

A method for the identification and evaluation of stay-green wheat variety

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Leaf senescence is synchronized with grain-filling in wheat. Delayed leaf senescence, or stay-green, can improve wheat yield. In this study, a method has been proposed to identify and evaluate the stay-green wheat. First, chlorophyll content of flag leaf was measured by SPAD-502 meter at three-day intervals from seven days after anthesis to physiological maturity. Meanwhile, green leaf area duration was visually recorded using 0–9 scale. Secondly, a mathematical model was simulated according to the above phenotypic dates. Thirdly, the senescence characteristic parameters were calculated, including the time from anthesis to (i) senescence start (T_s), (ii) complete senescence (T_o), and (iii) maximum rate of senescence (T_{MRS}), as well as the maximum rate of senescence. Finally, wheat varieties were divided into three stay-green types by hierarchical cluster analysis, including stay-green, early senescence and intermediate type. Based on this approach, a functional stay-green variety, Tainong 18 was screened. Tainong 18 with a delayed T_s and normal rate of senescence (RS) was similar to dSnR of type A under normal irrigation. However, it had earlier T_s and very small RS, and was similar to type B under drought stress. It can be used as a parent of crossing combinations in future wheat breeding, due to a longer photosynthetic activity and high yield potential.

Keywords: Cluster analysis, leaf senescence, stay-green variety, wheat.

DROUGHT stress is a major environmental factor which can severely limit the productivity of wheat, one of the most important cereal crops in the world^{1,2}. Therefore, it is necessary to develop wheat cultivars with superior adaptation to dry environment^{3,4}. Stay-green is an integrated drought-adaptation trait. The stay-green phenotype has been found to improve yield in some cereal crop species, including wheat (*Triticum aestivum* L.)^{5–7}, rice (*Oryza sativa*)^{8–11}, sorghum (*Sorghum bicolor*)^{12–14} and maize (*Zea mays*)^{15–17}, particularly under terminal drought stress. Plants with stay-green character are able to maintain green leaves for a longer duration after anthesis under drought stress condition and also longer photo-

synthetic activity. It has been reported that a two-day delay in the onset of senescence in *Lolium temulentum* L. increased the amount of carbon fixed by 11% (ref. 18). It has also been reported that stay-green sorghum hybrids produced 47% more post-anthesis biomass than their counterparts under terminal moisture deficit condition¹⁹. Therefore, it is important to select stay-green type for improving wheat adaptation to water-stressed environment.

Previously, stay-green traits of rice variety were estimated using the SPAD (soil and plant analyser development) values of heading stage and 30 days after heading²⁰. On the other hand, green leaf area at maturity was considered as an effective method for evaluating the stay-green traits in sorghum²¹. Two approaches, viz. difference in 0–9 scoring of green coloration (chlorophyll) of flag leaf and spike at the late dough stage and leaf area under greenness from the late milk stage until physiological maturity, were used to evaluate stay-green characteristics in wheat²². Henzell *et al.*²³ reported that there was significant difference of senescence rates between senescent and stay-green sorghum varieties on 29 April and 15 May in Australia. Lopes and Reynolds⁷ reported that stay-green in spring wheat could be determined by spectral reflectance measurements (normalized difference vegetation index) at physiological maturity. Obviously, stay-green characters of crops were evaluated in the above studies according to the phenotype value at one or a few growth stages. However, senescence in plant leaf is a programmed degeneration process; it is more reasonable and accurate to evaluate the stay-green character of crops by making tracks for changes of stay-green traits in the whole development process.

As is known, leaf colour gradually turns from green to yellow and green leaf area decreases from leaf tip to leaf base and from margins to the centre during leaf senescence. In the present study, chlorophyll content (SPAD values) and green leaf area duration (GLAD) of flag leaves in 16 wheat varieties were tested at three-day intervals from flowering to complete senescence. Their senescence curves were simulated, and the characteristic parameters of senescence were estimated. Stay-green wheat variety was obtained by hierarchical cluster analysis. Furthermore, change in chloroplast was the most significant at the cellular level during leaf senescence. So in

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order to verify the type of screened wheat variety, the chloroplast ultrastructure of stay-green and early senescence wheat leaves was analysed.

Materials and methods

Plant material and growth conditions

Sixteen wheat varieties were used for the analysis. Twelve of them (Jinmai 54, Jinmai 56, Jinmai 72, Jinmai 73, Nongda 92, Jinmai 61, Xin 9152, Tangmai 5012, S7073, S7074, Chang 6135 and Xindasui) were from the northern winter wheat area in China, three (Taishan 269, Lankao 1, Tainong 18) from the Yellow-Huai winter wheat area with stronger winter resistance also in China, and one (FRFSCD) from USA.

The seeds were sown in the farmland of Shanxi Agricultural University, China (37°25", 112°25") during 2012–2013 (16 wheat varieties) and 2013–2014 (Tainong 18 and Chang 6135). The experimental field was divided into two parts for different water environments, including normal and rainfed irrigation. The field design of each part consisted of randomized complete blocks with three replications. Each plot consisted of three rows, 2 m long with 0.25 m spacing between them. Sixty seeds per row were sown^{24,25}. The experimental soil was sandy with soil organic matter 12.5 g kg⁻¹, total nitrogen 2.2 g kg⁻¹, available phosphorus of 8.3 mg kg⁻¹ and available potassium 101.5 mg kg⁻¹. Before sowing, the test plots were irrigated well. After sowing, rainfed materials grew with a total of 206.8 mm rainfall during the whole growth period, was regarded as drought stress, while irrigated materials were applied to about 65 mm of water at the pre-overwintering, jointing and mid-grain filling stages, respectively.

Measurement of stay-green-related traits

For each wheat variety, the flowering date was recorded. Ten main stems which eared first were randomly selected from each plant and tagged. In 2012–2013, SPAD values and GLAD of flag leaves in the 16 wheat varieties were measured during the whole grain-filling period under normal irrigation and drought stress conditions. In 2013–2014, SPAD values and GLAD of flag leaves in Tainong 18 and Chang 6135 were measured during the whole grain-filling period under normal irrigation.

Chlorophyll content (SPAD values) in flag leaves of the main stem was measured using a Minolta Chlorophyll Meter (SPAD-502, Minolta Camera Co, Osaka, Japan), as described by Dwyer *et al.*²⁶. The SPAD values were tested at the upper, middle and base on each side of the midrib with three replications, at three-day intervals from seven days after anthesis (DAA) to complete senescence.

GLAD was tested as described previously with minor modifications¹. Wheat flag leaves were visually estimated using a 0–9 scale (9, whole leaf green; 8, 90% green leaf area; ..., 0, fully yellow). Similarly, green leaf area scores were recorded at three-day intervals from 7 DAA to senescence.

Calculation of senescence parameters

A nonlinear regression curve was fitted on the SPAD values and GLAD using a Gompertz statistical model, as follows

$$Y = k * \exp(-b * \exp(-a * t)), \quad (1)$$

where Y is a SPAD or GLAD at any given time after anthesis, k the point on the curve where the slope is maximum, t refers to DAA, and a and b are parameters which are determined by fitting experimental data.

Calculation of k , a and b : First, the initial values of curve parameters (k , a and b) were calculated by least square method. Secondly, the actual values of k , a and b were obtained using the SAS software. Thirdly, the actual values were substituted in Gompertz curve equation, and the change curves of SPAD values and GLAD for wheat flag leaves were obtained.

Characteristic parameters of senescence, including the time from anthesis to T_s , T_o and T_{MRS} , as well as MRS, were calculated by the first and second derivatives of the Gompertz curve. These characteristic parameters were calculated as

$$T_s = \frac{\ln b - \ln[\ln(k/95\%y)]}{a}, \quad (2)$$

$$T_o = \frac{\ln b - \ln[\ln(k/5\%y)]}{a}, \quad (3)$$

$$MRS = \frac{-ka}{e}, \quad (4)$$

$$T_{MRS} = \frac{\ln b}{a}. \quad (5)$$

Transmission electron microscopic analysis of chloroplasts

In 2014, flag leaves of main stem from Tainong 18 and Chang 6135 were detached at 17, 19, 21, 23, 25 DAA, and fixed with 2.5% glutaraldehyde fixative at 4°C.

Transmission electron microscopic analysis of chloroplasts was performed according to the method of Inada *et al.*²⁷, with minor modifications. First, small pieces of flag leaves (1 mm × 2 mm) from Tainong 18 and Chang 6135

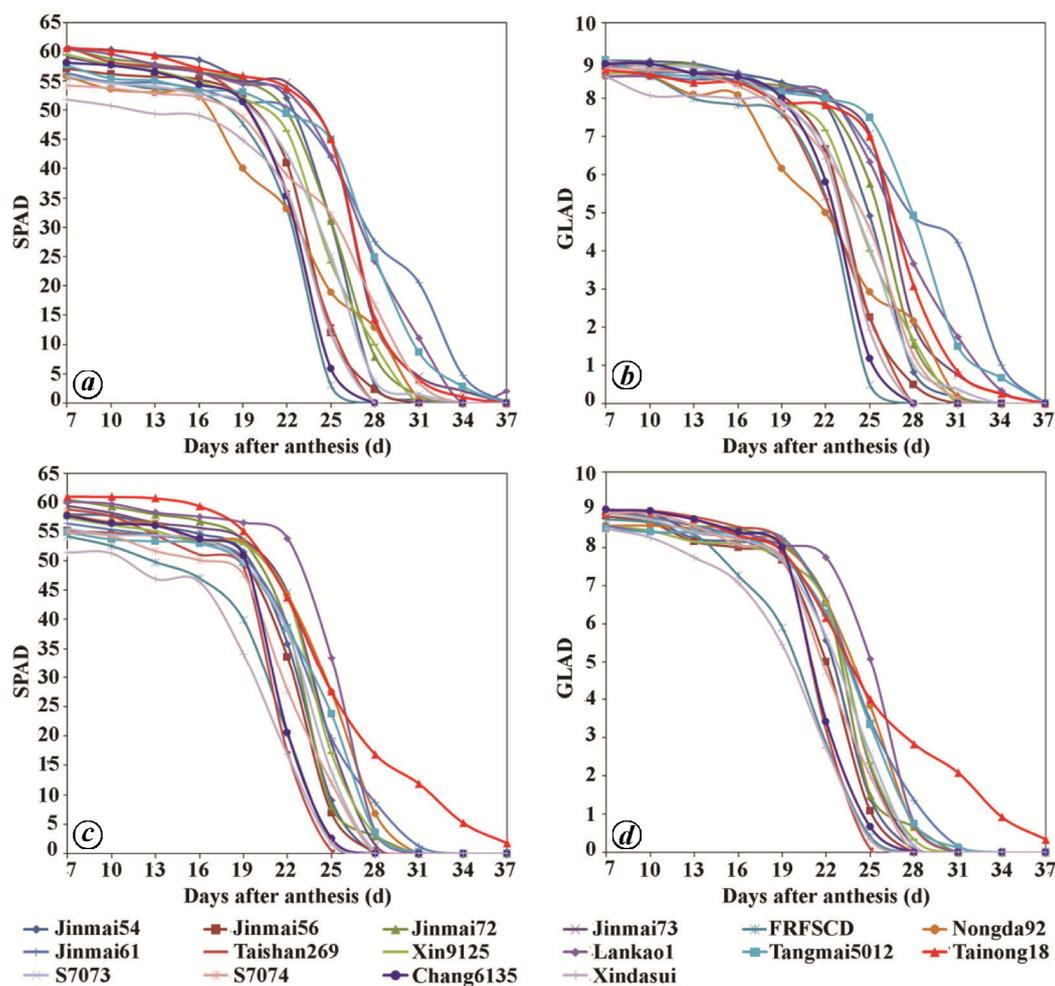


Figure 1. Senescence curves of flag leaves of 16 wheat varieties in 2012–2013. *a, b*, Change curves of chlorophyll content (SPAD) and green leaf area duration (GLAD) respectively, under normal irrigation. *c, d*, Change curves of SPAD and GLAD respectively, under drought stress.

were cut and fixed with 2.5% glutaraldehyde at 4°C for 24 h. The samples were washed three times with 100 mM phosphate buffer, pH 7.2, at 4°C for 25 min and post-fixed with 1% osmium tetroxide for 3 h, and then washed twice with phosphate buffer again for 25 min. Secondly, samples were dehydrated in a gradient series of ethanol, replaced by propylene oxide, and then embedded within Epon812 epoxy resin to polymerize at 37°C for 12 h, 45°C for 12 h and 60°C for 12 h, modified the embedded block at Zoom-stereo microscope, and cut the ultrathin sections (50–70 nm) on an ultramicrotome (Leica EM UC6, Japan). Finally, the sections were stained with uranyl acetate solution and lead citrate solution at 25°C for 30 min, washed thrice times with distilled water and then photographed under transmission electron microscope (JEOL-1400 EX, Japan) after drying.

Data analysis

The scatter plots were draw in Microsoft Excel 2007. Cluster analysis was carried out according to hierarchical

method. Microsoft Excel 2007 and SAS were used for data analysis.

Results

Senescence characteristics of different wheat varieties

SPAD values and GLAD showed ‘slow–fast–slow’ change trends (Figure 1). First, the rate of senescence (RS) was low before 22 DAA under normal irrigation and 19 DAA under drought stress; so the changes in SPAD values and GLAD were less. When RS increased, and SPAD values and GLAD decreased rapidly. Finally, when RS was close to zero at mature stage, SPAD values and GLAD were almost zero. However, there were significant differences of characteristic parameters of senescence among these wheat varieties.

There were marked differences of T_s in wheat varieties; the earliest was at 10 DAA (Nongda 92) and the last was at 22 DAA (Jinmai 73) under normal irrigation, while it ranged from 13 DAA (Xindasui) to 20 DAA (Lankao1)

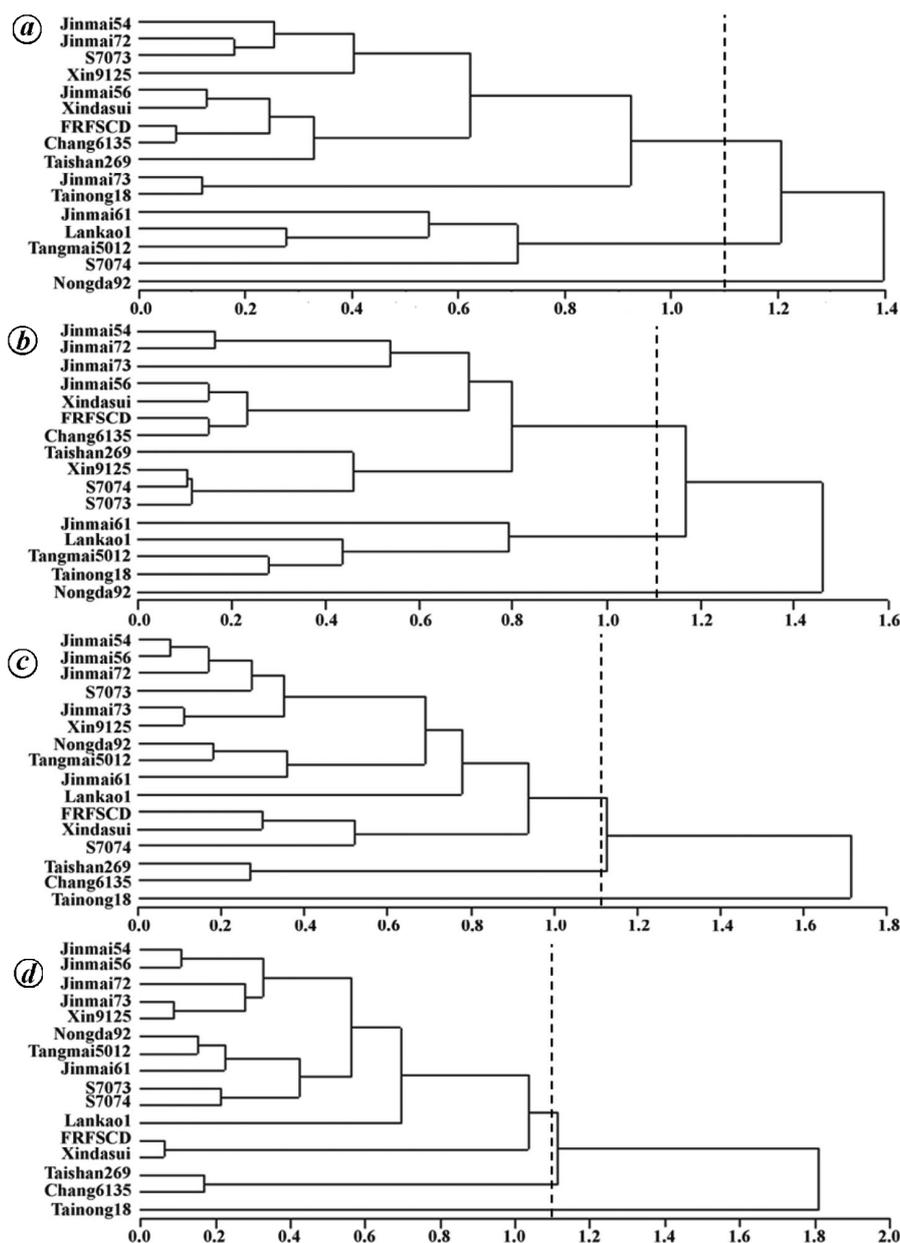


Figure 2. Hierarchical clustering of 16 wheat varieties using the average method in 2012–2013. Similarity coefficient = 1.1. *a, b*, Hierarchical clustering according to the time of senescence start (T_s), completed senescence (T_o), maximum rate of senescence (T_{MRS}) and maximum rate of senescence (MRS) of SPAD values and GLAD respectively, under normal irrigation. *c, d*, Hierarchical clustering according to T_s , T_o , T_{MRS} and MRS of SPAD and GLAD respectively, under drought stress.

under drought stress. The slope of non-regression lines was used to estimate RS. Obviously, Nongda 92 and Jinmai 73 had the minimum and maximum MRS under normal irrigation, while it was Chang 6135 and Tainong 18 respectively under drought stress. These characteristics eventually led to the differences in T_o . For example, T_o in Jinmai 61 (35 DAA) was 10 days later than that in Chang 6135 (25 DAA) under normal irrigation, and T_o in Tainong 18 (33 DAA) was 10 days later than that in Chang 6135 (23 DAA) under drought stress. Besides, T_{MRS} was also different. T_{MRS} of Jinmai 61 and Chang

6135 was at 30 and 23 DAA under normal irrigation, while that of Tainong 18 and Chang 6135 was at 28 and 22 DAA under drought stress respectively (Table 1).

Stay-green types of different wheat varieties

Under normal irrigation condition, based on T_s , T_o , T_{MRS} , and MRS of SPAD values and GLAD, 16 wheat varieties were clustered into three groups at a 1.1 similarity coefficient in 2012–2013 (Figure 2*a* and *b*). Among them,

Table 1. Senescence characteristic parameters of 16 wheat cultivars calculated using SPAD values and GLAD during flag leaves senescence in 2012–2013

Condition	Type	Variety	SPAD				GLAD				
			T_s/d	MRS	T_{MRS}/d	T_o/d	T_s/d	MRS	T_{MRS}/d	T_o/d	
Normal irrigation	Stay-green	Jinmai 61	17.22	4.64	30.33	35.21	17.89	0.72	31.18	36.12	
		Lankao 1	17.91	5.98	28.76	32.78	18.76	0.93	29.00	32.79	
		Tangmai 5012	19.72	6.57	28.99	32.42	21.58	1.19	29.52	32.45	
		Tainong 18	21.55	10.98	27.39	29.55	20.94	1.27	28.30	31.02	
		Mean	19.10	7.04	28.87	32.49	19.79	1.03	29.50	33.10	
	Intermediate	Jinmai 54	19.82	10.75	25.86	28.10	19.77	1.53	26.14	28.49	
		Jinmai 73	22.30	12.31	27.38	29.26	22.67	1.96	27.46	29.23	
		Jinmai 56	18.44	10.72	24.18	26.29	18.69	1.65	24.45	26.58	
		FRFSCD	17.72	10.85	23.20	25.22	18.31	1.80	23.36	25.22	
		Chang 6135	17.54	10.64	23.41	25.57	17.89	1.67	23.66	25.80	
		Taishan 269	15.99	8.22	23.89	26.81	15.78	1.18	24.03	27.08	
		Xindasui	17.64	8.44	24.18	26.60	19.34	1.79	24.37	26.23	
		Jinmai 72	18.64	8.48	26.23	29.03	20.31	1.47	26.84	29.26	
		Xin 9152	16.39	6.76	25.83	29.33	17.23	1.06	26.20	29.51	
		S7073	18.02	7.90	25.60	28.40	16.66	1.08	25.73	29.08	
		S7074	15.29	4.96	27.24	31.69	16.49	0.99	26.10	29.66	
		Mean	17.98	9.09	25.18	27.85	18.47	1.47	25.30	27.83	
		Early senescence	Nongda 92101	10.20	4.08	24.95	30.71	9.43	0.60	24.87	31.01
			Mean	10.20	4.08	24.95	30.71	9.43	0.60	24.87	31.01
		Drought stress	Stay-green	Tainong 18	11.00	4.28	26.88	33.08	9.08	0.53	27.14
Mean	11.00			4.28	26.88	33.08	9.08	0.53	27.14	34.62	
Intermediate	Jinmai 54		17.18	9.69	23.63	26.02	17.46	1.51	23.72	26.03	
	Jinmai 56		17.45	9.95	23.49	25.71	16.88	1.43	23.42	25.84	
	Jinmai 72		17.81	11.00	23.67	25.83	19.05	1.89	24.07	25.93	
	Jinmai 73		18.46	9.84	24.88	27.25	18.26	1.52	24.46	26.75	
	Xin 9152		18.85	10.38	24.71	26.88	18.86	1.59	24.57	26.68	
	Nongda 92101		17.55	7.45	25.89	28.98	17.91	1.23	25.56	28.38	
	Tangmai 5012		16.86	7.00	25.37	28.51	16.91	1.12	25.26	28.35	
	Jinmai 61		15.16	6.11	25.27	29.02	16.04	1.03	25.55	29.07	
	Lankao 1		20.62	11.79	26.08	28.10	20.69	1.64	26.31	28.38	
	FRFSCD		14.43	8.36	22.11	24.95	12.48	1.05	21.63	25.03	
	Xindasui		13.21	6.64	21.65	24.78	12.14	0.98	21.55	25.05	
	S7073		17.46	8.84	24.26	26.77	16.01	1.14	24.40	27.50	
	S7074		14.93	6.89	23.58	26.78	14.99	1.12	23.66	26.88	
	Mean		16.92	8.76	24.20	26.89	16.74	1.33	24.17	26.91	
	Early senescence		Chang 6135	17.99	15.37	21.99	23.47	17.90	2.31	22.08	23.63
			Taishan 269	17.20	13.44	21.76	23.45	18.16	2.55	21.97	23.38
			Mean	17.60	14.41	21.88	23.46	18.03	2.43	22.03	23.51

SPAD, Soil and plant analyser development; GLAD, Green leaf area duration; MRS, Maximum rate of senescence.

Jinmai 61, Lankao1, Tangmai 5012, and Tainong 18 were clustered into group I (stay-green type), Nongda 92 belonged to group II (early senescence type), and the remaining wheat varieties were under group III (intermediate type), including Jinmai 54, Jinmai 72, S7073, S7074, Xin 9152, Jinmai 56, Xindasui, FRFSCD, Chang 6135 and Taishan 269. For wheat varieties of the stay-green type, senescence started from about 17 to 22 DAA, ended from about 30 to 35 DAA, and their T_{MRS} was from about 27 to 30 DAA. For wheat varieties of early senescence type, SPAD values and GLAD began to reduce at about 10 DAA, T_{MRS} at about 25 DAA, and T_o at about 31 DAA. Obviously, chlorophyll content (SPAD values) and GLAD could be retained at a higher level for a longer

time by the stay-green varieties than those of early-senescence varieties (Table 1).

Under drought stress condition, according to T_s , T_o , T_{MRS} and MRS of SPAD values and GLAD, 16 wheat varieties were also divided into three types at a 1.1 similarity coefficient in 2012–2013 (Figure 2c and d). Type I had only one wheat variety, Tainong 18. Although senescence of wheat variety in this type started early, RS was smaller during the whole senescence process, and T_{MRS} (27 DAA) and T_o (33 DAA) occurred later; so this type was stay-green. Type II, early senescence type, included two wheat varieties, Taishan 269 and Chang 6135. T_s , T_o and T_{MRS} were at about 18, 23 and 22 DAA respectively. Although T_s in type II occurred later than that in type I,

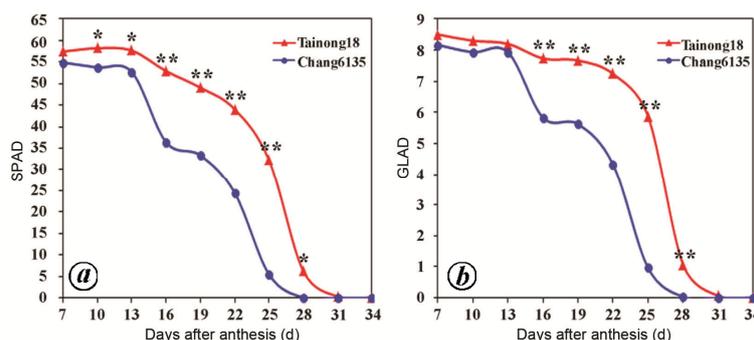


Figure 3. Senescence curves of flag leaves of Tainong 18 and Chang 6135 under normal irrigation in 2014. *a, b*, Change curves of (a) SPAD and (b) GLAD. * $P < 0.05$, ** $P < 0.01$.

RS was much higher and T_0 was earlier. Type III, the intermediate type, included the remaining 13 wheat varieties. Their senescence parameters were all between type I and type II (Table 1).

Obviously, Tainong 18 was classified into stay-green group under the two water environments, while Nongda 92 belonged to early senescence type under normal irrigation, and Taishan 269 and Chang 6135 were early senescence type under drought stress.

Further identification of stay-green wheat varieties

Two wheat varieties, Tainong 18 and Chang 6135, were selected from the stay-green and early senescence type respectively. A repetitive identification was undertaken in 2013–2014. Chlorophyll content (SPAD values) of flag leaves in the two varieties was similar and hardly changed before 13 DAA. However, chlorophyll content of leaves in Chang 6135 decreased rapidly from 52.68 at 13 DAA to 5.00 at 25 DAA, while chlorophyll content of leaves in Tainong 18 at 25 DAA was similar to that in Chang 6135 at 19 DAA. Tainong 18 began to senesce at 17.59 DAA, and ended at 29.34 DAA. Its T_{MRS} was at about 26.17 DAA. These characteristic parameters of senescence (T_s , T_0 and T_{MRS}) in Chang 6135 were at 8.24, 27.10 and 21.73 DAA respectively (Figure 3a). GLAD in Chang 6135 became zero at 25 DAA, whereas that of Tainong 18 showed little change before 25 DAA. T_s , T_0 and T_{MRS} were at 21.56, 28.78 and 26.83 DAA in Tainong 18 and 11.28, 27.14 and 22.8 DAA in Chang 6135 respectively (Figure 3b). In addition, SPAD values and GLAD were much higher in Tainong 18 than those in Chang 6135 during the whole senescence process, except for 7 and 31 DAA (Figure 3). Therefore, Tainong 18 was a stay-green type variety, while Chang 6135 belonged to early senescence type.

Changes in chloroplast structure in different stay-green types

In order to verify the above results and provide a basis for the availability of the method, changes of chloroplast

ultrastructure in Tainong 18 and Chang 6135 were compared. At 17 DAA, chloroplasts of Tainong 18 and Chang 6135 were well-differentiated with an ellipsoidal shape, smooth chloroplast membrane, well-organized chloroplast thylakoid, numerous granum stacks, and small starch granules (Figure 4A and a). From 19 to 25 DAA, the successive decomposition of chloroplast components took place in Tainong 18 and Chang 6135. At 19 DAA, plastoglobuli were found in Tainong 18 and Chang 6135, but the number and size in Chang 6135 was more than those in Tainong 18 (Figure 4B and b). At 21 DAA, the thylakoid stacks exhibited an irregular arrangement and the size of plastoglobuli showed a marked increase in Chang 6135 (Figure 4c), while chloroplast of Tainong 18 still maintained well-developed, including well-developed grana with numerous layers and stroma lamellae with a small amount of plastoglobuli (Figure 4C). At 23 DAA, chloroplast membranes began to degrade in Chang 6135, envelope membrane invariably ruptured, and a large number of thylakoids and starch granules disappeared with only a few small grana remaining (Figure 4d). The whole chloroplast structure of Chang 6135 was ruptured and all of the chloroplast components were completely decomposed at 25 DAA (Figure 4e). However, the chloroplast structure of Tainong 18 at 23 and 25 DAA was similar to that of Chang 6135 at 21 and 23 DAA respectively (Figure 4D and E). These findings indicated that the chloroplasts degradation in Tainong 18 occurred later than that in Chang 6135.

Discussion

In most of the previous studies, chlorophyll content was tested using spectrophotometry. This method gives accurately measurements, but we need to detach the leaves. Adu *et al.*¹ found that a single linear regression existed between SPAD values and chlorophyll content. In this study, chlorophyll content was measured using a SPAD meter. It could track SPAD values of *in vivo* leaves during senescence in real time, also it is quick, portable, and non-destructive. GLAD has been considered as a much better indicator for evaluating stay-green characteristics²⁸.

It was estimated by visual scoring in the present study. This approach is simple, rapid and convenient, and requires no specific equipment.

According to the relationship between chlorophyll content and photosynthetic activity, stay-greens were divided into five types, A, B, C, D and E^{18,29}. A and B were functional with longer time for chlorophyll and photosynthetic activity during the whole filling–graining period, while C, D and E were non-functional. In functional stay-greens, type A showed loss of chlorophyll and function at a normal rate with delayed initiation of senescence, such as XN901 in wheat²⁸. Theoretically, type A has three different forms, viz. (i) dSnR (delayed T_s , normal RS), (ii)

dSsR (delayed T_s , slow RS) and (iii) dSfR (delayed T_s , fast RS)⁶. Type B exhibits normal onset and a reduced rate of senescence, such as SNU-SG1 in rice³⁰, FS854 in maize^{18,31,32} and R16 in sorghum³³. In this study, under normal irrigation, compared with group II (early senescence type), group I (stay-green type) showed delayed T_s and T_0 and normal RS; so it was functional stay-green type, and similar to dSnR of type A, which had delayed onset of leaf senescence and longer photosynthetic activity. Under drought stress, T_s of group I (stay-green type) occurred earlier, but it had a slow rate resulting in a delayed T_0 . Therefore, it was also functional stay-green type, and similar to type B, which had small RS and longer photosynthetic activity. Furthermore, group I (Tainong 18) had higher SPAD values and GLAD than those of group II (Chang 6135) during the whole senescence process. The results of chloroplast ultrastructure indicated that chloroplast degradation in group I (Tainong 18) was later than that in group II (Chang 6135).

In recent years, there have been some reports on the relationship between crop productivity and stay-green. Thomas and Stoddart³⁴ assumed that delayed initiation of senescence will increase the yield. A positive correlation was shown between stay-green traits and grain yield in ten maize hybrids genotypes^{19,35}. A consistent result was obtained in 11 sorghum hybrid lines³⁵. Positive relationship between GLAD and plant dry mass was also reported for 11 genotypes of oilseed rape³⁶. In the present study, stay-green type (Tainong 18) had significantly higher grain number per spike, grain weight per spike, yield per plant, and thousand-grain weight than early senescence type (Chang 6135) (File S1). Therefore, due to a delayed T_s or slower RS, functional stay-greens can maintain green leaves for a longer duration and also longer photosynthetic activity³⁷. Further their grain yields were also found to increase.

In this study, based on SPAD values and GLAD tested during leaf senescence, we have developed an improved method for identifying and evaluating stay-green wheat varieties. A functional stay-green wheat variety was screened. This provided not only a method but also parent material for breeders to screen and develop stay-green varieties.

Conclusion

This study, describes an improved method for identifying and evaluating stay-green wheat varieties. First, the leaf senescence curve was simulated based on a series of phenotypic data. Secondly, the senescence characteristic parameters were calculated, including T_s , T_0 , T_{MRS} and MRS. Thirdly, wheat varieties were divided into three types by hierarchical cluster analysis, including stay-green, early senescence and intermediate type. According to this approach, a functional stay-green wheat variety, Tainong 18, was screened. It had a delayed T_s and normal RS, and was similar to dSnR of type A under normal irrigation.

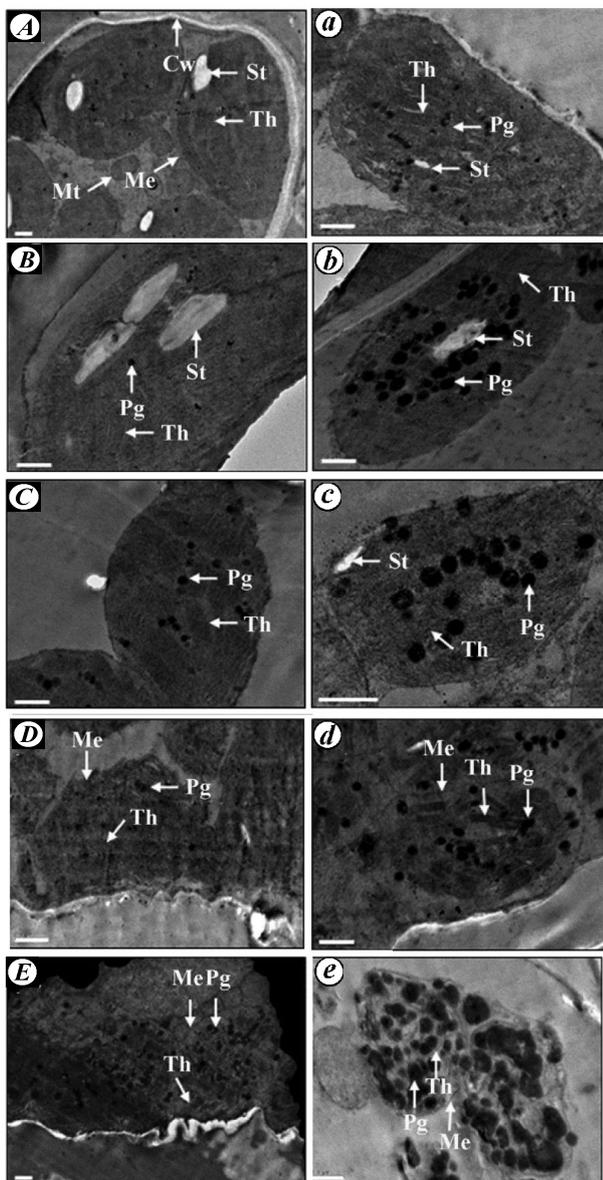


Figure 4. Transmission electron microscope images showing the ultrastructure of chloroplast at different stages of flag leaves in Tainong 18 and Chang 6135. A–E and a–e indicate chloroplast structure of Tainong 18 and Chang 6135, and correspond to 17, 19, 21, 23, 25 days after anthesis respectively.

However it had earlier T_s and small RS, and was similar to Type B under drought stress. It can be used as parent of crossing combinations in future wheat breeding, due to longer photosynthetic activity and high yield potential.

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