CHLOROPHYLL CONTENT MEASUREMENT OF DROUGHT TOLERANT WHEAT GENOTYPES SOWN AT NORMAL AND LATE CONDITIONS AS AN INDICATOR OF SPOT BLOTCH RESISTANCE

L. Aryal[1], S. M. Shrestha[2], G.B. Khatri-Chhetri[2] and D. Bhandari[1]

[1]Nepal Agriculture Research Council, Nepal, [2]Institute of Agriculture and Animal Sciences, Nepal

ABSTRACT

A field experiment was conducted at Agronomy block of the Institute of Agriculture and Animal Science (IAAS), Rampur to evaluate chlorophyll content of 20 drought tolerant wheat genotypes as a parameter of stay green character and spot blotch response. The experiment was laid out in a split plot design with three replications, considering 25 November as normal sowing and 15 December as late sowing dates. Of the tested genotypes, Aditya had highest SPAD value (46.67) with least AUDPC (146) on flag leaf on 25 November sowing and higher SPAD value (43.57) with lower AUDPC (161) on 15 December sowing. Similarly, CSISA DRYT 5204 and CSISA DRYT 5205 had moderate SPAD values with AUDPC value at par with Aditya. Aditya with low AUDPC possessed longer stay green days (118.3) on 25 November sowing and shorter green days (107.5) on 15 December sowing. The susceptible check variety RR-21 possessed shorter stay green days (113.6) with SPAD value (35.7) on 25 November sowing and 100.4 days of stay green with SPAD value (31.23) on 15 December sowing. Aditya also had highest grain yields and thousand kernel weights on both the 25 November and 15 December sowing. The results showed Aditya, CSISA DRYT 5204 and CSISA DYRT 5205 were having longer period of stay green with higher SPAD and low AUDPC values and thus these three genotypes can be recommended for late sown conditions.

Key words: Spot blotch, heat stress, stay green, SPAD value, AUDPC, TKW, grain yield.

INTRODUCTION

Successful cultivation of wheat in Eastern Gangetic plains of South Asia is challenged by two important factors. Among them Helminthosporium Leaf Blight (HLB), which is the complex of spot blotch and tan spot is the biotic factor and terminal heat stress is another abiotic factor which affects wheat kernel development (Rane *et al.,* 2000, Sharma *et al.,* 2007a).These two stresses are supposed to be associated for causing complication in the development of tolerant genotypes. Spot blotch has a worldwide distribution, but is particularly aggressive under conditions of high relative humidity and temperature associated with imbalanced soil fertility. 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).

Both spot blotch and heat stress are severe during the post-anthesis period when temperature increases slowly causing premature senescence of leaves, reduced grain filling duration, and lower kernel weight (Joshi *et al*., 2007 and Sharma *et al*., 2007a) which finally reduces the productivity of the crop. 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

Spot blotch 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). 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.

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 wheat in the late season,which are likely to be affected by both the spot blotch and terminal heat stress. One of the possible reasons for the slow progress in selection for spot blotch resistance and heat tolerance could be the positive association between the two, and their negative relationship with grain yield and thousand kernel weight (Sharma *et al*., 2007a, 2008). At temperatures higher than 26^{o}C, even resistant varieties show some degree of disease severity (Duveiller *et al*., 2005). Leaf chlorophyll content showed high genetic variation with yield under heat stress (Reynolds *et al*., 2007). Similarly, genotypes which possessed high chlorophyll content also showed spot blotch response accordingly (Rosyara *et al.,* 2009). Therefore, the present study was conducted

- to study variations in chlorophyll content of different genotypes as an indicator of spot blotch tolerance
- to find out effect of chlorophyll content on grain yield and thousand kernel weight of wheat genotypes

METHODOLOGY

Seed collection

Twenty released as well as pre-released drought tolerant wheat genotypes having different genetic background, yield potential, maturity and level of Spot blotch resistance were used in this study. The seeds of the genotypes were received from National Wheat Research Program (NWRP), Bhairahawa. Aditya and Bhrikuti were included as spot blotch resistant and RR-21 as spot blotch susceptible.

Experimental site

The experiment was conducted at the Agronomy Research Farm of the Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan, Nepal during November 2011 to April 2012. The site is situated at 27^{o}31’ north latitude and 84^{o}25’ east longitude with an elevation of 256 m above mean sea level. Climate of the location was subtropical and humid type. The maximum and minimum temperature recorded for the 25 November sowing day was 27^{o}C and 11^{o}C, respectively with 90% relative humidity (RH). For 15 December sowing day, the maximum and minimum temperature recorded was 20^{o}C and 8^{o}C, respectively with 88% RH. The 25 November sowing received total 171.6 mm of rain while 15 December sowing received total 306.7 mm of rain during the entire growth period of the wheat crops.

Design of the experiment plot and sowing

The field experiment was laid out in a split-plot design with three replications. Sowing date was taken as main plots and wheat genotypes as sub-plots. The size of individual plot was 1m x 1m. Four rows were drawn in each plot and continuous sowing was done at the spacing of 25cm between the rows @ 120kg/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 253m^2 (22 x 11.5 m). Chemical fertilizers were applied @ 120kg N, 60kg P_2O_5 and 40 kg K_2O ha^{-1}, as recommended by NWRP, was applied through urea, complexal and potash. The crop was grown under rainfed conditions.

Disease assessment:

Both single and double digit scoring were done for disease assessment. 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-Kazi *et al*., 1996). Three scorings at the interval of 6 days were done when the disease development started in penultimate and flag leaves.

Double digit scoring was done after anthesis 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 and Prescott, 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 scorings were done at the interval of 6 days. For each evaluation, percent disease severity was estimated based on the following formula:

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

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 formula (Das *et al*.,1992):

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

Chlorophyll content measurement

Portable chlorophyll meter SPAD-502 of Konica-Minolta company manufactured by Japan (Soil Plant Analysis Development) was used to measure the chlorophyll content (also known as stay green parameter). Ten effective tillers were tagged randomly from each plot and SPAD value was taken from three different part (leaf base, middle and top) of the flag leaf of each tillers and value was averaged for chlorophyll content reading. SPAD value was taken three times (2-3 days after disease scoring).

Yield attributes

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

SPAD, AUDPC values and days to flag leaf senescence

Interaction between the date of sowing and SPAD values of the wheat genotypes was highly significant. SPAD values ranged from 31.23 to 47.60. Highest SPAD value (47.60) was recorded in CSISA DRYT 5207 followed by CSISA DRYT 5226 (47.27), Aditya (46.67) and CSISA DRYT 5224 (45.43) on 25 November sowing. Lowest SPAD value (31.23) was recorded in RR-21 followed by CSISA DRYT 5217 (35.5), CSISA DRYT 5211 (35.67) on 15 December sowing. RR-21 had lower SPAD value (35.73) even on 25 November sowing. Aditya had higher SPAD value (43.57) even on 15 December sowing (Table 1).

Higher SPAD value for Aditya might be due to its longer flag leaf duration days and low disease severity. Lower SPAD with high AUDPC value for RR-21 suggests that the chlorophyll content of the genotypes decreases as the disease severity increases. High disease severity cause rapid necrosis of the flag leaf, thereby reducing chlorophyll content. Negative correlation between the SPAD value and AUDPC has been demonstrated by Rosyara *et al*., (2007, 2009) and Joshi *et al.,* (2007)

Interaction between the date of sowing and Area under Disease Progress Curve for Flag Leaf (AUDPC F) values of the wheat genotypes was highly significant. Highest AUDPC F value (815.7) was recorded in RR-21 followed by CSISA DRYT 5217 (796.3), CSISA DRYT 5202 (763.7) and CSISA DRYT 5219 (743.0) respectively on 15 December sowing. Lowest AUDPC F value (146.0) was recorded in Aditya, followed by CSISA DRYT 5205 (165.0), CSISA DRYT 5228 (167.7) and CSISA DRYT 5229 (168.3) respectively on 25 November sowing. Aditya had lower AUDPC F value (161.0) even on 15 December sowing (Table 1).

Interaction between the date of sowing and Days to Flag Leaf Senescence (DTFLS) values of wheat genotypes was significant. DTFLS value ranged from 121.7 to 100.4. Highest DTFLS value (121.7) was recorded in CSISA DRYT 5223 followed by CSISA DRYT 5229 (121.3), CSISA DRYT 5205 (120.4) and CSISA DRYT 5228 (120.3) respectively on 25 November sowing. Lowest DTFLS value (100.4) was recorded in RR-21, followed by CSISA DRYT 5207 (101.7), CSISA DRYT 5202 (102.1) and CSISA DRYT 5220 (103) respectively on 15 December sowing (Table 1).

Resistant genotypes having higher SPAD value took longer days for flag leaf senescence, susceptible genotypes which had shorter flag leaf senescence days had lower SPAD value and tolerant genotypes had moderate values. Similar results were obtained by Rosyara *et al.*, (2010).

Table 1: Mean SPAD and AUDPC values on different dates of sowing at Rampur, Chitwan, Nepal, 2011-2012

Genotypes	SPADValue 25 NOV	SPAD Value 15 DEC	DTFLS 25 NOV	DTFLS 15 DEC	AUDPC F 25 Nov	AUDPC F 15 DEC
CSISA DRYT 5202	40.87efghij	37.33lmno	117.3ef	102.1nop	482.0gh	763.7c
CSISA DRYT 5203	38.10klmn	36.53mno	119.3bcde	103.6lmno	451.0j	736.3de
CSISA DRYT 5204	44.73bc	39.97ghijk	119.7abcd	106.0hijk	181.7uvw	185.0uv
CSISA DRYT5205	45.27abc	41.50defgh	120.4abcd	104.6ijklm	165.0wx	194.0u
CSISA DRYT5207	47.60a	41.47defgh	118.4cdef	101.7op	249.7st	275.3qr
CSISA DRYT 5210	41.67defg	38.93hijklm	118.3def	104.5ijklm	241.7t	284.7q
CSISA DRYT 5211	40.80efghij	35.67no	118.3def	103.9klmno	491.3g	752.0cd
CSISA DRYT 5217	36.63mno	35.50o	116.3f	104.1jklmn	498.3g	796.3b
CSISA DRYT 5218	44.80bc	39.60ghijkl	119.0cde	103.8klmno	252.7st	265.3rs
CSISA DRYT 5219	43.03cdef	41.73defg	118.3def	103.9klmno	488.3gh	743.0d
CSISA DRYT 5220	44.97bc	38.80ijklm	118.5cdef	103.0mno	471.0hi	721.0e
CSISA DRYT 5223	42.03defg	37.30lmno	121.7a	106.7hi	332.0o	362.3m
CSISA DRYT 5224	45.43abc	41.70defg	120.7abc	105.7hijkl	343.0no	357.3mn
CSISA DRYT 5226	47.27ab	38.67ijklm	119.7abcd	107.3h	303.3p	358.0mn
CSISA DRYT 5227	43.27cde	38.93hijklm	119.7abcd	105.3hijkl	373.3m	391.0l
CSISA DRYT 5228	43.23cde	40.50fghijk	120.3abcd	106.3hij	167.7vwx	184.3uv
CSISA DRYT 5229	42.07defg	41.20defghi	121.3ab	106.4hi	168.3vwx	191.3u
Aditya	46.67ab	43.57cd	118.3def	107.5h	146.0y	161.0xy
Bhrikuti	45.13abc	38.37jklm	117.3ef	103.5lmno	409.0k	459.3ij
RR-21	35.73no	31.23p	113.6g	100.4p	569.0f	815.7a
LSD	2.20		1.93		16.69	
SEm(±)	0.78		0.68		5.92	
CV%	3.31		1.07		10.6	
Probability	0.00		0.014		0.00	

SPAD: Soil Plant Analysis Development; AUDPC F: Area Under Disease Progress Curve on Flag Leaf. DTFLS: Days to Flag Leaf Senescence. 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.

Grain yield and thousand kernel weight

Interaction between the dates of sowing and genotypes was not significant for grain yield (Table 2) though the early sown (25 November) wheat genotypes had the trend of higher grain yields than the late sown (15 December). This could be attributed to higher SPAD value for early sown and lower SPAD for late sown wheat genotypes. Aditya having higher SPAD value even at late sown had higher grain yield (2.90 t ha^{-1}). Positive correlation of AUDPC and SPAD with grain yield was also reported by Rosyara *et al.,* (2010).

Interaction between the dates of sowing and genotypes for thousand kernel weight (TKW) was highly significant (Table 2). The TKW ranged from 52.07 g and 47.61 g (Aditya) to 25.4 g and 22.33 g (CSISA DYRT 5217) on early and late sown wheat, respectively. Positive correlation between thousand kernel weight and SPAD values was also obtained by Sharma *et al*., (2007).

Table 2: Mean values of economic yield and thousand kernel weight on different dates of sowing at Rampur, Chitwan, Nepal, 2011-2012

Genotypes	EY(t ha^{-1}) 15 NOV	EY(t ha^{-1}) 15 DEC	TKW(g) 25 NOV	TKW(g) 15 DEC
CSISA DRYT 5202	1.81	1.45	40.03ef	30.63o
CSISA DRYT 5203	1.96	1.50	33.20lmn	27.50pq
CSISA DRYT 5204	2.80	2.64	43.03c	40.67e
CSISA DRYT 5205	2.84	2.50	38.05ghi	34.70kl
CSISA DRYT 5207	2.67	2.30	41.07de	37.87ghi
CSISA DRYT 5210	2.86	2.49	28.07p	25.87qr
CSISA DRYT 5211	1.77	1.38	32.40mno	24.91r
CSISA DRYT 5217	2.18	1.69	25.40r	22.33s
CSISA DRYT 5218	2.77	2.09	37.54ghi	35.07kl
CSISA DRYT 5219	2.20	1.61	42.93cd	31.32no
CSISA DRYT 5220	1.89	1.40	35.17kl	28.15p
CSISA DRYT 5223	2.23	1.50	38.61fgh	31.08o
CSISA DRYT 5224	2.79	1.70	37.37hij	32.53mno
CSISA DRYT 5226	2.39	2.23	37.83ghi	35.50jk
CSISA DRYT 5227	2.23	2.20	34.07klm	32.13mno
CSISA DRYT 5228	2.91	2.29	41.23cde	39.54efg
CSISA DRYT 5229	3.10	2.60	36.10ijk	34.60kl
Aditya	3.20	2.90	52.07a	47.61b
Bhrikuti	2.93	2.13	35.00kl	32.00no
RR-21	2.49	1.98	40.93e	32.47mno
LSD	0.39		1.79	
SEm(±)	1.14		0.63	
CV%	10.81		3.13	
Probability	Ns		0.00	

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.

Regression study between Area under Disease Progress curve in Flag Leaf (AUDPC F) and Days to Flag leaf Senescence (DTFLS)

There was highly negative significant correlation (r=-0.62**) between the DTFLS and AUDPC on flag leaf in first date and (r=-0.71**) in second date respectively (Figure 1). According to the coefficient of determination, about 38.8% flag leaf senescence was due to the flag leaf AUDPC on 25 November sowing and 50.5% leaf senescence was due to the flag leaf AUDPC on 15 December sowing. Positive correlation between the days to flag leaf senescence and severity of flag leaf was also reported by Rosyara (2002).

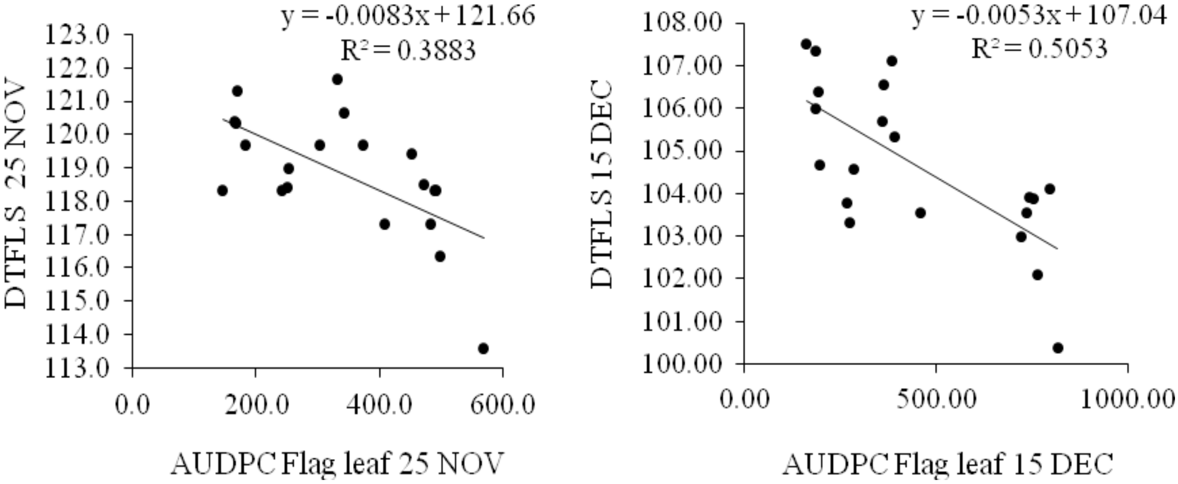

Figure 1. Estimated linear correlation between DTFLS and AUDPC on Flag leaf in two dates of sowing at Rampur, Chitwan, Nepal, 2011- 2012

CONCLUSION

The present study suggested that spot blotch disease reduced photosynthetic activity by reducing green leaf area and stimulating senescence in infected leaves. The wheat genotypes: Aditya, CSISA DRYT 5204 and CSISA DRYT 5205 having longer duration of stay green character with low disease severity can be recommended for late sowing conditions for sustainable wheat production.

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