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Research Paper

In-vitro test for seed borne incidence of wheat genotypes to *Bipolaris sorokiniana* at Chitwan, Nepal

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Abstract: A research was conducted in invitro to find out the variation in the incidence of seed borne of 20 different wheat genotypes to *Bipolaris sorokiniana*. Field experiment was carried out in Agronomy block of IAAS, Rampur, Chitwan in split plot design with three replications, considering 25 November as normal sowing and 15 December as late sowing dates. Seed borne incidence test was carried out before sowing and after harvest of the seeds in in-vitro in CRD design with four replications. Foliar blight and seed infection were highly positively correlated. RR-21, which had higher disease severity in both dates of sowing, had 32.5% seed infection on 25 November sowing and 41% on 15 December sowing. Aditya had less than 9% seed infection in both dates of sowing with low AUDPC value. From the experiment it is concluded that Aditya can be sown in late condition as it possess low AUDPC and low seed infection value. But RR-21 with high AUDPC value in normal and late sowing condition should not be suggested for any breeding program or cropping purpose because the infected seed harvested from highly severed genotype serves as a source of primary inoculums for the disease development.

Keywords: *Bipolaris sorokiniana*, Heat stress, Seed infection, AUDPC, *In-Vitro*

INTRODUCTION

Wheat (*Triticum aestivum* L.) cultivation in the warmer region of South Asia is constrained by several biotic and abiotic factors (Sharma and Duveiller, 2003) which has posed sharp decline in production and productivity. In wheat Helminthosporium leaf blight complex which generally occurs as a complex of spot blotch (caused by *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur (anamorph: *Bipolaris*

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sorokiniana) and tan spot (caused by *Pyrenophora tritici repentis* (Died.) is the threatful fungal foliar biotic factor and terminal heat stress during the latter stage of the crop growth is another abiotic factor causing significant yield loss and covering more than twenty five million hectares of land worldwide (Van Ginkel and Rajaram, 1998). HLB has been the main yield constraints of wheat since last decade after the intensity of rusts has been decreased due to the wide adoption of durable rusts resistant genotypes. The yield losses of 20 - 25% have been reported in farmer's field of Nepal (Duveiller, 2002, Shrestha *et al*, 1998). *Bipolaris sorokiniana* attacks almost all parts of wheat plants and causes spot blotch, root rot, black point, seedling blight and head blight (Duveiller and Gilchrist, 1994). Severity of disease has vertical relation to seed infection. In Nepal, seed germination was negatively correlated (r= -0.79) with seed infection that can reach up to 89% (Shrestha *et al.*, 1998). An experiment conducted) in vitro to determine the planting value of wheat seeds determined that during the germination of wheat seeds the pathogen transmitted from seed to plant and caused germination failure, coleoptiles infection and root infection (Sultana, 2012).

The pathogen has a worldwide distribution, but is particularly aggressive under conditions of high relative humidity and temperature associated with imbalanced soil fertility. Epidemiological factor is very important for the development of spot blotch and tan spot in the wheat growing season. The combined effects of high temperature, high relative humidity and long period >12 hours leaf wetness caused by rainfall, and dew are conducive to foliar blight development in the Indo-gangetic Plains where wheat is grown from November to April (Duveiller, 2004). In Asian context, spot blotch is more rapid and severe at 28ºC than at lower temperature (Nema and Joshi, 1973 and Singh *et al.*, 1998). Disease development becomes high after heading stage when temperature slowly increases.

The average yield loss due to spot blotch in South Asia and India has been estimated to be 19.6% and 15.5% respectively (Dubin and Van Ginkel, 1991). Average grain yield losses due to HLB associated with the 26 November, 11 December and 26 December seedling dates were 20%, 30% and 32% respectively in Nepalese situation (Duveiller *et al.,* 2005). Spot blotch causes 100% yield loss under severe infection conditions (Mehta, 1994). Higher yield loss at late sowing date is due to combined effect of higher temperature which favored spot blotch severity and terminal heat stress. *B. sorokiniana* is a seed borne disease. Saari (1984) reported if severe leaf infection is present and some rain occurs after heading, the percentage of grain infection may exceed 50% and becomes seed borne in nature.

Terminal heat stress has been shown to increase severity of spot blotch (Sharma and Duveiller, 2004). Drought is an expanding and creeping threat of world slowly taking hold of an area and tightening its grip with time (Mishra *et al*., 2002). About 60% wheat area in developing world (75 million hectare) is subjected to various abiotic stresses, of which 45 million hectare is affected by moisture stress (Parry *et al.*, 2005). The shift of rice -wheat cropping pattern from the normal date to delayed condition due to impact of climate change has made wheat growing farmers of Nepal reluctant to sow the seed in the late season which are surely to be attacked by combined effect of spot blotch and terminal heat stress at severe level. Bhandari (2001) stated that combined resistance to seed infection, root rot and spot blotch was not identified in any one genotype, which indicated that the resistance in different parts of wheat plant may be governed by different genes.

Therefore the present study was carried out to identify the best disease resistant and heat tolerant genotypes with the following objectives.

- to find out the severity of spot blotch at different dates of sowing
- to determine the level of resistance against seed borne infection by *Bipolaris sorokiniana*

MATERIALS AND METHOD

Seed collection

Twenty released as well as pre- released drought tolerant wheat genotypes seed differing in genetic background, yield potential, maturity and level of HLB resistance were included in the study which were collected from National Wheat Research Program (NWRP), Bhairahawa. Aditya and Bhrikuti were taken as spot blotch resistant and RR-21 as spot blotch susceptible.

In vitro seed infection test

Seed infection study after harvest of seed was carried out in lab. Four hundred seeds of each genotype were tested by placing 25 seeds per plate at equidistance in the sterilized petriplates in 1:8:16 ratio from center to periphery containing triple layers of blotting paper moistened with sterile distilled water.

Four plates or 100 seeds were considered as an experimental unit and were replicated 4 times in complete randomized design. The plates were incubated at 25 ± 1^{0}C for 5 days. After 5 days of incubation the seeds were observed under stereomicroscope and seed infection was recorded.

Table 1: List of twenty drought tolerant wheat genotypes included in the study at Rampur, Chitwan, Nepal, 2011- 2012

Accession No	Genotype/ Pedigree	Origin
CSISA DRYT5202	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1	Mexico
CSISA DRYT5203	MILAN//PRL/2*PASTOR/4/CROC_1/AE.SQUARROSA (213)//PGO/3/BAV92	Mexico
CSISA DRYT5204	CROC_1/AE.SQUARROSA (213)//PGO/3/CMH81.38/2*KAUZ/4/BERKUT	Mexico
CSISA DDRYT5205	MTRWA92.161/PRINIA/5/SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	Mexico
CSISA DRYT5207	ACHTAR*3//KANZ/KS85-8-5/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92	Mexico
CSISA DRYT5210	QG 78.5//2*INQALAB 91*2/TUKURU	Mexico
CSISA DRYT5211	HUANIL//2*WBLL1*2/KUKUNA	Mexico
CSISA DRYT5217	BABAX/LR42//BABAX/3/BABAX/LR42//BABAX/4/T.DICOCCON PI94625/AE.SQUARROSA (372)//3*PASTOR/5/T.DICOCCON PI94625/AE.SQUARROSA (372)//3*PASTOR	Mexico
CSISA DRYT5218	ONIX/4/MILAN/KAUZ//PRINIA/3/BAV92	Mexico

Accession No	Genotype/ Pedigree	Origin
CSISA DRYT5219	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//FRET2/6/MILAN/KAUZ//PRINIA/3/BAV92	Mexico
CSISA DRYT5220	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/FRET2/KUKUNA//FRET2/6/MILAN/KAUZ//PRINIA/3/BAV92	Mexico
CSISA DRYT5223	SOKOLL//PBW343*2/KUKUNA/3/ATTILA/PASTOR	Mexico
CSISA DRYT5224	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/BAV92	Mexico
CSISA DRYT5226	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//PRINIA/3/BAV92	Mexico
CSISA DRYT5227	MILAN/KAUZ//PRINIA/3/BAV92/4/WBLL1*2/KUKUNA	Mexico
CSISA DRYT5228	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/CASKOR/3/CROC_1/AE.SQUARROSA (224)//OPATA/7/PASTOR//MILAN/KAUZ/3/BAV92	Mexico
CSISA DYRT5229	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/CASKOR/3/CROC_1/AE.SQUARROSA (224)//OPATA/7/PASTOR//MILAN/KAUZ/3/BAV92	Mexico
Aditya		Nepal
Bhrikuti	(CDO/COC/3/PLO//Fury/Ana)	Nepal/ CMMYT
RR-21	(II 54-68/AN/3/YT54/N10B//LR64)	CIMMYT

Experimental details

Experimental site: The experiment was conducted in the research farm of Agronomy at the Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, Nepal. The site was situated at 27°31' North latitude and 84°25' east longitude with an elevation of 256m above mean sea level. Climate of the location was subtropical and humid type. The maximum and minimum temperature recorded for 25 November sowing day was 27°C and 11°C respectively with 90% relative humidity. For 15 December sowing day, the maximum and minimum temperature was 20°C and 8°C respectively with 88% relative humidity. November 25 sowing genotypes received total 171.6 mm rainfall while December 15 sowing genotype received total 306.7 mm rainfall water during the entire growth period.

Design of the experiment plot and sowing: The field experiment was laid out in split-plot design with three replications. Sowing dates was taken as main plots and wheat genotypes as sub- plots. The size of individual plot was 1m×1m. Seed sowing was done at the spacing of 25 cm between the rows @ 120 kg/ha. Distance between each replication was 1m. Distance between the main factors that is two date of sowing was 0.5 m. Border distance from all four sides was1m. Total gross area of the field was 22x11.5= 253m^2.Chemical fertilizers were applied @ 120kg N, 60 kg P2O5 and 40 kg K2O ha^{-1} as recommended by NWRP, through urea, complexal and potash. Crop was grown as rainfed.

Disease assessment: Both single and double digit scoring were done for the disease assessment at both date of sowing. Single digit scoring was done visually on flag leaf (F) and penultimate leaf (F-1) from 10 randomly selected single tillers per genotype in each replication by using standard diagram developed by CIMMYT (Muzeeb-KaaziA *et al.*, 1996). Three scoring at the interval of 6 days was done when the disease development started in penultimate and flag leaf respectively.

Double digit scoring after anthesis was evaluated using the double-digit scale (00 to 99) developed as a modification of Saari and Prescott's scale for assessing severity of foliar disease of wheat(Saari *et al*., 1975). The first digit (D_1) indicates disease progress in canopy height from the ground level and the second digit (D_2) refers to severity measured based on diseased leaf area. Both D_1 and D_2 are scored on a scale of 1 to 9. Five scoring were done at the interval of 6 days in both the seeding dates. For each evaluation, percent disease severity was estimated based on the formula.

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

The area under disease progress curve (AUDPC) was calculated by summarizing the progress of disease severity. AUDPC values from double digit and AUDPC from flag leaf (F) and penultimate leaf (F-1) were separately calculated by using the following

$$\text{AUDPC} = \sum_{i=1}^{n} (Y_{i+1} + Y_i) 0.5 (T_{i+1} - T_i)$$

formula given by Das *et al*. (1992).

Agronomic traits: After all the plants in the plot reached to maturity, they were hand harvested, threshed and grain weight and thousand kernels weight were recorded at 12% moisture level.

Data analysis: MS excel was used for data entry. The recorded data were subjected to analysis of variance and DMRT for the mean separations from the reference of Gomez and Gomez (1984). ANOVA was done at 1% and 5% level of significance to test the significance difference for each parameter.

RESULTS AND DISCUSSION

Seed infection: Seeds of all the tested genotypes were infected with *B. sorokiniana* however, the level of infection differed significantly Aditya, CSISA DRYT 5207 and CSISA DRYT 5218 had less than 10% seeds with *B. sorokiniana* and these genotypes were grouped under resistant (Table 2). RR-21, which was used as a susceptible check, had 30.5% infection and Bhrikuti, which was used as resistant check, had 22.5% infection and was categorized as moderately resistant.

Table 2: Category of the different genotypes on the basis of percentage seed borne inoculums in invitro condition, 2011-2012

Category	Seed infection %	Name of genotypes
Resistant	<10	Aditya(6.25%)
		CSISA DRYT 5207 (8.25%)
		CSISA DRYT 5218(8.25%)
		CSISA DRYT 5229 (10.25%)
Moderately resistant	10-25	CSISA DRYT 5227 (11.25%)
		CSISA DRYT 5228 (12%)
		CSISA DRYT 5204 (13%)
		CSISA DRYT 5217 (13.25%)
		CSISA DRYT 5220 (14.75%)
		CSISA DRYT 5210 (15.75%)
		CSISA DRYT 5211 (15.75%)
		CSISA DRYT 5219 (16%)
		CSISA DRYT 5224 (16.5%)

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	CSISA DRYT 5202 (18%) CSISA DRYT 5203 (18%) CSISA DRYT 5223 (19%) CSISA DRYT 5226 (20.75%) CSISA DRYT 5205 (21.25%) Bhrikuti (22.5%)
Susceptible 26-50	RR-21 (30.5%)

Higher incidence of *B. sorokiniana* in the susceptible genotype RR-21 (37%) was also found by Laila *et al.* (2010). Maximum incidence of seed infection in RR-21 proved that it is working as susceptible variety of spot blotch disease in the field.

Seed infection after harvest in relation to AUDPC

The tested wheat genotypes differed significantly for seed infection percentage at both dates of sowing ($p \leq 0.01$). The seed infection ranged from 7.25% (Log transformed value 0.850) to 41% (1.613). RR-21 had highest seed infection 41% (1.613) on 15 December sown and same genotype had 32.25% (1.508) on 25 November sown. Similarly CSISA DRYT 5202 had 31% (1.49), Bhrikuti 26% (1.41), and CSISA DRYT 5217 25% (1.39) seed infection on 15 December sown. Lowest seed infection was recorded in Aditya 7.25% (0.850) on 25 November and slightly higher 9% (0.94) on 15 December sown. Likewise CSISA DRYT 5207 had 10% (0.99) when sown on 25 November. Aditya having low AUDPC value also had low seed infection % as shown in Table 3.

Higher seed infection in RR-21 genotype along with high AUDPC value was also reported by Bhandari (2001). A significant correlation among the vertical spread of spot blotch, head blight and black point was reported by Racemakers (1987). Besides the disease, seed infection is also favored by the favorable environment condition where *B. sorokiniana* can infect leaves, stems, sheaths and even spike (Singh, 2002). The seed infection percentage of 15 December sowing was higher than the 25 November sowing, it might be due to the combined effect of higher disease severity and heavy rainfall before the harvest on second date sowing.

Table 3: Effect of dates of sowing in AUDPC value and seed infection percentage in wheat at Rampur, Chitwan, Nepal, 2011-2012

Genotypes	AUDPC DD 25 NOV	AUDPC DD 15 DEC	AUDPCFL 25 NOV	AUDPC FL 15 DEC	Seed infection 25 NOV in vitro	Seed infection 15 DEC in vitro
CSISADRYT 5202	615.9ghi	728.5bc	482.0gh	763.7c	1.380b	1.490b
CSISA DRYT5203	595.4ghijk	688.6cde	451.0j	736.3de	1.352bc	1.379cd
CSISA DRYT 5204	412.9o	506.3mn	181.7uvw	185.0uv	1.222def	1.289efg
CSISA DRYT 5205	401.4o	534.1lmn	165.0wx	194.0u	1.317bc	1.342cde
CSISA DRYT 5207	499.0n	631.7fgh	249.7st	275.3qr	0.9989h	1.105h
CSISA DRYT 5210	503.7mn	523.5lmn	241.7t	284.7q	1.320bc	1.384cd
CSISA DRYT 5211	618.6fgh	721.9bcd	491.3g	752.0cd	1.283cd	1.356cde
CSISA DRYT 5217	666.1ef	733.5bc	498.3g	796.3b	1.272cde	1.395c
CSISA DRYT 5218	492.4n	582.1hijk	252.7st	265.3rs	1.008h	1.113h
CSISA DRYT 5219	621.9fgh	680.8de	488.3gh	743.0d	1.273cde	1.321def
CSISA DRYT 5220	606.7ghij	679.7de	471.0hi	721.0e	1.145fg	1.265fg

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CSISA DRYT 5223	550.0klm	614.6ghi	332.0o	362.3m	1.326bc	1.349cde
CSISA DRYT 5224	549.1klm	622.1fgh	343.0no	357.3mn	1.194efg	1.318def
CSISA DRYT 5226	568.2ijkl	605.0ghij	303.3p	358.0mn	1.344bc	1.388cd
CSISA DRYT 5227	561.9jkl	614.9ghi	373.3m	391.0l	1.159fg	1.229g
CSISA DRYT 5228	403.5o	520.8lmn	167.7vwx	184.3uv	1.131g	1.229g
CSISA DRYT 5229	402.1o	524.5lmn	168.3vwx	191.3u	1.028h	1.103h
Aditya	394.1o	489.2n	146.0y	161.0xy	0.8504i	0.9474i
Bhrikuti	591.9ghijk	642.3efg	409.0k	459.3ij	1.319bc	1.414c
RR-21	752.8b	1073a	569.0f	815.7a	1.508a	1.613a
LSD	42.19		16.69		0.77	0.63
SEm(±)	14.98		5.92		0.02	0.22
CV%	4.41		10.6		4.82	3.43
Probability	0.00		0.00		0.00	0.00

AUDPC DD: Area Under Disease Progress Curve Double Digit; AUDPC FL: Area under Disease Progress Curve on flag leaf. Treatment means are separated by Duncan's Multiple Range Test (DMRT) and the columns represented by same letter (s) are not significantly different among each other at 5% level of significance.

Conclusion: The genotype with higher level of seed infection from the in vitro test showed higher level of disease severity in the field also. There was direct correlation between seed borne inoculums and spot blotch disease severity. Higher incidence of seed borne inoculums also causes the seedling abortion, lower seed germination and seedling symptom incidence. Even the resistant variety Adiyta showed increased seed borne incidence in late sown condition due to favorable disease condition, heat stress and combined with rainfall. So for less disease severity and lower seed infection it is better to sow seeds in normal planting date. However for the disease resistance in late sown condition Aditya, CSISA DYRT 5207, CSISA DYRT 5218 and CSISA DYRT 5229 can prove to be the good genetic source. These genotypes can be suggested to the farmers for late sowing condition. Also these genotypes may be used as resistant check in future research work on genotypes/ varietal screening against spot blotch.

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