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Research Article

Screening global set of barley germplasm for resistance to spot blotch disease in the warm-humid plains of Nepal

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Abstract

Spot blotch disease (SB) caused by *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*) is a major disease of barley and causes a significant yield loss under favorable conditions in the warm and humid areas. A global set of 340 barley germplasm obtained from ICARDA were evaluated for SB resistance under natural epiphytotic conditions along with two local checks (Bonus and Solu Uwa) at the research field of Nepal Agricultural Research Council, Rampur, Chitawan, Nepal for three consecutive years (2016-2018). Analysis of variance showed significant variation in Area Under Disease Progress Curve (AUDPC) of the studied genotypes in all the three years' experiments. The check varieties were susceptible to SB (AUDPC value ≥ 750) in all the experiments. In the first-year experiment, 28, 105, 98 and 109 genotypes had resistant (R, AUDPC <250), moderately resistant (MR, AUDPC 250-499), moderately susceptible (MS, AUDPC 500-749), and susceptible (S, AUDPC≥750) type of disease reaction, respectively. In the second year, 1, 42, 136 and 161 genotypes showed R, MR, MS, and S type of disease reaction. In the third-year, 69 genotypes had MR, 132 MS and 139 S type of disease reaction. Summarized across the years, about 27 % genotypes had MS to MR or R type of SB reaction. The study identified barley lines that can be a potential source of SB resistance for barley breeding programs.

Key words: AUDPC, *Bipolaris*, disease severity, *Hordeum vulgare*, susceptible

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the first cultivated food grains since human civilization. It is a major cereal grown in temperate climate with the production of 155.87 million mt across the world (FAO, 2019). Barley is primarily used for animal feed and to produce malt and alcoholic beverages (Tyagi *et al.,* 2020; Patial *et al.*, 2018). Also, it is a traditional food crop in East Africa, the Highlands of Himalayas, Tibet, Andean countries and in the Baltic States (Baik and Ullrich, 2008). Because of the wider adaptability, resilient nature and recent findings of the health benefits of barley grains, there is a renewed importance of the crop as a human food (Newton *et al.,* 2011; Idehen *et al.,* 2017). In Nepal, barley is traditionally grown in small areas throughout the country. As a food crop, it is common in the Himalayan highlands, especially in the western region where the production environments are harsh and food security is a major challenge (Pokharel *et al.,* 2016). In these areas, Nepal government has emphasized promoting indigenous crops, such as barley for food security (MALD, 2019). However, lack of suitable high-yielding and disease-resistant varieties are the major concern for adoption of barley in these areas.

Spot blotch disease of barley caused by *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*) (SB hereafter) is an old disease (Bovill *et al*., 2010) and the pathogen is well-known as a causative agent of several diseases of cultivated and wild plants. SB is often a seed-borne disease but can also be caused by the conidia in the soil and air (Gupta *et al*., 2017). The pathogen overwinters on crop residues and releases spores in the spring and wind and rain disperse the spores (Gyawali *et al*., 2018). The infected seeds are the major source of inoculum (Pandey *et al.,* 2008). The SB epidemics are higher in the areas characterized by average temperature above 17^0C and high relative humidity during the crop season (Mudi *et al.,* 2010; Mudi *et al*., 2016). The SB initially causes small, brown spots to appear on the leaves, sheaths, and glumes and these eventually expand into long, narrow, brown streaks striped with occasional deep brown lines across the lesions in a pattern that resembles a spot, yellow halo in the leaves (Manandhar *et al*., 2017). The diseased crop produces shriveled grains and decreased grain yield (Cane and Hampton, 1990) and the diseased grains degrade the quality of malt (Gyawali *et al*., 2018).

SB of barley is prevalent disease in the major barley cultivated area of the world, *viz*., Australia, Canada and United States (Knight *et al*., 2010; Gyawali *et al*., 2018) and causes significant yield loss, especially in the warm, humid production areas like that of North eastern Indo-Gangetic plains (Kumar *et al*., 2020), including Nepal. Yield loss of 10-30% is common when the weather condition is favorable for the disease development (Wang *et al*., 2017). The SB of barley can be controlled by fungicide applications (Devi *et al*., 2019; Kumar *et al*., 2014) but the use of resistant cultivar is the most economical and environment friendly approach for its control (Akhavan *et al*., 2016). The persistent challenge of SB disease in the warm humid environment requires an effective breeding program and diverse resistance sources. Hence, this study evaluated 342 global set of barley genotypes against SB disease and identified promising resistant germplasm lines.

2. Materials and Methods

2.1 Barley genotypes

A total of 342 barley genotypes that comprised of 340 diverse germplasm (Association mapping panel: AM-2014) obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA) and two checks (Bonus and Solu Uwa) from Nepal were evaluated. The Nepalese barley varieties Bonus (two-rowed, hulled type) and Solu Uwa (six-rowed, hull-less type) were developed and released for general cultivation in 1974 and 1990, respectively (NARC, 2020). These two checks are the only improved varieties currently grown in Nepal. The AM-2014 panel includes 230 genotypes from the ICARDA low input breeding program and 86 from the high-input breeding program. The other 24 genotypes were of mixed type. These include 280 hulled and 60 hull-less type and based on row pattern138 were two-row and 202 six-row type. In the AM-2014 panel, about 74% were advanced breeding lines, 9.5% were gene-bank accessions and 16.5% were barley varieties released by different breeding programs (India, Australia, USA, Canada, and Morocco). A detailed description of AM-2014 can be found in Amezrou *et al.* (2017).

2.2 Field experiments

Barley genotypes were evaluated in the research field of Nepal Agricultural Research Council, Rampur, Chitawan, Nepal. Rampur is situated at 84°20' E and 27°40' N and an altitude of 228-meter above the mean sea level. The location is characterized by a humid subtropical climate which is conducive for SB development (Figure 1). The experiment was laid out in augmented block design with two repeated checks. There were 17 blocks and 20 entries in each block. Planting was done on 4th December 2015, 1st December 2016, and 30th November 2017. Each genotype was sown on 1.25 m^2 plot (two rows of 2.5 m length). A row to row spacing was 25 cm and continuous seeding at the rate of 80 Kg ha^{-1} was practiced. Chemical fertilizers were applied @ 80 Kg N: 40 Kg P_2O_5: and 30 Kg K_2O ha^{-1}. Full doses of P_2O_5 in the form of diammonium phosphate and K_2O in the form of muriate of potash were supplied at sowing. Nitrogen in the form of urea was applied thrice in equal splits at sowing, at crown root initiation, and booting stages. Irrigation was applied as and when needed to keep the field moist and conducive for disease development. Standard crop management practices were followed.

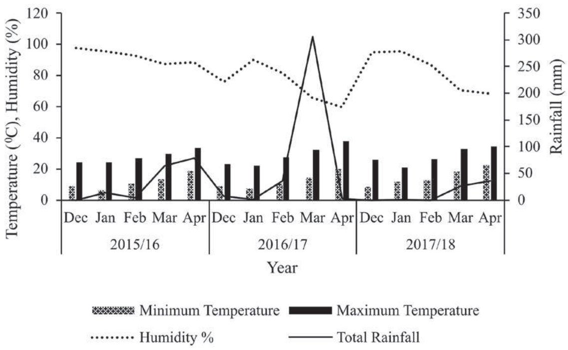

Figure 1. Weather data of the experimental field during the barley growing seasons of 2015-16, 2016-17 and 2017-18.

2.3 Disease assessment

Spot blotch disease observations were made during late boot stage, three times at ten days interval. Disease infection was scored using 1 to 9 scale (Saari and Prescott, 1975) and the double-digit scoring system (00-99) of Joshi *et al.* (2007). Disease severity was calculated by using the following formula (Turan *et al.*, 2020).

Disease severity (%) = $(D_1/9) \times (D_2/9) \times 100$

Where, first digit (D_1) shows the vertical progression of the disease in the plant and the second digit (D_2) indicates the disease severity. Area under the disease progress curve (AUDPC) was calculated using the following formula (Bocianowski *et al.*, 2020).

$$AUDPC = \sum_{i=1}^{n} \left\{ \left(\frac{y_i + y_{i-1}}{2} \right) (x_i - x_{i-1}) \right\}$$

Where, AUDPC is the area under disease progress curve, y_i is the percentage of visible infected area (y_i/100) at the i^{th} observation, x_i - day of the i^{th} observation, and n - the total number of observations.

As described by Joshi *et al.* (2007), the genotypes with AUDPC <250 were considered as resistant (R), 250-499 as moderately resistant (MR), 500-749 as moderately susceptible (MS) and >750 as susceptible (S).

2.4 Statistical analysis

The descriptive figure for the AUDPC data was obtained using Microsoft Excel. Analysis of variance for augmented design was carried out with the 'agricolae' package version 1.3-3 (Mendiburu, 2020), implemented in R environment (R Core Team, 2021).

3. Results and Discussion

The analysis of variance revealed highly significant differences ($p \leq 0.001$) among the entries for AUDPC in all the three years (Table 1). The two check varieties significantly differed for AUDPC values in 2017 and 2018 but not in 2016. The AUDPC of the 342 studied genotypes for 2016, 2017 and 2018; mean AUDPC and their disease reaction type (R, MR, MS or S) are presented in the supplementary Table S1. The frequency of genotypes with different reaction types for individual test year is given in Figure 2. The frequency of genotypes with MS and S type of reaction was higher in 2017 and 2018, whereas the genotypes with R and MR type of reaction was higher in 2016. Previous studies have shown variation in spot blotch (SB) severity and disease reaction type of barley genotypes across years (Singh *et al.*, 2014) and under natural and artificial disease inoculum conditions (Singh *et al.*, 2014). The conducive environment for SB disease in the test location (Figure 1) and the consistent susceptible disease reaction of the check varieties indicated that disease screening was effective under the natural epiphytotic condition. Subedi *et al.* (2020) also reported SB screening is effective under natural epiphytotic conditions at the present experiment site.

Table 1. Analysis of variation of the spot blotch disease - area under disease progress curve (AUDPC) of 342 barley genotypes during 2016, 2017 and 2018.

Source of variation	**df**	**MSS**		
		2016	2017	2018
Blocks (unadjusted)	16	207621	994235	263370
Entries (adjusted)	341	160098***	112087***	115242***
Check	1	24881	52773*	553866***
Check + Check vs. Augmented	340	160496***	112262***	113952***
Residuals	16	8377	7405	4777

*= significant ($p \leq 0.05$); ***= highly significant ($p \leq 0.001$); df= degree of freedom; MSS=mean sum of square

In 2016, about 8% (n=28) genotypes were resistant, 31% (n=105) genotypes were moderately resistant, 29% (n=98) were moderately susceptible and 32% (n=109) were susceptible to SB. The check varieties Bonus and Solu Uwa had a susceptible type of reaction with the mean AUDPC value of 1276.7 and 1222.6, respectively. In 2017, only one genotype (AM-321) showed a resistant reaction. There were 42 genotypes with MR reaction, 136 genotypes with MS reaction and 161 with S type of reaction. The number of susceptible genotypes were higher as compared to 2016 and 2018. The check variety Bonus (AUDPC=1212.8) and Solu Uwa (AUDPC=1134.0) were susceptible to SB. In 2018, about 20% (n=69) genotypes showed MR type of reaction, about 38% (n=132) MS type of reaction, and about 40% (n=139) showed S types of reaction. In this year, none of the genotypes had a R type of disease reaction. The check varieties Bonus and Solu Uwa were susceptible with the AUDPC value 1179.4 and 1434.6, respectively.

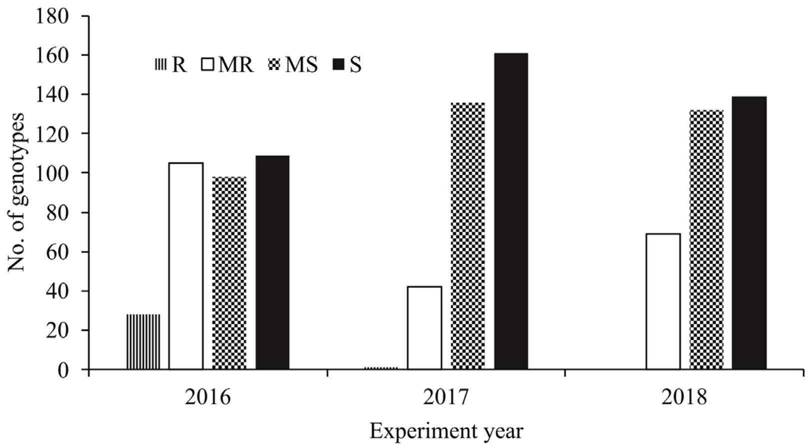

Figure 2. Frequency distribution of spot blotch reaction of 342 barley genotypes evaluated at Rampur, Chitwan under natural epiphytotic conditions in 2016, 2017 and 2018. Resistant (R), Moderately Resistant (MR), Moderately Susceptible (MS) and Susceptible (S).

The barley genotypes that have practical value for SB resistance breeding (R, MR or MS type of reaction across years) are summarized in Table 2. Of these, three genotypes- AM-128 (mean AUDPC=358), AM-188 (mean AUDPC=385) and AM-321 (mean AUDPC=270) showed a higher level of SB resistance. There were seven genotypes with R to MR reaction type and 24 genotypes with MR reaction type for at least two years (Table 2).

Table 2. Selected barley genotypes with resistant (R), moderately resistant (MR) or moderately susceptible (MS) type of spot blotch (SB) reaction across years (2016-2018).

Genotypes	Year 2016		Year 2017		Year 2018		Mean	
	AUDPC	Disease reaction	AUDPC	Disease reaction	AUDPC	Disease reaction	AUDPC	Disease reaction
AM-1	358	MR	420	MR	617	MS	465	MR
AM-4	358	MR	667	MS	660	MS	562	MS
AM-21	358	MR	562	MS	716	MS	545	MS
AM-24	426	MR	660	MS	580	MS	556	MS
AM-25	660	MS	562	MS	630	MS	617	MS
AM-30	500	MS	512	MS	716	MS	576	MS
AM-35	451	MR	685	MS	691	MS	609	MS
AM-42	407	MR	370	MR	593	MS	457	MR
AM-72	438	MR	562	MS	654	MS	551	MS
AM-82	309	MR	741	MS	580	MS	543	MS
AM-86	407	MR	568	MS	580	MS	519	MS
AM-87	660	MS	722	MS	531	MS	638	MS

AM-104	685	MS	691	MS	580	MS	652	MS
AM-105	272	MR	623	MS	630	MS	508	MS
AM-127	358	MR	617	MS	519	MS	498	MR
AM-128	235	R	432	MR	407	MR	358	MR
AM-130	407	MR	617	MS	630	MS	551	MS
AM-131	235	R	383	MR	636	MS	418	MR
AM-132	272	MR	580	MS	580	MS	477	MR
AM-142	660	MS	494	MR	673	MS	609	MS
AM-149	605	MS	586	MS	549	MS	580	MS
AM-156	309	MR	679	MS	383	MR	457	MR
AM-158	500	MS	642	MS	623	MS	588	MS
AM-160	309	MR	494	MR	654	MS	486	MR
AM-161	500	MS	457	MR	420	MR	459	MR
AM-162	235	R	568	MS	747	MS	516	MS
AM-164	235	R	531	MS	673	MS	479	MR
AM-166	500	MS	519	MS	358	MR	459	MR
AM-167	728	MS	407	MR	364	MR	500	MS
AM-168	667	MS	321	MR	617	MS	535	MS
AM-171	309	MR	401	MR	636	MS	449	MR
AM-174	358	MR	494	MR	685	MS	512	MS
AM-177	235	R	463	MR	679	MS	459	MR
AM-178	407	MR	253	MR	580	MS	414	MR
AM-180	636	MS	654	MS	636	MS	642	MS
AM-185	235	R	599	MS	667	MS	500	MS
AM-186	309	MR	568	MS	735	MS	537	MS
AM-187	580	MS	568	MS	500	MR	549	MS
AM-188	309	MR	401	MR	444	MR	385	MR
AM-198	358	MR	580	MS	549	MS	496	MR
AM-200	716	MS	383	MR	457	MR	519	MS
AM-201	235	R	580	MS	512	MS	442	MR
AM-202	660	MS	432	MR	654	MS	582	MS
AM-204	235	R	457	MR	580	MS	424	MR
AM-208	302	MR	432	MR	568	MS	434	MR
AM-211	235	R	284	MR	580	MS	366	MR
AM-212	580	MS	444	MR	549	MS	525	MS
AM-213	272	MR	648	MS	500	MR	473	MR
AM-215	691	MS	605	MS	432	MR	576	MS
AM-219	235	R	580	MS	562	MS	459	MR
AM-220	660	MS	667	MS	617	MS	648	MS
AM-222	580	MS	451	MR	383	MR	471	MR
AM-224	660	MS	556	MS	463	MR	560	MS
AM-225	272	MR	512	MS	605	MS	463	MR
AM-226	426	MR	617	MS	722	MS	588	MS
AM-230	358	MR	710	MS	611	MS	560	MS
AM-234	500	MS	568	MS	525	MS	531	MS

AM-236	235	R	525	MS	364	MR	374	MR
AM-238	309	MR	654	MS	265	MR	409	MR
AM-246	309	MR	593	MS	735	MS	545	MS
AM-249	407	MR	679	MS	383	MR	490	MR
AM-250	235	R	660	MS	383	MR	426	MR
AM-251	272	MR	642	MS	475	MR	463	MR
AM-254	630	MS	438	MR	525	MS	531	MS
AM-256	562	MS	704	MS	642	MS	636	MS
AM-257	377	MR	642	MS	494	MR	504	MS
AM-261	389	MR	531	MS	407	MR	442	MR
AM-262	531	MS	679	MS	623	MS	611	MS
AM-264	481	MR	654	MS	512	MS	549	MS
AM-265	407	MR	617	MS	531	MS	519	MS
AM-266	549	MS	444	MR	623	MS	539	MS
AM-267	358	MR	580	MS	654	MS	531	MS
AM-268	660	MS	512	MS	636	MS	603	MS
AM-270	642	MS	543	MS	500	MR	562	MS
AM-271	500	MS	617	MS	457	MR	525	MS
AM-279	667	MS	568	MS	457	MR	564	MS
AM-283	358	MR	494	MR	716	MS	523	MS
AM-284	272	MR	605	MS	580	MS	486	MR
AM-285	630	MS	562	MS	364	MR	519	MS
AM-286	235	R	611	MS	605	MS	484	MR
AM-291	309	MR	562	MS	512	MS	461	MR
AM-307	580	MS	642	MS	667	MS	630	MS
AM-309	500	MS	420	MR	580	MS	500	MS
AM-310	235	R	580	MS	716	MS	510	MS
AM-312	407	MR	568	MS	383	MR	453	MR
AM-315	407	MR	704	MS	469	MR	527	MS
AM-321	235	R	117	R	457	MR	270	MR
AM-322	407	MR	599	MS	414	MR	473	MR
AM-323	660	MS	673	MS	370	MR	568	MS
AM-327	272	MR	611	MS	432	MR	438	MR
AM-329	506	MS	537	MS	309	MR	451	MR
AM-330	235	R	660	MS	333	MR	409	MR
AM-334	660	MS	630	MS	475	MR	588	MS
AM-336	660	MS	512	MS	716	MS	630	MS
AM-339	574	MS	593	MS	512	MS	560	MS
AM-340	481	MR	543	MS	438	MR	488	MR
Bonus (Check)	1277	S	1213	S	1179	S	1223	S
Solu Uwa (Check)	1223	S	1134	S	1435	S	1264	S

AUDPC = Area Under Disease Progress Curve

Spot blotch of barley usually appearing in a warmer climate was not regarded as an important pathogen in Nepal where barley was initially grown mostly in the high-altitude cool areas. However, the pathogen became important after barley acreages started to expand in the warmer Terai plains and the disease is now considered a major challenge to barley production. The SB resistance level of barley varieties bred for the cool environments is unsatisfactory in the warm humid areas and require a significant improvement (Gyawali *et al.*, 2018). The current barley improvement activities in Nepal are primarily targeted for the cool areas and therefore need additional sources of SB resistance to incorporate satisfactory level of SB resistance in future barley varieties for the warm-humid areas.

Three years of disease screening results revealed variation for spot blotch resistance in the ICARDA germplasm (Association mapping panel- AM-2014). About 27% genotypes showed resistant to moderately susceptible type of reaction across the years (Table 2), indicating that the germplasm can be utilized for barley improvement program in Nepal. Previous study also showed variation of ICARDA barley germplasm for SB resistance in India and Tunisia (Visioni *et al.*, 2020; Kumar *et al.*, 2020). The genotypes identified in this study viz., AM-128, AM-188 and AM-321 that have high level of resistance and stable response to SB can be immediately utilized by the national barley improvement program. Further screening of the ICARDA germplasm (AM-2014) used in this study in different locations of Terai region of Nepal will provide a conclusive information regarding the germplasm reaction to the prevalent SB races in Nepal. Such information will be useful to the Nepalese and international barley improvement programs aiming to develop improved disease resistant varieties for the warm humid environment.

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Conflict of interest

The authors declare that they have no conflict of interest.

Compliance with ethical standards

The authors have followed ethical norms accepted by international scientific community and made every endeavor to prevent any infringements of the norms. No human participants or animals were used in the experiment.

Author contribution

BBP – Preparation of research proposal, carried out field experiments, data collection, data analysis, interpretation of the results and manuscript preparation; MMP – guided for research proposal preparation, field experimentation, data analysis and thorough guidance for manuscript preparation; SKG & DBT – guided for research proposal preparation, field experimentation, manuscript preparation; HKM – guided for field data collection; TRR, PA & ST helped to collect disease data; RPSV – helped for project proposal preparation and SG – project design, helped in germplasms import to Nepal, guided for field research, data collection, data analysis, interpretations of the data, results and manuscript preparation.

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