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ORIGINAL RESEARCH ARTICLE

Assessment of wheat genotypes for spot blotch (*Bipolaris sorokiniana* Sacc.) resistance under artificial epiphytotic conditions in the inner terai of Nepal

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ABSTRACT

Spot blotch, caused by (*Bipolaris sorokiniana* Sacc.) is a significant fungal disease leading to economic losses in wheat (*Triticum aestivum*), especially in regions with low soil fertility and warm, humid climates, such as the inner Terai of Nepal. A field experiment was conducted at the National Maize Research Program (NMRP), Rampur, Chitwan, during winter of 2021/022 with an aim of identifying wheat genotypes with spot blotch resistance under artificial epiphytotic conditions. Each genotype was sown in a single 2-meter long row alongside susceptible check genotypes (Agra and Morocco) at every 20th row. Aqueous spore suspension of *B. sorokiniana* was applied twice in border rows during the booting stage at 15-day intervals. Disease severity was assessed four times at five-day intervals using a double-digit scale based on the percentage blighted area on the flag and penultimate leaf, and the area under the disease progress curve (AUDPC) was calculated. The results categorized 43 genotypes as resistant, 127 as moderately resistants, 135 as moderately susceptible, 66 as susceptible, and 7 as highly susceptible based on AUDPC. Cluster analysis identified cluster 3 comprising 17 genotypes as superior in terms of disease resistance as well as agronomic parameters. NRN-34 emerged as the top-ranked genotype within this cluster, followed by NAL-73, NAL-94, NAL -12, NRN-34, NAL-57, NAL-43, NAL-82, and NAL-35, exhibiting lower AUDPC values and higher yield-attributing character values. This study will aid breeders in developing spot blotch-resistant and high-yielding wheat varieties by incorporating the identified promising genotypes into further breeding programs.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most extensively cultivated cereal crop globally, encompassing approximately 17% of the total cultivated land area (Basnet *et al.*, 2022). Nepal, despite ranking 39th in wheat production worldwide, contributes a mere 0.28% to global production (FAO, 2022). Despite being the third most produced staple crop in Nepal, wheat holds the second position in terms of consumption. The bulk of global wheat production is derived from two contemporary species: durum or tetraploid wheat (*T. turgidum* subsp. durum, 2n = 4x = 28, AABB) and common or hexaploid bread wheat (*Triticum aestivum*, 2n = 6x = 42, AABBDD) (Regmi *et al.*, 2021). Nepal's diverse geography permits wheat cultivation from the low-lying terai (100 masl) to the lofty mountain regions 2300 meter above sea level throughout the winter season. Wheat occupies a significant 23% of Nepal's total cultivated land area and contributes 19.13% to the total cereal crop production. The area,

production, and productivity of wheat during the year 2020/21 A.D. were 2,127,276 tons, 711,067 ha, and 2.99 tons/ha, respectively (MoALD, 2022). Wheat production has experienced a 2.65% decline in Nepal compared to the 2019/20 period, despite a 0.5% increase in cultivated area, resulting in a 3.14% reduction in productivity. This decline in productivity despite an increase in cultivation area suggests underlying challenges within the Nepalese wheat production system.

Among the biotic constraints challenging wheat productivity, spot blotch has emerged as a critical threat to wheat production in Nepal and globally (Nepal *et al.*, 2020). This disease, caused by the bio trophic fungal pathogen *Bipolaris sorokiniana* (Sacc.) Shoemaker (Teleomorph: *Cochliobolous sativus*, syn. *Helmonthosporium sativum)*, poses a significant challenge to wheat cultivation, leading to considerable economic losses (Aggarwal *et al.*, 2011). The pathogen, transmitted through seeds and soil, is responsible for several diseases in wheat and barley, including head blight, seedling blight, foliar blight/spot blotch, common root rot, and black point (Wiese, 1987). Spot blotch particularly thrives in warm and humid wheat cultivation regions, such as eastern India and Southeast Asia, including countries like Bangladesh, India, and Nepal (Joshi *et al.*, 2007). Its severity has escalated in Nepal's plains and foothills and is progressively spreading to the mid and hills of the country. In farmer's field settings, it results in a loss of up to 28% of grain production (Mahto, 2001); however, under severe epidemic conditions, the loss may increase up to 80% (Joshi & Chand, 2002). The impact of spot blotch on grain production can be substantial, with losses ranging from 28% under normal conditions to a staggering 80% during severe epidemics (Mahto, 2001; Joshi & Chand, 2002). The pathogen's detrimental effects encompass foot rot, grain discoloration, black point of kernels, blight of seedlings and heads, leaf spot, blotch, and blight, and sterility of spikes under severe infections (Ownley & Trigiano, 2016). At different stages of development, it targets grains, spikes, roots, leaves, and seedlings. Initial signs of spot blotch typically manifest during the seedling stage, with brown lesions appearing on various plant parts, which gradually expand to form necrotic patches or blotches (Gupta *et al.*, 2018). An erratic increase in infection is seen as the temperature rises (Duveiller, 2004). Crop management practices, soil fertility, plant growth stage, planting density, and meteorological conditions encountered by wheat throughout the later stages of the life cycle all typically have a major influence on the severity of this disease (Joshi *et al.*, 2007). Infection and pathogen multiplication rates are especially favored by warm temperatures between 18°C and 32°C and high relative humidity, which permit the canopy to stay wet for an extended period of time (Acharya *et al.*, 2011; Basnet *et al.*, 2022).

Spot blotch can be controlled with better cultural approaches, management of soil fertility, use of disease resistant varieties and effective fungicide usage. Despite extensive efforts at breeding, effective resistance to spot blotch has not been demonstrated by released wheat cultivars, with most cultivars exhibiting only partial resistance (Lozano *et al.*, 2022). Given the dynamic nature of climatic factors influencing disease severity, there is a pressing need to identify and develop novel sources of resistance to spot blotch in wheat (Sharma *et al.*, 2007). Since 1983, progress has been made in developing genotypes resistant to spot blotch, leading to decreased disease severity and associated yield declines (Joshi *et al.*, 2007). However, durable spot blotch-resistant varieties remain scarce in Nepal. Therefore, this study was undertaken with an aim to evaluate the severity of spot blotch disease across various wheat genotypes and identify resistant genotypes in the inner Terai condition at Rampur, Chitwan, Nepal. By assessing spot blotch resistance alongside its correlation with yield features, this research endeavors to identify suitable donors for spot blotch resistance with enhanced yield potential, thereby addressing a critical research gap.

MATERIALS AND METHODS

Experimental site

The field experiment was conducted at the agronomy research farm of the National Maize Research Program (NMRP), located in Rampur, Chitwan, Nepal (27° 39' 15" N latitude and 84° 21' 24" E longitude, 176.76 meters above mean sea level) (Figure 1). All laboratory activities related to pathogen culture were conducted in the plant pathology laboratory of NMRP.

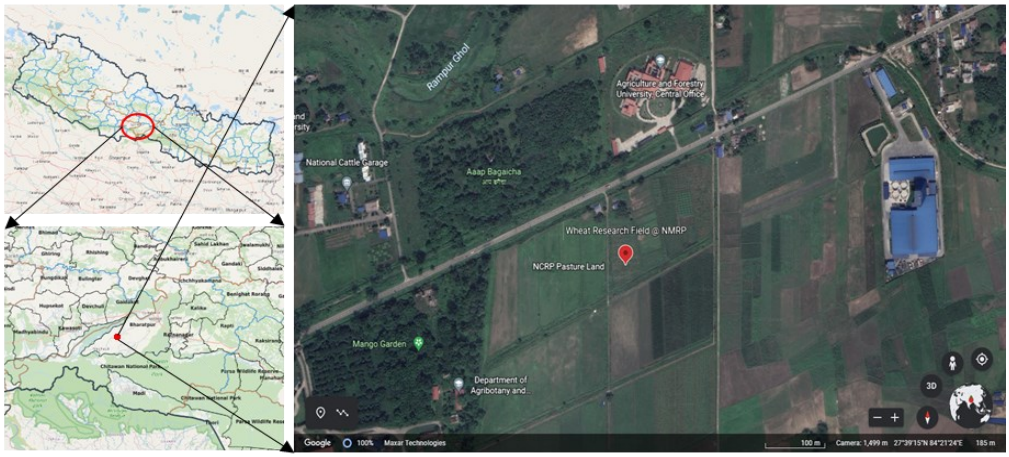

Figure 1. *Location of the experimental field in the agronomy research farm of the National Maize Research Program, Rampur, Chitwan, Nepal.*

Agrometeorological features of the experimental site

The experimental site represents the inner Terai region of Nepal, characterized by a subtropical and humid climate. Climatic data during the study period was recorded fortnightly (Figure 2).

Incubation of seeds for pathogen spore extraction

Single spores of *B. sorokiniana* were isolated from the seeds of the RR-21 genotype (a susceptible genotype against spot blotch). Initially, a 1% sodium hypochlorite solution was prepared. The seeds of RR-21 were first surface sterilized with the 1% sodium hypochlorite solution for about 30 seconds and washed with sterilized distilled water four times to remove any other surface contamination. Afterward, 10 seeds each were aseptically placed approximately equidistant from each other inside four petri plates over two layers of wet blotting paper. The Petri plates were then placed inside a BOD incubator at 25°C for 48 hours until prominent fungal growth with conidia was observed under a stereo binocular microscope.

Preparation of Potato Dextrose Agar (PDA) media and water agar

One liter of PDA media was prepared using 200 g of peeled potato pieces, 20 g of dextrose, 20 grams of agar, and the remaining distilled water. 500 ml of distilled water was taken in a beaker of 1 liter, 200 g of sliced potato pieces were added, and the mixture was then boiled over a Bunsen burner for about 20 minutes until the potato pieces became soft. The contents of the beaker were filtered out into another one-liter capacity beaker using a clean muslin cloth, and the remaining boiled potato pieces in the muslin cloth were also squeezed out into the beaker to create the potato infusion. Another beaker was also used simultaneously to slowly heat 500 ml of water and dissolve the 20 g each of dextrose and agar, facilitated by continuous stirring and gentle heat. The potato infusion and the dextrose and agar solution were mixed over the flame, and distilled water was added to increase the volume of the solution to one liter. Similarly, one-liter water agar was prepared by dissolving only 20 g of agar-agar in distilled water, facilitated by gentle heating.

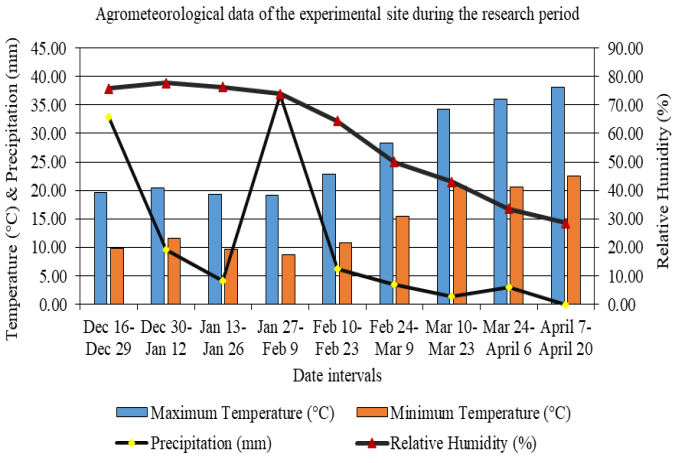

Figure 2. *Fortnightly climatic data from the experimental site throughout the research duration.*

The 1 liter of PDA and water agar were each dispensed into 4 Erlenmeyer flasks in equal amounts (250 ml), and the openings of the flasks were sealed with non-absorbent cotton and covered by aluminum foil. Similarly, the PDA media was also poured into four test tubes to prepare agar PDA slants and sealed with cotton plugs and aluminum foil. Then, the media and water agar were sterilized inside an autoclave for 15 minutes at 121°C and 15 psi pressure. Finally, after the sterilized PDA media and water agar had cooled down to about 50°C, they were removed from the autoclave, and a bactericide (streptomycin sulfate at 30 g/liter) was added to avoid any bacterial contamination. Then, 20 ml of the PDA media and water agar were poured into 8 sterilized petri plates each, allowed to solidify, and stored under the laminar flow until further use. The PDA slants were kept in a slanting position for solidification, alongside the PDA and water agar plates under the laminar flow.

Isolation of single spore and pure culture of *B. sorokiniana*

After 48 hours of seed incubation, single spores of *B. sorokiniana* were placed in eight petri plates containing water agar. Several circular markings were made in the water agar inside each agar plate by using a sterilized cork borer. The single spores were then removed from the surface of seeds inside the laminar flow viewing under a stereo binocular microscope using a sterilized inoculation needle and were placed in the water agar inside the markings made by the cork borer. Then the water agar plates containing the single spores were incubated for 24 hours at 25° C until the spores germinated. The water agar facilitates only the germination of the spores and not the growth. To facilitate the growth of mycelium and further production of conidia, the germinated single spores of the pathogen were selected and aseptically transferred from water agar to previously prepared PDA plates and slants using a sterilized inoculation loop. Then, the PDA plates were sealed with para-film and the slants with cotton plugs, labeled with the name of pathogen and date of inoculation, wrapped with aluminum foils, and incubated at 25° C for 10 days.

Mass culture of *B. sorokiniana*

Mass culture was also prepared in a completely aseptic condition. 1.5 kg of sorghum seeds were boiled with an equal amount of water (1.5 liters) for about 25 minutes, until the seeds became soft but not split, over a gas stove in a steel container. The excess water was drained, and about 500 grams of the sorghum seeds were transferred to each of three conical flasks of 1-liter capacity, and calcium carbonate was added at 4 g per 500 g of sorghum seeds (4 g in each conical flask) to remove the stickiness of the grains. Then, the mouths of the conical flasks were sealed with cotton plugs and aluminum foils and subjected to sterilization at 121°C and 15 psi pressure for 30 minutes inside an autoclave. After sterilization, the flasks were removed from the autoclave and cooled down to about 50°C under laminar flow. Under aseptic conditions inside the laminar flow, each flask was then inoculated with the PDA with mycelial growth

and conidia from previously prepared pure culture approximately at the center of the flask to ensure uniform radial growth and coverage of the seeds by the fungus. The mouths of the conical flasks were then sealed with cotton plugs and aluminum foil, and the inoculated sorghum seeds were placed inside an incubator at 25°C for 10 days.

Experimental materials

The experiment was conducted during the winter season from 16th December 2021 to 18th April 2022 to evaluate the resistance of 378 genotypes of wheat against spot blotch under artificial epiphytotic conditions in the inner terai condition of Nepal at NMRP, Rampur, Chitwan. The seeds of the genotypes were procured from the National Wheat Research Program (NWRP), Bhairahawa, Rupandehi, Nepal. A mixture of susceptible check genotypes (Agra and Morocco) was used, being highly susceptible to spot blotch disease of wheat. The list of genotypes used in the experiment is presented in (Table 1).

Table 1. List of wheat genotypes included in the experiment during winter 2021/022 at NMRP, Rampur, Chitwan, Nepal.

Treatments	Genotype	Treatments	Genotype	Treatments	Genotype	Treatments	Genotype
T_1	NRN-30	T_{37}	NRN-68	T_{73}	NRN-106	T_{109}	NAL-24
T_2	NRN-31	T_{38}	NRN-69	T_{74}	NRN-107	T_{110}	NAL-25
T_3	NRN-32	T_{39}	NRN-70	T_{75}	NRN-108	T_{111}	NAL-26
T_4	NRN-33	T_{40}	NRN-71	T_{76}	NRN-109	T_{112}	NAL-27
T_5	NRN-34	T_{41}	NRN-72	T_{77}	NRN-110	T_{113}	NAL-28
T_6	NRN-35	T_{42}	NRN-73	T_{78}	NRN-111	T_{114}	NAL-29
T_7	NRN-36	T_{43}	NRN-74	T_{79}	NRN-112	T_{115}	NAL-30
T_8	NRN-37	T_{44}	NRN-75	T_{80}	NRN-113	T_{116}	NAL-31
T_9	NRN-38	T_{45}	NRN-76	T_{81}	NRN-114	T_{117}	NAL-32
T_{10}	NRN-39	T_{46}	NRN-77	T_{82}	NRN-115	T_{118}	NAL-33
T_{11}	NRN-41	T_{47}	NRN-78	T_{83}	NRN-116	T_{119}	NAL-34
T_{12}	NRN-42	T_{48}	NRN-79	T_{84}	NRN-117	T_{120}	NAL-35
T_{13}	NRN-43	T_{49}	NRN-81	T_{85}	NRN-118	T_{121}	NAL-36
T_{14}	NRN-44	T_{50}	NRN-82	T_{86}	NRN-119	T_{122}	NAL-37
T_{15}	NRN-45	T_{51}	NRN-83	T_{87}	NAL-1	T_{123}	NAL-38
T_{16}	NRN-46	T_{52}	NRN-84	T_{88}	NAL-2	T_{124}	NAL-39
T_{17}	NRN-47	T_{53}	NRN-85	T_{89}	NAL-3	T_{125}	NAL-41
T_{18}	NRN-48	T_{54}	NRN-86	T_{90}	NAL-4	T_{126}	NAL-42
T_{19}	NRN-49	T_{55}	NRN-87	T_{91}	NAL-5	T_{127}	NAL-43
T_{20}	NRN-50	T_{56}	NRN-88	T_{92}	NAL-6	T_{128}	NAL-44
T_{21}	NRN-51	T_{57}	NRN-89	T_{93}	NAL-7	T_{129}	NAL-45
T_{22}	NRN-52	T_{58}	NRN-90	T_{94}	NAL-8	T_{130}	NAL-46
T_{23}	NRN-53	T_{59}	NRN-91	T_{95}	NAL-9	T_{131}	NAL-47
T_{24}	NRN-54	T_{60}	NRN-92	T_{96}	NAL-10	T_{132}	NAL-48
T_{25}	NRN-55	T_{61}	NRN-93	T_{97}	NAL-11	T_{133}	NAL-49
T_{26}	NRN-56	T_{62}	NRN-94	T_{98}	NAL-12	T_{134}	NAL-50
T_{27}	NRN-57	T_{63}	NRN-95	T_{99}	NAL-13	T_{135}	NAL-51
T_{28}	NRN-58	T_{64}	NRN-96	T_{100}	NAL-14	T_{136}	NAL-52
T_{29}	NRN-59	T_{65}	NRN-97	T_{101}	NAL-15	T_{137}	NAL-53
T_{30}	NRN-61	T_{66}	NRN-98	T_{102}	NAL-16	T_{138}	NAL-54
T_{31}	NRN-62	T_{67}	NRN-99	T_{103}	NAL-17	T_{139}	NAL-55
T_{32}	NRN-63	T_{68}	NRN-101	T_{104}	NAL-18	T_{140}	NAL-56
T_{33}	NRN-64	T_{69}	NRN-102	T_{105}	NAL-19	T_{141}	NAL-57
T_{34}	NRN-65	T_{70}	NRN-103	T_{106}	NAL-21	T_{142}	NAL-58
T_{35}	NRN-66	T_{71}	NRN-104	T_{107}	NAL-22	T_{143}	NAL-59
T_{36}	NRN-67	T_{72}	NRN-105	T_{108}	NAL-23	T_{144}	NAL-61

Table 1. *Contd.....*

Treatments	Genotype	Treatments	Genotype	Treatments	Genotype	Treatments	Genotype
T_{145}	NAL-62	T_{181}	NAL-99	T_{217}	NAL-147	T_{253}	NAL-187
T_{146}	NAL-63	T_{182}	NAL-101	T_{218}	NAL-148	T_{254}	NAL-188
T_{147}	NAL-64	T_{183}	NAL-102	T_{219}	NAL-149	T_{255}	NAL-189
T_{148}	NAL-65	T_{184}	NAL-103	T_{220}	NAL-152	T_{256}	NAL-190
T_{149}	NAL-66	T_{185}	NAL-104	T_{221}	NAL-153	T_{257}	NAL-191
T_{150}	NAL-67	T_{186}	NAL-105	T_{222}	NAL-154	T_{258}	NAL-192
T_{151}	NAL-68	T_{187}	NAL-106	T_{223}	NAL-155	T_{259}	NAL-193
T_{152}	NAL-69	T_{188}	NAL-107	T_{224}	NAL-156	T_{260}	NAL-194
T_{153}	NAL-70	T_{189}	NAL-110	T_{225}	NAL-157	T_{261}	NAL-195
T_{154}	NAL-71	T_{190}	NAL-112	T_{226}	NAL-158	T_{262}	NAL-196
T_{155}	NAL-72	T_{191}	NAL-113	T_{227}	NAL-159	T_{263}	NAL-197
T_{156}	NAL-73	T_{192}	NAL-114	T_{228}	NAL-161	T_{264}	NAL-198
T_{157}	NAL-74	T_{193}	NAL-115	T_{229}	NAL-162	T_{265}	NAL-199
T_{158}	NAL-75	T_{194}	NAL-116	T_{230}	NAL-163	T_{266}	NAL-201
T_{159}	NAL-76	T_{195}	NAL-117	T_{231}	NAL-164	T_{267}	NAL-202
T_{160}	NAL-77	T_{196}	NAL-118	T_{232}	NAL-165	T_{268}	NAL-203
T_{161}	NAL-78	T_{197}	NAL-119	T_{233}	NAL-166	T_{269}	NAL-204
T_{162}	NAL-79	T_{198}	NAL-121	T_{234}	NAL-167	T_{270}	NAL-205
T_{163}	NAL-81	T_{199}	NAL-122	T_{235}	NAL-168	T_{271}	NAL-206
T_{164}	NAL-82	T_{200}	NAL-123	T_{236}	NAL-169	T_{272}	NAL-207
T_{165}	NAL-83	T_{201}	NAL-124	T_{237}	NAL-170	T_{273}	NAL-208
T_{166}	NAL-84	T_{202}	NAL-125	T_{238}	NAL-171	T_{274}	NAL-209
T_{167}	NAL-85	T_{203}	NAL-126	T_{239}	NAL-172	T_{275}	NAL-210
T_{168}	NAL-86	T_{204}	NAL-127	T_{240}	NAL-173	T_{276}	NAL-211
T_{169}	NAL-87	T_{205}	NAL-128	T_{241}	NAL-174	T_{277}	NAL-212
T_{170}	NAL-88	T_{206}	NAL-129	T_{242}	NAL-175	T_{278}	NAL-213
T_{171}	NAL-89	T_{207}	NAL-130	T_{243}	NAL-176	T_{279}	NAL-214
T_{172}	NAL-90	T_{208}	NAL-137	T_{244}	NAL-177	T_{280}	NAL-215
T_{173}	NAL-91	T_{209}	NAL-138	T_{245}	NAL-178	T_{281}	NAL-216
T_{174}	NAL-92	T_{210}	NAL-139	T_{246}	NAL-179	T_{282}	NAL-217
T_{175}	NAL-93	T_{211}	NAL-141	T_{247}	NAL-181	T_{283}	NAL-218
T_{176}	NAL-94	T_{212}	NAL-142	T_{248}	NAL-182	T_{284}	NAL-221
T_{177}	NAL-95	T_{213}	NAL-143	T_{249}	NAL-183	T_{285}	NAL-222
T_{178}	NAL-96	T_{214}	NAL-144	T_{250}	NAL-184	T_{286}	NAL-223
T_{179}	NAL-97	T_{215}	NAL-145	T_{251}	NAL-185	T_{287}	NAL-224
T_{180}	NAL-98	T_{216}	NAL-146	T_{252}	NAL-186	T_{288}	NAL-225

Treatments	Genotype	Treatments	Genotype	Treatments	Genotype
T_{289}	NAL-226	T_{319}	NAL-257	T_{349}	NAL-289
T_{290}	NAL-227	T_{320}	NAL-258	T_{350}	NAL-290
T_{291}	NAL-228	T_{321}	NAL-259	T_{351}	NAL-291
T_{292}	NAL-229	T_{322}	NAL-261	T_{352}	NAL-292
T_{293}	NAL-230	T_{323}	NAL-262	T_{353}	NAL-293
T_{294}	NAL-231	T_{324}	NAL-263	T_{354}	NAL-294
T_{295}	NAL-232	T_{325}	NAL-264	T_{355}	NAL-295
T_{296}	NAL-233	T_{326}	NAL-265	T_{356}	NAL-296
T_{297}	NAL-234	T_{327}	NAL-266	T_{357}	NAL-297
T_{298}	NAL-235	T_{328}	NAL-267	T_{358}	NAL-298
T_{299}	NAL-236	T_{329}	NAL-268	T_{359}	NAL-299
T_{300}	NAL-237	T_{330}	NAL-269	T_{360}	NAL-301
T_{301}	NAL-238	T_{331}	NAL-270	T_{361}	NAL-302
T_{302}	NAL-239	T_{332}	NAL-271	T_{362}	NAL-303
T_{303}	NAL-241	T_{333}	NAL-272	T_{363}	NAL-304
T_{304}	NAL-242	T_{334}	NAL-273	T_{364}	NAL-305
T_{305}	NAL-243	T_{335}	NAL-274	T_{365}	NAL-306
T_{306}	NAL-244	T_{336}	NAL-275	T_{366}	NAL-307
T_{307}	NAL-245	T_{337}	NAL-276	T_{367}	NAL-308
T_{308}	NAL-246	T_{338}	NAL-277	T_{368}	NAL-309
T_{309}	NAL-247	T_{339}	NAL-278	T_{369}	NAL-310
T_{310}	NAL-248	T_{340}	NAL-279	T_{370}	NAL-311
T_{311}	NAL-249	T_{341}	NAL-281	T_{371}	NAL-312
T_{312}	NAL-250	T_{342}	NAL-282	T_{372}	NAL-313
T_{313}	NAL-251	T_{343}	NAL-283	T_{373}	NAL-314
T_{314}	NAL-252	T_{344}	NAL-284	T_{374}	NAL-315
T_{315}	NAL-253	T_{345}	NAL-285	T_{375}	NAL-316
T_{316}	NAL-254	T_{346}	NAL-286	T_{376}	NAL-317
T_{317}	NAL-255	T_{347}	NAL-287	T_{377}	NAL-318
T_{318}	NAL-256	T_{348}	NAL-288	T_{378}	NAL-319

Experimental design

The experimental field was laid out in an augmented design with six blocks, and seeds of each genotype were sown in a single row of 2 meters' length with a 25-cm distance between the rows. A single row of a mixture of susceptible check genotypes (Agra and Morocco) was sown at every 20th row and two rows between each block and around the field to provide a uniform source of inoculum throughout the field. In this way, 67 lines each were included in the first five blocks and 63 lines in the last block, for a total of 398 lines (378 test genotype lines and 20 susceptible check lines, as shown in (Figure 3). A space of 50 cm was maintained between each block besides the border rows of susceptible check genotypes.

Crop management in the field

Field preparation was carried out by using a tractor-mounted cultivator four times, followed by planking. Farmyard manure (FYM) at 10 tons/ha was applied, and N, P, and K at 100, 50, and 50 were applied via urea, Di-Ammonium Phosphate (DAP), and Murate of Potash (MOP), respectively, for soil fertilization. Half doses of nitrogen and full doses of potassium and phosphorus were applied as basal doses. One fourth dose of the remaining nitrogen was top-dressed 30 days after sowing (DAS), and after that, one eighth of the remaining nitrogen was top-dressed at 55 DAS and the final one eighth at 70 DAS. The experimental field was irrigated once at the crown root initiation stage and once immediately after the first inoculation of *B. sorokiniana* to provide a favorable environment for the pathogen to initiate spot blotch disease.

Inoculum preparation and field inoculation of *B. sorokiniana* spores

The ten-day-old mass culture of *B. sorokiniana* multiplied on sorghum seeds was used for inoculating the wheat field. The spore suspension was prepared in distilled water with a spore load of 50 to 75 per microscopic field (10X). Surfactant or sticker (teepol) was also added at 1 ml/liter to the suspension to enhance the attachment of the spores to the plant parts (especially leaves) and to improve the actual number of inoculums commencing the disease. To prevent the large solid particles from damaging the sprayer, the spore suspension was filtered through a clean muslin cloth. The spore suspension prepared in distilled water was checked under the microscope to ensure a concentration of 50–75 spores per microscopic field, and the final volume prepared was 30 liters. This suspension was sprayed twice on the border rows uniformly using a power sprayer in the late afternoon. The first inoculation was done at the booting stage of wheat, and the second inoculation was conducted after 15 days of the first inoculation.

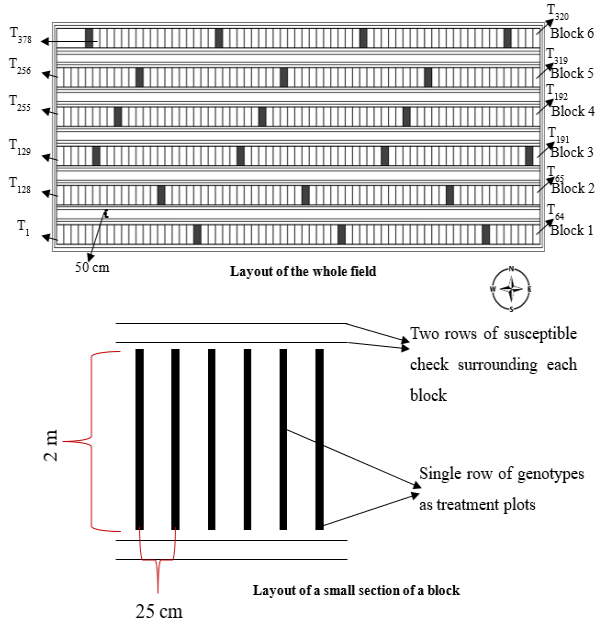

Figure 3. *Field layout of the experiment on wheat in augmented design during winter 2021/022 at NMRP, Rampur, Chitwan.*

Disease scoring

Following inoculation, the genotypes were routinely examined to document the disease severity. Disease severity was scored based on the percentage of diseased leaf area through visual observation on the flag leaf (F) and penultimate leaf (F-1) from 10 randomly selected single tillers of each genotype using the double-digit scale given by Eyal (1987) and Saari, (1998). The first scoring was conducted after ten days of inoculation and was repeated another three times at an interval of five days. So, altogether, four scorings were carried out, and the area under disease progress curve (AUDPC) value of each genotype was calculated.

Estimation of area under disease progress curve (AUDPC)

The following formula, provided by Das *et al.* (1992) was used to compute the AUDPC values based on infection of the flag leaf and penultimate leaf:

$$\text{AUDPC} = \sum_{i=1}^{n} \left(\frac{(Yi_{+1} + Y_i)}{2} (T_{i+1}.T_i) \right)$$

(Y_i = disease scored on ith date, T_i = date of ith scoring, n=no. of scorings).

The disease severity was calculated for each scoring by using the formula:

$$\text{Disease Severity \% (DS)} = \frac{D1 + D2}{2} x10$$

Where, D_1 = 1st digit (proportion of spot blotch infection on the flag leaf in the scale of 10), D_2 = 2nd digit (proportion of spot blotch infection on the penultimate leaf in the scale of 10).

Separate disease severity for each flag leaf and penultimate leaf observed visually at each scoring (which is simply the percentage of diseased leaf area) was also used to calculate their separate AUDPC values. Four AUDPC values, namely AUDPC0, AUDPC1, AUDPC2, and AUDPC3, were calculated using the data from four disease scorings and summed up to obtain the final total AUDPC. For the calculation of AUDPC0, the value of disease severity at that date was considered to be 0, and AUDPC1, AUDPC2, and AUDPC3 were calculated using the

disease severity recorded during the 4 scorings. Furthermore, genotypes were categorized into different resistance/ susceptibility categories, viz., resistant (AUDPC≤378.75), moderately resistant (AUDPC=378.75-648.75), moderately susceptible (AUDPC=648.75-918.75), susceptible (918.75-1188.75), and highly susceptible (AUDPC> 1188.75) based on the calculated maximum, minimum, and average AUDPC values of the genotypes included in this study. First, the range, i.e., the difference between the maximum and minimum AUDPC among the genotypes, was calculated. Then, the range was divided by 5 (i.e., the number of categories), and the value thus obtained was the difference between the upper and lower limits (270) of all the categories.

Observations of agronomic parameters

Days to heading (days): Days to heading were recorded for each genotype line (each plot) when 50% of the plants were observed with heads.

Days to maturity (days): The readings of days to maturity for each plot were taken when all the plants showed yellowish spikes.

Thousand kernel weight (g): After the harvest of the grains, 100 kernels of each of the genotype lines were counted, weighed, and later extrapolated to 1000 kernel weights after adjusting the moisture content of the grains to 12% using the following formula:

$$\text{TKW} = \frac{100 \text{ kernel weight} \times (100 - \text{observed moisture percentage})}{(100 - \text{standard moisture content}(12))} \times 10$$

Number of grains per spike: The number of grains in 10 spikes of each genotype was counted and then divided by 10 to obtain the number of grains per spike.

Kernel weight per spike (g): The kernels separated from 10 sample spikes were weighed, and the weight was divided by 10 to obtain the kernel weight per spike.

Spike length (cm): The length of each of the 10 spikes was measured using a scale in centimeters, and the average of the lengths was taken as the average spike length of each genotype.

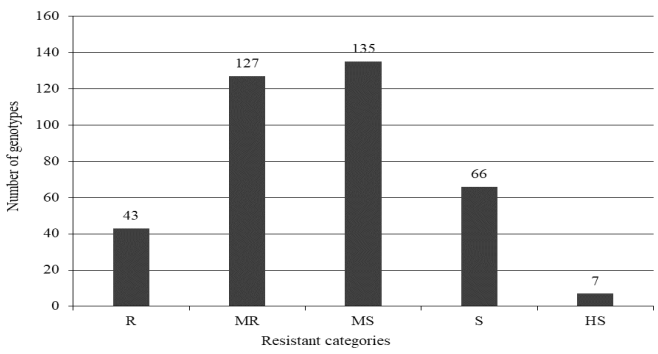

Figure 4. *Graphical representation of tested genotypes in each resistance categories based on AUDPC values.*

Statistical analysis

Microsoft Excel 365 was used to tabulate the data as well as to create correlation and regression graphs. Cluster analysis was performed via Minitab statistical software version 14, and correlation analysis was performed via IBM SPSS Statistics 26.

RESULTS AND DISCUSSION

Area under disease progress curve (AUDPC)

Three hundred and seventy-eight genotypes demonstrated different levels of resistance against spot blotch disease, as indicated by the AUDPC values. Categorized based on the AUDPC values of the genotypes as explained the in methodology section, out of the 378 genotypes tested under artificial epiphytotic conditions in the field, 43 of them viz., NAL-35, NAL-39, NAL-94, NAL-73, NRN-52, NAL-82, NAL-25, NAL-12, NRN -51, NAL-57, NRN-41, NAL-43, NRN-55, NRN-61, NRN-62, NRN-34, NRN-43, NRN-39, NAL-36, NAL-72, NAL-92, NAL-119, NAL-59, NAL-102, NAL-74, NAL-91, NAL-19, NAL-176, NRN-47, NAL-41, NRN-50, NAL-87, NAL-232, NRN-38, NAL-107, NAL-105, NAL-98, NAL-221, NAL-70, NAL-79, NAL-106, NAL-110, and NAL-213 were found to be resistant, 127 were found to be moderately resistant, 135 were moderately susceptible, 66 were susceptible and 7 genotypes namely NAL-144, NAL-215, NAL-309, NAL-143, NAL-146, NAL-313, and NAL-319 were highly susceptible as shown in Table 2 and Figure 4. The susceptible check genotype mixtures, Agra + Morocco demonstrated highly susceptible reaction with AUDPC of 1252. 20 and 3 of the genotypes, namely NAL-146, NAL-313, and NAL -319, were found to be more susceptible than the susceptible check, with AUDPC values of 1360, 1433.75, and 1458.75, respectively.

Correlation between and among disease and agronomic characters of wheat

AUDPC was highly and positively correlated with AUDPCF and AUDPC (Table 3), indicating a strong association between disease progression in flag and penultimate leaves. However, AUDPC, AUDPCF, and AUDPC (F-1) showed significant negative correlations with thousand kernel weight, days to maturity, days to heading, number of grains per spike, spike length, and kernel weight per spike. This suggests that higher disease severity adversely affects these yield-related and maturity traits. Thousand kernel weight exhibited positive correlations with the number of grains per spike, spike length, and kernel weight per spike. Similarly, days to maturity and days to heading were positively correlated with the number of grains per spike, spike length, and kernel weight per spike as shown in Table 3. These associations indicate that longer growth periods and larger spikes tend to result in heavier grains. The number of grains per spike was highly and positively correlated with spike length and kernel weight per spike, implying that longer spikes can accommodate more grains, leading to higher grain weights per spike.

Table 2. *Grouping of tested genotypes of wheat into 5 disease resistance categories based on AUDPC values.*

S. No.	Resistance categories based on	AUDPC range	Genotypes	No. of genotypes
1	Resistant (R)	<378.75	NAL-35, NAL-39, NAL-94, NAL-73, NRN-52, NAL-82, NAL-25, NAL-12, NRN-51, NAL-57, NRN-41, NAL-43, NRN-55, NRN-61, NRN-62, NRN-34, NRN-43, NRN-39, NAL-36, NAL-72, NAL-92, NAL-119, NAL-59, NAL-102, NAL-74, NAL-91, NAL-19, NAL-176, NRN-47, NAL-41, NRN-50, NAL-87, NAL-232, NRN-38, NAL-107, NAL-105, NAL-98, NAL-221, NAL-70, NAL-79, NAL-106, NAL-110, and NAL-213	43
2	Moderately Resistant (MR)	378.75 – 648.75	NRN-71, NAL-162, NAL-83, NAL-85, NAL-158, NAL-76, NAL-114, NAL-115, NRN-32, NAL-38, NAL-124, NAL-97, NAL-95, NRN-72, NAL-46, NRN-35, NAL-16, NAL-58, NRN-107, NAL-69, NRN-58, NRN-56, NAL-112, NRN-99, NAL-50, NAL-51, NAL-104, NRN-48, NAL-2, NRN-63, NAL-1, NAL-4, NRN-70, NRN-31, NAL-278, NRN-53, NRN-102, NRN-108, NAL-26, NAL-30, NAL-292, NAL-84, NAL-201, NRN-105, NRN-95, NAL-78, NRN-110, NRN-101, NAL-118, NAL-121, NAL-86, NAL-88, NAL-99, NAL-233, NRN-77, NAL-122, NRN-106, NAL-116, NAL-101, NAL-148, NRN-69, NRN-94, NAL-52, NRN-30, NRN-82, NAL-183, NAL-138, NAL-195, NAL-93, NRN-66, NAL-65, NAL-66, NAL-62, NAL-179, NAL-149, NRN-54, NRN-86, NAL-33, NAL-42, NAL-113, NRN-92, NAL-31, NAL-117, NRN-57, NRN-98, NAL-64, NAL-81, NRN-49, NAL-89, NAL-32, NRN-117, NRN-90, NRN-93, NRN-96, NAL-245, NAL-230, NAL-54, NRN-76, NAL-7, NAL-21, NAL-155, NRN-89, NAL-204, NRN-103, NAL-137, NAL-207, NAL-8, NAL-228, NAL-71, NAL-249, NRN-65, NAL-61, NAL-157, NAL-266, NRN-111, NAL-223, NRN-36, NAL-77, NRN-104, NAL-48, NAL-68, NAL-196, NAL-152, NAL-279, NRN-64, NRN-75, and NAL-203	127
3	Moderately Susceptible (MS)	648.75-918.75	NRN-97, NRN-83, NRN-46, NAL-63, NRN-67, NAL-186, NAL-283, NAL-49, NAL-67, NAL-184, NAL-29, NAL-307, NAL-311, NAL-227, NAL-153, NRN-79, NRN-74, NAL-3, NAL-256, NAL-27, NAL-90, NRN-91, NAL-224, NAL-188, NAL-199, NRN-33, NRN-73, NAL-172, NRN-59, NAL-271, NRN-45, NRN-118, NAL-189, NAL-315, NAL-244, NRN-81, NAL-193, NRN-109, NAL-243, NAL-287, NAL-282, NAL-128, NAL-173, NAL-45, NAL-125, NRN-114, NAL-175, NAL-55, NAL-56, NAL-44, NAL-190, NAL-166, NRN-119, NAL-103, NAL-192, NAL-37, NAL-28, NAL-177, NAL-229, NAL-265, NRN-88, NAL-194, NAL-165, NAL-276, NAL-75, NAL-206, NAL-127, NAL-6, NAL-142, NAL-210, NAL-182, NAL-202, NAL-208, NAL-241, NAL-273, NAL-277, NRN-78, NAL-214, NAL-238, NAL-237, NAL-261, NRN-85, NAL-156, NAL-34, NAL-130, NAL-167, NAL-164, NRN-37, NAL-258, NAL-139, NAL-217, NAL-301, NAL-14, NRN-87, NAL-178, NAL-181, NAL-231, NAL-295, NAL-303, NAL-191, NAL-262, NAL-305, NAL-159, NAL-234, NAL-53, NAL-318, NAL-306, NAL-275, NAL-310, NRN-68, NRN-84, NAL-168, NAL-197, NAL-252, NAL-225, NAL-268, NRN-113, NAL-286, NAL-47, NAL-270, NAL-212, NAL-290, NAL-257, NAL-17, NAL-289, NAL-264, NAL-298, NAL-293, NAL-284, NAL-13, NAL-296, NAL-218, NRN-112, NAL-205, and NAL-24	135
4	Susceptible (S)	918.75-1188.75	NAL-163, NAL-185, NAL-291, NRN-115, NAL-250, NAL-170, NAL-274, NAL-147, NAL-236, NAL-267, NAL-11, NAL-246, NAL-302, NAL-285, NAL-317, NAL-294, NRN-42, NRN-116, NAL-23, NAL-145, NRN-44, NAL-141, NAL-171, NAL-187, NAL-272, NAL-5, NAL-312, NAL-288, NAL-15, NAL-248, NAL-216, NAL-18, NAL-304, NAL-259, NAL-316, NAL-9, NAL-242, NAL-22, NAL-126, NAL-96, NAL-263, NAL-281, NAL-255, NAL-297, NAL-198, NAL-10, NAL-226, NAL-254, NAL-154, NAL-269, NAL-314, NAL-174, NAL-211, NAL-161, NAL-299, NAL-253, NAL-235, NAL-169, NAL-247, NAL-251, NAL-209, NAL-308, NAL-123, NAL-239, NAL-222, and NAL-129	66
5	Highly Susceptible (HS)	>1188.75	NAL-144, NAL-215, NAL-309, NAL-143, NAL-146, NAL-313, and NAL-319	7
			Total Genotypes	378

Correlation between AUDPC and thousand kernel weight of the tested wheat genotypes

Correlation analysis showed a highly significant (p≤ 0.01) moderate negative correlation between the AUDPC values of genotypes and days to heading (Figure 9), days to maturity (Figure 10), and number of grains per spike (Figure 6), with Pearson's correlation coefficient (r) equal to -0.555, -0.533, and -0.445, respectively. Moreover, a highly significant (p≤ 0.01) weak negative correlation was observed between AUDPC and kernel weight per spike (r = -0.398) (Figure 7), spike length (r = -0.380) (Figure 8), and thousand kernel weight (r = -0.249) (Figure 5). A highly significant negative correlation (p ≤ 0.01) was observed between AUDPC and thousand kernel weight (gm) (Pearson's correlation coefficient (r = -0.249) with a coefficient of determination (R^2) of 6.2%, which indicates that 6.2% of the variation observed in the thousand kernel weight is caused by the AUDPC (the disease progression) and the remaining proportion is due to other factors. Also, the linear regression equation shows that if there is a unit increase in AUDPC value, the thousand kernel weight decreases by 0.009 (Figure 5). Similar observations were made by (Basnet *et al.*, 2022); (Neupane *et al.*, 2013); (Sharma *et al.*, 2007) and (Tewari *et al.*, 2016), who reported the negative association between AUDPC and thousand kernel weight. However, contrasting result was obtained by Pandey *et al.*, (2018) who found a positive correlation between TKW and AUDPC.

Correlation between AUDPC and number of grains per spike among tested wheat genotypes

A highly significant negative correlation (p≤ 0.01) was observed between AUDPC and the number of grains per spike (r = -0.445), with a coefficient of determination (R^2) of 19.76%, which indicates that 19.76% of the variation observed in the number of grains per spike is caused by AUDPC (the disease progression) and the remaining proportion is due to other factors. Also, the linear regression equation shows that if there is a unit increase in the AUDPC value, the number of grains per spike decreases by 0.007 times (Figure 6). Basnet *et al.* (2022) and Tewari *et al.* (2016) also observed significant negative correlation between AUDPC and the total number of grains per spike. Basnet *et al.* (2022) further explained that it may be due to the lesser accumulation of photosynthates because of decreased photosynthetic area (especially leaves) caused by spot blotch.

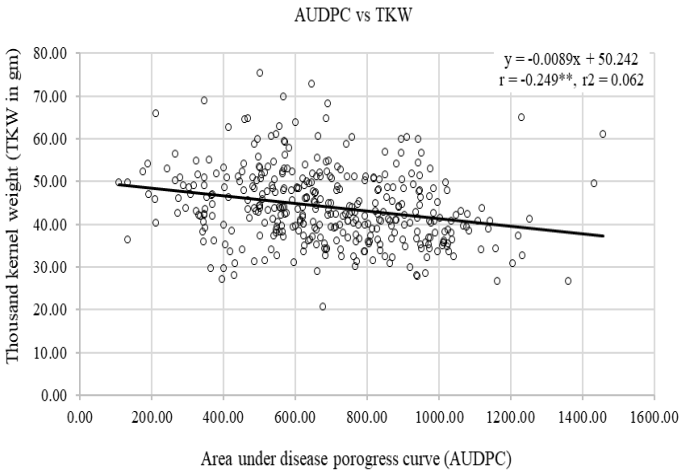

Figure 5. *Correlation between AUDPC and the thousand kernel weight of tested wheat genotypes during 2021/22.*

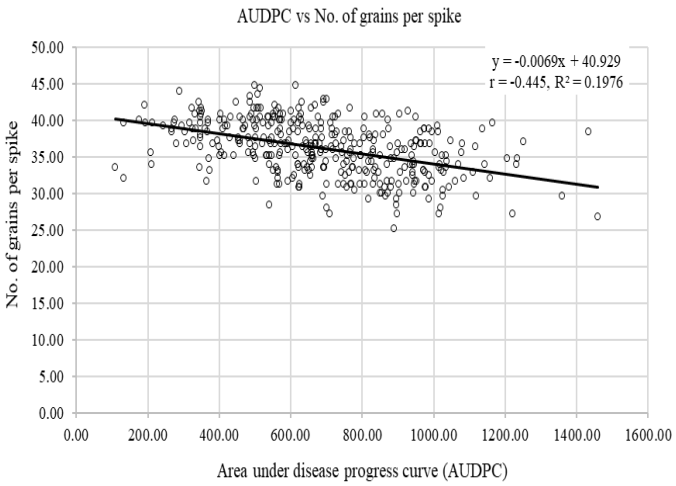

Figure 6. *Correlation between AUDPC and number of grains per spike among tested wheat genotypes during winter 2021/22 at Rampur, Chitwan.*

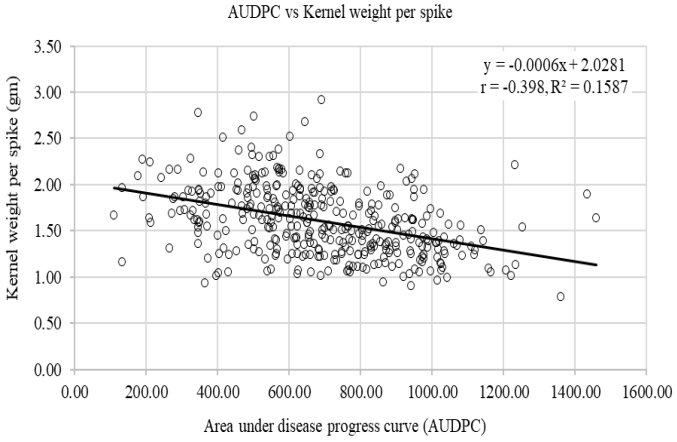

Figure 7. *Correlation between AUDPC and kernel weight per spike among 378 wheat genotypes during winter 2021/22 at Rampur, Chitwan.*

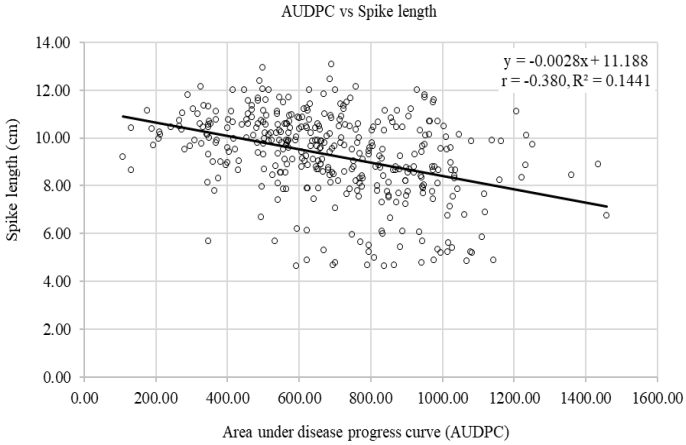

Figure 8. *Correlation between AUDPC and spike length among 378 wheat genotypes during winter 2021/22 at Rampur, Chitwan.*

Correlation between AUDPC and kernel weight per spike

A highly significant negative correlation (p≤ 0.01) was observed between AUDPC and kernel weight per spike (gm) (r = -0.398), with a coefficient of determination of 15.87%, which indicates that 15.87% of the variation observed in kernel weight per spike is caused by AUDPC (the disease progression) and the remaining proportion is due to other factors. Also, the linear regression equation shows that if there is a unit increase in AUDPC value, kernel weight per spike decreases by 0.0006 times (Figure 7).

Correlation between AUDPC and spike length spike

A highly significant negative correlation (p≤ 0.01) was observed between AUDPC and spike length (r = -0.380), with a coefficient of determination of 14.41%, which indicates that 14.41% of the variation observed in average spike length is caused by AUDPC (the disease progression) and the remaining proportion is due to other factors. Also, the linear regression equation shows that if there is a unit increase in the AUDPC value, the spike length decreases by 0.003 times (Figure 8). Paneru *et al.* (2020) also observed negative correlation between AUDPC and spike length.

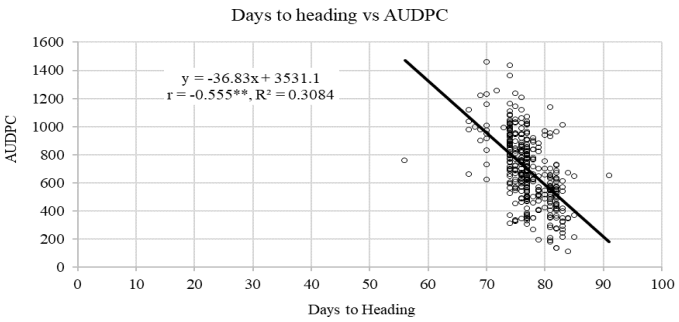

Figure 9. *Correlation between days to heading and AUDPC among 378 wheat genotypes during winter 2021/22 at Rampur, Chitwan.*

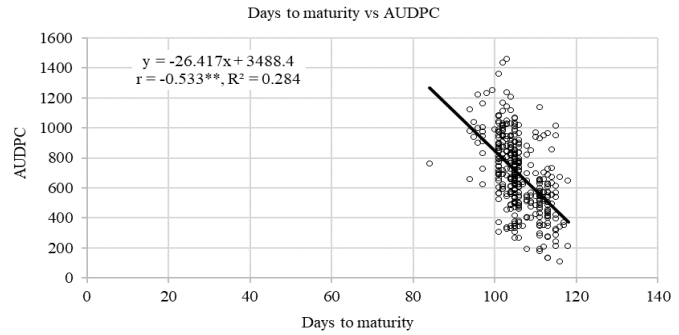

Figure 10. *Correlation between days to maturity and AUDPC among 378 wheat genotypes during winter 2021/22 at Rampur, Chitwan.*

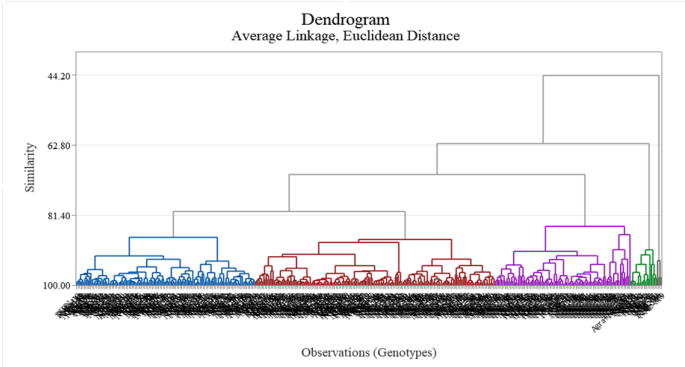

Figure 11. *Dendrogram showing grouping of the genotypes into 5 clusters based on different disease and agronomic parameters.*

Correlation between days to heading and AUDPC

A highly significant moderate negative correlation (p≤ 0.01) was observed between days to heading and AUDPC values (r = -0.555), with a coefficient of determination of 30.84%, which indicates that 30.84% of the variation observed in AUDPC (disease progression) is caused by days to heading and the remaining proportion is due to other factors. Also, the linear regression equation shows that if there is a unit increase in days to heading, the value of AUDPC decreases by 36.83 times (Figure 9). The findings are similar to the results reported by Pandey *et al.* (2018) and Tewari *et al.* (2016). The negative correlation of days to heading with the AUDPC value indicates that early heading leads to more severe disease development while late heading causes less severe disease development Pandey *et al.* (2018). Late-heading genotypes suffer from less severe disease, which may be explained by slower disease development due to the shorter time of exposure of plants to pathogens Duveiller *et al.* (1998).

Correlation between days to maturity and AUDPC

A highly significant negative correlation (p≤ 0.01) was observed between days to maturity and AUDPC values (r = -0.533), with a coefficient of determination of 28.4%, which indicates that 28.4% of the variation observed in AUDPC (disease progression) is caused by days to heading and the remaining proportion is due to other factors. Also, the linear regression equation shows that if there is a unit increase in days to maturity, the value of AUDPC decreases by 26.42 times (Figure 10). Early-maturing genotypes are more susceptible to disease, whereas late-maturing genotypes are more resistant Duveiller *et al.* (2005). A highly significant and strong positive correlation was observed between days to heading and days to maturity (r = +0.949). Table 3 indicates that late-heading genotypes go into maturity later and have a less severe disease. This result is similar to that of Neupane *et al.* (2013).

Cluster analysis based on different disease and agronomic parameters of wheat

The cluster analysis was conducted to categorize the 378 genotypes and 1 susceptible genotype mixture (Agra + Morocco) into 5 cluster groups based on different disease and yield parameters, viz., AUDPC, AUDPC of flag leaf, AUDPC of penultimate leaf, thousand kernel weight, average spike length, number of grains per spike, kernel weight per spike, days to maturity, and days to heading of all the genotypes (Figure 11). Cluster 1 is comprised of 116 genotypes, representing 30.61% of the total genotypes; Cluster 2 is comprised of 155 (40.9%) genotypes, Cluster 3 consisted of 17 (4.49%) genotypes, Cluster 4 comprised of 88 (23.22%) genotypes, and Cluster 5 comprised of 3 (0.79%) genotypes.

Cluster means of different disease and agronomic parameters of wheat genotypes belonging to different clusters

Cluster analysis demonstrated that cluster 3 is superior among all other clusters with the lowest cluster means of AUDPC

Table 3. Correlation between AUDPC, AUDPCF, AUDPCF-1 and agronomic characters of wheat during winter 2021/022 at NMRP, Rampur, Chitwan

	AUDPC	AUDPCF	AUDPC(F-1)	TKW (gm)	DTM (days)	DTH (days)	nGPS	SL (cm)	KWPS (g)
AUDPC	1	.952**	.945**	-.249**	-.533**	-.555**	-.445**	-.380**	-.398**
AUDPCF		1	.798**	-.254**	-.492**	-.524**	-.412**	-.378**	-.395**
AUDPC(F-1)			1	-.217**	-.520**	-.530**	-.431**	-.341**	-.360**
TKW (gm)				1	0.00	0.00	.139**	.298**	.868**
DTM (days)					1	.949**	.376**	.293**	.176**
DTH (days)						1	.372**	.278**	.154**
nGPS							1	.692**	.566**
SL (cm)								1	.559**
KWPS (g)									1

Note: * = significant at 5% level of significance (2-tailed), ** = significant at 1% level of significance (2-tailed), AUDPC = area under disease progress curve, AUDPCF = AUDPC of flag leaf, AUDPC(F-1) = AUDPC of penultimate leaf, TKW = thousand kernel weight, DTM = days to maturity, DTH = days to heading, nGPS = number of grains per spike, SL = spike length, KWPS = kernel weight per spike.

Table 4. Cluster means of different disease and yield parameters of genotypes belonging to different clusters.

Variable	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Grand centroid
AUDPCF	166.03	389.24	73.53	751.61	1311.67	398.20
AUDPC(F-1)	770.25	1048.24	369.27	1236.82	1523.33	980.25
Av. AUDPC	468.15	718.74	221.40	994.22	1417.50	689.23
SL (cm)	10.05	9.14	10.24	8.36	8.03	9.28
nGPS	38.17	35.89	38.21	33.87	31.60	36.19
KWPS	1.77	1.57	1.84	1.40	1.44	1.60
TKW (gm)	46.33	43.39	49.16	41.28	45.69	44.08
DTM (days)	108.75	105.15	111.53	102.77	102.00	105.96
DTH (days)	79.22	76.65	81.41	74.68	72.67	77.16

AUDPC = area under disease progress curve, AUDPCF = AUDPC of flag leaf, AUDPC(F-1) = AUDPC of penultimate leaf, Av. AUDPC = average AUDPC (of AUDPCF and AUDPC(F-1)), SL = spike length, nGPS = number of grains per spike, KWPS = kernel weight per spike, TKW = thousand kernel weight, DTM = days to maturity, DTH = days to heading.

Table 5. 17 superior genotypes in terms of disease and agronomic parameters of the tested genotypes belonging to cluster 3.

Genotype	AUDPCF	AUDPC (F-1)	Av. AUDPC	TKW (g)	n GPS	SL (cm)	KWPS (gm)	DTM (days)	DTH (days)
NRN-34	135.00	442.50	288.75	49.05	44.00	11.80	2.16	115	83
NRN-41	52.50	480.00	266.25	50.05	38.80	10.72	1.31	105	83
NRN-51	65.00	360.00	212.50	40.21	39.60	10.15	1.59	115	83
NRN-52	120.00	260.00	190.00	54.01	42.00	10.39	2.27	111	81
NRN-55	125.00	420.00	272.50	42.64	39.60	10.35	1.69	113	82
NRN-61	115.00	435.00	275.00	46.11	40.00	11.05	1.84	113	82
NRN-62	120.00	440.00	280.00	50.74	36.80	9.52	1.87	111	81
NAL-12	55.00	367.50	211.25	65.87	34.00	10.25	2.24	118	85
NAL-25	50.00	367.50	208.75	45.85	35.60	9.95	1.63	112	81
NAL-35	25.00	192.50	108.75	49.67	33.60	9.20	1.67	116	84
NAL-36	235.00	380.00	307.50	47.01	36.80	9.90	1.73	101	74
NAL-39	0.00	265.00	132.50	36.38	32.00	8.65	1.16	113	82
NAL-43	60.00	472.50	266.25	56.33	38.40	10.50	2.16	106	78
NAL-57	60.00	425.00	242.50	52.88	39.20	10.45	2.07	115	83
NAL-73	2.50	350.00	176.25	52.21	40.00	11.15	2.09	111	81
NAL-82	0.00	385.00	192.50	46.99	39.60	9.70	1.86	108	79
NAL-94	30.00	235.00	132.50	49.72	39.60	10.40	1.97	113	82

Note: AUDPC = Area under disease progress curve, AUDPCF = AUDPC of flag leaf, AUDPCF-1 = AUDPC of penultimate leaf, Av. AUDPC = Average of AUDPCF and AUDPCF-1, TKW = thousand kernel weight, nGPS = number of grains per spike, SL = spike length, KWPS = kernel weight per spike, DTM = days to maturity, DTH = days to heading.

(221.40), AUDPC of flag leaf (73.53), AUDPC of penultimate leaf (369.27), and the highest cluster means of thousand kernel weight (49.16 g), number of grains per spike (38.21), spike length (10.24 cm), kernel weight per spike (1.84), days to heading (81.41 days), and days to maturity (111.53 days), as shown in (Table 4). Moreover, Cluster 3 consisted of genotypes NAL-35, NAL-94, NAL-39, NAL-73, NRN-52, NAL-82, NAL-25, NAL-12, NRN-51, NAL-57, NRN-41, NAL-43, NRN-55, NRN-61, NRN-62, NRN-34, and NAL-36, which were superior in terms of all the parameters observed. Similarly, cluster 5 consisted of the genotypes NAL-146, NAL-313, and NAL-319 that are inferior in terms of all the disease parameters observed, i.e., AUDPC (1417.50), AUDPC of flag leaf (1311.67), and AUDPC of penultimate leaf (1523.33), and yield and growth stage parameters like number of grains per spike (31.60), average spike length (8.36 cm), days to heading (72.67 days), and days to maturity (102 days), but with the second lowest mean kernel weight per spike (1.44 g) and the third lowest mean thousand kernel weight (45.69 g).

Genotypes in terms of disease and agronomic parameters of the tested genotypes belonging to cluster 3

Cluster 3 consists of 17 genotypes that are superior to other genotypes tested based on disease parameters and different yield and growth traits (Table 5). Moreover, these 17 genotypes also fall among the 43 resistant genotypes categorized based on AUDPC earlier (Table 2). Among these 17 genotypes, NAL-12 was found to be the most resistant (AUDPC = 211.25) with the maximum TKW (65.87 g), followed by NAL-43 (56.33 g), NRN-52 (54.01 g), NAL-57 (52.88 g), NAL-73 (52.21 g), and NRN-62 (50.74 g). Similarly, the genotype NRN-34 was found to have the maximum number of grains per spike (44) followed by NRN-52 (42), NAL-73 (40), NRN-61 (40), NAL-94 (39.6), NAL-82 (39.6), NRN-55 (39.6), and NRN-51 (39.6). Moreover, the genotype NAL-12 demonstrated maximum days to heading (85 days) and maximum days to maturity (118 days), followed by NAL-35 (84 and 116 days), NRN-51 (115 and 83 days), NAL-57 (115 and 83 days), NRN-34 (113 and 82 days), NAL-94 (113 and 82 days), NAL-39 (113 and 82 days), NRN-55 (113 and 82 days), and NRN -61 (113 and 82 days). Furthermore, maximum spike length was observed in NRN-34 (11.80 cm), followed by NAL-73 (11.15 cm), NRN-61 (11.05 cm), NRN-41 (10.72 cm), NAL-43 (10.5 cm), and NAL-57 (10.45 cm). Lastly, kernel weight per spike was observed in NRN-52 (2.27 g), followed by NAL-12 (2.24 gm), NAL-43 (2.16 g), NRN-34 (2.16 g), NAL-73 (2.09 g), and NAL-57 (2.07 g).

Conclusion

43 genotypes were found to be resistant based on AUDPC among the 378 genotypes tested, Cluster analysis showed 17 of the 43 resistant genotypes to be superior to the remaining genotypes. NRN-34 was ranked best among the genotypes, followed by NAL-73, NAL-94, NAL-12, NRN-34, NAL-57, NAL-43, NAL-82, and NAL-35, based on their lower values of AUDPC and higher values of yield-attributing characters than all other genotypes. Though the disease severity of these genotypes increased over time, the higher values of yield-attributing characters suggested that selection of these genotypes can be useful for further breeding programs to develop improved and spot-blotch-resistant varieties and enhance the productivity of wheat in the inner Terai region of Nepal. To address the immediate challenge posed by spot blotch in Nepal, wheat genotypes that have consistently demonstrated tolerant reactions against the disease in various locations could be used as such, or resistance could be transferred using a cyclic breeding program into commercial varieties. This research could also be beneficial to these genotypes.

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DECLARATIONS

Authors contribution

Conceptualization, methodology: A.P., A.M. and B.S.B.G.; Software, validation: A.P. and A.B.; Investigation: A.P., A.M., A.I. and O.K..; Data curation: A.M. and O.K.; Writing -original draft preparation: A.P., A.M. and S.Y.; Writing-review and editing: A.P. and S.B.G.; Supervision: S.B.G., A.M. and S.Y. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

Acharya, K., Dutta, A. K., & Pradhan, P. (2011). *'Bipolaris sorokiniana'*(Sacc.) Shoem.: The most destructive wheat fungal pathogen in the warmer areas. *Australian Journal of Crop Science*, *5*(9), 1064–1071.

Aggarwal, R., Gupta, S., Banerjee, S., & Singh, V. B. (2011). Development of a SCAR marker for detection of *Bipolaris sorokiniana* causing spot blotch of wheat. *Canadian Journal of Microbiology*, *57*(11), 934–942. https://doi.org/10.1139/w11-089

Basnet, R., Shrestha, S. M., & Bandari, D. (2022). Performance of Wheat (*Triticum aestivum* L.) Genotypes for Spot Blotch (*Bipolaris sorokiniana* Sacc.) Resistance in Terai Condition of Nepal. *International Journal of Research and Review*, *9*(8), 894–906. https://doi.org/10.52403/ijrr.20220879

Basnet, R., Shrestha, S. M., Bandari, D., Manandhar, H. K., & Thapa, D. B. (2022). Evaluation of wheat (*Triticum aestivum* L.) genotypes for spot blotch (*Bipolaris sorokiniana* Sacc) resistance in terai condition of Nepal. *Archives of Agriculture and Environmental Science*, *7*(3), 440–449.

Das, M. K., Rajaram, S., Mundt, C. C., & Kronstad, W. E. (1992). Inheritance of Slow-Rusting Resistance to Leaf Rust in Wheat. *Crop Science*, *32*(6), 1452–1456. https://doi.org/10.2135/cropsci1992.0011183X003200060028x

Duveiller, E. (2004). Controlling Foliar Blights of Wheat in the Rice-Wheat Systems of Asia. *Plant Disease*, *88*(5), 552–556. https://doi.org/10.1094/PDIS.2004.88.5.552

Duveiller, E., Garcia, I., Franco, J., Toledo, J., Crossa, J., & Lopez, F. (1998). Evaluating spot blotch resistance of wheat: Improving disease assessment under controlled conditions and in the field. *Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot*, 171–181.

Duveiller, E., Kandel, Y. R., Sharma, R. C., & Shrestha, S. M. (2005). Epidemiology of Foliar Blights (Spot Blotch and Tan Spot) of Wheat in the Plains Bordering the Himalayas. *Phytopathology®*, *95*(3), 248–256. https://doi.org/10.1094/PHYTO-95-0248

Eyal, Z. (1987). The Septoria diseases of wheat: Concepts and methods of disease management. Cimmyt.

FAO. (2022). Food and Agriculture Organization. http://www.fao.org/faostat/en/No. of data/QC.

Gupta, P. K., Chand, R., Vasistha, N. K., Pandey, S. P., Kumar, U., Mishra, V. K., & Joshi, A. K. (2018). Spot blotch disease of wheat: The current status of research on genetics and breeding. *Plant Pathology*, *67*(3), 508–531. https://doi.org/10.1111/ppa.12781

Joshi, A. K., & Chand, R. (2002). Variation and inheritance of leaf angle, and its association with spot blotch (*Bipolaris sorokiniana*) severity in wheat (*Triticum aestivum*). *Euphytica*, *124*(3), 283–291. https://doi.org/10.1023/A:1015773404694

Joshi, A. K., Ortiz-Ferrara, G., Crossa, J., Singh, G., Alvarado, G., Bhatta, M. R., Duveiller, E., Sharma, R. C., Pandit, D. B., Siddique, A. B., Das, S. Y., Sharma, R. N., & Chand, R. (2007). Associations of Environments in South Asia Based on Spot Blotch Disease of Wheat Caused by *Cochliobolus sativus*. *Crop Science*, *47*(3), 1071–1081. https://doi.org/10.2135/cropsci2006.07.0477

Lozano-Ramirez, N., Dreisigacker, S., Sansaloni, C. P., He, X., Sandoval-Islas, J. S., Pérez-Rodríguez, P., Carballo, A. C., Diaz, C. N., Kishii, M., & Singh, P. K. (2022). Genome-wide association study for spot blotch resistance in synthetic hexaploid wheat. *Genes*, *13*(8), 1387.

Mahto, B. N. (2001). Effect of *helminthosporium* leaf blight on yield components and determination of resistance in selected wheat varieties. https://www.cabidigitallibrary.org/doi/full/10.5555/20023013416

MoALD. (2022). Agriculture Statistics | Publication Category | Ministry of Agriculture and Livestock Development. https://moald.gov.np/publication-types/agriculture-statistics/

Nepal, S., Shrestha, S. M., Manadhar, H. K., & Yadav, R. K. (2020). Field response of wheat genotypes to spot blotch under different sowing dates at Rampur, Chitwan, Nepal. *Journal of Agriculture and Forestry University*, 83–90.

Neupane, S. P., Ojha, B. R., Ghimire, S. K., Sah, S. K., Shrestha, S. M., & Puri, R. R. (2013). Morpho-physiology of wheat genotypes under different sowing dates as affected by *Helminthosporium* leaf blight and leaf rust in Chiwan, Nepal. *Agronomy Journal of Nepal*, *3*, 109–116.

Ownley, B. H., & Trigiano, R. N. (2016). *Plant pathology concepts and laboratory exercises*. CRC press.

Pandey, A., Paudel, R., Kafle, K., Sharma, M., Maharjan, N., Das, N., & Basnet, R. (2018). Varietal Screening of wheat genotypes against spot blotch disease (*Bipolaris sorokiniana*) under field condition at Bhairahawa, Nepal. *Journal of the Institute of Agriculture and Animal Science*, *35*(1), 267–276.

Paneru, A., Gahatraj, B., Poudel, M., Subedi, P., Sovana, G. C., & Paudel, R. (2020). Field Experiment to Evaluate the Response of Different Varieties of Wheat against Spot Blotch (*Bipolaris sorokiniana*) at Bhairahawa, Nepal. *Plant Pathology and Microbiology*, *11*, 525.

Regmi, S., Poudel, B., Ojha, B. R., Kharel, R., Joshi, P., Khanal, S., & Kandel, B. P. (2021). Estimation of genetic parameters of different wheat genotype traits in Chitwan, Nepal. *International Journal of Agronomy*, *2021*, 1–10.

Saari, E. E. (1998). Leaf blight disease and associated soil-borne fungal pathogens of wheat in South and South East Asia. *Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot*, 37–51.

Sharma, R. C., Duveiller, E., & Ortiz-Ferrara, G. (2007). Progress and challenge towards reducing wheat spot blotch threat in the Eastern Gangetic Plains of South Asia: Is climate change already taking its toll? *Field Crops Research*, *103*(2), 109–118.

Tewari, R., Jaiswal, J., Kumar, A., & Singh, P. (2016). Analysis of spot blotch resistant and its association with yield and its relation with yield and its related traits in bread wheat (*Triticum aestivum* L.) germplasm. *Growth*, *1*(2), 3.

Wiese, M. V. (1987). Compendium of wheat diseases. *American Phytopathological Society*. https://www.cabdirect.org/cabdirect/abstract/19901124690