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Expression analysis of hormonal pathways and defense associated genes in gamma-rays mutagenized wheat genotypes against combined stresses of spot blotch and terminal heat☆

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ABSTRACT

Wheat (*Tritium aestivum* L.) productivity is severely hampered by various pathogens and changing climatic conditions. Spot blotch and terminal heat stress are the major constraints of wheat production in the eastern Gangetic plains of India. To identify novel breeding sources and to understand underlying resistance mechanisms, forty-four gamma rays mutagenized wheat genotypes, derived from three different parents were screened under favourable agro-ecological conditions for spot blotch and terminal heat stress. Ten mutants showed reduced spot blotch infection calculated based on Area Under Disease Progress Curve (AUDPC), than their respective parents. The mutant TAW41 had the least infection (AUDPC: 354.32), significantly lower than its parent HD2967 (AUDPC: 675.51) and other checks. TAW41 also had a higher Normalized Difference Vegetation Index (NDVI) and chlorophyll content than the parent. Gene expression analysis of TAW41 showed differential accumulation of transcripts involved in hormonal pathways (Salicylic acid, Jasmonic acid, and ethylene) and other defense-associated genes, indicating that TAW41 might have unique resistance mechanism that facilitates this genotype to perform better against the combined stress of spot blotch and terminal heat. Hence, mutant TAW41 has been identified as a novel source of resistance that could be exploited in wheat improvement programmes to enhance tolerance to spot blotch and terminal heat stress.

1. Introduction

Wheat (*Triticum aestivum* L.) is a primary staple food crop of about half of India and 1/3rd of the global population [1]. However, its productivity is hampered by climate change and rapidly evolving new races of pathogens [2]. Among the various stresses in major wheat-growing regions of India, especially in the eastern Gangetic plains, spot blotch and terminal heat stress are the major constraints [3]. Spot blotch caused by *Bipolaris sorokiniana*, a hemibiotrophic fungal pathogen exhibits varied mechanisms such as heterokaryosis, multinucleate, nuclear migration (induces variability in the pathogen), and being asexually reproduced under warm and humid climate [4]. South-East Asia [5], North and Latin America, Africa [6], India [7], China [8], and Brazil [9] are among the major spot blotch affected areas where a significant reduction in wheat production was observed due to spot blotch disease. Average yield loss due to spot blotch was estimated to be in the range of 15–25%, however, higher losses are reported in micro-environments [10].

In addition, the proximity to the equator and late sowing of wheat in India exposes the crop to high temperatures, mostly above 30ºC during the grain filling stage, causing terminal heat stress [11]. It is estimated that 13.5 mha. area under wheat cultivation in India alone is affected by

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terminal heat stress [11]. Heat stress at the flowering stage causes pollen sterility, while during grain filling stage reduces grain filling rate which in turn declines grain weight and seed yield [12]. For every degree increase above 15 °C, susceptible wheat genotypes showed a 3% reduction in seed yield [13]. Additionally, the coincidence of warm and humid climatic conditions with the post-anthesis phase exaggerates spot blotch infection which usually happens in the rice-wheat cropping system of eastern Gangetic plains [14].

Therefore it is imperative to develop novel cultivars having better tolerance to both spot blotch and terminal heat stresses for this region [15]. Several quantitative trait loci (QTLs) conferring spot blotch resistance have been identified in wheat such as *Sb1*, *Sb2*, *Sb3*, *Sb4*, QSb.bhu-2B, QSb.bhu-5B, and QSb.bhu-7D [16], and many QTLs were also reported for tolerance to terminal heat stress [17]. In addition, many resistant and tolerant traits have been identified for spot blotch and terminal heat respectively [7]. However, there are gaps in our understanding of key defense regulation and the physiological basis of tolerance and susceptibility to combined stresses of spot blotch and terminal heat [18].

Previous studies on molecular defense response mechanisms against various abiotic and biotic stresses in wheat have shown the importance of different hormonal pathways and their cross-talks [18]. During spot blotch infection in wheat, resistant genotypes exhibited significant upregulation of salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) signaling genes, however, no significant induction for most of these genes were found in susceptible genotypes [19]. SA and JA play an important role in transcriptional reprogramming in plants as a defense response to counteract biotic and abiotic stresses [20]. Accumulation of SA during spot blotch infection in wheat showed a negative correlation with AUDPC [21]. Production of SA in turn results in the massive synthesis of PR proteins and collective action of SA and PR proteins ensures resistance against a wide range of pathogens [22].

Furthermore, Reactive oxygen species (ROS) involves majorly in signal transduction during infection and also in further prevention of infection [23]. However, the role of ROS as a signaling molecule or causing oxidative damage to the cell depends on the maintenance of equilibrium among ROS production and their scavenging [24]. Further, secondary metabolites like, phenolic acids participate in plant defense by acting as antimicrobial and antioxidants compounds [25]. Findings have independently confirmed the role of phenolic acids i.e., syringic acid for its inverse correlation with spot blotch disease progression in wheat, suggesting its role in defense signaling [21]. Evidence for the individual role of the various phyto-hormones and secondary metabolites is well established in model species like *Arabidopsis* [26] whereas, information on their response under field experiments involving combined spot blotch and terminal heat stresses in wheat is limited.

Therefore, lack of clear understanding of defense response regulations against these combined stresses [27] and limited availability of locally adapted resistant genotypes suggests to generate and characterize novel variation for combined stresses. Induced mutations play a critical role in creating new variations and also identifying key regulatory genes [28]. Thus, screening and characterization of diverse genetic sources including mutants for combined stresses of spot blotch and terminal heat would help in identifying new donor lines and their underlying defense mechanisms. Introgression of such novel alleles into elite lines could help in developing region-specific climate-resilient cultivars [29].

Thus, the present investigation aims to identify novel sources of resistance and to understand their tolerance mechanisms by studying expression dynamics of major hormonal, antioxidants, and senescence-related genes in mutant wheat genotypes grown under spot blotch and terminal heat stress conditions in the field. We identified a wheat mutant (TAW41) with tolerance to the spot blotch and terminal heat stresses with differential regulation of hormone signaling and biosynthesis genes in response to these stresses.

2. Materials and methods

2.1. Plant materials and growth conditions

Forty-four gamma rays induced mutant wheat genotypes abbreviated as TAW (Trombay Aestivum Wheat), derived from three parent varieties namely PBW677, HD2967, and Borlaug100 were obtained from Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai. These mutants were grown in the field station of the Institute of Agricultural Sciences of Banaras Hindu University (25.2677° N, 82.9913° E) from December to April 2021 in augmented block design with parents and local checks (HUW510, HUW234, and HUW468). Each genotype was planted in a three rows plot (row length: 3 m, spacing: 20 cm × 5 cm) with repeated checks at regular intervals for better comparison. Recommended package of practices was followed to raise the crop [30].

2.2. Pathogen inoculation, disease scoring, and data recordings

The experimental field was inoculated with a mixture of HD3069 (NCBI SRX7473632, NCCS-NCMR accession: MCC1572) and PUSA2 (NCBI SRX7473635, NCCS-NCMR accession: MCC1533) isolates of *Bipolaris sorokiniana*. For spot blotch infection, wheat plants were inoculated twice at 78th and 85th day, with the spore solution (10^4 spores ml^{-1}), whereas terminal heat (terminal heat) stress (31–36 ºC) was naturally coincided at post-anthesis stage of the crop. The humidity was maintained for uniform development of the spot blotch disease by flood irrigation. Wheat lines were scored for the spot blotch disease for 21 days with an interval of 7 days starting from initiation of flowering (Zadoks Growth stage-Z 59) to late milk stage (Z-77) [31]. A double-digit scale (00–99) [32] was employed to score the disease where the first digit designates vertical disease progress (D1) and second digit stands for disease severity (D2). The disease severity percentage (DS%) was calculated according to the formula, DS% = (D1/9) x (D2/9) x 100 [33]. The area under disease progress curve (AUDPC) was estimated using DS% based on the following formula [7]:

$$\text{AUDPC} = \sum_{i=1}^{n} \left[\{(Y_i + Y_{(i+1)})/2\} * \left(t_{(i+1)} - t_i\right)\right]$$

Where Y_i = disease level at time t_i, (t_{i+1}- t_i) = days between two disease scores, n = number of readings.

For control samples, uppermost fully emerged 62 days old flag leaves from 9 different plants were collected just before the first inoculation and immediately frozen in liquid nitrogen and stored at − 80 ºC for further analysis. Similarly, for treated (spot blotch+terminal heat) samples, the uppermost fully emerged infected flag leaves were collected during the last disease scoring to study the interaction between spot blotch and terminal heat stress. Parameters like normalized difference vegetation index (NDVI), chlorophyll content (Soil Plant Analysis Development (SPAD) values), canopy temperature depression (CTD) were recorded for 24 days post inoculation (dpi) with an interval of 4 days.

NDVI was recorded by hand-held crop sensor (Green seeker, Trimble Agriculture) as an average for each row between 11 AM to 2 PM, while leaf chlorophyll content was recorded by SPAD meter at a position 2/3rd of the distance between the leaf base and leaf apex of the uppermost fully expanded flag leaf. Additionally, CTD was recorded by an infrared thermometer on the same day of NDVI & SPAD recording at 2 pm. All other agronomic traits like days to heading, plant height (cm), number of tillers, spike length (cm), days to maturity, and thousand kernel weight were also recorded.

2.3. RNA extraction and cDNA synthesis

Four biological replicates of each condition (control and stress

treatment) were processed for RNA extraction. Total RNA was extracted using RNeasy plant mini kit (Qiagen, Germany) with on-column DNase treatment according to user instructions. RNA was quantified in Nanodrop™ spectrophotometer (Thermo Scientific) and integrity was checked on agarose gel. For cDNA synthesis, one microgram of total RNA was reverse transcribed through QuantiTect reverse transcription kit (Qiagen, Germany).

2.4. RT-qPCR and expression analysis

Quantitative real time-PCR was performed as described previously [34]. cDNA was diluted 10–15 folds for the expression analysis. KAPA SYBR FAST (Sigma-Aldrich) was used for RT-qPCR reactions. The reaction mixture was consisted of SYBR green (1x), forward and reverse primers (10 μM each), cDNA (20 ng), and nuclease-free water. All reactions were set up in duplicates for each sample. The actin gene [35] was used as an internal reference for normalization. Gene-specific primers for all the genes analyzed were collected from previously published literature (supplementary table 1). The RT-qPCR was performed in Rotor gene Q (Qiagen) with thermal cycle conditions of 95 °C for 3 min, 40 cycles of 95 °C for 5 s, and 61.5 °C for 30 s, and a final melting curve analysis was done as per default parameters. The relative expression data analysis was performed according to $2^{-\Delta\Delta CT}$ method [36] where ΔΔCT was calculated by comparing expression with control of parent HD2967.

2.5. Statistical analysis

The data were analyzed in IBM SPSS Statistics version 16.0 (SPSS Inc., Chicago, IL, USA). The treatment means were compared using one-way analysis of variance (ANOVA) and the Student-Newman-Keuls (SNK) and least significant difference (LSD) posthoc test with a 5% significance level ($p < 0.05$). While, principal component analysis (PCA) and heatmap analysis were performed using a publicly available online tool, ClustVis [37].

3. Results

3.1. Evaluation of wheat mutants against combined stress of spot blotch and terminal heat

Mutant genotypes derived from three parents namely PBW677, HD2967, and Borlaug100 were grown under field conditions and analyzed for spot blotch and terminal heat. Spot blotch infection calculated based on AUDPC showed a significant increase in all genotypes after inoculation (Supplementary table 2). Three mutant genotypes derived from PBW677 showed different response compared to PBW677. In them, TAW58 had a significantly higher disease level (AUDPC: 825) and TAW94 showed lower disease (AUDPC: 531). In case of HD2967 derived mutants, nine genotypes had shown different responses where, TAW41 and TAW134 showed significantly lower and higher spot blotch infection respectively. The level of spot blotch infection on mutant TAW41 was 47.5% lower than its parent HD2967 (Fig. 1). However, in Borlaug100 derived mutants, no lines showed significant difference from it, for spot blotch infection (Supplementary table 2).

We also used three checks HUW234, HUW468, and HUW510, in our study as these varieties are being popular among the farmers of the Northern Eastern Plain Zone. All these three check varieties showed significantly high disease infection. HUW510 was the most susceptible genotype that showed 137% more spot blotch infection than the mutant TAW41 (Fig. 1 and Supplementary table 2). The NDVI has been a reliable physiological parameter to check the performance of wheat genotypes against spot blotch infection [38] and terminal heat stress [39]. At the end of the experiment (where spot blotch and terminal heat stress were in peak), mutants of PBW677 showed NDVI ranging from 0.233

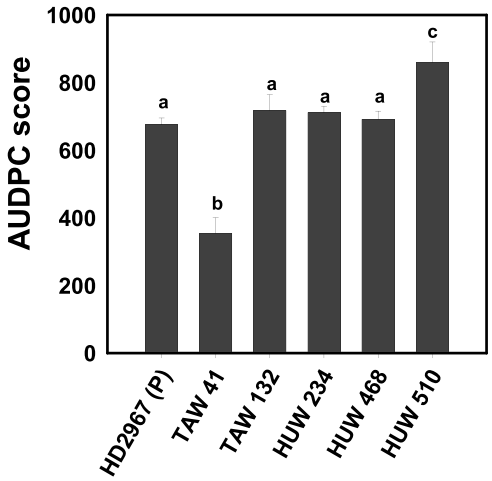

Fig. 1. Spot blotch infection in wheat genotypes grown under field conditions. Disease infection was calculated as area under disease progress curve (AUDPC). Data are means ± SE. The different letter above each bar indicates significant differences calculated by one-way ANOVA, $p < 0.05$ SNK test.

(TAW99) to 0.423 (TAW73) with ten mutants significantly differing from PBW677 (0.323) (Supplementary table 3). In HD2967 derived mutants, 26 genotypes were significantly different from it for NDVI, with a range of 0.207 (TAW115) to 0.59 (TAW122), while HD2967 had a 0.380 NDVI value. TAW41 also had significantly higher NDVI than HD2967 (34.2%) and HUW510 (155%) (Fig. 2a and Supplementary table 3). In case of the mutants of Borlaug100, only TAW147 (0.303) differed significantly from Borlaug100 (0.217). Further, NDVI values for all mutant genotypes derived from three parents were in the same range as with the mutants of HD2967.

In case of SPAD values, at last reading 10 mutant genotypes of PBW677, 27 of HD2967, and 3 of Borlaug100 differed significantly from their respective parents. Overall SPAD values ranged from 11.8 (TAW144) to 51.3 (TAW122) (Supplementary table 4). TAW41 also maintained significantly higher SPAD values with 22.2% and 64.5% more chlorophyll content than HD2967 and HUW510 respectively under the combined effect of spot blotch and terminal heat stress (Fig. 2b and Supplementary table 4). In of CTD, mean values ranged from 0.07 (TAW150) to 7.93 (TAW125), and 11 mutants of PBW677, 21 of HD2967, and none of Borlaug100 differed significantly from their respective parents (Supplementary table 5). TAW41 showed significant difference at the 12th and 16th day after treatment than the parent, whereas no difference was observed at the end of the stress treatment (Fig. 2c). Agronomic traits like days to heading, days to maturity, plant height, spike length, number of tillers, and thousand kernel weight were also recorded for all mutant lines and presented in Supplementary table 6.

Since the mutant genotype TAW 41 had the least disease infection (AUDPC: 354) and significantly higher vegetation index (NDVI: 0.51), this line was chosen for further analysis. To avoid the effect of genetic background, we chose another mutant genotype TAW132 that was also derived from HD2967. Although the TAW132 had the same background as TAW41, the mutant showed different response such as higher AUDPC (717) (Fig. 1 and Supplementary table 2) and lower NDVI (0.243) (Fig. 2a) than TAW41. No changes in SPAD and CTD was observed for TAW132 and TAW41 (Fig. 2b & c). Thus, in total four genotypes HD2967, TAW41, TAW132, and HUW510 were further analyzed for expression dynamics of phytohormones and other associated genes involved in spot blotch and heat stress tolerance.

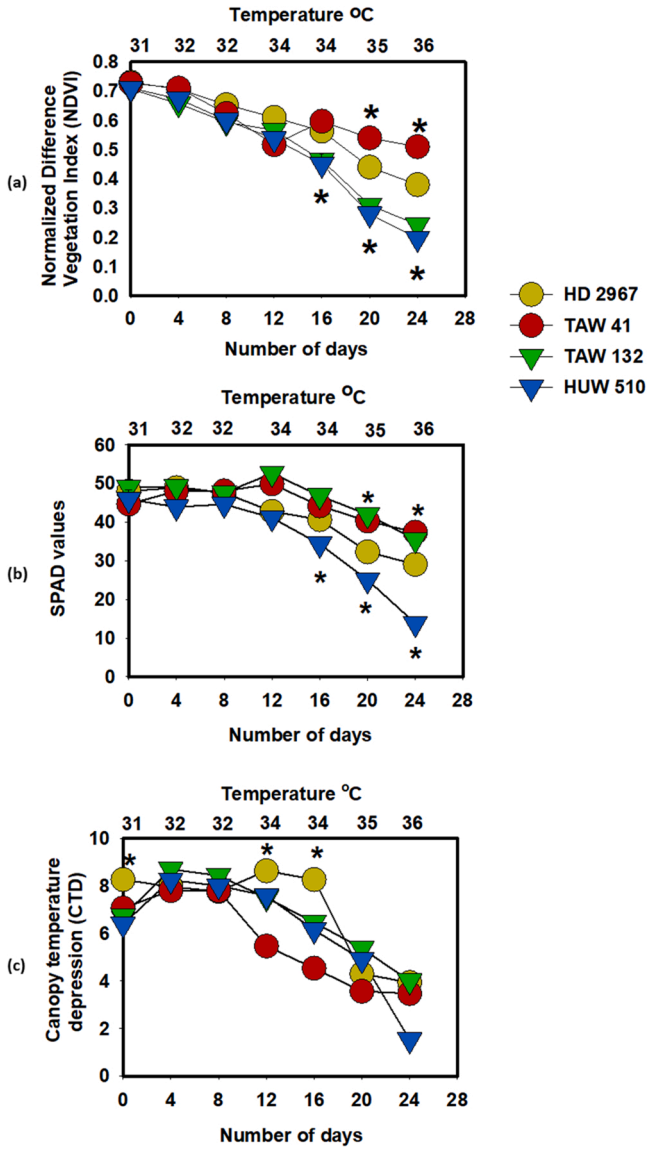

Fig. 2. : Analysis of NDVI, SPAD, and CTD in wheat genotypes under control and a combination of spot blotch and terminal heat stress. Time-course analysis of NDVI (a), SPAD (chlorophyll) (b), and CTD (c) in all four genotypes. The top X-axis of the plot highlights the corresponding atmospheric temperature of each reading. Data are means ± SE. Asterisks (*) indicates significant difference calculated by one-way ANOVA, $p < 0.05$ SNK test.

3.2. Expression dynamics: differential regulation of hormonal and other defense associated genes in TAW 41

3.2.1. Salicylic acid pathway and related genes

The role of SA is proven in the plant defense regulation process [40]. The host plant accumulates SA and activates downstream signaling cascades, as defense response to pathogen attack [41,42]. Pathogenesis-related (PR) genes are the important downstream components of systemic acquired resistance (SAR) in response to SA, JA, and ET [43–45]. Biosynthesis of SA in plants occurs via isochorismate (IC) and the phenylalanine ammonia-lyase (PAL) pathway [46]. We chose three PR genes (*PR1*, *PR1.1*, *and PR3*), an *NPR1* like gene (*NPR1*-3), and *PAL* (*PAL1*, *PAL2,* and *PAL3*) genes to check their expression pattern. These genes were analyzed in TAW41, HD2967, TAW132, and HUW 510 under control and treatment samples.

Expression analysis revealed that *NPR1*-3 gene expression pattern didn't show any significant variation between TAW41, TAW132, and HD2967, but it was significantly up-regulated in the control samples of HUW 510 (7.26 folds). Further, no significant variation was observed for *PR1*, *PR1.1,* and *PR3* genes between TAW41, TAW132 and HD2967. However, *PR1* and *PR1.1* showed significantly higher expression in the HUW510 treatment sample (32.9 and 11.41 folds respectively) compared to HD2967. Likewise, *PR3* gene expression was significantly higher in both control and treatment samples of HUW510. This expression pattern may be due to the higher susceptibility of HUW510 to spot blotch, which would have induced more *PR* genes in response to pathogen attack (Fig. 3a).

The scenario was quite different in phenylpropanoid pathway genes, where *PAL1*, *PAL2,* and *PAL3* were significantly down-regulated in TAW41 compared to parent HD2967. However, *PAL1* and *PAL3* expression didn't differ significantly between TAW41, TAW132, and HUW510. But *PAL2* expression was observed to be significantly higher in TAW132 and HD2967 compared to TAW41. With respect to expression dynamics of *PAL1*, *PAL2,* and *PAL3*, mutant genotype TAW41 differed significantly from HD2967 (Fig. 3a).

3.2.2. Genes associated to jasmonic acid (JA) metabolism

Jasmonate biosynthetic pathway initiates when linolenic acid is converted by successive actions of lipoxygenase (LOX), allene oxide synthase (AOS), and allene oxide cyclase (AOC) into the precursor 12-oxo-phytodienoic acid (OPDA), which is further reduced by OPDA-reductase 3 (*OPR3*) for the production of JA [47,48]. *MYC2*, a master switch transcription factor (TF) acts additively with its close homologs *MYC3* and *MYC4* and regulates JA responses for diverse stimuli [49]. Furthermore, many genes of the *WRKY* TF family are responsive to pathogen infection and phytohormones such as SA and JA [50]. Thus, three biosynthesis genes of JA (*AOS2, LOX2,* and *OPR3*) and two TFs *WRKY33* and *MYC2,* which regulate JA signaling and response were selected for the expression analysis [51]. Transcription factors *MYC2* and *WRKY33* were significantly downregulated in TAW41 compared to HD2967. In case of *WRKY33*, TAW41 showed significantly lower expression compared to TAW132 and HD2967 (Fig. 3b). However, there was no significant difference between TAW41 and TAW132 for *MYC2*.

Further *AOS2* expression was significantly downregulated in both samples of TAW41 compared to HD2967 (Fig. 3b), while its expression pattern in TAW132 considerably differed from HD2967 and TAW41. While *LOX2* gene showed an unusual expression pattern where its expression was mostly similar in HD2967 and TAW41, but TAW132 and HUW510 showed significantly higher expression, 223 and 152 fold changes in control and treatment samples of HUW510 respectively, and 89 and 68 fold changes in control and treatment samples of TAW132 respectively. As expected *OPR3* expression showed similar patterns of *AOS2*, as *OPR3* acts downstream to AOS and ACO in the JA biosynthesis pathway. Overall, all analyzed genes of JA were relatively down-regulated in TAW41 as compared to the parent HD2967.

3.2.3. Ethylene signaling and biosynthesis genes

Ethylene is known to stimulate various PR proteins or phytoalexins and rigidify cell walls by inducing phenylpropanoid pathway [52,53]. The 1-amminocyclopropane-1-carboxylic acid synthase (ACC synthase) and ACC oxidase (ACO) are the two major enzymes involved in the ethylene biosynthesis pathway.

Thus, two ACS genes (*ACS6* and *TaACS1*), one ACO gene (*ACO4*), a novel pathogen-induced ethylene-responsive factor (ERF) gene of wheat, *TaPIEP1* and ethylene signaling gene *TaEIL1* (Ethylene-Insensitive3-Like1-*EIN3*-Like1) were selected to check their expression dynamics in the test genotypes. All biosynthesis pathway genes *ACS6*, *TaACS1* and *ACO4* were downregulated in TAW41 compared to HD2967 (Fig. 3c). But there was no significant variation seen for these genes among the genotypes TAW41, TAW132, and HD2967. The expression of *ACS6* and *ACO4* significantly differed between control and treatment samples of HD2967. There was no significant variation in the expression of *TaPIEP1* gene among the genotypes. Though *TaEIL1* had lowest

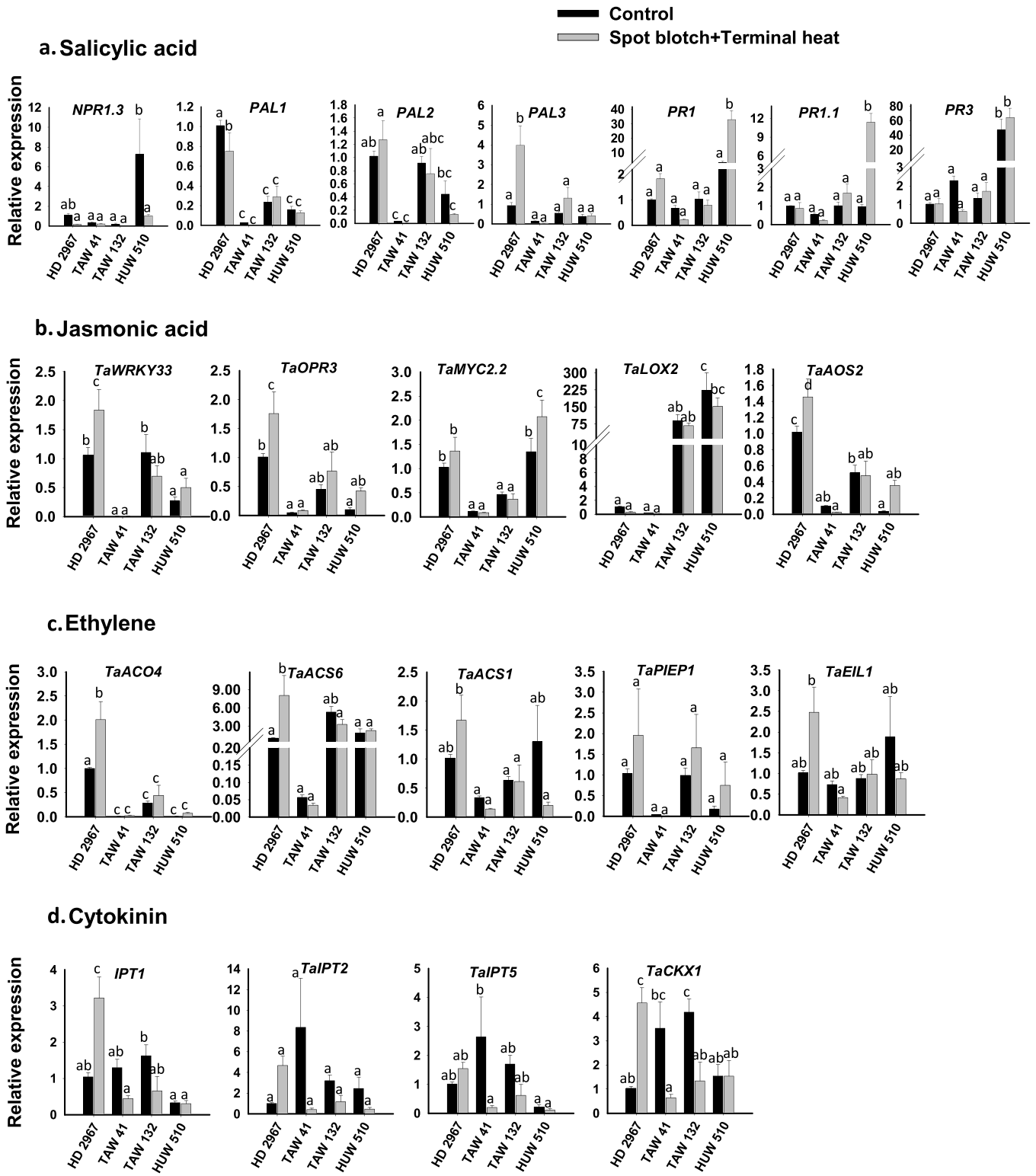

Fig. 3. Expression pattern of SA (3a), JA (3b), Ethylene (3c) and Cytokinin (3d) associated genes. Data are means ± SE. Different letter above each bar indicates significant difference calculated by one-way ANOVA, $p < 0.05$ SNK test.

expression (0.41 fold) in treatment sample of TAW41 but it didn't differ significantly from TAW41 control and TAW132. However, *TaEIL1* expression differed significantly between HD2967 treatment (2.47) and TAW41 treatment sample (0.41).

3.2.4. Cytokinin pathway and signaling genes

We checked the expression analysis of *IPT1*, *TaIPT2*, *TaIPT5,* and *TaCKX1* [cytokinin oxidase/dehydrogenase (CKX) which irreversibly degrades cytokinins and regulates its level)]. *IPT1* gene was significantly downregulated in TAW41 treatment sample compared to HD2967 treatment, while in control sample, there were no changes (Fig. 3d). But *IPT1* expression in TAW132 control differed significantly from HD2967 and TAW41 control. Gene *TaIPT2* did not show any significant variation, though its expression appeared to be higher in TAW41 control. But

TaIPT5 expression was significantly higher in control of TAW41 (2.6 folds) and it differed significantly from all the samples. For *TaCKX1* both TAW132 and TAW41 behaved similarly and both showed significantly higher expression in their control samples compared to respective treatment samples. In overview, *IPT1* and *TaCKX1* were significantly downregulated in TAW41 treatment compared to HD2967. While there was no significant variation observed in *TaIPT2* and *TaIPT5* between genotypes, *TaIPT5* showed higher expression in TAW41 control (Fig. 3d).

3.2.5. Antioxidant genes

The *Fe-SOD* (Superoxide dismutase) was relatively downregulated in both samples of TAW41 compared to HD2967. But it was upregulated significantly in control sample and downregulated in treatment sample of TAW132 compared to HD2967 control (Fig. 4a). There was no significant variation in the expression of catalase between HD2967, TAW41 and TAW132. However, Catalase was expressed relatively higher in control samples of HD2967, TAW41, TAW132 and HUW510 compared to their respective treatment samples. Expression of *APX* (Ascorbate peroxidase) differed significantly in TAW41 and TAW132

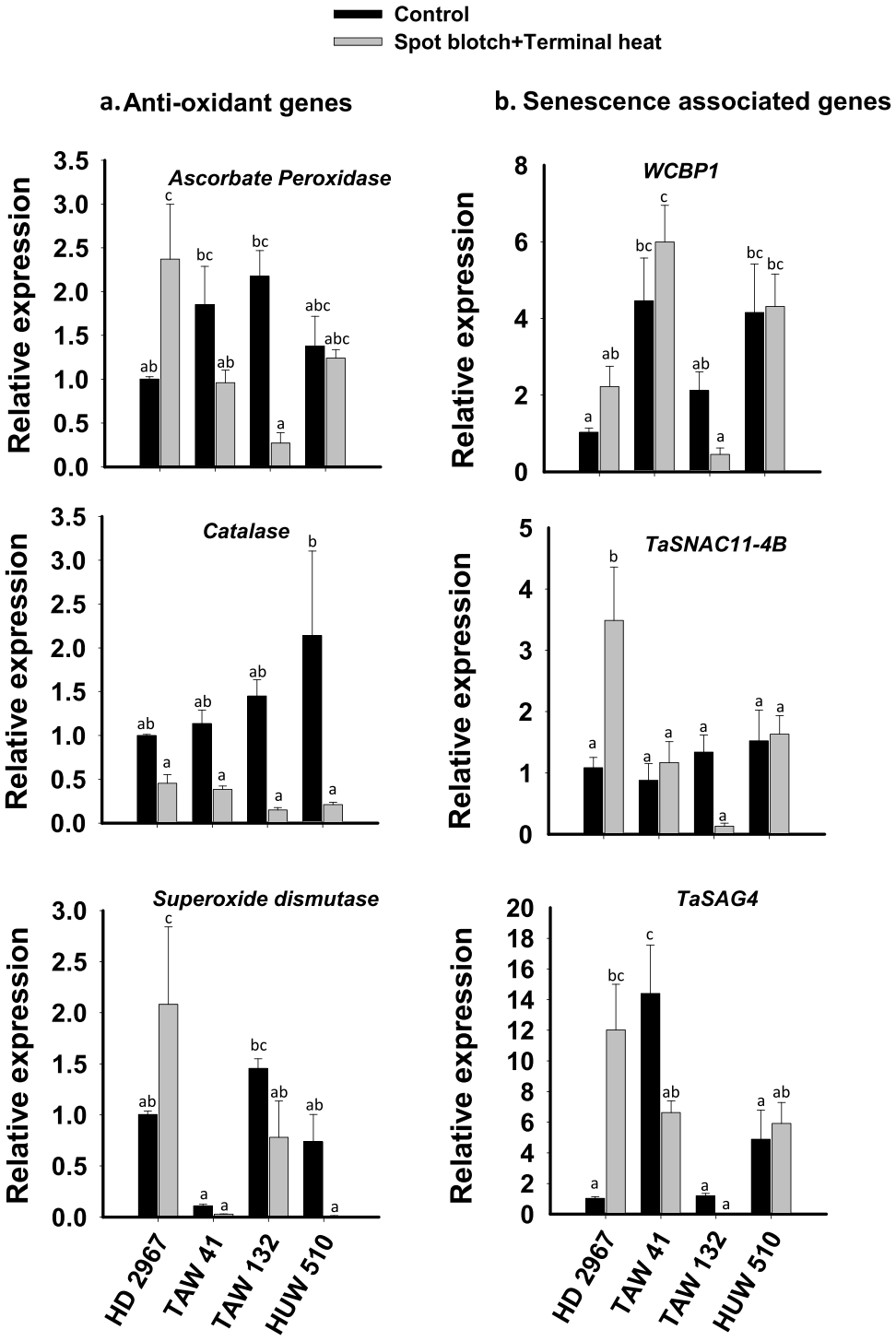

Fig. 4. Expression pattern of antioxidant enzymes genes (4a) and senescence-associated genes (4b). Data are means ± SE. Different letter above each bar indicates significant difference calculated by one-way ANOVA, $p < 0.05$ SNK test.

compared to HD2967. Its expression in control samples of TAW41 and TAW132 was significantly upregulated compared to control of HD2967. In brief, antioxidant enzymes weren't induced significantly in TAW41 treatment samples.

3.2.6. *Differential accumulation of senescence-associated genes in TAW41*

Mutant genotype TAW41 had relatively higher NDVI and lower AUDPC (Figs. 1 and 2). To support this observation, we analyzed the expression of a few senescence-associated genes in all four wheat genotypes. A wheat copper-binding protein (*WCBP1*) has been involved in leaf rust resistance and leaf senescence inhibition [54]. Similarly, *TaSAG4* and a *TaSNAC11–4B* (wheat NAC transcription factor regulating leaf senescence) have well-defined roles in the senescence process [55,56].

WCBP1, in treatment sample of TAW41 showed highest expression compared to other genotypes and it differed significantly from HD2967 and TAW132 (Fig. 4b). The control sample of TAW41 also had a significantly higher expression of *WCBP1* compared to HD2967 and TAW132. *TaSAG4* was significantly downregulated in the treatment sample of TAW41 compared to its control and treatment of HD2967. But its expression was highest in control of TAW41 compared to all other genotypes including check variety (Fig. 4b). There was no significant variation seen in the expression of *TaSNAC11–4B* between TAW41, TAW132, HUW510 and control of HD2967. However, HD2967 treatment sample showed significantly higher expression compared to all genotypes.

3.3. *Principal Component Analysis (PCA) and Heat map of differentially expressed genes*

PCA was performed with the observed gene expression differences in all the four wheat genotypes under control and treatment. In control, two principal components captured 84.6% variation and TAW41 was spotted far apart from HD2967 and HUW510 (Fig. 5a). Whereas, in the treatment of spot blotch and terminal heat, PCA components captured 88.1% of the total variation and TAW41 was spotted far apart from HD2967 but in opposite direction (Fig. 5b). This implies that TAW41 behaved genetically different in response to combined spot blotch and terminal heat stress compared to the parent HD2967. A summarized analysis of gene expression differences in all the four genotypes are given in the heat map where TAW41 showed a different pattern compared to HD2967 in both control and treatment (Supplementary figure 1).

4. Discussion

Spot blotch and terminal heat stresses are the two major constraints for wheat cultivation in the eastern Gangetic plain of India. Heat stress was reported to enhance disease infection, thus reducing the yields significantly. Crop plants were found to respond differently under combined stresses compared to individual stress [57,58]. Moreover, under field conditions, a crop season may witness several stresses in a given time period. Hence, screening diverse genotypes and understanding their defense mechanisms under combined stresses is essential to develop climate-resilient cultivars. Genetic diversity and new variation play a vital role in crop improvement and breeding programmes [59]. Mutations are quick sources of genetic variation and create new alleles. Therefore, in the present study, a set of mutant genotypes developed from agronomically superior cultivars through gamma-ray irradiation were screened against combined spot blotch and terminal heat stresses. The screening clearly showed significant genetic variation among the mutant genotypes for spot blotch and terminal heat stress tolerance. The experiments identified TAW41 as the best performing mutant genotype under combined stresses of spot blotch and terminal heat compared to parental genotypes and other check varieties. TAW41 mutant was also different at the molecular level where genes involved in various hormonal pathways/signaling were differentially expressed than the parent (Supplementary figure 1).

It is noteworthy that TAW41 is a longer duration genotype (85 days to heading and 132 days for maturation) than HD2967 (75 days to heading and 120 days to maturation) which raises a possibility that the resistance response of TAW41 against spot blotch and terminal heat might be due to a difference in developmental stage with its parental genotype. However, our results identified other mutant (TAW115) that flowered in 70 days and matured in 112; but it had a similar AUDPC (671) level like that of the parent (675.5). Similarly, another line TAW143 (heading 84 days and maturation 133 days) showed AUDPC value 684 (Supplementary table 2 & 6). The observation clearly implies that at the adult plant stage, this much difference in flowering and maturation time may not contribute to the resistance mechanisms against the combined stress of spot blotch and terminal heat in wheat. Lower thousand kernel weight of TAW41 than HD2967 may be due to its increased investment in tolerance and survival mechanisms under both stress conditions.

Hormonal homeostasis in TAW41 which showed the lowest AUDPC and relatively higher NDVI compared to other genotypes was significantly different from its parent HD2967. PR genes such as *PR1*, *PR2,* and

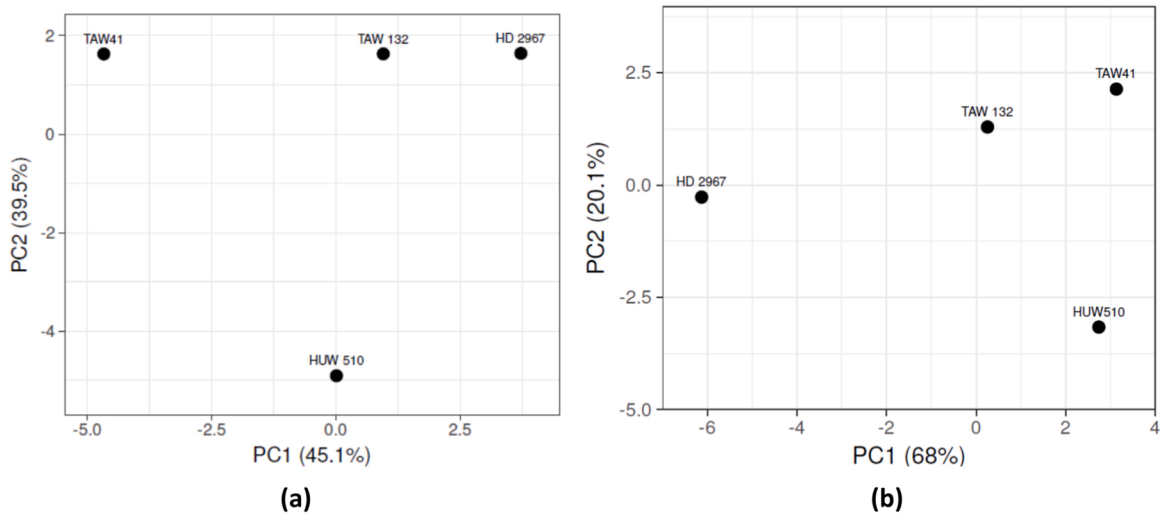

Fig. 5. : Principal component analysis (PCA) of differential gene expression data. Biplot analysis shows principal components (P1 and P2) that explain maximum variability in all the four genotypes under control (a) and stress treatment (b).

PR5 are often considered as marker genes for salicylate-induced plant defense response [60]. Further, *NPR1* (Non-expressor of pathogenesis-related genes1) plays a pivotal role in SAR [61] and master regulates plant defense signaling and cross-talks of SA and JA/Ethylene responses [62,63]. In this study, *PR* genes and *NPR1.3* genes did not show any significant variation between mutant genotype and parent, but *PAL* genes were significantly downregulated in TAW41 compared to HD2967. This could be due to cross-talk between abiotic (terminal heat) and biotic (spot blotch) stresses. Previous studies have reported that ABA-mediated water-deficit stress tolerance and SA-based immunity antagonized each other [64] and were also observed to be age-dependent [65]. Additionally, at higher temperatures, NLR (Nucleotide-binding domain leucine-rich repeat) nuclear accumulation (an important step in ETI) was found to underwent ABA-dependent suppression [66]. Further, in addition to *PR* genes, there could be other regulatory mechanisms facilitating defense response in TAW41.

Similarly, most of the JA genes except *LOX2* were significantly downregulated compared to parent HD2967 suggesting that TAW41 behaves differently from HD2967 under combined stress. Further JA regulators, *MYC2* and *WRKY33* were observed to be not induced significantly in TAW41 compared to HD2967. However, Anderson et al. showed antagonistic interaction between ABA and JA-ethylene signaling pathways, where endogenous and exogenous ABA suppressed transcript levels of JA-ethylene responsive defense genes [67]. Therefore, elucidating hormonal signaling cross-talk under combined stress remains a challenging task and seems to be important to develop stress-tolerant cultivars. Overall, JA signaling and pathway genes were observed to be less induced in TAW41. An exact reason is not clear; however, it is possible that the TAW41 might have suppressed its defense signaling components to activate tolerance mechanisms against the terminal heat stress which during crop season co-appears with spot blotch.

Ethylene pathway and signaling genes were differentially expressed in both TAW41 and TAW132 compared to HD2967. Ethylene regulator *TaEIL1* found to be significantly downregulated in TAW41 treatment compared to HD2967 treatment suggests that either ethylene is not induced in TAW41 treatment or it was suppressed by other pathways or cross-talks. ROS homeostasis and scavenging seems to be not well initiated and/or accumulated as mostly they were downregulated in treatment samples suggesting that plant may be investing more in defense and tolerance signaling.

Though there was no clear trend in cytokinin pathway genes but its relative downregulation in TAW41 treatment compared to its control and HD2967 treatment could be attributable to relatively low accumulation of senescence-associated genes and significantly higher expression of *WCBP1* in treatment sample of TAW41. Cytokinins are well known to delay leaf senescence in many plant species by promoting cell division and growth [68,69]. *WCBP1* is proven to be involved in stripe rust resistance and inhibiting leaf senescence [54]. Downregulation of senescence promoting genes *TaSAG4*, *TaSNAC11–4B* and upregulation of *WCBP1* in treatment samples of TAW41 could be probable possibility of low AUDPC values of TAW41. But further controlled environment studies on interaction with cytokinin, ABA, and senescence-associated genes could provide more clues about defense mechanisms under combined terminal heat and spot blotch stress.

Overall, hormonal homeostasis in TAW41 majorly differed from HD2967, and further investigation is needed to understand the expression patterns and cross-talks of cytokinin, *WCBP1,* and senescence-associated genes.

Based on the expression pattern of *PR*, *JA,* and ethylene genes in TAW41 treatment, there could be a possibility of some physical barriers or anatomical features and/or biochemical composition, hindering pathogen penetration or hyphal establishment. For example, trichomes create an unfavorable environment for the fungal spores to germinate and penetrate [70], similarly, lignin in leaves spatially restricts and limits their mobility [71]. Hence further microscopic observations and biochemical analysis of leaf could help in better understanding of the resistance mechanisms of TAW41.

The present study identified a novel source of resistance against combined stress to spot blotch and terminal heat stresses. The genotype TAW41 may further be utilized in the wheat breeding program to enhance the tolerance against these stresses. An extensive-expression analysis of hormonal pathway genes in the present study shows their role in tolerance mechanisms against spot blotch and terminal heat. The information will improve our understanding of tolerance mechanisms against both stresses in wheat. TAW41 may be utilized to develop mapping populations to identify regulatory components involved in tolerance mechanisms against the combined effect of spot blotch and terminal heat stresses in wheat.

5. Conclusion

Mutant genotypes derived from three parents showed considerable variation for AUDPC, NDVI, SPAD, CTD and other agronomic traits. Mutant TAW41 derived from HD2967 performed significantly better against combined spot blotch and terminal heat stress. Hormonal homeostasis in TAW41 differed significantly from its parent HD2967. A few genes were found to be significantly altered in TAW41 treatment that have proven roles in disease resistance. However, the exact mechanism by which TAW41 suppresses pathogen infection is not fully understood. A further holistic investigation including genetical, morphological and biochemical analysis could reveal more information.

CRediT authorship contribution statement

GMS, SRS and GS conducted experiments and performed data analysis. **GMS and SRS** prepared initial draft**. SB, UK, PB and SJJ** developed mutant genotypes, reviewed and edited MS. **RC and AKJ** conceptualize, reviewed & edited the draft; **VKM** conceptualize, supervised, reviewed & edited the draft; **SS:** design experiments, analyzed data, interpreted results and wrote and finalized the manuscript with the help of all authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.cpb.2021.100234.

References

[1] M. Acevedo, J.D. Zurn, G. Molero, P. Singh, The role of wheat in global food security. In Agricultural Development and Sustainable Intensification, Routledge, 2018, https://doi.org/10.4324/9780203733301-4.
[2] R.L. Villareal, Yield loss to spot blotch in spring bread wheat in warm nontraditional wheat production areas, Plant Dis. 79 (1995) 893.
[3] A.K. Joshi, B. Mishra, R. Chatrath, G. OrtizFerrara, R.P. Singh, Wheat improvement in India: present status emerging challenges and future prospects, Euphytica 157 (2007) 431–446.
[4] S.P. Pandey, S. Sharma, R. Chand, P. Shahi, A.K. Joshi, Clonal variability and its relevance in generation of new pathotypes in the spot blotch pathogen, Bipolaris sorokiniana., Curr. Microbiol. (n.d.). https://doi.org/doi: 10.1007/s00284–007-9034–3.
[5] E. Saari, Leaf blight disease and associated soil-borne fungal pathogens of wheat in South and South East Asia, in: E. Duveiller, . J. Dubin, J. Reeves, A. McNab (Eds.),

In Helminthosporium Blights Wheat Spot Blotch Tan Spot, CIMMYT, Mexico, 1998, pp. 37–51.
[6] L. Duczek, L. Jones-Flory, Relationships between common root rot, tillering, and yield loss in spring wheat and barley, Can. J. Plant Pathol. (1993) https://doi.org/doi: 10.1080/07060669309500816.
[7] A.K. Joshi, R. Chand, Variation and inheritance of leaf angle, and its association with spot blotch (Bipolaris sorokiniana) severity in wheat (Triticum aestivum), Euphytica 124 (2002) 283–291.
[8] N. Chang, Y. Wu, Incidence and current management of spot blotch of wheat in China, in: Blotch, Tan Spot (Eds.), In Helminthosporium Blights ofWheat: Spot Blotch and Tan Spot, Eds, CIMMYT, Mexico, 1998, pp. 119–125.
[9] Y. Mehta, Spot blotch (Bipolaris Sorokiniana)," in Seed Borne Disease and Seed Health, in: S.B. Mathurand, B.M. Cunfer (Eds.), Testing of Wheat, Institute of Seed Pathology for Developing Countries, 1993, pp. 105–112.
[10] Dubin, van Ginkel, The status of wheat diseases and disease research in warmer areas 125 (1991) 145.
[11] A.K. Joshi, G. Ortiz-Ferrara, J. Crossa, G. Singh, G. Alvarado, M.R. Bhatta, E. Duveiller, R.C. Sharma, D.B. Pandit, A.B. Siddique, S.Y. Das, R.N. Sharma, R. Chand, Associations of environments in south asia based on spot blotch disease of wheat caused by cochliobolus sativus, Crop Sci. 47 (2007) 1071–1081, https://doi.org/10.2135/CROPSCI2006.07.0477.
[12] S. Mondal, R.P. Singh, J. Crossa, J. Huerta-Espino, I. Sharma, R. Chatrath, G. P. Singh, V.S. Sohu, G.S. Mavi, V.S.P. Sukuru, I.K. Kalappanavar, Earliness in wheat: a key to adaptation under terminal and continual high temperature stress in South Asia, Crop. Res. 151 (2013) 19–26.
[13] I.F. Wardlaw, I.A. Dawson, P. Munibi, R. Fewster, The tolerance of wheat to high temperatures during reproductive growth. I. Survey procedures and general response patterns, Aust. J. Agric. Res. 40 (1989) 1–13.
[14] T. H, F. CJ, E. CA, Wheat cultivars Crop, adapted to post-heading high temperature stress, J. Agron. Crop 192 (2006) 111–120.
[15] A.K. Pandey, V.K. Mishra, R. Chand, S. Navathe, Crosses with spelt improve tolerance of South Asian spring wheat to spot blotch, terminal heat stress, and their combination, Sci. Rep. (2021) 1–12, https://doi.org/10.1038/s41598-021-85238-x.
[16] P.K. Gupta, R. Chand, N.K. Vasistha, S.P. Pandey, U. Kumar, V.K. Mishra, A. K. Joshi, Spot blotch disease of wheat: the current status of research on genetics and breeding, Plant Pathol. 67 (2018) 508–531, https://doi.org/10.1111/ppa.12781.
[17] S. Mohapatra, C. Chand, R. Navathe, S. Sharma, Histo-chemical and biochemical analysis reveals association of er1 mediated powdery mildew resistance and redox balance in pea, Plant Physiol. Biochem 106 (2016) 54–63, https://doi.org/10.1016/j.plaphy.2016.04.035.
[18] R. Sahu, P. Kundu, M. Shraff, M. Pradhan, V.K. Mishra, R. Chand, Understanding the defense-related mechanism during the wheat's interaction with fungal pathogens, Indian Phytopathol. 69 (2016) 203–205.
[19] R. Sahu, M. Sharaff, M. Pradhan, A. Sethi, T. Bandyopadhyay, V.K. Mishra, R. Chand, A.K. Chowdhury, A.K. Joshi, S.P. Pandey, Elucidation of defense-related signaling responses to spot blotch infection in bread wheat (Triticum aestivum L.), Plant J. 86 (2016) 35–49, https://doi.org/10.1111/tpj.13149.
[20] A. Herrera-Vásquez, P. Salinas, L. Holuigue, Salicylic acid and reactive oxygen species interplay in the transcriptional control of defense genes expression, Front. Plant Sci. 6 (2015) 1–9, https://doi.org/10.3389/FPLS.2015.00171.
[21] S. Sharma, R. Sahu, S. Navathe, V.K. Mishra, R. Chand, P.K. Singh, A.K. Joshi, S. P. Pandey, Natural variation in elicitation of defense-signaling associates to field resistance against the spot blotch disease in bread wheat (Triticum aestivum L.), Front. Plant Sci. 9 (2018) 1–18, https://doi.org/10.3389/fpls.2018.00636.
[22] D. Wang, N.D. Weaver, M. Kesarwani, X. Dong, Induction of protein secretory pathway is required for systemic acquired resistance, Science 308 (2005) 1036–1040, https://doi.org/10.1126/science.1108791.
[23] J. Kumar, P. Schäfer, R. Hückelhoven, G. Langen, H. Baltruschat, E. Stein, Bipolaris sorokiniana, a cereal pathogen of global concern: cytological and molecular approaches towards better control, Mol. Plant Pathol. 3 (2002) 185–195, https://doi.org/10.1046/j.1364-3703.2002.00120.x.
[24] P. Sharma, A.B. Jha, R.S. Dubey, M. Pessarakli, Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions, J. Bot. 2012 (2012) 1–26, https://doi.org/10.1155/2012/217037.
[25] D. Wuyts, N. Swennen, R. De Waele, Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behaviour of the plant-parasitic nematodes radopholus similis, pratylenchus penetrans and Meloidogyne incognita, Nematology 8 (2016) 89–101, https://doi.org/10.1163/156854106776179953.
[26] C.M.J. Pieterse, D. Van Der Does, C. Zamioudis, A. Leon-Reyes, S.C.M.Van Wees, Hormonal modulation of plant immunity, Annu. Rev. Cell Dev. Biol. 28 (2012) 489–521, https://doi.org/10.1146/annurev-cellbio-092910-154055.
[27] U.R. Rosyara, S.R. Subedi, S. Ram, E. Duveiller, Spot blotch and terminal heat stress tolerance in South Asian spring wheat genotypes, Acta Agron. Hung. 57 (2009) 425–435.
[28] J. Chaudhary, R. Deshmukh, H. Sonah, Mutagenesis approaches and their role in crop improvement, Plants 8 (2019), https://doi.org/10.3390/plants8110467.
[29] M.M. Mehta, Y.R. Riede, C.R. Campos, L.A.C. Cooli, Integrated management of major wheat diseases in Brazil: an example for southern cone region of Latin America, Crop Prot. 11 (1992) 517–524.
[30] A. Kumar, G.S. Randhir Singh, R.K. Sharma, M.S. Saharan, R.S. Chhokar, B.S. Tyagi, R. Sendhil, R. Chand, I. Sharma, Wheat Cultivation in India Pocket Guide, Project Director, DWR, Karnal, 2014. ⟨https://iiwbr.icar.gov.in/wp-content/uploads/2018/02/EB-52-Wheat-Cultivation-in-India-Pocket-Guide.pdf⟩.
[31] J.C. Zadoks, E. Board, A decimal code for the growth stages of cereals, Weed Res. 14 (1974).
[32] E. Saari, J. Prescott, Scale for appraising the foliar intensity of wheat diseases, Plant Dis. Rep. 59 (1975) 377–380.
[33] E. Duveiller, Y.R. Kandel, R.C. Sharma, S.M. Shrestha, Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas, Phytopathology 95 (2005) 248–256, https://doi.org/10.1094/PHYTO-95-0248.
[34] S. Sharma, B. Choudhary, S. Yadav, A. Mishra, V.K. Mishra, R. Chand, C. Chen, S. P. Pandey, Metabolite profiling identifiedpipecolic acid as an important component of peanut seed resistance against Aspergillus flavus infection, J. Hazard. Mater. 404 (2021), 124155.
[35] Z. Zhang, W. Yao, N. Dong, H. Liang, H. Liu, R. Huang, A novel ERF transcription activator in wheat and its induction kinetics after pathogen and hormone treatments, J. Exp. Bot. 58 (2007) 2993–3003, https://doi.org/10.1093/jxb/erm151.
[36] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative CT method, Nat. Protoc. 3 (2008) 1101–1108, https://doi.org/10.1038/nprot.2008.73.
[37] T. Metsalu, J. Vilo, ClustVis: a web tool for visualizing clustering of multivariate data using principal component analysis and heatmap, Nucleic Acids Res. 43 (2015) 566–570, https://doi.org/10.1093/nar/gkv468.
[38] S. Kumar, M.S. Röder, R.P. Singh, S. Kumar, R. Chand, A.K. Joshi, U. Kumar, Mapping of spot blotch disease resistance using NDVI as a substitute to visual observation in wheat (Triticum aestivum L.), Mol. Breed. 36 (2016), https://doi.org/10.1007/s11032-016-0515-6.
[39] H. Hazratkulova, C. Ram, Sharma, S.A. Likulov, S.I. Slomov, T. Uldashev, Z. Z. Iyaev, Z.K. Halikulov, Z.Z. Iyadullae, Urok, V.J. Ozef, Analysis of genotypic variation for normalized difference vegetation index and its relationship with grain yield in winter wheat under terminal heat stress 721 (2012) 716–721, https://doi.org/10.1111/pbr.12003.
[40] H. Lu, Dissection of salicylic acid-mediated defense signaling networks, Plant Signal. Behav. 4 (2009) 713–717, https://doi.org/10.4161/psb.4.8.9173.
[41] A. Corina Vlot, D.A. Dempsey, D.F. Klessig, Salicylic acid, a multifaceted hormone to combat disease, Annu. Rev. Phytopathol. 47 (2009) 177–206, https://doi.org/10.1146/annurev.phyto.050908.135202.
[42] J.P. Métraux Systemic Acquired Resistance Brenner's Encycl. Genet. Second Ed. 2013 627 629 doi: 10.1016/B978-0-12-374984-0.01509-6.
[43] J. Zhang, X. Du, Q. Wang, X. Chen, D. Lv, K. Xu, S. Qu, Z. Zhang, Expression of pathogenesis related genes in response to salicylic acid, methyl jasmonate and 1-aminocyclopropane-1-carboxylic acid in Malus hupehensis (Pamp.) Rehd, BMC Res. Notes 3 (2010), https://doi.org/10.1186/1756-0500-3-208.
[44] M. Ohshima, H. Itoh, M. Matsuoka, T. Murakami, Y. Ohashi, Analysis of stress-induced or salicylic acid-induced expression of the pathogenesis-related 1a protein gene in transgenic tobacco, Plant Cell 2 (1990) 95–106, https://doi.org/10.1105/tpc.2.2.95.
[45] N.A. Boccardo, M.E. Segretin, I. Hernandez, F.G. Mirkin, O. Chacón, Y. Lopez, O. Borrás-Hidalgo, F.F. Bravo-Almonacid, Expression of pathogenesis-related proteins in transplastomic tobacco plants confers resistance to filamentous pathogens under field trials, Sci. Rep. 9 (2019), https://doi.org/10.1038/s41598-019-39568-6.
[46] D.M.A. Dempsey, A.C. Vlot, M.C. Wildermuth, D.F. Klessig, Salicylic acid biosynthesis and metabolism, Arabidopsis Book 9 (2011).
[47] A. Schaller, A. Stintzi, Enzymes in jasmonate biosynthesis–structure, function, regulation, Phytochemistry 70 (2009) 1532–1538.
[48] M. Hazman, M. Sühnel, S. Schäfer, J. Zumsteg, A. Lesot, F. Beltran, V. Marquis, L. Herrgott, L. Miesch, M. Riemann, T. Heitz, Characterization of Jasmonoyl-Isoleucine (JA-Ile) hormonal catabolic pathways in rice wound salt stress, Rice 12 (2019), https://doi.org/10.1186/s12284-019-0303-0.
[49] E. Breeze, Master mycs:MYC2, the jasmonate signaling master switch, Plant Cell 31 (2019) 9–10, https://doi.org/10.1105/tpc.19.00004.
[50] X. Chen, C. Li, H. Wang, Z. Guo, WRKY transcription factors: evolution, binding, and action, Phytopathol. Res. 1 (2019).
[51] Z. Zheng, S.A. Qamar, Z. Chen, T. Mengiste, Arabidopsis WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens, Plant J. 48 (2006) 592–605, https://doi.org/10.1111/j.1365-313X.2006.02901.x.
[52] M. Arshad, W.T.J. Frankenberger, Ethylene, Agricultural Sources and Applications, Kluwer Academic/Plenum Publishers, 2002.
[53] F.B. Abeles, P.W. Morgan, J.M.E. Saltveit, Ethylene in Plant Biology, 1992.
[54] X. Li, T. Liu, W. Chen, S. Zhong, H. Zhang, Z. Tang, Z. Chang, L. Wang, M. Zhang, L. Li, H. Rao, Z. Ren, P. Luo, Wheat WCBP1 encodes a putative copper-binding protein involved in stripe rust resistance and inhibition of leaf senescence, BMC Plant Biol. 15 (2015) 1–15, https://doi.org/10.1186/s12870-015-0612-4.
[55] Z. Zhang, C. Liu, Y. Guo, Wheat transcription factor tasnac11-4b positively regulates leaf senescence through promoting ros production in transgenic arabidopsis, Int. J. Mol. Sci. 21 (2020) 1–18, https://doi.org/10.3390/ijms21207672.
[56] Y. Luo, D. Pang, M. Jin, J. Chen, X. Kong, W. Li, Y. Chang, Y. Li, Z. Wang, Identification of plant hormones and candidate hub genes regulating flag leaf senescence in wheat response to water deficit stress at the grain-filling stage, Plant Direct 3 (2019) 1–23, https://doi.org/10.1002/pld3.152.
[57] P. Pandey, V. Ramegowda, M. Senthil-Kumar, Shared and unique responses of plants to multiple individual stresses and stress combinations: physiological and molecular mechanisms, Front. Plant Sci. 6 (2015) 1–14, https://doi.org/10.3389/fpls.2015.00723.
[58] V. Ramegowda, M. Senthil-Kumar, The interactive effects of simultaneous biotic and abiotic stresses on plants: Mechanistic understanding from drought and

pathogen combination, J. Plant Physiol. 176 (2015) 47–54, https://doi.org/10.1016/j.jplph.2014.11.008.
[59] G.M. Singh, J. Xu, D. Schaefer, R. Day, Z. Wang, F. Zhang, Maize diversity for fall armyworm resistance in a warming world, Crop Sci. (2021), https://doi.org/10.1002/csc2.20649.
[60] H.J. Linthorst, L.C.Van Loon, Pathogenesis-related proteins of plants, Crit. Rev. Plant Sci. (1991) 123–150.
[61] C.M.J. Pieterse, S.C.M. Van Wees, J.A. Van Pelt, M. Knoester, R. Laan, H. Gerrits, P. J. Weisbeek, L.C. Van Loon, A. Novel, A novel signaling pathway controlling induced systemic resistance in arabidopsis published by: American Society of Plant Biologists (ASPB) Stable, Plant Cell 10 (2016) 1571–1580. ⟨http://www.jstor.org/stable/3870620⟩.
[62] H. Cao, J. Glazebrook, J.D. Clarke, S. Volko, X. Dong, The arabidopsis NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats, Cell 88 (1997) 57–63, https://doi.org/10.1016/S0092-8674(00)81858-9.
[63] R. Backer, S. Naidoo, N. van den Berg, The Nonexpressor of pathogenesis-related genes 1 (NPR1) and related family: mechanistic insights in plant disease resistance, Front. Plant Sci. 10 (2019) 1–21, https://doi.org/10.3389/fpls.2019.00102.
[64] R. Finkelstein, Abscisic acid synthesis and response, Arabidopsis Book. 11 (2013).
[65] M.L. Berens, K.W. Wolinska, S. Spaepen, J. Ziegler, T. Nobori, A. Nair, V. Krüler, T. M. Winkelmüller, Y. Wang, A. Mine, D. Becker, R. Garrido-Oter, P. Schulze-Lefert, K. Tsuda, Balancing trade-offs between biotic and abiotic stress responses through leaf age-dependent variation in stress hormone cross-talk, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 2364–2373, https://doi.org/10.1073/pnas.1817233116.
[66] Y. Saijo, E.P. iian Loo, Plant immunity in signal integration between biotic and abiotic stress responses, New Phytol. 225 (2020) 87–104, https://doi.org/10.1111/nph.15989.
[67] J.P. Anderson, E. Badruzsaufari, P.M. Schenk, J.M. Manners, O.J. Desmond, C. Ehlert, D.J. Maclean, P.R. Ebert, K. Kazan, Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in arabidopsis, Plant Cell 16 (2004) 3460–3479, https://doi.org/10.1105/tpc.104.025833.
[68] R.M. Rivero, M. Kojima, A. Gepstein, H. Sakakibara, R. Mittler, S. Gepstein, E. Blumwald, Delayed leaf senescence induces extreme drought tolerance in a flowering plant, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 19631–19636, https://doi.org/10.1073/pnas.0709453104.
[69] P.R.H. Robson, I.S. Donnison, K. Wang, B. Frame, S.E. Pegg, A. Thomas, H. Thomas, Leaf senescence is delayed in maize expressing the agrobacterium IPT gene under the control of a novel maize senescence-enhanced promoter, Plant Biotechnol. J. 2 (2004) 101–112, https://doi.org/10.1111/j.1467-7652.2004.00054.x.
[70] A. Duba, K. Goriewa-Duba, U. Wachowska, K. Głowacka, M. Wiwart, The associations between leaf morphology, phenylalanine ammonia lyase activity, reactive oxygen species, and fusarium resistance in selected species of wheat with different ploidy levels, Plants 8 (2019), https://doi.org/10.3390/plants8100360.
[71] M. Lee, H.S. Jeon, S.H. Kim, J.H. Chung, D. Roppolo, H. Lee, H.J. Cho, Y. Tobimatsu, J. Ralph, O.K. Park, Lignin-based barrier restricts pathogens to the infection site and confers resistance in plants, EMBO J. 38 (2019) 1–17, https://doi.org/10.15252/embj.2019101948.