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# Photochemical Efficiency and SPAD Value as Indirect Selection Criteria for Combined Selection of Spot Blotch and Terminal Heat Stress in Wheat

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## Abstract

Terminal heat and spot blotch caused by *Cochliobolus sativus* are important stresses causing significant wheat (*Triticum aestivum* L.) yield losses in the south Asian plains. Recent studies have shown that chlorophyll-related traits are correlated with heat stress and spot blotch resistance in wheat. This study was conducted to evaluate leaf photochemical efficiency and leaf greenness (measured as SPAD value) for combined selection of spot blotch and terminal heat stress. The efficiency of photosystem II was measured as ratio of variable to maximal chlorophyll fluorescence, $F_v/F_m$, using chlorophyll fluorometer build on pulse modulation principle. The study was conducted in three spring wheat populations derived by crossing spot blotch–resistant wheat genotypes 'Milan/Shanghai#7', 'Chirya.3' and 'NL971' with a susceptible cultivar 'BL 1473'. The $F_3$ and $F_4$ generations were grown under natural epiphytotics of spot blotch either in optimal or in terminal heat stress conditions at Rampur, Nepal. The heritability ($h^2$) of $F_v/F_m$, SPAD measurements and their genetic correlation with 1000-kernel weight (TKW) and area under disease progress curve (AUDPC) were estimated. The $h^2$ estimates for $F_v/F_m$ and SPAD measurements were moderate to high. In addition, AUDPC and TKW showed low to high genetic correlation with these traits. These findings suggest that $F_v/F_m$ and SPAD measurements could be used as complementary traits in selecting for spot blotch resistance and heat tolerance in wheat.

## Introduction

Spot blotch, caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur [anamorph: *Bipolaris sorokiniana* (Sacc.) Shoemaker], and heat are important stresses affecting wheat production in the non-traditional warm wheat-growing regions of South Asia (Sharma et al. 2007). Both of these stresses are severe during grain filling causing loss of photosynthetically active tissues, reduced chlorophyll content and increased senescence (Randall and Moss 1990; Mercado et al. 2003; Joshi et al. 2007). Terminal heat stress was reported to increase spot blotch severity in south Asia (Sharma and Duveiller 2004; Sharma et al. 2007).

Efficiency of photosynthesis has been a good indicator of plant response to abiotic and biotic stresses (Huang 2006). Measurement of gas exchange might be more time consuming and costly particularly in context of breeding applications (Earl and Tollenaar 1999). Alternatively, surrogate traits such as chlorophyll fluorescence (Earl and Tollenaar 1998; Ying et al. 2002; Earl and Davis 2003) and chlorophyll content (Araus et al. 1997) have practical application for indirectly assessing leaf photosynthetic rates. Measurement of photochemical efficiency ($F_v/F_m$) requires only a few seconds; large number of measurements can be performed per day with a single instrument. This will greatly improve the sampling resolution over gas exchange techniques (Earl and Tollenaar 1999).

Previous studies reported that photochemical efficiency and leaf greenness measurements are positively associated with heat stress tolerance. The $F_v$:$F_m$ ratio (measurement of quantum yield potential of photosynthesis or maximal photochemical efficiency of PSII) has been shown to be a reliable indicator of stress (Krause and Weise 1991) and associated with heat stress tolerance (Liu and Huang 2000). The measurement of photochemical efficiency was useful in assessing photosynthetic responses to heat (Sayed 2003) and disease stresses (Duraes et al. 2001; Bassanezi et al. 2002; Chaerle et al. 2007) in wheat and other crops. Mercado et al. (2003) reported similar effects of heat stress and spot blotch on chlorophyll fluorescence in a growth chamber study. Moffatt et al. (1990a,b) reported that wheat genotypes differed in chlorophyll fluorescence values and showed a high general combining ability for this trait.

Chlorophyll content was negatively correlated with heat susceptibility index in wheat-alien amphiploids (Yang et al. 2002). Leaf chlorophyll content during grain filling showed a high genetic correlation with grain yield in heat stressed environments (Reynolds 1994; Reynolds et al. 1998). In high-radiation environments, high chlorophyll content could also be an indicator of a low photo-inhibition of photosynthesis (Farquhar et al. 1989). Portable equipment for indirect measurement of chlorophyll content (SPAD value) is used in different studies. The SPAD value showed association with photosynthesis and photosynthesis-related parameters (Araus et al. 1997). Leaf chlorophyll or SPAD value depression during grain filling was associated with tolerance to spot blotch (Rosyara et al. 2007) and heat stress (Liu and Huang 2000). Generally, the tolerant genotypes showed slower decline in SPAD value than the susceptible ones.

Over the past 15 years, progress from direct selection for spot blotch resistance in wheat has been slow (Duveiller and Sharma 2009). Besides spot blotch, heat stress has emerged as a major challenge. There is a need for identifying traits that could assist in combined selection for both of these traits This study was conducted to evaluate leaf photochemical efficiency (measured as $F_v/F_m$) and leaf greenness (measured as SPAD value) for combined selection of spot blotch and terminal heat stress.

## Materials and Methods

### Crossing and population development

Three spot blotch–resistant genotypes 'Milan/Shanghai#7' (VS73.600/MIRLO/3/BOW//YE/TRF/4/Shanghai#7), 'NL971' (Mrng/Buc//Blo/Pvn/3/Pjb81) and 'Chirya.3' (CS/*Th cu*//Vee/3/Ald/Pvn/4/Ningmai No.4/Oleson//Ald/Yangmai No. 4) were crossed with a susceptible genotype 'BL 1473' (Nepal 297/NL531). BL 1473 is a commercial cultivar, however, susceptible to spot blotch (Duveiller et al. 2005; Rosyara et al. 2007). The rest three genotypes are resistant to spot blotch and developed at CIMMYT, Mexico (Neupane et al. 2007). Crosses were made in the 2003 wheat-growing season (November to March) and resulting seeds were advanced to $F_1$ and $F_2$ generations during 2004 and 2005. The research was conducted at Rampur, Chitwan, Nepal which is located at 27°40'N and 84°19'E, 228 m above sea level.

### Evaluation in $F_3$ generation

Three heads per $F_2$ plant were harvested. Of the three heads harvested from each plant, one head was grown under optimal (non-heat stressed) while another under late (terminal heat stressed) planting conditions. The population size in $F_3$ was 216 for BL 1473 × Milan/Sanghai #7, 216 for BL 1473 × Chirya.3 and 177 for BL 1473 × NL971 under both optimal and late seeding conditions. The length of $F_3$ head rows was 1 m and spaced at 0.25 m (row to row spacing) with ~20 seeds per row. The experiment was arranged in three replicates in a randomized complete block design. The two parents were grown after every 20 lines as checks. The trial was seeded on 27 November and 29 December 2005 for optimal and late planting conditions, respectively. Normal wheat planting time in the region is 15–30 November.

Main tillers of five plants per row were tagged for disease and physiological trait measurements. Three spot blotch disease readings (measured as percentage of diseased leaf area on flag leaf) were recorded at 5–7 days intervals. The first disease scoring was accomplished when most susceptible genotypes had more than 30% of diseased leaf area on flag leaf. The resulting readings were used to calculate the area under the disease progress curve (AUDPC) using the formula outlined by Duveiller et al. (2005).

Handheld chlorophyll fluorometer (model: OS-30P; Manufacturer: Opti-Sciences, Inc., Hudson, NH, USA) was used to measure photochemical efficiency ($F_v/F_m$). The leaves were held by 60 × 15 mm dark-adoption clips (Opti-Sciences, Inc) at least 15 min before recording to achieve flush out of assimilates. The devise had a slider for shielding or exposing the leaf surface to light as required. The area exposed when the slider was opened was approximately 71 mm$^2$.

$$F_v/F_m = (\text{Maximum Fluorescence} - \text{Minimum Fluorescence})/\text{Maximal Fluorescence}$$

In each flag leaf, three measurements (one each at tip, middle and base) were recorded and averaged. Three readings were taken at 5–7 days interval with 2 days after spot blotch readings. The resulting scores were used to calculate the area under the chlorophyll fluorescence ($F_v/F_m$) decline curve (AUFDC) using the following formula –

$$\text{AUFDC} = \sum_{i=1}^{n-1} [(\frac{F_{(i+1)} + F_i}{2})](T_{(i+1)} - T_i)$$

where $F_i$ = $F_v/F_m$ value on the $i^{th}$ date, $T_i$ = $i^{th}$ day, $n$ = number of dates of recording $F_v/F_m$ value, The last observation of $F_v/F_m$, denoted here as $F_v/F_{m\ (L)}$, was analysed as a separate variable.

Minolta chlorophyll meter (Model: SPAD-502) was used for non-destructive measurement of chlorophyll content of flag leaf over time (Rosyara et al. 2007). The leaf greenness was recorded in Minolta company-defined SPAD values. In each flag leaf, five measurements were recorded at different positions starting from tip to base of each leaf and averaged (Rosyara et al. 2007). SPAD readings were recorded three times, 1–2 days after disease scoring. The readings were used to calculate the area under SPAD value decline curve (AUSDC) as previously described by Rosyara et al. (2007).

At maturity, plants in individual plots were harvested and threshed separately. One thousand randomly selected kernels were counted for each plot and weighed to record 1000-kernel weight (TKW).

Top and bottom 15% ranking $F_3$ lines for $F_v/F_m$ and SPAD value were independently selected both under optimal and under late planting conditions. The selected lines were advanced to $F_4$ generation.

**Evaluation in $F_4$ generation**

The $F_4$ lines were evaluated in 1.5 $m^2$ plots consisting of three rows of 2 m length planted at 0.25 m spacing. The parents were grown after every 20 lines as checks in each population. The experiment was planted in three replications for the populations studied.

The trial was seeded on 29 November in case of optimal and 30th December for late planting conditions in 2006. Chlorophyll content, chlorophyll fluorescence ($F_v/F_m$) and disease data were measured as discussed earlier for $F_3$ generation.

**Trial management**

The trials were managed under natural disease infection because a high and uniform spot blotch pressure occurred in both years and no supplementary artificial inoculation was needed. Spot blotch–susceptible variety Sonalika was sown as spreader row around and in between blocks to give uniform natural inoculum pressure to the population. Similarly, late planting was carried out to create terminal heat stress (Sharma et al. 2008). Terminal heat stress is consequence of rising temperature towards maturity in south Asian lowland environments.

In each trial, fertilizers were applied at the rate of 120 kg N, 60 kg $P_2O_5$ and 40 kg $K_2O$ per hectare. Nitrogen was used in split doses. One hundred kilograms of nitrogen was broadcasted as basal dose, whereas the rest 20 kg was top-dressed at maximum tillering stage. All other nutrients were applied at seeding time. Hand weeding was performed to keep the plots free of weeds. Irrigations were applied as required, based on soil moisture condition.

**Data analysis**

Daily mean, minimum and maximum temperature data were obtained from the weather stations situated near experimental plots. Crop growing degree days (GDD) were calculated by averaging maximum and minimum temperature (°C) for each day. Base temperature used was 0°C. Cumulative GDD was calculated from germination of seedlings to physiological maturity following Cao and Moss (1989). Average temperatures and cumulative GDD were compared between timely and late planted wheat to assess severity of heat stress.

Realized $h^2$ estimates were obtained using the means of the 15% of the $F_3$ progeny lines with the lowest value and the 15% with the highest value compared to their $F_4$ progeny means and using the following formula outlined by Guthrie et al. (1984):

Table 1
Means for TKW, area under disease progress, SPAD value, area under SPAD decline curve, photochemical efficiency ($F_v/F_m$) and AUDPC for three $F_3$ and $F_4$ wheat populations and their parents

| | | Normal | | | | | | Heat stressed | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parents/cross | Year | TKW | AUDPC | $SR_A$ | AUSDC | $F_v/F_{m\,(L)}$ | AUFDC | TKW | AUDPC | $SR_A$ | AUSDC | $F_v/F_{m\,(L)}$ | AUFDC |
| Parents | | | | | | | | | | | | | |
| BL 1473 | 2006 | 38.1 | 621 | 40.2 | 441 | 0.52 | 7.7 | 37.1 | 802 | 41.4 | 405 | 0.35 | 5.4 |
| | 2007 | 39.2 | 629 | 41.3 | 434 | 0.49 | 7.8 | 38.2 | 813 | 40.5 | 399 | 0.38 | 5.8 |
| MS7 | 2006 | 43.2 | 120 | 44.6 | 723 | 0.72 | 11.2 | 41.4 | 135 | 43.2 | 704 | 0.66 | 9.8 |
| | 2007 | 44,4 | 115 | 45.7 | 732 | 0.73 | 12.2 | 43,4 | 138 | 44.8 | 701 | 0.67 | 10.1 |
| Chirya.3 | 2006 | 42,7 | 121 | 45.2 | 724 | 0.72 | 10.4 | 41,5 | 129 | 46.5 | 718 | 0.65 | 9.2 |
| | 2007 | 44.7 | 124 | 44.4 | 714 | 0.71 | 10.6 | 43.4 | 130 | 44.5 | 706 | 0.66 | 9.5 |
| NL971 | 2006 | 40.2 | 338 | 43.9 | 683 | 0.68 | 9.8 | 39.2 | 376 | 43.3 | 666 | 0.63 | 8.9 |
| | 2007 | 41.2 | 202 | 42.1 | 676 | 0.66 | 9.7 | 40.2 | 302 | 42.4 | 656 | 0.62 | 8.2 |
| $LSD_{0.05}$ | | 1.2 | 234 | 3.4 | 118 | 0.18 | 2.3 | 1.6 | 246 | 2.7 | 110 | 0.17 | 2.9 |
| Populations | | | | | | | | | | | | | |
| BL 1473 × MS 7 – $F_3$ | 2006 | 40.2 ± 3.1 | 354 ± 58 | 43.2 ± 3.1 | 589 ± 102 | 0.62 ± 0.08 | 9.2 ± 2.0 | 37.2 ± 4.2 | 418 ± 62 | 41.2 ± 4.2 | 489 ± 105 | 0.46 ± 0.12 | 7.2 ± 1.6 |
| BL 1473 × MS 7 – $F_4$ | 2007 | 39.2 ± 3.3 | 368 ± 48 | 44.0 ± 2.8 | 592 ± 98 | 0.61 ± 0.11 | 10.1 ± 1.8 | 36.2 ± 3.8 | 476 ± 58 | 42.0 ± 3.4 | 492 ± 92 | 0.44 ± 0.12 | 7.5 ± 2.2 |
| BL 1473 × Chirya.3 – $F_3$ | 2006 | 41.5 ± 2.4 | 346 ± 29 | 43.0 ± 4.2 | 582 ± 106 | 0.59 ± 0.14 | 10.0 ± 1.6 | 40.5 ± 5.4 | 446 ± 69 | 41.0 ± 4.3 | 462 ± 93 | 0.47 ± 0.16 | 7.0 ± 1.7 |
| BL 1473 × Chirya.3 – $F_4$ | 2007 | 40.5 ± 1.4 | 370 ± 38 | 42.8 ± 3.2 | 576 ± 101 | 0.61 ± 0.09 | 9.8 ± 1.8 | 38.5 ± 3.4 | 482 ± 66 | 40.8 ± 4.3 | 470 ± 99 | 0.46 ± 0.17 | 7.6 ± 2.1 |
| BL 1473 × NL971 – $F_3$ | 2006 | 39.2 ± 2.6 | 423 ± 78 | 43.6 ± 4.5 | 510 ± 110 | 0.56 ± 0.15 | 8.8 ± 2.2 | 35.9 ± 2.6 | 498 ± 90 | 42.6 ± 3.8 | 424 ± 98 | 0.42 ± 0.18 | 6.8 ± 2.4 |
| BL 1473 × NL 971 – $F_4$ | 2007 | 39.8 ± 4.1 | 412 ± 65 | 42.4 ± 5.1 | 507 ± 195 | 0.59 ± 0.15 | 8.1 ± 2.3 | 34.9 ± 4.6 | 502 ± 92 | 39.4 ± 4.2 | 403 ± 101 | 0.42 ± 0.15 | 6.4 ± 2.1 |

MS 7, Milan/Shanghai#7; $SR_A$, SPAD value at anthesis; AUSDC, area under SPAD decline curve; $F_v/F_{m\,(L)}$, is the lowest $F_v/F_m$ value; AUFDC, area under $F_v/F_m$ progress curve; ± indicates standard deviation of the populations; AUDPC, area under disease progress curve. TKW, 1000-kernel weight.

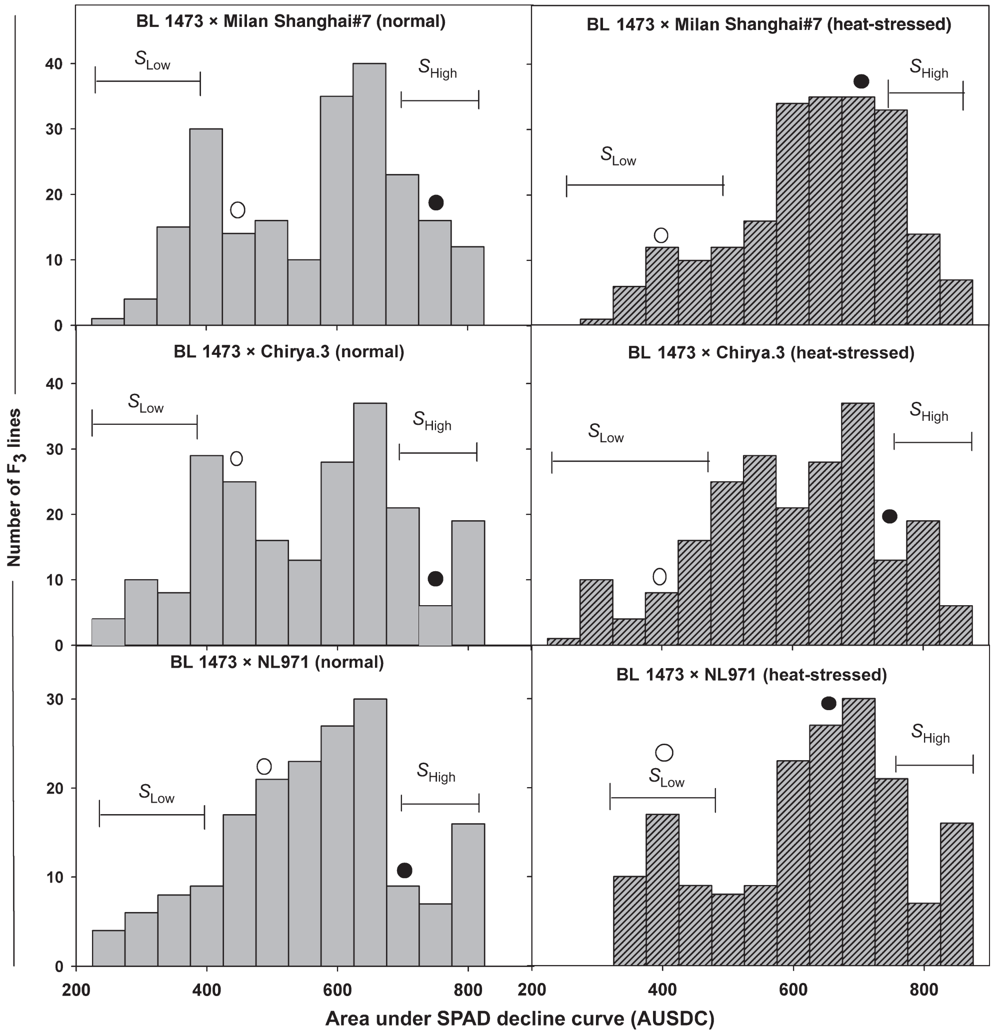


Fig. 1 Frequency distribution of $F_3$ lines and selected high ($S_{High}$) and selected low ($S_{Low}$) lines for area under SPAD decline curve (AUSDC) evaluated under normal and late planting conditions during 2006 at Rampur, Chitwan, Nepal. The selected high and low fractions are denoted with horizontal bars. Open circle (○) indicates position of mean of BL 1473 parent whereas solid circle (●) represents the mean for the second parent Milan/Shanghai#7, Chirya.3 or NL971

$$h_R^2 = \left(\frac{\overline{F_{4high}} - \overline{F_{4low}}}{\overline{F_{3high}} - \overline{F_{3low}}}\right) \times 100$$

where $\overline{F_{3high}}$ and $\overline{F_{3low}}$ are the respective means of the 15% lines selected for high and the 15% lines selected for low value of individual traits under consideration in the $F_3$ generation, and $\overline{F_{4high}}$ and $\overline{F_{4low}}$ are their corresponding mean values in the $F_4$ generation. Genetic correlation was calculated using SAS (2003) using parents and offspring covariances as outlined by Sexton (2004).

## Results

### Weather and heat stress

Late planting created high level of terminal heat stress in both years as indicated by average daily temperature greater than optimal temperature and crop GDD. Under normal planting conditions, the average daily temperature exceeded 25°C for 3 days in 2006 and 4 days in 2007, in contrast to 23 days in 2006 and 25 days in 2007 under late planting conditions. Similarly, GDD values for normal planting were 2417 and 2412°C in 2006 and 2007, respectively; the corresponding values for the late planting were 2992 and 3012°C.

### Disease severity

Foliar blight severity was high in both years as shown by AUDPC value of susceptible genotype BL 1473 (Table 1). Disease symptoms were uniformly visible in plants and leaf samples confirmed presence of *C. sativus* and no other disease was present. The parent genotypes greatly differed for AUDPC (Table 1).

### Physiological traits

In each of the three populations under both normal and late seeding, the $F_3$ lines were continuously distributed for AUSDC (Fig. 1) and AUFDC (Fig. 2),

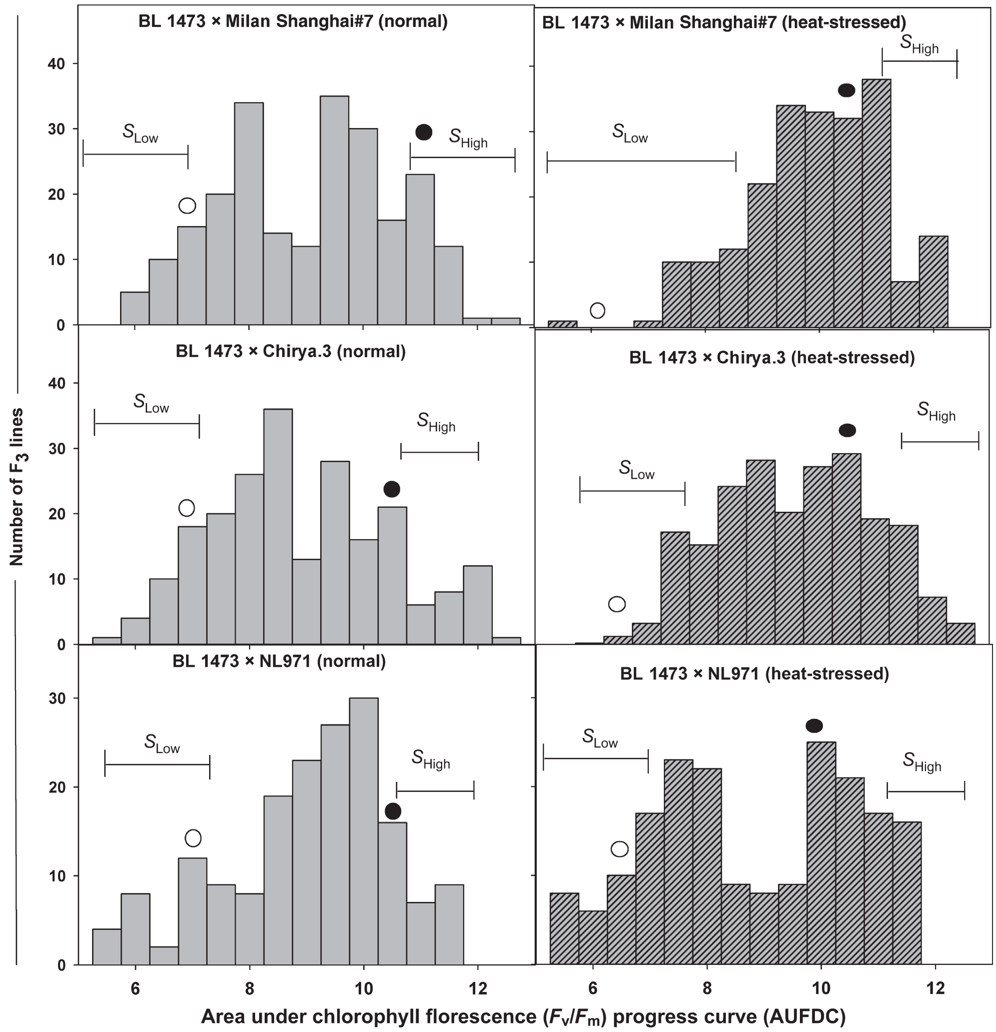


Fig. 2 Frequency distribution of $F_3$ lines and selected high ($S_{High}$) and selected low ($S_{Low}$) lines for area under chlorophyll florescence ($F_v/F_m$) progress curve (AUFDC) evaluated under normal and late planting conditions during 2006 at Rampur, Chitwan, Nepal. The selected high and low fractions are denoted with horizontal bars. Open circle (○) indicates position of mean of BL 1473 parent whereas solid circle (●) represents the mean for the second parent either Milan/Shanghai 7, Chirya.3 or NL971

suggesting quantitative inheritance of the two traits in these populations. Positive as well as negative transgressive segregants were observed for all of the traits studied (Figs 1–3).

**Photochemical efficiency**

The $F_v/F_m$ measurements showed a declining trend in all genotypes under both seeding conditions (Fig. 4), which was expected. The lowest $F_v/F_m$ was attained in the last date of observation. However, the rate of decline was higher in the susceptible than resistant genotypes. Similarly, normal planting conditions had a slower decline in $F_v/F_m$ than late planting. The $F_v/F_{m\ (L)}$ and AUFDC were negatively correlated with spot blotch severity (Table 2). The spot blotch–resistant genotypes – Milan Shanghai#7, Chirya.3 and NL971 – were able to maintain high $F_v/F_m$ value under stressed conditions. AUFDC showed a stronger genetic correlation with grain yield and TKW than with spot blotch. Lowest chlorophyll fluorescence ($F_v/F_{m\ (L)}$) showed high genetic correlation with AUDPC and TKW both under normal and under heat stressed conditions (Table 2).

The heritability estimates were high for AUFDC (range: 0.55–0.89) and $F_v/F_{m\ (L)}$ (range 0.53–0.79) both under normal and under heat stressed conditions (Table 2).

**SPAD value**

The difference between resistant and susceptible genotypes in the SPAD value taken just after anthesis ($SR_A$) was non-significant. There was progressive decline of chlorophyll content after anthesis (Fig. 4). Difference in SPAD value among genotypes became prominent with development of grain and disease. The resistant genotypes had higher AUSDC than the susceptible genotype both under heat stressed and under non-stressed conditions (Table 1). The AUSDC was positively correlated with TKW and negatively with AUDPC (Table 2). Both $SR_A$ ($h^2$ range: 0.69–0.76)

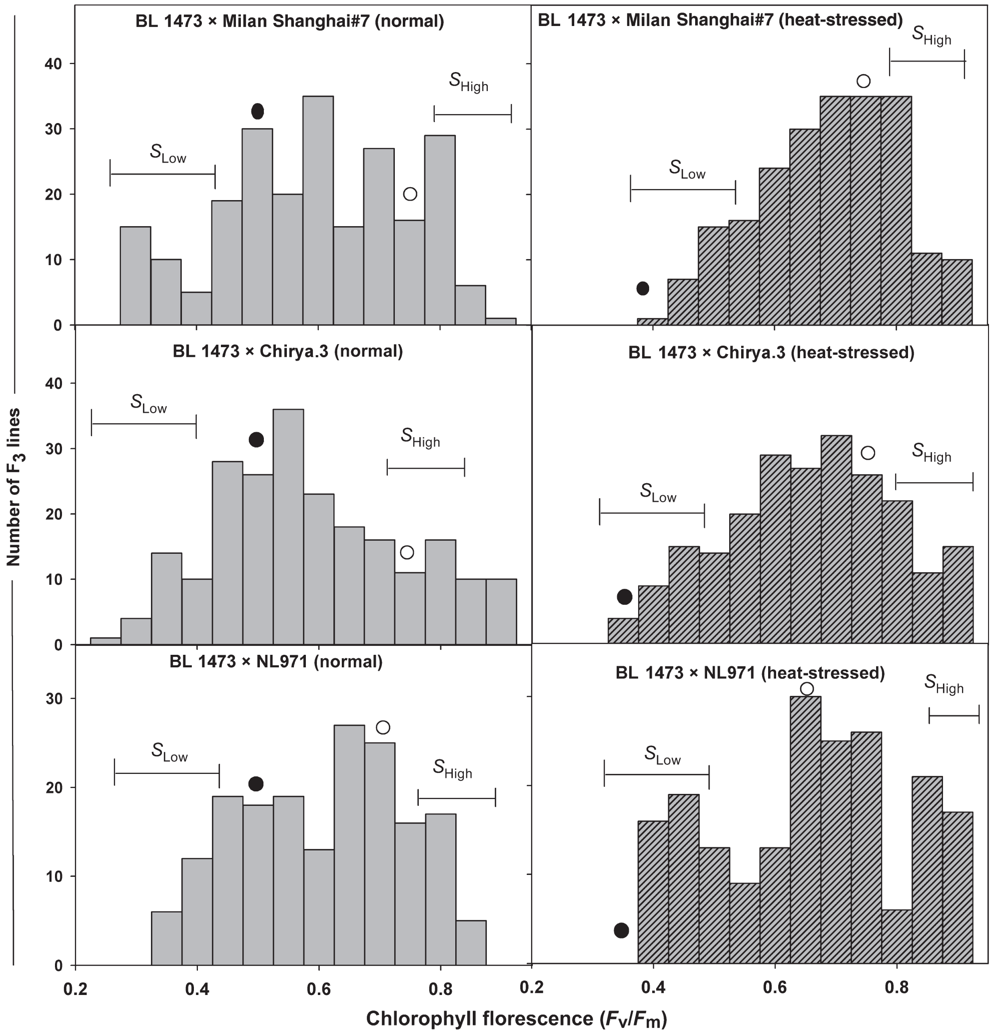


Fig. 3 Frequency distribution of $F_3$ lines and selected high ($S_{High}$) and selected low ($S_{Low}$) lines for Chlorophyll Florescence ($F_v/F_m$) evaluated under normal and late planting conditions during 2006 at Rampur, Chitwan, Nepal. The selected high and low fractions are denoted with horizontal bars. Open circle (○) indicates position of mean of BL 1473 parent whereas solid circle (●) represents the mean for the second parent Milan/Shanghai#7, Chirya.3 or NL971

and AUSDC ($h^2$ range: 0.73–0.86) showed high heritability under normal and heat stressed conditions (Table 2).

## Discussion

The low disease severity on Chirya.3 and Milan Shanghai#7 and moderate value on NL971 were consistent with the results of previous studies (Duveiller et al. 2005; Neupane et al. 2007).

The $F_v/F_m$ and SPAD value showed continuous distribution inconsistent with previous studies (Araus et al., 1998; Collaku and Harrison 2005). Reduced $F_v/F_m$ due to both stresses indicated their adverse effect on quantum yield potential of photosynthesis or maximal photochemical efficiency of Photosystem II. Reduced $F_v/F_m$ due to heat stress is a well-known phenomenon (Moffatt et al. 1990a,b; Bassanezi et al. 2002; Sayed 2003; Earl and Davis 2003). Besides, our study showed that spot blotch had also adversely affected $F_v/F_m$ in susceptible genotypes. In consistence with our results, different chlorophyll fluorescence parameters have been suggested as a disease resistance or severity screening tool for other host–pathogen systems (Duraes et al. 2001; Sayed 2003; Chaerle et al. 2007). Also, chlorophyll fluorescence was found to be affected by both heat stress and spot blotch (Mercado et al. 2003). Phenotypic variation for photochemical efficiency is well known. Royo et al. (2000) observed variation for chlorophyll fluorescence parameters during the grain filling period. Chlorophyll fluorescence was found as a good marker for economic yield in barley and was suggested as a selection criterion due to its high heritability (Planchon et al. 1989).

The result of this study differs from previous findings showing low to moderate heritability estimates of chlorophyll content under other conditions (Collaku and Harrison 2005). In fact, SPAD measurement is indirect measure of leaf chlorophyll content but is good indirect estimator of concentration of photosynthetically important pigments. Leaf colour, glaucous-

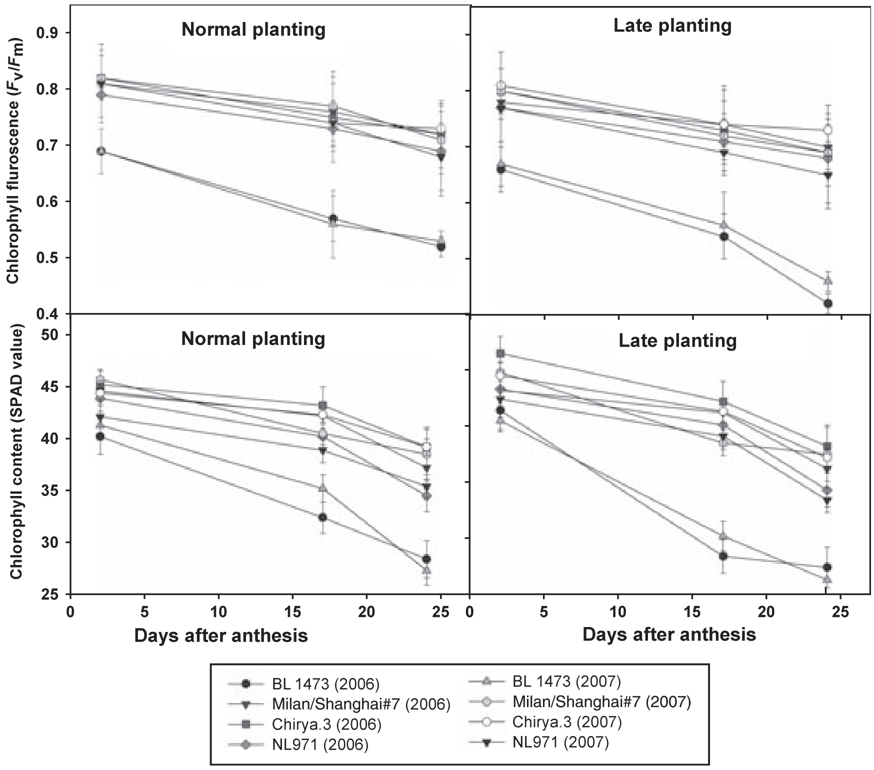


Fig. 4 Effect of spot blotch on photosynthetic quantum yield of PSII ($F_v/F_m$(L)), and chlorophyll content (SPAD value) of wheat genotypes after anthesis in wheat genotypes evaluated during 2006–2007 at Rampur, Chitwan, Nepal. Bar represents standard error of means

Table 2
Genetic correlation of SPAD value and photochemical efficiency ($F_v/F_m$)–related traits with AUDPC and 1000-kernel weight under natural epiphytotics of spot blotch and heat stress on wheat evaluated during 2006–2007 at Rampur, Chitwan, Nepal

| | Normal | | | | Heat stressed | | | |
|---|---|---|---|---|---|---|---|---|
| Cross | AUSDC | $SR_A$ | AUFDC | $F_v/F_{m\ (L)}$ | AUSDC | $SR_A$ | AUFDC | $F_v/F_{m\ (L)}$ |
| Genetic correlation with AUDPC | | | | | | | | |
| BL 1473 × MS7 | −0.72** | −0.11 | −0.82** | −0.70** | −0.70** | −0.10 | −0.84** | −0.73** |
| BL 1473 × Chirya.3 | −0.74** | −0.20 | −0.79** | −0.72** | −0.76** | −0.08 | −0.89** | −0.70** |
| BL 1473 × NL971 | −0.74** | −0.18 | −0.84** | −0.69** | −0.79** | −0.06 | −0.80** | −0.65** |
| Genetic correlation with 1000-kernel weight | | | | | | | | |
| BL 1473 × MS 7 | 0.76** | 0.18 | 0.84** | 0.69** | 0.72** | 0.18 | 0.81** | 0.74** |
| BL 1473 × Chirya.3 | 0.76** | 0.23 | 0.83** | 0.65** | 0.72** | 0.21 | 0.82** | 0.73** |
| BL 1473 × NL971 | 0.78** | 0.26 | 0.80** | 0.75** | 0.79** | 0.16 | 0.79** | 0.74** |
| Heritability | | | | | | | | |
| BL 1473 × MS 7 | 0.86 | 0.72 | 0.89 | 0.79 | 0.82 | 0.76 | 0.86 | 0.72 |
| BL 1473 × Chirya.3 | 0.80 | 0.76 | 0.83 | 0.69 | 0.82 | 0.69 | 0.84 | 0.71 |
| BL 1473 × NL971 | 0.74 | 0.70 | 0.55 | 0.58 | 0.73 | 0.73 | 0.82 | 0.53 |

MS7, Milan/Shanghai#7; $SR_A$, SPAD value at anthesis; AUSDC, area under SPAD decline curve; $F_v/F_{m\ (L)}$, lowest chlorophyll fluorescence $F_v/F_m$ value; AUFDC, area under chlorophyll fluorescence ($F_v/F_m$) progress curve; AUDPC, area under disease progress curve. *$P < 0.05$; **$P < 0.01$.

ness and pubescence contribute to stress avoidance, by reducing radiation absorbed by plants and increasing crop albedo. In our study, both heat and disease stress reduced chlorophyll content of leaves. It is well established that loss in chlorophyll content is closely associated with heat-induced damage to thylakoids (Ristic et al. 2007). Thylakoid membranes and PS II are considered the most heat-labile cell structures. Thylakoids harbour chlorophyll, a portion of which is associated with the proteins of PS II. Damage to thylakoids caused by heat is expected to result in a loss of chlorophyll. Postanthesis decline of chlorophyll content has been found to be associated with heat stress (Reynolds et al. 2007; del Blanco et al. 2000) and spot blotch (Mercado et al. 2003; Rosyara et al. 2007). The results of this study are consistent with the findings of these earlier studies.

Breeders are always in search of traits that are good predictors of yield under both stressed as well as non-stressed situations. A useful trait should be genetically correlated with the target traits of breeders' interest under normal and/or stressed conditions. In addition, measurements of the trait should be quick and cost effective. Labour requirement restricts using photosynthesis measurements as indicative of many stresses in the plants (Earl and Tollenaar 1999).

The availability of recent models of chlorophyll fluorometer allows non-destructive measurements of $F_v/F_m$ on the same leaf over time. Although measurement of chlorophyll fluorescence is faster than gas exchange measurements, still breeders' measuring device to score a large number of plants or lines per day. More advanced measurement techniques, such as remote sensing or thermal and visible/near infrared imaging system might be useful for indirect estimation of leaf chlorophyll content (Babar et al. 2006) and chlorophyll fluorescence (Moya et al. 2004) in future.

While applying physiological tools, one important decision required is how many readings should be taken precisely to represent the extent of stress to plants. There could be two alternative ways to employ these physiological traits: (i) taking a single reading when the traits show highest level of variation among genotypes, thus differentiating high from low without confounding effect of other physiological processes such as natural senescence and (ii) recording three or more readings during grain filling and calculate a progress curve including chlorophyll fluorescence and chlorophyll content. The concept of area under progress or decline curve has been found useful to integrate multiple observations for other physiological processes (Rosyara et al. 2007; Wu et al. 2006) in addition to the common use of AUDPC in plant pathology to assess disease evolution. The results indicate that taking multiple readings and calculating progress or decline curve are more informative than single reading.

The high genetic correlations of chlorophyll content and photochemical efficiency–related traits with AUDPC and TKW under both normal and heat stressed condition show that these traits are linked. Such results can be explained by the effect of both stresses on photosynthesis in leaves. Also, the high heritability estimates under both heat stress and non-stressed conditions suggest that these traits can potentially serve as complementary indicators to select high performing genotypes in spot blotch and terminal heat stress conditions.

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**References**

Araus JL, Amaro T, Zuhair Y, Nachit M. (1997) Effect of leaf structure and water status on carbon isotope discrimination in field grown-durum wheat. *Plant Cell Environ* **20**:1484–1494.

Araus JL, Amaro T, Voltas J, Nakkoul H, Nachit MM. (1998) Chlorophyll fluorescence as a selection criterion for grain yield in durum wheat under Mediterranean conditions. *Field Crops Res* **55**:209–223.

Babar MA, Reynolds MP, van Ginkel M, Klatt AR, Raun WR, Stone ML. (2006) Spectral reflectance to estimate genetic variation for in-season biomass, leaf chlorophyll, and canopy temperature in wheat. *Crop Sci* **46**:1046–1057.

Bassanezi RB, Amorim L, Bergamin Filho A, Berger RD. (2002) Gas exchange and emission of chlorophyll fluorescence during the monocycle of rust, angular leaf spot and anthracnose on bean leaves as a function of their trophic characteristics. *J Phytopathol* **150**:37–47.

del Blanco IA, Rajaram S, Kronstad WE, Reynolds MP. (2000) Physiological performance of synthetic hexaploid wheat-derived populations. *Crop Sci* **40**:1257–1263.

Cao W, Moss DN. (1989) Day length effect on leaf emergence and phyllochron in wheat and barley. *Crop Sci* **29**:1021–1025.

Chaerle L, Hagenbeek D, Bruyne ED, Van Der Straeten D. (2007) Chlorophyll fluorescence imaging for disease-resistance screening of sugar beet. *Plant Cell Tiss Organ Cult* **91**:97–106.

Collaku A, Harrison SA. (2005) Heritability of waterlogging tolerance in wheat. *Crop Sci* **45**:722–727.

Duraes FOM, Gama EEG, Malchaes PC, Marriel IM, Casela CR, Oliveira AC, Luchiari A Jr, Shanahan JF. (2001) The usefulness of chlorophyll fluorescence in screening for disease resistance, water stress tolerance, aluminum toxicity, and use efficiency in maize. *Proceedings of Seventh Eastern and Southern African Regional Maize Conference 11th–15th Feb, 2001*, pp 356–360.

Duveiller EM, Sharma RC. (2009) Genetic improvement and crop management strategies to minimize yield losses in warm non-traditional wheat growing areas due to spot blotch pathogen *Cochliobolus sativus*. *J Phytopathol* **157**:521–534.

Duveiller E, Kandel YR, Sharma RC, Shrestha SM. (2005) Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. *Phytopathology* **95**:248–256.

Earl HJ, Davis RF. (2003) Effect of drought stress on leaf and whole canopy radiation use efficiency and yield of maize. *Agron J* **95**:688–696.

Earl HJ, Tollenaar M. (1998) Relationship between thylakoid electron transport and photosynthetic $CO_2$ uptake in leaves of three maize (*Zea mays* L.) hybrids. *Photosynth Res* **58**:245–257.

Earl HJ, Tollenaar M. (1999) Using chlorophyll fluorometry to compare photosynthetic performance of commercial maize (*Zea mays* L.) hybrids in the field. *Field Crops Res* **61**:201–210.

Farquhar GD, Wong SC, Evans JR, Hubick KT. (1989) Photosynthesis and gas exchange. In: Jones HG, Flowers TJ, Jones MB. (eds) *Plants Under Stress*. Cambridge, Cambridge University Press, pp 47–69.

Guthrie DA, Smith EL, Mcnew RW. (1984) Selection for high and low grain protein in six winter wheat crosses. *Crop Sci* **24**:1097–1100.

Huang B. (2006) *Plant–Environment Interactions*. Boca Raton, FL, CRC Press.

Joshi A, Kumari M, Singh V, Reddy C, Kumar S, Rane J, Chand R. (2007) Stay green trait: variation, inheritance and its association with spot blotch resistance in spring wheat (*Triticum aestivum* L.). *Euphytica* **153**:59–71.

Krause GH, Weise E. (1991) Chlorophyll fluorescence and photosynthesis: the basis. *Annu Rev Plant Physiol* **136**:472–479.

Liu X, Huang B. (2000) Heat stress injury in relation to membrane lipid peroxidation in creeping Bentgrass. *Crop Sci* **40**:503–510.

Mercado D, Renard ME, Maraite H, Duveiller E. (2003) Chlorophyll content and chlorophyll fluorescence as indicators of resilience to temperature stress in wheat and its relationship with resistance to *Bipolaris sorokiniana*. In: Rasmussen JB, Friesen TL, Ali S. (eds) *Proceedings of the International Wheat Tan Spot and Spot Blotch Workshop*, Bemidji, North Dakota State University, 21–24 July 2002, pp 60–63.

Moffatt JM, Sears RG, Paulsen GM. (1990a) Wheat high temperature tolerance during reproductive growth. I. Evaluation by chlorophyll fluorescence. *Crop Sci* **30**:881–885.

Moffatt JM, Sears RG, Cox TS, Paulsen GM. (1990b) Wheat high temperature tolerance during reproductive growth: II. Genetic analysis of chlorophyll fluorescence. *Crop Sci* **30**:886–889.

Moya I, Camenen L, Evain S, Goulas Y, Cerovic ZG, Latouche G, Flexas J, Ounis A. (2004) A new instrument for passive remote sensing 1. Measurements of sunlight-induced chlorophyll fluorescence. *Remote Sen Env* **91**:186–197.

Neupane RB, Sharma RC, Duveiller E, Ortiz-Ferrara G, Ojha BR, Rosyara UR, Bhandari D, Bhatta MR. (2007) Major gene controls of field resistance to Spot Blotch in wheat genotypes 'Milan/Shanghai #7' and 'Chirya.3'. *Plant Dis* **91**:692–697.

Planchon C, Sarrafi A, Ecochard R. (1989) Chlorophyll fluorescence transient as a genetic marker of productivity in barley. *Euphytica* **42**:269–273.

Randall PJ, Moss HJ. (1990) Some effects of temperature regime during grain filling on wheat quality. *Aust J Agric Res* **41**:603–617.

Reynolds MP. (1994) Summary of data from the 1st and 2nd International Heat Stress Genotype Experiment. In: Saunders DA, Hettel GP. (eds) *Wheat in Heat Stressed Environments: Irrigated, Dry Areas and Rice Farming Systems. Proceedings of the International Conference, Wheat in Hot, Dry, Irrigated Environments*. CIMMYT, DF, Mexico.

Reynolds MP, Singh RP, Ibrahim A, Ageeb OAA, Larqué-Saavedra A, Quick JS. (1998) Evaluating physiological traits to complement empirical selection for wheat in warm environments. *Euphytica* **100**:84–95.

Reynolds MP, Saint Pierre C, Saad ASI, Vargas M, Condon AG. (2007) Evaluating potential genetic gains in wheat associated with stress-adaptive trait expression in elite genetic resources under drought and heat stress. *Crop Sci* **47**:172–189.

Ristic Z, Bukovnik U, Vara Prasad PV. (2007) Correlation between heat stability of thylakoid membranes and loss of chlorophyll in winter wheat under heat stress. *Crop Sci* **47**:2067–2073.

Rosyara UR, Pant K, Duveiller E, Sharma RC. (2007) Variation in chlorophyll content, anatomical traits and agronomic performance of wheat genotypes differing in spot blotch resistance under natural epiphytotic conditions. *Aust Pl Path* **36**:245–251.

Royo C, García del Moral LF, Aparicio N, Villegas D, Casadesús J, Araus JL (2000) Tools for improving the efficiency of durum wheat selection under Mediterranean conditions. In: *Seminar on Durum Wheat Improvement in the Mediterranean Region: New Challenges, Zaragoza (Spain), 12–14 Apr 2000*, CIHEAM-IAMZ, Zaragoza, Spain.

SAS. (2003) *SAS 9.1 for Windows*. Cary, NC, USA, SAS Institute.

Sayed OH. (2003) Chlorophyll fluorescence as a tool in cereal crop research. *Photosynthetica* **41**:321–330.

Sexton AM. (2004) *Genetic Analysis of Complex Traits Using SAS*. Cary, NC, USA, SAS Institute.

Sharma RC, Duveiller E. (2004) Effect of Helminthosporium leaf blight on performance of timely and late-seeded wheat under optimal and stressed levels of soil fertility and moisture. *Field Crops Res* **89**:205–218.

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

Sharma RC, Tiwary AK, Ortiz-Ferrara G. (2008) Reduction in kernel weight as a potential indirect selection criterion for wheat grain yield under terminal heat stress. *Plant Breed* **127**:241–248.

Wu B, Yue Z, Peiji G. (2006) Estimation of cellobiohydrolase I activity by numerical differentiation of dynamic ultraviolet spectroscopy. *Acta Biochim Biophys Sin* **38**:372–378.

Yang J, Sears RG, Gill BS, Paulsen GM. (2002) Growth and senescence characteristics associated with tolerance of wheat-alien amphiploids to high temperature under controlled conditions. *Euphytica* **126**:185–193.

Ying J, Lee EA, Tollenaar M. (2002) Response of leaf photosynthesis during the grain-filling period of maize to duration of cold exposure, acclimation, and incident PPFD. *Crop Sci* **42**:1164–1172.