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RELATIONSHIP AMONG WATER USE EFFICIENCY, CANOPY TEMPERATURE, CHLOROPHYLL CONTENT AND SPOT BLOTCH (*COCHLIOBOLUS SATIVUS*) RESISTANCE IN DIVERSE WHEAT (*TRITICUM AESTIVUM* L.) GERMPLASM

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Abstract

The food security vision for 2050 has front- lined wheat as the major conduit to feed the global populace estimated at 9.2 billion. Pakistan ranks 6th in world population and among the top ten countries for wheat production, but annual yield productivity appears to be stagnated due to prevalence of various biotic and abiotic stresses. More recently the incidence of a new foliar wheat disease spot blotch (*Cochliobolus sativus*) in drought and heat stressed areas of Pakistan has necessitated that a new look on both water use efficiency and spot blotch resistance be taken. Hence this study has attempted to establish a relationship between these two stress influencing constraints. One hundred lines were assessed for intrinsic water use efficiency, canopy temperature, chlorophyll concentration index and spot blotch resistance. Rates of photosynthesis (A) and transpiration (E), stomatal conductance (gs) and internal CO_2 (Ci) were estimated using the Infrared Gas Analyzer (IRGA). Chlorophyll concentration index and canopy temperature were also measured to determine the extent of physiological changes under disease pressure. Spot blotch presence was estimated using the standard double digit disease scoring scale. Our results have revealed a direct relationship among water use efficiency, canopy temperature, chlorophyll concentration index and spot blotch resistance. Keeping three growth stages (GS: 63, GS: 69 and GS: 77) as a source of variation, data analyses have shown a significant difference amongst the studied attributes. Structural model equation revealed that about 87.8% variability was explained by the studied attributes. Looking at significance of mean square values of % severity, 17 wheat lines were found spot blotch resistant. These lines are valuable breeding stocks for wheat improvement for hot ecological niches within Pakistan and globally where higher incidence of spot blotch prevails.

Key words: Wheat, Spot blotch, Structure equation model (SEM), Canopy temperature, Water use efficiency.

Introduction

Wheat is one of the earliest cultivated food crops and for approximately 6000 years has been the basic staple food of major civilizations. It is an important food grain source for humans and is a close third to rice and corn in total world production (Monneveux *et al*., 2012). Increasing food demands for secure and healthy nutrition can be achieved by the production of high yielding wheat varieties through development of diverse genetic stocks by incorporating wheat wild relatives as a genetic resource for biotic/abiotic stress resistances. Commercial breeding programs are utilizing such unique approaches (Mujeeb-Kazi and Hettel, 1995; Mujeeb-Kazi, 2006; Mizuno *et al*., 2010; Yang, *et al*., 2010; Ogbonnaya, *et al*., 2013), and are an impetus for researchers to augment the prevalent narrow genetic diversity.

Wheat genetic variability is vital for conventional crop improvement and faced with a huge yield gap it is crucial that the variation is enhanced to maximize yield via new alleles. Mujeeb-Kazi *et al*. (2009) have demonstrated this variability augmentation strategy to combat biotic and abiotic stresses. Among biotic stresses spot blotch caused by *Cochliobolus sativus* (asexual: *Bipolaris sorokiniana*) is a major production constraint in warm humid regions. It has been pronounced threat during the last two decades especially in warmer wheat growing regions. Yield losses due to spot blotch of wheat have been reported to be as high as 85% from Zambia (Raemakers, 1988) and 40 % from field trails in Philippines (Lapis, 1985). Grain quality also gets severely affected as has been observed in experimentation at Londrina, Brazil with highly susceptible cultivars where losses ranged from 79 to 87% (Hetzler *et al*., 1991). The Indian subcontinent on-farm studies revealed that Nepal faced 16% crop destruction and Bangladesh about 15% damage due to spot blotch (Saari, 1998). In Pakistan during 2009 in the southern Punjab this disease surfaced the first time and caused massive yield losses. The popular variety Bhakkar-2001 in cultivation was highly susceptible and went out of cultivation due to this disease immediately (Rattu *et al*., 2011; Iftikhar *et al*., 2012). This occurrence opened up the necessity for researchers to consider this as a potent research objective in wheat improvement.

Biotic stress (Spot blotch) a foliar disease imparting dark brown irregular lesions with chlorotic margins on leaves (Acharya *et al*., 2011) can alter physiological functions by decreasing photosynthetic capacity (Tas and Tas, 2007). Physiological performance and disease resistance can be introduced simultaneously using

phenotypic tools in breeding (Lopes *et al*., 2010). Yield potential can be increased through photosynthetic capacity by increasing biomass (Reynolds *et al*., 2009). Hence physiological characters such as stomatal conductance (Ci), internal CO_2 concentration could be studied (Reynolds *et al*., 2000).

Using canopy temperature as a selection criterion to improve stress tolerance, Saint Pierre *et al*., (2010) studied genetic basis and association of canopy temperature with yield. It has also been used as a screening tool in previous years (Araus *et al*., 2003; Olivares-Vilegas *et al*., 2007).

Water use efficiency (WUE) indicates optimal carbon gain by transpiring water per unit area (Schroeder *et al*., 2001) so is the ratio between photosynthesis and transpiration (Blum, 2005). Using water use efficiency, stomatal conductance and photosynthesis as screening tools, distinguishable fungal effects can be predicted (Swarthout *et al*., 2009).

The above preamble necessitated designing the present study; so that a large set of 100 genotypes for better physiological traits along with spot blotch resistance could be evaluated. Keeping in view these objectives, a relationship among area under disease progress curve (AUDPC), area under canopy temperature curve (AUCTC), area under chlorophyll concentration index curve (AUCCIC) and water use efficiency (WUE) have been identified in a multivariate regression model equation and elucidated with a path diagram. Present study has thus enabled us to pre-screen our germplasm on the basis of WUE, CT and CCI. Purpose of this pre-screening was to focus on broad spectrum resistance regarding biotic and abiotic stress interactions. As there are some master regulators that relate biotic and abiotic stress responses in plants, so there must be a holistic approach to identify and utilize broad spectrum stress tolerant genetic stocks (Atkinson and Urwin, 2012) and wisely stay away from mono-trait focused breeding efforts.

Materials and Methods

A total of one hundred lines of wheat diverse germplasm adapted from CIMMYT nurseries in 2012-13 were sown on November 18, in three randomized complete blocks in the experimental field at National Agricultural Research Center (NARC), Islamabad (30º40'12 N and 73º7'33 E) during 2013 and 2014 crop cycle under four irrigations. Each line was sown in two rows of two meter with 30cm row to row and 5cm plant to plant distance and given standard recommended agronomic practices. One row of a highly susceptible variety "Ciano" was also planted as a border and after each 10 lines within the plots as spreader rows to maximize disease pressure.

Artificial epiphytotic conditions were created by a uniform spray of aggressive pure spore culture of *Bipolaris sorokiniana* having a density more than 10^{-4} spores/ml provided by the Crop Disease Research Institute (CDRI) of National Agricultural Research Center (NARC) Pakistan at two different growth stages GS:37 (flag leaf just visible) and GS: 65 (pollination half complete).

The double digit (00-99) rating scale (Mujeeb-Kazi *et al*., 2007) was used to determine % disease severity of each genotype at three different growth stages of the Zadoks *et al*., 1974 scale viz. GS:63 (beginning of anthesis), GS:69 (anthesis complete) and GS:77 (Late milking) with the formula:

$$\% \text{ Age severity} = D1/9 \times D2/9 \times 100$$

where, Digit 1 (D1) = Height of infection; Digit 2 (D2) = Severity of infection displayed by all leaves of a genotype.

Chlorophyll Concentration Index (CCI) and Canopy Temperature (CT) were measured using Minolta SPAD-502 chlorophyll meter and infrared thermometer MIKRON (IR-MAN), respectively according to the protocols of Pask *et al*. (2012) at growth stages (GS: 63, GS:69, GS:77). Rates of photosynthesis (A), transpiration (E); internal CO_2 (C_i) and sub-stomatal conductance (gs) were estimated using Infra-red gas analyzer (IRGA) of the make LC-Pro+ on GS: 36 (6^{th} node detectable) to evaluate inherent physiological potential of each genotype. Water use efficiency (WUE) was calculated by the ratio of A and E (Swarthout *et al*., 2009).

$$\text{AUDPC} = \sum_{i=1}^{n} [\{(Y_i + Y_{(i+1)})/2\} \times (t_{(i+1)} - t_i)]$$

Disease severity was maximum on the susceptible check at GS: 77, hence severity estimated at this stage was used as the severity basis of each genotype. Area under disease progress curve (Roelf *et al*., 1992) was calculated as:

where, Y_i = disease level at time t_i , $t_{(i+1)}$ - t_i = time (days) between two disease scores, n = number of dates on which spot blotch was noted.

$$\text{AUCCIC} = \sum_{i=1}^{n} [\{(Y_i + Y_{(i+1)})/2\} \times (t_{(i+1)} - t_i)]$$

Kumar *et al*. (2009) assessed AUDPC and used the same formula to estimate (LAUG) leaf Area under greenness (Kumar *et al*., 2010). Following these studies, two more attributes, area under canopy temperature curve (AUCTC) and area under chlorophyll concentration index curve (AUCCIC), were agglomerated to permit better observation of changes in canopy temperature and chlorophyll concentration with disease progress over three different stages when severity was recorded.

Incorporated were the following: Y_i = Chlorophyll concentration index at time t_i, $t_{(i+1)} - t_i$ = time (days) between two readings, n = number of dates on which chlorophyll concentration index was estimated.

$$\text{AUCTC} = \sum_{i=1}^{n} [\{(Y_i + Y_{(i+1)})/2\} \times (t_{(i+1)} - t_i)]$$

Y_i = Canopy temperature at time t_i, $t_{(i+1)} - t_i$ = time (days) between two readings, n = number of dates on which chlorophyll concentration index was estimated.

Data were analyzed statistically for Descriptive Statistics and Pearson's Correlation using XLSTAT (2014). Analysis of Variance (ANOVA) was done and histogram was constructed using Statistica 12. The multivariate regression model equation and path diagram were developed using LISREL 9.1 software.

Results and Discussion

Descriptive statistics of studied attributes among 100 genotypes (Table 1) indicating the mean and standard deviation. Standard error, Variance and range has also been calculated to check the general behavior of the variation in germplasm with respect to the studied traits.

Rates of photosynthesis (A) and transpiration (E) were significantly correlated to each other and with water use efficiency (WUE) (Table 2). Chlorophyll concentration, canopy temperature and disease severity found significantly correlated and their means squares found remarkably significant during GS: 63, GS: 69 and GS: 77 (Table 2). Correlation of canopy temperature and chlorophyll concentration with stress has also been previously reported by Lopes *et al*. (2010) and Steinmeyer *et al*. (2013) but the present study highlighting the least square measures (Fig. 2) through effective hypothesis decomposition indicating pattern of change during three growth stages.

Analysis of variance (Table 3) by keeping growth stages as a source of variation showed that severity varied and had increased significantly with each growth stage. Continuous and steep increase in AUDPC during GS: 63 and GS:69 was observed (Fig. 1) indicating a severe infection rate and at the same time remarkable decrease in chlorophyll content revealed the effect of infection on photosynthesis. Chlorophyll content concentrations in plants are useful attributes to assess stress severity (Mercado *et al*., 2003; Nawaz *et al*., 2013). Analysis of variance has made it clear that there is a significant difference between the chlorophyll contents at these three growth stages at which the disease prevailed with high epidemics.

Significant difference in canopy temperature at each growth stage but not within genotypes was observed. Hence this variation and increase in canopy temperature at the subsequent three growth stages caused disease establishment.

To improve water-use efficiency of rain-fed and irrigated crop production has been a dire need and breeding for higher water-use efficiency might be an upcoming solution (Condon *et al*., 2004). In the present study water-use efficiency was calculated as a ratio of photosynthesis and transpiration rates and taken as an independent estimate in the multivariate regression model to develop the structural equation and path diagram.

Through one-way analysis of variance by keeping the genotype as a source of variation, only % severity was found significantly varying within genotypes showing differential response towards infection (Table 3).

Table 1. Descriptive statistics of studied attributes among 100 genotypes of data obtained from experimental study at NARC in 2013-14.

Description	A	E	Ci	gs	WUE	AUCTC	AUCCIC	% Severity	AUDPC
Mean	11.61	1.93	96.83	0.23	6.09	1102.65	1141.40	38.75	1039.51
Standard error	0.27	0.04	5.01	0.13	0.14	2.78	3.04	1.91	50.29
Standard deviation	2.72	0.39	50.10	1.29	1.39	27.84	30.44	19.14	502.86
Sample variance	7.42	0.16	2509.67	1.66	1.93	774.98	926.82	366.35	252865.21
Range	12.77	2.08	298.00	12.96	8.65	150.00	165.49	83.95	2972.25
Minimum	4.95	1.05	13.00	0.04	2.36	1027.50	1068.52	4.94	120.30
Maximum	17.72	3.13	311.00	13.00	11.01	1177.50	1234.01	88.89	3092.55

Table 2. Pearson's correlation coefficient of studied traits among 100 genotypes of data obtained from experimental study at NARC in 2013-14.

Variables	A	E	Ci	gs	WUE	% Severity	AUDPC	AUCTC
E	0.566							
Ci	-0.562	-0.009						
gs	0.066	0.093	0.000					
WUE	0.569	-0.330	-0.620	-0.019				
% Severity	0.095	-0.071	-0.035	-0.084	0.168			
AUDPC	0.046	-0.074	-0.019	-0.051	0.121	0.936		
AUCTC	0.023	0.032	-0.097	-0.028	0.029	-0.201	-0.207	
AUCCIC	0.080	-0.016	-0.111	-0.079	0.132	0.445	0.400	0.788

WUE: Water use efficiency (μmole CO_2/mmole H_2O); A = Photosynthetic rate (μ mol m^{-2} s^{-1}); E = Transpiration rate (m mole m^{-2} s^{-1}); C_I = Sub-stomatal CO2 (vpm); gs = Stomatal conductance of H_2O; CCI = Chlorophyll concentration index; CT = Canopy temperature (^oC)

Table 3. Univariate results of One-way analysis of variance for % Severity, canopy temperature (CT) and chlorophyll concentration index (CCI) studied at three different growth stages among 100 genotypes (p<0.05).

SOV	df	% Severity	CT	CCI
Growth Stages	2	20608.16***	2019.04***	9643.78***
Genotype	99	206533.99***	1.107	81.75

*** = (p<0.001)

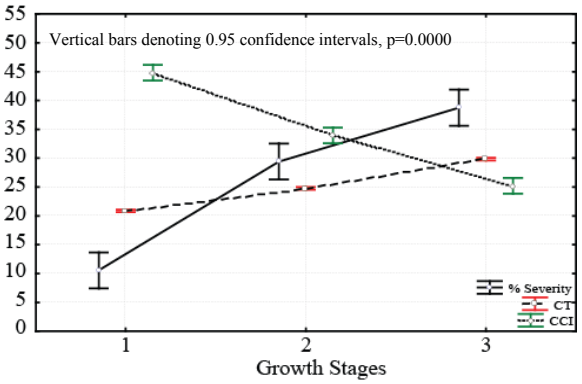


Fig. 1. Growth stages; least square mean (effective hypothesis decomposition for % severity, CT and CCI.

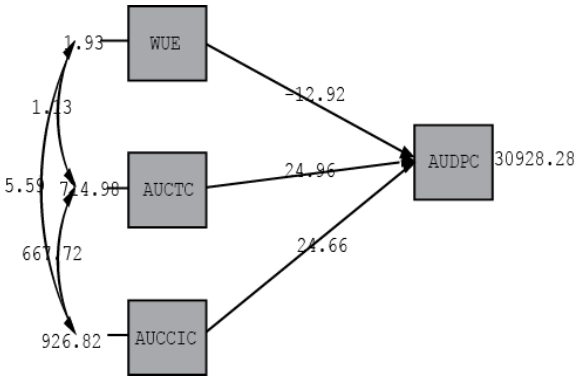
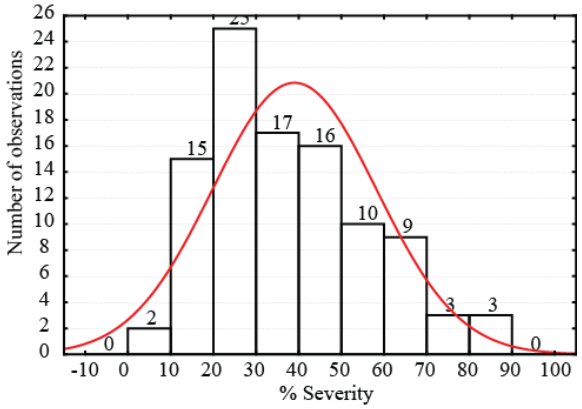


Fig. 2. Histogram showing frequency distribution of % severity of 100 genotypes.



% Severity	Number of genotypes	Infection response
Up to 20 %	17	Resistant (R)
21 to 40 %	42	Moderately Resistant (MR)
41 to 60 %	35	Moderately Susceptible (MS)
Above 60 %	15	Susceptible

Fig. 3. Path diagram with structural equation of the model highlighting inter-relationship among dependent (area under disease progress curve AUDPC) and independent (water use efficiency WUE, area under canopy temperature curve AUCTC and area under chlorophyll concentration index curve, AUCCIC) estimates structure equation of the Model: AUDPC = 494.39 - 12.92 * WUE - 24.96 * AUCTC + 24.66*AUCCIC
Errorvar.= 30928.28, R^2 = 0.878

Looking through the % severity, ANOVA results enabled us to sort out some spot blotch resistant genotypes (Table 4). Cultivar 'Chirya 3' reported earlier in literature as a standard resistant genotype remained at the top with lowest % severity (4.94) and AUDPC (120.3). Further, an advanced backcross derived line with Pavon, CS/TH.SC, Kauz and Milan as ancestors showed resistance (% Severity = 9.88, AUDPC = 287.1) against spot blotch. Duveiller *et al*. (2005) and Neupane *et al*. (2007) have already declared Milan and Chirya 3 as spot blotch resistant genotypes. The line with alien introgression of *Thinopyrum curvifolium* (Mujeeb-Kazi *et al*., 2008) as in 'Chirya 3' and with synthetic wheat in ancestory also proved to be resistant (Ogbonnaya *et al*., 2013). In our study a synthetic wheat derived line backcrosse with 'Pastor' showed resistance with 14.8 % disease severity. Some other genotypes (Fig. 1) resistant to spot blotch were also identified and will be further used in breeding plus be registered internationally.

Evidence of the relationship between AUDPC as dependent factor and AUCCIC, AUCCT and WUE enabled us to predict the extent of disease severity based on physiological measurements (Fig. 3.). A model was set up by taking WUE, AUCCIC and AUCCT as exogenous variables regressing the AUDPC taken as endogenous estimate. Structure equation was developed hence the model was found fit with 87.8% variability explained. Our results confirmed that WUE, AUCTC and AUCCI can be the selected criterion to predict AUDPC.

The multidisciplinary integration of observational possibilities have provided optimism the we may be able to study effectively major crop production traits in wheat simultaneously where we give emphasis here to hot areas where spot blotch is also prevalent. Such an unison of traits is essential to pyramid stress in a positive holistic manner allowing us the latitude to select promising segregates that emanate within the populations of the recombination breeding programs. It is also encouraging that the best lines selected (Table 4) have provided sources that have contributed to resistant types and are spread across the *Triticeae* gene pools with *Ae. tauschii* of the D-genome and *T. dicoccon* (AABB) placed in the primary pool coupled with *Th. scirpeum* and *Th. curvifolium* of the difficult to genetically exploit belonging to the tertiary gene pool.

As a way forward for this study has provided additional impetus to selectively exploit other *Triticeae* genetic resources where our greater initial priorities should be on the primary gene pool wild progenitor accessions that readily allow homologous transfers and thus wild deliver practical varietal outputs more swiftly.

It is encouraging to see that spot blotch resistant lines (Table 4) have inherent genomic diversity and have performed well under hot climatic regimes. This endorses an earlier report by one of us (Mujeeb-Kazi *et al*., 2006) where it was suggested that spot blotch resistant lines may also possess heat tolerance as seen here with some of our new tested germplasm entries. This will open up further work as heat tolerance breeding for wheat is at a high priority for the crops improvement in Pakistan due to swift climatic change events.

Table 4. Ten genotypes with the lowest AUDPC and % severity.

Genotype ID	Pedigree	A	E	C.I	GS	WUE	AUCTC	AUCCIC	% Severity	AUDPC
2	CHIRYA.3	10.17	1.71	68	0.07	5.95	1117.5	1122.44	4.94	120.3
15	CS/TH.SC//3*PVN/3/MIRLO/BUC/4/URES/JUN//KAUZ/5/HUITES/6/YANAC/7/CS/TH.SC//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI	9.45	1.71	311	0.08	5.53	1125	1134.88	9.88	287.1
99	WBLL1/KUKUNA//TACUPETO F2001/6/PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/YR/4/TRAP#1	15.81	2.29	48	0.14	6.9	1095	1109.81	14.81	351.825
82	BECARD/KACHU	13.39	2.05	23	0.09	6.53	1087.5	1102.31	14.81	379.5
37	VORB/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//3*PASTOR	11.47	2.23	163	0.15	5.14	1080	1099.75	19.75	407.4
95	KZA/4/2*WBLL1//KAUZ/2*STAR/3/BAV92/RAYON	11.51	1.88	81	0.1	6.12	1132.5	1151.02	18.52	407.475
85	PBW343*2/KHVAKI//JUCHI	12.95	2.03	14	0.09	6.38	1162.5	1181.02	18.52	426
33	SKAUZ*2/FCT´S´//VORB	9.97	1.27	13	0.06	7.85	1132.5	1151.02	18.52	453.675
83	ALTAR 84/AE.SQUARROSA (221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/MILAN/S87230//BAV92	12.09	2.11	72	0.1	5.73	1147.5	1166.02	18.52	481.575
90	TACUPETOF2001/6/OASIS/5*BORL95/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI/7/ROLF07	11.42	1.74	90	0.11	6.56	1147.5	1166.02	18.52	481.575

WUE = Water use efficiency (µmole CO_2/mmole H_2O)

A = Photosynthetic rate (µ mol m^{-2} s^{-1}); E = Transpiration rate (m mole m^{-2} s^{-1}); C_1 = Sub-stomatal CO2 (vpm); gs = Stomatal conductance of H_2O; CCI = Chlorophyll concentration index; CT = Canopy temperature (^oC)

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