Eur J Plant Pathol (2011) 131:249–260
DOI 10.1007/s10658-011-9803-5

Assessing genetic resistance to spot blotch, *Stagonospora nodorum* blotch and tan spot in wheat from Nepal

B. N. Mahto · S. Gurung · Tika B. Adhikari

Accepted: 5 May 2011 / Published online: 26 May 2011

Abstract Fungal leaf spot diseases of wheat (*Triticum aestivum* L.) in Nepal cause significant yield reduction. Although field testing has identified a few partially resistant cultivars, most wheat grown in Nepal lacks adequate resistance to leaf spot diseases. During 2009–2010, 116 local and commercial spring wheat cultivars and advanced breeding lines were selected from multi-year field experiments in Nepal and evaluated for seedling resistance to three leaf spot diseases: spot blotch, *Stagonospora nodorum* blotch (SNB) and tan spot races 1 and 5 (two of the most prevalent races) in the growth chambers at North Dakota State University, Fargo, ND, USA. The wheat cultivars and lines were artificially inoculated with individual pathogens or races at the two-leaf stage and disease reactions were evaluated 6 to 10 days after inoculation (DAI). Results indicated that 30%, 31%, 19% and 10% of the tested wheat cultivars and lines were resistant to spot blotch, SNB, tan spot races 1 and 5, respectively. Six advanced breeding lines (SW89-5422, BL 2127 = DANIAL88/HLB30//NL297, BL 3033, FILIN/IRENA/5/CNDO/R143//ENTE/MEXI-2/3/AE. SQUA (TAUS)/4WEAVER, GAN/AE.SQUARROSA (236)//DOY1/AE.SQUARROSA(447)/3/MAIZ/4/INQALAB91, Mayoor//TK SN1081/Ae. Squarrosa (222)/3/FCT, were resistant to spot blotch, SNB and tan spot race 1. Similarly, two wheat cultivars Chirya 3 and Chirya 7 were resistant to spot blotch, and tan spot races 1 and 5. The resistant wheat lines identified in this study represent potentially useful and untapped sources of resistance to multiple leaf spot diseases and should be utilized in wheat breeding programs in Nepal in order to develop wheat cultivars with broad-spectrum resistance.

Keywords *Cochliobolus sativus* · Disease resistance · *Phaeosphaeria nodorum* · *Pyrenophora tritici-repentis* · *Triticum aestivum* L.

Abbreviations

CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo (International Maize and Wheat Improvement Center)
DAI	Days after inoculation
NARC	Nepal Agricultural Research Council
NDSU	North Dakota State University
NWRP	Nepal Wheat Research Program
PTR	*Pyrenophora tritici-repentis*
RCBD	Randomized complete block design
SNB	*Stagonospora nodorum* blotch

B. N. Mahto · S. Gurung · T. B. Adhikari (✉)
Department of Plant Pathology,
North Dakota State University,
NDSU Dept. 7660, P. O. Box 6050, Fargo,
ND 58108, USA
e-mail: tika.adhikari@ndsu.edu

Present Address:
B. N. Mahto
Division of Plant Pathology,
Nepal Agricultural Research Council (NARC),
Khumaltar,
Lalitpur, Nepal

Introduction

Spot blotch, caused by *Cochliobolus sativus* [(Ito & Kurib.) drechs. ex Dastur (*Bipolaris sorokiniana* (Sacc.) Shoemaker (*Helminthosporium sativum* Pammel, King and Bakke)], and tan spot, caused by *Pyrenophora tritici-repentis* (Died.) Drechs., are two of the most important biological constraints limiting wheat production in South Asia, including Nepal (Dubin and van Ginkel 1991; Dubin and Duveiller 2000; Duveiller 2002). *Stagonospora nodorum* blotch (SNB), caused by *Phaeosphaeria nodorum* (E. Müller) Hedjaroude (anamorph: *Stagonospora nodorum* (Berk.) E. Castellani & E.G. Germano), is another destructive disease of wheat worldwide (Eyal 1999). Compared to spot blotch and tan spot, SNB has not yet been a serious problem in Nepal. However, these leaf spot diseases together can cause yield losses as high as 50% (Riede et al. 1996).

Recently, epidemics of spot blotch and tan spot in wheat have increased in many parts of Nepal. This trend may be due to changes in climate, cultivation practices, or pathogen populations (De Wolf et al. 1998; Ortiz et al. 2008). Although the application of fungicides is one of the strategies to manage these diseases (Dubin and Duveiller 2000), resource-poor farmers find the practice unprofitable and unsafe. Host plant resistance is an alternative approach to manage leaf spot diseases of wheat (Eyal 1999; Duveiller 2002; Sharma et al. 2004b). In the past decades, the National Wheat Research Program (NWRP) and other institutions have conducted research on wheat breeding, either independently or in collaboration with the International Maize and Wheat Improvement Center (CIMMYT), South Asia regional office in Kathmandu, Nepal. However, progress in breeding wheat for resistance to leaf spot diseases has been slow. Although considerable progress has been made on the epidemiological aspects (Duveiller et al. 2005) and multi-location testing of wheat against spot blotch in Bangladesh, India and Nepal (Sharma et al. 2004b), information on multiple disease resistance is still lacking.

Sources of resistance to multiple leaf spot diseases have been identified in wheat in other countries (Ali et al. 2008; Friesen et al. 2008; Gurung et al. 2009; Singh et al. 2006). Although complete resistance has not been found, a few cultivars with partial resistant to multiple leaf spot diseases exist. Resistance to spot blotch in wheat has been evaluated in field trails (Dubin and Duveiller 2000; Duveiller 2002; Sharma and Duveiller 2007; Sharma et al. 2004a, b); however, a systematic assessment of wheat germplasm has not been conducted for reactions to spot blotch, SNB, and tan spot in Nepal.

Most of the wheat cultivars grown in Nepal were from Brazil, China, CIMMYT (Mexico), and Zambia. It is still unclear whether or not wheat cultivars and lines currently available in Nepal are resistant to multiple leaf spot diseases. Recent studies have also shown that yield reductions from multiple leaf spot diseases of wheat were more than 20% (Duveiller et al. 2005; Sharma and Duveiller 2007). Therefore, there is an urgent need to search for resistant wheat germplasm that can be crossed with commercial cultivars possessing low to moderate levels of resistance to leaf spot diseases. The main objective of this study was to evaluate and identify new sources of resistance to spot blotch, SNB and tan spot in Nepalese wheat germplasm.

Materials and methods

Selection of wheat cultivars

A total of 116 promising spring wheat germplasm were selected from foliar diseases screening tested over several years (between 2000 and 2008) at NWRP, Bhairahwa, Rupandehi, Nepal. Seeds of local wheat cultivars, commercial varieties, and advanced breeding lines were shipped to North Dakota State University (NDSU), Fargo, ND from Nepal.

Sowing and plant growing conditions

The plastic cones (3.8 cm in diameter and 20 cm long) (Stuewe and Sons, Inc., Tangent, OR) were filled with Fison Sunshine Mix #1 (Fison Horticulture, Vancouver, BC) and seeds of each cultivar were planted in a cone. Each cone was fertilized with 2.5 g of a slow-release commercial fertilizer (Multicote 14-14-16, N-P-K, Sungro Horticulture Distribution Inc., Bellevue, WA) at planting. Three seeds were planted in each cone, and each cultivar was planted in three cones. All cones were placed in plastic trays (Town for Stuewe and Sons, Inc.,). Unless otherwise stated, Salamouni and ND495 were used in all experiments as resistant and susceptible checks, respectively. Seedlings were grown in a

greenhouse and temperatures were maintained between 22 and 25°C with 16 h of supplemental light provided by a 400 W Lucalox LU400 sodium vapor (General Electric Co., Cleveland, OH). All experiments were conducted at NDSU, Fargo, ND from 2009 to 2010.

Evaluation for spot blotch resistance

A virulent isolate CS 45 of *C. sativus* collected during the 2009 season from Bhairahwa, Nepal (Mahto et al. 2010) was used. Inoculum was prepared by placing dried mycelia plugs on the centre of 10-cm Petri plates containing potato dextrose agar (PDA) supplemented with V8 juice (V8-PDA, 150 ml V8 juice, 10 g Difco PDA, 10 g Difco agar, 3 g calcium carbonate, and 850 ml distilled water). The plates with fungal cultures were incubated at 21°C with continuous fluorescent light for 10 days. Conidia were harvested by adding 25 ml sterile distilled water to each plate and by scraping the agar surface with a platinum loop. This conidial suspension was filtered through four layers of cheese-cloth to remove mycelial fragments and was adjusted to 3000 conidia ml^{-1} using a haemocytometer. To facilitate the dispersion and adherence of conidia over the leaf surface, Tween 20 (polyoxyethylene-20-sorbitan monolaurate) was added (100 μl/l) to the conidial suspension. Seedlings were spray-inoculated with a conidial suspension until runoff 14 days after planting. Chirya 3 (Sharma and Duveiller 2007), and ND495 were planted as checks to verify resistance and susceptible reactions, respectively. Following inoculation, seedlings were transferred into a mist chamber at 21°C with 100% relative humidity and misted for 16 s every 4 minutes to provide continuous leaf wetness. After 24 h, seedlings were moved into a growth chamber with a 22/18°C (day/night) temperature and a 16-h photoperiod. The spot blotch severity, expressed as infection response (IR), was assessed on the second leaf of each seedling 10 days after inoculation (DAI) using the 1 to 9 rating scale (Fetch and Steffenson 1999), where median disease scores 1 to 4 were resistant (R) and median disease scores > 4 were susceptible (S).

Evaluation for SNB resistance

To evaluate resistance to SNB, seedlings were inoculated with isolate Sn2000 of *P. nodorum* (Ali et al. 2008; Gurung et al. 2009). This isolate was originally collected from a wheat field in North Dakota and maintained at the Department of Plant Pathology, NDSU. Two dried mycelia plugs were placed in the centre of 10-cm Petri plates containing V8-PDA to produce pycnidiospores. The plates with fungal cultures were incubated at 21°C in darkness for 5 days. Pycniodospores were collected by adding 30 ml sterile distilled water to each plate and scraping the agar surface with a platinum loop. The pycnidial suspension was filtered through four layers of cheese-cloth and the pycniodospore concentration was adjusted to 1×10^6 ml^{-1} using a haemocytometer. Tween 20 was added (100 ul/l) to the inoculum suspension to facilitate uniform dispersion of the pycnidia over the leaf surface. Fully expanded the second leaves of two-week-old seedlings were spray-inoculated with the pycnidial suspension until runoff. Following inoculation, seedlings were moved into a mist chamber, incubated for 24 h to initiate infection, and later transferred into a growth chamber with a 22/18°C (day/night) temperature and a 16-h photoperiod. Disease reactions were assessed on the second leaf of each seedling 8 DAI using a rating scale of 0 to 5 (Liu et al. 2004). On the scale, median disease scores ≤ 2 were R and median disease scores > 2 were S.

Evaluation for tan spot resistance

For tan spot race 1 and race 5, seedlings were inoculated separately with the prevalent tan spot race 1 (isolate # Pti2) and race 5 (isolate # DW7). Isolate # Pti2 was originally collected from South Dakota while isolate # DW7 was collected from a durum wheat field in North Dakota in 1998. Inocula of both races 1 and 5 were prepared according to methods as described previously (Lamari and Bernier 1989). A single-dried mycelium plug of each isolate was placed in the centre of 10-cm Petri plates containing V8-PDA. The plates with fungal cultures were incubated at 21°C in darkness for 5 days, and then 20 ml sterile distilled water was added to each plate. The mycelial growth was flattened with the bottom of sterile glass tube. Excess water was drained and the plates were further incubated under continuous light for 24 h at 21°C, followed by 24 h in darkness at 16°C. Conidia were obtained by adding 25 ml sterile distilled water to each plate and by scraping the agar surface with a platinum loop. Each conidial suspension was filtered

through two layers of cheese-cloth and the concentration adjusted to 3000 conidia ml^{-1} using a hemacytometer. Tween 20 was added (100 μl/l) to the suspension to facilitate dispersion of the inoculum over the leaf surface. Two-week-old seedlings were spray-inoculated with the conidial suspension until runoff. Following inoculation, the seedlings were transferred into a mist chamber, incubated for 24 h to initiate infection and later moved into a growth chamber with a 22/18°C (day/night) temperature and a 16-h photoperiod. Between 6–7 DAI, disease reactions were assessed on the second leaf of each seedling using a 1 to 5 scale lesion-type rating system (Lamari and Bernier (1989), where median disease scores of 1.0 to 2.0 were R and median disease scores above 2.0 were S.

Experimental design and data analysis

Each experiment was conducted in a randomized complete block design (RCBD) and replicated three times. Each treatment consisted of three seedlings per replication (total of nine seedlings per cultivar). For each seedling, the second inoculated-leaf was assessed and considered as an experimental unit. Disease scores of each disease was analyzed separately using nonparametric analysis. Median, mean rank (R_{ij}), relative treatment effects (p_{ij}) and 95% confidence intervals (CI) were calculated using PROC MIXED procedure in SAS Version 9.2 (SAS Institute, Cary, NC, 2010).

Results

Resistance to spot blotch

Chirya 3 is moderately resistant (median disease score= 3), while ND495 is highly susceptible (median disease score=7) to *C. sativus* (Table 1). Of the wheat cultivars and lines tested, 30% were resistant, and the remaining lines were susceptible (Fig. 1a). Among susceptible cultivars, 14 were highly susceptible with median disease scores >7.0 (*data not shown*). Eleven resistant wheat breeding lines: AE. SQ (205)/5/BP10*3/4/IAS55*4/CT14/23/3/IAS5S*4/EG.AUS//IAS55*4/ALD, NL 922 = AE. SQ(205)/5/BR 12*3/4/IAS55*4/CI 14123/IAS55*4/EGA.US//IAS 55*4, CHIRYA 7, FILIN/IRENA/5/CNDO/R143//ENTE/MEXI-2/3/AE.SQUA(TAUS)/4WEAVER, BL 2069 = NL 297*4/NL505, Mayoor//TK SN1081/Ae.Squarrosa(222)/3/FCT, BL 3033, BL 3036, BH 1150, BL 4155, BL1496/MILAN/3/CROC_1AE.SQUARROSA(205)//KAUZ had median disease scores <3.0 (Table 1). Two advanced breeding lines: ALTAR84/AE.SQUARROSA(221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLLI, CHEN/AEGLOPSSQUARROSA(TAUS)//BCN/3/BAV92 were highly resistant and had a median disease score of 2. The remaining 22 resistant lines had a median disease score of 4 (Table 1).

Resistance to SNB

Of the 116 wheat cultivars and lines evaluated, 69% were susceptible and 31% were resistant to SNB (Fig. 1b). Among susceptible cultivars, 34% were highly susceptible to SNB with median disease scores >4 (data not shown). Twelve breeding lines: BL 1905 = ZSH12/HLB19/NL297, AE.SQ (205)/5/BP10*3/4/IAS55*4/CT14/23/3/IAS5S*4/EG.AUS//IAS55*4/ALD, BL 1910 = ZSH23/HLB15//NL297, NL 923 = (205)/5/BR 2*3/4/IAS55*4/CI14123/3/IAS 55*4/EG.AUS/IAS 55*4, SW89-5193, SW89-5422, BL 2631 = PBW 343/CHIRYA 3, (205)/5/BR/2*3/4/IAS55*4/CI14/23/3/IAS55*4/EG.AUS//IAS55*4/ALD, GAN/AE.SQUARROSA(236)//DOY1/AE.SQUARROSA(447)/3/MAIZ/4/INQALAB91, BL 2827, BL 2710, ALTAR84/AE.SQUARROSA(221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLLI) were highly resistant to SNB, with an median disease score of 1.0. The remaining 24 resistant lines had a median disease score of 2 (Table 2).

Resistance to tan spot races 1 and 5

Salamouni was resistant to both races 1 and 5 (median disease score=2), while susceptible check ND495 was susceptible to both races 1 and 5 of *P. tritici-repentis* with a median disease score of 5 (Tables 3 and 4). Of 116 wheat cultivars and lines assessed, 19% were resistant and 81% were susceptible to race 1 (Fig. 1c). Five wheat lines: Mayoor//TK SN1081/Ae.Squarrosa(222)/3/FCT, NL 750, SW89-5193, SW89-5422, FILIN/IRENA/5/CNDO/R143//ENTE/MEXI-2/3/AE.SQUA(TAUS)/4WEAVER were highly resistant to race 1 and had a median disease score of 1.0 (Table 3). Among resistant lines, seventeen lines had median disease scores of 2 (Table 3).

Table 1 Wheat cultivars resistant to *Cochliobolus sativus*[a]

Name of entry or pedigree	Median[b]	R_{ij}[c]	p_{ij}[d]	95% CI for p_{ij}[e]
BL 2127 = DANIAL88/HLB30//NL297	4	299.9	0.3	(0.237, 0.341)
AE.SQ(205)/5/BP10*3/4/IAS55*4/CT14/23/3/IAS5S*4/EG.AUS//IAS55*4/ALD	3	253.2	0.2	(0.136, 0.392)
NL 922 = AE.SQ(205)/5/BR 12*3/4/IAS55*4/CI 14123/IAS55*4/EGA.US//IAS 55*4	3	150.5	0.1	(0.096, 0.210)
CHIRYA 7	3	225.7	0.2	(0.189, 0.361)
FILIN/IRENA/5/CNDO/R143//ENTE/MEXI-2/3/AE.SQUA(TAUS)/4WEAVER	3	63.8	0.1	(0.041, 0.089)
BL 2069 = NL 297*4/NL505	3	73.6	0.1	(0.052, 0.092)
GAN/AE.SQUARROSA(236)//DOY1/AE.SQUARROSA(447)/3/MAIZ/4/INQALAB91	4	354.8	0.3	(0.267, 0.419)
Mayoor//TK SN1081/Ae.Squarrosa (222)/3/FCT	3	154.9	0.1	(0.090, 0.233)
Yangmai No.6	4	283.5	0.3	(0.141, 0.458)
BL 3033	3	142.6	0.1	(0.085, 0.210)
BL 3036	3	162.9	0.2	(0.100, 0.233)
BH 1150	3	225.7	0.2	(0.189, 0.561)
FILIN	4	238.9	0.2	(0.154, 0.324)
NL 1012	4	191.2	0.2	(0.163, 0.478)
BL 1048	4	307.1	0.3	(0.130, 0.250)
BL 3704	4	259.3	0.2	(0.217, 0.383)
NL 1051	4	361.9	0.3	(0.178, 0.363)
NL 1055	4	327.4	0.3	(0.254, 0.451)
NL 1047	4	211.5	0.2	(0.249, 0.384)
BL 4004	4	251.3	0.2	(0.289, 0.449)
BL 4092	4	279.6	0.3	(0.164, 0.336)
BL 4107	4	299.9	0.3	(0.205, 0.340)
BL 4123	4	299.9	0.3	(0.237, 0.341)
BL 4154	4	252.2	0.2	(0.237, 0.341)
AC8528/WBLLI	4	299.9	0.3	(0.204, 0.282)
ALTAR84/AE.SQUARROSA(221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLLI	2	18.0	0.0	(0.237, 0.341)
BL1496/MILAN/3/CROC_1AE.SQUARROSA(205)//KAUZ	3	93.9	0.1	(0.013, 0.021)
CHEN/AEGLOPSSQUARROSA(TAUS)//BCN/3/BAV92	2	49.8	0.0	(0.023, 0.068)
SW89-5422	4	211.5	0.2	(0.233, 0.476)
BL 4155	3	130.2	0.1	(0.151, 0.265)
BL 4162	4	259.3	0.2	(0.082, 0.183)
WK 1204	4	259.3	0.2	(0.178, 0.333)
WK 1182	4	327.4	0.3	(0.249, 0.384)
WK 1625	4	307.1	0.3	(0.217, 0.383)
WK 1622	4	305.5	0.3	(0.194, 0.413)
Chriya 3 (resistant check)	3	49.8	0.1	(0.038, 0.068)
ND 495 (susceptible check)	7	851.6	0.8	(0.702, 0.891)

[a] Seedlings were scored for disease reactions 10 days after inoculation with *Cochliobolus sativus* (isolate CS 45) according to a 1 to 9 rating scale (Fetch and Steffenson 1999), where median disease scores between 1 to 4 are resistant (R) and median disease scores > 4 were susceptible (S). Chirya 3 and ND495 were used as resistant and susceptible checks, respectively

[b] Median

[c] Mean rank (R_{ij})

[d] Relative treatment effects (p_{ij}) and

[e] 95% confidence intervals (CI) were calculated from nonparametric analysis using PROC MIXED procedure in SAS (Version 9.2, SAS Institute Inc., Cary, NC)

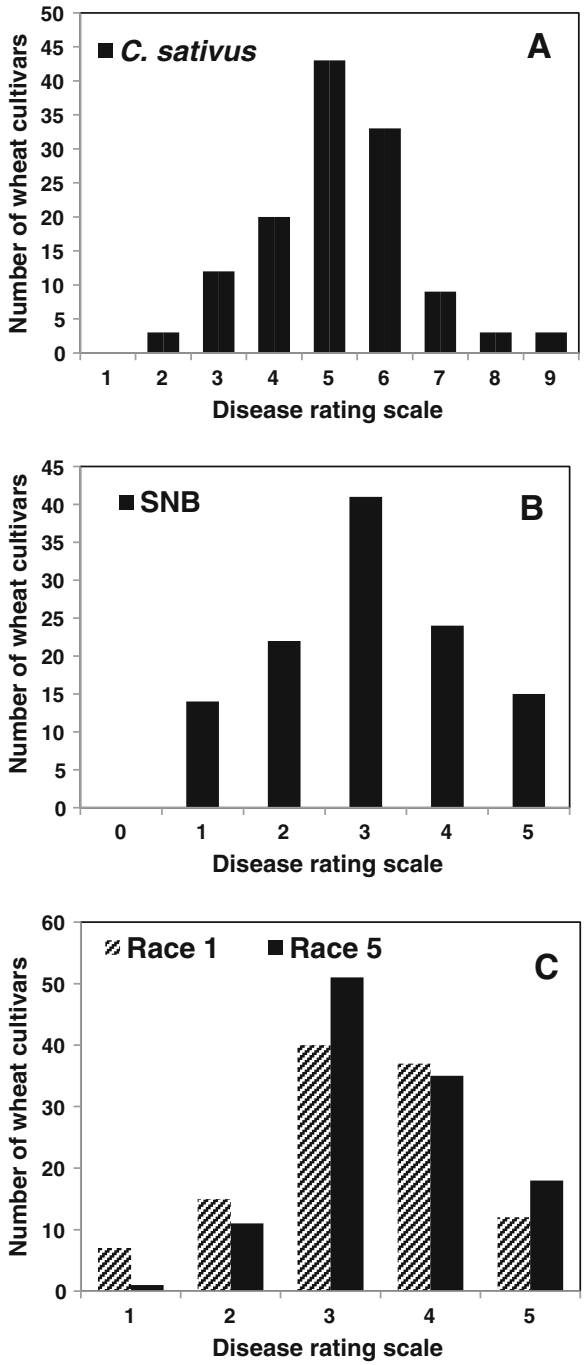

Fig. 1 Disease reactions of spring wheat cultivars and lines (n=116) to *Cochliobolus sativus* (**A**); *Phaeosphaeria nodorum* (**B**), and *Pyrenophora tritici-repentis* races 1 and 5 (**C**)

Among the 116 wheat cultivars and lines evaluated for race 5, 16% were highly susceptible, while 10% were resistant to race 5 (Fig. 1c). Among resistant lines, only one wheat line 'BL 2047 = DANIAL 88/HLB 25//NL 297' had a median disease score of 1 (Table 4), and the remaining 11 resistant lines exhibited median disease scores of 2 (Table 4).

Resistance to multiple leaf spot diseases

Of the wheat cultivars and lines, 27 exhibited resistance to more than one disease or pathogen race (Table 5). Among these, six wheat lines, SW89-5422, BL 2127 = DANIAL88/HLB30//NL297, BL 3033, FILIN/IRENA/5/CNDO/R143//ENTE/MEXI-2/3/AE.SQUA(TAUS)/4WEAVER, GAN/AE.SQUARROSA(236)//DOY1/AE.SQUARROSA(447)/3/MAIZ/4/INQALAB91, Mayoor//TK SN1081/Ae.Squarrosa(222)/3/FCT were resistant to spot blotch, SNB and tan spot race 1 (Table 5). Similarly, two wheat cultivars Chirya 3 and Chirya 7 were resistant to spot blotch, tan spot races 1 and 5 (Table 5).

Discussion

In this study, 116 spring wheat cultivars and advanced breeding lines selected from the field studies were evaluated against multiple leaf spot diseases under a controlled environment. We identified new sources of resistance to spot blotch, SNB, and tan spot races 1 and 5 in the existing wheat germplasm. Most of these wheat cultivars or elite lines had not been previously evaluated for these diseases in wheat. These results showed differences in disease reactions, indicating that genetic variations exist among wheat cultivars.

Evaluating wheat cultivars to individual diseases in the fields is difficult because both spot blotch and tan spot produce similar types of lesions, are frequently observed in the same lesions, and are often difficult to identify with the naked eye (Duveiller et al. 2005). In addition, assessing field resistance to diseases is further complicated by environmental conditions, the amount of natural inoculum, and competition between pathogens (Duveiller et al. 2005; Sharma and Duveiller 2007; Sharma et al. 2004a, b). Thus, evaluating seedling resistance in wheat against leaf spot diseases under artificial inoculation in controlled environments is rapid, and accurate. Large numbers of wheat germplasm lines have been previously evaluated for seedling resistance to leaf spot diseases (Ali et al. 2008; Gurung et al. 2009; Singh et al. 2006). Seedling

Table 2 Wheat cultivars resistant to *Stagonospora nodorum*[a]

Name of entry or pedigree	Median[b]	R_{ij}[c]	p_{ij}[d]	95% CI for p_{ij}[e]
BL 1905 = ZSH12/HLB19/NL297	1	188.6	0.2	(0.114, 0.273)
AE.SQ(205)/5/BP10*3/4/IAS55*4/CT14/23/3/IAS5S*4/EG.AUS//IAS55*4/ALD	1	134.8	0.1	(0.076, 0.211)
BL 1910 = ZSH23/HLB15//NL297	1	250.9	0.2	(0.128, 0.404)
BL 2127 = DANIAL88/HLB30//NL297	2	269.2	0.3	(0.196, 0.329)
KLAT/SOREN//PSN/3/BOW/4/VEE#5.10/5/CNO67	2	215.4	0.2	(0.138, 0.296)
NL 923 = (205)/5/BR 2*3/4/IAS55*4/CI14123/3/IAS 55*4/EG.AUS/IAS 55*4	1	81.0	0.1	(0.069, 0.085)
NL 922 = AE.SQ(205)/5/BR 12*3/4/IAS55*4/CI 14123/IAS55*4/EGA.US//IAS 55*4	2	269.2	0.3	(0.196, 0.329)
BL 1970 = NL297*2/DANIAL88//HLB18	2	304.7	0.3	(0.182, 0.431)
Seri M 82(X2)//A6/GLEN	2	246.6	0.2	(0.146, 0.365)
UP 2472	2	273.3	0.3	(0.170, 0.379)
PBW 343	2	385.3	0.4	(0.295, 0.448)
BL 2631 = PBW 343/CHIRYA 3	1	161.7	0.2	(0.093, 0.245)
SW89-5193	1	161.7	0.2	(0.093, 0.246)
SW89-5422	1	188.6	0.2	(0.114, 0.273)
FILIN/IRENA/S/CNDO/R143//ENTE/MEXI-2/3/AE SQUA(TAUS)/4WEAVER	2	242.3	0.2	(0.165, 0.315)
(205)/5/BR/2*3/4/IAS55*4/CI14/23/3/IAS55*4/EG.AUS//IAS55*4/ALD	1	219.7	0.2	(0.117, 0.347)
BL 2069 = NL 297*4/NL505	2	215.4	0.2	(0.138, 0.296)
BL 2140 = NL 297*4/LR15	2	335.8	0.3	(0.199, 0.474)
GAN/AE.SQUARROSA(236)//DOY1/AE.SQUARROSA(447)/3/MAIZ/4/INQALAB91	1	250.9	0.2	(0.128, 0.404)
Mayoor//TK SN1081/Ae.Squarrosa(222)/3/FCT	2	215.4	0.2	(0.138, 0.296)
BL 3033	2	385.3	0.4	(0.295, 0.448)
BL3036	2	327.3	0.3	(0.237, 0.399)
BL 2730 = NL 297/Sabuf	2	277.8	0.3	(0.154, 0.418)
BL 2827	1	192.8	0.2	(0.095, 0.328)
BL 2844	2	393.9	0.4	(0.252, 0.519)
BL 2710	1	165.9	0.2	(0.075, 0.307)
MILAN/SHA7	2	447.7	0.4	(0.339, 0.567)
NG8201/KAUZ	2	215.4	0.2	(0.138, 0.296)
BL 3623	2	300.4	0.3	(0.201, 0.392)
NL 1047	2	273.5	0.3	(0.170, 0.379)
BL 3961	2	327.3	0.3	(0.237, 0.399)
ALTAR84/AE.SQUARROSA(221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLLI	1	81.0	0.1	(0.069, 0.085)
CHEN/AEGLOPSSQUARROSA(TAUS)//BCN/3/BAV92..	2	242.3	0.2	(0.165, 0.315)
BL 4148	2	273.5	0.3	(0.170, 0.379)
WK 1204	2	470.9	0.3	(0.339, 0.365)
Pasang Lhamu	2	246.6	0.2	(0.142, 0.365)
Salamouni (resistant check)	1	165.9	0.2	(0.075, 0.307)
ND495 (susceptible check)	5	902.4	0.9	(0.802, 0.908)

[a] Seedlings were rated for disease reactions 8 days after inoculation with *Stagonospora nodorum* blotch (isolate Sn 2000) according to a 0 to 5 rating scale (Liu et al. 2004), where median disease scores ≤ 2 = resistant (R) and > 2 = susceptible (S). Salamouni and ND495 were used as resistant and susceptible checks, respectively

[b] Median

[c] Mean rank (R_{ij})

[d] Relative treatment effects (p_{ij}) and

[e] 95% confidence intervals (CI) were calculated from nonparametric analysis using PROC MIXED procedure in SAS (Version 9.2, SAS Institute Inc., Cary, NC)

Table 3 Wheat cultivars resistant to *Pyrenophora tritici-repentis* race 1[a]

Name of entry or pedigree	Median[b]	R_{ij}[c]	p_{ij}[d]	95% CI for p_{ij}[e]
SW89-5422/NL 251	2	210.7	0.2	(0.149, 0.266)
BL 2127 = DANIAL88/HLB30//NL297	2	131.3	0.1	(0.087, 0.177)
Mayoor//TK SN1081/Ae.Squarrosa(222)/3/FCT	1	99.2	0.1	(0.057, 0.153)
NL 750	1	98.3	0.1	(0.036, 0.230)
BL 1965 = ZSH/2/HLB 19//2*NEPAL 297	2	194.6	0.2	(0.127, 0.264)
BL 1970 = NL297*2/DANIAL88//HLB18	2	273.0	0.3	(0.183, 0.356)
Seri M 82(X2)//A6/GLEN	2	131.3	0.1	(0.087, 0.177)
CHIRYA 3	2	163.4	0.2	(0.127, 0.189)
CHIRYA 7	2	147.4	0.1	(0.105, 0.185)
GISUZ/SABUF	2	162.5	0.2	(0.090, 0.255)
BL 2731 = NEPAL 297/SABUF	2	209.7	0.2	(0.121, 0.313)
BL 2631 = PBW 343/CHIRYA 3	2	210.7	0.2	(0.149, 0.266)
SW89-5193	1	130.4	0.1	(0.060, 0.242)
SW89-5422	1	99.2	0.1	(0.057, 0.153)
FILIN/IRENA/5/CNDO/R143//ENTE/MEXI-2/3/AE.SQUA(TAUS)/4WEAVER	1	83.2	0.1	(0.045, 0.137)
(205)/5/BR/2*3/4/IAS55*4/CI14/23/3/IAS55*4/EG.AUS//IAS55*4/ALD	2	210.7	0.2	(0.149, 0.266)
GAN/AE.SQUARROSA(236)//DOY1/AE.SQUARROSA(447)/3/MAIZ/4/INQALAB91	2	256.9	0.2	(0.160, 0.356)
Yangmai No.6	2	304.2	0.3	(0.208, 0.390)
BL 3033	2	210.7	0.2	(0.149, 0.266)
BL 2907	2	179.5	0.2	(0.161, 0.181)
TURACO	2	115.3	0.1	(0.071, 0.166)
ALD/COC//URES/3/MILAN/SHA7	1	114.3	0.1	(0.047, 0.236)
Salamouni (resistant check)	2	273.0	0.3	(0.183, 0.356)
ND495 (susceptible check)	5	982.5	0.9	(0.934, 0.946)

[a] Seedlings were rated for disease reactions 6–7 days after inoculation with *Pyrenophora tritici-repentis* race 1 according to 1 to 5 scale (Lamari and Bernier 1989), where median disease scores ≤ 2 = resistant (R) and > 2 = susceptible (S). Salamouni and ND495 were used as resistant and susceptible checks, respectively

[b] Median

[c] Mean rank (R_{ij})

[d] Relative treatment effects (p_{ij}) and

[e] 95% confidence intervals (CI) were calculated from nonparametric analysis using PROC MIXED procedure in SAS (Version 9.2, SAS Institute Inc., Cary, NC)

resistance to SNB was compared with adult plant resistance and correlations in disease reactions were observed between greenhouse and field experiments (Engle et al. 2006). Additionally, the same QTL were found to be associated with seedling and adult plant resistance to SNB (Friesen et al. 2009). After the Green Revolution, the Mexican semi-dwarf wheats became most popular in South Asia and were extensively grown over large areas, resulting in wheat populations more vulnerable to foliar leaf spot diseases (Chaurasia et al. 1999). A few wheat cultivars tested in fields also showed resistance to spot blotch (Sharma and Duveiller 2007). Of 116 wheat cultivars and lines assessed, six lines: SW89-5422, BL 2127 = DANIAL88/HLB30//NL297, BL 3033, FILIN/IRENA/5/CNDO/R143//ENTE/MEXI-2/3/AE.SQUA(TAUS)/4WEAVER, GAN/AE.SQUARROSA(236)//DOY1/AE.SQUARROSA(447)/3/MAIZ/4/INQALAB91, Mayoor//TK SN1081/Ae.Squarrosa(222)/3/FCT were resistant to spot blotch, SNB, and tan spot race 1. The line SW89-5422 has also shown stable resistance to spot blotch in Nepal, India and Bangladesh (Sharma et al. 2004b). Most of the resistant lines found in this study differ in their agronomical characters and differ in plant

Table 4 Wheat cultivars resistant to *Pyrenophora tritici-repentis* race 5[a]

Name of entry or pedigree	Median[b]	R_{ij}[c]	p_{ij}[d]	95% CI for p_{ij}[e]
BL 2047 = DANIAL 88/HLB 25//NL 297	1	68.3	0.1	(0.022, 0.185)
CHIRYA 3	2	105.8	0.1	(0.055, 0.177)
HLB 19 (Mayoor)	2	169.3	0.2	(0.091, 0.270)
HLB 25 = Mayoor = CS/A.CURV.//GLEN/3/ALD/PVN	2	74.0	0.1	(0.064, 0.076)
BL 2731 = NEPAL 297/SABUF	2	51.5	0.0	(0.032, 0.073)
NANJING 8508/3/CHUM18//JUP/BJY	2	137.6	0.1	(0.070, 0.231)
TURACO	2	74.0	0.1	(0.064, 0.076)
ALD/COC//URES/3/MILAN/SHA7	2	74.0	0.1	(0.064, 0.076)
BL 3539	2	105.8	0.1	(0.055, 0.177)
BL 1048	2	105.8	0.1	(0.055, 0.177)
BL 3704	2	169.3	0.2	(0.091, 0.270)
NL 1086	2	44.0	0.0	(0.025, 0.069)
Salamouni (resistant check)	2	260.0	0.3	(0.233, 0.255)
ND495 (susceptible check)	5	968.0	0.9	(0.583, 0.739)

[a] Seedlings were rated for disease reactions 6–7 days after inoculation with *Pyrenophora tritici-repentis* race 5 according to 1 to 5 scale (Lamari and Bernier 1989), where median disease scores ≤ 2 = resistant (R) and > 2 = susceptible (S). Salamouni and ND495 were used as resistant and susceptible checks, respectively

[b] Median

[c] Mean rank (R_{ij})

[d] Relative treatment effects (p_{ij}) and

[e] 95% confidence intervals (CI) were calculated from nonparametric analysis using PROC MIXED procedure in SAS (Version 9.2, SAS Institute Inc., Cary, NC)

type, maturity, yield potential, and genetic background (Rosyara et al. 2008).

Two wheat cultivars: Chirya 3 (CS/A.Curv./Glenn81/3/Al d/Pvn/Ning Mai #4/Olesen/Ald/Yang mai#4) and Chirya 7 were developed by CIMMYT (Dubin et al. 1998) and introduced into South Asia, including Nepal (Dubin and Bimb 1991; Dubin et al. 1998). These two wheat cultivars were resistant to spot blotch and tan spot races 1 and 5 in this study. Resistance in Chirya 7 was derived from *Agropyron curvifolium* (*Thinopyrum curvifolium*) as a synthetic hexaploid (Mujeeb-Kazi et al. 1996). These cultivars identified as resistant in this study have *Thinopyron curvifolium* as their donor species. Chirya 3 and Mayoor showed high levels of resistance to spot blotch in fields (Ragiba et al. 2004). Resistance to spot blotch in Chirya 3 and Milan/Shangai # 7 was reported to be controlled by a single dominant gene (Neupane et al. 2007) and had the lowest disease severity and the highest grain yield (Sharma and Duveiller 2007). Intriguingly, only Chirya 3 was found to be resistant in this study, but Milan/Shangai # 7 was moderately susceptible to spot blotch. One of the possible reasons for this discrepancy could be the difference in sources of resistance to spot blotch. It is also possible that the resistance gene in Milan/Shangai # 7 may be functional at the adult plant stage in field conditions, but it may remain ineffective at the seedling stage. This hypothesis needs to be examined in future studies.

Resistance to spot blotch is mainly quantitatively inherited (Dubin and Van Ginkel 1991; Kumar et al. 2009, 2010). Joshi et al. (2004) identified resistant sources governed by 3 to 4 additive genes. An association between microsatellite markers and spot blotch resistance also was reported in the F_7 progeny from a cross between the resistant cultivar G162 (CIMMYT CID 93926) and the susceptible cultivar Sonalika (Sharma et al. 2007). Kumar et al. (2009) mapped the QTL for spot blotch resistance in a cross between Yangmai No. 6 and Sonalika and identified four QTL mapped them on wheat chromosomes 2A, 2B, 5B and 6D. These novel QTL were designated as *QSb.bhu-2A*, *QSb.bhu-2B*, *QSb.bhu-5B* and *QSb.bhu-6D* (Kumar et al. 2009). Using another mapping

Table 5 Summary of wheat cultivars resistant to two or more leaf spot diseases

Name of entry or pedigree	Disease reaction			
	C. sativus[a]	SNB[b]	Tan spot race 1[c]	Tan spot race 5[d]
(205)/5/BR/2*3/4/IAS55*4/CI14/23/3/IAS55 *4/EG.AUS//IAS55*4/ALD	S	R	R	S
AE.SQ(205)/5/BP10*3/4/IAS55*4/CT14/23/3/IAS5S *4/EG.AUS//IAS55*4/ALD	R	R	S	S
ALD/COC//URES/3/MILAN/SHA7	S	S	R	R
ALTAR84/AE.SQUARROSA(221)//3*BORL95/3/URES/ JUN//KAUZ/4/WBLLI	R	R	S	S
BL 1048	R	S	S	R
BL 1970 = NL297*2/DANIAL88//HLB18	S	R	R	S
BL 2069 = NL 297*4/NL505	R	R	S	S
BL 2127 = DANIAL88/HLB30//NL297	R	R	R	S
BL 2631 = PBW 343/CHIRYA-3	S	R	R	S
BL 2731 = NEPAL 297/SABUF	S	S	R	R
BL 3033	R	R	R	S
BL 3704	R	S	S	R
BL 4148	R	R	S	S
CHEN/AEGLOPSSQUARROSA(TAUS)//BCN/3/BAV92..	R	R	S	S
CHIRYA 3	R	S	R	R
CHIRYA 7	R	S	R	R
FILIN/IRENA/5/CNDO/R143//ENTE/MEXI-2/3/AE.SQUA (TAUS)/4WEAVER	R	R	R	S
GAN/AE.SQUARROSA(236)//DOY1/AE.SQUARROSA(447)/ 3/MAIZ/4/INQALAB91	R	R	R	S
Mayoor//TK SN1081/Ae.Squarrosa(222)/3/FCT	R	R	R	S
NL 1047	R	R	S	S
NL 922 = AE.SQ(205)/5/BR 12*3/4/IAS55*4/CI 14123/IAS55 *4/EGA.US//IAS 55*4	R	R	S	S
Seri M 82(X2)//A6/GLEN	S	R	R	S
SW89-5193	S	R	R	S
SW89-5422	R	R	R	S
TURACO	S	S	R	S
WK 1204	R	R	S	S
Yangmai No.6	R	S	R	S
Salamouni (resistant check)	-e	R	R	R
ND 495 (susceptible check)	-	S	S	S

[a] For spot blotch, seedlings were rated for disease reactions 10 days after inoculation with *Cochliobolus sativus* isolate CS 45 according to a 1 to 9 rating scale (Fetch and Steffenson 1999), where meadian disease scores between 1 and 4 = resistant (R) and > 4 = susceptible (S)

[b] *Stagonospora nodorum* blotch (SNB). Seedlings were rated for disease reaction 8 days after inoculation with isolate Sn 2000 according to a 0 to 5 rating scale (Liu et al. 2004), where median disease scores ≤ 2 = resistant (R) and > 2 = susceptible (S)

[c] For tan spot, seedlings were rated for disease reaction 6–7 days after inoculation with *Pyrenophora tritici-repentis* race 1 according to a 1 to 5 rating scale (Lamari and Bernier 1989), where median disease scores ≤ 2 = resistant (R) and > 2 = susceptible (S)

[d] For tan spot, seedlings were rated for disease reactions 6–7 days after inoculation with *Pyrenophora tritici-repentis* race 5 according to a 1 to 5 disease scale (Lamari and Bernier 1989), where median disease scores ≤ 2 = resistant (R) and > 2 = susceptible (S)

[e] Not determined

population, Kumar et al. (2010) identified an additional four QTL mapped on the chromosomes 2A, 2B, 5B, and 7D. Although 30% of the wheat cultivars tested were resistant to spot blotch in this study, only a few wheat cultivars such as Yangmai # 6, Ning 8201 and Chirya 3 have been used to develop mapping populations (Kumar et al. 2009, 2010). Therefore, other resistance sources identified here could contain additional useful genes for resistance to spot blotch in the future.

S. nodorum produces several host-selective toxins (HSTs) that operate with dominant sensitivity genes in an inverse gene-for-gene relationship (Friesen et al. 2006; Liu et al. 2004). Breeding wheat for resistance to SNB has been difficult for plant breeders due to presence of only few major genes for resistance but several minor QTL have also been identified on wheat genomes (Friesen and Faris 2010).

P. tritici-repentis also produces different HSTs on sensitive wheat cultivars (Strelkov and Lamari 2003). Resistance to tan spot has been postulated to be inherited both qualitatively and quantitatively (Singh et al. 2010). Eight pathogenic races of *P. tritici-repentis* have been identified, and these races produce three HSTs such as Ptr ToxA, Ptr ToxB, and Ptr ToxC (Strelkov and Lamari 2003; Singh et al. 2010). At least six recessive genes (*Tsr 1* to *Tsr 6*) have been identified on wheat chromosomes by classical genetic analysis and mapped with different molecular markers (Singh et al. 2010). Similarly, race-specific and race non-specific QTL have been mapped on different wheat chromosomes (Friesen and Faris 2004; Singh et al. 2008a, b). The newly identified resistant lines in this study could be potential sources of resistance to both tan spot and SNB and can be utilized directly or through backcrossing to broaden the genetic basis of resistance to tan spot and SNB diseases in wheat in Nepal.

In summary, six advanced breeding lines (SW89-5422, BL 2127 = DANIAL88/HLB30//NL297, BL 3033, FILIN/IRENA/5/CNDO/R143//ENTE/MEXI-2/3/AE.SQUA(TAUS)/4WEAVER, GAN/AE.SQUARROSA(236)//DOY1/AE.SQUARROSA(447)/3/MAIZ/4/INQALAB91, Mayoor//TKSN1081/Ae.Squarrosa (222)/3/FCT identified in this study can provide excellent sources of resistance to the major leaf spot diseases of wheat. These elite lines can be utilized directly as wheat cultivars in Nepal or as resistance sources in crosses to develop new wheat cultivars with resistance to multiple leaf spot diseases. Additionally, resistant lines should be used as parents to develop mapping populations in wheat improvement programs in Nepal. Further studies related to the inheritance and allelic relationships are necessary to confirm whether or not the newly identified resistance sources have known or novel genes.

Acknowledgements B. N. Mahto was supported by the Council for International Exchange of Scholars *(CIES)*, Institute of International Education *(IIE)*, the U. S. Fulbright Core Program, Washington, D. C., USA and the Fulbright Commission Nepal, Gyaneshwor, Kathmandu, Nepal, and Nepal Agricultural Research Council (NARC), Nepal. We thank M. R. Bhatta and J. M. Hansen for their technical help. We are grateful to Mike Bonman and Mary Pull for editing the manuscript.

References

Ali, S., Singh, P. K., McMullen, M. P., Mergoum, M., & Adhikari, T. B. (2008). Resistance to multiple leaf spot diseases in wheat. *Euphytica, 159*, 167–179.

Chaurasia, S., Joshi, A. K., Dhari, R., & Chand, R. (1999). Resistance to foliar blight of wheat: a search. *Genetic Resources and Crop Evolution, 46*, 469–475.

De Wolf, E. D., Effertz, R. J., Ali, S., & Francl, L. J. (1998). Vistas of tan spot research. *Canadian Journal of Plant Pathology, 20*, 349–370.

Dubin, H. J., & Bimb, H. P. (1991). Effects of soil and foliar treatments on yield and disease of wheat in low land of Nepal. In D. A. Saunders & G. P. Hettel (Eds.), *Wheat for non-traditional warm areas* (pp. 484–485). CIMMYT, Mexico, D. F.

Dubin, H. J., & Duveiller, E. (2000). Helminthosporium leaf blights of wheat: integrated control and prospects for the future. Proceeding of International Conference on Integrated Plant Disease Management. Sustainable Agriculture Indian Phytopathological Society, New Delhi, India (pp. 575–579).

Dubin, H. J., & van Ginkel, M. (1991). The status of wheat diseases and disease research in the warm areas. In D. A. Saunders (Ed.), *Wheat for the non-traditional warm areas* (pp. 125–145). Mexico, D. F: CIMMYT.

Dubin, H. J., Arun, B., Begum, S. N., Bhatta, M. R., Dhari, R., Goel, L. B., Joshi, A. K., Khanna, B. M., Malakar, P. K., Pokhrel, D. R., Rahman, M. M., Saha, N. K., Shaheed, M. A., Sharma, R. C., Singh, A. K., Singh, R. M., Singh, R. V., Vargas, M., & Verma, P. C. (1998). Results of the South Asia regional Helminthosporium leaf blight and yield experiments, 1993–94. In E. Duveiller, H. J. Dubin, J. Reeves, & A. McNab (Eds.), *Helminthosporium blights of wheat: spot blotch and tan spot* (pp. 182–187). CIMMYT, Mexico, D. F.

Duveiller, E. (2002). Helminthosporium blights of wheat: challenges and strategies for a better disease control. Advances of Wheat Breeding in China. China Science and

Technology Press, Jinan, Shandong, People's Republic of China (pp. 57–66).

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*, 248–256.

Engle, J. S., Lipps, P. E., & Minyo, R. J., Jr. (2006). Reaction of commercial soft red winter wheat cultivars to *Stagonospora nodorum* in the greenhouse and field. *Plant Disease, 90*, 576–582.

Eyal, Z. (1999). Breeding for resistance to Septoria and Stagonospora diseases in wheat. In J. A. Lucas, P. Bowyer, & H. M. Anderson (Eds.), *Septoria in cereals: a study of pathosystem* (pp. 332–344). Wallingford: CABI Publishing.

Fetch, T. G., Jr., & Steffenson, B. J. (1999). Rating scales for assessing infection responses of barley infected with *Cochliobolus sativus*. *Plant Disease, 83*, 213–217.

Friesen, T. L., & Faris, J. D. (2004). Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. *Theoretical & Applied Genetics, 109*, 464–471.

Friesen, T. L., & Faris, J. D. (2010). Characterization of the wheat-Stagonospora nodorum disease system: what is the molecular basis of this quantitative necrotrophic disease interaction. *Canadian Journal of Plant Pathology, 32*, 20–28.

Friesen, T. L., Stukenbrock, E. H., Liu, Z. H., Meinhardt, S. W., Ling, H., & Faris, J. D. (2006). Emergence of a new disease as a result of interspecific virulence gene transfer. *Nature Genetics, 38*, 953–956.

Friesen, T. L., Xu, S. S., & Harris, M. O. (2008). Stem rust, tan spot, Stagonospora nodorum blotch, and Hessian fly resistance in Langdon durum-*Aegilops tauschii* synthetic hexaploid wheat lines. *Crop Science, 48*, 1062–1070.

Friesen, T. L., Chu, C.-G., Liu, Z. H., Xu, S. S., Halley, S., & Faris, J. D. (2009). Host-selective toxins produced by *Stagonospora nodorum* confer disease susceptibility in adult wheat plants under field conditions. *Theoretical & Applied Genetics, 118*, 1489–1497.

Gurung, S., Bonman, J. M., Ali, A., Patel, J., Myrfield, M., Mergoum, M., et al. (2009). New and diverse sources of multiple disease resistance in wheat. *Crop Science, 49*, 1655–1666.

Joshi, A. K., Kumar, S., Chand, R., & Ortiz-Ferrara, G. (2004). Inheritance of resistance to spot blotch caused by *Bipolaris sorokinian*a in spring wheat. *Plant Breeding, 123*, 213–219.

Kumar, U., Joshi, A. K., Kumar, S., Chand, U., & Röder, M. S. (2009). Mapping of resistance to spot blotch disease caused by *Bipolaris sorokiniana* in spring wheat. *Theoretical & Applied Genetics, 118*, 783–782.

Kumar, U., Joshi, A. K., Kumar, S., Chand, U., & Röder, M. S. (2010). Quantitative trait loci for resistance to spot blotch caused by *Bipolaris sorokiniana* in wheat (*T. aestivum* L.) lines 'Ning 8201' and 'Chirya 3'. *Molecular Breeding*, doi:10.1007/s11032-009-9388-2.

Lamari, L., & Bernier, C. C. (1989). Toxin of *Pyrenophora tritici repentis*: Host-specificity, significance in disease, and inheritance of host reaction. *Phytopathology, 79*, 740–744.

Liu, Z. H., Friesen, T. L., Rasmussen, J. B., Ali, S., Meinhardt, S. W., & Faris, J. D. (2004). Quantitative trait loci analysis and mapping of seedling resistance to *Stagonospora nodorum* leaf blotch in wheat. *Phytopathology, 94*, 1061–1067.

Mahto, B. N., Gurung, S., & Adhikari, T. B. (2010). Morphological and pathogenic variability of *Cochliobolus sativus* from Nepal. (Abstract). *Phytopathology, 100*, S76.

Mujeeb-Kazi, A., Pena, R. J., & Delgadu, R. (1996). Novel genetic diversity for biotic stress resistance in *Triticum aestivum* L. Focus on *Cochliobolus sativus*. *The* 2nd I*nt. Crop Science Congress. Crop productivity and sustainability shaping the future.17-24 November 1996,* New Delhi, India.

Neupane, R. B., Sharma, R. C., Duveiller, E., Ortiz-Ferrara, G., Ojha, B. R., Rosyara, U. R., et al. (2007). Major gene controls of field resistance to spot blotch in wheat genotypes "Milan/Shanghai#7' and "Chirya. 3'. *Plant Disease, 91*, 692–697.

Ortiz, R., Sayre, K. D., Govaerts, B., Gupta, R., Subbarao, G. V., Ban, T., et al. (2008). Climate change: can wheat beat the heat? *Agriculture, Ecosystems & Environment, 126*, 46–58.

Ragiba, M., Prabhu, K. V., & Singh, R. B. (2004). Recessive genes controlling resistance to Helminthosporium leaf blight of wheat in synthetic hexaploid wheat. *Plant Breeding, 123*, 389–391.

Riede, C. R., Francl, L. J., Anderson, J. A., Jordahl, J. G., & Meinhardt, S. W. (1996). Additional sources of resistance to tan spot of wheat. *Crop Science, 36*, 771–777.

Rosyara, U. R., Ghimire, A. A., Subedi, S., & Sharma, R. C. (2008). Variation in South Asian wheat germplasm for seedling drought tolerance traits. *Plant genetic Resiurces: Characterization and Utilization, 1*, 88–93.

SAS Institute Inc. 2010. SAS OnlineDoc, Version 9.2, SAS Institute, Cary, NC, USA.

Sharma, R. C., & Duveiller, E. (2007). Advancement toward new spot blotch resistant wheats in South Asia. *Crop Science, 47*, 961–968.

Sharma, R. C., Duveiller, E., Gyawali, S., Shrestha, S. M., Chaudhary, N. K., & Bhatta, M. R. (2004). Resistance to Helminthosporium leaf blight and agronomic performance of spring wheat genotypes of diverse origins. *Euphytica, 139*, 33–44.

Sharma, R. C., Duveiller, E., Ahmed, F., Arun, B., Bhandari, D., Bhatta, M. R., et al. (2004). Helminthosporium leaf blight resistance and agronomic performance of wheat genotypes across warm regions of South Asia. *Plant Breeding, 123*, 520–524.

Sharma, R. C., Duveiller, E., & Jacquemin, J. M. (2007). Microsatellite markers associated with spot blotch resistance in spring wheat. *Journal of Phytopathology, 155*, 316–319.

Singh, P. K., Mergoum, M., Ali, S., Adhikari, T. B., Elias, E. M., & Hughes, G. R. (2006). Identification of new sources of resistance to tan spot, Stagonospora nodorum blotch, and Septoria tritici blotch of wheat. *Crop Science, 46*, 2047–2053.

Singh, S., Bockus, W. W., Sharma, I., & Bowden, R. L. (2008). A novel source of resistance in wheat to *Pyrenophora triticirepentis* race 1. *Plant Disease, 92*, 91–95.

Singh, P. K., Mergoum, M., Ali, S., Adhikari, T. B., & Hughes, G. R. (2008). Genetic analysis of resistance to *Pyrenophora tritici-repentis* races 1 and 5 in tetraploid and hexaploid wheat. *Phytopathology, 98*, 702–708.

Singh, P. K., Singh, R. P., Duveiller, E., Mergoum, M., Adhikari, T. B., & Elias, E. M. (2010). Genetics of wheat—*Pyrenophora tritici-repentis* interactions. *Euphytica, 171*, 1–13.

Strelkov, S. E., & Lamari, L. (2003). Host-parasite interactions in tan spot (*Pyrenophora tritici-repentis*) of wheat. *Canadian Journal of Plant Pathology, 25*, 339–349.