CSIRO PUBLISHING

Functional Plant Biology, 2014, **41**, 1035–1048
http://dx.doi.org/10.1071/FP14052

Phenotyping novel stay-green traits to capture genetic variation in senescence dynamics

John T. Christopher[A,F], *Mathieu Veyradier*[B], *Andrew K. Borrell*[C], *Greg Harvey*[D], *Susan Fletcher*[D] *and Karine Chenu*[E]

[A]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation (QAAFI), Leslie Research Facility, PO Box 2282, Toowoomba, Qld 4350, Australia.
[B]Department of Agriculture, Fisheries and Forestry Queensland (DAFF), 203 Tor St., Toowoomba, Qld 4350, Australia.
[C]The University of Queensland, QAAFI, Hermitage Research Facility, MS 508, Warwick, Qld 4370, Australia.
[D]DAFF, Leslie Research Facility, PO Box 2282, Toowoomba, Qld 4350, Australia.
[E]The University of Queensland, QAAFI, 203 Tor St., Toowoomba, Qld 4350, Australia.
[F]Corresponding author. Email: j.christopher@uq.edu.au

This paper originates from a presentation at the Interdrought IV Conference, Perth, Australia, 2–6 September 2013.

Abstract. Stay-green plants retain green leaves longer after anthesis and can have improved yield, particularly under water limitation. As senescence is a dynamic process, genotypes with different senescence patterns may exhibit similar final normalised difference vegetative index (NDVI). By monitoring NDVI from as early as awn emergence to maturity, we demonstrate that analysing senescence dynamics improves insight into genotypic stay-green variation. A senescence evaluation tool was developed to fit a logistic function to NDVI data and used to analyse data from three environments for a wheat (*Triticum aestivum* L.) population whose lines contrast for stay-green. Key stay-green traits were estimated including, maximum NDVI, senescence rate and a trait integrating NDVI variation after anthesis, as well as the timing from anthesis to onset, midpoint and conclusion of senescence. The integrative trait and the timing to onset and mid-senescence exhibited high positive correlations with yield and a high heritability in the three studied environments. Senescence rate was correlated with yield in some environments, whereas maximum NDVI was associated with yield in a drought-stressed environment. Where resources preclude frequent measurements, we found that NDVI measurements may be restricted to the period of rapid senescence, but caution is required when dealing with lines of different phenology. In contrast, regular monitoring during the whole period after flowering allows the estimation of senescence dynamics traits that may be reliably compared across genotypes and environments. We anticipate that selection for stay-green traits will enhance genetic progress towards high-yielding, stay-green germplasm.

Additional keywords: drought, leaf senescence, *Triticum aestivum*, wheat.

Received 18 February 2014, accepted 17 June 2014, published online 19 August 2014

Introduction

Plants with a stay-green phenotype retain green leaf area for longer after anthesis than standard genotypes. The stay-green phenotype has been linked to higher yield in several cereal crop species, including wheat (*Triticum aestivum* L.) and sorghum (*Sorghum bicolor* (L.) Moench), particularly under terminal drought stress (recently reviewed in Gregersen *et al*. 2013). Hence, stay-green is considered an important potential target phenotype to improve crop adaptation to water-stressed environments (Borrell *et al*. 2006; Christopher *et al*. 2008; Lopes and Reynolds 2012; Jordan *et al*. 2012).

Stay-green has been assessed in the field using several techniques. Visual stay-green ratings can be rapid and require no specific equipment. The downside of such phenotyping approaches is that visual ratings are inevitably subjective and methods can differ significantly between researchers. This complicates comparison of results from independent studies. Visual assessments have been performed for the number of green leaves per culm (Haussmann *et al*. 1999), greenness of all fertile shoots (Foulkes *et al*. 2007) and greenness of just the flag leaf and peduncle (Joshi *et al*. 2007). More objective methods have also been applied, with measurements of

individual leaves using instruments such as the Minolta SPAD meter (Harris *et al*. 2007; Christopher *et al*. 2008). However, such methods can be time-consuming and impractical for measurements of large numbers of genotypes, as they require repeated measurements of multiple leaves to provide reliable information for a single trial plot. Recently, methods have been developed using normalised difference vegetative index (NDVI) measurements from devices such as the hand-held Greenseeker (NTech Industries, Ukiah, CA, USA) (Lopes and Reynolds 2012; Kipp *et al*. 2014). The advantage of such methods is that they provide objective, integrated measurements of canopy greenness and only take a few seconds to measure a field plot (versus several minutes required for SPAD measurements). NDVI-based methods can provide measurements of hundreds of field plots in an hour and thus can be used to screen large numbers of plots (e.g. breeding populations or structured populations for genetic studies). As they are nondestructive and relatively rapid, repeated measurements can be made on the same plots over the period of crop development. Studies reporting NDVI-based measurements of the stay-green phenotype commonly rely on measurements from one or a few time points late in the crop cycle (e.g. Lopes and Reynolds 2012). However, are measurements of NDVI at one or a few time points sufficient for selection decisions? Given the potential offered by NDVI methods, what is the most efficient way to detect genetic stay-green variation in terms of the timing and frequency of measurement and the integration of the data?

To capture senescence dynamics, linear regressions have been fitted to measurements taken when plants are fully green as well as close to maturity (Harris *et al*. 2007; Lopes and Reynolds 2012). However, traits such as senescence onset vary between genotypes and the date of measurements could thus impact the results. In addition, the dynamics of senescence probably follows a nonlinear pattern (Vijayalakshmi *et al*. 2010).

The aims of this study were (i) to propose a method to phenotype stay-green dynamics, (ii) to determine whether there is potential advantage in taking multiple NDVI measurements during the crop life cycle from before anthesis to maturity, and (iii) to investigate cheaper alternative methods for high-throughput phenotyping. In particular, we wished to answer several questions of interest for crop improvement phenotyping. Can measurements on senescence dynamics give additional information about genetic variation for stay-green? Conversely, can information from one or a few time points be used to select for stay-green in breeding programs? If so, how many measurements are needed and when should they be taken? To address these questions, NDVI was measured on lines of a population contrasting for stay-green that were grown in three field environments in southern Queensland, Australia.

Materials and methods

Plant material

NDVI was measured on a population of 184 doubled haploids derived from the bread wheat (*Triticum aestivum* L.) cultivars SeriM82 and Hartog, plus the parents and a small number of controls. SeriM82 is a high-yielding drought-tolerant CIMMYT line derived from the Veeri cross (Sivapalan *et al*. 2000, 2001; Olivares-Villegas *et al*. 2007). Hartog is a locally-adapted line derived from the CIMMYT cross Pavon. SeriM82 and Hartog have been shown to differ in yield adaptation to water-limited environments, in root architecture and in stay-green (Manschadi *et al*. 2006, 2010; Christopher *et al*. 2008). The 184 lines studied here were selected from a larger population, based on their similarity in height and maturity date (Christopher *et al*. 2013).

Field sites and treatments

Field trials were established during three seasons (2010, 2011 and 2012) at two sites in southern Queensland, Australia: Gatton (GAT: 27°54′S 152°34′E, 89 m above sea level) in 2010; Warwick (WAR: 28°21′S 152°10′E, 480 m above sea level) in 2011 and 2012. Experiment names are derived from a combination of location ('GAT' or 'WAR') and year (e.g. '10' for 2010) such that the trial at Warwick in 2011 is designated 'WAR11'. Heavy alkaline cracking clay soils with high moisture-holding capacity and no known subsoil constraints predominated at both sites. These environments are typical of the northern subtropical cereal cropping region of Australia.

Crops were sown in 2 m × 6 m plots with a row spacing of 25 cm and a target population density of 100 plants m^{-2}. Soil tests were performed before planting to estimate soil N and P levels as well as parasitic nematode densities. Nematode densities were below levels known to cause damage to sensitive wheat cultivars. Luxury levels of nutrients were applied using 120 kg ha^{-1} urea before sowing and 40 kg ha^{-1} of Starter Z (Granulock ® Z Extra, Incitec Pivot Ltd, Southbank, Vic., Australia) containing 10.5 N, 19.5 P, 2.2 S and 2.2 Zn (%w/w) at sowing. Weeds and diseases were controlled as necessary. Crops were sown into soil near field capacity at WAR11 and WAR12. At GAT10, soil was irrigated to field capacity by addition of 25 mm immediately after sowing, with an additional 42 mm of supplementary irrigation applied immediately before anthesis.

Trial design

All three trials were designed as partially replicated row–column experiments (Cullis *et al*. 2006). The designs included an underlying component for autocorrelation in the column and row direction. All designs were generated using *lmmdesign* (Butler *et al*. 2008). At the GAT10 trial, 198 lines were grown in a 20 columns × 13 rows layout grouped into two replicate blocks. The two parents (SeriM82 and Hartog) plus 60 other lines were replicated twice, providing ~30% of replicated material. At WAR11, 183 lines were grown in 8 columns × 33 rows grouped in two replicate blocks. SeriM82 and Hartog were each replicated five times, but 73 other lines were replicated twice, providing ~40% of replicated material. At WAR12, 198 lines were grown in 8 columns × 33 rows grouped in two replicate blocks. SeriM82 and Hartog were each replicated five times, but 58 other lines were replicated twice, providing ~30% of replicated material.

Measurements

Emergence scores were taken to ensure plots were well established. For each plot, Zadoks stages were recorded weekly to determine flowering date (Zadoks code 65; Zadoks

et al. 1974). NDVI was measured weekly for each plot starting from awn emergence (Zadoks code 49) in Warwick 2012 and just before anthesis (Zadoks code 60) at the other two trials up until after maturity using a hand-held Greenseeker model 505 (NTech Industries). At the end of the experiment, grain was harvested using a small plot harvester to estimate yield. Plots affected by disease were removed from the analysis.

Estimation of stay-green traits

A wheat senescence evaluation tool was developed in the computer package R (R Development Core Team 2013). This application fits a logistic function (Eqn 1; Fig. 1*a*) to NDVI data centred at the anthesis for each plot. To enable proper fits to the data, an extra NDVI value corresponding to the final NDVI of the fully senesced crop was added after maturity, at 1000 degree-days (°Cd) after the last measurement date. The NDVI value of this extra point corresponds to the minimum between 0.15 and the minimum NDVI recorded for the plot considered.

$$NVDI = N_{\text{final}} + \left(\frac{N_{\text{green\_max}}}{1 + \left(\frac{t}{TFN50^{SR}} \right)} \right), \quad (1)$$

where N_{final} is the final NDVI of the dead plants, $N_{\text{green\_max}}$ corresponds to the difference in NDVI between the maximum and final values (i.e. maximum NDVI (N_{max}) is equal to $N_{\text{final}} + N_{\text{green\_max}}$), *TFN50* is the thermal time from anthesis to loss of 50% of $N_{\text{green\_max}}$, *SR* is an indicator of the senescence rate and t is the thermal time from the plot anthesis (Fig. 1, Table 1).

The stay-green evaluation tool was used to estimate stay-green traits for each plot (Table 1; Fig. 1; Eqn 1), including maximum NDVI (N_{max}), and the thermal time from anthesis to (a) senescence onset (which corresponds to the thermal time

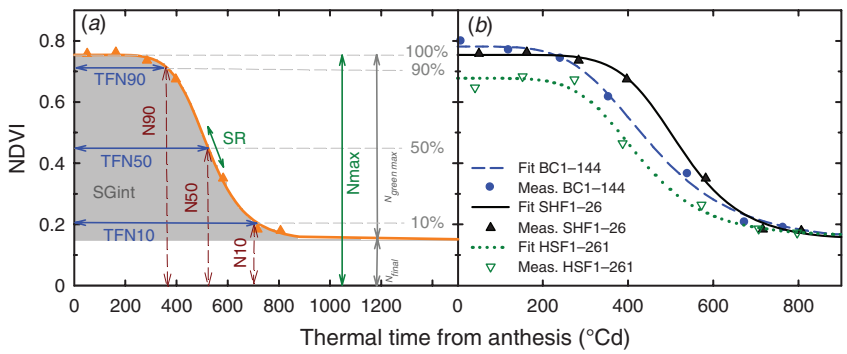

Fig. 1. Change in canopy greenness in wheat over time presented for (*a*) an illustration of the fitted logistic curve and the estimated stay-green traits and for (*b*) three contrasting genotypes BC1–144, SHF1–26 and HSF1–261 at Warwick in 2011 (WAR11). In (*b*), symbols represent measured experimental normalised difference vegetative index (NDVI) values ('Meas.'), whereas the lines represent the regression fitted using Eqn 1 ('Fit'). A list of the stay-green traits and their descriptions are given in Table 1.

Table 1. Abbreviations and descriptions of stay-green traits and normalised difference vegetative index (NDVI) estimates at regular intervals in wheat

Stay-green traits were estimated for each plot of each genotype from a logistic function (Eqn 1) fitted to NDVI field data centred at anthesis. Fig. 1 provides a graphical representation of these traits. NDVI estimates were calculated every 100 degree-days (°Cd) from anthesis to 1100°Cd after anthesis

Abbreviation	Stay-green traits	Description
N_{max}	Maximum leaf greenness	Maximum NDVI value usually near or before anthesis ($N_{\text{final}} + N_{\text{green\_max}}$)
TFN90	Senescence onset	Thermal time from anthesis to 90% of $N_{\text{green\_max}}$
TFN50	Midpoint of senescence	Thermal time from anthesis to 50% of $N_{\text{green\_max}}$
TFN10	Senescence concluding	Thermal time from anthesis to 10% of $N_{\text{green\_max}}$
SR	Indicator of senescence rate	Indicated the rate of NDVI decrease; see Eqn 1
SGint	Stay-green integral	NDVI integral from anthesis to senescence completion
N90	NDVI at senescence onset	NDVI value at TFN90
N50	NDVI at midpoint of senescence	NDVI value at TFN50
N10	NDVI at senescence concluding	NDVI value at TFN10
SG100 to SG1100	NDVI estimates at various times	NDVI values at 100°Cd intervals from 100°Cd to 1100°Cd after anthesis estimated from Eqn 1

when N_{green_max} dropped by 10%; TFN90), (b) the midpoint of senescence (TFN50; i.e. a drop of 50% from N_{green_max}) and (c) senescence nearing conclusion (TFN10; i.e. a drop of 90% from N_{green_max}). In addition, NDVI values for each plot were estimated at the thermal times TFN90, TFN50 and TFN10, and were designated 'N90', 'N50' and 'N10', respectively (Table 1). The indicator of the senescence rate (SR) was estimated as described in Eqn 1. The stay-green integral ('SGint') was calculated as the integral from anthesis to 1500°Cd after anthesis (i.e. after the completion of senescence in all environments). Thus, SGint corresponds to the area under the stay-green curve from anthesis until the completion of senescence (grey area in Fig. 1*a*) and is related to the overall green-leaf area retention of the crop after anthesis.

In order to determine when NDVI was most closely correlated with yield, correlations were estimated at 100°Cd intervals from 100°Cd to 1100°Cd after anthesis (Table 1), a thermal period of 100°Cd corresponding approximately to a weekly NDVI sampling period in these trials.

In order to assess the value of a simpler and more direct phenotyping method, the correlation between yield and NDVI was also determined for direct NDVI measurements at actual sampling times. Sampling times were expressed relative to the flowering date of a single reference genotype, Hartog.

Genetic correlations of stay-green traits and NDVI measurements with yield

For each trial, a series of separate bivariate linear mixed models were used to calculate the genetic correlation between yield and the stay-green traits, yield and NDVI measurements, as well as yield and each of the NDVI estimates that were calculated at 100°Cd intervals using the logistic function (Eqn 1). In the analysis model, genotypes were considered to be a random effect, since the interest was in the variation in genotype ranking between yield and NDVI. Each bivariate model estimated the genetic correlation between the two traits, yield and NDVI, allowing for heterogeneous variance for each trait. The correlation and heterogeneity are denoted by 'corh' in the base model detailed below. Since yield and NDVI were measured in the same field plot, correlation and separate variances were also included at the observational unit (field plot) level (denoted 'rcov' in the base model). The experimental design was based on two replicate blocks, denoted 'Block' in the base model. In addition, spatial variation was modelled through a separable autoregressive structure for both rows and columns at the residual level, and any extraneous field trends due to rows and columns in the field were included, following the method of Gilmour *et al*. (1997).

The base ASReml-R syntax was:

```
fixed = ~Trait,
random = ~corh(Trait) : Genotype + Block,
rcov = ~corh(Trait) : ar1(Row):ar1(Column).
```

Genetic correlations between NDVI measurements and between stay-green traits

To assess the possible change in genotype ranking across weekly NDVI measurements, a multitrait analysis was applied using a factor analytic approach (Smith *et al*. 2001). Because the NDVI measurements were taken repeatedly across time from the same plot, an ante-dependence covariance structure was applied at the field plot level, thus accounting for any covariance (correlation) between plot measurements over time. The residual term also included a separable autoregressive model for spatial variation over the rows and columns in the field. In addition, terms associated with the field rows and columns were included, which were specific for each site and measurement time. The set of measurement times is grouped and denoted as 'Trait' in the syntax below. Each trial was analysed separately.

The base ASReml-R syntax was:

```
fixed = ~Trait,
random = ~fa(Trait, k) : Genotype + Block,
rcov = ~ante(Trait) : ar1(Row):ar1(Column),
```

where k is the number of factor loadings that explain a significant amount of variation in the data.

The factor analytic approach was also used to determine the level of agreement between the traits TFN90, TFN50, TFN10, Nmax, SGint, SR, N90, N50 and N10.

For each trait, the statistical model provided the best linear unbiased predictors of the genetic effects. The residual maximum likelihood (Patterson and Thompson 1971) algorithm was used to provide estimates of the variance components.

Data were analysed with ASReml-R (Gilmour *et al*. 1999; Butler *et al*. 2009) using R software.

Results

Yield varied between genotypes and environments

For all trials, the deep clay soils held substantial plant-available soil moisture at sowing from the previous summer fallow, as typically observed in this cropping environment (Table 2). In-crop rainfall varied between trials from 216 mm at GAT10 down to 109 mm at WAR12 (Table 2). At GAT10 and WAR11, in-crop rainfall, stored soil moisture at sowing (plus irrigation at GAT10) resulted in little or no moisture stress during the growing season (corresponding to environment Type 1 from Chenu *et al*. 2013).

Table 2. Year, site, trial name (Trial ID), sowing date (Sow), irrigation (Irri, in mm), date of Hartog anthesis (DHA), plant-available soil moisture at sowing (PAW, in mm), in-crop rainfall (ICR, mm), average daily (Ave T) and cumulative incident radiation (Radn, in MJ m^{-2}) for three wheat trials in southern Queensland

GAT10, trial at Gatton in 2010; WAR11, trial at Warwick in 2011; WAR12, trial at Warwick in 2012

Year	Site	Trial ID	Sow	DHA	PAW	Irri	ICR	Ave T	Radn
2010	Gatton	GAT10	26 May	29 August	191	67	216	15.7	1808
2011	Warwick	WAR11	24 June	2 October	256	0	187	13.2	2447
2012	Warwick	WAR12	22 June	7 October	255	0	109	13.8	2649

Minor stress occurred for only a short period before anthesis at WAR11. In contrast, WAR12 experienced moisture stress from before anthesis to just after the middle of the grain filling period, which was relieved only by late rainfall (environment Type 3 from Chenu *et al.* 2013). Frequent in-season rainfall and prolonged periods of cloud cover reduced radiation at GAT10, particularly during the grain filling period (Table 2). The reduced radiation contributed to a lower site mean yield at GAT10 (464 g^{-2}) compared with WAR11 (567 g^{-2}; Table 3). Lack of rainfall leading up to anthesis and during the early grain filling period affected yield at WAR12, leading to the lowest site mean yield (415 g^{-2}; Tables 2 and 3). Site mean yields at WAR11 and WAR12 were above the district average commercial yield of 300–350 g^{-2} (Potgieter *et al*. 2002, 2004). Significant yield variation was observed between genotypes, for all environments ($P \leq 0.05$).

Genotypic differences in stay-green traits indicate variation in stay-green expression

The logistic function in Eqn 1 provided a close fit to the experimental data (e.g. Fig. 1*b*). Coefficients of determination (r^2) averaged more than 0.99 overall and were greater than 0.86 for all plots. Correspondingly, the residual mean square error and coefficient of variation of error were low, with an overall average of 0.027 and 0.062, respectively.

The dynamics of senescence varied between genotypes (Fig. 1; Table 3), with significant genetic variation ($P \leq 0.05$) observed for all stay-green traits (Table 1; Fig. 1) in all environments except for TFN10 at GAT10 and N50 at WAR11 ($P \leq 0.05$; Table 3). Some examples of the types of variation observed are illustrated in Fig. 1*b* for three genotypes grown at WAR11. Here, HSF1–261 had a relatively low N_{max} (~0.7) versus BC1–144 and SHF1–26 (~0.8). HSF1–261 had the earliest onset of senescence (TFN90) and was also the earliest genotype to lose 50% NDVI with TFN50 at 428°Cd from anthesis versus BC1–144 and SHF1–26 at 456°Cd and 524°Cd, respectively (Fig. 1*b*). In contrast, final NDVI was similar for the three genotypes.

Times taken to reach certain stages of senescence were highest in the wettest environment (GAT10) and lowest in the stressed environment (WAR12, Table 3) where maturity was also reached earlier. For example, the time taken for NDVI to fall to specific levels as estimated such as TFN90, TFN50 and TFN10 were greatest at GAT10 and least at WAR12. Similarly, mean N_{max} values and mean NDVI values reached at specific points during senescence (e.g. N90, N50 and N10) were also generally greater at GAT10 than at WAR11 and WAR12 (Table 3).

Broad-sense heritability for yield was higher in GAT10 than in the other two environments (Tables 2 and 3). Several traits exhibited greater heritability than yield in certain environments (Table 3). For example, SGint exhibited heritability of 77% and 61% at GAT10 and WAR11 respectively, versus 66% and 49% for yield. Similarly, TFN90 and TFN50 exhibited relatively high heritability, with values ranging between 58% and 85% (Table 3). In contrast, N_{max} had a high heritability of 72% at WAR12 but lower values in the other two environments. SR had heritability values of 78% and 67% at WAR11 and WAR12 but only 37% at GAT10. N90 and N50 heritabilities were moderate, ranging from 42% to 59%, whereas N10 exhibited low heritability at each environment, probability due to low genetic variation in final NDVI.

In contrast to stay-green traits, time to anthesis was not significantly correlated with yield at any of the trials ($P > 0.05$), despite significant variation between genotypes at all three trials ($P < 0.05$). Height was significantly negatively correlated with yield only at GAT10 despite also exhibiting significant variation between genotypes at each site ($P < 0.05$). The small s.e. for height and time to flowering at each site indicate that the selection for these traits has reduced the range of variation within sites as targeted (Table 2).

Table 3. Mean predicted values (best linear unbiased predictors; Pred.), s.e. and broad-sense heritability (h^2) for wheat yield, height and thermal time from sowing to anthesis as well as the stay-green traits estimated from fitted logistic curves for each field plot at Gatton (GAT10) and Warwick (WAR11 and WAR12) in southern Queensland

NA, the estimated genetic variance for these traits in these environments was close to zero; N_{max}, maximum leaf greenness; TFN90, onset of senescence; TFN50, midpoint of senescence; TFN10, conclusion of senescence; SR, senescence rate; N90, normalised difference vegetative index (NDVI) at the onset of senescence; N50, NVDI at the midpoint of senescence; N10, NDVI at the conclusion of senescence; SGint, stay-green integral

Trait	GAT10			WAR11			WAR12		
	Pred.	s.e.	h^2	Pred.	s.e.	h^2	Pred.	s.e.	h^2
Yield ($g\,m^{-2}$)	464	11	66	567	10	49	415	10	63
Height (cm)	104	0.5	77	90	1.5	93	74	1.9	86
Anthesis (°Cd)	1251	3.8	81	1126	9.3	77	1259	1.2	74
Nmax	0.87	0.002	38	0.76	0.006	35	0.72	0.01	72
TFN90	454	4.4	73	384	7.62	78	110	29.8	61
TFN50	696	6.8	85	555	9.79	72	316	23.9	58
TFN10	1097	38.0	NA	749	9.95	29	644	41.4	48
SR	5.28	0.123	37	7.01	0.27	78	5.67	0.69	67
N90	0.80	0.001	42	0.70	0.005	12	0.66	0.01	58
N50	0.51	0.001	59	0.452	0.004	NA	0.42	0.01	55
N10	0.23	0.003	39	0.20	0.002	24	0.19	0.01	34
SGint	524	9.1	77	343	5.06	61	382	20.0	50

Strong genetic correlations were observed between traits involved in early to mid-senescence

Several high correlations between traits were observed at all three sites, particularly for traits affecting early to mid-senescence (Table 4). For instance, there was a consistent and strong positive correlation between the timing of senescence onset (TFN90) and mid-senescence (TFN50), and, to a lesser degree, between NDVI values close to anthesis (N_{max}), at senescence onset (N90) and at mid-senescence (N50). Correlations among N_{max}, N90 and N50 probably arose as N90 and N50 were defined as set proportions of N_{green_max} (Fig. 1), whereas N_{final} varied little. However, correlations between early to mid-senescence NDVI values and their timing (i.e. N90 *vs.* TFN90 and N50 *vs.* TFN50) were weak in WAR11 and WAR12, but were strongly negative in the irrigated trial at GAT10. Well watered genotypes with high NDVI around anthesis in GAT10 tended to have a slower senescence rate, as indicated by the strong negative correlation between N_{max} and SR in this environment.

Correlations between traits influencing early to mid-senescence versus late senescence were less consistent. The timing of senescence onset (TFN90), although strongly positively correlated with mid-senescence (TFN50), generally exhibited weak correlation with the timing of concluding senescence (TFN10; Table 4). Limited genetic variation was observed in NDVI value towards the end of the crop cycle, partly explaining the weak correlations observed between N90 and N10, and between TFN90 and TFN10 in all environments. Both TFN90 and TFN50 exhibited correlations with N10 that varied from strongly negative at GAT10 to positive at WAR11 and WAR12, indicating a genotype by environment interaction. These results suggest that traits measured late in senescence are not necessarily dependent on those measured early and may respond differently to environmental variations.

SR was moderately to strongly positively correlated with TFN90 in all three environments (Table 4). These correlations are consistent with the fact that increases in the time to senescence onset, as estimated by TFN90, did not necessarily correspond with similar increases in the time to senescence concluding (TFN10), leading to increased SR. An example of this relationship is illustrated by the senescence patterns of the three genotypes represented in Fig. 1*b*.

Increases in SGint generally corresponded with increased TFN90 and TFN50 values. SGint correlations with TFN90 and TFN50 were strong at GAT10 and WAR11, but weaker in the stressed environment of WAR12, indicating variation in stay-green patterns among genotypes and environments.

Table 4. Correlations among stay-green traits in wheat at three environments in southern Queensland

GAT10, trial at Gatton in 2010; WAR11, trial at Warwick in 2011; WAR12, trial at Warwick in 2012; N_{max}, maximum leaf greenness; TFN90, onset of senescence; TFN50, midpoint of senescence; TFN10, conclusion of senescence; SR, senescence rate; N90, normalised difference vegetative index (NDVI) at the onset of senescence; N50, NDVI at the midpoint of senescence; N10, NDVI at the conclusion of senescence; SGint, stay-green integral; NA, not applicable, as the estimated genetic variance for these traits in these environments was close to zero

Trial	Trait	N_{max}	TFN90	TFN50	TFN10	SR	N90	N50	N10
GAT10	TFN90	–0.55	–	–	–	–	–	–	–
	TFN50	–0.23	0.94	–	–	–	–	–	–
	TFN10	NA	NA	NA	–	–	–	–	–
	SR	–0.85	0.45	0.17	NA	–	–	–	–
	N90	0.99	–0.64	–0.33	NA	–0.84	–	–	–
	N50	0.67	–0.99	–0.88	NA	–0.55	0.74	–	–
	N10	–0.01	–0.83	–0.97	NA	0.03	0.10	0.74	–
	SGint	0.01	0.83	0.97	NA	–0.03	–0.10	–0.74	–1.00
WAR11	TFN90	–0.15	–	–	–	–	–	–	–
	TFN50	–0.02	0.99	–	–	–	–	–	–
	TFN10	0.56	–0.20	–0.12	–	–	–	–	–
	SR	–0.24	0.92	0.89	–0.24	–	–	–	–
	N90	1.00	–0.13	0.00	0.56	–0.23	–	–	–
	N50	NA	NA	NA	NA	NA	NA	–	–
	N10	0.13	0.55	0.58	0.01	0.48	0.14	NA	–
	SGint	0.52	0.74	0.82	0.20	0.61	0.54	NA	0.55
WAR12	TFN90	0.02	–	–	–	–	–	–	–
	TFN50	0.14	0.95	–	–	–	–	–	–
	TFN10	0.30	–0.40	–0.10	–	–	–	–	–
	SR	–0.11	0.93	0.81	–0.55	–	–	–	–
	N90	1.00	0.04	0.16	0.28	–0.08	–	–	–
	N50	0.96	0.21	0.26	0.08	0.11	0.97	–	–
	N10	0.25	0.70	0.49	–0.73	0.75	0.28	0.49	–
	SGint	0.78	0.44	0.65	0.43	0.23	0.78	0.74	0.17

Colour legend	–1.00 to –0.75	–0.74 to –0.50	–0.49 to –0.25	–0.24 to 0.00	0.00 to 0.24	0.25 to 0.49	0.50 to 0.75	0.75 to 1.00

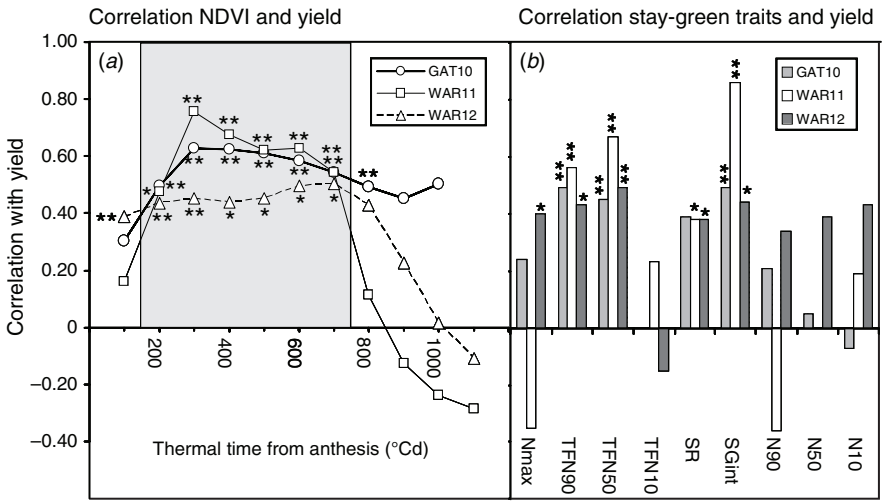

Fig. 2. Genetic correlations between wheat yield and (*a*) normalised difference vegetative index (NDVI) values after anthesis estimated at intervals of 100 degree-days (°Cd) from the fitted logistic function (Eqn 1) and (*b*) stay-green traits. Data are for three trials in southern Queensland (Table 1). Probability of correlations are indicated at less than 0.05 (*) and 0.01 (**) levels. The grey-shaded box in (*a*) represents the period when correlations between yield and NDVI were significant in all three environments ($P \leq 0.05$). The absence of a bar in (*b*) indicates that estimated genetic variance was close to zero for the trait.

Traits expressed during early to mid-senescence correlated with yield

Many stay-green traits were significantly positively correlated with yield (Fig. 2). Traits influencing early to mid-senescence were particularly well correlated with yield, with, for instance, significant positive correlations observed for TFN90 and TFN50 in all three environments (Fig. 2*b*). N_{max} was also significantly correlated with yield but only in the stressed environment of WAR12 ($P \leq 0.05$). By contrast, traits related to late senescence (TFN10, N10) were not significantly correlated with yield in any trial.

In contrast to TFN90 and TFN50, which indicate the timing of certain stages of senescence, NDVI values at those stages (N90 and N50, respectively) were not significantly correlated with yield (Fig. 2*b*, $P \leq 0.05$). N10 was also not significantly correlated with yield in any environment. These results are partly explained by the fact that N90, N50 and N10 are defined as fractions of N_{green_max} (Fig. 1), which is the difference between (i) N_{max}, which was not significantly correlated with yield in GAT10 and WAR11, and (ii) N_{final}, which varied little.

Traits related to the more general senescence pattern varied in their correlation with yield. SR was significantly positively correlated with yield in the environments at WAR11 and WAR12 but not at GAT10 ($P \leq 0.05$). However, SGint, which tends to integrate the senescence pattern overall, was one of the traits most consistently correlated with yield in all three environments (Fig. 2*b*).

NDVI during the rapid phase of senescence correlated significantly with yield

To determine when the correlation between NDVI and yield is strongest, NDVI was estimated from the fitted logistic function for individual genotypes every 100°Cd from anthesis onwards (Fig. 2*a*). The strongest correlations were 0.76, observed at 300°Cd after anthesis for WAR11, 0.63 at 300°Cd for GAT10 and 0.50 at 700°Cd for WAR12 (Fig. 2). The correlation between yield and the extrapolated NDVI values was positive during most of the period after anthesis. The correlation was significant at all three environments from 200°Cd to 700°Cd after anthesis (i.e. during the period of rapid senescence common to all trials; $P \leq 0.05$, Fig. 3).

A single time-point measurement can indicate stay-green phenotype

As resources may limit the frequency of field observations, we wished to establish whether significant correlations with yield could be detected with a limited number of measurements and, if so, at which dates. To examine this possibility, the correlations between yield and NDVI measurements were calculated for NDVI values expressed relative to the anthesis date of the standard genotype Hartog (Fig. 4).

Significant positive correlations between yield and NDVI measurements were observed for different dates of measurements in each environment (Fig. 4). As for the NDVI values estimated with the logistic function, genetic correlations were strongest for times when crops were rapidly senescing. In contrast, for dates before the onset of rapid senescence, or when senescence was near completion, NDVI was often not significantly correlated with yield (Figs 4 and 5). The timing of the rapid senescence period relative to the anthesis date of Hartog varied with environment (Fig. 5) but, in these experiments, included a common period from 16 to 33 days after Hartog anthesis (Figs 4 and 5). This suggests that repeated measurements of NDVI and the fitting of the logistic function may not always be required to find significant correlations with yield if measured during the period of

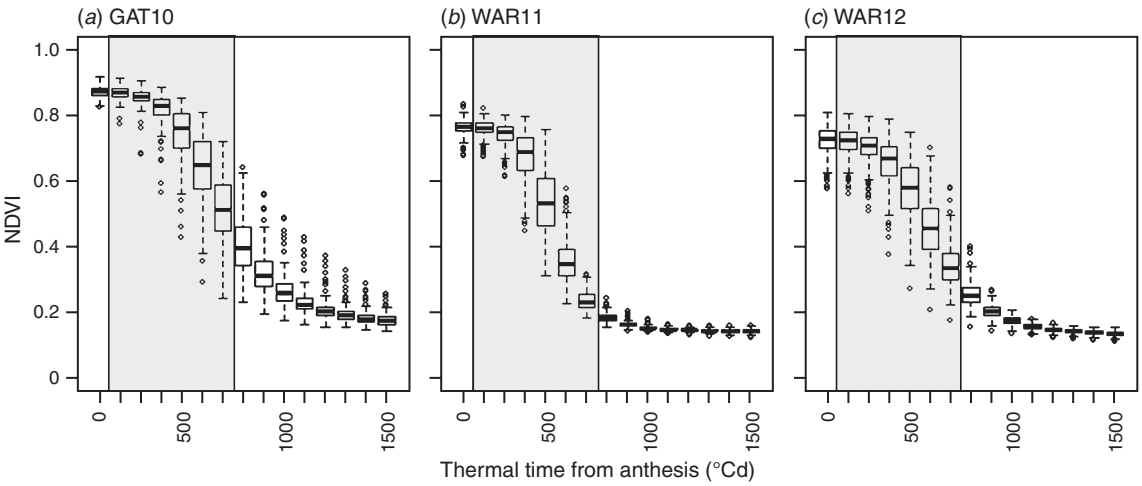

Fig. 3. Box plots of normalised difference vegetative index (NDVI) values in wheat estimated from the fitted logistic function (Eqn 1) at intervals of 100 degree-days (°Cd) after anthesis for three trials in southern Queensland at (*a*) Gatton in 2010 (GAT10), (*b*) Warwick in 2011 (WAR11) and (*c*) Warwick in 2012 (WAR12). The grey-shaded boxes represent the period when yield was significantly ($P \leq 0.05$) correlated with NDVI at all three trials, ~200−700°Cd after anthesis, as indicated in Fig. 2*a*. For the boxplot, the line in the middle of the boxes represents the median value for the data, the upper edge of the boxes represents the 75th percentile, and the lower edge represents the 25th percentile. The whiskers correspond to 1.5 times the interquartile range (IQR, the difference between the 75th and 25th percentiles) or to the most extreme observed value, whichever is smallest. Dots above or below the whiskers represent individual values outside this range.

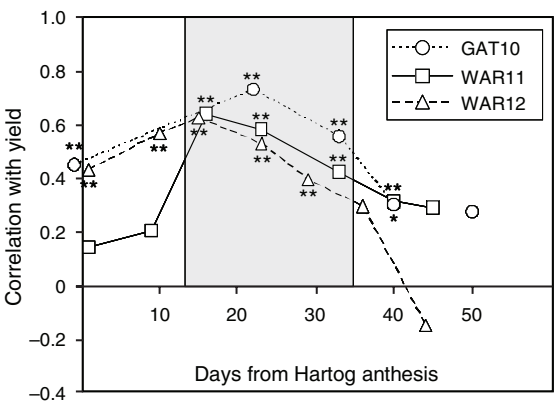

Fig. 4. Genetic correlations between wheat yield and normalised difference vegetative index (NDVI) measurements for three environments in southern Queensland. Dates of observation are expressed in days relative to Hartog anthesis. The probability of correlations are indicated at less than 0.05 (*) and 0.01 (**) levels. The grey-shaded box represents the period when correlations between yield and measured NDVI were significant ($P \leq 0.05$) at all trials, ~15–33 days after the anthesis of Hartog.

rapid senescence. However, it must be cautioned that these are results from only three environments and come from a population of lines selected to have similar flowering dates (Christopher *et al*. 2013; Table 1).

Genotype ranking for NDVI can vary over the cycle

In each of the three environments, positive correlations were observed between NDVI measurements (best linear unbiased predictors) taken on different dates from near anthesis onwards (Fig. 5; Table 5), namely from –1 to 40 (days after Hartog anthesis) DHA at GAT10, from –4 to 40 DHA at WAR11 and from –12 to 36 DHA at WAR12. These correlations were moderate to high at GAT10 (ranging from 0.54 to 0.94) and WAR12 (0.41–0.98) but some were lower at WAR11 (0.26–0.96).

Correlations between NDVI measured at different dates within the common rapid senescence phase, where NDVI was significantly correlated with yield, were consistently strong and positive in all three environments (grey shaded boxes in Fig. 5 and bold italicised values in Table 5). During this period, the correlations were 0.89 at GAT10, 0.60–0.84 at WAR11 and 0.86–0.98 at WAR12 (Table 5). These observations indicate that NDVI measurements at a single date during the common rapid senescence phase are likely to capture genotypic variation. However, lower genetic correlations were observed when comparing values from one date with those from earlier or later dates, suggesting that changes in ranking (crossovers) might occur during the crop cycle.

Genotype rankings for NDVI value can change during the suggested rapid senescence sampling period (Figs 6 and 7). For instance, genotypes like SHF1–2 have a relatively high NDVI at anthesis and during early grain filling, but senesce rapidly and have relatively low NDVI later (Fig. 6). In contrast, a genotype like HSF1–294 had a low relative NDVI at early stages but a slow senescence rate that allowed it to retain green leaves longer. Thus the ranking between these genotypes changes from early to late grain filling.

To examine how frequently NDVI measurements taken on different dates during the rapid senescence phase provide inconsistent genotype rankings, genotype ranks for NDVI at the start and the end of the common rapid senescence period were compared (Fig. 7). At GAT10, NDVI rankings at the two

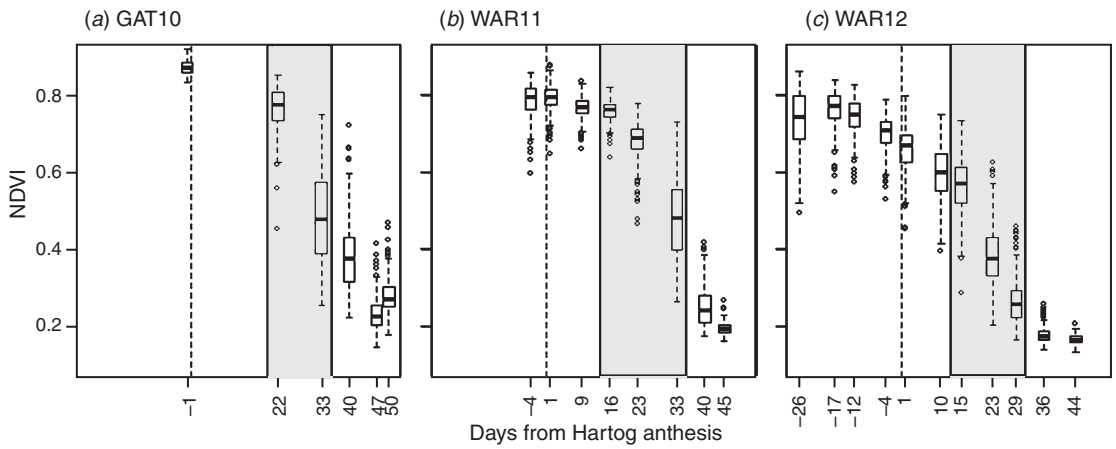

Fig. 5. Box plots of normalised difference vegetative index (NDVI) (best linear unbiased predictors) measured on particular dates for three trials of wheat in southern Queensland at (*a*) Gatton in 2010 (GAT10), (*b*) Warwick in 2011 (WAR11) and (*c*) Warwick in 2012 (WAR12). Dates are expressed relative to the anthesis of the standard genotype Hartog. The grey-shaded boxes represent the common period of significant correlation ($P \leq 0.05$) between NDVI and yield corresponding to that in Fig. 4. Dashed lines indicate the anthesis date of Hartog. For the boxplot, the line in the middle of the boxes represents the median value for the data, while the upper and lower edges of the boxes represent the 75th and 25th percentiles, respectively. The whiskers correspond to 1.5 times the interquartile range (IQR, the difference between the 75th and 25th percentiles) or to the most extreme observed value, whichever is smallest. Dots above or below the whiskers represent individual values outside this range.

Table 5. Genetic correlations between normalised difference vegetative index (NDVI) measurements taken on various dates and broad-sense –heritability at those dates (h^2) for wheat grown in three environments in southern Queensland

Correlations were calculated between dates relative to the date of Hartog anthesis (DHA). Dates during the period when correlations between NDVI and yield were significant ($P \leq 0.05$) in all environments (Fig. 5) are highlighted grey with bold text and values for correlations between NDVI values taken on these dates are given in bold italics

GAT10	DHA				−1				**22**	33	40	h^2
	−1											74
	22				0.54							85
	33				0.69				***0.89***			89
	40				0.68				0.63	0.88		79
	50				0.70				0.56	0.87	0.94	35
WAR11	DHA				−4	1	8	16	23	33	40	
	−4											32
	1				0.77							50
	8				0.35	0.29						45
	16				0.69	0.58	0.26					47
	23				0.80	0.68	0.30	***0.60***				67
	33				0.96	0.80	0.36	***0.72***	***0.84***			80
	40				0.77	0.64	0.29	0.57	0.67	0.80		86
WAR12	DHA	−26	−17	−12	−4	1	10	**15**	**23**	29	36	
	−26											46
	−17	0.93										67
	−12	0.57	0.83									61
	−4	0.64	0.86	0.95								64
	1	0.31	0.65	0.93	0.89							69
	10	0.20	0.51	0.83	0.80	0.88						53
	15	−0.02	0.27	0.64	0.61	0.74	0.89					57
	23	−0.32	−0.01	0.46	0.41	0.63	0.86	***0.86***				58
	29	−0.16	0.13	0.54	0.51	0.66	0.91	***0.88***	***0.98***			54
	36	0.13	0.41	0.70	0.66	0.77	0.78	0.69	0.65	0.67		51
	44	0.55	0.55	0.37	0.37	0.29	−0.05	−0.18	−0.4	−0.4	0.05	45

Colour legend	−1.00 to −0.75	−0.74 to −0.50	−0.49 to −0.25	−0.24 to 0.00	0.00 to 0.24	0.25 to 0.49	0.50 to 0.75	0.75 to 1.00

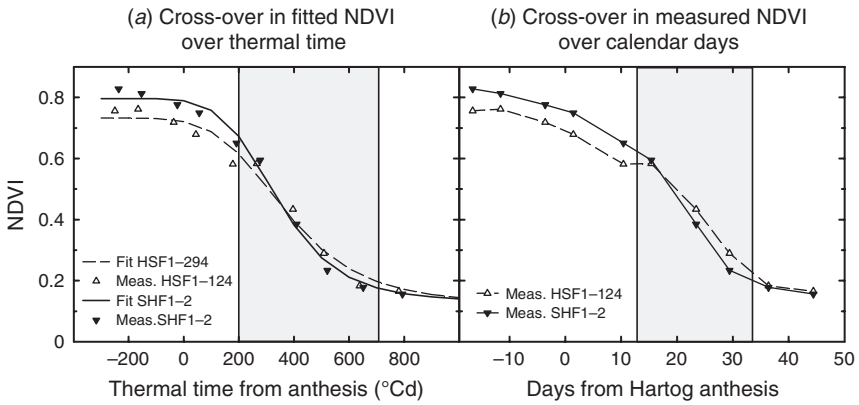

Fig. 6. Change in canopy greenness over time presented for two contrasting wheat genotypes: SHF1–2 and HSF1–294 at Warwick in 2012 (WAR12) for (*a*) periods of 100 degree-days (°Cd) after the anthesis of each genotype and (*b*) days from Hartog anthesis. The common window for rapid senescence in all environments, as illustrated in Figs 3 and 5, is represented by grey-shaded boxes. Symbols represent measured normalised difference vegetative index (NDVI) values ('Meas.'), whereas the lines in (*a*) represent the fitted logistic from Eqn 1 ('Fit').

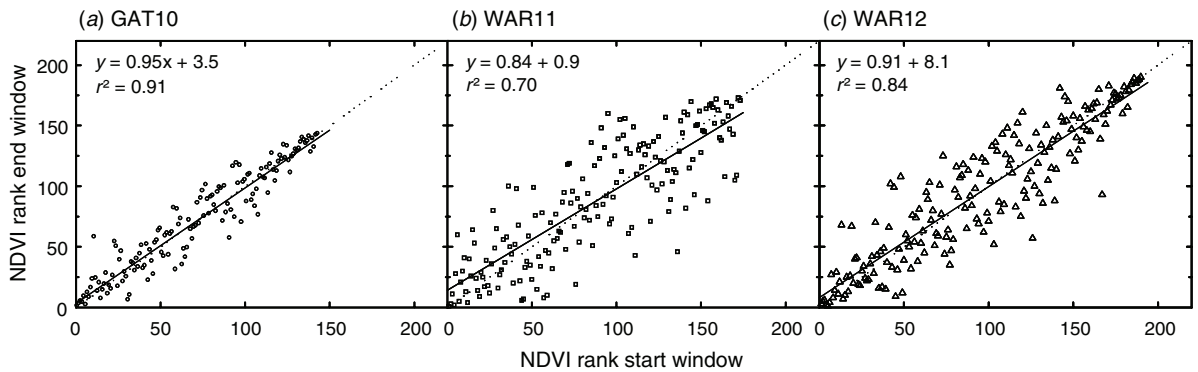

Fig. 7. Comparison of wheat genotype ranking for normalised difference vegetative index (NDVI) measured at the start and end of the common rapid senescence phase when NDVI was significantly correlated with yield at all three environments (as represented by the grey-shaded boxes in Fig. 5) for (*a*) Gatton in 2010 (GAT10), (*b*) Warwick in 2011 (WAR11) and (*c*) Warwick in 2012 (WAR12). Thus, for (*a*) GAT10, NDVI rankings for 22 days from Hartog anthesis (DHA) are plotted against those for 33 DHA; for (*b*) WAR11, NDVI rankings for 16 DHA are plotted against 33 DHA and for (*c*) WAR12, NDVI rankings for 15 DHA are plotted against 29 DHA. The linear regression fitted to the actual distribution is indicated by the solid line, whereas the dotted lines represent the 1 : 1 line.

measurement dates within this period were generally well correlated, exhibiting a regression line with a slope close to 1 at 0.95 and an r^2 value of 0.91 (Fig. 7*a*). However, a small number of genotypes diverged widely from the 1 : 1 line, indicating a large change in rank between the two dates (Fig. 7). At WAR11 and WAR12, the degree of correlation between first and last measurement dates was less consistent than at GAT10. At Warwick, a greater number of genotypes changed ranking for NDVI so that a lower r^2 of 0.70 and 0.84 was observed at WAR11 and WAR12, respectively. The estimated slopes for the regression lines at these trials also deviated slightly further from the expected 1 : 1 ratio than they did at GAT10, with slopes of 0.84 and 0.91 for WAR11 and WAR12, respectively. The changes in ranking indicate that selection of genotypes with high NDVI based on measurements from a single date could lead to quite different outcomes depending on which date is used.

Genetic relationships between stay-green indicators and yield varied across environments

To further examine the genetic relationships between (i) stay-green traits, (ii) NDVI measurements during the common period of rapid senescence and (iii) yield, a principal components analysis was conducted for each trial (Fig. 8). In all environments, SGint was consistently and closely related to yield, as the vector for this trait has a similar direction and a similar magnitude to the yield vector (Fig. 8). TFN90 and TFN50 were also positively related to yield at all sites but not as closely as SGint. NDVI measurements taken during the common rapid senescence window were also related to yield in all three environments but most closely at GAT10 and WAR12. In contrast, TFN10 exhibited little relationship, as the vector for TFN10 is close to perpendicular to that for yield. Finally, SR and N_{max} appeared to have little relationship with

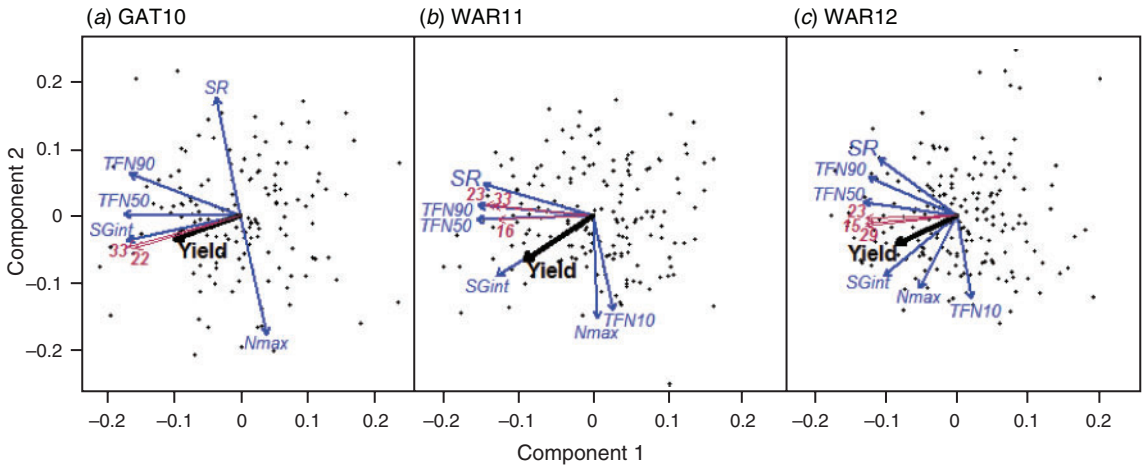

Fig. 8. Principal component analysis performed for wheat grown at (*a*) Gatton in 2010 (GAT10), (*b*) Warwick in 2011 (WAR11) and (*c*) Warwick in 2012 (WAR12). The two principal components plotted here explained 85%, 77% and 76% of the variation in the data at GAT10, WAR11 and WAR12, respectively. Points represent individual genotypes while the direction and magnitude of the vectors represent the effects, in relation to the first two principal components, of yield (black vector), stay-green traits (maximum NDVI (N_{max}), onset of senescence (TFN90), midpoint of senescence (TFN50), conclusion of senescence (TFN10), senescence rate (SR) and stay-green integral (SGint); blue vectors) and stay-green measurements during the common period of rapid senescence (NDVI measured at 22 and 33 days from Hartog anthesis (DHA) in GAT10; 16, 23 and 33 DHA in WAR11; and 15, 23 and 29 DHA in WAR12; mauve vectors).

yield at GAT10, whereas they had some degree of relationship with yield at WAR11 and WAR12 (Fig. 8*a*). Overall, the trait that related most closely to yield was SGint.

Discussion

We aimed to propose a method to phenotype stay-green dynamics and to determine whether taking repeated periodic NDVI measurements of a cereal crop from before anthesis to maturity offers an advantage when studying genetic variability in the expression of the stay-green phenotype. We also wished to investigate a cheaper alternative method by determining whether measurements taken at a single or at a few dates could be used to select for stay-green in breeding programs.

A new method to monitor and analyse the dynamics of canopy greenness with improved detection and interpretation of genotypic variation in stay-green

The method developed in this study used NDVI to monitor canopy greenness over time to infer multiple traits related to senescence dynamics. Using an NTech Greenseeker allowed rapid plot measurements and could be applied to an entire population. This approach provided a more detailed understanding of genotypic variation in stay-green traits than previous methods and characterised the whole senescence pattern for each genotype.

Significant variation between genotypes was observed for all stay-green traits in at least some environments. Differences in canopy greenness during early grain filling appeared to be important drivers in the dynamics of senescence and also in yield. For example, the time from anthesis to the onset of senescence (TFN90) and to the midpoint of senescence (TFN50) were significantly correlated with yield, whereas the time from anthesis to senescence concluding (TFN10) was poorly correlated with yield. This suggests that the 'journey' to complete senescence is more important than the 'destination', which agrees with previous studies where delayed onset or a reduced rate of senescence were proposed as major contributors to the stay-green phenotype (Thomas and Smart 1993).

SGint, an indicator of green-leaf retention over the whole period after anthesis, was strongly correlated with yield in all environments (Fig. 2). This integrated trait was also closely aligned with yield in terms of genetic variation (Fig. 8) and thus appears to be a valuable trait for studying genetic variation in stay-green.

The stay-green traits examined in this study were chosen to be biologically meaningful but were also at least partly redundant (not all independent). Correlations between traits (Table 4) confirmed, for instance, that the timing to reach mid-senescence (TFN50) was highly correlated with the timing of senescence onset (TFN90) and with SR. However, limited correlations were found between the onset (TFN90) and the conclusion of senescence (TFN10). This led at least partially to variation in SR.

Several stay-green traits defined in previous studies have analogues among the traits examined here. For example, the average rate of senescence calculated by Lopes and Reynolds (2012) with a linear regression may be considered an analogue of SR calculated from a logistic function here. In both studies, the rate of senescence was significantly correlated with yield. However, we found other parameters with stronger correlations, such as TFN90, TFN50 and SGint (Fig. 2). Lopes and Reynolds (2012) also found that NDVI near maturity significantly correlated with yield, which was also the case in some instances in our trials (e.g. 29 DHA WAR12; Figs 4 and 5). However, we found greater correlations with yield when

measuring NDVI during the period of rapid senescence than near maturity.

A different nonlinear model has been applied in the past to visual leaf senescence scores for single-culmed wheat plants grown in a growth chamber (Vijayalakshmi *et al*. 2010). However, the study did not capture the initial leaf greenness, as the score was arbitrarily set to an initial value of 10. Nor did the fitted exponential function capture the slow-down in senescence at the end of the grain filling period. Additionally, the use of single culms in growth chambers did not allow direct, meaningful crop yield comparisons. However, these authors' 'time from anthesis to 50% loss of leaf greenness' and 'maximum rate of senescence' are analogous to our TFN50 and SR, respectively, and were significantly affected by heat stress. In this study, we demonstrated that TFN50 and SR are also useful for assessing field-grown plants and can correlate with yield (Fig. 2).

Stay-green traits varied in their response to the environment

A stay-green phenotype can lead to either positive or negative effects on yield. Positive effects have been reported in crops like sorghum, maize (*Zea mays* L.) and wheat, especially in water-limited environments (reviewed in Jordan *et al*. 2012; Gregersen *et al*. 2013). For example, in sorghum under drought conditions after anthesis, stay-green has been shown to be associated with increased stem mass (Borrell *et al*. 2000) and lodging resistance (Henzell *et al*. 1984; Woodfin *et al*. 1988; Rosenow and Clark 1995). However, under specific conditions and for certain types of stay-green, the stay-green phenotype can have deleterious consequences. For example, stay-green can be linked to problems of lodging and inefficient conversion of biomass to grain in certain well watered conditions (reviewed in Yang and Zhang 2006).

In the studied wheat population, the correlation between certain stay-green traits and yield varied between environments. TFN90, TFN50 and SGint were strongly positively correlated with yield in all three environments and had similar or higher broad-sense heritability to yield. In contrast, SR was significantly correlated with yield in only two environments (WAR11 and WAR12), whereas N_{max} correlated significantly with yield only at the stressed environment at WAR12. Reflectance-based measurements of NDVI, such as those used by the NTech Greenseeker often become saturated at a high leaf area index near anthesis. This may have reduced variation for N_{max}, especially in GAT10 and WAR11, and have weakened the correlation between N_{max} and yield. In the environments where correlations with yield were high, both SR and N_{max} also exhibited relatively high broad-sense heritability.

The genotype by environment interactions observed for stay-green traits suggest that certain traits such as TFN90, TFN50 and SGint may be useful to select for broad adaptation, whereas others, such as SR and N_{max}, may select for more specific adaptation. However, the restricted number of environments studied here does not allow decisive conclusions about environment-specific trait expression. Further research is warranted to confirm these results for a wider range of environments and to identify traits of interest for specific environmental types (e.g. Chenu *et al.* 2013; Chenu 2014).

Genotypic variation in stay-green expression likely arises from differences in the underlying mechanisms

Although the mechanisms and genetic controls underlying the identified traits were not examined here, it is important to understand that stay-green phenotypes can be emergent properties, resulting from the effects of other physiological processes. In water-limited conditions, for example, sorghum stay-green has been shown to arise when plants have access to more water during the grain filling period (Borrell *et al*. 2006; Borrell *et al*. 2014*a*, 2014*b*). Stay-green can arise from an increase in accessible water (e.g. more deep roots), a reduction in plant transpiration (e.g. smaller canopy area) or an increase in the water-use efficiency (e.g. greater transpiration efficiency). Differences in nitrogen dynamics can also underpin the stay-green phenotype, as shown in sorghum (Borrell and Hammer 2000; van Oosterom *et al*. 2010*a*, 2010*b*) and, in turn, variation in nitrogen dynamics is likely underpinned by differences in canopy development, root architecture and water uptake. Hence, the differences we observed in the dynamics of senescence are likely to point back to differences in the underlying physiological mechanisms.

The identified traits provide an objective framework for characterising variation in stay-green expression

Regular NDVI monitoring, expressed relative to anthesis and analysed using a logistic function, makes use of data from the entire senescence sequence to provide information on specific, definable stay-green traits. This allows objective comparison of specific aspects of senescence between genotypes and potentially reduces the confounding effects of variations in other traits such as phenology.

The traits identified in this study provide a framework for discussing specific aspects of the dynamics of senescence. For example, N_{max}, N90 and TFN90 are specific to the early stages of senescence; SR, N50 and TFN50 characterise the middle phase; whereas N10 and TFN10 are related to the concluding phase. It is likely that specific underlying mechanisms of stay-green will affect the various stages of senescence in different ways. Detailed information on the expression of stay-grain traits could therefore aid understanding of underlying mechanisms and their effects.

Single NDVI measurements can identify high-yielding stay-green phenotypes in a population with similar phenology

Our results suggest that a single measurement taken during the period of most rapid senescence is sufficient to select for stay-green genotypes with superior yield for a population where genotypes have similar flowering dates. However, this may not be the case in breeding trials where height and phenology are generally highly variable. Even in our population selected for flowering time and plant height, genotype rankings for NDVI differ markedly depending on the stage of senescence when measurements were conducted (Figs 6 and 7). In our study, correlations with yield were greatest during the period of

rapid senescence common to all experiments, ~200°Cd to 700°Cd after anthesis. However, a stress (e.g. drought) can significantly influence the timing of this period. The period of rapid senescence varies greatly with environment as well as with genotype, which can make the exact period of rapid senescence harder to predict.

Thus, genotype selection, experimental design and analysis methods need to be carefully considered to allow confident interpretation of data from NDVI measurements taken at one or a few time points. In contrast, regular monitoring of NDVI allows reliable capture of the period of rapid senescence for all genotypes and environments.

Conclusion

Monitoring canopy greenness using NDVI measurements has great potential to facilitate the selection of high yielding stay-green phenotypes in large populations in the field. Regular monitoring followed by analysis using a logistic model provides improved understanding of the variation in senescence patterns between individual genotypes both within environments and across environments. However, where resources do not allow for frequent periodic measurements, selections may be made using experiments carefully designed to account for variation in phenology, with NDVI measurements at a single or a few targeted dates during the phase of rapid senescence.

The most consistent genetic relationship with yield was found for SGint, an integrated indicator of stay-green, over the whole period after anthesis. This trait and the timing of onset (TFN90) and mid-senescence (TFN50) were strongly correlated with yield in all tested environments and often had heritability similar to or higher than that of yield, making them potentially useful to detect high-yielding stay-green phenotypes in a wide range of environments. Other traits, such as N_{max} and SR, appeared more correlated with yield in a subset of environments. Further research is warranted to confirm whether selection for certain stay-green traits could assist in breeding for greater productivity in specific environments, as well as to investigate the underlying physiological mechanisms and genetic controls of stay-green.

Acknowledgements

The authors acknowledge the financial support of the Grains Research and Development Corporation, the University of Queensland and the Queensland Department of Agriculture, Fisheries and Forestry.

References

Borrell AK, Hammer GL (2000) Nitrogen dynamics and the physiological basis of stay-green in sorghum. *Crop Science* **40**, 1295–1307. doi:10.2135/cropsci2000.4051295x

Borrell AK, Hammer GL, Henzell G (2000) Does maintaining green leaf area in sorghum improve yield under drought? 2. Dry matter production and yield. *Crop Science* **40**, 1037–1048. doi:10.2135/cropsci2000.4041037x

Borrell AK, Jordan DJ, Mullet J, Henzell RG, Hammer G (2006) Chapter 10: Drought adaptation in sorghum. In 'Drought adaptation in cereals'. (Ed. J-M Ribaut) pp. 335–399. (Haworth Press Inc.: New York)

Borrell AK, van Oosterom EJ, Mullet JE, George-Jaeggli B, Jordan DR, Klein PE, Hammer GL (2014*a*) Stay-green alleles enhance grain yield in sorghum under drought by modifying canopy development and enhancing water uptake. *New Phytologist*, in press. doi:10.1111/nph.12869

Borrell AK, Mullet JE, George-Jaeggli B, van Oosterom EJ, Hammer GL, Klein PE, Jordan DR (2014*b*) Drought adaptation of stay-green cereals associated with canopy development, leaf anatomy, root growth and water uptake. *Journal of Experimental Botany,* in press. sdoi:10.1093/jxb/eru232

Butler DG, Eccleston JA, Cullis BR (2008) On an approximate optimality criterion for the design of field experiments under spatial dependence. *Australian and New Zealand Journal of Statistics* **50**, 295–307. doi:10.1111/j.1467-842X.2008.00518.x

Butler DG, Cullis BR, Gilmour AR, Gogel BJ (2009) 'ASReml-R reference manual, release 3.' Technical report. (Queensland Department of Primary Industries: Brisbane, Australia)

Chenu K (2014) Characterising the crop environment – nature, significance and applications. In 'Crop physiology. Applications for genetic improvement and agronomy'. 2nd edn. (Eds VO Sadras, DF Calderini) In press. (Academic Press)

Chenu K, Deihimfard R, Chapman SC (2013) Large-scale characterization of drought pattern: a continent-wide modelling approach applied to the Australian wheatbelt spatial and temporal trends. *New Phytologist* **198**, 801–820. doi:10.1111/nph.12192

Christopher JT, Manschadi AM, Hammer GL, Borrell AK (2008) Developmental and physiological traits associated with high yield and stay-green phenotype in wheat. *Australian Journal of Agricultural Research* **59**, 354–364. doi:10.1071/AR07193

Christopher JT, Christopher MJ, Jennings R, Jones S, Fletcher S, Borrell AK, Manschadi AM, Jordan D, Mace E, Hammer GL (2013) QTL for root angle and number in a population developed from bread wheats (*Triticum aestivum*) with contrasting adaptation to water-limited environments. *Theoretical and Applied Genetics* **126**, 1563–1574. doi:10.1007/s00122-013-2074-0

Cullis BR, Smith AB, Coombes NE (2006) On the design of early generation variety trials with correlated data. *Journal of Agricultural Biological & Environmental Statistics* **11**, 381–393. doi:10.1198/108571106X154443

Foulkes MJ, Sylvester-Bradley R, Weightman R, Snape J (2007) Identifying physiological traits associated with improved drought resistance in winter wheat. *Field Crops Research* **103**, 11–24. doi:10.1016/j.fcr.2007.04.007

Gilmour AR, Cullis BR, Verbyla AP (1997) Accounting for natural and extraneous variation in the analysis of field experiments. *Journal of Agricultural Biological & Environmental Statistics* **2**, 269–293. doi:10.2307/1400446

Gilmour AR, Cullis BR, Welham SJ, Thompson R (1999) 'ASRemL reference manual.' Biometrics Bulletin 3 (NSW Agriculture: Orange, NSW)

Gregersen PL, Culetic A, Boschian L, Krupinska K (2013) Plant senescence and crop productivity. *Plant Molecular Biology* **82**, 603–622. doi:10.1007/s11103-013-0013-8

Harris K, Subudhi P, Borrell A, Jordan D, Rosenow D, Nguyen H, Klein P, Muller J (2007) Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. *Journal of Experimental Botany* **58**, 327–338. doi:10.1093/jxb/erl225

Haussmann BIG, Obilana AB, Ayiecho PO, Blum A, Schipprack W, Geiger HH (1999) Quantitative genetic parameters of sorghum (*Sorghum bicolor* (L.) Moench) grown in semi-arid Kenya. *Euphytica* **105**, 109–118. doi:10.1023/A:1003469528461

Henzell RG, Dodman RL, Done AA, Brengman RL, Mayers PE (1984). Lodging, stalk rot, and root rot in sorghum in Australia. In 'Sorghum root and stalk diseases, a critical review. Proceedings of the consultative group discussion of research needs and strategies for control of sorghum root and stalk diseases; Bellagio, Italy; 27 November–2 December 1983'. (Ed LK Mughogho) pp. 225–235. (ICRISAT: Patancheru)

Jordan DR, Hunt CH, Cruickshank AW, Borrell AK, Henzell RG (2012) The value of the stay-green trait in sorghum. *Crop Science* **52**, 1153–1161. doi:10.2135/cropsci2011.06.0326

Joshi AK, Kumari M, Singh VP, Reddy CM, Kumar S, Rane J, Chand R (2007) Stay green trait: variation, inheritance and its association with spot blotch resistance in spring wheat (*Triticum aestivum* L.). *Euphytica* **153**, 59–71. doi:10.1007/s10681-006-9235-z

Kipp S, Mistele B, Schmidhalter U (2014) Identification of stay-green and early senescence phenotypes in high-yielding winter wheat, and their relationship to grain yield and grain protein concentration using high-throughput phenotyping techniques. *Functional Plant Biology* **41**, 227–235. doi:10.1071/FP13221

Lopes MS, Reynolds MP (2012) Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology. *Journal of Experimental Botany* **63**, 3789–3798. doi:10.1093/jxb/ers071

Manschadi AM, Christopher JT, deVoil P, Hammer GL (2006) The role of root architectural traits in adaptation of wheat to water-limited environments. *Functional Plant Biology* **33**, 823–837. doi:10.1071/FP06055

Manschadi AM, Christopher JT, Hammer GL, deVoil P (2010) Experimental and modelling studies of drought-adaptive root architectural traits in wheat (*Triticum aestivum* L.). *Plant Biosystems* **144**(2), 458–462. doi:10.1080/11263501003731805

Olivares-Villegas JJ, Reynolds MP, McDonald GK (2007) Drought-adaptive attributes in the Seri/Babax hexaploid wheat populations. *Functional Plant Biology* **34**, 189–203. doi:10.1071/FP06148

Patterson HD, Thompson R (1971) Recovery of interblock information when block sizes are unequal. *Biometrika* **31**, 100–109.

Potgieter AB, Hammer GL, Butler D (2002) Spatial and temporal patterns in Australian wheat yield and their relationship with ENSO. *Australian Journal of Agricultural Research* **53**, 77–89. doi:10.1071/AR01002

Potgieter A, Meinke H, Doherty A (2004) Likely impact of climate change on wheat and sorghum in production in central Queensland. In 'New directions for a diverse planet: Proceedings for the 4th International Crop Science Congress, Brisbane, Australia, 26 September–1 October 2004.' (Eds RA Fischer *et al*.)

R Development Core Team (2013) 'R: a language and environment for statistical computing.' (R Foundation for Statistical Computing: Vienna) Available online at: http: //www.r-project.org/[Verified 8 July 2014].

Rosenow DT, Clark LE (1995). Drought and lodging resistance for a quality sorghum crop. In 'Proceedings of the 50th Annual Corn and Sorghum Industry Research Conference,' 6–7 December 1995, Chicago. pp. 82–97. (American Seed Trade Association: Washington, DC)

Sivapalan S, O'Brien L, Ortiz-Ferrara G, Hollamby GJ, Barclay I, Martin PJ (2000) An adaptation analysis of Australian and CIMMYT/ICARDA wheat germplasm in Australian production environments. *Australian Journal of Agricultural Research* **51**, 903–915. doi:10.1071/AR99188

Sivapalan S, O'Brien L, Ortiz-Ferrara G, Hollamby GJ, Barclay I, Martin PJ (2001) Yield performance and adaptation of some Australian and CIMMYT/ICARDA developed wheat genotypes in the West Asia North Africa (WANA) region. *Australian Journal of Agricultural Research* **52**, 661–670. doi:10.1071/AR00115

Smith AB, Cullis BR, Thompson R (2001) Analyzing variety by environment data using multiplicative mixed models and adjustments for spatial field trends. *Biometrics* **57**, 1138–1147. doi:10.1111/j.0006-341X.2001.01138.x

Thomas H, Smart CM (1993) Crops that stay green. *Annals of Applied Biology* **123**, 193–219. doi:10.1111/j.1744-7348.1993.tb04086.x

van Oosterom EJ, Borrell AK, Chapman SC, Broad IJ, Hammer GL (2010*a*) Functional dynamics of the nitrogen balance of sorghum I. N demand of vegetative plant parts. *Field Crops Research* **115**, 19–28. doi:10.1016/j.fcr.2009.09.018

van Oosterom EJ, Chapman SC, Borrell AK, Broad IJ, Hammer GL (2010*b*) Functional dynamics of the nitrogen balance of sorghum II. Grain filling period. *Field Crops Research* **115**, 29–38. doi:10.1016/j.fcr.2009.09.019

Vijayalakshmi K, Fritz AK, Paulsen GM, Bai GH, Pandravada S, Gill BS (2010) Modelling and mapping QTL for senescence-related traits in winter wheat under high temperature. *Molecular Breeding* **26**, 163–175. doi:10.1007/s11032-009-9366-8

Woodfin CA, Rosenow DT, Clark LE 1988. Association between the stay-green trait and lodging resistance in sorghum. In 'Agronomy abstracts.' p. 102. (American Society of Agronomy: Madison)

Yang J, Zhang J (2006) Grain filling of cereals under soil drying. *New Phytologist* **169**, 223–236. doi:10.1111/j.1469-8137.2005.01597.x

Zadoks JC, Changi TT, Konzak CF (1974) A decimal code for the growth stages of cereals. *Weed Research* **14**, 415–421. doi:10.1111/j.1365-3180.1974.tb01084.x