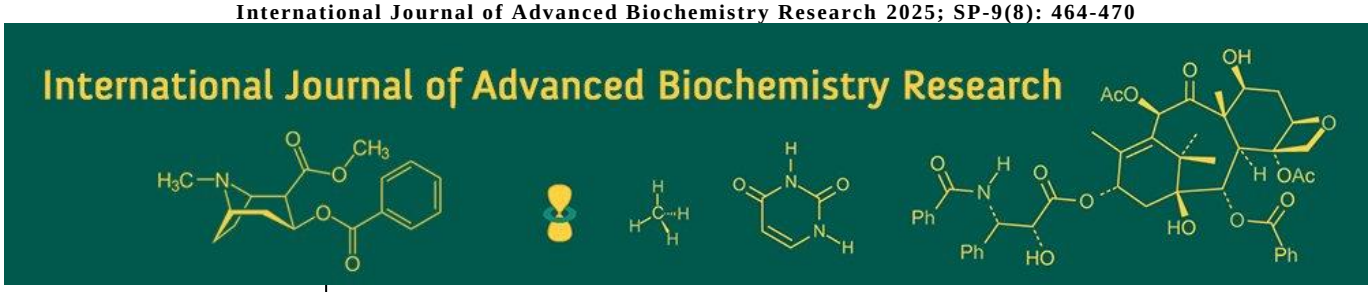

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Screening of elite germplasm of wheat against *Bipolaris sorokiniana* disease resistance under terminal heat stress

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Abstract
Spot blotch (*Bipolaris sorokiniana*) and terminal heat stress are critical biotic and abiotic stresses that substantially limits wheat (Triticum aestivum L.) yields in the Eastern Indo-Gangetic Plains (EGP) of South Asia. The present study evaluated 104 wheat genotypes, sourced from both national and international sources, for their resistance to spot blotch disease and tolerance to terminal heat stress under field conditions. A range of physiological parameters, including disease severity, SPAD index, NDVI, canopy temperature, and canopy temperature depression, were monitored on a weekly basis, and their correlations with Area Under Disease Progress Curve (AUDPC) and yield were statistically analysed. AUDPC demonstrated a significant negative correlation with yield. Based on these findings, genotypes such as ACC-311 and ACC-306 emerged as promising genotypes exhibiting resistance to spot blotch and tolerance to heat stress. These genotypes hold considerable potential for use in breeding programs aimed at developing heat-and disease-tolerant wheat varieties, thereby contributing to enhanced wheat productivity in the region, particularly in the context of climate change-induced challenges.

Keywords: Wheat, spot blotch, terminal heat stress, AUDPC, genotype screening, climate resilience

Introduction
Wheat (*Triticum aestivum* L.) is one of the most widely grown cereal crops and a cornerstone of global food security nourishing over 2.5 billion people globally and providing the majority of calories, earning it the title of the "king of cereals" (Aditya *et al.*, 2024) [2]. Wheat originated in south-western Asia (Giraldo *et al.*, 2019) [7].

Food and Agriculture Organization (FAO) estimates for the year 2022, 770 million metric tons of wheat are produced on 221 million hectares of area worldwide (FAOSTAT, 2022) [6]. In 2022, the globe's major wheat producing nations are the European Union, China, India, Russia, United States and Canada (United States Department of Agriculture, 2022) [21].

In India, wheat crop ranks second in terms of total production next to rice. In 2021-2022, wheat was cultivated on around 30.54 million hectares of land, yielding productivity and production of 3484 kg/ha and 106.84 million tons, respectively Uttar Pradesh was the leading wheat producing state in India in 2021-2022, followed by Madhya Pradesh, Punjab, Haryana and Rajasthan (ICAR-IIWBR, 2022) [10].

Despite these remarkable output numbers, wheat production must rise to meet a projected world food demand of almost 9 billion people by 2050 (United Nations, 2019) [6]. Minimizing yield losses from biotic and abiotic stress is one of the key tactics for raising production (Khan *et al.*, 2022) [12]. The Eastern Indo-Gangetic Plains (EGP) in India, encompassing key wheat-producing regions such as Bihar, West Bengal, eastern Uttar Pradesh, and adjacent areas, represent a pivotal agricultural zone for wheat cultivation. However, wheat production in this region is increasingly vulnerable to a combination of biotic and abiotic stresses, with spot blotch disease and terminal heat stress emerging as particularly critical constraints to crop yield and productivity (Pandey *et al.*, 2021) [13].

Terminal heat stress, occurring during the reproductive and grain-filling stages of wheat growth, has become a dominant limiting factor to wheat production within the EGP.

Exposure to elevated temperatures exceeding 30 °C during these key growth phases accelerates plant senescence, restricts photosynthetic activity, shortens the grain-filling period, and significant reductions in yield potential (Rane *et al.*, 2000). Many reports suggest that for every 1 °C increase in temperature above optimal levels during the grain-filling phase, wheat yield may decline by approximately 3-5% (Singh *et al.*, 2022) [10].

Exacerbating the adverse effects of heat stress is the concurrent occurrence of spot blotch disease, caused by the pathogenic fungus *Bipolaris sorokiniana* (Sacc.) Shoem, also known by its teleomorph *Cochliobolus sativus*. This foliar blight represents a significant global threat to wheat production, contributing to substantial yield losses. The pathogen affects plant productivity primarily by reducing the photosynthetic efficiency, which in turn limits overall crop growth and development (Chowdhury *et al.*, 2013) [4]. Spot blotch disease thrives in the warm, humid microclimate that characterizes the late-season growth phase of wheat, thereby adverse impact of heat stress. The combined impact of these stresses intensifies the challenge of maintaining wheat productivity under such conditions (Acharya *et al.*, 2011) [1]. Addressing this dual threat necessitates the identification and cultivation of wheat genotypes capable of withstanding elevated temperatures while simultaneously offering resistance to disease pressure. Physiological parameters such as chlorophyll retention (SPAD), canopy temperature (CT), canopy temperature depression (CTD), and NDVI have proven effective in screening for heat-tolerant genotypes under field conditions (Chaubey *et al.*, 2023) [3].

In West Bengal, the districts Murshidabad, Cooch Behar have been identified as hotspots for spot blotch development, with significant disease outbreaks in recent years. Notably, during the 2020-21 season, Murshidabad experienced a disease severity of 59.26% (Hooi *et al.*, 2023) [9].

The objective of this study is to evaluate the effects of terminal heat stress in conjunction with spot blotch disease pressure on the agronomic and physiological performance of spring wheat genotypes. Through a comprehensive multi-parameter evaluation approach, this research aims to identify wheat genotypes with enhanced resilience to climate-induced stresses, particularly those that are suited to late-sown conditions where terminal heat stress is most pronounced.

Materials and Methods

Experimental site

The field experiment was conducted at the university research farm of Uttar Banga Krishi Viswavidyalaya, located in Cooch Behar, West Bengal, during the Rabi seasons of 2022-23 and 2023-24. The site is situated at a latitude of 26°19' N and longitude of 89°23' E, with an elevation of 43 meters above mean sea level (AMSL).

Screening of genotypes

A total of 104 wheat genotypes were collected from both national and international sources for this study. All genotypes were sown late to expose them to terminal heat stress, with two replications. Late sowing conditions are favourable for both biotic stresses, particularly spot blotch disease, and abiotic stress, especially terminal heat stress (Tiwari *et al.*, 2012) [19]. Each genotype was planted in four rows of 2 meters in length with a row spacing of 20 cm. The experimental layout followed a randomized block design with two replications, with resistant checks, BHU35 and DBW187. susceptible check variety Sonalika sown along the border. Standard agronomic practices were followed, including fertilizer application (120:60:60 NPK/ha), weeding, and irrigation at critical growth stages, such as crown root initiation, tillering, flowering, milking, and dough stages, to ensure optimal crop growth and development (Singh, 1986) [18].

Details of morpho-physiological parameters perform during screening of genotypes

In the present study, Morpho-Physiological traits were assessed to evaluate the performance of wheat genotypes. These traits included the area under the Disease Progress Curve (AUDPC), plot yield, the area under the SPAD decline curve (AUSDC), the area under the NDVI decline curve (AUNDC), the area under the Canopy Temperature Progress Curve (AUCTPC), and the area under the Canopy Temperature Depression Progress Curve (AUCTDPC), all of which were computed for each genotype.

To ensure comprehensive assessment, each genotype was scored for various morpho-physiological parameters at five distinct growth stages, based on the Zadoks growth scale GS 50-59 (heading), GS 60-69 (flowering), GS 70-77 (milking stage), GS 78-83 (soft dough), and GS 84-87 (hard dough) (Zadoks *et al.*, 1974) [22].

Disease assessment

Disease severity was visually evaluated using a double-digit scale ranging from 00 to 99, as established by Saari and Prescott (1975) [16]. Disease scoring began at the post-flowering stage of wheat plants and was carried out at 7-day intervals throughout the growing season.

% Severity = (D1/9) (D2/9) *100

D1 indicates the vertical progress of the disease from bottom towards the top of the plant.

D2 indicates the horizontal infection. *i.e*. the total infected area of the leaf.

The AUDPC was computed by aggregating the disease severity across successive time points, The AUDPC calculation was carried out using the following formula outlined by Duveiller and Sharma (2005) [5].

$$AUDPC = \Sigma\ 1/2\ (S_i\text{-}S_{i\text{-}1})\ d$$

Si = Disease severity at the end of time i, k = Number of successive evaluations of spot blotch disease severity, d = Interval between two observations

Canopy temperature (CT)

In this experiment, canopy temperature (CT) was measured using a handheld infrared thermometer (LT300, www.instrument.com), starting from the post-anthesis period and continuing at 7-day intervals throughout the growing season. To quantify the cumulative effect of canopy temperature across time, the Area Under the Canopy Temperature Progress Curve (AUCTPC) was calculated, as described by Rosyara *et al.* (2010) [15]. The formula for calculating AUCTPC is as follows:

$$AUCTPC = \sum_{i-1}^{n-1}[(Xi + Xi + 1)/2] (t\,i + t\,i-1)]$$

Where, Xi is the Canopy Temperature on the ith date, the ti is the ith day and n is the number of scoring days.

Canopy temperature depression (CTD)

While recording the canopy temperatures of various genotypes, the ambient atmospheric temperature was measured at intervals of 5 to 7 minutes. This data was primarily utilized to calculate the Canopy Temperature Depression (CTD) in the heat-tolerant field. To capture the accumulated response of the genotype in terms of canopy temperature depression, the Area Under the Canopy Temperature Depression Progress Curve (AUCTDPC) was computed, as per the methodology outlined by Rosyara *et al.* (2010) [15]. The AUCTDPC is determined using the following formula:

$$AUCTDPC = \sum_{i-1}^{n-1}[(Xi + Xi + 1)/2] (t\,i + t\,i-1)]$$

Where,

Xi is the Canopy Temperature on the ith date, the ti is the ith day and n is the number of scoring days.

Chlorophyll meter (SPAD)

The Soil Plant Analysis Development (SPAD) values were measured using a SPAD meter (Konica Minolta SPAD-502 plus, Japan), with readings taken exclusively from the flag leaf. These measurements commenced during the post-anthesis period and were recorded at seven-day intervals. To assess the cumulative effect of the stay-green trait in the genotype, the Area Under the SPAD Decline Curve (AUSDC) was calculated, following the methodology described by Rosyara *et al.* (2010) [15]. The formula used to calculate AUSDC is as follows:

$$AUSDPC = \sum_{i-1}^{n-1}[(Xi + Xi + 1)/2] (t\,i+1-t\,i)]$$

Where

Xi is the SPAD value on ith date, the ti is the ith day and n is the number of scoring days.

Normalized difference vegetation index (NDVI)

In the present experiment, the Normalized Difference Vegetation Index (NDVI) was recorded using a handheld sensor (LT300, www.instrumart.com). Data collection commenced during the post-anthesis period and was performed at seven-day intervals on clear, sunny days between 8:00 a.m. and 10:00 a.m. To represent the cumulative response of the genotype in terms of NDVI, the Normalized Difference Vegetation Index Progress Curve (NDVIPC) was calculated by Rosyara *et al.* (2010) [15]. The formula for calculating the NDVIPC is as follows:

$$NDVIPC = \sum_{i-1}^{n-1}[(Xi + Xi + 1)/2] (t\,i + t\,i-1)]$$

Where

Xi is the NDVI Value on the ith date, the ti is the ith day and n is the number of scoring days.

Plot grain yield

In this experiment, the full plot was harvested and then left for the sun dry. After that the grain yield was recorded in a good functional weighing balance.

Statistical analysis

Observations were made on five morpho-physiological parameters during the 2022-23 and 2023-24 growing seasons. The data, averaged across both years, were subjected to statistical analysis using SAS software to perform Analysis of Variance (ANOVA) for the Area Under Disease Progress Curve (AUDPC) and yield. K-means clustering analysis was conducted using IBM SPSS software (Hammer, 2001) [8]. Furthermore, Scree plots and Principal Component Analysis (PCA) biplots for various morphological and physiological parameters were generated using the RStudio statistical package.

Results and Discussion

Analysis of variance for plot yield and AUDPC

Plot yield

Genotypic variation was identified as the primary factor influencing plot seed yield as mentioned in Table1. The effect of genotype on plot yield was highly significant, as indicated by a p-value < 0.05 and a statistically significant F-value. Moreover, the lack of a significant interaction between genotype and year suggests that the genotypic effect on seed yield is consistent across the two experimental years.

AUDPC

Similarly, genotype made a highly significant influence on the Area Under Disease Progress Curve (AUDPC), confirming that genotypic differences significantly impact disease progression as mentioned in Table 1. This is reflected by the p-value < 0.05 and a statistically significant F-value. However, the significant interaction between genotype and year indicates that the expression of disease resistance is modulated by two years environmental conditions, highlighting the importance of environmental factors in determining genotype performance in relation to disease resistance.

Table 1: Analysis of Variance for plot yield and AUDPC

Source	DF	Sum of Squares (SS)	Mean Square (MS)	F value	Prob > F
Plot yield					
Genotype	102	1640531.1	16083.6382	6.9396	<.0001*
Genotype x year	102	8885.6	87.1137255	0.0376	1.0000
AUDPC					
Genotype	102	12217940	119783.725	6.8268	<.0001*
Genotype*year	102	2398280	23512.549	1.3400	0.0395*

K-means clustering analysis

Clustering of genotypes was performed based on the mean Area under Disease Progress Curve (AUDPC) and mean yield values. The cluster analysis revealed that Cluster 1 exhibited the best performance in terms of disease resistance and grain yield among all clusters. This cluster includes the genotypes ACC-311, ACC-306 and ACC653 demonstrated strong performance. The mean AUDPC of BHU35 (check variety) and DBW187 (check variety) was 1162.38, indicating high disease pressure over the two years of the trial. In contrast, Genotypes like ACC-314, ACC-588 and ACC-623 exhibited higher susceptibility to the disease as mentioned in Table 2.

Comparison of AUDPC and yield values in resistant and susceptible genotypes with check Varieties

Three representative genotypes selected based on their AUDPC and plot yield values. ACC 306, ACC 311 and ACC653 exhibited lower AUDPC values and higher plot yields compared to the other genotypes, making them stand out in terms of both disease resistance and yield performance as mentioned in Table 3. In contrast, the susceptible genotypes, including ACC-588, ACC-314 and ACC-623, exhibited significantly higher AUDPC values and lower plot yields, indicating their greater susceptibility to the disease. The check varieties, DBW-187 and BHU-35, also showed relatively high disease progression and lower yields compared to the resistant genotypes (Fig.1).

Table 2: Clusters of genotypes based on a combination of mean AUDPC and plot seed yield for heat tolerant trial

Cluster No.	AUDPC	Plot Yield	Cluster genotype	Genotypes of heat tolerant trial
1	714.4	1083.48	3	ACC-306, ACC-311, ACC-653
2	861.73	1048.75	7	ACC-172, ACC-202, ACC-229, ACC-313, ACC-614, ACC-648, ACC-651
3	1064.45	881.28	8	ACC-483, ACC-524, ACC-534, ACC-591, ACC-622, ACC-630, ACC-642, ACC-652
4	1171.41	800.06	17	ACC-299, ACC-453, ACC-493, ACC-494, ACC-501, ACC-552, ACC-565, ACC-612, ACC-625, ACC-628, ACC-638, ACC-639, ACC-640, ACC-645, ACC-649, BHU-35©, DBW-187(C)
5	1223.08	875.46	22	ACC-230, ACC-297, ACC-341, ACC-420, ACC-465, ACC-487, ACC-554, ACC-563, ACC-569, ACC-590, ACC-593, ACC-615, ACC-620, ACC-627, ACC-634, ACC-643, ACC-646, ACC-650, ACC-654, ACC-656, ACC-657, ACC-677
6	1233.75	727.82	10	ACC-196, ACC-437, ACC-478, ACC-491, ACC-525, ACC-583, ACC-589, ACC-594, ACC-621, ACC-631
7	1273.25	802.95	12	ACC-367, ACC-461, ACC-618, ACC-626, ACC-629, ACC-633, ACC-635, ACC-637, ACC-644, ACC-647, ACC-658, ACC-676
8	1301.29	881.14	8	ACC-279, ACC-283, ACC-328, ACC-481, ACC-495, ACC-507, ACC-542, ACC-613
9	1373.62	727.24	12	ACC-173, ACC-475, ACC-510, ACC-511, ACC-616, ACC-617, ACC-619, ACC-624, ACC-632, ACC-636, ACC-641, ACC-655
10	1704.99	658.29	3	ACC-314, ACC-588, ACC-623

Table 3: Comparison of AUDPC and yield values in resistant and susceptible genotypes with check Varieties

Genotype	AUDPC	Plot Yield(g/1.6m^2)
ACC-306	676.24	1089.25
ACC-311	715.12	1096.74
ACC-229	907.41	1058.6
ACC-614	917.13	1037.43
ACC-534	1101.85	914.32
DBW-187(C)	1131.02	804.04
ACC-591	1134.26	896.92
BHU-35©	1152.19	851.22
ACC-589	1272.53	741.92
ACC-196	1273.61	719.32
ACC-637	1308.18	768.32
ACC-637	1308.18	768.32
ACC-676	1320.06	792.52
ACC-676	1320.06	792.52
ACC-507	1334.11	949.24
ACC-283	1360.03	889.72
ACC-624	1401.08	661.12
ACC-636	1405.4	669.12
ACC-588	1686.26	672.25
ACC-314	1718.67	665.37

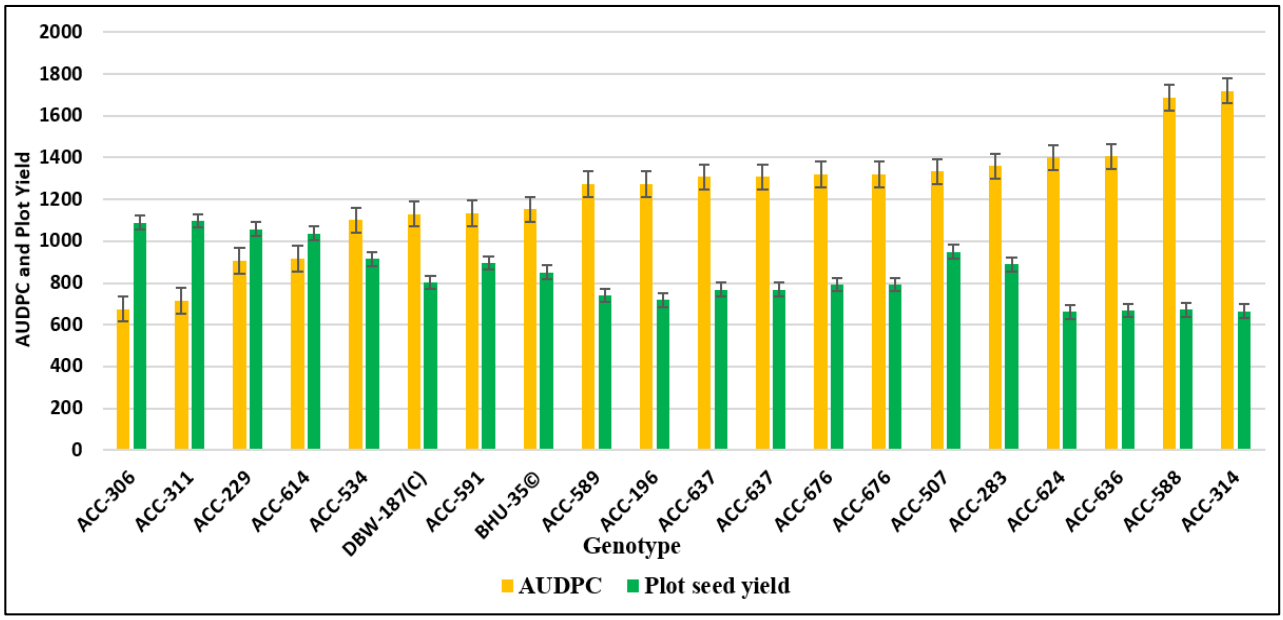

Fig 1: Graphical representation of selected genotypes mean AUDPC and plot seed yield

Scree plot for different morphological-physiological parameters

The scree plot indicated that PC1 and PC2 are the most important components, covered a large portion of the variance of the total variance, explaining 78.5% and 8.4%, respectively. Together, these two components captured 86.9% of the variance in the data. Most of the meaningful variation in the dataset was explained by these two dimensions. On the other hand, the remaining components (PC3 to PC6) contributed much less to the total variance as mentioned in Fig.2.

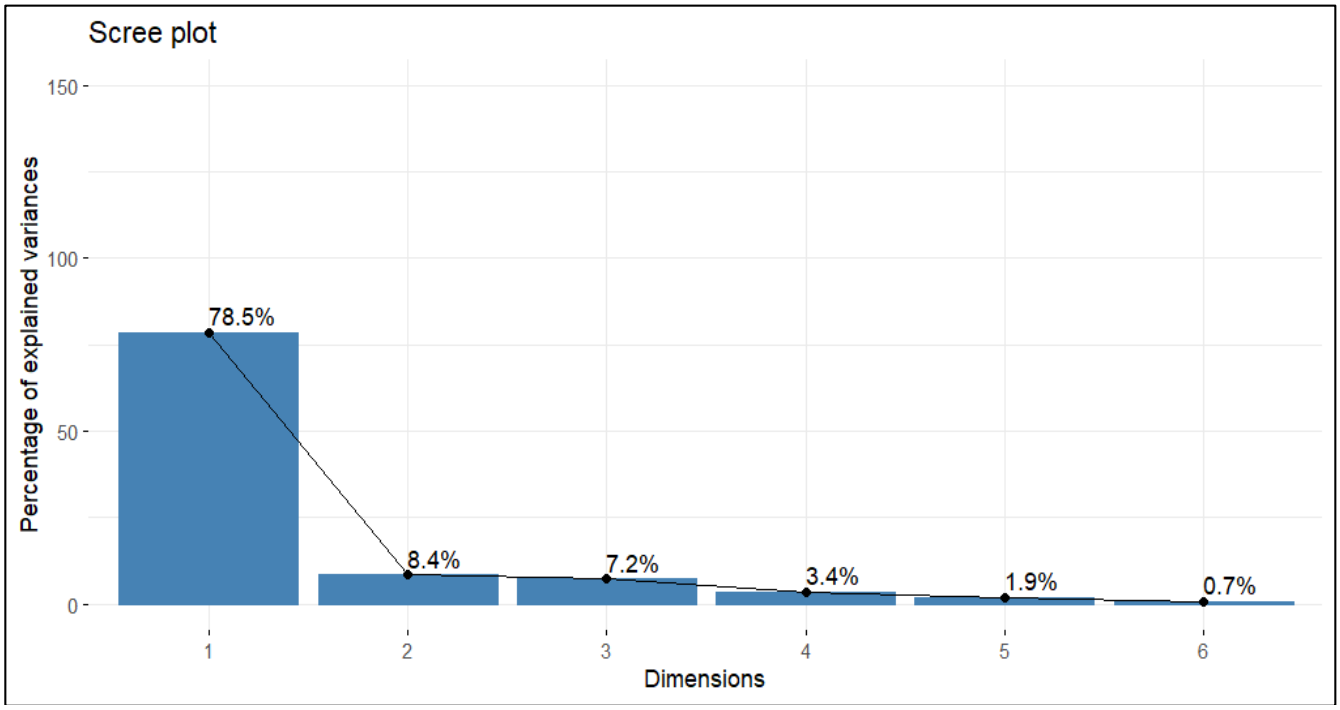

Fig 2: Scree plot for different morphological-physiological parameters

Biplot-Principal Component Analysis for different morphological-physiological parameters

The biplot have showed that PCA (Principal Component Analysis) results of the genotypes with respect to multiple parameters such as AUDPC, seed yield, AUSDC, AUCTP, AUCTDPC and NDVIPC. The genotypes ACC-306 and ACC-311, represented by points 11 and 12 on the plot, are positioned on the negative side of PC1. This indicates that they are more resistant to disease progression (AUDPC) and chlorophyll loss (AUSDC), despite having moderate yields as mentioned in Fig.3.

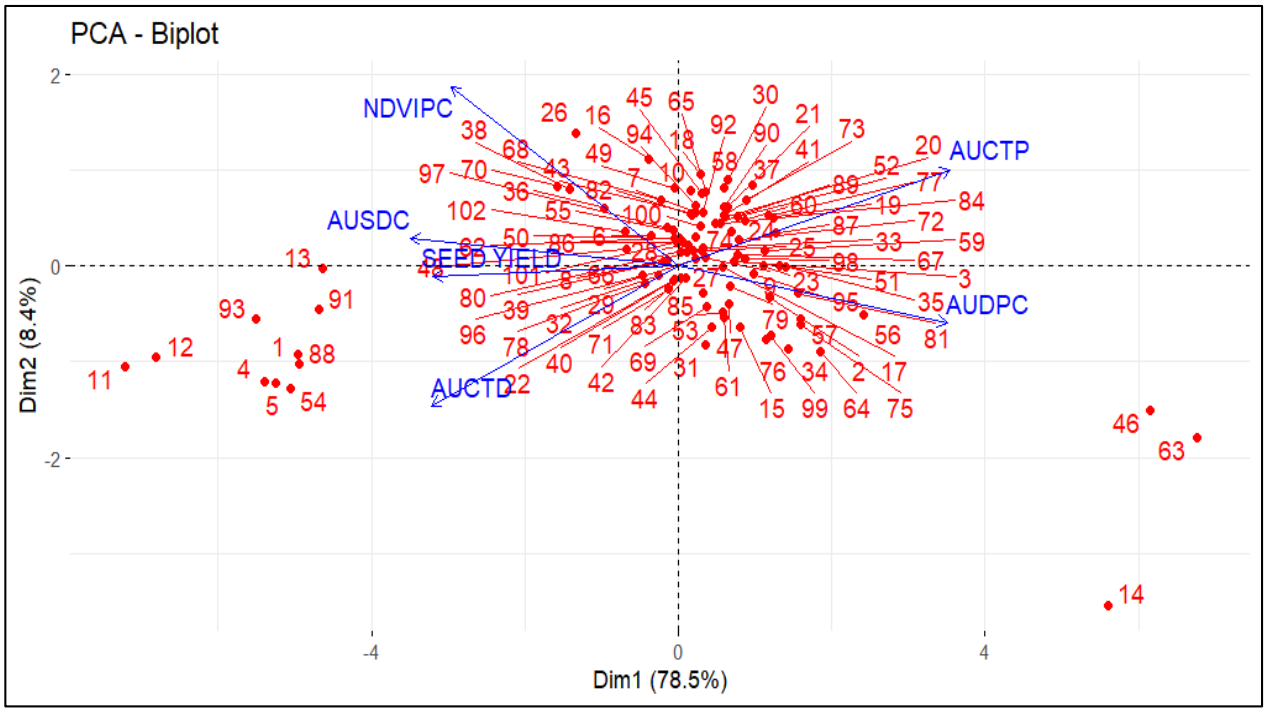

Fig 3: Biplot-Principal Component Analysis for different morphological-physiological parameters

Table 3: Pedigree of most promising genotypes after screening of genotypes

Genotypes	Pedigree
ACC-306	VEE#8//JUP/BJY/3/F3.71/TRM/4/BCN/5/KAUZ
ACC-311	MNCH/3*BCN

Conclusion

One hundred four spring wheat genotypes belonging to Indian and CIMMYT wheat programs were evaluated for terminal heat stress and resistance to spot blotch caused by *Bipolaris sorokiniana* in 2022-23 and 2023-2024 crop season. Parameters like AUDPC, grain yield, NDVI, SPAD, canopy temperature and canopy temperature depression were recorded and based on the above parameters and few genotypes like ACC-311 and ACC 306 were identified to be promising against both the stresses terminal heat stress and resistance to spot blotch.

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