

Heritability of stay green traits and association with spot blotch resistance in three spring wheat populations

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ABSTRACT

Spot blotch (caused by *Cochliobolus sativus*) is a serious constraint to wheat production in tropical and subtropical wheat areas causing severe yield reductions. Combined with other biotic stresses, spot blotch causes chlorophyll reduction of leaves and premature senescence. Stay green property has been considered as trait of interest for development of cultivars in stressed environments. Some of CIMMYT's new synthetic wheat has been found to possess high level of resistance and stay green traits. Studies are limiting about heritability and genetic association of stay green traits with spot blotch in populations derived from such synthetic wheats. This study was done in three spring wheat population derived three spot blotch resistant wheat genotypes 'Milan/Shanghai # 7', 'Chirya.3' and 'NL 971' crossed with 'BL1473', a susceptible wheat cultivar. Both stay green related traits as well as disease severity was evaluated in F2 and F3 generations during 2005-2006 at Rampur, Chitwan, Nepal. Area under SPAD progress curve (AUSDC) ($r = -0.72^{**}$ to -0.88^{**}) and Flag leaf duration ($r = -0.56^{**}$ to -0.69^{**}) showed high phenotypic and genetic correlation whereas Chlorophyll content at anthesis (CCA), days to heading, days to anthesis, days to maturity and days peduncle dryness showed low and non-significant correlation with Area Under Disease Progress Curve (AUDPC). Moderate to high heritability estimates were observed for AUDPC (0.52 to 0.74), chlorophyll content at anthesis (0.77 to 0.81), AUSDC (0.68 to 0.88), flag leaf duration (0.62 to 0.72), days to maturity (0.88 to 0.94) and days to peduncle dryness (0.42 to 66). The results show that AUDPC, AUSDC and flag leaf duration has potential complementary trait for selection whereas independent selection for maturity related traits is possible.

Key words: Bipolaris sorokinina, *Cochliobolus sativus*, Chlorophyll content, Stay green trait, trait association

INTRODUCTION

The spot blotch of wheat (also called Helminthosporium leaf blight, HLB or foliar blight) has been a most important disease in warm wheat-growing regions affecting livelihood of millions of wheat growing small farmers (ALAM *et al.*, 1994; DUBIN and VAN GINKEL, 1991; DUVEILLER and GILCHRIST, 1994; DUVEILLER *et al.*, 1998; LAPIS, 1985; SHARMA *et al.*, 2003). Spot blotch, caused by *Cochliobolus sativus* (Ito and Kurbayzshi) Drechsler ex Dastur, is severe threat to wheat cultivation causes 20% to 100% grain

yield losses (DUVEILLER and GILCHRIST, 1994; MEHETA, 1998). Important progress has been made in recent years in identification of source of resistance in wheat breeding programs of South Asia (SHARMA and DUVEILLER, 2007; DUVEILLER and SHARMA, 2005; SIDDIQUE *et al.*, 2006). Nevertheless currently released cultivars show substantial grain yield reductions (SHARMA and DUVEILLER, 2006). The symptoms associated with the disease are progressive blighting of leaves and accelerated post-anthesis senescence.

In tropical environments post-anthesis heat stress is another important constraint to wheat

production causing reduced photosynthesis and premature senescence. Spot blotch and heat stress affect at least twenty five million hectares of land worldwide (DUVEILLER and GILCHRIST, 1994), resulting in premature senescence of the leaves and reduced grain filling duration (DUVEILLER and GILCHRIST, 1994; JOSHI *et al.*, 2007; MERCADO *et al.*, 2003). Late planting (resulting in post-anthesis heat stress) was found to increase spot blotch severity (SHARMA and DUVEILLER, 2004, ROSYARA *et al.*, 2008).

Stay green trait has been considered as important traits that reflect heat stress tolerance. Stay green is property of a variant in which senescence is delayed compared with a standard reference genotype (THOMAS and HOWARTH, 2000). Stay green trait is considered very important trait under stressed conditions that allows plants to retain their leaves in the active photosynthetic state. A positive phenotypic correlation between stay green trait and HLB severity has been found (ROSYARA *et al.*, 2007, JOSHI *et al.*, 2007). Similarly, a negative correlation was found between spot blotch and grain fill duration (GILCHRIST and PFEIFFER, 1991).

Currently breeding programs in South Asia are in stage of using new Synthetic hexaploids, obtained by crossing tetraploid wheat and *Aegilops tauschii* Coss, as resistant donor for spot blotch (DUVEILLER and SHARMA, 2005; DUVEILLER *et al.*, 2005). This is assumed that they might increase level of resistance as well as increase tolerance to other abiotic stresses including heat stress.

For breeding applications, combined selection for both heat stress and spot blotch resistance has been suggested, although associated suitable trait(s) is largely unknown. Considering associated physiological traits in addition to conventional traits might facilitate to overcome the limitation of conventional yield based selection in stressed environments (REYNOLDS *et al.*, 2001; ROSYARA *et al.*, 2007). Differences among genotypes were observed between resistant and susceptible genotypes for chlorophyll decline measurements as well as flag leaf anatomy (ROSYARA *et al.*, 2007). Canopy temperature depression has been suggested as selection criteria for selection of spot blotch resistance and heat stress tolerance (ROSYARA *et al.*, 2008). For early generations and small plots this trait is impractical to measure with current available technology.

Studies are limited studying genetics of stay green traits in association with spot blotch resistance in heat stressed environments. The objective of this study was to study association of different stay green related traits with field resistance to spot blotch.

MATERIALS AND METHODS

Plant material

Three spot blotch resistant genotypes 'Milan/Shanghai #7' (VS73.600/MIRLO /3/ BOW//YE/TRF/4/ Shanghai #7), 'NL 971' (pedigree: Mrng/Buc//Blo/Pvn/3/Pjb 81), and 'Chirya.3' (pedigree: CS/Th cul/Vee/3/Ald/Pvn/4/Ningmai No.4/Oleson//Ald/ Yangmai No. 4), were crossed with susceptible genotype 'BL1473' (pedigree: Nepal 297/NL531). BL 1473 is commercial cultivar from Nepal (SHARMA *et al.*, 2004) whereas other three genotypes are CIMMYT synthetic wheat genotypes (DUVEILLER and SHARMA, 2005; DUVEILLER and SHARMA, 2007; SHARMA *et al.*, 2004; SHARMA *et al.*, 2007). These resistant lines genotypes high as well as stable level of resistance and stay green property. Crosses were made in the 2003 wheat-growing main season (November to March) and the F₁ plants were grown in an off-season, high-altitude field nursery in 2004 (June to October) at Marpha, Nepal (28°43'_N and 83°15'_E, 2,900 meters above sea level in the Himalayas) to produce the F₂ seed. F₂ and F₃ generations were evaluated at Rampur, Chitwan, Nepal (27°40'_N and 84°19'_E at 228 meters above sea level) during 2006-2007. Relative humidity in Rampur is usually higher than elsewhere in the Nepal lowlands, known as the *Tarai*. This favors the early onset of spot blotch epidemics, with the first lesions (1 to 2 mm) already visible about 4 weeks after sowing. The site is considered as hot spot for the disease with severe epiphytotics in past several years.

The population F₂ population consisted of 174 individuals for BL 1473 x Milan Shanghai # 7, 211 individuals for BL 1473 x Chirya.3 and 206 for BL 1473 x NL 971. Similarly the F₃ population size was 216 head rows for BL 1473 x Milan Shanghai # 7, 216 head rows for BL 1473 x Chirya.3 and 177 head rows for BL 1473 x NL 971. Along with F₂ and F₃ populations, sixty rows of all four parents were grown as parental checks.

The F₂ seeds were space planted in 4m rows with seed to seed spacing 20cm and row to row spacing 25 cm along with their parents. Seeds from each F₂ head were planted as F₃ head rows of 1m long rows with 0.25 m row to row spacing. Parents of the particular crosses were planted after every 20 progeny rows. Both generations were replicated three times in a randomized complete block arrangement. Sonalika (most susceptible genotype for foliar blight) was sown as spreader rows around and in between the blocks to give uniform natural inoculum pressure to the pop-

ulation. The trial was seeded on 26 November for F₂ and 27 November for F₃.

Fertilizers were applied @ 120 N, 60 Kg P₂O₅, and 40 Kg K₂O per hectare. Split application of nitrogen was done 100 Kg as basal broadcasted and rest 20 Kg as top-dressed at active tillering stage. Plots were kept free of weeds by hand weeding. The trials were managed under natural disease infection as there was high uniform inoculum pressure no artificial inoculation was needed. The experimental field had no residue from the previous wheat crop. The field remained submerged in floodwater for several weeks in August and September 2004, as is the case in most years in the lowlands of Nepal grown to rice.

Stay green and spot blotch resistance

Observations for stay green traits were based on individual F₂ plant whereas for F₃ rows were an average of five randomly selected plants per row. Flag leaf was scored visually three times at five to six days of interval when susceptible genotypes received more than 25% of area of disease in their flag leaf. The AUDPC was calculated using the following formula (DAS *et al.*, 1992).

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left[\left(\frac{X_{(i+1)} + X_i}{2} \right) (T_{(i+1)} - T_i) \right]$$

Where,

X_i = Disease severity on the ith date

T_i = Date on which the disease was scored

n = number of dates on which disease was recorded

Non-destructive measurement of chlorophyll content on the tagged flag leaves was measured using Minolta Chlorophyll Meter (SPAD-502) (ROSYARA *et al.*, 2007). In each F₂ plant five random readings of the flag leaf were taken to get average value for the plant for each date of observations. In F₃ head rows, five main tillers 25 random readings in different flag leaves were done to represent average progeny row value. There were three dates of observations, just 1-2 day after disease scoring. All three dates of observations were used to calculate the AUSDC using the following formula (ROSYARA *et al.*, 2007).

$$\text{AUSPC} = \sum_{i=1}^{n-1} \left[\left(\frac{S_{(i+1)} + S_i}{2} \right) (T_{(i+1)} - T_i) \right]$$

Where,

X_i = SPAD value on the ith date

T_i = ith day

n = number of dates of recording SPAD value

Days to flag leaf emergence was recorded when more than 90% of the plants in a row have flag leaf completely emerged (unfolded). Death of flag leaf was recorded when 90% of flag leaves in progeny row completely died. Flag leaf duration was calculated as difference between days of flag leaf emergence to days to flag leaf death.

Each entry was considered to have headed when approximately 90% of the shoots have the entire spike out of the flag leaf. Similarly, the days to anthesis was recorded when 90% of the shoots had the entire spike going to anthesis. Days to peduncle drying was recorded when more than 90% of the plants in a row had peduncles dried. Each panicle was threshed individually and grains were counted to calculate grains per spike. Randomly sampled hundred grains from each progeny rows were counted and weighed twice to record the hundred grain weight.

Heritability

Frequency distribution was calculated using the SAS (1990) software. Those progeny lines having mean disease severities greater than one standard deviation below or above individual parental means were considered as Transgressive segregates (RONALD *et al.*, 1999). Heritability (h^2) was estimated using two methods: offspring – realized heritability (h^2_R) and parent-offspring regression (h^2_{op}) (FALCONER and MACKAY, 1996). Estimates of h^2_{op} were made by regressing progeny means in the F₃ generation on means of the F₂ parents using SAS (1990) software. Because the different generations were grown in different years, h^2_{op} estimates of spot blotch severity were computed according to the procedural details given by FOOLAD and JONES (1992) to reduce the scaling effects of different environments. Genetic correlation was calculated using PROC GLM using parents and offspring covariances as outlined by SEXTON (2004).

Realized h^2 estimates were obtained using the means of the 20% of the F₃ progeny lines with the lowest disease scores and the 20% with the highest scores compared to their F₂ progeny means and using the following formula outlined by GUTHRIE *et al.* (1984):

$$h^2_R = \left(\frac{\overline{F_3 \text{ high}} - \overline{F_3 \text{ low}}}{\overline{F_2 \text{ high}} - \overline{F_2 \text{ low}}} \right) \times 100$$

where $\overline{F_3 \text{ high}}$ and $\overline{F_3 \text{ low}}$ are the respective means of the 20% lines selected for high and the 20% lines selected for low disease scores in the F₃ generation; and $\overline{F_2 \text{ high}}$ and $\overline{F_2 \text{ low}}$ are their mean values in the F₂ generation.

RESULTS

Foliar blight

Foliar blight severity was very high during the study period, as shown by AUDPC value of susceptible parents BL 1473 (table 1). Spot blotch was observed as early as the second week of February, after wheat reached the heading stage. The disease symptoms were uniformly vis-

ible on all plants in research area. Toward maturity, disease severity on the susceptible parent reached 100% but was below 30% on the resistant parents. Isolates from representative samples showed presence of *C. sativus* on diseased leaf lesions and no other disease were appeared during the study period.

The resistant parents had low AUDPC values then the susceptible parent, BL 1473. The NL971 had considerably higher AUDPC in contrast to other two resistant genotypes. Frequency distribution of AUDPC value shows a negative skewness showing dominance of resistant over susceptible in both generations and in all three populations (fig. 1). Few lines exceeded the parental mean values showing transgressive segregations

for resistance. Frequency distribution of all traits showed continuous frequency distribution and majority of them were either positively or negatively skewed. Transgressive segregants were observed for all of the traits studied with a possibility to find stay green variant.

Beside spot blotch the parents showed differences in other traits including flag leaf duration, Chlorophyll content of flag leaves at anthesis, maturity, and days to peduncle dryness (table 1). Resistant genotypes were having greener leaves, low post anthesis chlorophyll decline values, longer flag leaf duration, longer grain filling duration and longer peduncle life (table 1).

The traits studied and AUDPC showed wide variation from low to high genetic and phenotypic

TABLE 1
Properties of parents involved in the study done at Rampur, Nepal in 2005-2006

Traits	BL 1473	Milan / Shanghai #7	Chirya.3	NL 971
Area Under Disease Progress Curve	621	120	121	338
Chlorophyll content at Anthesis (SPAD value)	42	44	45	43
Area Under SPAD Progress Curve	441	723	724	683
Flag leaf duration (days)	39	51	52	46
Days to heading	72	82	83	82
Days to anthesis	74	84	84	83
Days to maturity	114	125	126	124
Days to peduncle dryness	116	127	128	128

TABLE 2
Correlation between Area under disease progress curve (AUDPC), stay green and maturity related traits in three spring wheat populations evaluated during 2005-2006 at Rampur, Chitwan, Nepal

Trait	Gen	CCA (SPAD value)	AUSDC	FLD (days)	DH	DA	DM	DPD
Phenotypic correlation								
BL 1473 x MS 7	F ₂	-.39	-.61	-.45	-.27	-.30	-.41	-.37
	F ₃	-.26	-.71	-.56	-.45	-.44	-.53	-.42
BL 1473 x Chirya.3	F ₂	-.61	-.79	-.58	-.36	-.38	-.32	-.29
	F ₃	-.19	-.71	-.54	-.25	-.25	-.26	-.27
BL 1473 x NL 971	F ₂	-.57	-.68	-.47	-.36	-.35	-.40	-.34
	F ₃	-.21	-.69	-.48	-.42	-.41	-.53	-.35
Genetic correlation								
BL 1473 x MS 7	–	-.19	-.72	-.65	ns	-.18	-.21	-.27
BL 1473 x Chirya.3	–	-.24	-.81	-.69	ns	-.19	ns	ns
BL 1473 x NL 971	–	-.27	-.88	-.56	-.19	ns	-.31	-.24

Abbreviations: Gen – Generation, CCA – Chlorophyll content at Anthesis, AUSDC – Area under SPAD decline curve, FLD – Flag leaf duration, DH- Days to heading, DA- Days to anthesis, DM – Days to maturity, DPD – Days to peduncle dryness, MS 7 – Milan / Shanghai # 7 ns = p > 0.05, Only significant correlation (p < 0.05) are shown.

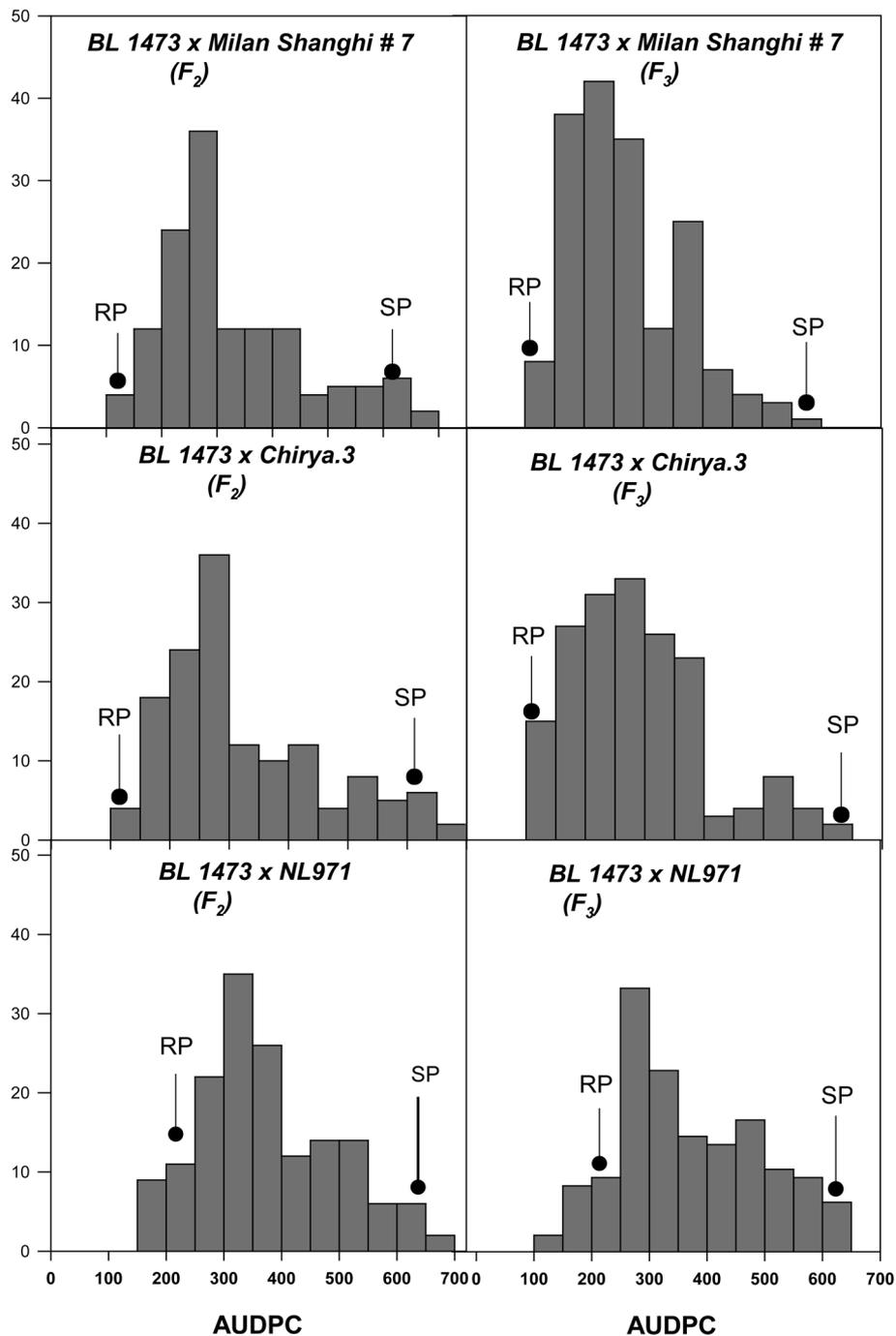


FIGURE 1 - Frequency distribution for Area Under Disease Progress Curve (AUDPC) in populations studied. RP - Resistant parent; SP - Susceptible parent.

correlation (table 2). Among the traits studied AUDPC, a measure of post anthesis decline in chlorophyll content of flag leaf, showed strong negative correlation with AUDPC (table 2). The flag leaf life, as flag leaf duration was negatively correlated with AUDPC showing that resistant

genotypes have longer flag leaf life than susceptible genotypes (table 2). Also, flag leaf duration had moderate genetic correlation with AUDPC (table 2). There was weak negative correlation between chlorophyll content at anthesis and AUDPC (table 2). At anthesis plants were disease free, thus the

Table 3
Heritability of Area under disease progress curve (AUDPC), stay green and maturity related traits under natural epiphytotics of spring wheat evaluated during 2005-2006 at Rampur, Chitwan, Nepal

Cross		AUDPC	HKW	CCA	AUSDC	FLD	DM	DPD
BL 1473 x MS 7	h^2_R	0.74	0.42	0.81	0.88	0.68	0.91	0.58
	h^2_{op}	0.68	0.44	0.78	0.81	0.66	0.88	0.66
BL 1473 x Chirya.3	h^2_R	0.72	0.48	0.78	0.81	0.72	0.94	0.42
	h^2_{op}	0.69	0.42	0.82	0.73	0.68	0.93	0.58
BL 1473 x NL 971	h^2_R	0.52	0.29	0.77	0.68	0.62	0.93	0.52
	h^2_{op}	0.54	0.34	0.80	0.72	0.72	0.94	0.42

Abbreviations: h^2_R – realized heritability h^2_{op} – heritability based on parent offspring correlation, AUDPC- Area Under Disease Progress Curve, HKW-Hundred Kernel Weight, CCA- Chlorophyll content at anthesis, DPD- Days to Peduncle Drying, AUSDC- Area Under SPAD decline curve, FLD- Flag leaf duration, DM-Days to Maturity, MS 7 – Milan / Shanghai # 7

difference show genetic difference in chlorophyll content of genotypes. The AUDPC showed weak but significant negative correlation with maturity related traits which include days to heading, anthesis, maturity, and peduncle dryness.

Heritability

Heritability estimates based on offspring – parent regression and parent-offspring regression ranged from 0.52 to 0.74 for AUDPC, 0.77 to 0.81 chlorophyll content at anthesis, 0.68 to 0.88 for AUSDC, 0.62 to 0.72 for flag leaf duration, 0.88 to 0.94 for days to maturity and 0.42 to 66 for days to peduncle dryness (table 3). The higher estimates under foliar blight stressed conditions illustrate selection could be effective for the traits studied under spot blotch stressed tropical environments.

DISCUSSION

Recently synthetic hexaploid wheats [derived from so-called «wide» crosses between a *Triticum turgidum* var. durum (Desf.) Husnot and *Triticum tauschii* (Coss.) Schmal] are identified as important resistance source to spot blotch resistance. Wild ancestors of common wheat (*Triticum aestivum* L.) have been reported to have higher maximum photosynthetic rates than modern wheat cultivars (AUSTIN *et al.*, 1989; CARVER and NEVO, 1990). Several synthetic-derived lines have showed higher photosynthetic rates (DEL BLANCO *et al.*, 2000). Addition-

ally synthetic hexaploids have proven useful as a source of resistance or tolerance to biotic and abiotic stresses (GORHAM, 1990; LIMIN and FOWLER, 1993).

The results show that the genotypes Milan / Shangahi # 7, Chirya.3 and NL 971 confirming the results of previous studies (DUVEILLER and SHARMA, 2005; DUVEILLER and SHARMA, 2007; DUVEILLER *et al.*, 2005; JOSHI *et al.*, 2004, NEUPANE *et al.*, 2007). This further confirms the positive phenotypic correlation has between found between stay green trait and AUDPC (JOSHI *et al.*, 2007). AUSDC showed strong negative correlation with spot blotch (ROSYARA *et al.*, 2007). Significant genetic correlation in this study further suggests that the association was at genetic level. The possible explanation of such phenomena could be by maintenance of green coloration, stay green genotypes may resist the growth and development of other diseases especially those caused by facultative pathogens such as the spot blotch pathogen (MERCADO *et al.*, 2003). It seems that the increased senescence due to the disease was combined effect of heat stress and spot blotch. Under a growth chamber study MERCADO *et al.* (2003) found similar symptoms of senescence in heat stressed and spot blotch affected wheat plants. Thus a general prediction from this result could be existence of some common genetic mechanism to resist the pathogen development as well as stay green.

Sustained chlorophyll concentrations during grain filling duration have be observed an efficient indicator of heat tolerance in wheat (REYNOLDS *et al.*, 1994). High-temperature stress causes inhibition of chlorophyll biosynthesis and

induces leaf senescence (YANG *et al.*, 2002). Even if for timely sown, wheat faces some degree of heat stress mostly temperatures greater than 35°C occur during grain filling in south Asian Gangetic plains. In such situations heat stress susceptibility might induce senescence and attack of facultative pathogens. Combined effect of spot blotch and heat stress could increase the senescence of flag leaf and flag leaf life is subsequently shortened.

The study showed that genotypes having trait associated expressed lower severities to spot blotch than those with non stay green traits. The results could be interpreted by making three alternative hypothesis – i) highly resistant lines were remaining green over grain filling period thus demonstrating stay green property or ii) heat susceptible lines start losing energy to defense quickly and becomes more vulnerable to the disease or iii) there might be a common defense mechanism that plays against the disease and heat stress. It is not very clear that what is playing major role, nevertheless other types of stress have been found to aggravate the disease (SHARMA and DUVEILLER, 2004). It seems that disease comes a little early before there is substantial increase in temperature and heat stress becomes more prominent in later growth stages only.

Generally heritability of traits studied were fairly high heritability (table 3). A fairly high heritability for AUPDC indicates possibility of finding large effect genes for the trait of interest in the genotypes studied and indicating selection for AUDPC it self is effective in the population studied. Wide variation in heritability for spot blotch resistance has been documented, SHARMA *et al.* (2006) estimated low to high heritability in populations involving resistant genotypes 'Attila', 'Chirya.7', 'G162', and 'SW89.5422'. Moreover AUSDC has comparatively higher heritability as well as genotypic and phenotypic correlation with AUDPC (table 2). As AUSDC is not supposed to be function of spot blotch resistance only, has potential to act as integrative trait for multiple stress tolerance that come together with spot blotch. Considering a high genetic correlation of AUSDC with AUDPC, higher heritability than AUDPC, and potential indicator of heat stress tolerance, could be a suitable trait of interest in selection of genotypes with high physiological profile under stressed conditions.

The results show genetic association of stay

green traits with spot blotch resistance and selection for such traits is effective. Thus the traits evaluated hold a promise as integrated selection criteria for screening foliar blight and heat stress tolerance in irrigated spring wheat grown in tropical environments. In addition the maturity related traits were not found high genetic correlation with AUDPC, showing possibility for combining earliness and resistance together in a single genotype.

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