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# Effect of gamma-aminobutyric acid on resistance against stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) in wheat cultivars

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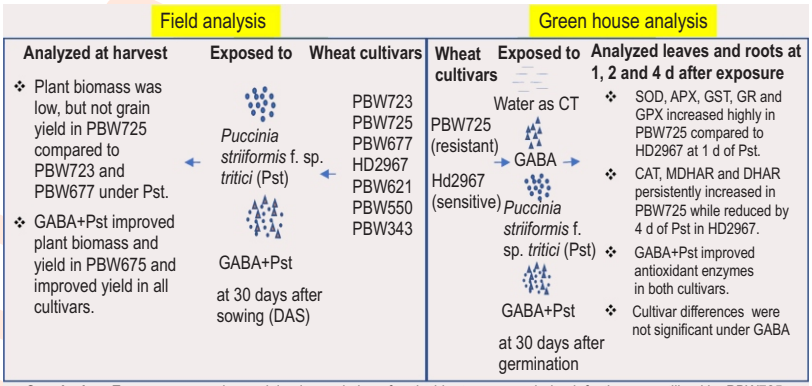
## Abstract

**Aim:** The present study was undertaken to screen wheat cultivars for resistance against stripe rust and the improvement of resistance by exogenous gamma-aminobutyric acid (GABA) under field conditions and studying two contrasting cultivars for antioxidant related defense activities at 1, 2 and 4 d of GABA, *Puccinia striiformis* f. sp. *tritici* (Pst) and GABA+Pst treatments given to one-month-old seedlings.

**Methodology:** Wheat cultivars (PBW723, PBW725, PBW677, PBW550, PBW343, PBW621, HD2967) were grown and exposed to *Puccinia striiformis* f. sp. *tritici* (Pst) and GABA+Pst in fields and studied for yield parameters. Subsequently, two cultivars found varying in resistance (PBW725 and HD2967) were analyzed for antioxidant enzymes in leaves and roots at 1, 2, and 4 day of GABA, Pst, and GABA+Pst and water as control given to 30 days old seedlings in the greenhouse.




**Conclusion:** Energy conservation and timely regulation of antioxidant enzymes during infection was utilized by PBW725 against stripe rust. GABA deficiency can be a reason for poor regulation of antioxidants in HD2967 under Pst. GABA application improved resistance against stripe rust in wheat.

**Results:** Field analysis found that the most resistant cultivar PBW725 reduced vegetative growth but not yield compared to other resistant cultivars (PBW723 and PBW677) under Pst and improved both growth and yield under GABA+Pst. GABA+Pst improved yield parameters in other cultivars also. In defense analysis, superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione-S-transferase (GST) increased in the leaves of PBW725 while only APX increased in HD2967 at 1 d of Pst. In roots also, SOD and GST increased 2-fold in PBW725 while only GST increased 1.3-fold in HD2967 at 1 d of Pst. There was persistence of catalase (CAT), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR) during Pst in the leaves of PBW 725 while CAT decreased at 4 d in HD2967. In roots also, CAT and MDHAR increased at 4 days of Pstin PBW725 while DHAR decreased in HD2967. Under GABA, cultivar differences were not significant. GABA+Pst improved deficient antioxidant enzymes in HD2967.

**Interpretation:** Energy conservation and timely regulation of antioxidant enzymes during infection was utilized by PBW725 against stripe rust. GABA deficiency can be a reason for poor regulation of antioxidants in HD2967 under Pst. GABA application improved resistance against stripe rust in wheat.

**Key words:** Antioxidant, Ascorbate, Gamma-aminobutyric acid, Glutathione, Stripe rust, *Triticum aestivum*

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## Introduction

Stripe rust (yellow rust) of wheat caused by *Puccinia striiformis* f. sp. *tritici* (Pst) is one of the three wheat rust diseases principally found in the wheat grown in a cooler environment. It causes powdery yellow stripes on leaves. Pst completes its life cycle in two different and unrelated host species, *i.e.,* wheat and *Berberis* spp. The infection process of Pst starts with spore attachment to host plant, and then germination, formation of appressorium, and obtaining nutrients by haustorium formation and host invasion. This pathogen is a biotrophic fungus that spreads potentially across long distances, contributing to sudden disease epidemics in new areas (Hovmøller *et al.,* 2011). The world has seen 51 major stripe rust epidemics from 1939 to 2016, which has caused 2% yield losses to total crop failure, and the epidemic occurred more frequently in Pakistan, India, China, Nepal, Yemen, Ethiopia, Uzbekistan, Kenya, Australia, the United Kingdom, the United States, Chile, New Zealand, Ecuador, Peru, Colombia, and Mexico (Chen *et al.,* 2020a; Jamil *et al.,* 2020).

The frequent outbreaks of Pst highlighted the need for an effective control strategy. Plants are adapted to use ROS as a stress signal to activate antioxidant response under biotic stresses like abiotic stresses (Gadjev *et al.,* 2006). Enzymatic and non-enzymatic antioxidants increased under different biotic stresses in plants (Sharma *et al.,* 2012). Thus, the ROS scavenging systems can have an essential role in managing ROS generated in response to pathogens (Sharma *et al.,* 2012). Comprehensive reviews have indicated that plant responds to different stresses through common mechanisms where ROS and cellular redox state maintained by enzymatic and non-enzymatic antioxidants, act as both sensor and buffer against environmental perturbations including both abiotic and biotic (Perez and Brown, 2014). However, compared to abiotic stresses, antioxidant activity is less studied under biotic stresses. Gamma (γ)-aminobutyric acid (GABA), a non-protein amino acid, plays a critical role in plant development and stress. Several studies found that GABA can alleviate the deleterious effects of different abiotic stresses (reviewed by Sheteiwy *et al.,* 2019; Suhel *et al.,* 2022), and some studies found GABA improving biotic stresses also (Yu *et al.,* 2014; Yang *et al.,* 2017; van Rensburg and Van den Ende, 2020).Yu *et al.* (2014) found effective alleviation of stress effects of *Penicillium expansumin* harvested pear fruits upon GABA treatments where GABA activated antioxidant and defense activities.

Exogenous GABA induced resistance to the alternaria rot caused by *Alternaria alternata* in tomato fruits by increasing antioxidant enzymes and GABA-shunt (Yang *et al.,* 2017).GABA priming has produced resistance in *Arabidopsis thaliana* against *Botrytis cinerea* by reducing ROS and increasing antioxidant activity (van Rensburg and Van den Ende, 2020). To the best of our knowledge, GABA improving wheat response against stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (Pst) has not been reported. In general, high levels of defense proteins under pathogen infection have been related to resistance in studies comparing resistant and susceptible cultivars. But some studies have indicated that regulation of defense proteins varied over time between resistant and susceptible cultivars. PR proteins, beta-1,3 glucanase, and chitinase induced late by high amount in susceptible while early by high amount in resistant response against wheat blast (Tufan *et al.,* 2009). The same has been reported under *Septoria tritici* in wheat (Shetty *et al.,* 2009). Some reports have found that some PR proteins increased late by high amount in resistant versus susceptible response. Peroxidases, and ribonuclease increased late by high amount in resistant compared to susceptible response against Pst in wheat (Bozkurt *et al.,* 2010). Thus, it is important to study defense response over time of infection while comparing resistant and susceptible cultivars, however, such studies are less reported in literature. Thus, keeping all these views in mind, this study was planned to screen wheat cultivars for resistance against stripe rust and the improvement of resistance by exogenous GABA under field conditions, and further studying two contrasting cultivars for antioxidant related defense activities at 1, 2 and 4 d of GABA, *Puccinia striiformis* f. sp. *tritici* (Pst) and GABA+Pst treatments given to one-month-old seedlings.

## Materials and Methods

**Screening of cultivars under field conditions:** Seeds of wheat (*Triticum aestivum* L.) cultivars PBW723, PBW677, PBW725, HD2967, PBW621, PBW550, PBW343 were obtained from the Department of Plant Breeding and Genetics and sown in fields of the Department of Plant Pathology, Punjab Agricultural University. Seeds were sown in split plot-RCBD with each plot size of one $m^2$. Cultivar was taken as the main plot while treatments as subplots. Two treatments were used, *Puccinia striiformis* f. sp. *tritici* (Pst) and GABA+Pst. Untreated and GABA controls were not included as their purity was hard to maintain under field conditions due to wind-blown spores from nearby infected plots. Treatments were applied at 30 d after sowing (DAS), for which 1 mM GABA was sprayed to GABA+Pst plots, then after one h, Pst was applied to all plots (Pst and GABA+Pst).

For Pst application, a single pot having artificially inoculated wheat seedlings (of HD2967) with the pathogen (mixture of pathotypes prevalent in the area included 238 S 119, 110 S 119, 46S 119, 78S 84), was kept in each plot for initiating the infection. Suspension made by taking freshly harvested Pst uredospores in water ($10^9$ $ml^{-1}$) was sprayed in the evening for two consecutive days. GABA-treatment was repeated at 50 DAS where 1 mM GABA was again sprayed to GABA+Pst plots but Pst treatment was not repeated. Each sprayed solution was used @ 120 ml per plot. The concentration of GABA was decided from literature reports as well as by performing laboratory experiments. One mM GABA was found highly effective concentration from the range in pear fruits (Yu *et al.,* 2014) and rice (Nayyar *et al.,* 2014). Screening of cultivars under field conditions was done for two years 2017-18 and 2018-19. In 2017-18, data of disease incidence and severity of lower and upper leaf was collected at 85 DAS while plant above-ground dry biomass, number of seeds per ear, 50-seed weight, and plot yield were

collected at the time of harvest. Measurement of plot yield was repeated in the next year (2018-19). Incidence was checked by looking at each plot manually and writing % of the plot affected with disease. Severity at upper leaf and lower leaf was checked for % of the leaf area affected based on the modified Cobb scale (Peterson *et al*., 1948), for 10 randomly selected plants from each plot. The upper and lower leaf was the first or second leaf from the top and bottom of the plant, respectively.

**Screening of cultivars under greenhouse conditions:** PBW725 and HD2967, varying in resistance against stripe rust and the famous growing varieties of the region, were selected for biochemical analysis. Seeds were sown in earthen pots in the greenhouse. Treatments (CT, GABA, Pst, GABA+Pst) were applied to 30 d old seedlings where 1 mM GABA was sprayed to GABA and GABA+Pst pots, then after one hour, suspension of freshly harvested uredospores of Pst in water ($10^9$ $ml^{-1}$) was sprayed to Pst and GABA+Pst pots, and water was sprayed to control (CT) pots. Inoculated pots were provided saturated vapor pressure (100% humidity) for successful germination of uredospores and infection. The pots were then shifted outside in isolation for further development of disease. Data were collected from leaves and roots at 1, 2, and 4 d post-treatment.

**Estimation of antioxidant enzymes:** Activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) were estimated as mentioned (Yang *et al.,* 2008). These were commonly extracted in 50 mM potassium phosphate buffer pH 7, 1 mM EDTA, 2% PVP and 0.05% triton-X-100. SOD was assayed in 50 mM Tris HCL pH 8.5, 1.42 % triton-X-100, 25 uM NBT and 1.5 mM pyragallol at 550 nm. Reading without extract was used as blank. One SOD unit was defined as the amount of enzyme required to cause 50% inhibition of NBT-reduction of blank. APX was assayed in 50 mM potassium phosphate buffer pH 7, 0.1 mM EDTA, 0.3 mM ascorbate and 1 mM $H_2O_2$ at 290 nm and activity was calculated using molar extinction coefficient of ascorbate of 2.8 $mM^{-1}$ $cm^{-1}$. CAT was assayed in 25 mM $H_2O_2$ and 50 mM potassium phosphate buffer of pH 7.0 at 240 nm and activity was calculated using a molar extinction coefficient of $H_2O_2$ as 0.0394 $mM^{-1}$ $cm^{-1}$. GR was assayed in 100 mM potassium phosphate buffer (pH 7.0),1.0 mM oxidized glutathione (GSSG), and 0.1 mM NADPH at 340 nm and activity was calculated using a molar extinction coefficient of NADPH as 6.22 $mM^{-1}$ $cm^{-1}$.

Glutathione peroxidase (GPX), glutathione-S-transferase (GST) were estimated following the protocols of (Gapińska *et al.,* 2008). Both enzymes were commonly extracted in 50 mM sodium phosphate buffer pH 7.0, 1 mM EDTA, 1% PVP. GPX was assayed in 50 mM potassium phosphate buffer pH 7.0, 2 mM EDTA, 0.15 mM NADPH, 0.3 mM t-butylhydroperoxide, 0.44 units of glutathione reductase (GR) and 2.5 mM GSH at 340 nm. The activity was calculated using a molar extinction coefficient of NADPH as 6.22 $mM^{-1}$ $cm^{-1}$. GST was assayed in 100 mM potassium phosphate buffer pH 6.25, 1 mM CDNB (1-chloro-2,4-dinitrobenzene) and 2 mM GSH at 340 nm. The activity was calculated using a molar extinction coefficient of glutathione conjugated-CDNB as 9.6 $mM^{-1}$ $cm^{-1}$. Monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) were measured by protocol derived (Doulis *et al.,* 1997; Liu *et al.,* 2012; Mishra *et al.,* 2013). Both enzymes were commonly extracted in 100 mM potassium phosphate buffer pH 7.5, 1 mM EDTA, 2% PVP where 1 mM ascorbate was added to buffer just before use. MDHAR was assayed in 50 mM potassium phosphate buffer pH 7.5, 10 mM NaCl, 2 mM $MgCl_2$, 400 mM sucrose, 2.5 mM ascorbate, 0.14 units of ascorbate oxidase and 0.10 mM NADH at 340 nm.The activity was calculated using a molar extinction coefficient of NADH as 6.22 $mM^{-1}$ $cm^{-1}$. DHAR was assayed in 50 mM potassium phosphate buffer pH 7 with 0.1 mM EDTA, 0.2 mM dehydroascorbate and 2.5 mM reduced glutathione (GSH) at 265 nm. The activity was calculated using a molar extinction coefficient of ascorbate as 14 $mM^{-1}$ $cm^{-1}$.

**Statistical analyses:** All analyses were performed in three independent biological replicates and the values were presented in mean ± S. D. Data were analyzed by Fisher's L. S. D.at $p < 0.05$ using DSASTAAT.XLX (Onofri and Pannacci, 2014).

## Results and Discussion

In field analysis, cultivars were compared with one another under Pst to know the resistance of the cultivar against stripe rust. GABA+Pst was compared to Pst in each cultivar to know if GABA application can improve response of the cultivar against stripe rust. Incidence of rust (Fig. 1a) was highest in PBW343/HD2967 (100%/93%) under Pst but reduced in HD2967 (80%) under GABA+Pst. Severity of upper and lower leaf (Fig. 1b, c) was highest in PBW343 (47/95) under Pst but reduced (37/89) under GABA+Pst. Severity reduced in HD2967/PBW550 also (from 28/26 to 17/14 in upper leaf and 73/76 to 57/70 in lower leaf) under GABA+Pst. Plant above ground biomass (Fig. 1d) was lowest in PBW343/PBW550 (2.10/2.79) under Pst but improved (3.7) in PBW550 under GABA+Pst. Plant above ground biomass (Fig. 1d) and number of seeds per ear (Fig. 1e) were low (3.99 and 43) in PBW725 compared to PBW723/PBW677 (5.06 and 51.8/4.80 and 50.5) under Pst but improved greatly in PBW725 (5.32 and 52.8) under GABA+Pst. However, 50-seed weight (Fig. 1f) was similar among PBW723/PBW725/PBW677 under Pst.

The number of seeds per ear were improved in HD2967 (from 38 to 51) and PBW550 (from 39.6 to 53.07) under GABA+Pst. Plot yield was statistically similar among PBW725/PBW723/PBW677 (725.8/691.9/734.5) in 2017-18 (Fig. 1g) while highest in PBW725 (714.7) in 2018-19 (Fig. 1h) under Pst. PBW677 showed low yield in 2018-19 (484.7) compared to 2017-18 (734.5) under Pst. Plot yield improved in PBW723/PBW725/PBW677/PBW550 in 2017-18 while in all cultivars in 2018-19 under GABA+Pst. The present study showed the improvement effects of GABA against stripe rust in wheat. PBW725 reduced vegetative growth but not grain yield under Pst but improved both under GABA+Pst. PBW725 might be conserving energy and preferring defense over the growth under

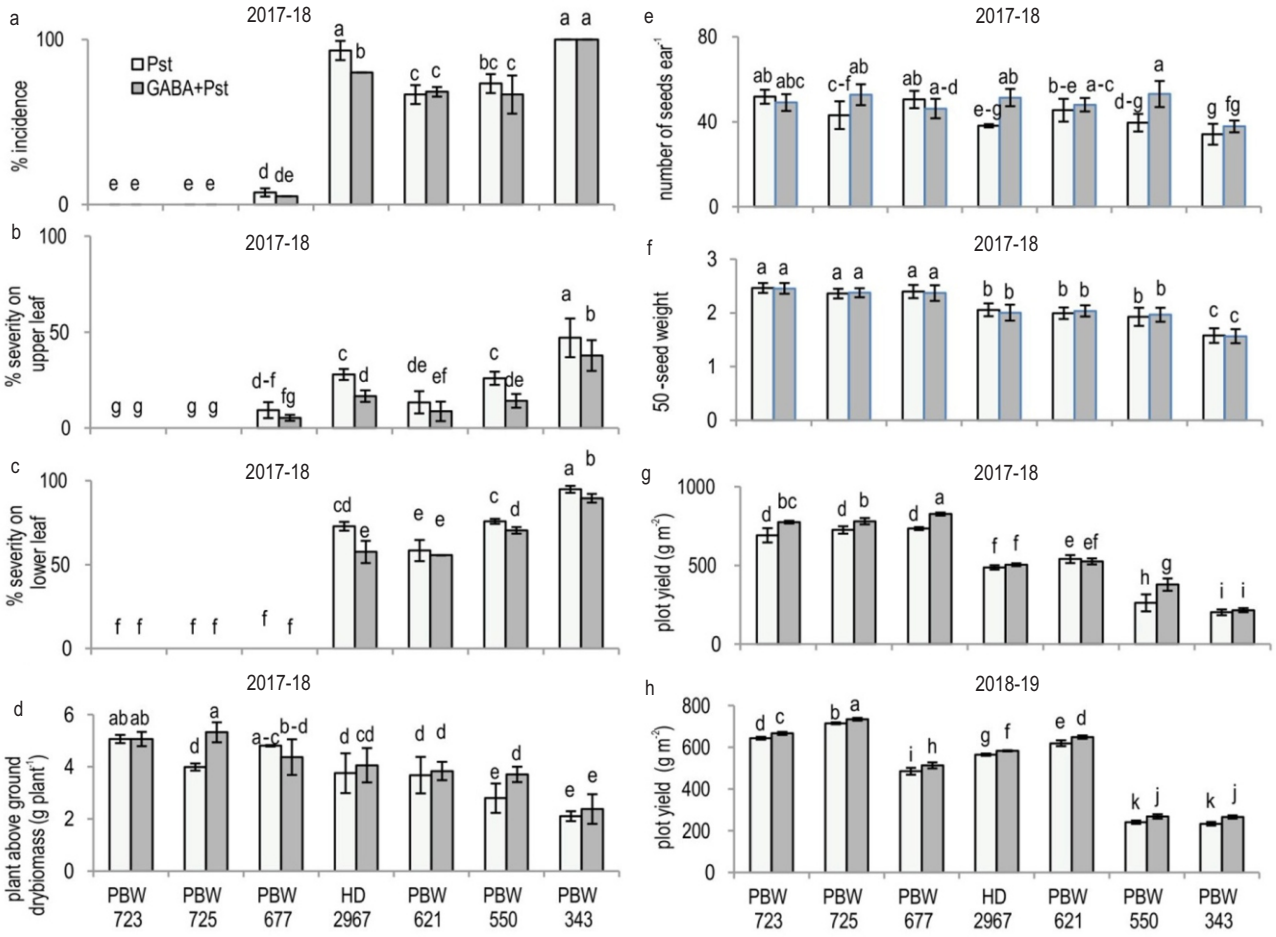


**Fig. 1:** GABA improving stripe rust response under field conditions in wheat cultivars PBW723, PBW725, PBW677, HD2967, PBW621, PBW550, and PBW343. Each data point is mean ± S.D. (n=3). Incidence and severity measured at 85 DAS while other data collected at harvest at P. A.U. Ludhiana during 2017-18 and 2018-19. Different alphabets represent the significant difference (Fisher's L.S.D. at $p < 0.05$).

Pst but improved both defense and growth under GABA+Pst. Jiang *et al.* (2017) reported that in response to pathogen attacks, plants prioritize defense reactions generally at the expense of plant growth. Phytohormone signaling pathways are associated with the stunted plant growth caused by blast disease in rice seedlings (Jiang *et al.,* 2017). Like other foliar diseases, yield losses under stripe rust can be due loss of photosynthesis thus reduced carbohydrate availability for grain filling (Devdas *et al.,* 2014; Chen *et al.,* 2015). Mineral uptake and remobilization of minerals from vegetative tissue to grains after anthesis can also be reduced by stripe rust (Devdas *et al.,* 2014).

In biochemical analysis, individual application of GABA was compared to water control (CT) to know the regulation of biochemical activity by GABA under controlled conditions. Pst was compared to CT to know the level of biochemical activity under Pst. GABA+Pst was compared to Pst to know if GABA improves biochemical activity under infected state. SOD (Table 1) increased at 1 and 2 d in leaves and roots of PBW 725 while at 2 and 4 d in HD2967 under Pst. GABA+Pst improved SOD at 2 d in the roots of HD2967 and 4 d in the leaves of PBW725. Individually GABA increased SOD in leaves and roots of both cultivars at different stages. APX (Table 1) increased by more amount in the leaves of PBW725 than of HD 2967 at 1 d under Pst. In roots, APX increased in HD2967 only at 2 and 4 d under Pst.

GABA+Pst improved APX at 4 d in the leaves of PBW725 only. Individually GABA increased APX in leaves and roots of both cultivars at different stages. GST (Table 1) increased under Pst at all three stages in the leaves of PBW725 while at 2 and 4 d in HD2967 and in roots also, fold increase at 1 d was high (2.1-fold) in PBW725 compared to HD2967 (1.3-fold). GABA+Pst improved GST greatly in HD2967, in leaves at 2 d and roots at 2 and 4 d. Individually GABA increased GST in both cultivars at different stages. GR increased at 2 d in the leaves of PBW725 while 2 and 4 d in HD2967 (Fig. 2a), and in roots (Fig. 2b), GR increased at 1 and 2 d in PBW725, while 2 and 4 d in HD2967 under Pst. GABA+Pst improved GR only at 2 d in the roots of HD2967, while

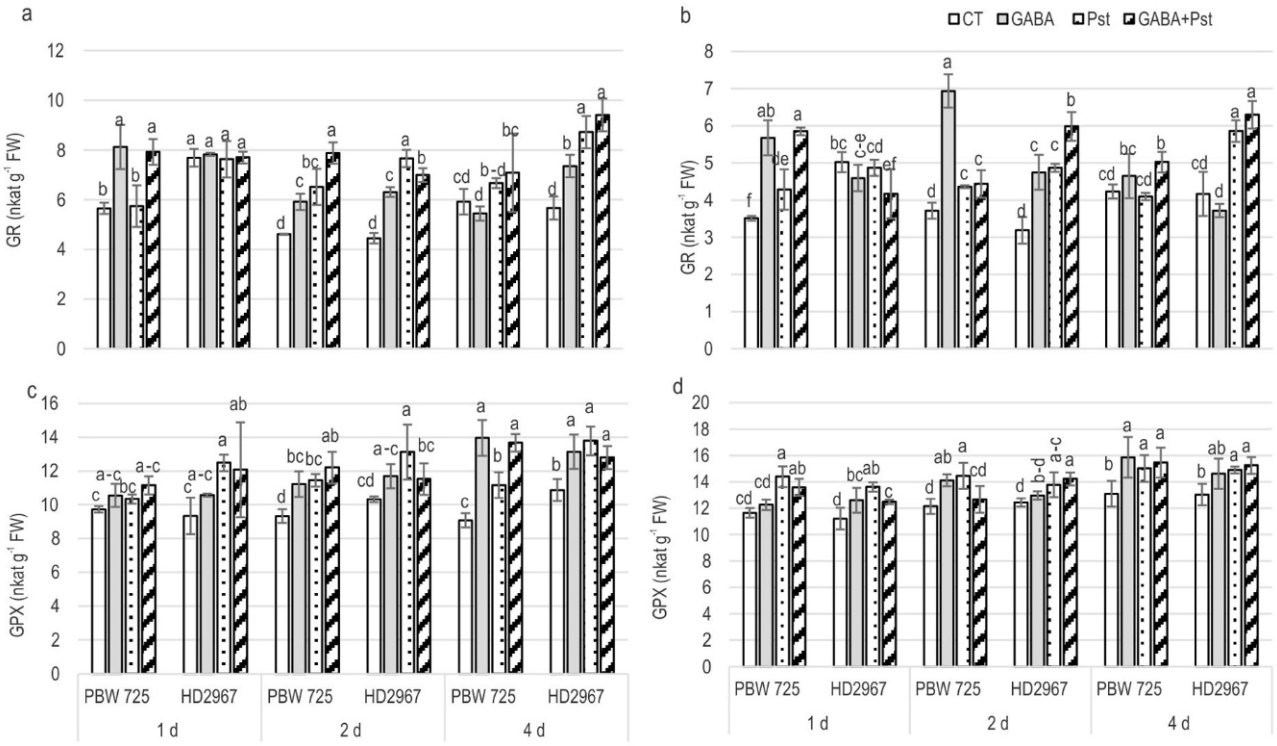


**Fig. 2:** GABA improvement of GR (a, b) and GPX (nkat g-1 FW) (c, d) under stripe rust(Puccinia striiformisf. sp. tritici (Pst)) in wheat. Data collected from leaves (a, c) and roots (b, d) at 1, 2, and 4 d post-treatment of GABA, Pst, GABA+Pst, and water (CT) applied to 30 d-old seedlings of wheat cultivars PBW725 and HD2967. Each data point represents mean ± S. D. (n=3). Data collected at P. A. U. Ludhiana during 2017-18. Different alphabets at each stage (1, 2, 4 d) represent significant difference (Fisher's L. S. D. at $p < 0.05$).

at different stages in leaves and roots of PBW725. Individually GABA increased GR in both cultivars at different stages. GPX increased almost at all three stages in leaves (Fig. 2c) and roots (Fig. 2d) of both cultivars under Pst but fold increase at 1 and 2 d was relatively high (24% and 20%) in the roots of PBW725 compared to HD2967 (21% and 10%). GABA+Pst improved GPX activity at 4 d in the leaves of PBW725 only. Individually GABA increased GPX in leaves and roots of both cultivars at different stages. Therefore, early high induction of antioxidant enzymes specially SOD, APX, and GST related to stripe rust resistance while late increase was seen in susceptible cultivar HD2967.

Early signaling events in response to pathogen may be the part of PTI (pattern triggered immunity) and are the major contributing factor for effective resistance in plants, and this way, plants can overcome suppressors produced by pathogens and suppress pathogen growth at early stage of infection. However, late induction indicated partial resistance of ETI (effector triggered immunity) which is probably induced when pathogen has grown to certain level inside plants (Hauck *et al.,* 2003; Tufan *et al.,* 2009). Early and high induction of SOD, APX, CAT and POX related to resistance against bacterial (*Xanthomonas*) infection in mungbean (Farahani and Taghavi, 2016). APX, GR, and GPX related resistance against Pst race CYR32 in wheat cultivar CM 42 (Chen *et al.,* 2020b). The SOD, CAT, and GST levels related to resistance against leaf rust caused by *Puccinia recondita* f. sp. *triticiin* wheat (Ivanov *et al.,* 2005). SOD activity decreased in the leaves of susceptible wheat cultivar HD 2329 following stripe rust (*Puccinia striiformis* f. sp. *tritici*) infection (Asthir *et al.,* 2011).High production of antioxidant enzymes, SOD, was found crucial in triggering the host defense mechanism against Pst at seedling and adult plant stage in wheat (Kiani *et al.,* 2021).

CAT (Table 2) increased consistently at all three stages in the leaves of PBW725 while decreased by about 30% at 4 d in HD2967 under Pst but improved by same amount under GABA+Pst. In roots, CAT increased at 2 and 4 d in PBW725 while 1 and 2 d in HD2967. GABA+Pst improved CAT at 2 d in leaves and roots of PBW725 by large amount. Individually GABA increased CAT in the roots of both cultivars and leaves of HD2967 at different stages. Under Pst, MDHAR (Table 2) increased in leaves of both cultivars but increase at 4 d was high in PBW725 and in roots, MDHAR increased at 2 and 4 d in PBW725 while 1 and 2 d in HD2967. GABA+Pst improved MDHAR in roots of both cultivars at 4 d. Individually GABA increased MDHAR in both cultivars at different stages. DHAR (Table 2) increased by 50% at 2 d and 65% at 4 d in the leaves of PBW725 but not raised at any stage in HD2967 under Pst and similarly in roots, DHAR decreased at 4d in HD2967 but not in PBW725. GABA+Pst did not improve DHAR in both cultivars. Individually GABA increased

**Table 1:** GABA regulation of SOD, APX and GST in wheat under stripe rust (*Puccinia striiformis* f. sp. *tritici* (Pst))

| | | SOD units $g^{-1}$ f.wt. | | | APX (nkat $g^{-1}$ f.wt.) | | | GST (nkat $g^{-1}$ f.wt.) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cultivars** | **Treatments** | **1 d** | **2 d** | **4 d** | **1 d** | **2 d** | **4 d** | **1 d** | **2 d** | **4 d** |
| | | **leaves** | | | | | | | | |
| PBW 725 | CT | 14.24 | 5.5 | 6.53 | 76.9 | 65.5 | 72 | 2.52 | 2.45 | 3.28 |
| (resistant) | GABA | 11.6 | 8.41 | 10.66 | 86.5 | 82.6 | 105.4 | 4.02 | 4 | 4.68 |
| | Pst | 23.6 | 9.74 | 7.49 | 130.2 | 121 | 131.7 | 3.59 | 4.15 | 4.09 |
| | GABA+Pst | 10 | 10.28 | 9.32 | 142.7 | 120.2 | 165.7 | 3.26 | 4.34 | 4.4 |
| HD2967 | CT | 15.66 | 7.85 | 8.17 | 110.6 | 78 | 62.1 | 3.33 | 3.21 | 2.64 |
| (susceptible) | GABA | 29.73 | 10.53 | 8.9 | 97 | 75.4 | 91.4 | 4.18 | 4.39 | 3.68 |
| | Pst | 11.63 | 9.15 | 13.83 | 144.7 | 139.2 | 136.9 | 3.75 | 4.37 | 4.17 |
| | GABA+Pst | 8 | 9.83 | 12 | 128.5 | 94.9 | 90.3 | 4.13 | 5.17 | 4.24 |
| **L. S. D. (p≤0.05)** | **6.62** | **1.88** | **1.57** | **27.6** | **15.8** | **14.9** | **0.5** | **0.73** | **0.53** | |
| | | **roots** | | | | | | | | |
| PBW 725 | CT | 12.98 | 7.98 | 7.6 | 80.9 | 66.5 | 57.4 | 2.97 | 3.97 | 4.45 |
| (resistant) | GABA | 21.57 | 9.43 | 7.69 | 81.6 | 58.9 | 81 | 3.51 | 5.16 | 5.83 |
| | Pst | 21.73 | 11.86 | 10.53 | 86.7 | 67.6 | 63.9 | 6.2 | 5.32 | 5.17 |
| | GABA+Pst | 10.19 | 11.86 | 10.99 | 81 | 78.7 | 62.4 | 4.96 | 5.01 | 5.89 |
| HD2967 | CT | 29.49 | 8 | 7.92 | 83.7 | 64.9 | 72.9 | 4.46 | 4.06 | 5.43 |
| (susceptible) | GABA | 33.66 | 12.9 | 8.78 | 100.9 | 80 | 65.6 | 6.18 | 6.07 | 7.3 |
| | Pst | 14.77 | 11.53 | 14.59 | 90.4 | 78.3 | 115.6 | 6.02 | 6 | 6.87 |
| | GABA+Pst | 10.83 | 14.36 | 10.03 | 90.2 | 90.3 | 100.6 | 5.35 | 7.08 | 10.23 |
| **L. S. D. (p≤0.05)** | **7.6** | **1.97** | **4.4** | **14.2** | **12.6** | **17.1** | **0.92** | **0.95** | **0.93** | |

Values are mean of three biological replicates; Data collected at 1, 2 and 4 d post treatment during 2017-18 at P. A. U., Ludhiana

**Table 2:** GABA regulation of CAT, MDHAR and DHAR in wheat under stripe rust (*Puccinia striiformis* f. sp. *tritici* (Pst)

| | | CAT (nkat $g^{-1}$ f.wt.) | | | MDHAR (nkat $g^{-1}$ f.wt.) | | | DHAR (nkat $g^{-1}$ f.wt.) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cultivars** | **Treatments** | **1 d** | **2 d** | **4 d** | **1 d** | **2 d** | **4 d** | **1 d** | **2 d** | **4 d** |
| | | **leaves** | | | | | | | | |
| PBW 725 | CT | 25.5 | 25.93 | 28.54 | 2.28 | 1.77 | 1.1 | 2.62 | 2.45 | 2.11 |
| (resistant) | GABA | 26.39 | 21.2 | 26.76 | 1.27 | 2.17 | 1.95 | 1.94 | 2.87 | 3.58 |
| | Pst | 30.54 | 29.28 | 35.64 | 2.58 | 2.26 | 1.93 | 2.57 | 3.76 | 3.47 |
| | GABA+Pst | 28.76 | 36.42 | 19.66 | 1.25 | 2.04 | 1.88 | 2.5 | 2.77 | 1.89 |
| HD2967 | CT | 24.04 | 17.61 | 23.62 | 1.23 | 1.77 | 1.4 | 2.12 | 2.84 | 2.56 |
| (susceptible) | GABA | 29.22 | 30.54 | 23.57 | 1.6 | 2.35 | 1.54 | 3.37 | 2.84 | 2.87 |
| | Pst | 31.77 | 37 | 17.99 | 2.35 | 2.4 | 2.01 | 2.21 | 2.64 | 3.09 |
| | GABA+Pst | 30.3 | 37.1 | 24.9 | 1.28 | 1.71 | 1.64 | 2.44 | 2.39 | 2.31 |
| **L. S. D. (p≤0.05)** | **2.33** | **1.84** | **2.94** | **0.18** | **0.32** | **0.21** | **0.60** | **0.45** | **0.70** | |
| | | **roots** | | | | | | | | |
| PBW 725 | CT | 1.84 | 2.08 | 1.63 | 0.91 | 0.74 | 0.6 | 0.38 | 0.6 | 0.4 |
| (resistant) | GABA | 1.54 | 2.66 | 2.79 | 1.02 | 1.26 | 0.94 | 0.48 | 0.78 | 0.49 |
| | Pst | 1.98 | 3.35 | 2.61 | 1.04 | 1.45 | 0.9 | 0.43 | 0.61 | 0.48 |
| | GABA+Pst | 1.79 | 6.74 | 1.79 | 1.08 | 0.95 | 1.17 | 0.35 | 0.63 | 0.47 |
| HD2967 | CT | 1.74 | 2.8 | 3.14 | 0.91 | 0.88 | 0.89 | 0.53 | 0.74 | 0.74 |
| (susceptible) | GABA | 1.36 | 5.07 | 4.21 | 1.09 | 1.27 | 0.91 | 0.81 | 0.62 | 0.66 |
| | Pst | 2.18 | 3.7 | 3.02 | 1.12 | 1.19 | 0.96 | 0.57 | 0.71 | 0.55 |
| | GABA+Pst | 1.16 | 2.29 | 2.59 | 0.84 | 1.07 | 1.14 | 0.4 | 0.59 | 0.48 |
| **L. S. D. (p≤0.05)** | **0.39** | **0.64** | **0.25** | **0.15** | **0.14** | **0.17** | **0.08** | **0.08** | **0.14** | |

Values are mean of three biological replicates; Data collected at 1, 2 and 4 d post treatmentduring 2017-18 at P. A. U., Ludhiana

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DHAR in both cultivars at different stages. In the present study, persistence of CAT, DHAR, and MDHAR related stripe rust resistance while failure at 4 d of Pst was found in HD2967.

One reason could be that these enzymes may be more effective antioxidants, thus their selection over other antioxidants by plant may save plant resources and energy during infection. CAT is known for its high efficiency and is the bulk removal of $H_2O_2$ (Cruz De Carvalho, 2008). Regeneration of ascorbate by MDHAR and DHAR could be important for removal of ROS during late infection. DHAR is reported to increase under increased stress when ascorbate gets deficient or dehydroascorbate over accumulates (Pérez-López *et al.,* 2009; Kour and Zhawar, 2018). MDHAR gene has been found to contribute to adult plant resistance against stripe rust through ROS metabolism in wheat (Feng *et al.,* 2014). Progressive increase of CAT related to adult plant resistance during stripe rust in wheat (Esmail *et al.*, 2020).

CAT and DHAR levels related to resistance towards powdery mildew infection in near-isogenic barley lines with varying types of resistance (Harrach *et al.,* 2008). It was clear from field experiments that HD2967 was mildly resistant to stripe rust. In antioxidant analysis also, though some antioxidant enzymes became deficient, but other antioxidant enzymes increased by 4 d of Pst. HD2967 is the released variety of 2011 and had Yr2, Yr7, Yr24, Yr64 RS (seedling resistance)/AS (all stage resistance) genes, and Yr 30 APR (adult plant resistance) gene, and PBW725 released in 2015 and has Yr2, Yr15, Yr24, Yr26, Yr64 RS/AS genes, and Yr29 and Yr30 APR genes (Rani *et al.,* 2019). HD2967 had developed susceptibility only to new races of stripe rust. Another important observation in the present study was that both cultivars did not differ for regulation of antioxidant enzymes under GABA but differed under Pst, thus, GABA deficiency may be a reason of poor regulated antioxidant enzymes under Pst in HD2967 (Kaur and Zhawar, 2021).

The present study concluded that timely regulation of antioxidant enzymes and conservation of energy may be the important weapons against stripe rust in wheat. Failure of these events in susceptible plant may be due to the deficiency of important regulators like GABA. GABA application can improve response of susceptible wheat cultivar against stripe rust.

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