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Stay green trait: variation, inheritance and its association with spot blotch resistance in spring wheat (*Triticum aestivum* L.)

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Abstract One thousand four hundred and seven spring wheat germplasm lines belonging to Indian and CIMMYT wheat programs were evaluated for stay green (SG) trait and resistance to spot blotch caused by *Bipolaris sorokiniana* during three consecutive crop seasons, 1999–2000, 2000–2001 and 2001–2002. Disease severity was recorded at six different growth stages beginning from tillering to late milk stage. SG trait was measured by following two approaches: difference for 0–9 scoring of green coloration (chlorophyll) of flag leaf and spike at the late dough stage (GS 87) and a new approach of leaf area under greenness (LAUG). Germplasm lines showed a wide range (7–89) for LAUG and were grouped into four viz., SG, moderately stay green, moderately non-stay green and non-stay green (NSG). However, very few (2.2%) lines showed high expression of SG trait, i.e., LAUG >60. LAUG appeared to be a better measure of SG trait than a 0–9 scale. Mean spot blotch ratings of SG genotypes were significantly lower than those of NSG genotypes at all growth stages. Two spot blotch resistant genotypes (Chirya 3 and Chirya 7) having strong expressions of SG trait were crossed with NSG, spot blotch susceptible cv. Sonalika. Individually threshed F_2 plants were used to advance the generations. SG trait and spot blotch severity were recorded in the parents and F_1, F_3, F_4, F_5, F_6 and F_{6-7} generations under disease-protected and inoculated conditions. SG trait in the F_1 generation was intermediate and showed absence of dominance. Evaluation of progenies (202–207) in the segregating generations revealed that SG trait was under the control of around four additive genes. Lines homozygous for SG trait in F_4, F_5, F_6 and F_{6-7} generations showed significantly lower mean area under disease progress curve (AUDPC) for spot blotch than those with NSG expression. A positive correlation (0.73) between SG trait and AUDPC further indicated a positive influence of SG on severity of spot blotch. The study established that

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variation for SG trait exists in spring wheat; around four additive genes control its inheritance in the crosses studied and there is positive association between SG trait and resistance to spot blotch.

Keywords Stay green trait · Inheritance · Variation · Wheat · *Triticum aestivum* · *Bipolaris sorokiniana* · Spot blotch · Resistance

Abbreviations

SG	Stay green
MSG	Moderately stay green
MNSG	Moderately non-stay green
NSG	Non-stay green
LAUG	Leaf area under greenness

Introduction

Stay green (SG) is the general term given to a variant in which senescence (normally apparent to the eye as loss of chlorophyll) is delayed compared with a standard reference genotype (Thomas and Howarth 2000). It is considered an important trait that allows plants to retain their leaves in the active photosynthetic state when subjected to stress conditions. Positive correlation of SG trait with high grain yield has been found in many crops. In durum wheat, a SG mutant has been associated with increased leaf area, rate and duration of grain filling and photosynthetic competence (Spano et al. 2003). SG duration of flag leaf and harvest index showed positive correlations with water-use efficiency during grain formation of wheat (Gorny and Garczynski 2002). During grain maturation in wheat, green and viable leaves significantly contribute photosynthates to developing grain (Thorne 1982). Since there is a strong association between the duration of photosynthetically active leaf area and grain yield (Rawson et al. 1983), selection for SG is expected to have a significant implication in productivity of wheat particularly under harsh environments (Reynolds et al. 1999).

The presence of genetic variation for the timing and rate of leaf senescence, between both species and genotypes has been reported in a number of crop species, including cereals (Thomas and Smart 1993). The genetic basis of SG trait has been studied in crops like soybean, sorghum and sunflower. In wheat, only one gene with two alleles was reported to control the SG trait. This gene had high heritability and showed partial dominance with additive effect (Silva et al. 2000).

Stay green is also known to display good association with resistance to stem rot (Evangelista and Tangonan 1990) suggesting that SG leaves remain photosynthetically active even under biotic stress conditions. By maintaining green coloration, SG trait may resist the growth and development of other diseases especially those caused by facultative pathogens such as the spot blotch pathogen [*Bipolaris sorokiniana* (Sacc.) Shoem (syn. *Helminthosporium sativum*, teleomorph *Cochliobolous sativus*)] and this needs further investigation (Mercado et al. 2003). Spot blotch is considered the most important disease in the warmer and humid growing regions of the world (Joshi et al. 2004a, b; Pandey et al. 2005; Duveiller et al. 2005). 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). To control this disease an integrated approach is considered necessary with host resistance as a major component (Joshi and Chand 2002). Since a vast wheat area is affected by spot botch, even a marginal reduction in disease level may be of significance for wheat growing areas, especially those in developing countries.

Despite the importance of SG, this trait has largely remained uninvestigated in an important crop like wheat. Therefore, the present investigation was undertaken to determine the variation and genetics of SG trait and its association with spot blotch severity in wheat so as to help wheat breeders define selection strategies to manipulate SG and to exploit the associated advantages especially those related to reducing spot blotch incidence. The study also emphasizes a novel method to assess SG expression.

Materials and methods

Germplasm

Two sets of 1407 diverse wheat (*Triticum aestivum*) lines were evaluated for SG and resistance to spot blotch. One set (protected from disease) was used for evaluation of SG expression, while the other (inoculated) was for measuring spot blotch severity under artificial epiphytotic conditions. The 1407 wheat lines included in the first experiment were previously studied to determine sources of resistance to spot blotch (Chaurasia et al. 1999), and associations of plant height, days to maturity (Joshi et al. 2002), leaf angle (Joshi and Chand 2002) and leaf tip necrosis (Joshi et al. 2004a) with spot blotch severity.

Each line was hand sown in three replications of a randomized complete block design in a paired row plot of 3 m length with 25 cm spacing between the rows and 5 cm between seeds at the research station of Banaras Hindu University, Varanasi, India (25.2°N and 83.0°E) for three consecutive seasons, 1999–2000, 2000–2001 and 2001–2002. The maximum difference in the crop durations of genotypes selected for this study was 21 days (117–138 days). Therefore, to avoid variation if any due to crop phenology and to ensure that plants were at a similar developmental stage at the time of recording observations, planting in each year was staggered between 5th and 25th of December. Since it was not possible to do staggered plantings on each day, lines were divided into five groups (<120, 121–125, 126–130, 131–135 and >135 days) and were planted at intervals of 5 days. The delayed planting allowed the post anthesis stages to coincide with warmer temperatures during March that enhance the development of spot blotch (Joshi et al. 2004a).

Standard agronomic practices recommended for normal fertility (120 kg N:60 kg P_2O_5:40 kg - K_2O ha^{-1}) were followed. Full rates of K_2O and P_2O_5 were applied at the time of sowing. Nitrogen was supplied in split applications, 60 kg N ha^{-1} at sowing, 30 kg N ha^{-1} at the first irrigation (21 days after sowing) and 30 kg N ha^{-1} at the second irrigation (45 days after sowing). A total of five irrigations were given in each year.

In the set used for SG evaluation, fungicide Tilt (propiconazole; [1-{[2-(2,4-dichlophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl}-1H-1,2,4-triazole]) @ 625 g a.i. ha^{-1} was applied at two growth stages (Zadoks et al. 1974) GS 54, 69 to prevent spot blotch and leaf rust, the two most important diseases of eastern India. The other set that was used for evaluation of spot botch severity, an artificial epiphytotic described in following sections was created for spot blotch.

Crosses

In our initial study during 1998–1999 crop season (unpublished data) Chirya 3 and Chirya 7 showed significantly superior SG expression as well as resistance (<3 score on 0–9 scale) to spot blotch. These two SG spot blotch resistant genotypes were crossed with the NSG spot blotch susceptible genotype Sonalika (>8 score). Sonalika was formerly recommended for cultivation under normal (November) to late (December) sown, irrigated conditions of the North Eastern Plains Zone. The F_3 generation was obtained by harvesting 202–207 space sown random F_2 plants during the 2001–2002 season. The F_4, F_5 and F_6 generations were derived by harvesting single random plants from each line in each generation (Singh and Rajaram 1992; Joshi et al. 2004a, b). Each progeny row of the F_6 generation was bulk harvested to obtain the F_{6-7} generation. Off-season nurseries were used to expedite generation enhancement.

All the generations (F_1, F_3, F_4, F_5, F_6 and F_{6-7}) of the two crosses were grown under protected and artificially inoculated conditions. The F_3 and F_4 progeny lines were evaluated during 2002–2003, F_5 and F_6 during 2003–2004, while the F_{6-7} lines were evaluated during the 2004–2005 crop season. Based on the number of days to maturity of the progeny rows observed in the F_4 generation, differential sowings were carried out in the F_5, F_6 and F_{6-7} generations to synchronize the growth stages between progeny rows, thereby attempting to nullify the growth stage × environment interaction (Joshi et al. 2002). In each of the generations that were planted in three replications, each genotype was sown in a single 3 m row with 30 cm between rows and approximately 40–50 plants per row. Similar agronomic practices

as described for germplasm were followed. For segregating generations, planting in each year was done during the second week of December. Spreader rows of A-9-30-1 were also planted in the alleyways of the experimental plots 2 weeks prior to sowing the experiment to induce disease development.

Rows comprising of parental genotypes were included at the beginning and at the end of the experimental plots and also after every 20 rows. Expression of SG trait (in the protected set) and spot blotch severity (in inoculated set) was recorded for the parental genotypes and all the progeny rows in different generations (F_3, F_4, F_5, F_6 and F_{6-7}). For each line, observations on all plants in a row were recorded.

SG assessment

Stay green trait was measured using two approaches (i) difference of leaf and spike greenness scores on a 0–9 scale (modified version of the 1–10 scale of Silva et al. 2000), and (ii) a new parameter ''leaf area under greenness'' (LAUG). In the first approach, SG trait was recorded on the basis of visual scores (0–9 scale) for both flag leaf and spike at the late dough (GS 87) stage. The difference between flag leaf and spike scores was considered to group genotypes as SG (<3–6), moderately stay green (MSG) (>2–<3), moderately non-stay green (MNSG) (>1–<2) and non-stay green (NSG) (0–<1).

In the second approach, LAUG was determined using a modified formula given for leaf area under decline (LAUD) (Joshi 2003). This approach was based on the method employed for estimating AUDPC (van der Plank 1963; Roelfs et al. 1992). In the LAUD approach scores for green area of the flag leaf and that of spikes were estimated visually on a 1–100 scale at 4-day intervals starting from late milk to physiological maturity and the ratio of green area under spike and flag leaf at time t_i was used as Y_i. However, in the present approach (LAUG), a 0–9 scale was used instead of 1–100 and Y_i was taken as the difference of green area under spike and flag leaf. The LAUG approach was found better than LAUD for two reasons; firstly, it did not change rank of lines due to inclusion or separation of one or two readings, and secondly, unlike the previous approach (LAUD) the higher scoring lines were SG while those displaying lower scores were NSG types. The formula used for calculating LAUG was:

$$\text{LAUG} = \sum_{i=1}^{a} \left[\left\{ \frac{Y_i + Y_{(i+1)}}{2} \right\} \times \left(t_{(i+1)} - t_i \right) \right]$$

where, Y_i = difference of green area under spike and flag leaf (0–9 scale) at time t_i, $t_{(i+1)} - t_i$ = time (days) between two consecutive readings.

Green areas of the flag leaf and spikes were estimated visually on a 0–9 scale at intervals of about 4 days. Green area in the flag leaf and spike was recorded from late milk stage (Zadok's GS 77) until physiological maturity marked by complete loss of green color in both flag leaf and spike. For longer duration lines, readings were more than five, hence, only the last five readings were considered for calculation.

Spot blotch inoculation and assessment

A pure culture of a local isolate of *B. sorokiniana* identified at this center (registered in Auckland, New Zealand, No. ICMP 13584) and known to be highly aggressive, was used in all studies (Joshi and Chand 2002). The inoculum was multiplied on wheat grains and spores were harvested in water (Misra 1973). The disease was initiated by uniformly spraying the spreader rows and all other plots during the evening hours with a spore-water suspension (10^4 spores ml^{-1}) at tillering, flag leaf emergence and anthesis (Chaurasia et al. 1999; Joshi et al. 2002). The field was frequently irrigated to maintain high humidity and promote disease development.

A spot blotch score for each germplasm was evaluated on ten random plants following a 0–9 scale (Saari and Prescott 1975) at six growth stages, viz., GS 25, 37, 47, 57, 69 and 77 (Zadoks et al. 1974). Disease severity (%) was also recorded for each genotype. Disease evaluations were done on the same ten plants throughout each season and were averaged to generate a final score for each genotype. Genotypes scoring 1–3 were considered to be resistant, 4–5 moderately resistant, 6–7 moderately susceptible and 8–9

susceptible. Area under disease progress curve (AUDPC) based on disease severity over time (Van der Plank 1963) was estimated from:

$$\text{AUDPC} = \sum_{i=1}^{a}\left[\left\{\frac{Y_i + Y_{(i+1)}}{2}\right\} \times \left(t_{(i+1)} - t_i\right)\right]$$

where, Y_i = disease level at time t_i, $t_{(i+1)} - t_i$ = time (days) between two sequential disease scores.

Analyses were done by SAS (1997) using planting date (five groups) as a covariate.

The number of genes for SG was estimated using chi-square analysis (Singh and Rajaram 1992) as well as a quantitative approach in which Wright's (1968) modified formula (Singh et al. 1995) was applied. For chi-square analysis, F_3 lines were grouped into four classes viz., (i) homozygous/homogeneous for the SG parental response, (ii) homozygous/homogeneous for the NSG parental response, (iii) either segregating or homozygous for scores lower than the SG, but more than the NSG parent and (iv) segregating with scores reaching the level of the NSG susceptible parent. In F_4, F_5, F_6 and F_{6-7}, lines were grouped into three classes by merging the last two categories mentioned above (Singh and Rajaram 1992; Singh et al. 1995). The F_6 and F_{6-7} lines were also analyzed by grouping them into two categories, i.e., SG-intermediate and NSG followed by chi-square analysis.

The quantitative approach (Wright 1968) was also followed in the F_6 and F_{6-7} generations to confirm the number of genes. Separate analyses of variance were conducted for each generation (F_6 and F_{6-7}) of each cross following RCBD (Steel and Torrie 1960) to estimate heritabilities for spot blotch response. Narrow sense heritabilities were estimated using the formula of Singh and Chaudhary (1977) and Fehr (1987) ($h^2 = \sigma^2_g/\sigma^2_p$; where, h^2 = heritability estimate, σ^2_g is genotypic variance and σ^2_p is phenotypic variance; $\sigma^2_g = (\sigma^2_L - \sigma^2_E)/r$ and $\sigma^2_p = \sigma^2_g + \sigma^2_E$; σ^2_L = variance of the F_6 and F_{6-7} lines, σ^2_E = error variance and r = number of replications). Although the genetic variance used in the formula to calculate heritability was the total genetic variance of the progeny lines, the heritability estimate was considered to be narrow sense because the dominance variance was negligible and the confounding effect of the additive by additive genetic variance can be included in the heritability estimate at the F_6 level of inbreeding (Singh et al. 1995). The minimum number of genes controlling SG was verified using the modified formula (Singh et al. 1995; Joshi et al. 2004a), $n = (\text{GR})^2/(R \times \sigma^2_g)$, where n = minimum number of genes, GR = genotypic range and σ^2_g = genetic variance of the segregating generation and the factor $R = (8/(2-1/2^g))$. As reported by Singh et al. (1995), the modification for the level of inbreeding in the original formula is based on Cockerham (1983). The value of g in F_6 was 4, therefore, the value of R was 4.13 in this generation. GR was estimated by two different methods (Singh et al. 1995; Joshi et al. 2004a). In the first method, GR was the range of segregating generation line means, while in the second GR was the range of segregating generation line means multiplied by heritability. The second method was expected to be more precise since the use of heritability eliminates the influence of environment on the expression of the SG trait (Mulitze and Baker 1985).

The unpaired t-test was also performed to compare the spot blotch AUDPC of the SG and NSG homogeneous/homozygous progeny lines in four different (F_4, F_5, F_6 and F_{6-7}) generations of the two crosses.

Correlation between SG and spot blotch severity

The phenotypic correlation between SG trait and AUDPC was estimated using the AUDPC values and SG scores (LAUG) of the lines homozygous for SG trait in the segregating generations of the two crosses.

Results

Germplasm evaluation

The analysis of variance of the 1407 germplasm and elite breeding lines indicated that there was significant variation for SG trait (Table 1) and the germplasm was divided into four phenotypic groups based on SG scores (Table 2). Observations on LAUG revealed that the

Table 1 Mean squares for the expression of stay green trait in 1407 germplasm/elite breeding lines tested by LAUG and 0–9 scale for 3 years

Source	*df*	Mean squares			
		Stay green		Spot blotch scores	
		LAUG	0–9 scale	AUDPC	0–9 scale
Year	2	11,386.77*	19.73*	117,776.5*	19.75*
Rep (year)	6	4,163.52*	4.23*	427,241.5*	81.61*
Stay green group	3	703,303.29*	3,601.6*	87,477,508.8*	16,818.55*
Year × stay green group	6	5,831.68*	12.51*	306,690.1*	58.65*
Maturity group	1	2,219.55*	13.58*	1,379,146.3*	287.91*
Error	12,644	36.82	0.49	8,873.1	1.76

*Significant at $P < 0.01$

Table 2 Mean scores of stay green and non-stay green germplasm/elite breeding lines tested under field conditions for 3 years

Stay green response	Mean LAUG	Genotypes		Mean difference of visual scores of leaf and spike in 0–9 scale	Genotypes	
		No.	%		No.	%
Stay green	73.05	31	2.20	4.13	58	4.12
Moderately stay green	50.54	102	7.25	1.83	156	11.09
Moderately non-stay green	31.37	376	26.72	1.12	351	24.95
Non-stay green	12.30	898	63.82	0.24	842	59.84
Total		1,407	100.0		1,407	100.0
LSD (1%)	5.60			0.65		

majority of lines were NSG (63.8%), followed by MNSG (26.7%), MSG (7.2%) and SG types (2.2%). The evaluations following 0–9 scoring at late dough stage gave relatively higher frequency for SG and moderately SG lines than obtained using LAUG approach. No line appeared to be heterogeneous for the SG trait.

Statistical analysis showed that SG and MSG lines displayed significantly lower mean scores for spot blotch than NSG or MNSG lines (Tables 1, 3). None of the stay green and MSG lines was susceptible, although around 18% of MSG lines (1.2% of total germplasm) displayed moderate susceptibility. In comparison, 12 and 15% MNSG and NSG lines were susceptible (Table 3). SG lines also maintained a low response to spot blotch until a very late stage of plant growth, whereas NSG lines succumbed much earlier (Table 4).

Inheritance of SG trait

In comparison to the SG and NSG parents, the F_1's were semi-SG (Table 5). In the F_3 generations, lines with parental type expressions were observed in very low frequencies (Table 6). The test for goodness of fit suggested that the observed distributions among the progenies of the three crosses corresponded to those expected for segregation of four independent loci (Table 6). A similar trend was noticed in the F_4, F_5, F_6 and F_{6-7} generations of both crosses (Table 7). Cross (Chirya 7 × Sonalika) gave goodness of fit for both three and four gene segregations in F_3, but in subsequent generations, four genes were implicated. When progeny lines were grouped into two categories by merging SG-intermediate and NSG in the F_6 and F_{6-7} generations (analysis not shown), the chi-square values again suggested the same number of genes. The distribution of F_3 and F_6 progeny rows in these crosses (Fig. 1) also indicated that genes controlling stay green trait interacted in an additive manner.

The heritability estimates for the stay green trait in the segregating generations were moderate and ranged from 0.75 to 0.80 (Table 8). The results of the modified formula of Wright (1968), using 0–9 scale, as well as LAUG values, in the F_6 and F_{6-7} generations, showed that the number of

Table 3 Spot blotch response of wheat germplasm at late milk (77) stage divided into four classes of stay green expression under field conditions

Response	Disease score[a]	No. (and %) of genotypes[b]				Total	% of total
		Stay green	Semi-stay green	Semi-non-stay green	Non-stay green		
Resistant	1–3	19 (1.35)	12 (0.85)	11 (0.78)	5 (0.36)	47	3.34
Moderately resistant	4–5	12 (0.85)	72 (5.12)	66 (4.69)	311 (22.10)	341	24.24
Moderately susceptible	6–7	0 (0.00)	18 (1.28)	123 (8.74)	367 (26.08)	508	36.11
Susceptible	8–9	0 (0.00)	0 (0.00)	176 (12.51)	215 (15.28)	391	36.32
Total		31	102	376	898	1,407	100.0
% of total		2.20	7.25	26.72	63.82	100.0	
Mean final severity		3.1	4.6	5.9	7.1		
LSD at 5 and 1%		1.22 and 1.28					
Mean AUDPC		327.56	838.33	1,343.44	1,757.30		
LSD at 5 and 1%		87.03 and 91.47					

[a]0–9, Saari–Prescott scale (Saari and Prescott 1975)

[b]Figures in parenthesis represent % values for 1407 genotypes

Table 4 Mean severity of spot blotch and AUDPC in genotypes with different stay green expressions at six growth stages

Stay green type	Growth stage and mean spot blotch severity (%)						Mean AUDPC
	25	37	47	57	69	77	
Stay green	5.1	7.2	9.6	14.5	21.8	30.3	327.56
Moderately stay green	8.6	12.3	18.3	26.1	36.7	46.0	838.33
Moderately non-stay green	12.6	19.2	28.5	38.9	46.1	57.2	1,343.44
Non-stay green	18.9	24.6	41.2	52.4	63.7	71.2	1,757.30
Mean	11.30	15.83	24.40	32.98	42.08	51.18	1,066.65
LSD at 5%							87.03
1%							91.47

genes controlling stay green was around four (Table 9). Although method I (where heritability was not used) displayed higher estimates, method II (in which heritability was used) suggested gene numbers matching those indicated by chi-square tests.

SG trait and spot blotch severity in the segregating generations

Mean AUDPC values of the homozygous lines for SG and NSG traits in the F_4, F_5, F_6 and F_{6-7} generations of the two crosses are presented in Table 10. The mean AUDPC values of SG and NSG types were significantly different in all generations of both crosses. Although some NSG progeny lines possessed low AUDPC, none of the SG progeny lines displayed high AUDPC. Transgressive segregants were not recorded for resistance to the pathogen. The phenotypic correlation between SG and AUDPC values was +0.73.

Discussion

Variability in the SG trait

The evaluation of 1407 germplasm and elite breeding lines showed that substantial variation existed for the SG trait in wheat (Table 1). The analysis of variance for SG trait showed significant differences for this trait. Silva et al. (2000) evaluated changes in stem, leaf and head color in different phases of development of wheat. Some workers (Xu et al. 2000) measured SG expression using a 1–5 scale based on the degree of leaf and plant death at physiological maturity in the field under post flowering drought stress. The Minolta Chlorophyll Meter SPAD-502 (Minolta Camera

Table 5 Pedigree, mean stay green expressions and spot blotch severity of parents and F_1s used in the genetic analysis

Parent	Pedigree	LAUG score		*Spot blotch severity (%)*	
		2000–2001	2001–2002	*2000–2001*	2001–2002
Chirya 3 (SG-R)	CS/TH.CU//GLEN/3/ALD/PVN/4/NINGMAI No. 4/OLESON//ALD/YANGMAI No. 4 (selection history: CIGM87.116)	78.27 ± 3.68	77.76 ± 3.21	25.9 ± 3.4	26.1 ± 2.8
Chirya 7 (SG-R)	(CS/TH.CU//GLEN/3/ALD/PVN/4/NINGMAI No. 4/OLESON//ALD/YANGMAI No. 4 (CIMMYT CID No. 66176)	81.04 ± 3.89	76.38 ± 3.42	26.1 ± 3.0	28.1 ± 3.3
Sonalika (NSG-S)	II54-368/AN/3/YT54/N10B//LR64 (II18427-4R-1M)	13.92 ± 1.17	13.04 ± 1.34	90.0 ± 9.6	88.3 ± 8.9
Cross I	Chirya 3 × Sonalika	46.85 ± 2.47	47.85 ± 2.40	48.3 ± 4.6	51.6 ± 4.9
Cross II	Chirya 7 × Sonalika	48.45 ± 2.58	48.86 ± 1.96	53.2 ± 4.8	55.7 ± 5.4

SG-R stay green resistant, *NSG-S* non-stay green susceptible

Co. Ltd) has also been used to measure total leaf chlorophyll content (Xu et al. 2000). Since measurements with a Minolta Chlorophyll Meter SPAD-502 recorded in the present investigation were not consistent, the data were not used for analysis. Hence a new parameter, LAUG, for evaluating SG was adopted since it takes into consideration, the gradual loss of chlorophyll in leaves relative to spikes that occurs during grain filling.

Inheritance of the SG trait

Compared to the parents, F_1's (Table 4) had a semi-SG phenotype indicating no dominance. Because the crosses involved parents with contrasting SG expressions and genes interacted in an additive manner, only extreme homozygotes were expected to display parental type behavior in the segregating generations. The fact that relatively few lines in F_3 and subsequent generations were scored equally to the parents indicated that the SG trait was controlled by a number of genes acting additively. Based on the way by which lines were scored the expected frequencies of parental SG, non-parental and parental NSG classes in F_3 would be 1.563:96.874:1.563 if three genes were involved and in F_4, the corresponding numbers would be 5.273:89.454:5.273. The tests of goodness of fit suggested that the observed distributions for the two crosses involved segregation at around four independent loci (Tables 6, 7). The quantitative approach followed in the F_6 and F_{6-7} generations to estimate the number of genes confirmed these estimates.

Physiological traits that may have implications on yield potential in wheat include those controlling translocation of non-structural carbohydrates from stems to grain (Rane and Nagarajan 2000) and the ability to maintain green leaf area duration (SG) throughout grain filling (Jenner and Rathjen 1975; Reynolds et al. 2001). While both of these traits play a crucial role in grain development, particularly when assimilates are limited, physiological studies have indicated that genotypes with high grain yield depend less on stem reserves compared to low yielding ones (Austin et al. 1980). In durum wheat, four mutants with delayed leaf senescence maintained

Table 6 Goodness of fit of the observed and hypothesized ratios for stay green class frequencies of F_3 lines generated from the two crosses

Cross	Observed ratio in F_3				Hypothesized ratio	χ^2 Value	*P* value	Gene number
	HSG[a]	Seg R[b]	Seg S[c]	HNSGT[d]				
Chirya 3 × Sonalika	3	135	62	2	1:174:80:1	8.11	0.04	4
Chirya 7 × Sonalika	2	137	65	3	1:174:80:1	7.79	0.05	4
					1:36:26:1	8.45	0.04	3

[a]Homozygous for stay green parental type (homozygous for all stay green alleles, i.e., AABBCCDD)

[b]Segregating or homozygous for stay green levels higher than that of stay green parent but less than that of non-stay green parent (homozygous for at least one stay green locus)

[c]Segregating with stay green levels equivalent to the non-stay green parent (heterozygous for at least one locus and homozygous for non-stay green alleles at other loci)

[d]Homozygous for non-stay green parental type (homozygous, lacking all stay green alleles, i.e., aabbccdd)

Table 7 Results of goodness of fit to the gene ratio observed in F_4, F_5, F_6 and F_{6-7} lines from crosses between genotypes having stay green and non-stay green traits

Resistant parents	Observed ratio			Hypothesized ratio (approximate)	χ^2 Value	*P* value	Gene number
	HSG[a]	Seg[b]	HNSG[c]				
Chirya 3 × Sonalika							
F_4	5	195	2	1.977:96.044:1.977	1.25	0.53	4
F_5	4	192	6	3.663:92.672:3.663	1.95	0.38	4
F_6 (2003–2004)	6	188	8	4.827:90.344: 4.827	0.95	0.62	4
F_{6-7} (2004–2005)	7	184	11	4.827:90.344: 4.827	1.92	0.38	4
Chirya 7 × Sonalika							
F_4	3	200	4	1.977:96.044:1.977	0.30	0.86	4
F_5	4	197	6	3.663:92.672:3.663	2.16	0.34	4
F_6 (2003–2004)	10	188	9	4.827:90.344: 4.827	0.10	0.95	4
F_{6-7} (2004–2005)	9	187	11	4.827:90.344: 4.827	0.20	0.90	4

[a]Homozygous for stay green parental type (homozygous for all stay green alleles, i.e., AABBCCDD)

[b]Segregating or homozygous for stay green expression different from the parent

[c]Homozygous for non-stay green parental type (aabbccdd)

photosynthetic competence for longer periods than parental lines, and also had higher seed weights and grain yields per plant than the parents (Spano et al. 2003). In subtropical environments like the eastern Gangetic Plains of India, where wheat occupies around 10 million ha, temperatures greater than 35°C occur during grain filling. This leads to enhanced senescence and reduced productivity. While measures suggested for delaying senescence of wheat leaves in order to enhance grain yield (Benbella and Paulsen 1998) are yet to be tested for economic viability, improved cultivars with SG attributes provide a better option for drought and high temperature environments.

Association of SG and spot blotch severity

The evaluation of a large number of wheat lines showed that genotypes having SG trait expressed lower severities to spot blotch than those with NSG trait. This was reflected in three ways; (i) significant superiority of SG or MSG lines over NSG or MNSG lines for spot blotch response (Table 2), (ii) maintenance of disease free leaves by SG leaves until later growth stages (Table 3) and (iii) significantly superior resistance expressed by homozygous SG lines in the segregating generations of two crosses (Table 10).

The low spot blotch scores of SG genotypes and a positive phenotypic correlation between SG

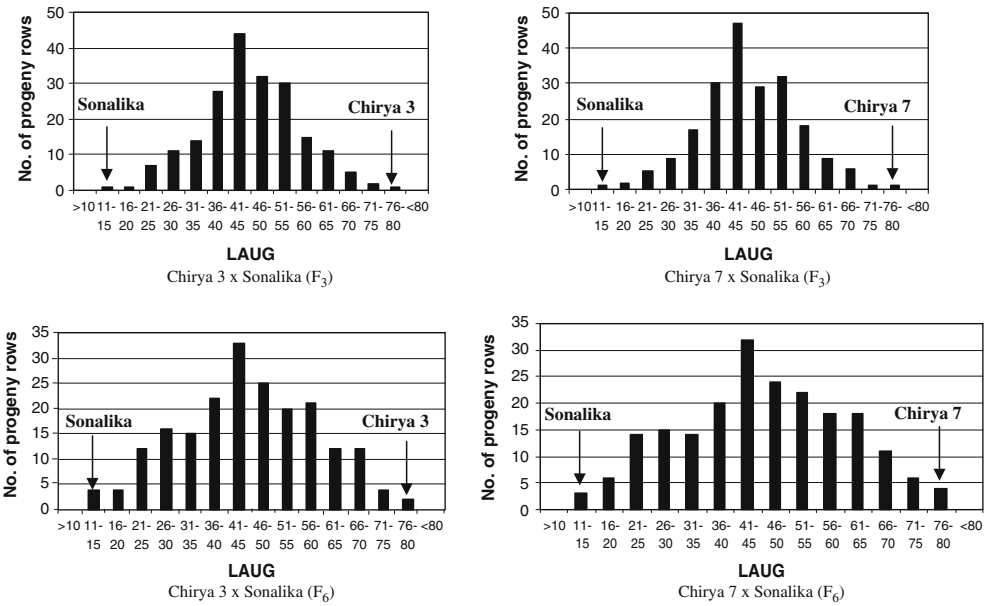

Fig. 1 Distribution of mean LAUG of F_3 and F_6 lines of crosses between stay green genotypes and Sonalika

Table 8 Mean response and LSD of F_6 and F_{6-7} lines to stay green trait and heritability estimates for crosses between stay green parents and ''Sonalika'' tested during 2003–2004 and 2004–2005

Cross	No. of F_6/F_{6-7} lines	*Stay green expression*						Heritability	
		LAUG			Visual score			LAUG	Visual score
		Range	Mean	LSD	Range	Mean	LSD		
Chirya 3 × Sonalika	202 (F_6)	10.62–79.13	39.58 ± 5.56	10.90	0.66–6.39	3.45 ± 0.47	0.92	0.76	0.77
	202 (F_{6-7})	11.54–73.97	40.45 ± 5.15	10.09	0.79–6.32	3.53 ± 0.48	0.95	0.78	0.75
Chirya 7 × Sonalika	207 (F_6)	10.90–82.44	44.96 ± 5.48	10.75	0.68–6.80	3.86 ± 0.51	1.00	0.80	0.77
	207 (F_{6-7})	9.74–77.67	38.24 ± 5.85	11.47	0.84–6.33	3.39 ± 0.47	0.92	0.75	0.76

Table 9 Estimate of maximum number of effective genes contributing to stay green in crosses between stay green parents and ''Sonalika'' using Wrights (1968) formula modified for F_6/F_{6-7} generations (adapted from Singh et al. 1995)

Cross	No. of F_6 and F_{6-7} lines	Number of genes			
		LAUG		0–9 scale	
		Method I	Method II	Method I	Method II
Chirya 3 × Sonalika	202 (F_6)	7.72	4.46	6.93	4.14
	202 (F_{6-7})	7.05	4.25	6.84	3.88
Chirya 7 × Sonalika	207 (F_6)	6.86	4.39	6.90	4.08
	207 (F_{6-7})	7.05	4.01	6.77	3.93

and AUDPC (+0.73) suggested that SG trait contributed positively in reducing severity of spot blotch. Although, other indirect effects may also have contributed, the presence of low disease severities in SG lines indicated the presence of a close genetic association between the two traits. The mean low AUDPC scores of SG progenies might partly be due to the delayed breakdown of chlorophyll and hence resistance to the growth and development of the facultative pathogen.

Table 10 Mean AUDPC scores of progeny rows homozygous for stay green and non-stay green expression in F_4, F_5, F_6 and F_{6-7} generations of two crosses

No. of Progeny	F_4		F_5		F_6		F_{6-7}	
Rows	Stay green	Non-stay green	Stay green	Non-stay green	Stay green	Non-stay green	Stay green	Non-stay green
Chirya 3 × Sonalika								
1.	224	1,820	245	980	343	682	227	822
2.	364	775	259	405	350	525	350	425
3.	332		315	1,348	241	840	297	805
4.	276		210	1,418	297	1,382	311	717
5.	203			1,610	469	1,470	245	525
6.				1,453	490	1,347	245	1,137
7.						470	189	1,750
8.						1,820		1,540
9.								1,417
10.								1,487
11.								1,452
Mean	280.0	1,297.5	257.3	1,202.1	365.2	1,067.2	266.5	1,098.2
t Value	3.62* (P = 0.001)		4.17* (P = 0.001)		3.35* (P = 0.001)		4.74* (P = 0.001)	
Chirya 7 × Sonalika								
1.	294	805	360.5	752	329	805	262	752
2.	378	787	378	682	504	717	311	682
3.	364	1,190	210	560	315	525	210	435
4.		1,680	343	840	350	335	245	240
5.				1,470	241	787	210	822
6.				1,365	297	1,820	399	980
7.					469	1,347	469	1,522
8.					455	1,470	525	1,452
9.					227	1,610	297	1,715
10.					350			1,260
11.								1,837
Mean	345.3	1,115.6	322.8	945.0	353.8	1,046.4	325.5	1,063.6
t Value	3.09* (P = 0.001)		3.18* (P = 0.001)		4.12* (P = 0.001)		4.09* (P = 0.001)	

*Significant value

Temperature is one of the factors associated with increased infection and disease development caused by *B. sorokiniana* (Chaurasia et al. 2000; Duveiller et al. 2005) and *Pyrenophora tritici repentis* on wheat (Hosford et al. 1987). The maintenance of green coloration by plants enables them to maintain lower canopy temperatures (Kumari et al. 2006) which may be less favorable for pathogens requiring relatively higher temperatures for faster growth and development.

The present study indicated the presence of substantial variation in the SG trait in wheat and the LAUG approach is suggested as an appropriate way to measure it. The presence of a few additive genes for SG trait indicates that its selection is possible by growing moderately large segregating populations. Most importantly, there is positive association of the SG trait and spot blotch resistance that needs to exploited for developing spot blotch resistant wheat.

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