



## Research article

# New genetic resources for aphid resistance were identified from a newly developed wheat mutant library

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## ABSTRACT

Reports on development of resilient wheat mutants to aphid infestation—causing heavy losses to wheat production in many parts of the world, are scanty. The present study aimed to identify genetic diversity of wheat mutants in terms of varying degree of resistance to aphid infestation which can help protect wheat crop, improve yields and enhance food security. Resistance response to aphid infestation was studied on newly developed 33 wheat mutants, developed through irradiating seed of an elite wheat cultivar “Punjab-11” with gamma radiations, during three normal growing seasons at two sites. Data on various traits including aphid count per plant, biochemical traits, physiological traits and grain yield was recorded. Meteorological data was also collected to unravel the impact of environmental conditions on aphid infestation on wheat plants. Minimum average aphid infestation was found on Pb-M-2725, Pb-M-2550, and Pb-M-2719 as compared to the wild type. High yielding mutants Pb-M-1323, Pb-M-59, and Pb-M-1272 supported the moderate aphid infestation. The prevailing temperature up to 25 °C showed positive correlation (0.25) with aphid count. Among biochemical traits, POD (0.34), TSP (0.33), TFA (0.324) exhibited a high positive correlation with aphid count. In addition, CAT (0.31), TSS (0.294), and proline content (0.293) also showed a positive correlation with aphid count. However, all physiological traits depicted negative correlation with aphid count, while, a very weak correlation (0.12) was found between mean aphid count and grain yield. In PCA biplots, the biochemical variables clustered together with aphid count, while physiological variables grouped with grain yield. Biochemical parameters contributed most, towards first dimension of the PCA (48.6%) as compared to the physiological variables (13%). The FAMD revealed that mutant lines were major contributor towards total variation; Pb-M-1027, Pb-M-1323, Pb-M-59 were found to be the most diverse lines. The PCA revealed that biochemical parameters played a significant role in explaining variations in aphid resistance, emphasizing their importance in aphid defense

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mechanisms. The identified mutants can be utilized by the international wheat community for getting insight into the molecular circuits of resistant mechanism against aphids as well as for designing new KASP markers. This study also highlights the importance of considering both genetic and environmental factors in the development of resilient wheat varieties and pave the way for further investigations into the molecular mechanisms underpinning aphid resistance in wheat.

## 1. Introduction

The world escalating human population demands corresponding increase in food supply [1]. Bread wheat (*Triticum aestivum*) holds the position as 2nd most cultivated crop worldwide after maize in the regions provided by sufficient nutrients and fertilizers [2] and fulfills the food demands of 35% world's population [3]. In Pakistan, wheat per hectare yield is lower as compared with other developed countries owing to several biotic and abiotic constraints [4]. The biotic stresses have destructive impacts on wheat plant growth which ultimately results in lower yield and production. Among biotic factors, insect pests have severe impacts on the crop yield in wheat consequently affecting the economy of a country [5]. Furthermore, the growth and development of populations of insect pests is greatly affected by warm climatic conditions; extreme temperatures (high and low) favor pest outbreaks causing huge damage to plant growth and productivity [6,7]. So far, 10,000 insect species have been documented which damage the crop plants used as food by the human beings [8]. Hence, pest population dynamics due to the changing climate pose a big challenge for sustainable wheat production [9]. Insects are poikilothermic, hence, an increase in temperature may affect their reproduction, sustained survival and the dynamics of populations, and also the relationships with the other insect pests including their predators [10]. Climate change may create new ecological niches for insects for establishing as pest in new geographic regions (FAO, 2020). The increase in temperature not only retards plant growth but also poses positive impact on population of insect pests including wheat aphids [11]. Thus, aphids can be new potential threat to wheat productivities worldwide, especially for countries where the impact of climate change is going on.

Aphids damage plants indirectly by transmitting viruses which negatively impact the plant growth and development. The grain aphids are most destructive pests of wheat around the world. The depression in yield of wheat and barley may exceed 95% if left the infestations of *D. noxia* unchecked [12]. In Pakistan, among all species of wheat aphid, *S. avenae*, *R. padi* and *S. graminum*, are the most destructive species causing 80% reduction in wheat yield by transmitting many fungal and viral infections [13–15]. Yield loss in wheat due to aphid infestation ranges from 7.9 to 34.2%. However, yield reduction due to direct damage is 10–50% and indirect damage results in 20–80% reduction in wheat yield [16]. Moreover, the extent of damage also depends upon crop growth stage, aphid infestation intensity as well as population density of insects [17]. Hence, climatic conditions influence the distribution and diversity of aphid's population which ultimately lead to the positive or negative impacts on wheat yield [18].

During a lengthy parallel evolution of host plant resistance and insect pests helped plant to evolve the defensive strategies to combat and escape the invasion of the insect pests [19]. Host-plant resistance invoked by chemical and physical mechanisms safeguards the plants through evolving mechanisms including host plant tolerance, antibiosis (inhibitory effects on insect reproduction), antixenosis (aphid non-preference by the host plant), or the pyramiding of all these mechanisms [20,21]. In antibiosis, host plant produces harmful chemicals that inhibit the growth and reproduction of insects that attack on the plant. Antixenosis affects the desirability of host plant by the insects. Although various insecticides have been applied for controlling the infestation of aphid population, however, evolution of resistance in pest species against these insecticides render these chemical ineffective [22,23]. Also, these chemicals are lethal for non-targets insects as well as adding pollution in the environment. To increase the crop yield and minimize the risk of food security, a sustainable and eco-friendly approach is direly needed [24]. Improving genetic resilience in wheat has been the most durable and effective strategy for combating aphids [25]. Because of its shorter life cycle and high fecundity rate may lead to the evolution of new resistant breaking strains, hence, new sources of resistance are direly needed to mitigate the potential threat of newly evolved strains.

A better insight into the mechanism of resistance to aphid is pre-requisite for designing new strategies for developing resistance against aphid in wheat. Several physiological and biochemical process including signal transduction are triggered immediately after the infestation of insects. These reactions result in the synthesis of few enzymes and secondary metabolites for combating the infestation of insect pests [26]. For example, phenylalanine ammonia-lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO) are synthesized in host plant for combating the pests [27]. It was shown that after the infestation of aphids, several proteins including ubiquitin, superoxide dismutase (SOD) and ascorbate peroxidase (APX) were differentially expressed [28].

To the extent of our knowledge, there is not a single variety which was bred for resistance to *R. padi*, *S. avenae* or *M. dirhodum*, however, partial resistance was observed in few varieties [29,30]. Therefore, in this experiment, wheat mutant lines developed by irradiating a wheat variety "Punjab-11" with gamma rays were characterized for their response to aphid infestation. Characterization and screening of a mutant population of wheat was performed under field conditions. The study was aimed at exploring variations induced in wheat mutants for aphid resistance and also variable responses of leaf physiological and biochemical traits under aphid stress. Findings of the present study provided a valuable reference and germplasm for using in future breeding for developing aphid-resistant wheat cultivars.

## 2. Materials and methods

### 2.1. Plant material

A gamma irradiated mutant population comprising of 33 mutants along with wild type “Punjab-11” were characterized for various biochemical and physiological traits as well as resistance responses to aphid infestation under natural field conditions for three consecutive normal wheat growing seasons at two locations.

### 2.2. Field trials/experimental design

The field study for observing the aphid infestation on wheat mutant lines was carried out at two sites i.e., farm fields of the National Institute for Biotechnology and Genetic Engineering (NIBGE) and Nuclear Institute for Agriculture and Biology (NIAB) for three consecutive years (2018–2020). The seed of all mutant lines along with “Punjab-11” were sown through dibbler. A randomized complete block design (RCBD) with three replications was applied for sowing all the trials. The row-to-row distance was 30 cm while hill-to-hill distance was 10 cm. Standard agronomic practices were followed throughout the season. The mutant lines as well as wild type were individually harvested at maturity.

### 2.3. Measuring aphid populations

The extent of aphid infestation was estimated based upon visual observations of prevailing aphid species. The number of aphids were counted on each main tiller of randomly tagged plants (five) on weekly and biweekly basis. The field evaluation for aphid was started during third week of February when aphid infestation started appearing and continued till second week of March when aphid population diminished. Meteorological data such as maximum temperature, minimum temperature, pan evaporation, relative humidity for (Feb–March) was received from the observatory of plant physiology section, Agronomic Research Institute, AARI, Faisalabad, Pakistan. Meteorological data were analyzed to study the impact and association of different weather parameters on growth and infestation of aphids.

### 2.4. Biochemical assays

To study the biochemical basis of aphid resistance, biochemical assays were performed. Fresh leaf samples were collected from the mutants and wild type in ice. A well-established protocol was adopted to measure the total soluble proteins (TSPs) [31]. The absorbance of reaction mixture was estimated through using a spectrophotometer at 620 nm. The bovine serum albumin (BSA) was used to draw a standard curve to estimate TSPs. The TSSs contents were determined by adopting a published protocol [32] followed by recording the absorbance at 625 nm through a spectrophotometer. Lastly, standard curve developed for various concentrations of glucose was used to determine the concentration of TSSs [33]. The TFAs were measured by following a standard protocol [34]. The absorbance of solution was measured at 570 nm using a spectrophotometer. The standard curve of leucine was used to estimate the concentration of TFAs.

#### 2.4.1. Enzymatic assays

Activity of superoxide dismutase (SOD) was determined by applying a well devised assay [35]. The SOD inhibits the reduction of photochemical in nitro blue tetrazolium (NBT) at 560 nm. The inhibition rate was used to estimate the activity of SOD. The cocktail was constituted by adding phosphate buffer (0.95 cm<sup>3</sup>, 50 mM), methionine (0.5 cm<sup>3</sup>, 13 mM), NBT (1 cm<sup>3</sup>, 50 μM), EDTA (0.5 cm<sup>3</sup>, 75 mM), riboflavin (0.005), enzyme extract (50 μg/mL) and de-ionized H<sub>2</sub>O (0.25 mL). The chemical reaction was induced by irradiating the reaction mixture with a fluorescent lamp (30V) for 15 min. The SOD activity was monitored by determining the inhibition rate in NBT photochemical activity and non-irradiated reaction at 560 nm. Peroxidase activity (POD) was measured by deploying a well-established protocol [36]. The solution was prepared by dissolving phosphate buffer (50 mM), guaiacol (20 mM), H<sub>2</sub>O<sub>2</sub> (40 mM), and enzyme extract (0.1 mL). The oxidation of guaiacol and peroxidation of H<sub>2</sub>O<sub>2</sub> were estimated through an extinction coefficient 2.47 mM<sup>-1</sup> cm<sup>-1</sup> to determine the POD activity. The concentration of catalase (CAT) was estimated by following a well described procedure [36]. The reaction mixture was prepared by mixing the phosphate buffer (50 mM), 5.9 mM hydrogen per oxide (H<sub>2</sub>O<sub>2</sub>), and 0.1 mL extract by making 3 mL final volume. Finally, the catalase activity was estimated by the rate of H<sub>2</sub>O<sub>2</sub> decomposition at 240 nm after every 20 s. The ascorbate peroxidase (APX) was measured by following a standard protocol [37]. The reaction mixture was prepared by dissolving 50 mM phosphate buffer, 0.1 mM sodium EDTA, 12 mM H<sub>2</sub>O<sub>2</sub>, 0.25 mM ascorbic acid, and leaf extract in a final volume of 1 mL. After every 20s, the rate of ascorbate oxidation at 470 nm was monitored to estimate the APX activity.

#### 2.4.2. Non-enzymatic assays

The malondialdehyde concentration (MDA, μmol g<sup>-1</sup> FW), was measured using a standard protocol [38]. The leaf extract was prepared by adding 1 g leaf sample into 3 cm<sup>3</sup> trichloroacetic acid solution (TCA) (0.1%w/v), and then was centrifuged for 15 min at 20,000 rpm. Then, 0.5 cm<sup>3</sup> supernatant was added into 3 cm<sup>3</sup> thiobarbituric acid (0.5%) that was prepared in TCA (20%). The resultant mixture was incubated at 95 °C for 1 h followed by centrifugation at 10,000 rpm for 10 min. The MDA concentration was estimated from changing optical densities of supernatant at 532 nm and 600 nm using an extinction coefficient.

## 2.5. Physiological parameters

We determined the physiological traits including photosynthetic rate, photosynthetic absorbance rate (PAR) at leaf surface, substomatal conductance and transpiration rate of all mutant lines and wild type. Data were recorded using Infrared Gas Analyzer (IRGA) LcPro-SD portable Photosynthetic System. Data was collected during the day time from five randomly selected plants of each mutant line.

## 2.6. Statistical analysis

The statistical tests for determining the significance of the traits were conducted through deploying R-4.2.0 for win (<http://cran.r-project.org/>) (accessed on May 23, 2022). Mean, median, standard deviation, standard error and interquartile range were calculated using dplyr and parameters packages in R-4.3.1 (<http://cran.r-project.org/>). One-way ANOVA was applied to measure variations in various studied traits of the studied mutant lines. Boxplots were prepared using ggplot2 [39] for presenting the collected data in graphical presentation. The mean data regarding number of aphids infested on each of the mutant line was calculated by pooling the whole data collected from two locations during the three seasons. The extent of correlations among different traits and aphid infestation was determined by estimating the correlation coefficients. The correlation coefficient matrix was visualized through ggpairs function of the packages GGally and ggplot2 [40]. The principal component analysis (PCA) was conducted through deploying the packages ggplot2, factoextra and FactoMineR [41] for reducing the dimensionality of the data sets. To explore the data further, multiple factor analysis and factor analysis of the mixed data were performed using ggplot2, factoextra and FactoMineR. Cluster analysis was performed to understand the difference in the performances of mutant lines and wild type. All the variables including biochemical, physiological parameters, grain yield and aphid count was used to conduct hierarchical clustering. Circos dendrograms and correlation heatmap were constructed using gplots and circlize packages in R-4.3.1 (<http://cran.r-project.org/>).

## 3. Results

### 3.1. Descriptive statistics for all traits under study

In the present study, mean, median, standard deviation (SD), standard error (SE) and interquartile range (IQR) were calculated for all traits including aphid count, grain yield, biochemical and physiological parameters using various statistical tools (Table 2). The data was tested for normal distribution. The effects of locations were found non-significant. Therefore, data of all sites and years were pooled and used in further analysis (Supplementary Table 1). The mean number of aphid count was 5.75 with standard deviation and standard error of 3.17 and 0.22, respectively. The interquartile range for aphid count was 3.40. The mean grain yield was 3871.25 (Kg $ha^{-1}$ ) with standard deviation of 446.04 and standard error 31.23. The IQR value for grain yield was 683.62. Similarly, mean TSP (total soluble protein) was 23.17 with standard deviation of 7.80 and IQR for TSP was 13.91. The mean TFA (total free amino acid) was 25.51 with SD (7.91) and SE (0.71) (Table 2). Among antioxidant enzymes, SOD (superoxide dismutase) and POD (peroxidase) estimated mean values of 68.35 and 101.56, respectively. The mean value for APX (ascorbate peroxidase) was 100.19 having SD (11.05) and SE (0.77). Other biochemical parameters also depicted substantial variations (Table 2). Among physiological parameters, PAR at leaf surface estimated a mean value of 1547.42 with SD (416.74) and SE (29.18). The IQR value for PAR at leaf surface was 631.75. The mean values for sub-stomatal CO<sub>2</sub> were 1071.67 with SD (380.50) and SE (26.64). Similarly, mean of transpiration and photosynthetic rate were 707.69 and 51.46, respectively (Table 2).

### 3.2. Variability in biochemical and physiological parameters among mutant lines

The box plot analysis was performed for all traits including biochemical, physiological parameters, grain yield and mean aphid count to explore the variability of these parameters among the mutant lines and wild type. The basic idea lying behind this analysis was

**Table 1**  
Meteorological data of three years (2019–22).

Date	Average Temperature (°C)	Average relative humidity %	Average pan evaporation (mm)
February 19, 2019	12.25	76.00	0.55
February 26, 2019	14.50	75.50	0.55
March 05, 2019	16.25	65.50	1.00
March 13, 2019	17.25	57.00	0.90
February 19, 2020	18.10	58.00	0.90
February 26, 2020	19.60	70.50	0.75
March 05, 2020	17.75	77.00	0.95
March 13, 2020	17.40	72.50	1.15
February 19, 2021	17.75	71.50	0.75
February 26, 2021	22.25	64.00	1.15
March 05, 2021	21.75	50.50	1.75
March 13, 2021	21.50	63.00	1.75

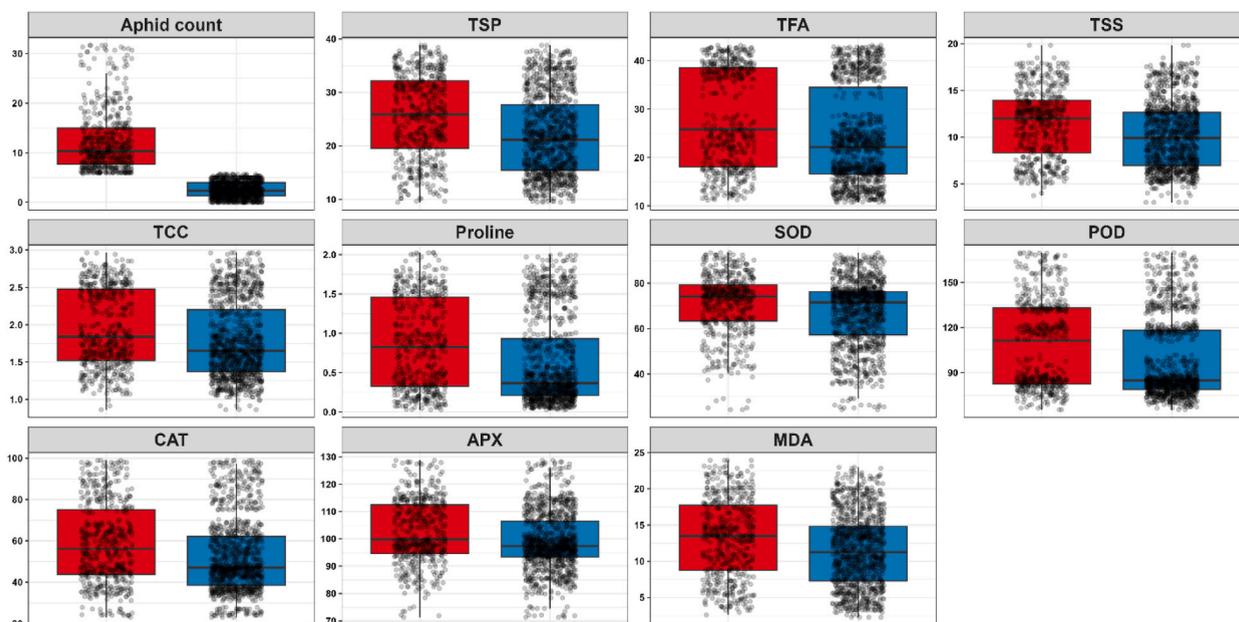
**Table 2**

Mean, median, standard deviation (SD), standard error (SE) and interquartile range (IQR) of all the quantitative variables used in the study.

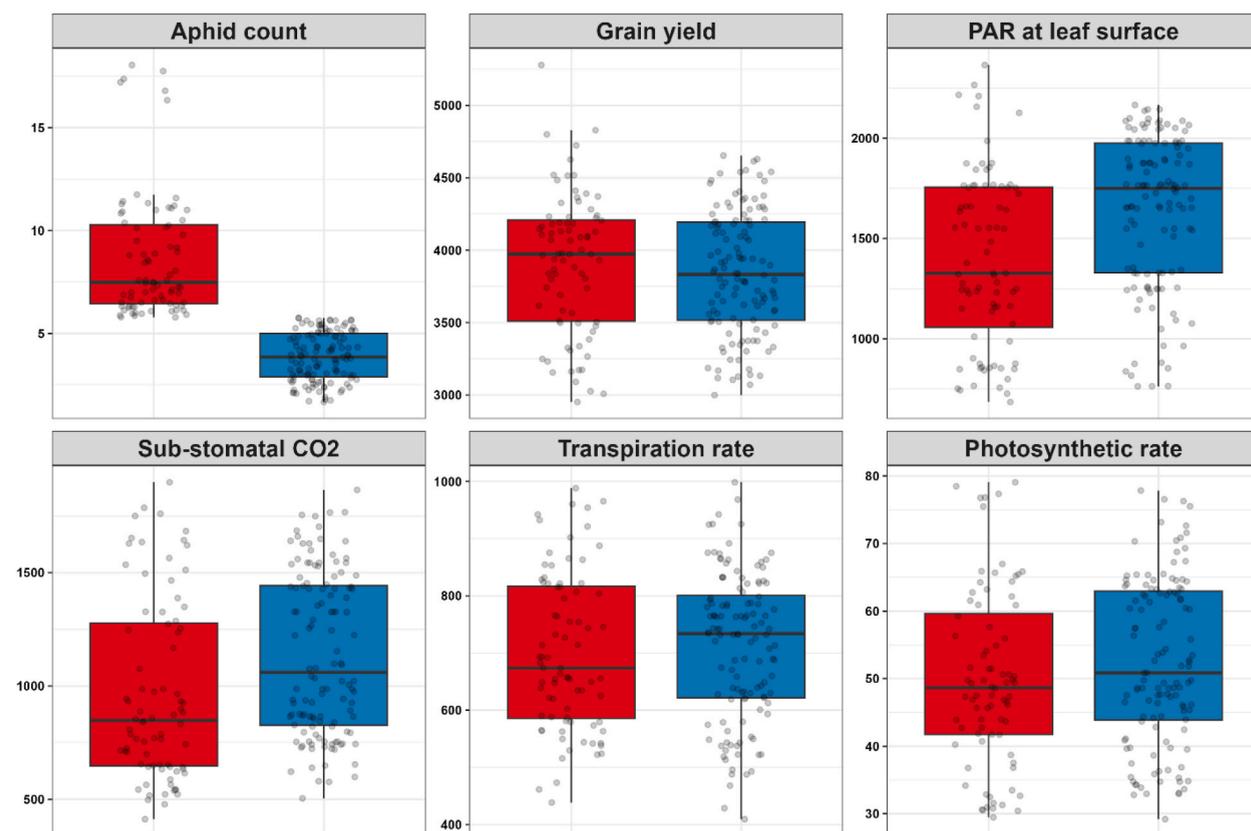
Traits	Mean	Median	SD	SE	IQR
Aphid count	5.75	5.21	3.17	0.22	3.4
Grain yield (kg ha <sup>-1</sup> )	3871.25	3904.51	446.04	31.23	683.62
TSP (mg/g fw)	23.17	22.46	7.8	0.55	13.91
TFA (mg/g fw)	25.51	22.92	10.19	0.71	20.18
TSS (mg/g fw)	10.59	10.78	3.59	0.25	5.81
TCC (mg/g fw)	1.84	1.7	0.52	0.04	0.94
Proline (mg/g fw)	0.71	0.47	0.57	0.04	1.04
SOD (Unit/g fw)	68.35	72.6	14.28	1	17.71
POD (Unit/g fw)	101.56	86.08	28.1	1.97	40.74
CAT (Unit/g fw)	54.58	48.61	18.73	1.31	24.22
APX (Unit/g fw)	100.19	98.04	11.05	0.77	14.53
MDA (umol g <sup>-1</sup> fw)	12.04	12.15	5.19	0.36	8.14
PAR at leaf surface	1547.42	1653.70	416.74	29.18	631.75
Sub-stomatal CO <sub>2</sub>	1071.67	964.72	380.5	26.64	675.64
Transpiration rate	707.69	720.1	131.33	9.19	203.65
Photosynthetic rate	51.46	49.42	12.55	0.88	19.82

TSP: total soluble protein, TFA: total free amino acid, TSS: total soluble sugar, TCC: total chlorophyll content, SOD: superoxide dismutase, POD: peroxidase, CAT: catalase, APX: ascorbate peroxidase, MDA: melondialdehyde

to study the distribution pattern of all the traits with reference to mean aphid count. The mutant lines were classified into those that have above average aphid count and those that have below average aphid count. The average aphid count per plant was 5.75 (Table 2). The boxplot revealed that the median values of all the biochemical variables were higher for mutant lines with “above average” aphid count as compared to the mutant lines with “below average” aphid count (Fig. 1). A positive trend between mean aphid count and biochemical parameters was observed. Hence biochemical constitution of the leaves was directly influenced by aphid infestation and vice versa. However, an opposite trend was observed for physiological traits as the median values of physiological variables were less for the mutant lines with “above average” aphid count per plant as compared to the mutant lines with “below average” aphid count per plant (Fig. 2). Grain yield was not found to be significantly correlated with aphid count. However, the physiological variables were found to be negatively correlated, and biochemical traits were found to be positively correlated with the average aphid count per plant (Figs. 1 and 2).



**Fig. 1.** Box plot of all the biochemical variables colored according to average aphid count per plant. Average of the aphid count per plant was calculated to be 5.75. Values corresponding to the mutant lines that have above average aphid count are colored red while those of below average aphid count are colored as blue. Numbers on y-axis in each plot are representing the range of values for corresponding traits.



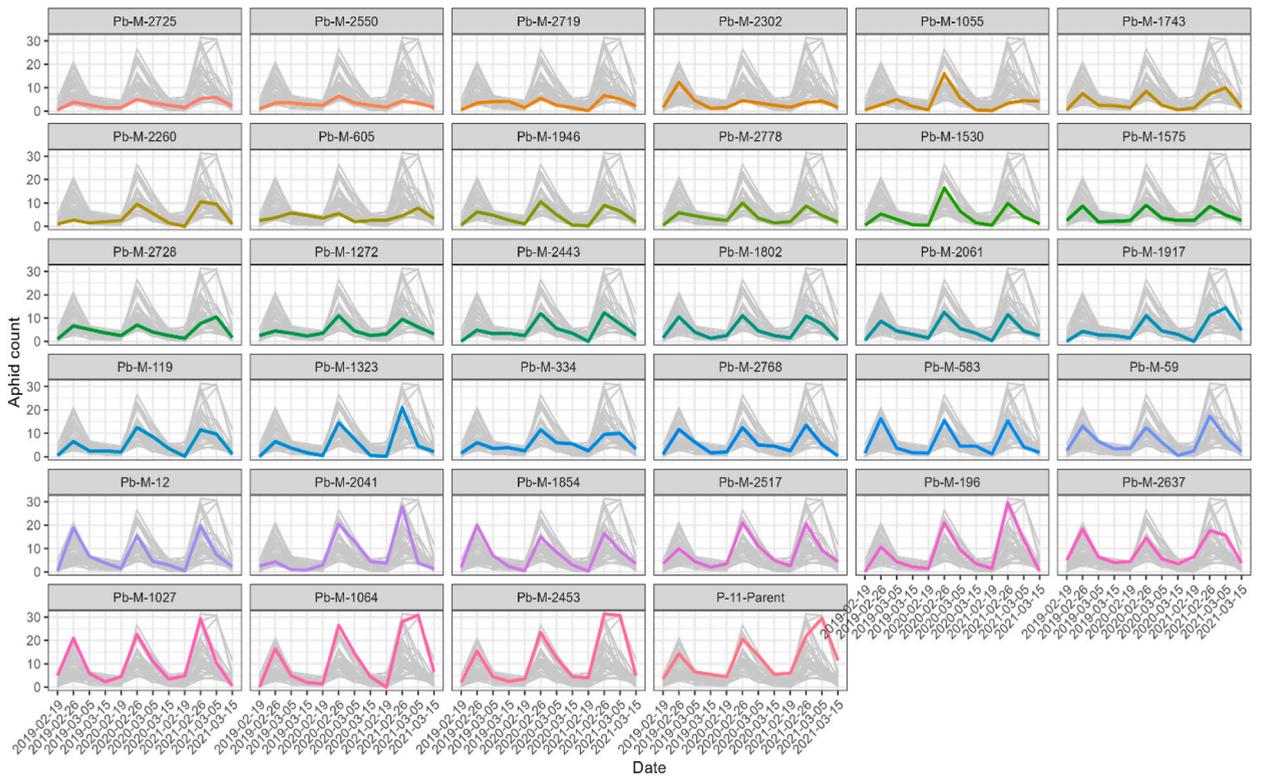
**Fig. 2.** Box plot of all the physiological variables and grain yield colored according to average aphid count per plant. Average of the aphid count per plant was calculated to be 5.75. Values corresponding to the mutant lines that have above average aphid count are colored red while those of below average aphid count are colored as blue. Numbers on y-axis in each plot are representing the range of values for corresponding traits.

### 3.3. Mean aphid infestation on wheat mutant lines and wild type

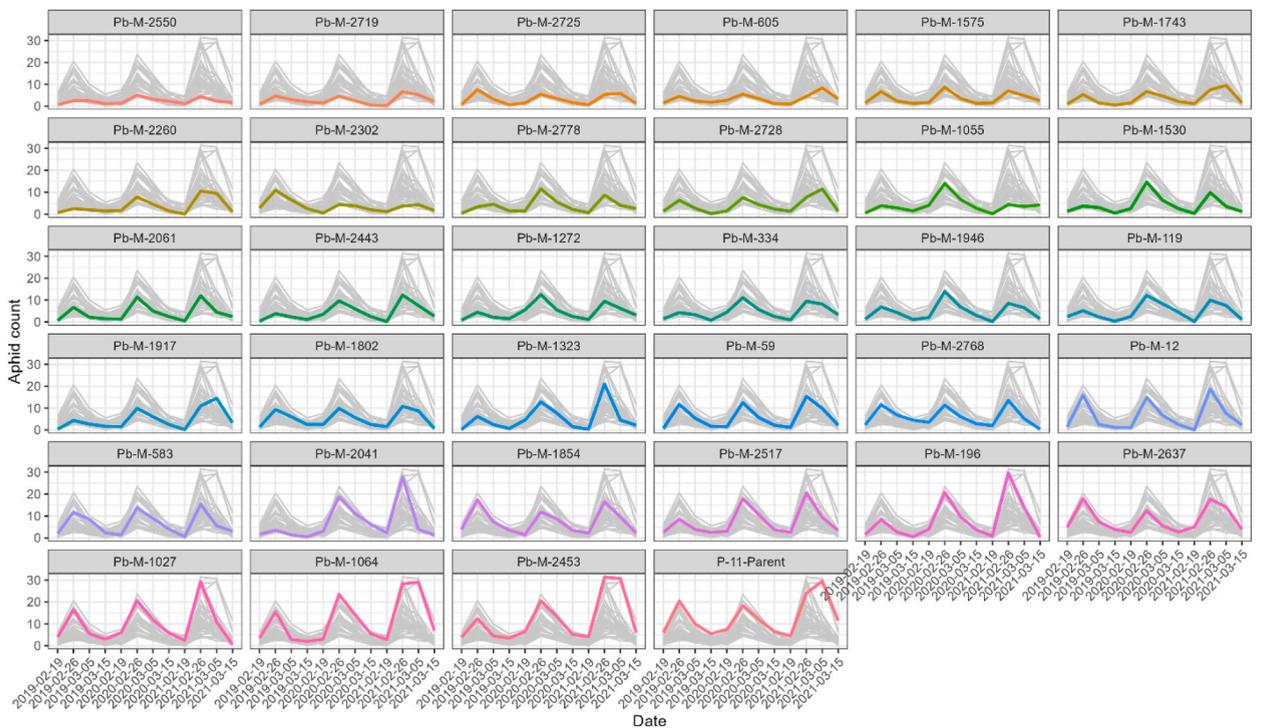
All mutant lines and wild type expressed variable response against aphid infestation. Density of aphid population was dependent on mutant lines, dates of data collection as well as years of field trials. Aphid infestation started during 3rd week of Feb, gradually reaching to the maximum during 4th week of Feb and then started declining till the mid of March as highest peaks were observed during 4th week of Feb during all years (Figs. 3 and 4). However, aphid infestation was relatively low in 2019 during all time intervals of data collection as compared to the aphid infestation observed in 2020 and 2021 (Supplementary Tab. 2.1; 2.2). Varying number of aphids were counted on various mutant lines throughout multiple time intervals over the course of three years and two sites (NIBGE and NIAB). At NIBGE site, minimum number of mean aphids were observed on Pb-M-2725, Pb-M-2550, Pb-M-2719, Pb-M-2302, Pb-M-1055, and Pb-M-1743. However, maximum number of aphids were found on Pb-11 (wild type) followed by Pb-M-2453, Pb-M-1064, and Pb-M-1027 (Fig. 3). Similarly, at NIAB site, lowest aphids count was observed on Pb-M-2550, Pb-M-2719, Pb-M-2725, Pb-M-605, Pb-M-1575 and Pb-M-1743. However, maximum number of aphid was observed on Pb-M-1027, Pb-M-1064, Pb-M-2453, and Pb-11 (wild type) (Fig. 4). Mutant lines including Pb-M-2550, Pb-M-2719, Pb-M-2725, Pb-M-605, Pb-M-1027, and Pb-M-2453 depicted a consistent response at both sites (Figs. 3 and 4).

### 3.4. Cumulative number of aphids at both sites (NIBGE and NIAB) infested the wheat mutant lines and wild type

The cumulative number of aphids of both sites revealed significant variations among all the mutant lines showing varying number of aphid infestation at different time intervals as well as years. However, 4th week of Feb, was relatively the hotspot for aphid infestation during all years (Fig. 5). Moreover, aphid infestation was slightly higher in 2021 and 2020 as compared to the infestation in 2019. In addition to the 4th week of Feb, aphid infestation was also higher during 1st week of March in 2021 and 2020. During 2019, at peak infestation period (4th week of Feb), maximum number of aphids were observed on Pb-M-1027 (19.2) followed by Pb-M-1854 and Pb-M-2637 which both attracted 17.7 number of aphids. The minimum mean number of aphids were counted on Pb-M-2260 (2.5), Pb-M-1055 (3.00), Pb-M-2550 (3.2), Pb-M-2243 (3.3), Pb-M-605 (3.3), Pb-M-2041 (3.7), and Pb-M-1530 (4.2) (Fig. 5). The average number of aphids observed on Pb-11 (wild type) was 12.2. During 2020, at peak infestation period, minimum average aphid count was observed on Pb-M-2260 (4.3), Pb-M-2719 (5.00), Pb-M-605 (5.2), and Pb-M-2725 (5.5), however, maximum number of aphids were



**Fig. 3.** Distribution pattern of number of aphids on all mutant lines at different time intervals spanning three years at NIBGE site. Mutant lines are arranged according to their mean aphid count from low to high aphid count.



**Fig. 4.** Distribution pattern of number of aphids on all mutant lines at different time intervals spanning three years at NIAB site. Mutant lines are arranged according to their mean aphid count from low to high aphid count.

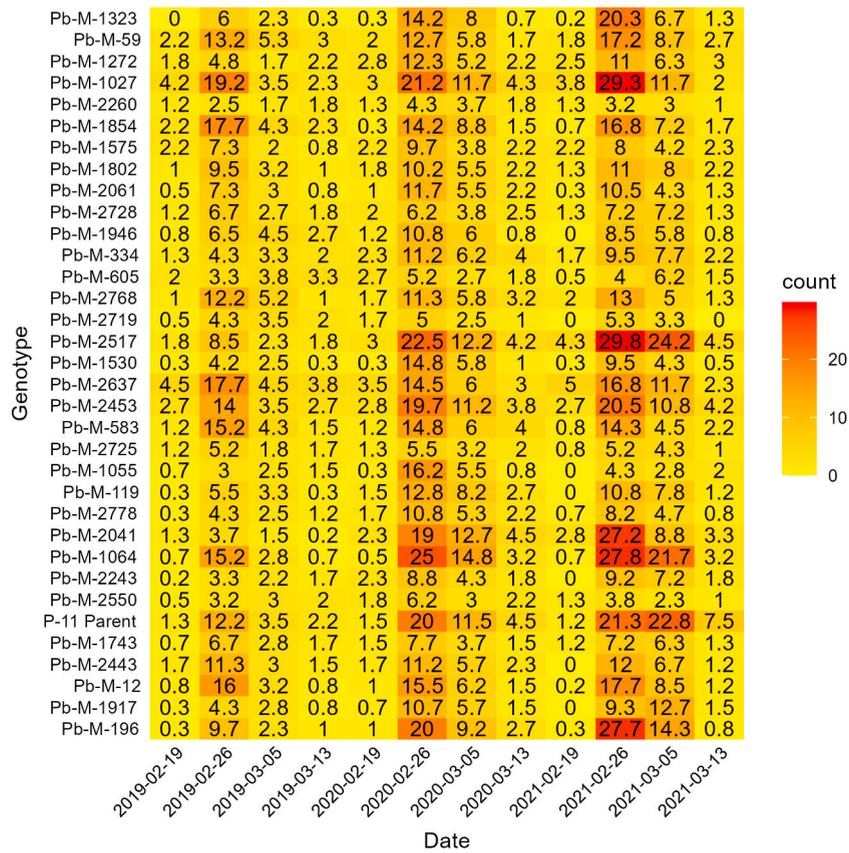


Fig. 5. Average number of aphids across three years and two sites on all mutant lines and wild type (Pb-11). The tiles are colored from yellow (for lower aphid count) to red (for higher aphid count).

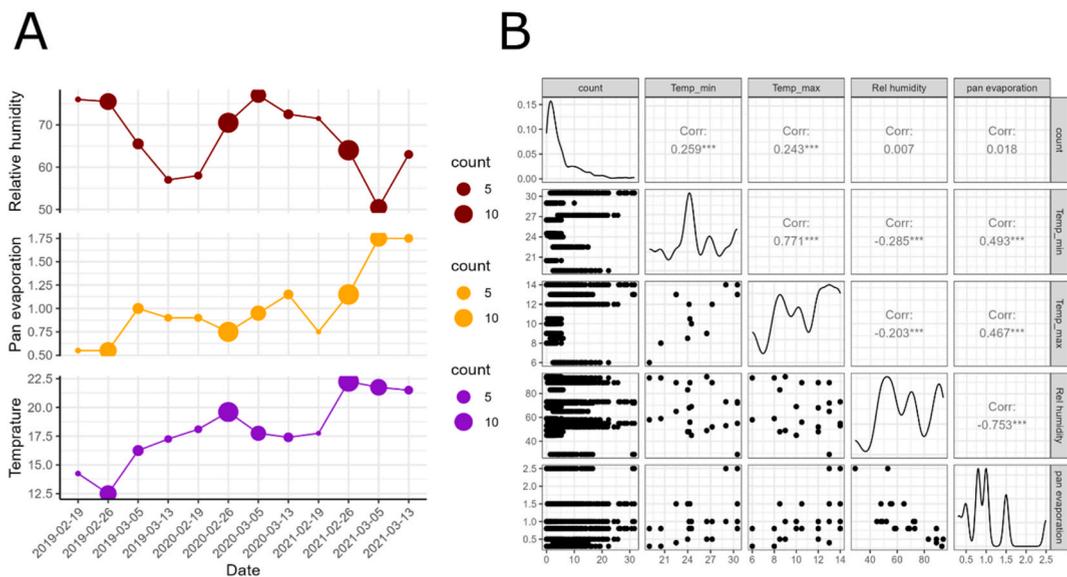


Fig. 6. (a) Average temperature, relative humidity, and pan evaporation for Feb and March during field trial from 2019 to 2021 (b) Correlation between aphid count and meteorological parameters. Correlation coefficient was calculated using Pearson correlation.

found on Pb-M-1064 (25.00) followed by Pb-M-2517 (22.50) and Pb-M-1027 (21.20). The wild type recorded 20.00 number of aphids. In the year 2021, the lowest aphid count found on Pb-M-2260 (3.2), Pb-M-2550 (3.8), Pb-M-605 (4.00), Pb-M-1055 (4.3), and Pb-M-2725 (5.2) (as shown in Fig. 5). Maximum number of aphids were observed on Pb-M-2517 (29.8), Pb-M-1027 (29.3), Pb-M-1064 (27.8) and Pb-M-196 (27.7), while wild type attracted 21.3 number of aphids during peak infestation period. Overall, mutant lines including Pb-M-2725, Pb-M-2260, Pb-M-2550, Pb-M-2719 and Pb-M-605 were found to be the most resistant lines attracting lowest number of aphids. On the other hand, most susceptible lines were Pb-M-1064, Pb-M-1027, and P-11 parent (Fig. 5).

3.5. Correlation studies among meteorological attributes, biochemical and physiological parameters with mean aphid count and grain yield

3.5.1. Correlation of temperature, humidity and pan evaporation with aphid count

Meteorological data including temperature, relative humidity and pan evaporation were analyzed to explore the impact of temperature and humidity on aphid infestation (Fig. 6a). Relative humidity ranged from 24 to 94 with the mean and median values of 66.75 and 66.5, respectively. Pan evaporation ranged from 0.3 to 2.5 with the mean and median at 1.01 and 0.9, respectively. The minimum temperature ranged from 6 °C to 14 °C with a mean value of 10.83 °C and standard deviation of 2.52 °C. The observed meteorological factors showed varying degree of correlation with the aphid count. Aphid count depicted a positive correlation (0.25) with average day temperature within given period, however, aphid count was negatively correlated with relative humidity (-0.58) and pan evaporation (-0.63) (Fig. 6b). The average temperature during peak infestation period in 2019 was slightly lower than the temperature in 2020 and 2021 (Table 1). However, average relative humidity during peak infestation across three years (2019–21) was 75.5%, 70.5% and 64%, respectively (Table 1).

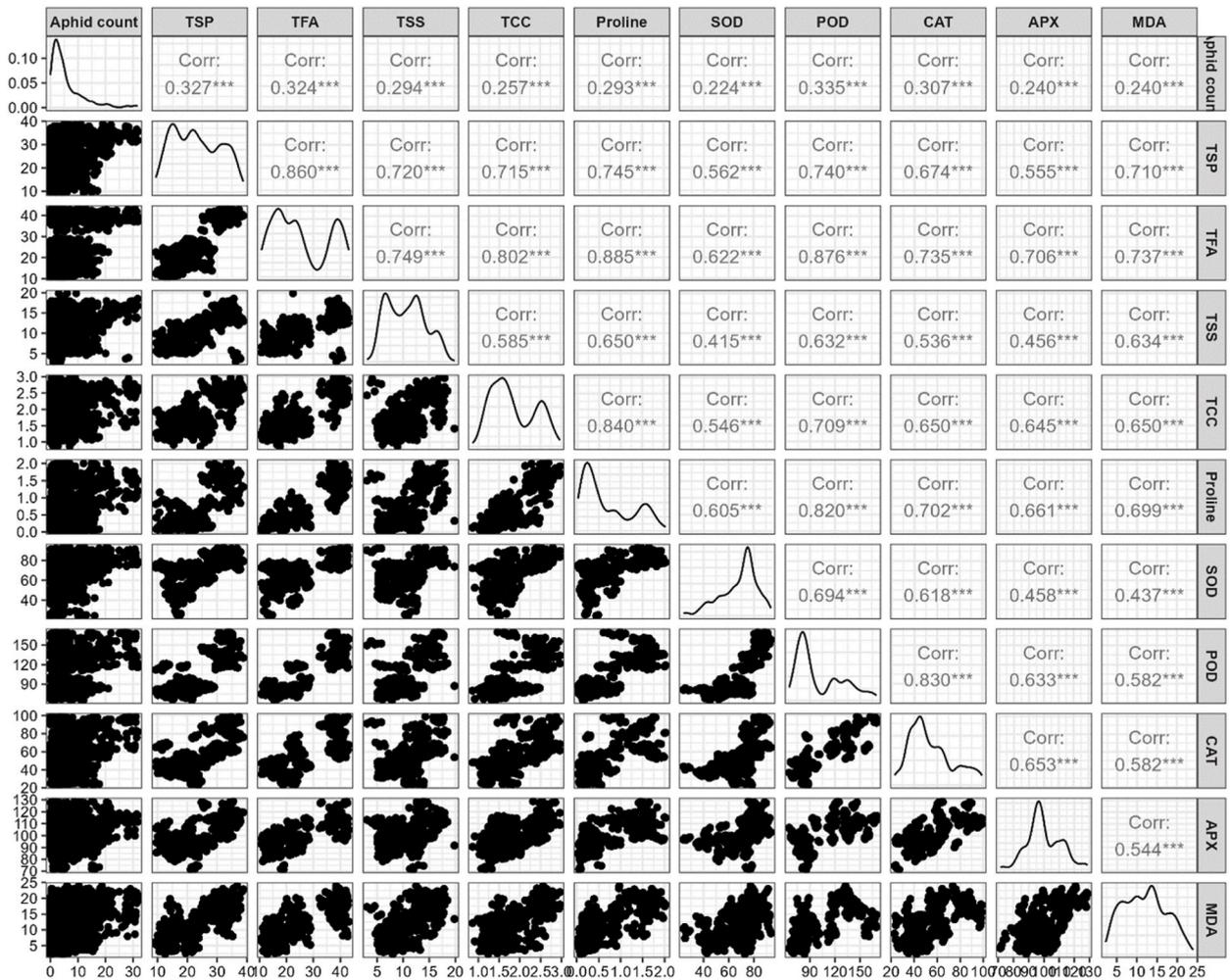


Fig. 7. Combined correlation between average number of aphids and biochemical parameters across three years (2019–2021) and two sites (NIBG and NIAB). Black dots and diagonal curves are representing the extent of correlation coefficients among all traits.

### 3.5.2. Correlation analysis among aphid count and biochemical parameters

Correlation coefficient between mean aphid count and biochemical parameters were estimated (Supplementary Table 3). All biochemical parameters including TFA, TSS, TSP, TCC, Proline, SOD, POD, APX, CAT, and MDA exhibited a positive correlation with mean aphid count at both sites (NIBGE and NIAB) during three years (Figs. 7 and 8). However, POD (0.34), TSP (0.33), TFA (0.324) exhibited high correlation with mean aphid count. In addition, CAT (0.31), TSS (0.294), and proline content (0.293) also showed a significant positive correlation with mean aphid count (Fig. 7). The lowest positive correlation of aphid count was found with APX (0.24) and MDA (0.24). At NIBGE site, aphid count exhibited highest correlation with POD (0.34) followed by TSP (0.33) and TFA (0.32). Moreover, CAT (0.31) and TSS (0.31) also depicted strong correlation with aphid count (Fig. 8). At NIAB site, aphid count demonstrated maximum correlation with TSP, TFA, and POD, these depicted a correlation of 0.33. The CAT and proline depicted a correlation value of (0.30) with aphid count (Fig. 8).

### 3.5.3. Correlation between mean aphid count, physiological parameters and grain yield

Correlation coefficient was estimated between mean aphid count, physiological parameters and grain yield (Supplementary Table 4). All physiological parameters including PAR at leaf surface (−0.28), sub-stomatal conductance (−0.24), transpiration rate (−0.14) and photosynthetic rate (−0.12) depicted a negative correlation with mean aphid count at both sites (NIBGE and NIAB) over three years (2019–2021) (Fig. 9). However, a very weak positive correlation (0.12) was found between mean aphid count and grain yield. Grain yield depicted positive correlation with all physiological traits i.e., sub-stomatal conductance (0.27), transpiration rate (0.24), PAR at leaf surface (0.21) (Fig. 9).

### 3.6. Hierarchical clustering of mutant lines and wild type based upon all traits under study

Hierarchical clustering of all the mutant lines and wild type revealed that mutant lines were clustered into five main clusters depending upon grain yield index and aphid count (Fig. 10; Fig. 13). For example, high yielding mutant lines Pb-M-1323, Pb-M-59, Pb-M-1027, Pb-M-1272 and Pb-M-2728 were clustered together. Some other high yielding mutant lines including Pb-M-583, Pb-M-1575, Pb-M-605, Pb-M-2719, Pb-M-2302, Pb-M-2061, Pb-M-1854, Pb-M-2517, Pb-M-119, Pb-M-2768, Pb-M-2637 and Pb-M-1946 were

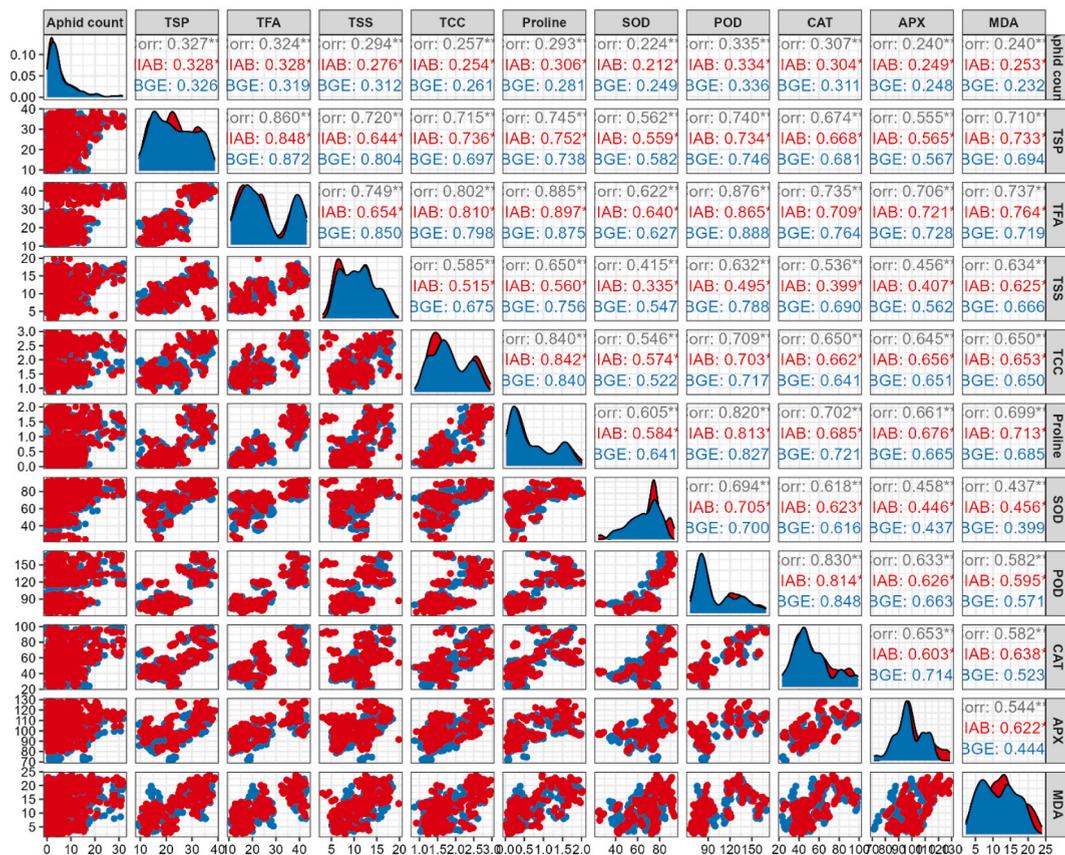


Fig. 8. Correlation between mean aphid count and biochemical parameters across two sites (NIBGE and NIAB). Red dots are explaining the extent of correlation coefficient for traits collected at NIAB site and blue dots are explaining the extent of correlation coefficient for traits collected at NIBGE site. The diagonal matrix also represents the correlation peaks for NIBGE (blue in color) and NIAB (red in color) sites.

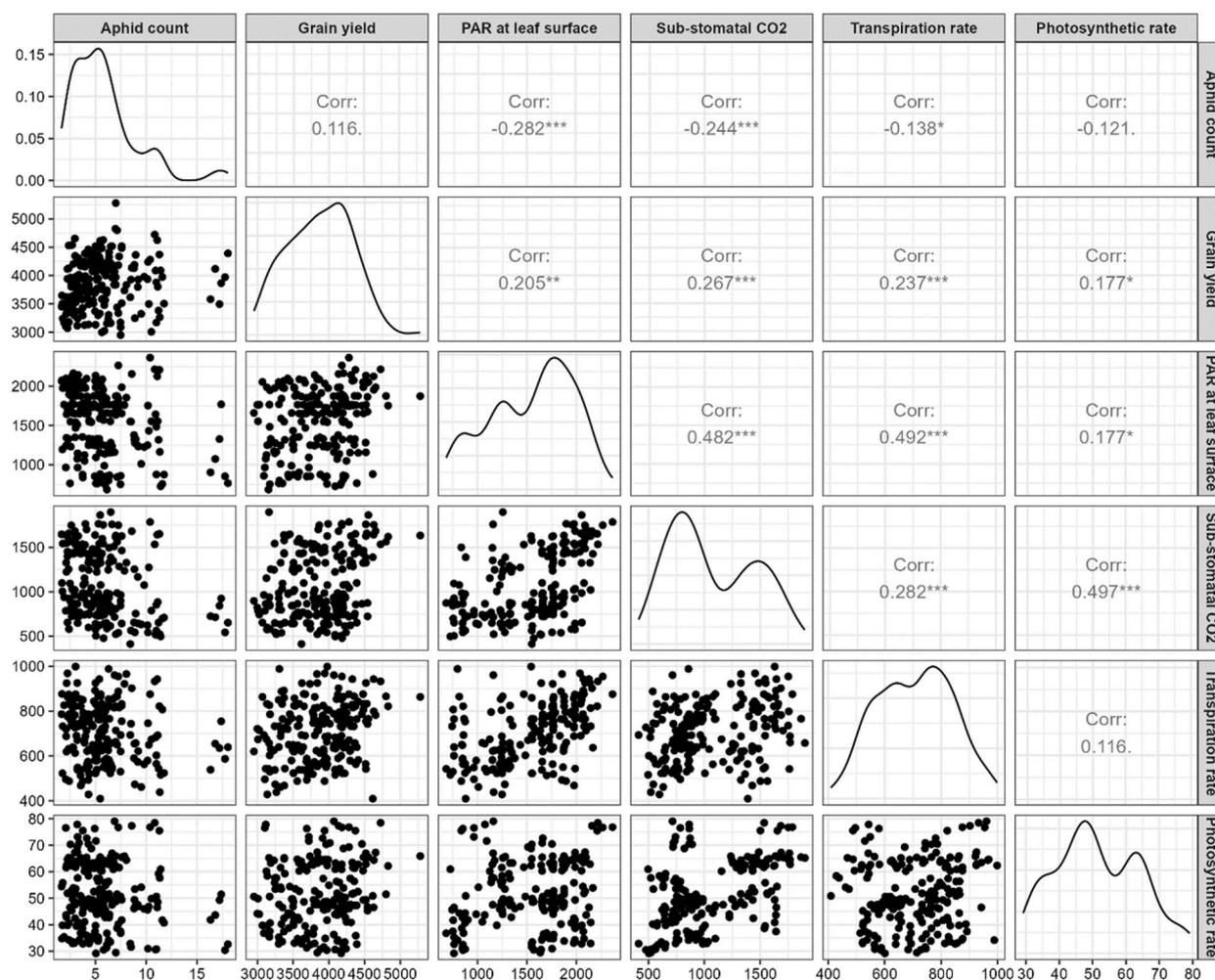


Fig. 9. Combined correlation between mean aphid count, grain yield and physiological parameters across two sites (NIBGE and NIAB) and three years (2019–2021). Black dots and diagonal curves are representing the extent of correlation coefficients among all traits.

clustered together. Mutant lines Pb-M-2453, Pb-M-334, Pb-M-196, Pb-M-12, Pb-M-1064 and Pb-M-1743 were grouped together. Similarly, Pb-M-1055, Pb-M-2725, Pb-M-2728, Pb-M-1530, Pb-M-1802 and Pb-M-2041 made a separate cluster. Some other mutant lines, Pb-M-2260, Pb-M-2550, Pb-M-1917 and Pb-M-2443 having relatively low aphid count were grouped together (Figs. 10 and 11). Mutant lines showing high aphid count were clustered together non-exclusively which revealed a complex underlying interaction of the aphid count and biochemical traits. It is revealed that mutant lines showing relatively high aphid count also expressed high values for biochemical traits (Figs. 10 and 11). It also supported the correlation analysis that most of the biochemical variables were very strongly correlated to aphid count, and mutant lines with lowest aphid count were dispersed randomly in smaller sub-clusters. Moreover, mutant lines with low aphid count had low values of the biochemical variables, relatively (Figs. 10 and 11) but depicted higher values of physiological parameters (Figs. 12 and 13).

By deploying principal component analysis (PCA), multiple factorial analysis (MFA), and factor analysis of mixed data (FAMD), we were able to gain a more comprehensive understanding of how different variables impact the overall variation in the data and identify patterns in the data that may not have been obvious otherwise.

### 3.7. Analysis of data using principal component analysis (PCA)

The PCA partitioned the total variability within the dataset across ten principal components (PCA1; PCA10). Notably, the first component (PCA1) accounted for substantial variation by contributing 48.6% of the total variation in the data, while PCA2 contributed 13% towards total variability within dataset (Fig. 14A). The variation contributed by first two components (PCA1 and PCA2) was further explored based upon contribution of different traits including biochemical and physiological traits, aphid count and grain yield (Fig. 14B and C). In PCA1, major variation was contributed by biochemical parameters as compared to the physiological variables. However, maximum contribution was made by the TFA followed by proline content and POD than that of any other biochemical



**Fig. 10.** Circular map showing distribution of aphid count, biochemical traits, and grain yield with respect to mutant lines. Mutant lines were clustered using hierarchical clustering after calculating correlation coefficients using Pearson correlation.

parameter (Fig. 14C and D). Grain yield was the least contributing trait towards total variability accounted for PCA1. Moreover, in both PCAs (PCA1 and PCA2), all the biochemical variables were clustered together with aphid count, while physiological variables were grouped together with grain yield (Fig. 14E and F). There was a correlation observed between a high aphid count and certain biochemical traits, whereas a high grain yield appeared to be associated with a preference for physiological traits with higher values (Fig. 14E and F).

### 3.8. Identification of variation pattern among the studied traits by deploying multiple factor analysis (MFA)

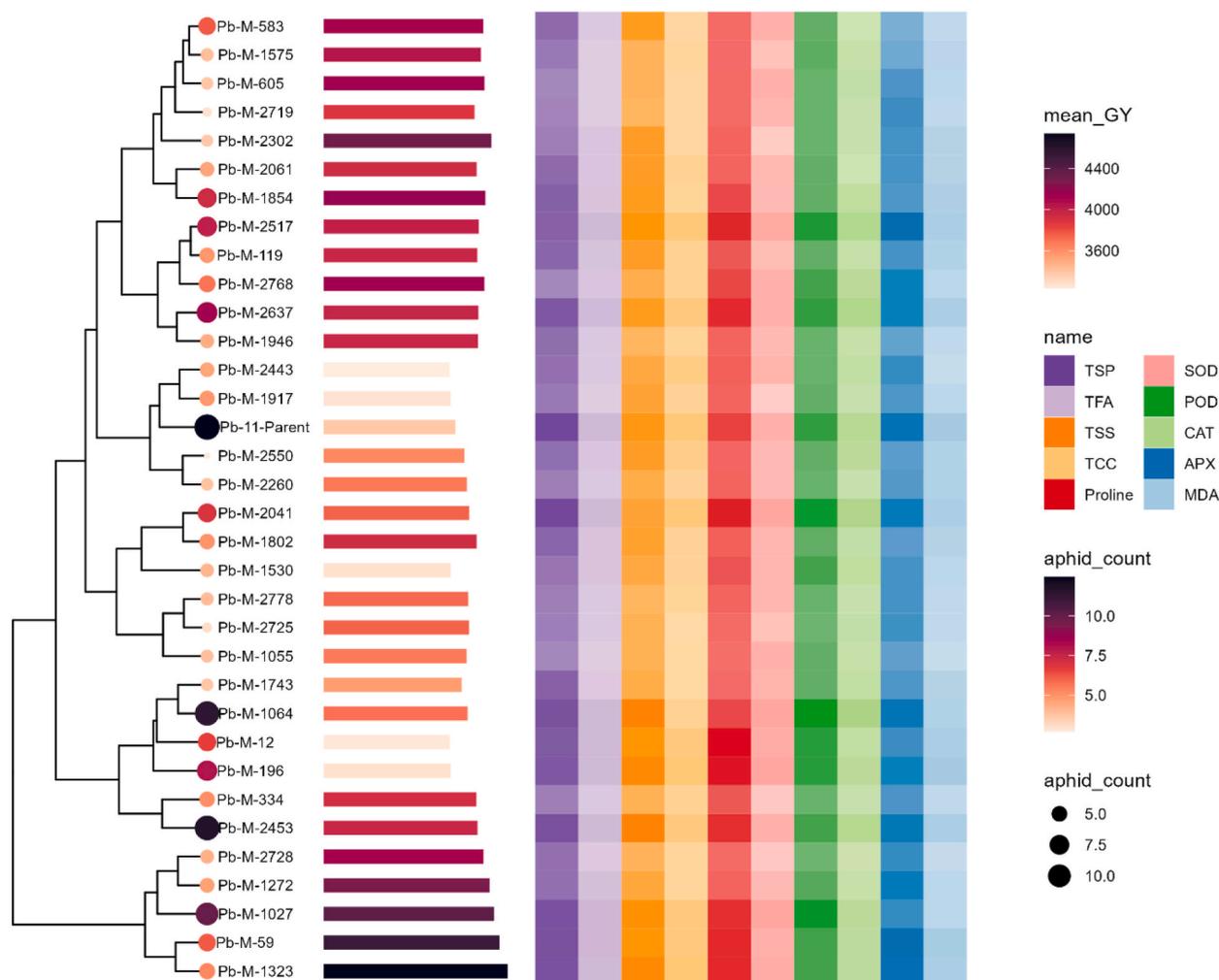
All the quantitative variables were clustered into three groups; biochemical, physiological and outcome. The outcome group contained grain yield and aphid count. Categorical variables such as mutant lines, location, and year were grouped into LLY (location lines years). The variations explained by dimensions of MFA were less as compared to that of PCA possibly due to the incorporation of categorical variables in the MFA (Fig. 15A). In MFA, maximum variation was contributed by the LLY group which comprised of categorical variables including mutant lines, year and location (Fig. 15B). Mutant lines were clustered together into different groups according to their similarity index. For instance, Pb-M-1323, Pb-M-59, and Pb-M-2768 clustered together. Likewise, Pb-M-119, Pb-M-1272, and Pb-M-2443 were clustered into another group (Fig. 15C). In MFA, the maximum contribution towards total variation was made by aphid count. Biochemical and physiological parameters contributed almost equally; however, grain yield was least contributing factor (Fig. 15D). The quantitative variables in MFA were correlated but the contributions of different variables differed than that of measured using PCA. Biochemical parameters clustered with aphid count while physiological parameters clustered with grain yield (Fig. 15E and F).

### 3.9. Factor analysis of mixed data (FAMD)

The first dimension of FAMD contributed 16.9% variation while second dimension contributed 5.7%. The mutant lines with higher grain yield were majorly found at the top of the FAMD biplot (Fig. 16A). The contribution of quantitative variables in FAMD was similar to that of PCA (Fig. 16C). However, FAMD revealed that the highest contribution toward total variability within the dataset was offered by mutant lines substantially, as shown by “Mutants” (Fig. 16B). Among the mutant lines, the top two highest yielding lines (Pb-M-1323 and Pb-M-1027) offered maximum contribution to the first and second dimensions of FAMD. Moreover, mutant lines Pb-M-1027, Pb-M-1323, Pb-M-59 were found to be the most diverse lines as compared to the remaining mutant lines and wild type (Fig. 16D).

### 3.10. Mean yield performance of mutant lines and mean number of aphids

Sorting all the mutant lines according to their median yield irrespective of the year and location revealed that Pb-M-1323, Pb-M-59, Pb-M-1272, Pb-M-1027, and Pb-M-1854 were the highest yielding mutant lines. However, Pb-M-2443, Pb-M-1530, Pb-M-12, Pb-M-196



**Fig. 11.** Correlation heatmap among aphid count, grain yield and biochemical parameters. Variables were clustered using hierarchical clustering after calculating correlation coefficients using Pearson correlation.

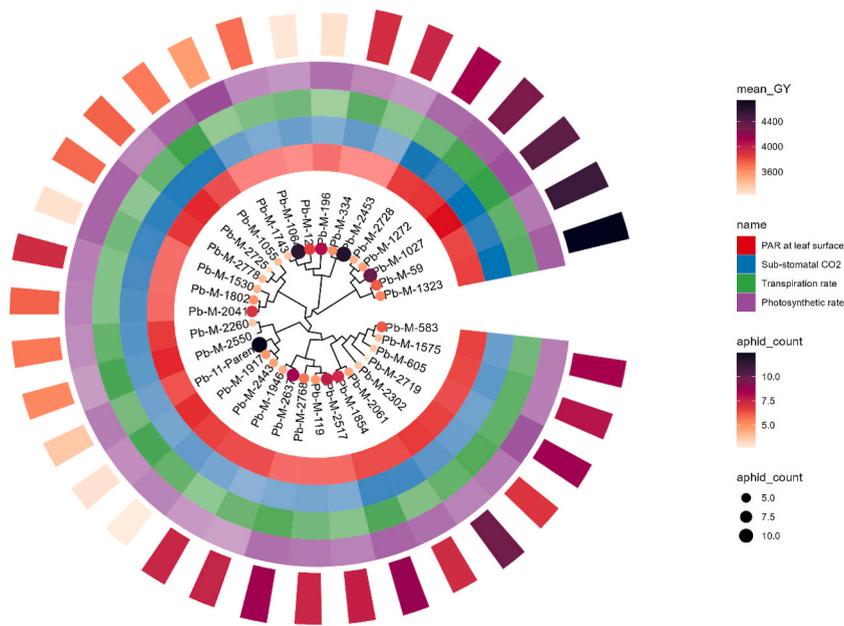
and Pb-11 parent were the least yielding (Fig. 17). It was revealed that out of 33 mutant lines, 28 mutant lines gave yield higher than that of wild type while only five mutant lines produced lower yield as compared to the wild type. As for as the impact of aphid infestation on grain yield was concerned, the top yielding mutant lines such as Pb-M-1323 (5.28), Pb-M-59 (6.16), Pb-M-1272 (4.64), Pb-M-1027 (9.9), and Pb-M-1854 (7.32) depicted varying mean aphid count. However, Pb-M-1027 exhibited high yield in spite of high aphid count (9.9) (Fig. 17). On the other hand, mutant lines showing lowest grain yield, i.e., Pb-M-2443 (4.57), Pb-M-12 (6.62), Pb-M-1530 (4.14), Pb-M-196 (8.08), and Pb-M-1917 (4.91) also exhibited varying number of aphids. Highest mean aphid count (12.45) was observed on Pb-11 parent (wild type) followed by Pb-M-2453 (11.74) and Pb-M-1064 (11.38). While, lowest mean aphid count was observed on Pb-M-2550 (2.71), Pb-M-2719 (2.96), Pb-M-2725 (3.05), Pb-M-2302 (3.62), and Pb-M-605 (3.69) (Fig. 17).

## 4. Discussion

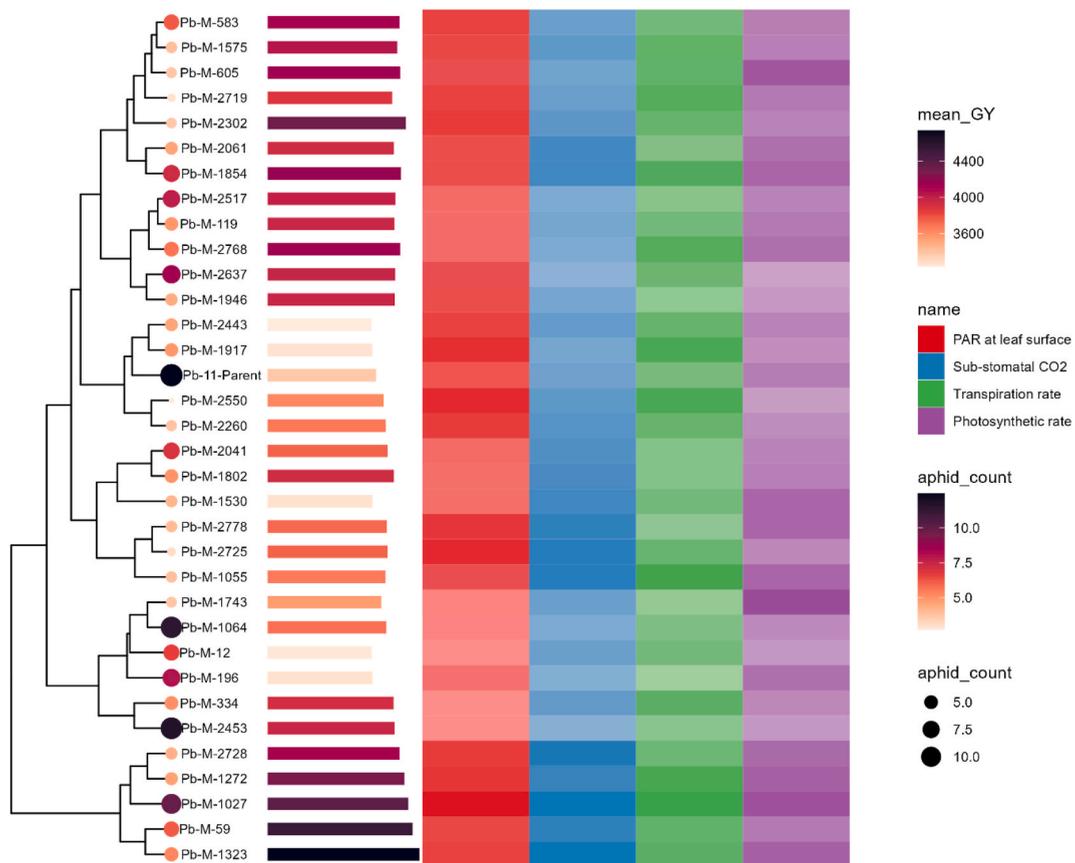
### 4.1. Characterization and screening of mutant lines against aphid infestation under field conditions

To know the extent of variability in various traits in the available germplasm sources is one of the pre-requisites before initiating any breeding program. In this regard, extensive characterization of germplasm by conducting field trials in various ecological zones may help to know the genetic potential of genotypes-accessions. Such efforts will pave the way of developing new resilient wheat varieties [42]. Hence, for enriching the germplasm, it is important to have enough diversity in germplasm which can be utilized for transferring new alleles into the newly developed cultivars [43].

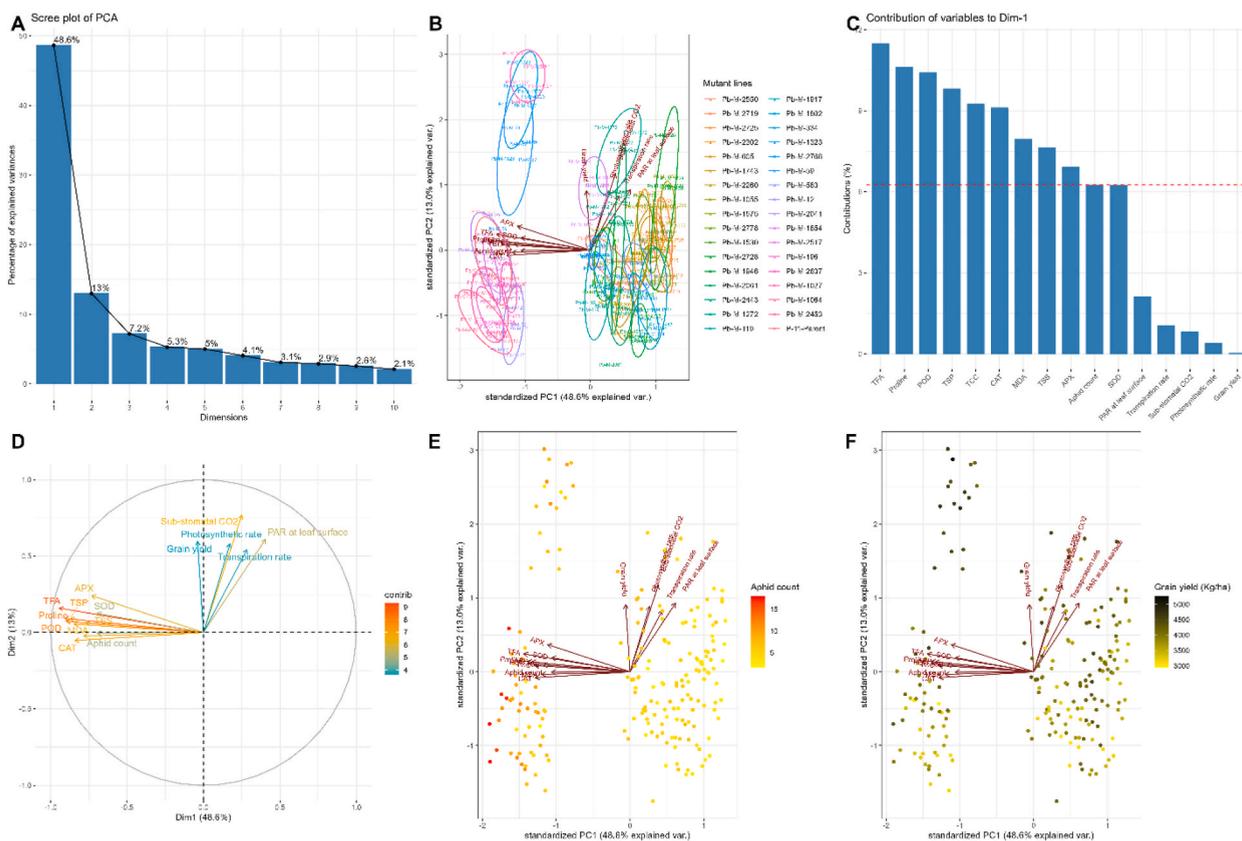
Infestation of insect pests including aphids has been found to be the most destructive in reducing the wheat production and quality of wheat flour [44–46]. The aphids remove photo assimilates and act as vector for several plant viruses [47,48]. The cereal aphids act as vectors for transmitting the barley yellow dwarf virus (BYDV) [46,49]. Screening and identification of newly developed wheat



**Fig. 12.** Circular map showing distribution of aphid count, physiological traits and grain yield with respect to mutant lines. Mutant lines were clustered using hierarchical clustering after calculating correlation coefficients using Pearson correlation.



**Fig. 13.** Correlation heatmap among mutant lines, grain yield, aphid count and physiological parameters followed by hierarchical clustering. Heatmap is annotated by aphid count, physiological parameters, and grain yield differentiated by colors keeping median as a threshold.

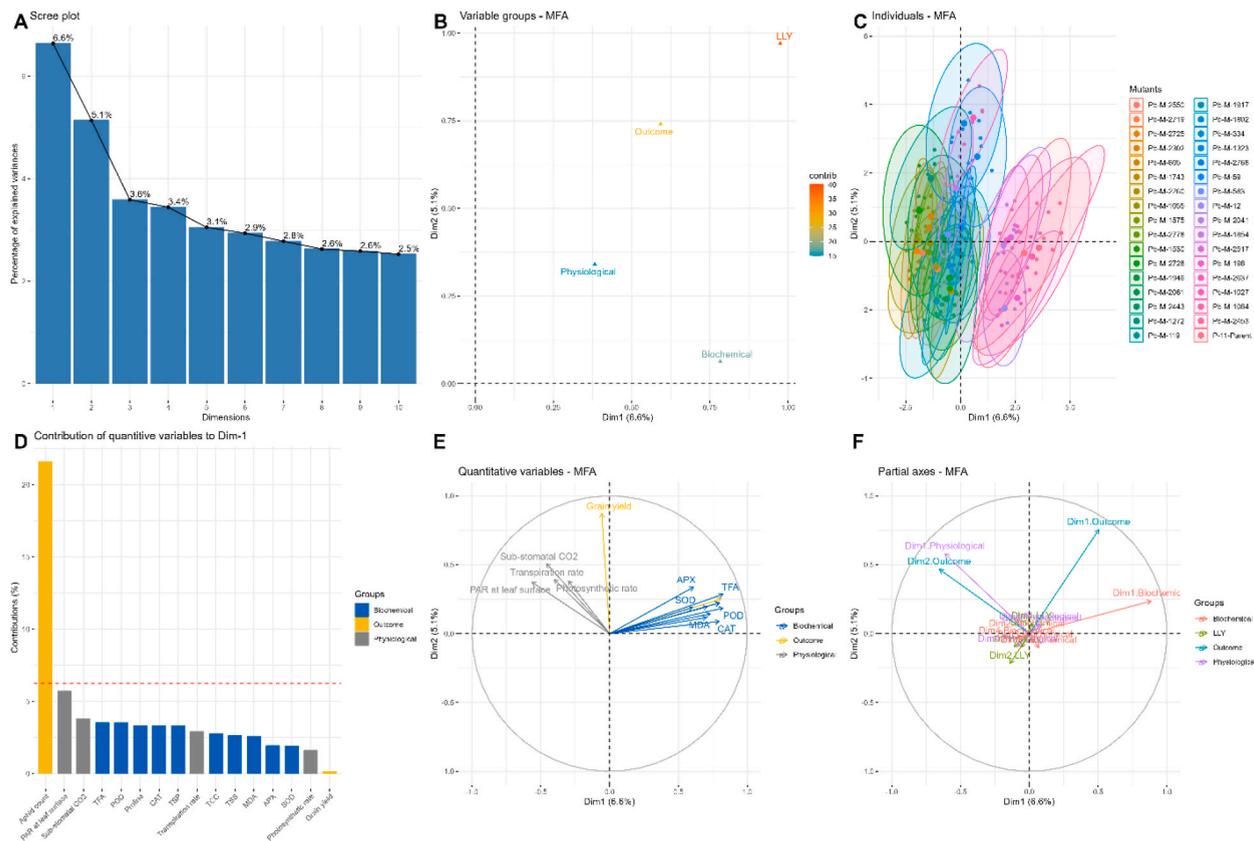


**Fig. 14.** Principal component analysis. A: The percentage of the variance explained by top 10 PCAs in the data. B: Biplot of the PCA showing PCA1 and PCA2, observations are colored by mutant lines. C: The percentage of contribution offered by different variables in PCA1. D: PCA variables plot colored by contribution of each trait in PCA1 and PCA2. Color ranges from blue (lower contribution) to red (higher contribution). E: The biplot of PCA1 based upon aphid count (observations are colored by aphid count) F: The biplot of PCA1 based upon grain yield (observations are colored by grain yield).

varieties for their response to aphid infestation has been remained a major target of researchers and breeders since several years. Therefore, to determine the resistance response of newly developed wheat mutant lines to aphid under natural field conditions, we conducted a study over a period of three years at two different sites (NIBGE and NIAB). By comparing the number of aphids on the mutant lines to the number of aphids on wild-type, we determined the resistance response of each mutant line against aphid infestation. The aphid infestation was tracked at regular intervals following the first detection of aphid infestation. In addition, the impact of biochemical and physiological traits as well as weather parameters on aphid infestation was also studied. This study provides valuable insights into the molecular mechanisms underlying plant resistance to aphids and can enhance the development of more resilient crop varieties.

Plants have adapted several mechanisms (antibiosis and antixenosis) to combat the aphid attack, however, antibiosis was found to be the most effective [21]. Antixenotic mechanism influences the feeding behavior of invading insect as well as preference of insect for infestation on a particular plant species. While, antibiosis impacts the aphid survival on host plant by retarding its growth, development and reproductive potential. Moreover, presence of wax on plant surface can potentially change the interaction of aphid with plant which was demonstrated in oat [28,50]. Application of extract containing wax lessen the deterrence of aphid infestation on a triticale cultivar through increasing aphid mortality. Presence of trichomes on plant organs acts as a barrier against the invading aphids and restricts their movement [51].

In the present study, varying level of resistance was observed among the mutant lines as compared to the wild type. A correlation was also found between aphid count and meteorological factors, particularly the average daily temperature. Our results indicate that both genetic factors and environmental conditions, including weather parameters and planting locations, significantly influenced the variable aphid counts in mutant lines. At NIBGE site, minimum number of aphids were observed on Pb-M-2725, Pb-M-2550, Pb-M-2719, Pb-M-2302, Pb-M-1055, and Pb-M-1743 (Fig. 3). Similarly, at NIAB site, mutant lines with lowest aphids count were Pb-M-2550, Pb-M-2719, Pb-M-2725, Pb-M-605, Pb-M-1575 and Pb-M-1743. The maximum number of aphids was observed on Pb-M-1027, Pb-M-1064, Pb-M-2453, and Pb-11 (wild type) (Figs. 3 and 4) suggesting that these mutant lines are more vulnerable to aphid infestation. Mutant lines including Pb-M-2550, Pb-M-2719, Pb-M-2725, and Pb-M-605, consistently demonstrated reduced aphid infestation at both sites during the normal wheat growing seasons (Figs. 3 and 4). The prevalence of lower number of aphids on these mutant lines

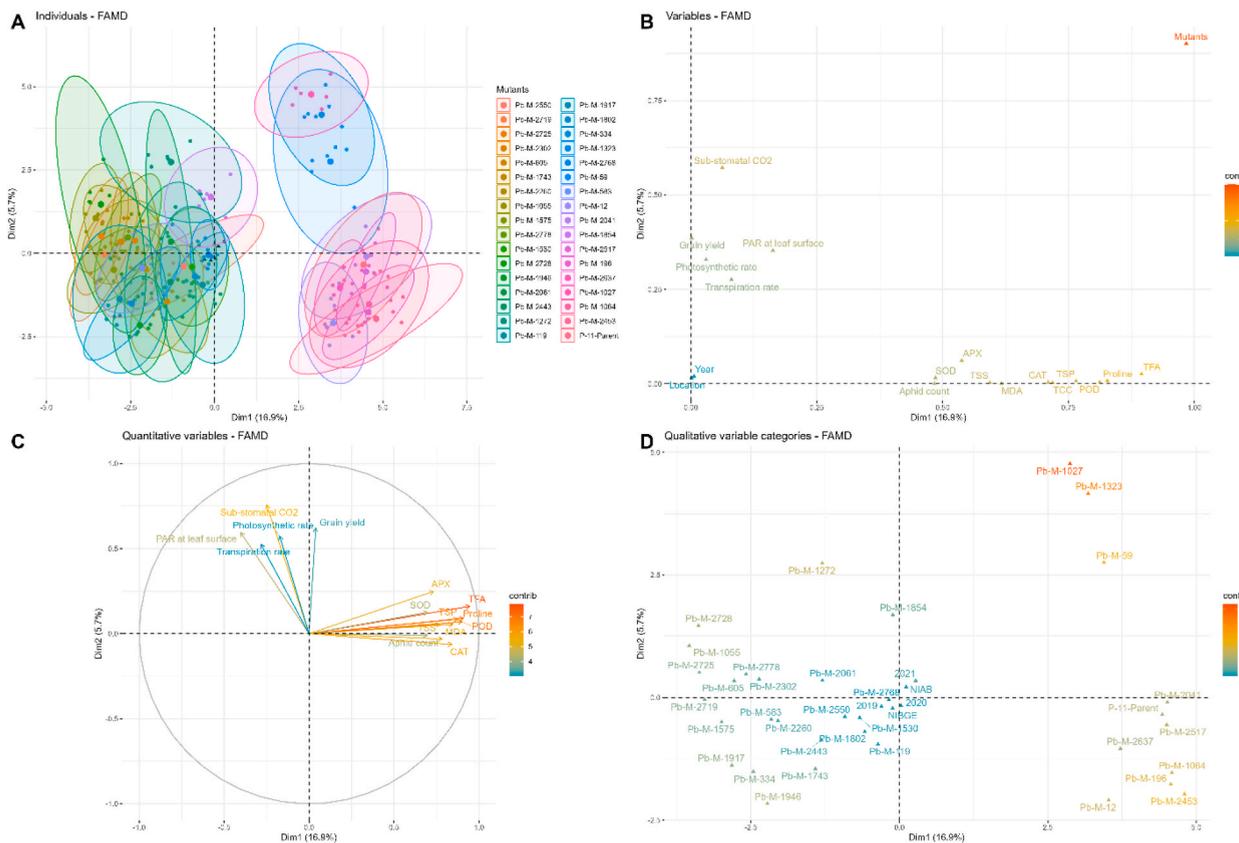


**Fig. 15.** Multiple factor analysis. A) The percentage of the variance explained by top 10 PCAs in the data. B) Contribution of the groups. There were four groups i) Biochemical that include biochemical variables ii) Physiological that include physiological variables iii) Outcome that include grain yield and aphid count and iv) LLY that include mutant lines, location and year. C) MFA biplot colored by mutant lines. D) The percentage of contribution offered by different quantitative variables in MFA1. Bars are colored by different groups as mentioned in 11B. E) MFA variables plot colored by different groups. The length of the variables corresponds to the contribution of that variable in MFA. F) Partial axis plot of MFA. Lines are colored by groups. The length of the lines corresponds to their contribution.

may be attributed to different genetic mutations compared to the other mutants and wild type. It is also possible that these mutant lines possess unique characteristics that make them less susceptible to aphid infestations, potentially linked to their environmental adaptability and plant resistance mechanisms. Moreover, resistance to aphids can manifest in different ways, such as antixenosis or antibiosis, or a combination of both. Antixenosis, in particular, was due to the release of semiochemical and/or visual cues that discourage aphids from landing on the host plant [21,52]. Also, synthesis of pheromones may vary between crop genotypes [53,54] that can affect the aphid behavior [52]. In another study, 64 genotypes of wheat were evaluated for their resistance to aphid infestation under field conditions. The aphid count per tiller varied significantly among all the studied genotypes [55]. Consistent results indicating substantial variation among genotypes concerning aphid populations were also documented [56–59]. This consistency with previous studies suggests that aphid resistance is a complex trait influenced by genetic variations among different wheat mutant lines. Extensive research is required to thoroughly investigate the genetic mechanisms underlying resistance and susceptibility in these mutant lines.

By monitoring the number of aphids on mutant lines over a certain period of time, we were able to gain insight into the rate of infestation and identify different time intervals which can be exploited for designing effective pest management strategies. In the present study, the maximum aphid count was observed during 4th week of February during all field trials conducted during normal wheat growing seasons of 2019–2021 (Fig. 5). However, the intensity of aphid infestation was relatively low in 2019 as compared to the subsequent seasons, which may be attributed to the lower average temperature (14.50 °C) in 2019 than that of the other years (2020, and 2021) (Table 1). Notably, it was due to an increase in average temperature of 5 °C and 8 °C during corresponding time intervals in 2020 and 2021, respectively. Thus, an increase in temperature supported the aphid infestation in wheat mutant lines (Fig. 5). However, temperature above 25 °C caused significant reduction in aphid population as the optimal temperature range for aphid growth is 15°C–25 °C [60]. In few other studies, temperature above 28 °C showed the negative effect on aphid growth [61,62]. Similar findings of fluctuation with temperature in aphids population were also reported [63,64].

The present study showed the population dynamics of aphids in relation to crop growth stages. It was found that the aphid count was maximum during the 4th week of February followed by gradual and steady decrease in March (Supplementary Tab. 2.1 and 2.2).



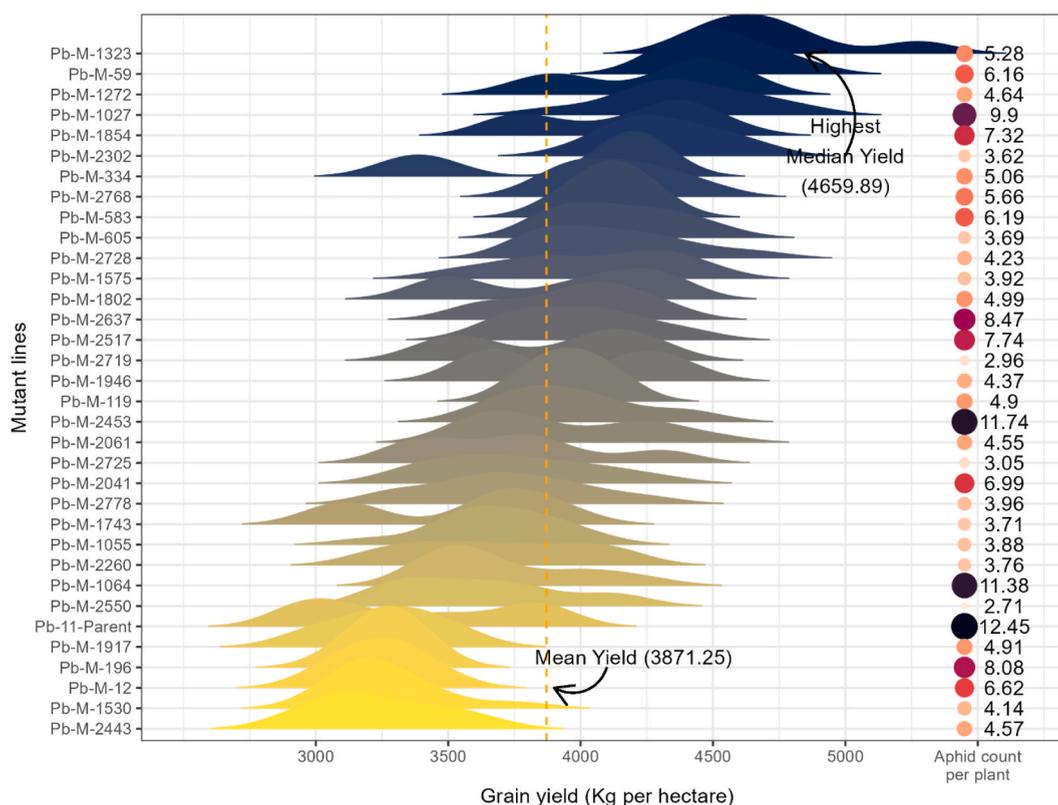
**Fig. 16.** Factor analysis of mixed data. A: Individuals plot for FAMD colored by mutant lines. B, C, D: Contribution of all variables, quantitative and qualitative variables respectively.

At peak infestation, the crop was at heading stage. During 2019, minimum aphids were counted on Pb-M-2260 (2.5), Pb-M-1055 (3.00), Pb-M-2243 (3.3), Pb-M-605 (3.3), Pb-M-2041 (3.7), and Pb-M-1530 (4.2) (Fig. 5). During 2020, lowest aphid count was observed on Pb-M-2260 (4.3), Pb-M-2719 (5.00), Pb-M-605 (5.2), and Pb-M-2725 (5.5). In the year 2021, lowest aphid count found on Pb-M-2260 (3.2), Pb-M-2550 (3.8), Pb-M-605 (4.00), Pb-M-1055 (4.3), and Pb-M-2725 (5.2) (as shown in Fig. 5). Moreover, aphid infestation was slightly higher in 2021 and 2020 as compared to the infestation in 2019. Overall, mutant lines including Pb-M-2725, Pb-M-2260, Pb-M-2550, Pb-M-2719 and Pb-M-605 were found to be the most resistant lines attracting lowest number of aphids. On the other hand, most susceptible lines were Pb-M-1064, Pb-M-1027, and P-11 parent (Fig. 5).

The infestation began during the 3rd week of February, when the crop was in the booting stage, and gradually declined towards mid-March when the crop entered its grain formation/reproductive stage. This suggests that the highest levels of aphid infestation occurred during the booting/heading/tillering stage of vegetative growth. Additionally, the aphid count fluctuated significantly among the mutant lines, with some lines found to be more tolerant to infestation than the wild type and others attracting a higher number of aphids. It clearly showed that these differences resulted due to different mutations induced in each mutant line. Our findings are consistent with earlier studies conducted in Pakistan and other countries [65–68]. In contrary, high number of aphids were observed in March [4,69,70]. It was reported that aphid populations decline at the ear emergence stage, typically between the 9th and 16th of March [16]. Additionally, other researchers have reported that as the crop matures, aphid populations was disappeared by the last week of March [14]. This contradiction was probably due to an increase in temperature in this particular region which let the crop to reach heading stage much earlier (1st week of March) than that of the reported in previous studies. This research highlights the importance of especially understanding the population dynamics of aphids in relation to crop growth and environmental factors, especially the high temperature. These findings can help in developing effective pest management strategies. Further research is needed to understand the population dynamics of aphids in different regions, climates, and cropping systems.

#### 4.2. Exploring the impact of biochemical and physiological factors on aphid infestation

Aphids, upon landing on a plant, probe different sites for getting first-hand knowledge about the internal biochemical and physiological properties of the substrate. After landing on plant, aphid feeding may lead to an increase in the flow of nutrients at the infested tissues [71]. The preferred food source for aphid proliferation and development are the amino acids present in phloem sap [72, 73]. Thus, plant biochemistry and physiology at the time of infestation impacts the survival, growth and development of aphid



**Fig. 17.** Ridge plot for grain yield and average aphid count of all mutant lines and wild type. Lines with maximum median yield are colored dark blue, all other lines with median yield more than the average yield of all the lines are colored blue while lines that have a median yield less than the average yield are colored yellow. Circles at the right of the plot shows the aphid count. The circle size represents the aphid count. Color gradient is from red for lower aphid count to black for higher aphid count. Number along the circles are mean aphid count per plant.

population on wheat plant. In the present study, all biochemical parameters including TFA, TSS, TSP, TCC, Proline, SOD, POD, APX, CAT, and MDA exhibited strongly positive correlation with mean aphid count at both sites (NIBGE and NIAB) (Figs. 7 and 8). However, POD (0.34), TSP (0.33), TFA (0.324) exhibited high correlation with mean aphid count. In addition, CAT (0.31), TSS (0.294), and proline content (0.293) also showed significant positive correlation with mean aphid count (Fig. 7).

The positive correlation between various biochemical parameters (e.g., proline, CAT, SOD, POD, APX, TFAs, TSS) and aphid counts in our study suggests a dynamic interplay between plant physiological responses and aphid infestation. Aphid-induced stress in plants can trigger the upregulation of these biochemical compounds, serving as a defense mechanism against oxidative stress and nutrient loss caused by aphids [74]. Additionally, the attractive nutritional profile of host plants with elevated levels of these compounds may draw aphids. This intricate relationship underscores the complex nature of plant-aphid interactions and offers valuable insights into potential targets for more effective pest management and crop protection strategies, warranting further research into the specific underlying mechanisms governing these dynamics.

In contrast to the present findings, POD was found in large amount in resistant cultivars, but earlier its excessive amount was reported in aphid-sensitive cultivars [75]. It was reported that phenolic metabolites are toxic to insects. These metabolites also play a role in signal transduction pathways. In response to these signals, plant synthesizes the secondary metabolites including CAT, SOD and POD which are poisonous to insects [76]. In another study, enhanced concentration of phenolic compounds for combating the bird-cherry oat aphid was found in mature grains of hard red winter wheat [77]. Consistent with our results, fluctuation in amino acid concentrations after aphid infestation was reported in host plant [78]. In the present study, high accumulation of soluble sugars was found in wheat leaves infested with aphids as compared to the healthy mutants (Supplementary Table 1). However, in another study, infected leaves expressed low concentration of soluble sugars [48]. It was concluded that susceptible genotypes expressed high protein contents and free amino acids—providing congenial conditions for the growth and development of aphids. In our study, high expression of antibiosis against aphids was found positively associated with a high accumulation of nutritional components, such as soluble proteins, total free amino acids (Supplementary Table 1). Another study revealed that during early stages of insect-plant interaction, insect feeding and host plant factors stimulate each other, ultimately leading to an increase in metabolic efficiency of the host plant which also triggers the growth rate of insect population [79]. When a plant's photosynthetic tissues are damaged, the plant triggers defense mechanisms to protect itself. This response enhances metabolic processes to offset the harm inflicted by plant-eating insects [74]. The present research suggests that understanding the biochemical and physiological properties of wheat

plants can help in understanding the resistance mechanisms against aphids and aid in developing pest management strategies.

Aphid-infested plants were found to be less efficient in utilizing the sun light which results in reduced chlorophyll fluorescence as well as photosynthetic rate. Aphid infestation on wheat can cause significant reductions in gas-exchange and chlorophyll fluorescence [80]. In the present study, aphid count was negatively correlated with physiological parameters including PAR at leaf surface, sub-stomatal conductance, photosynthetic rate and transpiration rate (Fig. 9). However, sub-stomatal conductance depicted strong correlation (0.45) with aphid count and aphid injury affected physiological responses and resulted in reduced photosynthetic and transpiration rates (Supplementary Table 1). Decline in photosynthetic rate may indicate a metabolic shift toward the production of an induced compound at the expense of photosynthesis. Although the mechanism of impact of aphid infestation on photosynthesis is still unclear, it was reported that aphid can negatively impact the photosynthetic rate and chlorophyll content in wheat [16,81]. This is likely due to the physical damage caused by the pests to the cell walls and chloroplasts, as well as the toxic effects of their saliva. Our findings in this experiment have implications beyond the aphid-wheat system we examined. Addressing the mechanism of antibiosis at the cost of plant metabolism (i.e., photosynthesis) is another important research question that follows from this research. In our study, we found a non-significant positive correlation between aphid count and grain yield, suggesting that aphid infestation did not directly impact crop yield. Further research is essential to understand the intricate interactions between pest infestations and plant physiological processes for effective pest management and crop enhancement.

## 5. Conclusions

Present study addresses the scarcity of reports regarding the development of resilient wheat mutants against aphid infestation, a significant threat to wheat production worldwide. By assessing 33 wheat mutants developed through gamma radiation, we aimed to identify genetic diversity in terms of resistance to aphids and its impact on wheat crop, yield improvement, and food security. This study has shown that exposure to radiation is an effective method for creating novel mutants conferring resistance to aphid infestation. The study evaluated the level of resistance among mutant lines and characterized them using biochemical and physiological parameters. The study also assessed the relationship between aphid infestation, biochemical parameters, physiological parameters, and grain yield. Results showed that the accumulation of certain biochemical traits correlated with aphid infestation. The dough and ripening stages were found to be the most susceptible growth periods for aphid attacks, occurring during mid-February to mid-March. Specific mutant lines such as Pb-M-2719, Pb-M-2725, Pb-M-2550, Pb-M-2260, Pb-M-1575, and Pb-M-605 were found to be the most resistant, while Pb-M-1027, Pb-M-1064 and wildtype were most susceptible to aphid attack. This study can be used to devise a management strategy for the control of aphid and could be used as potential breeding material for meeting the objectives of breeders. Furthermore, a high-yielding mutant line Pb-M-1323 could be crossed with Pb-M-2725, which showed the lowest aphid count, so that both high yield and aphid resistance could be combined into a single line. This study provides a significant contribution to the field of crop protection and breeding, as it could lead to the development of more resilient and productive wheat cultivars.

## Ethics declaration

Review and/or approval by an ethics committee was not needed for this study because we conducted this study on certain mutant lines of wheat plants developed by our own group. We didn't carry out any sort of research on animals.

## Data availability statement

The data from the manuscript can be found within the article, as well as in the Supplementary Files. Data associated with our study has not been deposited to public repository.

## Funding

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## CRediT authorship contribution statement

**Sana Zulfiqar:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Shumila Ishfaq:** Methodology. **Sayyad Ali Raza Bukhari:** Software, Formal analysis. **Muhammad Sajjad:** Writing – review & editing. **Muhammad Akhtar:** Resources. **Dongcheng Liu:** Writing – review & editing, Resources, Conceptualization. **Mehboob-ur Rahman:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization.

## Declaration of competing interest

The authors had no conflict of interest. Additionally, the funding agency did not play any role in planning experiments, data collection, explaining results, or writing the manuscript. The funders also had no role in making decisions regarding publishing the results.

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## Appendix A. Supporting information

Table S1: Mean data for all traits under study, Table S2.1: Mean Aphid count on all mutant lines at NIBGE, Table S2.2: Mean aphid count on all mutant lines at NIAB, Table S3: Correlation coefficient between mean aphid count and biochemical parameters, Table S4: Correlation coefficient between aphid count, grain yield and physiological parameters.

## Appendix B. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e26529>.

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