adding 0.1 ml of enzyme extract to a mixture of 0.6 ml of 50 mM potassium phosphate buffer (pH 8.0), 0.1 ml of EDTA, 0.1 ml of H_2O_2 and 0.1 ml of guaiacol, and changes in absorbance were recorded immediately at 470 nm at 30 seconds interval for two minutes. 0.1 mL of enzyme extract was added to 0.6 ml of 50 mM potassium phosphate buffer (pH 8.0), 0.1 ml of EDTA, 0.1 ml of H_2O_2 and 0.1 ml of ascorbate mixture and changes in absorbance were recorded immediately at 290 nm at 30 seconds interval for two minutes to measure APX activity.

Determination of Hydrogen Peroxide (H_2O_2) and Malondialdehyde (MDA) Activity

Malondialdehyde (MDA) and Hydrogen peroxide (H_2O_2) content were measured according to the protocol by [27, 28]. Briefly, 0.1 g leaf tissue (with similar age, and young expanded leaf) was ground into powder with liquid nitrogen, and then the powder was put into a tube containing 1 ml 0.1% (w/v) TCA and mixed by inverting the tube to homogenize the leaf tissue. Homogenized samples were centrifuged at 10,000 rpm for 10 min, and then the supernatants were transferred to a new tube. After centrifugation, the supernatants were kept in dark for 1 h after mixing with phosphate buffer (10 mM, pH 7.0) and potassium iodide (1 M) (in the ratio of 0.5 ml 0.5 ml 1 ml). Absorbance of the resulting solution was recorded at 390 nm. For MDA content measurement, 4 ml of 20% TCA containing 0.5% TBA was added to the supernatant and mixed well. The mixture was boiled at 95°C for 15 min and quickly cooled on ice (TBA can interact with MDA and resulted into red compound in acidic buffer, so the content of MDA can be calculated by measuring the density of the resulting red compound with

spectrophotometer at 532 nm. All the steps were performed at 4 °C except absorbance measurement.

Statistical Analysis

All experiments were performed with at least three biological samples and three time repetition unless mentioned. Data analysis was performed using the Statistical tools (Student's t-test) of Microsoft Excel software.

RESULTS

Assessment of Wheat Genotypes for Blast Tolerance

A number of sixteen wheat varieties/cultivars were grown in plastic tray filled up with soil (Fig. 1b) at plant growth room, Department of Genetics and Plant Breeding. The collected blast pathogen was grown on oat agar media (Fig. 1a) and conidial suspension were sprayed on twenty one days old seedlings and scoring were done seven days after inoculation (Fig. 1 c,d). On the basis of the system score 0 indicated 0% leaf area infection and score 9 denoted more than 65% leaf area infection. In score 8 extensive leaf curling and browning of upper and middle leaves were prevalent. Plants were generally stunted and many plants were died. On the other hand in score 3 several lesions were randomly scattered within the plot and the lesions number were ranged from 1 to 4. In case of control plant, no lesions were developed. Among the cultivars tested, BARI Gom 33 scored as three (3); BARI Gom 20, BARI Gom 25 and BARI Gom 29 scored as eight (8); and others were scored as seven (7). (Table 1)

Evaluation of Yield and its Attributing Traits in Tolerant and Susceptible Cultivars

The selected moderately tolerant variety BARI Gom 33 along with BARI Gom 25 (Score 8) and BARI Gom 31 (score 7) were also grown in the experimental field laboratory of Genetics & Plant Breeding department under natural growth condition

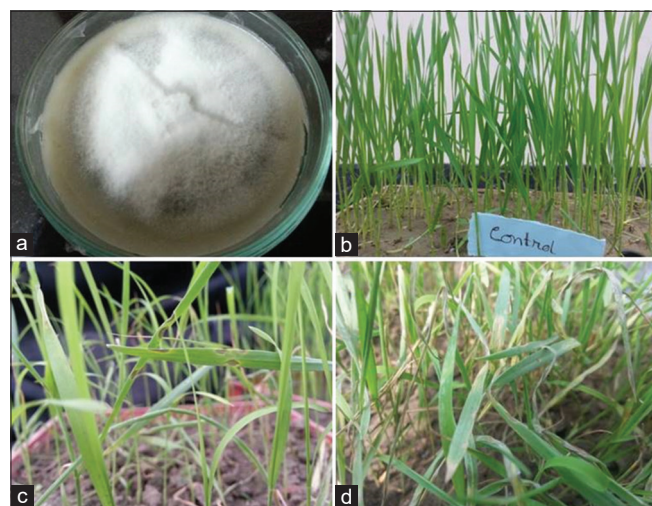


Figure 1 Assessment of wheat genotypes tolerance to *Magnaporthe oryzae* pv *Triticum*. a) Inocula preparation; b) Growing of plant in plastic tray; c, d) Plant after inoculation and scoring stage

for evaluation of normal yield and yield contributing traits such as days to 50% flowering, spikelets per spike, grains per spike, 100-grain weight. There is no marked difference among the genotypes in terms of tested traits under normal growth condition. The maximum value were obtained for spikelets number per spike in BARI Gom 25, grain number per spike in BARI Gom 31, 100-grain weight in BARI Gom 33 and yield per plant in BARI Gom 33 and minimum yield per plant were obtained in BARI Gom 31 (Table 2). Other some varieties were also tested for those characters (data not shown), but their performance was also not better than BARI Gom 33, BARI Gom 25.

H₂O₂ (Hydrogen Peroxide) and MDA (Malondialdehyde) Content; Antioxidant Enzyme Activity

Upon inoculation of blast pathogen the content of H₂O₂, MDA were tested in both tolerant and susceptible genotypes. It was found that accumulation of both compound were less in BARI Gom 33, high in BARI Gom 25 and BARI Gom 31 after seven days of inoculation (Figure 2a). The different antioxidant enzyme (APX, CAT, POD) involved in the detoxification of oxidative stress were also estimated and in all cases the activity is high in tolerant genotypes BARI Gom 33 as compared to susceptible variety BARI Gom 25 and BARI Gom 31 (Figure 2b).

DISCUSSION

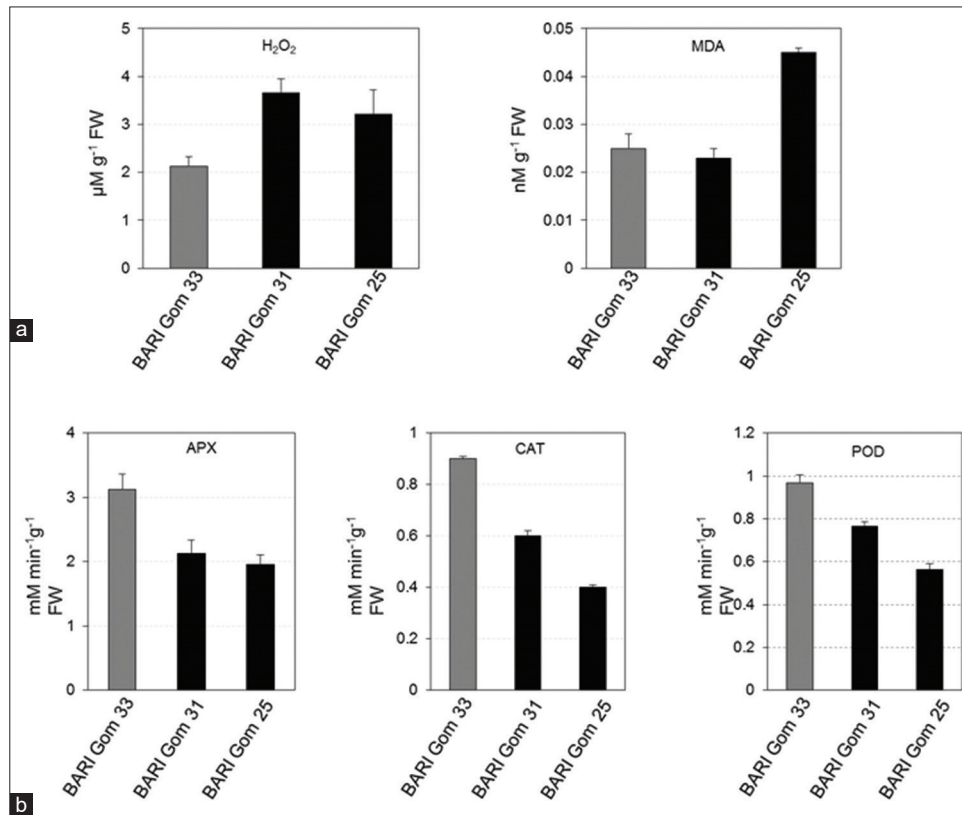
A number of total sixteen wheat varieties including some recent cultivars were collected from BARI and undergoes for their disease resistance at seedling stage and also phenotypic evaluation for yield and yield contributing characters were performed under normal field condition. The evaluations of tolerance among the collected cultivars were measured by the Standard Evaluation System (SES) [24]. Upon inoculation of *M. oryzae* pv *triticum*, plant showed stunted growth and developed lesions on the leaf. Among the cultivars, BARI Gom 20 (Gourav), BARI Gom 25 and BARI Gom 29 were found more susceptible to MoT as it scored 8 (Table 1). According to SES, in score 7, tips of most upper leaves were curling, upper and middle leaves were brown and most of the leaves were going to be dead. Kheri, Kanchan, BARI Gom 19 (Sourav), BARI Gom

Table 1: Assessment of wheat cultivars in terms of leaf injury score after inoculating wheat blast pathogen

Name of cultivar/variety	Disease score	Name of cultivar/variety	Disease score
Kheri	7	BARI Gom 27	7
Kanchan	7	BARI Gom 28	7
BARI Gom 19 (Sourav)	7	BARI Gom 29	8
BARI Gom 20 (Gourav)	8	BARI Gom 30	7
BARI Gom 21 (Shatabdi)	7	BARI Gom 31	7
		(BAW-1182)	
BARI Gom 22 (Sufi)	7	BARI Gom 32	7
		(BAW-1202)	
BARI Gom 25	8	BARI Gom 33	3
		(BAW-1260)	
BARI Gom 26	7	BROUCK-100	7
		(BAW-1300)	

Table 2: Evaluation of tolerant and susceptible cultivars in terms of yield and its contributing traits

Variety / Cultivar	Days to 50 % flowering	Spikelets/spike	Grains/spike	100-grain weight (g)	Yield/plant (g)
BARI Gom 33 (BAW-1260)	69	19	51	3.5	9.5
BARI Gom 31 (BAW-1120)	70	18	59	3.0	8.5
BARI Gom 25	67	21	47	3.3	9.0

**Figure 2** Antioxidant activity in response to disease infection a) MDA and H₂O₂ content b) APX, CAT and POD activity in moderately tolerant and susceptible genotype under disease condition

21 (Shatabdi), BARI Gom 22 (Sufi), BARI Gom 26, BARI Gom 27, BARI Gom 28, BARI Gom 30, BARI Gom 31 (BAW-1182), BARI Gom 32 (BAW-1202), BROUCK 100 (BAW-1300) were found susceptible to MoT as it scored 7 [24]. On the other hand in score 3 several lesions were randomly scattered within the plot and the lesions number were ranged from 1 to 4. Only BARI Gom 33 showed resistance to MoT as it scored 3 [24], having similarity to the finding of other researcher [29]. The partially tolerant BARI Gom 33 showed similar rather better performance in terms of yield and yield contributing traits under natural field condition which is also similar to the results of [29] indicating their greater performance under normal and stressed condition.

The identified tolerant and susceptible varieties were biochemically analyzed to understand the response of antioxidant system in tolerance mechanism. Biotic stress generally leads to an overproduction of reactive oxygen species (ROS) like H₂O₂ and MDA which are responsible for oxidative stress. During pathogenic infection, ROS accumulation is important defensive mechanism [30] which mainly happened due to imbalance between their production and host defense responses that cause damage to the host. Plant cell activate

a number of enzymatic and non-enzymatic systems to cope the situation and maintain constant level in cells [31]. In the present study, higher accumulation of hydrogen peroxide (H₂O₂) during infection by MoT contributed to higher concentration of malonaldehyde (MDA) in BARI Gom 31 and BARI Gom 25 as compared to BARI Gom 33. In case of tomato plants infected by *Fusarium oxysporum*, overaccumulation of ROS also results in increased lipid peroxidation [32]. Oxidative stress related cellular damage can be estimated by lipid peroxidation products, in which MDA is a great indicator. Cellular stress also occurred due to some toxins produced by pathogen that are cytotoxic to cell in rice [33] and can cause rapid lipid peroxidation and cellular damage [34]. The lower enzymatic activity of APX, CAT and POD were observed in BARI Gom 25 and BARI Gom 31, but higher in BARI Gom 33. The higher activity of those enzymes in BARI Gom 33 during pathogen infection resulted in the lower concentrations of H₂O₂, and subsequently reduced the cellular damages caused by ROS. Therefore, the difference in the activity of CAT, APX and SOD in tolerant and susceptible cultivars suggested that these enzymes played a major role for resistance in BARI Gom 33 to blast. The higher catalase activity was also observed in resistant

lines of maize against *Aspergillus flavus* than susceptible one [30]; in tomato leaf against *B. cinereo* infection resulting in reduced lesion formation [35] and their involvement against abiotic stresses was also documented [35]. Peroxidase (POX) activity is very much important in wheat cultivars against *Rhizoctonia cerealis* and also detrimental to resistance during late stages of infection against *P. oryzae* [36], is very much similar to the present study which showed higher level of POD activity in BARI Gom 33 during pathogen infection. The enzyme APX removed H₂O₂ from chloroplast, peroxisomes and mitochondria [37] and their increased activity have been well described during fungal infection [38]. In present study increased APX activity in BARI Gom 33 also suggested their role in defense mechanism. Increased of both transcript and activity of APZ was also observed in barley leaves inoculated by *B. graminis* [39]. The higher activity of APX, CAT and POD in BARI Gom 33 compared to BARI Gom 25 and BARI Gom 31 during pathogen infection resulted in lower accumulation of H₂O₂ and subsequently reduced MDA content restricting cellular damage caused by ROS. The result of the present study indicated the presence of a more efficient antioxidative defense mechanism in BARI Gom 33 which reduced the cellular damage by the removal of excess ROS accumulation during the infection of *M. oryzae* pv *tritricum*, contributing partially resistance to blast. Further research may aimed to develop biochemical marker using antioxidant system in breeding program to select/develop blast resistant wheat variety suitable for blast prone areas in the world.

CONCLUSION

Among the sixteen cultivars of wheat tested against *M. oryzae* pv *tritricum*, blast severity in BARI Gom 33 is lower and the phenotypic performance of BARI Gom 33 in terms of yield and yield contributing traits is almost same or better in some extent compare to all other varieties. BARI Gom 33 exhibited an increased antioxidant enzyme activity than susceptible cultivars BARI Gom 25 and BARI Gom 31, contributed to lower concentration of reactive oxygen species (ROS) limits the cellular damage during the infection process of MoT, thus showing greater resistance to blast in wheat.

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Author's Contribution

GHMS Design, formulation, supervision of experiment and writing of manuscript. AH Conducting experiment, lab analysis, writing of manuscript. CSB Conducting experiments, collection and analysis of data, writing of manuscript. AM Inocula preparation, supervision.

Competing Interests

The authors have declared that no competing interests exist

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