https://doi.org/10.56093/ijas.v90i5.104358

Indian Journal of Agricultural Sciences **90** (5): 909–13, May 2020/Article

Identification of resistant genotypes and representative environments for spot blotch (*Biploris sorokiniana*) in barley (*Hordeum vulgare*)

VISHNU KUMAR*, SUDHEER KUMAR, S P SINGH, S S VAISH, DEEPSHIKHA, J B KHAN, A S KHARUB and G P SINGH

ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana 132 001, India

Received: 24 January 2019; Accepted: 03 September 2019

ABSTRACT

A set of 31 barley genotypes was evaluated at four locations, viz. Kanpur, Pantnagar, Varanasi and Faizabad for two years (2016 and 2017) against spot blotch [*Biploris sorokiniana* (Sacc.) Shoem.] resistance. The combined analysis of variance revealed significant influence of year (5.64%) and location (18.08%) effects with significant genotypic effects (29.05%). Location-wise, the highest susceptibility was observed at Varanasi (51.61%) followed by Kanpur (19.35%) whereas, the moderately susceptible reactions were obtained at Faizabad (67.74 %) and Kanpur (61.29%) locations. In heritability adjusted GGE biplot analysis, the ratio of (G+G × E)/(E+G+G × E) was estimated as 71.77 % and substantiated usefulness of generated biplots to elucidate resistant and stable genotypes with location discrimination. In AMMI analysis, the initial two principal components (PCs) cumulatively explained 90.7 % of total variation with individual scores of 51.8 % and 38.9 %, respectively. The triangular image of the initial three PCs depicted scattered pattern for disease reaction and varied response for the locations. The weather parameters, viz. maximum temperature, minimum temperature and sunshine hr/day showed strong correlations 0.87**, 0.83** and 0.64* with spot blotch severity. The genotypes, DWRB 180, PL 891 and DWRB 190 were found promising and suggested to be used in future resistance breeding and spot blotch genetic studies. The locations Varanasi and Faizabad were found type 2 discriminative and representative environments for spot blotch.

Key words: AMMI, Barley, HA-GGE, Spot blotch

Barley is utilized for food, feed and malting purposes and India contributed 34.86 % of the total South-Asian barley production with average productivity of 27.15 q/ha during 2017 (Kumar *et al.* 2018). Spot blotch is an economically important biotic stress in barley and major constraint in productivity enhancement. The disease is caused by ascomycetous hemi-biotrophic filamentous fungus *Cochliobolus sativus* (Ito and Kurib.) Drechsler ex Dastur [anamorph: *Bipolaris sorokiniana* (Sacc.) Shoem.] (Wang *et al.* 2017). Due to the warm and humid climate, north eastern Indo-Gangetic plains and the parts of eastern Uttar Pradesh, Bihar and West Bengal are highly prone for foliar blight incidences. However, with the changing climate and varying weather parameters the disease is also expanding horizons in the north western plains in India (Singh *et al.* 2014). The disease is devastating and infection prior to heading stage can cause marked yield reductions (40%) by damaging tender tillers in barley (Kuldeep *et al.* 2008). Chemical control for the disease is not eco-friendly and less affordable by small and marginal resource poor farmers, who cultivate barley due to its low input requirement and ability to withstand under drought and saline soils. Therefore, inbuilt genetic resistance is sustainable, eco-safe and widely accepted mechanism to check the spot blotch losses and to get better malting quality in barley.

The development of spot blotch resistant genotypes is an arduous objective and further influence of weather parameters, isolate dynamics, plant canopy and compounded effects of genotype by environment interaction (G × E) further aggravates this situation. Besides, it is also imperative to know environmental behavior and representativeness along with the identification of resistant sources through evaluation of genotypes in possible spatial and temporal variations for harnessing future resistance breeding programs. Under Indian condition, few studies have been delineated resistance sources but are not effective due to the evaluation at single location and ignorance of G × E (Chand *et al.* 2008, Prasad *et al.* 2013). Therefore, the present investigation was conducted to identify resistant genotypes for spot blotch through multi-location biplot evaluation and approximation of discriminating and representative environments for accelerating future breeding programs.

MATERIALS AND METHODS

To study spot blotch (*Biploris sorokiniana*) resistance

*Corresponding author e-mail-vishnupbg@gmail.com

and environmental discrimination a set of 31 genotypes was evaluated at four hot spots namely, Kanpur (E1), Pantnagar (E2), Varanasi (E3) and Faizabad (E4) during 2016 and 2017. The locations E1, E3 and E4 are geographically part of north eastern Indo-Gangetic plains, while the centre Pantnagar encompasses in north western plains zone. The experimental materials consisted of standard cultivars, advance breeding strains and elite check varieties. Each genotype was grown in a single row of one meter length with row to row spacing of 25 cm in augmented design and all the recommended agronomic package of practices, except higher irrigation (for higher humidity) were followed to raise the good crop. The infector rows of mixture of susceptible check RD 2786 were grown peripheral sides of the experiments and were repeated after every 20 genotypes to maximize disease pressure.

The considered locations are natural hot spots for spot blotch but spreader rows were artificially inoculated to ensure maximum disease pressure. The sorghum grains were used for isolate multiplication and spores were harvested in water (Prasad *et al.* 2013, Kuldeep *et al.* 2008). The spore suspension (approx.10^4 spores/ml) having the surfactant Tween 20 was inoculated evenly at different growth stages (Zadok *et al.* 1974); tillering (25), flag leaf emergence (37) and anthesis (65) during evening hours. The observations of disease severity were recorded thrice at flowering (55), early milk (73) and soft dough (85) growth stages on the basis of complete row reactions. The spot blotch severity was recorded based on per cent infected area with spot blotch on the flag leaf and penultimate (flag-1) leaf in double digit scale (00-99) (Prasad *et al.* 2013, Singh *et al.* 2014). In the double digit score, first digit depicted flag leaf per cent area covered by the disease and second digit denoted the flag-1 leaf disease severity, respectively.

The combined analysis of variance and G × E analysis using additive main effects and multiplicative interactions (AMMI) and heritability adjusted GGE biplots (HA-GGE) were carried out with R software version 3.4.3., where year source of variation was considered as replication for analysis purposes. For disease resistance stability an index namely Resistance Stability Index was estimated as sum of AMMI stability value (RASV) and average disease score (ADS) of respective genotypes. The genotypes with low RSI were considered resistant and stable.

RESULTS AND DISCUSSION

Location-wise, the highest susceptible reactions were observed at Varanasi (51.61%) followed by Kanpur (19.35%) whereas, the moderately susceptible observations were obtained at Faizabad (67.74 %) and Kanpur (61.29%) locations. The mean disease score over two years depicted resistant to moderately resistant for the genotypes DWRB 180 (24) and DWRB 190 (35) whereas, the check variety RD 2786 (78) and genotype RD 2927 (78) showed higher disease severity followed by the genotype RD 2941 (68) for foliar blight. The combined analysis of variance revealed significant influence of year (5.64 %) and location (18.08 %) effects on disease severity with significant genotypic effects (29.05 %). The significant G × E mean squares explained 16.92 % of the total variation and indicated to exclude confounding role of location effect before selecting stable and resistant spot blotch genotypes. Varied disease pressure at different locations indicated possibility of different spot blotch isolates and environmental interaction across the locations with changed weather regimes. The reports on existence of spot blotch isolates namely 29B and RCBHUBR1857 (most aggressive) from Varanasi and other north eastern Indian parts and prevalence of isolate BS2 in north western plains (Pantnagar) further substantiated our findings (Bashyal *et al*. 2012, Kuldeep *et al*. 2008, Singh *et al*. 2014).

Location-wise frequency distribution depicted wide spectrum and high disease pressure across the locations. Out of 31 tested genotypes, 52 % genotypes showed highly susceptible disease reactions (>78) at Varanasi and 42% genotypes were recorded with moderately susceptible (>58) double digit disease score. Whereas, at Faizabad and Kanpur, 68 % and 61% genotypes exhibited moderately susceptible reactions for spot blotch, respectively. The weather parameters, viz. maximum temperature, minimum temperature and sunshine hr/day showed strong positive correlations 0.87**, 0.83** and 0.64* with the spot blotch severity. The analysis of core weather parameters, viz. temperature, humidity, sunshine hours suggested that hot and humid weather favoured disease development and hampered resistance for spot blotch. Prasad *et al.* (2013) also reported that the dense plants canopies, high environmental temperature and availability of free moisture are congenial and associated with the disease development. Moreover, abundant free moisture on leaf surface tends to reduced crop evapo-transpiration and thereby increased tissue moisture is conducive for fungal growth (Huber and Gillespie 1992).

Similarly, between locations associations were also computed and all the locations depicted positive significant correlations. The highest correlation coefficient was observed between Faizabad and Varanasi (0.70**) followed by Kanpur and Faizabad (0.55**) and Pantnagar (0.48**) and Faizabad locations, respectively. The significant G × E and location effects warranted excluding confounding environmental variations and therefore genotypic performance and stability were studied using HA-GGE and AMMI models for spot blotch resistance.

Heritability adjusted GGE (HA-GGE) analysis

The partitioning of genotype (G) + genotype by environment interaction (G × E) revealed that initial two principal components (PCs) attributed 78.36 % of total variation, representing adequately approximation of environment centred data. The ratio of (G+G × E)/(E+G+G × E) was estimated as 71.77 % and indicated high usefulness of HA-GGE biplot analysis to elucidate resistant and stable genotypes with location discrimination for spot blotch. Yang *et al.* (2009) summarized that the initial two PCs should explain approximately 60% of the (G + G × E) variability

and the ratio of (G+G × E)/(E+G+G × E) should account for more than 10% for the usefulness of biplots. In which-won-where view, an irregular shaped small size polygon was obtained and four locations fell into two distinct sectors. The size of the polygon was small due to the higher number of susceptible genotypes and narrow variability among the tested genotypes. The location Pantnagar depicted separate location marker in the which-won-where biplot. The vertices of the irregular polygon represented the genotype markers, viz. g7 (DWRB 180), g27 (RD 2941) and g29 (VLB 147) located farthest away from the biplot origin in different directions.

The average environment coordinates (AEC) were proportional to the two rank approximations, where abscissa denoted main genotypic effects and directed towards susceptible genotypes. Based on AEC projections, the genotypes g7 (DWRB 180), g8 (DWRB 190) and g17 (PL891) were considered resistant and stable, whereas the genotypes g19 (RD 2786), g23 (RD 2927), g27 (RD 2941) and g18 (RD 2715) were regarded as highly susceptible for spot blotch (Fig 1). Yan and Holland (2010) based on heritability adjusted scaling method demonstrated that the vector length of an environment indicates the square root heritability (√H) and the cosine of the angle between two environments represents the genetic correlation (r) between them. In the present investigation, the location Kanpur had the longest vector followed by Faizabad, Varanasi and Pantnagar. Based on acute genetic correlations and vector length the locations Faizabad and Varanasi were found highly discriminative and representative for disease screening and initiating concrete breeding efforts.

Additive main effects and multiplicative interactions (AMMI) analysis

In AMMI analysis, the initial two principal components (PCs) cumulatively explained 90.7 % of total variation with individual scores of 51.8 % and 38.9 %, respectively. The triangular image of the initial three PCs depicted scattered pattern for disease reaction and varied response for the locations. Based on the initial three PCs the locations Faizabad and Varanasi were grouped together, while Kanpur and Pantnagar exhibited differential behaviour. The genotypes, viz. g7 (DWRB 180), g8 (DWRB 190) and g17 (PL 891) were observed with low mean disease scores, while the genotypes g18 (RD 2715), g19 (RD 2786), g23 (RD 2927) and g27 (RD 2941) showed high mean disease scores on the abscissa (Fig 2). The AMMI1 biplot depicted that the environment Varanasi was most favourable with high additive disease scores for spot blotch severity.

Resistance stability index (RSI)

Further in addition to AMMI1 and AMMI2 biplots, resistance stability index (RSI) based on sums of AMMI stability value (ASV) and mean disease scores was also estimated to identify stable genotypes with low disease infection (Table 1). The RSI scores substantiated that the genotype DWRB 180 (g7) followed by PL 891 (g17) and DWRB 190 (g8) were consistent and desirable across the locations for foliar blight resistance.

Here, HA-GGE biplot indicated existence of two mega environments and the locations Faizabad and Varanasi were found most discriminating and representative type 2 environment. Yan *et al*. (2007) explained that the locations having long vectors and acute angles with target environment axis (TEA) are most suitable and called as Type 2 environments. The locations having long vectors but obtuse angles from TEA are Type 3 environments (e.g. Kanpur- E1), which are useful only in culling unstable genotypes. Based on AMMI 2 and HA-GGE biplots outputs, we suggest germplasm characterization, international foliar

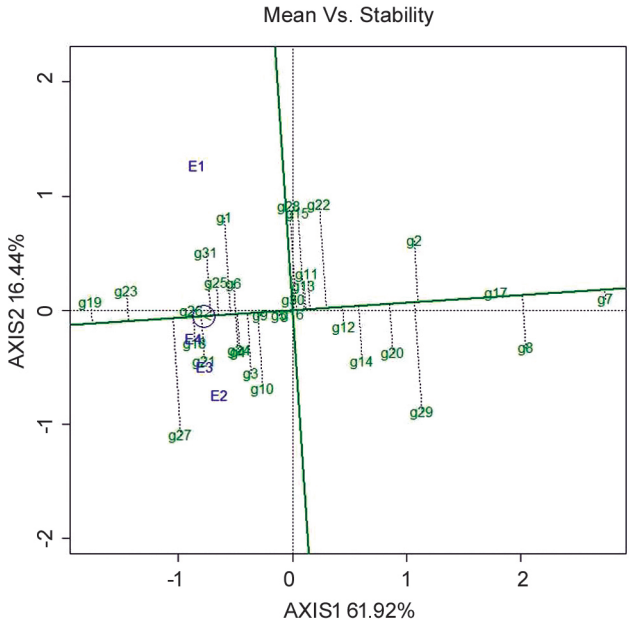

Fig 1 Mean vs. stability view of HA-GGE biplot for spot blotch.

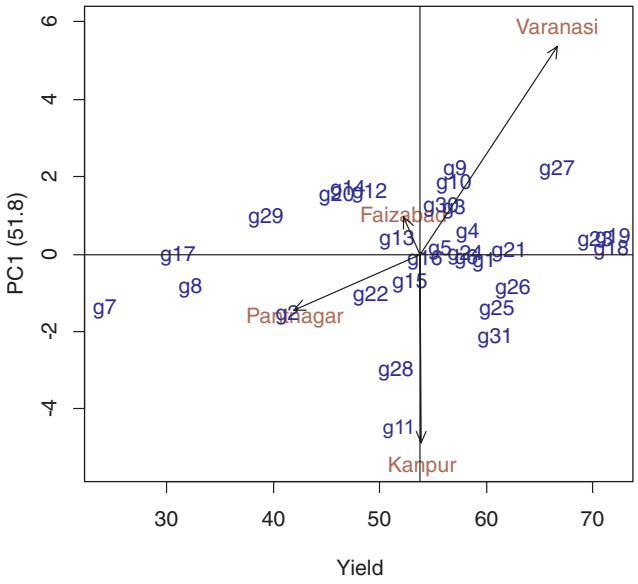

Fig 2 AMMI1 biplot based on additive mean disease scores vs. PC1.

Table 1 Resistance stability index for spot blotch in barley

Genotype	Code	Parentage	AMMI stability value (ASV)	Average disease score (ADS)	Resistance stability index (RSI)	Rank RSI
DWRB180	g7	Selection from P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA 1/6/M111	2.33	24	26.33	1
PL891	g17	IBON 343/12th HSBN-176	0.06	35	35.06	2
DWRB190	g8	Selection from M104/TOCTE//OROSUS/PETUNIA1	1.81	34	35.81	3
RD2899	g20	RD2592/RD2035//RD2715	1.86	46	47.86	4
KB1318	g14	LAKHAN/JB137	2.00	46	48.00	5
VLB147	g29	INDIANUR(2009-10)-42	2.64	46	48.64	6
BH1014	g2	EIBGN-34/DWRUB52	1.79	47	48.79	7
NDB1173	g16	BYTLRA 3-(1994-95)/NDB217	0.17	57	57.17	8
DWRB101	g5	DWR28/BH581	0.63	57	57.63	9
K603	g13	K257/C138	0.83	57	57.83	10
BH902	g3	BH495/RD2552	1.61	57	58.61	11
Jyoti	g12	K 12/C 251	1.89	57	58.89	12
VLB149	g30	INDIANUR(2009-10)-51	2.26	57	59.26	13
RD2917	g22	PL705/RD2668//DWR46	2.39	57	59.39	14
HUB113	g10	KARAN280/C138	2.55	57	59.55	15
RD2944	g28	UPB1020/RD2651	3.55	57	60.55	16
HUB247	g11	RD2618/RD2660	5.57	57	62.57	17
RD2930	g24	BH935/PL838	0.40	67	67.40	18
DWRB137	g6	DWR28/DWRUB64	0.97	67	67.97	19
RD2907	g21	RD103/RD2518//RD2592	1.10	67	68.10	20
BH946	g4	BHMS22A/BH549//RD2552	1.15	67	68.15	21
RD2715	g 18	RD387/BH602//RD2035	0.64	68	68.64	22
RD2927	g 23	RD2624/RD2696	1.31	68	69.31	23
RD2937	g26	RD2552/K958	1.39	68	69.39	24
VLB118	g31	14th EMBSN-9313	2.44	67	69.44	25
RD2935	g25	RD2624/RD2696	1.58	68	69.58	26
DWRUB52	g9	DWR17/K551	2.94	67	69.94	27
Lakhan	g15	K12/IB226	3.03	67	70.03	28
Azad	g1	K12/K19	3.08	68	71.08	29
RD2941	g27	DWRUB49/RD2615	3.10	68	71.10	30
RD2786	g19	RD2634/NDB1020//K425	1.19	78	79.19	31

blight nurseries screening and RIL evaluation at Faizabad and Varanasi to save resources and under limited seed conditions.

Leng *et al.* (2016) reported that barley genotype NDB 112 (CIho11531) derived from the cross CIho7117-77/ Kindred was effective almost for five decades in USA and circumvent yield losses with subsequent lineage in six row cultivars. However, non-availability of resistant sources and ignorance of role of G × E are major constraints to control spot blotch in India. Here, after rigorous screening in temporal and spatial means only three genotypes namely DWRB 180, DWRB 190 and PL 891 were found moderately resistant for spot blotch. The AMMI biplots and HA-GGE biplots depicted these genotypes stable and consistent over the environments. These genotypes have certain mechanism to reduce phytotoxicity of metabolites of sesquiterpene nature, which cause cell membrane disruption, chlorosis, inhibition of mitochondrial electron transport and oxidative phosphorylation etc. (Aggarwal *et al.* 2011, Bashyal *et al.* 2012). The resistant stability indices (RSI) of DWRB 180 (26.33), PL 891 (35.06) and DWRB 190 (35.81) were comparatively lower and in turn supported the use of these genotypes in breeding, mapping population development and genetic studies. The direct involvement of mean disease score in RSI explained that the resistant genotypes have lower proportion of disease severity and minimal contribution in RSI than susceptible cultivars and can be culled easily based on their respective ranks for

resistance and stability.

In the light of present study, the barley genotypes namely DWRB 180, DWRB 190 and PL 891 were found promising for spot blotch and to be used extensively in future breeding programs. Further, the locations Varanasi and Faizabad were delineated type 2 discriminative and representative environments for germplasm screening, RIL evaluation and conduction of national and international disease nurseries for spot blotch in India.

ACKNOWLEDGEMENTS

Authors express sincere thanks to all the technical and field staff of the coordinating centres for their kind support during experimentation and disease data recording.

REFERENCES

Aggarwal R, Gupta S, Singh V B and Sharma S. 2011. Microbial detoxification of pathotoxin produced by spot blotch pathogen *Bipolaris sorokiniana* infecting wheat. *Journal of Plant Biochemistry and Biotechnology* **20**(1): 66–73.

Bashyal B M, Chand R, Prasad L C and Joshi A K. 2012. Influence of growth stage on helminthosporol toxin of *Bipolaris sorokiniana* of barley (*H. vulgare* L.). *Indian Journal of Agricultural Sciences* **82** (8): 724–26.

Chand R, Sen D, Prasad K D, Singh A K, Bashyal B M, Prasad L C and Joshi A K. 2008. Screening for disease resistance in barley cultivars against *Bipolaris sorokiniana* using callus culture method. *Indian Journal of Experimental Biology* **46**: 249–53.

Huber L and Gillespie T J. 1992. Modeling leaf wetness in relation to plant disease epidemiology. *Annual Review of Phytopathology* **30**: 533–77.

Kuldeep T, Nandan R, Kumar U, Prasad L C, Chand R and Joshi A K. 2008. Inheritance and identification of molecular markers associated with spot blotch (*Cochliobolus sativus* L.) resistance through microsatellites analysis in barley. *Genetics and Molecular Biology* **31:**734-42.

Kumar V, Kharub A S and Singh G P. 2018. Additive main effects and multiplicative interaction and yield stability index for genotype by environment analysis and wider adaptability in barley. *Cereal Research Communications* **46** (2):365-75.

Leng Y, Wang R, Ali S, Zhao M and Zhong S. 2016. Sources and genetics of spot blotch resistance to a new pathotype of *Cochliobolus sativus* in the USDA Small Grains Collection. *Plant Disease* **100:**1988–93.

Prasad R, Prasad L C, Chand R and Joshi A K. 2013. Assessment of diversity for resistance to spot blotch disease and its association with certain phenotypic traits in barely. *Field Crops Research* **154:**195–200.

Singh S, Singh H, Sharma A, Meeta M, Singh B, Joshi N, Grover P, Al-Yassin and Kumar S. 2014. Inheritance of spot blotch resistance in barley (*Hordeum vulgare* L.). *Canadian Journal of Plant Science* **94**: 1203-09.

Wang R, Leng Y, Ali S, Wang M and Zhong S. 2017. Genome-wide association mapping of spot blotch resistance to three different pathotypes of *Cochliobolus sativus* in the USDA barley core collection. *Molecular Breeding* **4**: 37-44.

Yan W and Holland J B. 2010. A heritability-adjusted GGE biplot for test environment evaluation. *Euphytica* **171**: 355–69.

Yan W, Kang M S, Ma B, Woods S and Cornelius P L. 2007. GGE biplot vs. AMMI analysis of genotype-by-environment data. *Crop Science* **47**: 643-53.

Yang R C, Crossa J, Cornelius P L and Burgueno J. 2009. Biplot analysis of genotype × environment interaction: Proceed with caution. *Crop Science* **49**: 1564-76.

Zadoks J C, Chang T T and Konzak C F. 1974. A decimal code for the growth stages of cereals. *Weed Research* **14**: 415–21.