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Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature

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Abstract Senescence is a genetically programmed and environmentally influenced process resulting in the destruction of chlorophyll and remobilization of nutrients to younger or reproductive parts of plants. Delayed senescence, or stay-green, contributes to a long grain-filling period and stable yield under stress. To model senescence and identify quantitative trait loci (QTL) for the trait, a population of recombinant inbred lines (RIL) from a cross between winter wheat cultivars, 'Ventnor' and 'Karl 92' was evaluated for heat tolerance under optimum temperature of 20/15°C (day/night) and continuous heat stress of 30/25°C from 10 days after anthesis (DAA) until maturity. Ventnor is a heat-tolerant cultivar and Karl 92 is a relatively heat-susceptible cultivar. Green leaf area was measured and used to model percent greenness retained over the reproductive period. Chlorophyll content and chlorophyll fluorescence were recorded on flag leaves. Senescence was converted to a quantitative trait using the model. Based on the modeled parameters, the RILs were categorized into three groups. When senescence-related traits were evaluated, nine QTL for heat tolerance were found on chromosome 2A, two each on chromosomes 6A and 6B and one each on chromosome 3A, 3B, and 7A. Both parents contributed favorable alleles for most of the senescence-related traits. Microsatellite markers *Xgwm356* and *Xgwm5* prominently linked to the senescence-related traits may be useful in marker-assisted breeding. These and the linked AFLP (amplified fragment length polymorphism) markers *XCGT.TGCG-349*, *XCGT.GTG-343*, and *XCGT.CTCG-406,* if converted to STS (sequence tagged sites), can be used for further molecular dissection of the QTL for post-anthesis heat tolerance.

Keywords Post-anthesis heat stress · Senescence · Modeling · Molecular markers · Marker-assisted breeding · QTL mapping

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Introduction

Senescence is internally programmed cell death and is affected by environmental factors like abiotic and

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biotic stress (Buchanan-Wollaston 1997; Noodén et al. 1997; Chandler 2001). Senescence includes loss of chlorophyll content and a decline in photosynthetic capability of the leaf. However, environmental factors can cause early loss of photosynthetic capacity in the life cycle of the plant and result in premature senescence. Abiotic stress, such as high temperature during and after flowering, may cause premature senescence, resulting in poor grain quality and loss of yield (Xu et al. 2000a; Jiang et al. 2004). Genetic variation exists in the capacity of many species to withstand heat stress and retain their green leaf area (Thomas and Howarth 2000).

Stay-green refers to delayed senescence during post-anthesis stages of plant development (Thomas and Howarth 2000). Stay-green may correspond to either the delayed onset of senescence or a slower rate of senescence (Thomas and Smart 1993). Photosynthesis in wheat contributes 80–90% of assimilates for grain filling under optimum temperature conditions (Evans et al. 1975). Therefore, premature senescence and the rate of senescence may be important factors that influence yield potential under stress (Gentinetta et al. 1986; Evans 1993; Thomas and Howarth 2000). In many crop species, stay-green plants have better quality of foliage, higher chlorophyll content, and greater resistance to pests and diseases (Ambler et al. 1987; Thomas and Smart 1993; Xu et al. 2000a).

Most studies across plant species indicate that stay-green is a quantitative trait. In sorghum, Xu et al. (2000b) found three major QTL for stay-green; however, other studies reported QTL on two to nine chromosomes (Tuinstra et al. 1997; Crasta et al. 1999; Subudhi et al. 2000; Tao et al. 2000; Kebede et al. 2001; Haussamann et al. 2002; Sanchez et al. 2002). In rice (*Oryza sativa* L.), a stay-green mutant controlled by a single recessive nuclear gene, *sgr* (*t*), was mapped to the long arm of chromosome 9 (Cha et al. 2002). Jiang et al. (2004) found 46 QTL with main effects and other QTL with minor effects distributed on all 12 rice chromosomes. Bertin and Gallais (2001) reported stay-green QTL on chromosome 10 of maize.

In winter wheat, QTL associated with flag leaf senescence were detected on the long arms of chromosomes 2D under drought stress and 2B under irrigated conditions (Verma et al. 2004). However, QTL influencing senescence traits under high temperature in wheat have not been reported to date. The objectives of this study were to (1) characterize the senescence-related traits in response to heat stress, and (2) identify QTL for those traits using a RIL population of winter wheat.

Materials and methods

Plant materials and experimental design

A population of F_7 recombinant inbred lines (RIL) was derived from a cross between Ventnor (a heat-tolerant hard white Australian winter wheat cultivar) and Karl 92 (a heat-sensitive hard red winter wheat cultivar from the USA) (Yang et al. 2002). A total of 101 $F_{6:7}$ RILs were developed by single seed descent in the green house at Kansas State University, Manhattan, KS, USA. RILs were phenotyped as single culms (the main tiller) by clipping all other tillers as they appeared (Wardlaw 2002). The chambers with optimum temperature were set at 20/15°C (day/night), 50/70% relative humidity, 16-h photoperiod, and light intensity of 420 μmol m^{-2} s^{-1}, as suggested by Yang et al. (2002). Flowering date was recorded when 50% of the spikelets reached anthesis. The RILs for the high temperature treatment were placed in chambers held at 30/25°C, with the same humidity and day length as the control, from 10 DAA to physiological maturity. Plants were well-watered. The soil mix was silt loam consisting 1.7 g N, 0.11 g P, and 1.4 g K/kg soil, gypsum (4.0 g/kg soil), perlite (63.0 g/kg soil), and peat moss (400.0 g/kg soil). The RILs were randomized within each of the three replications in a split plot experimental design, with planting data as a blocking factor and growth chambers as the experimental units within each temperature regime. In each experimental unit, one plant per RIL was grown.

Trait characterization

Green leaf area duration (GLAD) was visually estimated using a 0–10 scale, where 0 was complete senescence and 10 was 100% green leaf area. The plants were rated across all leaves. Green leaf area scores were recorded at 3-d intervals from 10 DAA to physiological maturity. A non-linear regression curve was fitted on the green leaf area data using a

Gompertz statistical model (Seber and Wild 1989). The model used in the analysis is as follows:

$$Y = \alpha\left\{1 - e^{[-b(\text{time}-c)]}\right\}$$

where Y is the response variable, α refers to the point of inflection where GLAD is assumed to be 100% and leaf senescence is initiated, b refers to the slope of the curve at any given time after initiation of senescence, and c refers to the point on the curve where the slope is maximum.

The empirical graph plotted between time in days and GLAD, shows percent green leaf area at different points in time (Fig. 1). For certain RILs, the point of inflection could have occurred before the first measurement of senescence 10 DAA, in which case the GLAD measurements were used to extrapolate α.

The GLAD estimates over time were converted to percent green leaf area and the modeled traits related to progression of senescence were estimated as: (i) 75% green (75%G) which refers to the time interval from 100% green leaf area to 75% green leaf area or, conversely, the duration for achieving 25% senescence, (ii) 25% green (25%G) which refers to the duration for achieving 75% senescence, (iii) 50% green (50%G) refers to the time interval between 75%G and 25%G, (iv) maximum rate of senescence (MRS) which refers to the point of the regression curve where the slope of the curve was maximum, indicating the plant had reached its maximum rate of senescence, (v) time to maximum rate of senescence (TMRS) which is the duration between point of inflection (α) and point when plant reaches MRS, (vi) percent green at maximum senescence (PGMS) which refers to the percent green leaf area at the point when the plant reaches MRS.

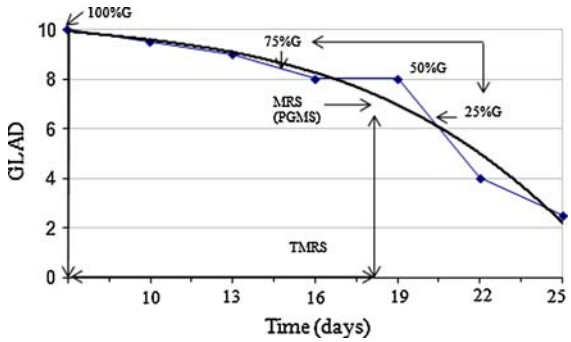


Fig. 1 Empirical graph illustrating the non-linear Gompertz regression model for senescence. Green leaf area duration (*GLAD*) from 10 DAA to physiological maturity was fitted using the model. Senescence traits estimated were: (i) 75% *green* (75%G), (ii) 25% *green* (25%G), (iii) 50% *green* (50%G), (iv) maximum rate of senescence (*MRS*), (v) time to maximum rate of senescence (*TMRS*), and (vi) percent greenness at maximum senescence (PGMS)

Percentage chlorophyll content (SPAD) was estimated for all treatments using a Model 502 SPAD meter (Minolta, Plainfield, IL). The readings were taken about 5 cm from the base of the abaxial surface of the flag leaf at 10 and 16 DAA. Differences between SPAD units at 10 DAA and 16 DAA were used in the analysis. Chlorophyll fluorescence (Fv/Fm) was estimated using a Chlorophyll fluorescence meter (Fluorescence Monitoring System, Hansatech Instruments, Norfolk, England). Readings were recorded at 10 and 16 DAA on the flag leaf. Differences between the Fv/Fm ratios at 10ADD and 16 DAA were used in the analysis.

Marker analysis and map development

The DNA was extracted from all RILs and the two parents using the CTAB method (Saghai-Maroof et al. 1984). The RIL population was genotyped with 450 markers to construct a genetic linkage map. The markers included 259 amplified fragment length polymorphisms (AFLPs), 190 simple sequence repeats (SSRs), and an expression sequence tag (EST, *RZ876*). The EST is part of the coding sequence of the chloroplast elongation factor (EF-Tu) gene from rice BAC clone AP004023 (Liang et al. 2007). The sequences of the forward and reverse primers were 5′-CGTTCTTGCTTGCTGTTGAGG-3′ and 5′-AGG GCGGTAACCAGGGAAAAAC-3′, respectively. The AFLP reactions and DNA template preparation for *PstI*/*MseI* fragments followed Vos et al. (1995) with some modifications. Selective amplifications were performed using 59 unique primer combinations. A linkage map was constructed using Mapmaker/EXP for UNIX version 3 (Whitehead Institute, Cambridge, MA). A Chi-square test was performed to test the markers for 1:1 segregation ratio. A maximum genetic distance of 30 cM (Kosambi units) and a LOD value of 6 were the thresholds used to establish linkage among markers on a chromosome. The position of anchored SSR markers was based on published consensus maps (Somers et al. 2004). Other markers were assigned to linkage groups based on their linkage to the anchored markers in the framework map.

QTL analysis

QTL Cartographer version 2.0 (Basten et al. 2002) was used for QTL analysis. Composite interval mapping (CIM) was conducted to detect the QTL. The co-factors, defined as the markers closest to the peaks in the LOD profile above the significance threshold, were identified by simple interval mapping with a window size of 5 cM. A QTL was considered to be significant when the LOD value exceeded 2.5. Resources and tools at http://www.gramene.org/ (Release #27, Liang et al. 2007) were used for comparative mapping.

Statistical procedures

Analysis of variance and least square means of all traits were estimated using the statistical procedure Proc. Mixed of SAS version 8.2 (SAS Inst. Inc. 1999). Correlations for all the traits were performed using Pearson's correlation in the statistical procedure Proc. Corr., and non-linear regressions were determined by the Gompertz model (SAS Inst. Inc. 1999).

Results

Analysis of variance for senescence-related traits

Significant differences in the senescence-related traits were observed between high and optimal temperature treatments ($\alpha = 0.001$) and among RILs ($\alpha \leq 0.05$) at all stages evaluated (Table 1). Treatment by entry interactions were significant ($\alpha \leq 0.05$) for MRS, TMRS, 50%G, SPAD, and Fv/Fm. Differences between the replications were not significant except for 75%G and TMRS ($\alpha \leq 0.05$). Within each temperature treatment, the RILs differed significantly in 50%G, MRS and TMRS. A non-crossover interaction (not shown) was observed for all traits except SPAD, indicating that genotypes that stayed green under optimum conditions also stayed green under high temperature. The interaction for SPAD was an orderly crossover type, indicating a change in the rank order of genotypes from optimum to high temperature conditions.

Categorization and modeling of senescence

The RILs can be broadly separated into three groups based on three measures of senescence, TMRS, MRS and PGMS. The average trait values for each group of RILs and their parents are listed in Table 2. Group 1 included lines which can be considered heat tolerant. The heat-tolerant parent, Ventnor, was a member of Group 1. Group 2 included lines with moderate levels of heat susceptibility. Karl 92, was a member of Group 2. Group 3 included lines with the highest levels of heat susceptibility. Ventnor took 8.5 more days to reach MRS and retained 6.9% greater PGMS than Karl 92 did. Karl 92 showed a 8.3% higher rate of senescence than Ventnor at the point of MRS.

The non-linear regression curves obtained from the model for Karl 92 and Ventnor, the parents of RIL population, are shown in Fig. 2. There is a gradual progression in senescence of both parents under normal temperatures (Fig. 2a, c). The curves under high temperature showed accelerated senescence in both parents due to heat stress (Fig. 2b, d). Ventnor had longer green leaf area duration and a shorter rate of senescence than that of Karl 92.

Correlation among related traits

Significant negative correlations were observed between MRS and all other traits except Fv/Fm under high-temperatures (Table 3). The significant positive correlations were observed between TMRS and PGMS and between SPAD and Fv/Fm ($\alpha < 0.001$). However, correlation was not significant between Fv/Fm and any other senescence traits.

QTL for senescence-related traits

A linkage map consisting of 222 markers covering all 21 wheat chromosomes was generated for analysis of QTL associated with senescence-related traits (Fig. 3). Composite interval mapping identified QTL for senescence-related traits on most homoeologous groups except group 1. Positive additive effect values indicate that favorable alleles were contributed by Ventnor.

Under optimum conditions, 47 and 41.7% of total variation was explained by markers linked to TMRS

Table 1 Variance analysis on senescence related traits for RIL population derived from a cross between Karl and Ventnor

Source of variation	*df*	*75%G*	*25%G*	*50%G*	MRS	TMRS	PGMS	SPAD	Fv/Fm
Treatment (optimum vs. stress)	1	25,027.319***	483,971.836***	288,885.386***	38,927.022***	72,814.423***	76,119.369***	756.678***	0.212***
Replicates	2	330.279**	2,998.218^{NS}	1,734.354^{NS}	97.123^{NS}	599.024*	353.685^{NS}	6.486^{NS}	0.476^{NS}
Entry (RILs)	103	76.100*	2,113.539***	1,797.203**	178.113***	203.538***	323.572***	29.914**	0.009*
Treatment*Entry	103	58.441^{NS}	1,688.581^{NS}	1,487.106**	145.695***	152.311*	171.507^{NS}	26.892*	0.009**
Optimum	103	3,046.896**	20.860***	328.328**	5.258^{NS}	0.003^{NS}			
Stress	103	213.797**	300.467***	24.174**	51.125**	0.014**			
Error	404	55.306	1,159.600	1,069.486	97.973	108.678	155.530	19.006	0.007

75%G 75% green, *25%G* 25% green, *50%G* 50% green, *MRS* maximum rate of senescence, *TMRS* time to maximum rate of senescence, *PGMS* percent greenness at maximum senescence, *SPAD* chlorophyll content, *Fv/Fm* chlorophyll fluorescence

*, **, *** Significant at $\alpha = 0.05$, 0.01, 0.001 and respectively. *NS* non significant

Table 2 Grouping of RILs and parents based on the three calculated traits for senescence maximum rate of senescence (MRS), time to maximum rate of senescence (TMRS) and percent greenness at maximum senescence (PGMS) under high temperature

Group	MRS (%/d)	TMRS (d)	PGMS (%)
Group 1	4.6	11.5	43.5
Group 2	12.1	12.9	35.7
Group 3	20.5	11.3	29.9
Ventnor	3.9	17.0	45.7
Karl 92	12.2	8.5	38.8

The values presented are averages of all lines that represent the group

and 25%G, respectively (Table 4). The variation explained by the marker linked to SPAD was the lowest (9%). The QTL for 50%G (*Q50%G^{o}.ksu-4B; Q50%G^{o}.ksu-5D*) and PGMS (*QPgms^{o}.ksu-4B; QPgms^{o}.ksu-5D*) were co-localized with markers *Xgwm368* and *Xgwm292* on chromosome 4B and 5D, respectively. Similarly, a QTL linked to 25%G (*Q25%G^{o}.ksu-7D*) and TMRS (*QTmrs^{o}.ksu-7D*) were co-localized with the marker *Xgwm111* on chromosome 7D. A high proportion of variation (30%) was explained by the QTL for 75%G (*Q75%G^{o}.ksu-5A*) linked to the marker *XCGA.CGCT-485* on chromosome 5A. Favorable alleles for 75%G and 25%G were from Karl 92, while favorable alleles for SPAD were from Ventnor. Favorable alleles for the remaining traits were contributed by both parents.

Under heat stress conditions, the proportion of trait variation explained by markers ranged from 11.2% for Fv/Fm to 83% for TMRS (Table 4). *Xgwm356* and *XCGT.TGCG-349* on chromosome 2A span a QTL region for the traits 25%G, 50%G, 75%G, MRS and TMRS. *Q75%G^{h}.ksu-3B* was co-localized with *XCGT.CTCG-146* on chromosome 3B. The *QTmrs^{h}.ksu-6A* on chromosome 6A and *QTmrs^{h}.ksu-6B* on chromosome 6B were co-localized with markers *XCGT.GTG-343* and *XCGT.CTCG-406*, respectively. The *QPgms^{h}.ksu-3A* on chromosome 3A was co-localized with *Xgwm5*. Favorable alleles for 50%G and Fv/Fm were from Ventnor and both parents contributed favorable alleles for all other traits. PGMS was mapped on chromosome 3A linked to *Xgwm5* and chromosome 6A linked to the marker *XCGT.GTG-343* that also associated with the QTL for 50%G and TMRS under heat stress conditions.

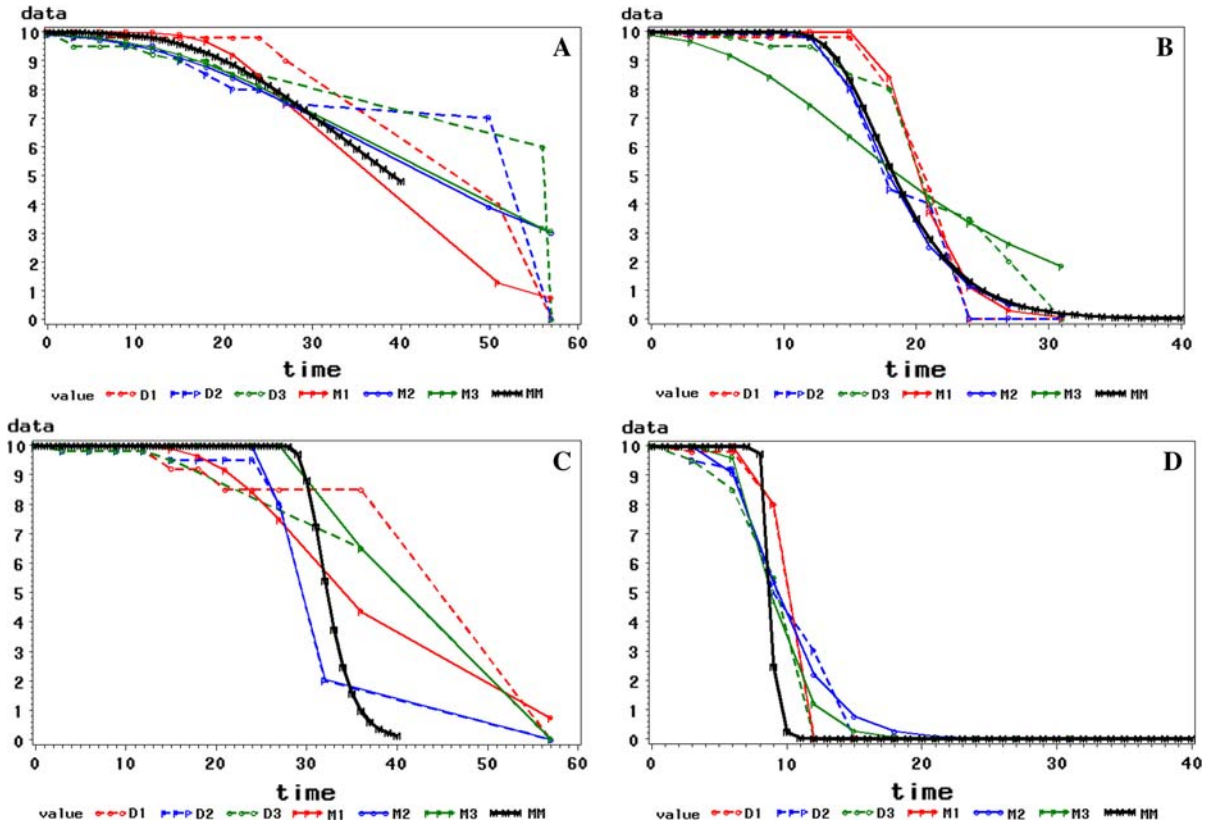


Fig. 2 Non-linear regression curves fitted over the rating for green leaf area duration (*GLAD*) with a range of 10–0, and time (days) for the parents Ventnor and Karl 92 studied under high temperature. Time zero equals 10 DAA. *Dotted lines* (D1, D2, and D3) indicate the actual data points joined together, and the *solid lines* (M1, M2, and M3) in the *same color* represents the model fitted on the replicates. The *solid black line* represents the model fitted over the average of the three replicates. Figures **a** and **b** are Ventnor under optimum and high temperature conditions, respectively; **c** and **d** are Karl 92 under optimum and high temperature conditions, respectively

Table 3 Pearson's correlation coefficients in RIL population under high temperature

Trait[a]	MRS	TMRS	SPAD	Fv/Fm
75%G	−0.558***	0.810***	0.285**	0.180^{NS}
25%G	−0.478***	0.903***	0.022^{NS}	-0.024^{NS}
50%G	−0.402***	0.811***	-0.035^{NS}	-0.063^{NS}
PGMS	−0.658***	0.832***	0.054^{NS}	-0.048^{NS}
MRS	1.000	−0.592***	−0.226*	-0.090^{NS}
TMRS	−0.592***	1.000	0.154^{NS}	0.071^{NS}
SPAD	−0.226*	0.154^{NS}	1.000	0.439***

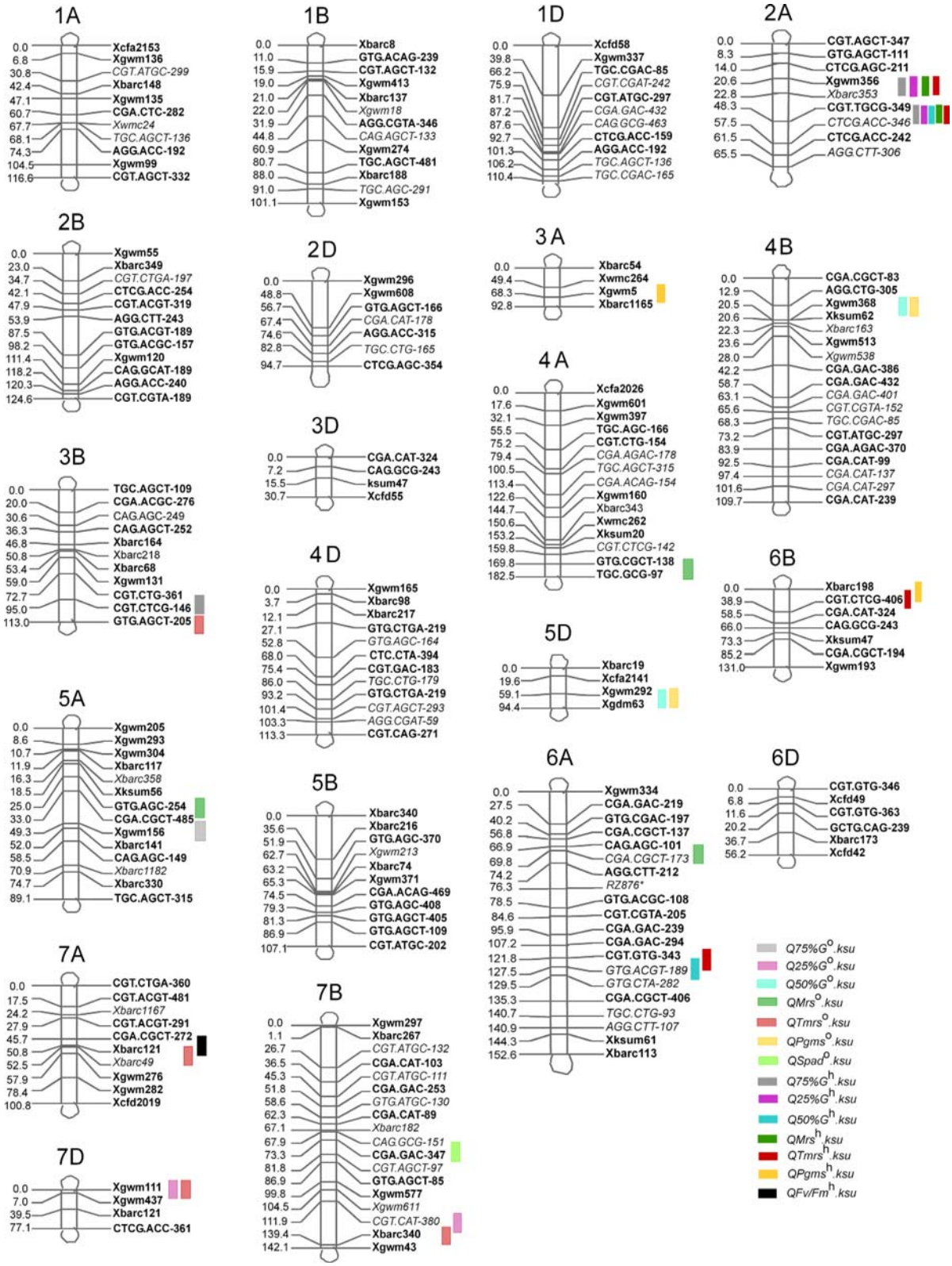
[a] *75%G* 75% green, *25%G* 25% green, *50%G* 50% green, *MRS* maximum rate of senescence, *TMRS* time to maximum rate of senescence, *PGMS* percent greenness at maximum senescence, *SPAD* chlorophyll content, *Fv/Fm* chlorophyll fluorescence

*, **, *** Significant at $\alpha = 0.05$, 0.01, and 0.001, respectively. *NS* non significant

Discussion

Monocarpic leaf senescence is a natural response in wheat as sink structures get established and plants

Fig. 3 Genetic linkage maps of 21 chromosomes used for RIL QTL analysis. The marker names are listed on the *right*, and map distances in centimorgans (cM) are listed on the left of the chromosome. Framework markers are represented in *bold* and placement markers are represented in *italics*. Markers beginning with "X" are SSR, *RZ876** is an EST derived from the coding sequence of putative chloroplast elongation factor-Tu in rice, and the others are AFLP markers denoted by their selective base combinations and size (bp) of the band. Abbreviations for traits: *Q75%G.ksu* 75%G; *Q25%G.ksu* 25%G; *Q50%G.ksu* 50%G; *QMrs.ksu* maximum rate of senescence; *QTmrs.ksu* time to maximum rate of senescence; *QPgms.ksu* percent *greenness* at maximum senescence; *QSpad.ksu* chlorophyll content; *QFv/Fm.ksu* chlorophyll fluorescence. [o] and striped bars indicate QTL found under optimum conditions; [h] and *solid bars* indicate QTL found under heat stress conditions


1A
0.0 Xcfa2153
6.8 Xgwm136
30.8 CGT.ATGC-299
42.4 Xbarc148
47.1 Xgwm135
60.7 CGA.CTC-282
67.7 Xwmc24
68.1 TGC.AGCT-136
74.3 AGG.ACC-192
104.5 Xgwm99
116.6 CGT.AGCT-332
1B
0.0 Xbarc8
11.0 GTG.ACAG-239
15.9 CGT.AGCT-132
19.0 Xgwm413
21.0 Xbarc137
22.0 Xgwm18
31.9 AGG.CGTA-346
44.8 CAG.AGCT-133
60.9 Xgwm274
80.7 TGC.AGCT-481
88.0 Xbarc188
91.0 TGC.AGC-291
101.1 Xgwm153
1D
0.0 Xcfd58
39.8 Xgwm337
66.2 TGC.CGAC-85
75.9 CGT.CGAT-242
81.7 CGT.ATGC-297
87.2 CGA.GAC-432
87.6 CAG.GCG-463
92.7 CTCG.ACC-159
101.3 AGG.ACC-192
106.2 TGC.AGCT-136
110.4 TGC.CGAC-165
2A
0.0 CGT.AGCT-347
8.3 GTG.AGCT-111
14.0 CTCG.AGC-211
20.6 Xgwm356
22.8 Xbarc353
48.3 CGT.TGCG-349
57.5 CTCG.ACC-346
61.5 CTCG.ACC-242
65.5 AGG.CTT-306
2B
0.0 Xgwm55
23.0 Xbarc349
34.7 CGT.CTGA-197
42.1 CTCG.ACC-254
47.9 CGT.ACGT-319
53.9 AGG.CTT-243
87.5 GTG.ACGT-189
98.2 GTG.ACGC-157
111.4 Xgwm120
118.2 CAG.GCAT-189
120.3 AGG.ACC-240
124.6 CGT.CGTA-189
2D
0.0 Xgwm296
48.8 Xgwm608
56.7 GTG.AGCT-166
67.4 CGA.CAT-178
74.6 AGG.ACC-315
82.8 TGC.CTG-165
94.7 CTCG.AGC-354
3A
0.0 Xbarc54
49.4 Xwmc264
68.3 Xgwm5
92.8 Xbarc1165
4B
0.0 CGA.CGCT-83
12.9 AGG.CTG-305
20.5 Xgwm368
20.6 Xksum62
22.3 Xbarc163
23.6 Xgwm513
28.0 Xgwm538
42.2 CGA.GAC-386
58.7 CGA.GAC-432
63.1 CGA.GAC-401
65.6 CGT.CGTA-152
68.3 TGC.CGAC-85
73.2 CGT.ATGC-297
83.9 CGA.AGAC-370
92.5 CGA.CAT-99
97.4 CGA.CAT-137
101.6 CGA.CAT-297
109.7 CGA.CAT-239
3B
0.0 TGC.AGCT-109
20.0 CGA.ACGC-276
30.6 CAG.AGC-249
36.3 CAG.AGCT-252
46.8 Xbarc164
50.8 Xbarc218
53.4 Xbarc68
59.0 Xgwm131
72.7 CGT.CTG-361
95.0 CGT.CTCG-146
113.0 GTG.AGCT-205
3D
0.0 CGA.CAT-324
7.2 CAG.GCG-243
15.5 ksum47
30.7 Xcfd55
4A
0.0 Xcfa2026
17.6 Xgwm601
32.1 Xgwm397
55.5 TGC.AGC-166
75.2 CGT.CTG-154
79.4 CGA.AGAC-178
100.5 TGC.AGCT-315
113.4 CGA.ACAG-154
122.6 Xgwm160
144.7 Xbarc343
150.6 Xwmc262
153.2 Xksum20
159.8 CGT.CTCG-142
169.8 GTG.CGCT-138
182.5 TGC.GCG-97
4D
0.0 Xgwm165
3.7 Xbarc98
12.1 Xbarc217
27.1 GTG.CTGA-219
52.8 GTG.AGC-164
68.0 CTC.CTA-394
75.4 CGT.GAC-183
86.0 TGC.CTG-179
93.2 GTG.CTGA-219
101.4 CGT.AGCT-293
103.3 AGG.CGAT-59
113.3 CGT.CAG-271
5D
0.0 Xbarc19
19.6 Xcfa2141
59.1 Xgwm292
94.4 Xgdm63
6B
0.0 Xbarc198
38.9 CGT.CTCG-406
58.5 CGA.CAT-324
66.0 CAG.GCG-243
73.3 Xksum47
85.2 CGA.CGCT-194
131.0 Xgwm193
5A
0.0 Xgwm205
8.6 Xgwm293
10.7 Xgwm304
11.9 Xbarc117
16.3 Xbarc358
18.5 Xksum56
25.0 GTG.AGC-254
33.0 CGA.CGCT-485
49.3 Xgwm156
52.0 Xbarc141
58.5 CAG.AGC-149
70.9 Xbarc1182
74.7 Xbarc330
89.1 TGC.AGCT-315
5B
0.0 Xbarc340
35.6 Xbarc216
51.9 GTG.AGC-370
62.7 Xgwm213
63.2 Xbarc74
65.3 Xgwm371
74.5 CGA.ACAG-469
79.3 GTG.AGC-408
81.3 GTG.AGCT-405
86.9 GTG.AGCT-109
107.1 CGT.ATGC-202
6A
0.0 Xgwm334
27.5 CGA.GAC-219
40.2 GTG.CGAC-197
56.8 CGA.CGCT-137
66.9 CAG.AGC-101
69.8 CGA.CGCT-173
74.2 AGG.CTT-212
76.3 RZ876*
78.5 GTG.ACGC-108
84.6 CGT.CGTA-205
95.9 CGA.GAC-239
107.2 CGA.GAC-294
121.8 CGT.GTG-343
127.5 GTG.ACGT-189
129.5 GTG.CTA-282
135.3 CGA.CGCT-406
140.7 TGC.CTG-93
140.9 AGG.CTT-107
144.3 Xksum61
152.6 Xbarc113
6D
0.0 CGT.GTG-346
6.8 Xcfd49
11.6 CGT.GTG-363
20.2 GCTG.CAG-239
36.7 Xbarc173
56.2 Xcfd42
7A
0.0 CGT.CTGA-360
17.5 CGT.ACGT-481
24.2 Xbarc1167
27.9 CGT.ACGT-291
45.7 CGA.CGCT-272
50.8 Xbarc121
52.5 Xbarc49
57.9 Xgwm276
78.4 Xgwm282
100.8 Xcfd2019
7B
0.0 Xgwm297
1.1 Xbarc267
26.7 CGT.ATGC-132
36.5 CGA.CAT-103
45.3 CGT.ATGC-111
51.8 CGA.GAC-253
58.6 GTG.ATGC-130
62.3 CGA.CAT-89
67.1 Xbarc182
67.9 CAG.GCG-151
73.3 CGA.GAC-347
81.8 CGT.AGCT-97
86.9 GTG.AGCT-85
99.8 Xgwm577
104.5 Xgwm611
111.9 CGT.CAT-380
139.4 Xbarc340
142.1 Xgwm43
7D
0.0 Xgwm111
7.0 Xgwm437
39.5 Xbarc121
77.1 CTCG.ACC-361
Q75%G°.ksu
Q25%G°.ksu
Q50%G°.ksu
QMrs°.ksu
QTmrs°.ksu
QPgms°.ksu
QSpad°.ksu
Q75%Gʰ.ksu
Q25%Gʰ.ksu
Q50%Gʰ.ksu
QMrsʰ.ksu
QTmrsʰ.ksu
QPgmsʰ.ksu
QFv/Fmʰ.ksu

re-mobilize resources and nutrients towards actively developing kernels (Thomas and Howarth 2000). However, under post-flowering abiotic stress such as heat or drought, this process of leaf senescence is accelerated thus putting a pressure on source availability for current photosynthates which, along with re-mobilized sugars and proteins are important contributors to kernel weight and hence the ultimate realized yield (Tuinstra et al. 1997). While yield and yield component traits are directly quantifiable during harvest, senescence is a relatively complex trait to quantify primarily because of its progressive nature with time. The relative rate of senescence and the period of accelerated senescence due to post-flowering heat stress would vary between the RILs and hence cannot be properly quantified by using senescence measurements at any one time point. Modeling for senescence using the derived parameters of MRS, TMRS and PGMS makes it possible to quantify the progressive nature of accelerated leaf senescence under post-flowering heat stress. Variation for these traits within the RILs can then be used to map QTLs associated with senescence and as a response to post-flowering heat stress. Lines which are able to maintain greater green leaf area under post-flowering high temperature stress would have lower MRS, longer TMRS and higher PGMS compared to more senescent lines. The extended green leaf duration in tolerant lines could result in a higher rate of current photosynthates that could then translate to a larger assimilate pool for re-mobilization to sink tissues and could ultimately result in higher yield.

The Fv/Fm and SPAD measurements used in this study were intended to measure the photosynthetic efficiency of the plants indirectly. Light absorbed by the leaf at a normal physiological temperature is used in the photochemical act and the balance is dissipated as heat or reemitted as chlorophyll fluorescence. Chlorophyll fluorescence is estimated as Fv/Fm and reflects thylakoid membrane integrity. Fv (variable fluorescence) is the difference between Fo and Fm where, Fo is the fluorescence at ground state which rises to its maximum value (Fm) upon an application of a saturated flash of light (Moffatt et al. 1990). In healthy leaves, the Fv/Fm ratio is close to 0.8. A lower value indicates that a proportion of PSII reaction centers are damaged. This phenomenon is called as photo-inhibition, and is observed in the plants under stress conditions (Fracheboud et al. 2002).

Wheat is a naturally tillering plant and differences in tiller number exist between Karl and Ventnor (data not shown). Hence, the RILs derived from their cross are segregating for the number of tillers. In order to avoid the confounding effects of tiller number on yield and senescence traits under post-flowering high temperature stress and to accurately quantify all the traits, all phenotypic measurements were taken on the main culm with multiple replicates in growth chambers under precise control of temperature, humidity and light intensity.

The RILs in this experiment varied significantly in senescence related traits under both optimum and high temperature regimes. Similar results have been reported in previous studies for plant responses to post-flowering stress (Chen et al. 2002; Mohammadi et al. 2004). In this study, Ventnor, the tolerant parent, had longer duration of photosynthetic activity, higher chlorophyll content and higher Fv/Fm ratio under both optimum and high temperature conditions. This was in agreement with previous studies (Al-Khatib and Paulsen 1990; Yang et al. 2002).

After 10 days of continuous heat stress on RILs, GLAD was measured on a progressive scale at 3-day intervals. Senescence is a continuous process as the plant moves into reproductive phase, sink potential gets established and senescence is accelerated to remobilize nutrients to the growing kernels. The visible manifestation of these processes is the breakdown of chlorophyll, which typically starts from the leaf margins and progresses towards the interior of the leaf blade. Imposition of heat stress accelerates senescence (Thomas and Howarth 2000). Gompertz's analysis was used to model the senescence pattern in the RILs. By using the model, the non-linear regression curve obtained can be extrapolated to detect the point of time when the plant was 100% green. Since the heat stress was imposed from 10DAA, for accuracy purpose only 75%G, 50%G and 25%G were included with key emphasis on MRS, TMRS and PGMS. Senescence could be broadly categorized as low, exemplified by lines in Group 1; moderate as seen in Group 2; or high as in Group 3 (Table 3) based on differences between RILs for the traits MRS, TMRS and PGMS. Group 1 has the lowest MRS and also took the longest duration after anthesis to reach this high rate of senescence (TMRS). The heat tolerant parent Ventnor was in this group. Karl 92 was in group 2 and had higher

Table 4 Putative quantitative trait loci (QTL) detected for senescence related traits in the RIL population derived from the cross Ventnor/Karl 92

Trait[a]	QTL[o/h,b]	Marker[c]	Distance[d] (cM)	LOD	A[e]	R^2	Total R^2 (%)
75%G	*Q75%G^o.ksu-5A*	CGA.CGCT-485 Xgwm156	3.9	4.8	−4.37	0.30	30.0
	Q75%G^h.ksu-2A	Xgwm356 CGT.TGCG-349	0.1	4.1	−0.74	0.17	
	Q75%G^h.ksu-2A	CGT.TGCG-349 CTCG.ACC-242	9.5	4.8	0.93	0.26	
	Q75%G^h.ksu- 3B	CGT.CTCG-146 GTG.AGCT-205	0	3.5	−0.56	0.10	53.0
25%G	*Q25%G^o.ksu-7B*	Xgwm577 Xbarc340	32	3.2	−15.90	0.21	
	Q25%G^o.ksu-7D	Xgwm111 Xgwm437	2.0	2.5	−12.27	0.12	41.7
	Q25%G^h.ksu-2A	Xgwm356 CGT.TGCG-349	0.3	2.7	−3.10	0.10	
	Q25%G^h.ksu-2A	CGT.TGCG-349 CTCG.ACC-242	9.5	4.3	3.92	0.18	28.0
50%G	*Q50%G^o.ksu-4B*	Xgwm368 Xksum62	0	3.1	−10.41	0.10	
	Q50%G^o.ksu-5D	Xgwm292 Xgdm63	34	2.5	9.97	0.09	19.0
	Q50%G^h.ksu-2A	CGT.TGCG-349 CTCG.ACC-242	9.5	2.9	3.03	0.12	
	Q50%G^h.ksu-6A	CGT.GTG-343 CGA.CGCT-406	3.9	14.4	17.09	0.51	63.0
MRS	*QMrs^o.ksu-4A*	GTG.CGCT-138 TGC.GCG-97	12	2.4	0.84	0.09	
	QMrs^o.ksu-5A	GTG.AGC-254 CGA.CGCT-485	8	4.6	−1.09	0.12	
	QMrs^o.ksu-6A	CAG.AGC-101 AGG.CTT-212	4	6.4	2.31	0.26	47.0
	QMrs^h.ksu-2A	Xgwm356 CGT.TGCG-349	0.3	4.6	−3.65	0.19	
	QMrs^h.ksu-2A	CGT.TGCG-349 CTCG.ACC-242	11.6	4.8	3.87	0.21	40.0
TMRS	*QTmrs^o.ksu-3B*	CGT.CTCG-146 GTG.AGCT-206	8	2.4	4.49	0.18	
	QTmrs^o.ksu-7A	Xbarc121 CTCG.ACC-361	0	2.9	3.17	0.09	
	QTmrs^o.ksu-7B	Xbarc340 Xgwm43	0	3.3	3.73	0.12	
	QTmrs^o.ksu-7D	Xgwm111 Xgwm437	0	3.1	−3.46	0.10	49.1

Table 4 continued

Trait[a]	QTL[o/h,b]	Marker[c]	Distance[d] (cM)	LOD	A[e]	R^2	Total R^2 (%)
	QTmrs^h.ksu-2A	Xgwm356	0.1	4.4	−1.25	0.17	
		CGT.TGCG-349					
	QTmrs^h.ksu-6A	CGT.GTG-343	0	2.9	2.49	0.30	
		CGA.CGCT-406					
	QTmrs^h.ksu-6B	CGT.CTCG-406	0	2.8	−0.84	0.08	55.0
		CGA.CAT-324					
PGMS	*QPgms^o.ksu-4B*	Xgwm368	0	4.7	5.85	0.17	
		Xksum62					
	QPgms^o.ksu-5D	Xgwm292	33	2.5	−3.63	0.10	26.4
		Xgdm63					
	QPgms^h.ksu-3A	Xgwm5	0	2.5	−2.84	0.08	
		Xbarc1165					
	QPgms^h.ksu-6B	Xbarc198	36	2.4	3.38	0.10	36.4
		CGT.CTCG-406					
SPAD	*QSpad^o.ksu-7B*	CGA.GAC-347	0	2.6	0.43	0.09	9.0
		GTG.AGCT-85					
Fv/Fm	*QFv/Fm^h.ksu-7A*	CGA.CGCT-272	4.1	2.8	0.02	0.11	11.2
		Xbarc121					

[a] *75%G* 75% green, *25%G* 25% green, *50%G* 50% green, *MRS* maximum rate of senescence, *TMRS* time to maximum rate of senescence, *PGMS* percent greenness at maximum senescence, *SPAD* chlorophyll content, *Fv/Fm* chlorophyll fluorescence

[b] O represents that QTL found under optimum and H represents that QTL found under heat stress conditions

[c] Flanking markers to the putative QTL, first marker is the left flanking marker and second marker is the right flanking marker

[d] Genetic distance between the most likely position of the putative QTL and its left flanking marker

[e] A, the additive effect. A positive value indicates the Ventnor allele having a positive effect on the trait, and a negative value indicates Karl 92 allele having positive effect on the trait

MRS and lesser PGMS than Ventnor. The traits MRS, TMRS and PGMS are useful in distinguishing the RILs for their senescence response under post-anthesis high temperature conditions and could be used to measure variability in leaf senescence.

The SPAD chlorophyll readings had significantly negative correlations with maximum rate of senescence, indicating that the chlorophyll content and photosynthetic ability of the plant were maintained longer at lower senescence rates. This result was similar to that from functional stay-green mutants of durum wheat, which had delayed leaf senescence and longer photosynthetic competence (Spano et al. 2003). Similarly plants with stay-green trait have stable chlorophyll content (SPAD) and higher Fv/Fm ratio, as exemplified by Ventnor over Karl 92 (Al-Khatib and Paulsen 1990). In the present study the RILs derived from Ventnor and Karl 92 also showed a positive correlation between chlorophyll fluorescence and chlorophyll content. Since the SPAD or Fv/Fm readings were measured at a single time point, they may not completely correlate with traits that are related to senescence over a cumulative period like MRS, TMRS and PGMS.

Composite interval mapping in the RIL population showed that QTL for senescence-related traits were distributed on most of the chromosomes under optimum conditions, while they were mostly on chromosomes 2A, 6A and 6B under heat stress. Different QTL for senescence-related traits were identified under optimum and heat stress conditions, indicating different set of genes getting activated under heat stress conditions. The QTL *Q75%G^h.ksu-2A*, *Q25%G^h.ksu-2A*, *QMrs^h.ksu-2A*, and *QTmrs^h.ksu-2A* associated with markers *Xgwm356* and *XCGT.TGCG-349* on chromosome 2A and *Q50%G^h.ksu-6A*, and *QTmrs^h.ksu-6A* associated

with marker *XCGT.GTG-343* on chromosome 6A with a prominent effect on slowing down senescence under heat stress likely enhance stay-green.

Previous studies on stay-green in wheat and other species under abiotic stress have been conducted at a given point of time. Although stay-green rating at a given point of time may be of value, getting a rating of senescence over the period of crop development from flowering to physiological maturity could be more useful in characterizing behavior of RILs under stress as senescence is a continuous response and is influenced by the level of tolerance the germplasm has to post-anthesis heat stress. The method we used in this study to model visual rating of GLAD over the reproductive growth phase and to map traits related to senescence can provide quantitative characterization of stay-green trait. Using this model we have described three predictive traits MRS, TMRS and PGMS which characterize the progress of senescence and have ability to distinguish different RILs with respect to accelerated senescence under post-anthesis heat stress. Ventnor contributed the favorable alleles for interval between markers *XCGT.TGCG-349* and *XCTCG.ACC-242* for QTL corresponding to $QMrs^{h}.ksu\text{-}2A$, and $QTmrs^{h}.ksu\text{-}2A$. Ventnor also contributed the favorable alleles for QTL at the interval on chromosome 6A between markers *XCGT.GTG-343* and *XCGA.CGCT-406* associated with $QTmrs^{h}.ksu\text{-}6A$; and the interval on chromosome 6B between markers *Xbarc198* and *XCGT.CTCG-406* associated with $QPgms^{h}.ksu\text{-}6B$. It appears that the post-anthesis heat tolerance in Ventnor is due to QTL contributing towards the traits MRS and PGMS.

According to the genome synteny (Devos and Gale 1997; Sorrells et al. 2003), homoeologous wheat chromosome 6 is syntenic to chromosome 2 in rice and chromosomes 4, and 5 in maize. Similarly, homoeologous wheat chromosome 3 is syntenic to chromosomes 3 and 8 in maize. $QPgms^{h}.ksu\text{-}6B$ associated with marker *Xbarc198* is present in the deletion bin 6BS5-0.76 at an arm length of 0.38%. Within the same bin, the locus for grain protein gene (*Gpc-B1*) was mapped (Distelfeld et al. 2006). The *Gpc-B1* gene has been cloned and found to have multiple pleiotropic effects on grain protein content, progression of leaf senescence, remobilization of nutrients, and grain development (Uauy et al. 2006).

XCGT.GTG-343, linked to QTL for 50%G and TMRS, is mapped at 76.3 cM distal to EST *RZ876*. According to Bhadula et al. (2001), heat stress in maize induces an enhanced synthesis of chloroplast elongation factor (EF-Tu) and plays an important role in thermo-tolerance. In GenBank (National Center for Biotechnology Information) the BAC clone AP004023 from the physical map of rice chromosome 2 that were mapped between 23,050 and 23,146 kb had 16 putative genes (TIGR Rice Genome Annotation). One of those genes was a chloroplast elongation factor Tu (EF-Tu) spanning between 23,160 and 23,108 kb on the BAC clone. This gene corresponded to the EST *RZ876* that mapped at 91.1 cM in the rice genetic linkage map (Cheng et al. 2001). The PCR primers designed from the coding sequence of putative EF-Tu gene were used on the RIL population in the present study. The EST *RZ876* in the wheat genetic linkage map showed significant linkage to 50%G, and explained 6.9% of the variation for the trait (data not shown).

Marker *Xgwm5* on chromosome 3A associated with QTL $QPgms^{h}.ksu\text{-}3A$ was physically mapped to deletion bin 3AL3-0.42 (Qi et al. 2003; Sourdille et al. 2004). In the wheat consensus map (Somers et al. 2004), this marker is located at 72 cM from the proximal end. Marker *Xcdo54* is distal to *Xgwm5* at 74.00 cM apart. *Xcdo54* also was mapped to maize bin 8.03 on chromosome 8. This bin is associated with gene for light harvesting chlorophyll a/b binding protein 3 (*lhcb3*) and a QTL for leaf senescence as possible insights in the mode of action for genes underlying $QPgms^{h}.ksu\text{-}3A$.

Conclusions

Leaf senescence is a complex process. The modeling of senescence-related traits over the reproductive period allowed the characterization of senescence or stay-green in a quantitative manner. Among the senescence-related traits, MRS, TMRS and PGMS are key traits for senescence. 50%G can also be considered when using the traits for selection under heat stress. Under high temperature, QTL for senescence-related traits were mainly mapped on chromosomes 2A, 6A and 6B. Especially the regions on chromosome 2A close to the markers *Xgwm356* and *XCGT.TGCG-349* were populated with QTL for the senescence-related traits; and the regions close to marker CGT.GTG-343 on chromosome 6A and marker

XCGT.CTCG-406 on chromosome 6B accounted for the variation for the 50%G and co-localized with the TMRS. All positive alleles for trait 50%G were from Ventnor indicating a significant contribution to the stay-green from Ventnor. The prominent markers *Xgwm356* and *Xgwm5* may be useful in marker-assisted breeding and together with *XCGT.TGCG-349*, *XCGT.GTG-343*, and *XCGT.CTCG-406* provide markers for further molecular dissection of the QTL for senescence-related traits in wheat.

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