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Genetic analysis and assessment of stay-green traits in hybrids of temperate and tropical maize germplasm

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Abstract

Leaf stay-green is a trait in which plants retain leaves with green tissue from flowering through physiological maturity in maize. To assess whether this characteristic improved grain filling in maize, we analyzed five stay-green traits, five grain yield-related traits, and three photosynthetic parameters in 10 hybrids derived from an incomplete diallele cross mating design between five temperate and two tropical maize germplasm inbred lines. The hybrids were grown in three different environments. The degree of stay-green (DSG) was significantly positively correlated with grain yield, ear length, number of grains per row, number of visible green leaves at maturity (MNL), ratio of visible source leaves (RSL), and photosynthetic potential at maturity (MPP). The highest correlation between traits was between RSL and MNL. All five stay-green traits were significantly positively correlated with net photosynthetic rate (Pn). RSL had the highest broad-sense heritability among the stay-green traits evaluated. Our findings suggest that RSL is an important stay-green trait and can be used as a selection criterion for improving stay-green traits in maize breeding programs.

Introduction

Maize (*Zea mays* L) is one of the most important grain crops and plays a crucial role in global food security. Factors such as global climate change and ecological deterioration have led to increased pressure to stabilize global maize production (Nellemann et al, 2009). Leaf stay-green is a trait in which leaves retain green tissue from flowering through physiological maturity instead of senescing and may be important to grain filling in maize. Prior studies indicated that early leaf senescence in during the maize grain-filling period may lead to significant decreases in yield (Ma and Dwyer, 1998) and that improvements in leaf stay-green characteristics could significantly increase the production efficiency of commercial maize varieties (Lee and Tollenaar, 2007; Duvick and Cassman, 1999). Lee and Tollenaar (2007) used source–sink theory to study the physiological basis of increased maize yields in the United States from 1939 to 2005. Their results revealed that the source capacity increased significantly, especially visual and functional stay-green. Stay-green has been reported to prolong the effective grain-filling period and enhance resistance to lodging, disease, and drought (Cavalieri and Smith, 1985; Gentineua et al, 1986; Thomas and Smart, 1993; Bekavac, 1998; Thomas and Howarth, 2000; Monneveux et al, 2006). Biotic and abiotic stresses (such as drought) are known to induce leaf senescence, leading to low yields (Lim et al, 2007). Consequently, how to improve crop leaf stay-green traits under stress conditions has attracted the attention of many scientists (Christopher et al, 2008; Tien and Tohru, 2009).

Stay-green (also called non-yellowing or non-senescence) was originally discovered in mutants isolated from several plants (Thomas and Smart, 1993; Lim et al, 2007). Stay-green crops can be divided into five different types (Thomas and Howarth, 2000). The understanding of leaf senescence has expanded rapidly in the last two decades (Lim et al, 2007). Studies in sorghum [*Sorghum bicolor* L (Moench)] have identified four stay-green quantitative trait loci (QTLs) (Xu et al, 2000). Moreover, the stay-green trait has become an important indicator in breeding drought-resistant sorghum in Australia and the United States (Borrell et al, 2000). However, research on leaf stay-green in maize is not as advanced as in sorghum.

Bekavac et al (1998) proposed a quantitative measure, the degree of stay-green (DSG). Duvick and Cassman (1999) suggested leaf stay-green could be classified into nine levels in maize. Moreover, Liu et al (2003) proposed the evaluation of leaf stay-green by counting the number of green leaves and the chlorophyll content at maturity (MCL). The maize stay-green QTLs that have been detected require further verification, because, for example, Mo17 was correlated

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with no-senescence and senescence inbred lines in two different studies (Beavis et al, 1994; Zheng et al, 2007).

In the past 80 years, genetic improvement of maize has mainly progressed by using different heterotic groups of temperate germplasm, such as the Reid and Lancaster groups. Thus, the genetic basis of temperate maize varieties has become narrower (Meng et al, 2010; Reif et al, 2005; Smith et al, 2004) and may threaten future maize production. In contrast, tropical maize germplasm have many advantages (e.g., stay-green traits, resistance to abiotic stress) that could broaden the genetic basis of modern temperate maize varieties and improve their stay-green and stress resistance characteristics (Goodman, 1999; Chen and Fan, 2001). In the past 20 years in China, more than 100 new cultivars have been bred using tropical germplasm. For example, X178, a parental line of Nongda 108 hybrid cultivars, was bred from Pioneer hybrids from the United States with a tropical genetic background (Teng et al, 2004). These cultivars have been planted throughout the maize belt in China and now cover approximately 12 million hectares (Wang and Jing, 2004).

Although the use of tropical maize germplasm to improve temperate strains of maize has achieved much success, few studies have genetically analyzed stay-green traits in tropical maize. Therefore, the objectives of this study were to investigate the relationships among stay-green traits, grain yield, and photosynthetic parameters and to evaluate the heritability of stay-green traits in temperate-tropical hybrids of maize.

Materials and Methods

Plant materials

Ten temperate-tropical hybrids were generated using five temperate and two tropical maize inbred lines (Table 1) and an incomplete diallele cross mating design (Huang and Liu, 1980). The 10 hybrids were planted separately at Banan, Chongqing, China (29°24’19.00”N 106°32’11.31”E; 220 m altitude; average annual temperature: 18.7°C; average annual rainfall: 1160 mm) and Yaan, Sichuan, China (30°0’29”N 103°1’52”E; 620 m altitude; average annual temperature: 16.2°C: average annual rainfall: 1,774.3 mm) in 2010 and in 2011 at Banan, Chongqing, China. The trials used a randomized complete block design with three replicates per trial. Each hybrid was planted in two 3.67 m rows that were 0.9 m apart at an overall density of 48,000 plants ha^{-1}.

Measurement of stay-green traits

Five plants were randomly selected and tagged in each plot. The number of green leaves and leaf length (cm), leaf width (cm), and the chlorophyll content (CHL) of ear leaves were assessed at flowering and post-flowering stages (10, 20, 30, 40, 45, and 50 d after flowering), and at physiological maturity. CHL was measured using a SPAD 502 Plus Chlorophyll Meter (Konica Minolta, Osaka, Japan).

Plants were characterized by (1) green leaf area per plant (in cm^2; = average leaf length × average leaf width at widest point × 0.75) (Montgomery, 1911); (2) DSG= (green leaf area at physiological maturity / maximum leaf area at flowering stage × 100%); (3) the ratio of visible source leaves (RSL = number of visible source leaves at physiological maturity / number of visible source leaves at flowering stage), where visible source leaves included the first leaf under the ear and all leaves from the ear to the tassel (Figure 1), only leaves with > 50% green tissue area were counted (Supplementary Figure 1); and (4) the number of visible green leaves at physiological maturity (MNL; the number of leaves with green tissue area greater than 50%).

Finally, (5) photosynthetic potential at maturity

Table 1 - Maize inbred lines used to generate temperate–tropical hybrids to examine the stay-green trait.

Inbred line	Heterotic group	Germplasm adaptability	Source of germplasm	Breeding hybrid variety	Parental line in incomplete diallele cross
478	Reid	Temperate	Maize Research Institute, Shandong, Laizhou, China	YeDan13	P2
Mo17	Lancaster	Temperate	United States	ZhongDan2	P2
Huangzao4	Spt-H	Temperate	Beijing Academy of Agriculture Sciences, China	YanDan14	P2
340	Luda	Temperate	Dandong Academy of Agriculture Sciences, Liaoning, China	YeDan13	P2
Jiao51	Opvs	Temperate	From southwestern China; obtained from Guizhou Academy of Agriculture Cadres, Guiyang, China	JiaoSan Dan Cross	P2
B31-3	Suwan-1	Tropical	Originally from Thailand; obtained from the Maize Research Institute, Chongqing Academy of Agriculture Sciences, China	Yudan32	P1
YP8007	Tuxpeno	Tropical	Originally from Thailand; obtained from the Maize Research Institute, Chongqing Academy of Agriculture Sciences, China	YuS1101	P1

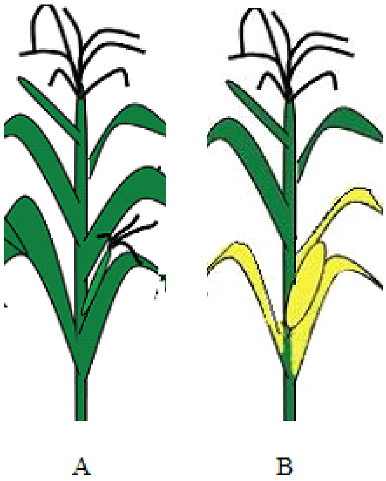

Figure 1 - Number of visible source leaves.
A - six leaves visible at flowering; B - three leaves visible at maturity.

(MPP) (m^2 day ha^{-1}) was calculated as (L1 + L2) × (T2 – T1) / 20,000, where L1 is the photosynthetic leaf area (cm^2 ha^{-1}) 5 d before physiological maturity, L2 is the photosynthetic leaf area (cm^2 ha^{-1}) at physiological maturity, T1 is the duration (in days) from flowering to 5 d before physiological maturity, and T2 is the duration (in days) from flowering to physiological maturity.

Photosynthetic parameters

The net photosynthetic rate (Pn; μmol CO_2 m^{-2} s^{-1}), stomatal conductance (Gs; mmol H_2O m^{-2} s^{-1}), and transpiration rate (Tr; mmol H_2O m^{-2} s^{-1}) were measured at the flowering, grain-filling, and maturity stages using an LI-6400 portable photosynthesis system (LI-COR Scientific, Lincoln, NE, USA).

Grain yield traits

Maize plants were harvested manually, and 10 harvested ears from each row were randomly selected and sun dried. Ear length (EL), rows per ear (ER), grains per row (RGN), 100-kernel weight (HKW), and grain yield/plot (PYG) were measured. Grain water content was kept below 13% by sunshine and natural wind.

Data analysis

Analysis of variance of each stay-green trait in the multi-environment trials was performed using the GLM procedure implemented in SAS v. 9.1 (SAS Institute, Cary, NC, USA). Correlations between stay-green traits, grain yield, and photosynthetic parameters were evaluated using the CORR process in SAS. The components of variance originally from the various variation were estimated using the VARCOMP process in SAS, and then broad-sense heritability was calculated using the formula:

$$h_B^2\% = \frac{\sigma_G^2}{\sigma_P^2} \times 100$$

where σ^2_G is genotype variance and σ^2_P is phenotypic variance.

Results

Genetic analysis of stay-green trait variance

Analysis of variance of five stay-green traits, including DSG (range: 13.5–29.6%, average: 20.9%); MNL (range: 1.4–5.1, average: 3.2); MPP (range: 4.27 × 10^4 – 14.06 × 10^4 M2 day ha^{-1}, average: 8.26 × 10^4 M2 day ha^{-1}); RSL (range: 0.17–0.64, average: 0.40); and CHL at physiological maturity (MCL; range: 28.2–44.4, average: 37.3) revealed that they differed significantly among the 10 temperate–tropical hybrids (Table 2). DSG, MPP, and MCL differed significantly between environments. With the exception of DSG, all of the stay-green traits showed interactions between the temperate–tropical hybrid and the environment.

Correlation among stay-green traits

There was a significant positive correlation between DSG and MNL ($p \leq 0.01$), MPP ($p \leq 0.01$), and RSL ($p \leq 0.01$) (Table 3). MCL was not correlated with any other stay-green trait. The highest correlation coefficient was between RSL and MNL ($p \leq 0.01$). RSL was also correlated with MPP ($0.01 < p \leq 0.05$). The RSL regression equation DSG = 11.203 + RSL × 24.249 was obtained using stepwise multiple regression analysis of DSG and the other four traits.

Table 2 - Analysis of variance of stay-green traits in 10 temperate–tropical hybrids of maize.

Source of variation	Degrees of freedom	Mean square				
		DSG	MNL	RSL	MPP	MCL
Block within environment	6	—	—	—	—	—
Environment	2	754.47**	2.12	0.06	257.26**	913.55**
Hybrid combination	9	239.43*	10.12**	0.17**	63.19**	330.55**
P1 parent	1	1102.22**	37.25**	0.74**	113.25**	325.81*
P2 parent	4	154.08	5.35(*)	0.06	96.31**	571.09**
Interaction between parents (P1 × P2)	4	109.09	8.10*	0.13*	17.54(*)	91.20
Interaction between hybrid combination and environment	18	133.06	4.31(*)	0.07(*)	32.27**	204.97**
Error	54	96.14	2.45	0.04	7.13	61.28

DSG - degree of stay-green trait; MCL - chlorophyll content at maturity; MNL - number of visible green leaves at maturity; MPP - photosynthetic potential at maturity; RSL - ratio of visible source leaves at physiological maturity vs. at flowering; (*) $0.05 < P \leq 0.10$; * $0.01 < P \leq 0.05$; ** $P \leq 0.01$.

Table 3 - Correlation coefficients between stay-green traits in temperate–tropical hybrids of maize.

Trait	DSG	MNL	MPP	RSL
MNL	0.515**			
MPP	0.497**	0.384*		
RSL	0.542**	0.979**	0.368*	
MCL	–0.265	0.043	0.282	0.064

DSG - degree of stay-green trait; MCL - chlorophyll content at maturity; MNL - number of visible green leaves at maturity; MPP - photosynthetic potential at maturity; RSL - ratio of visible source leaves at physiological maturity vs. at flowering; * 0.01 < P ≤ 0.05; ** P ≤ 0.01.

Correlation between stay-green traits and grain yield components

There was a significant positive correlation between DSG and PYG (p ≤ 0.01), EL (p ≤ 0.01), and RGN (p ≤ 0.01) (Table 4). There were significant negative correlations between MPP and HKW (0.01 < p ≤ 0.05), MCL and RGN (0.01 < p ≤ 0.05), and MCL and PGY (p ≤ 0.01).

Correlation between stay-green traits and photosynthetic parameters

There was a significant positive correlation between all five stay-green traits and net photosynthetic rate (Pn) (Table 5). The correlation coefficients between Pn and DSG, MPP (both p ≤ 0.01), MCL, RSL, MNL (all 0.01 < p ≤ 0.05), and MNL (p ≤ 0.01) were significantly positively correlated to Gs, but the other three stay-green traits were not. MNL was significantly positively correlated to Tr (0.01 < p ≤ 0.05), but the other four stay-green traits were not correlated with Tr.

Broad-sense heritability of stay-green traits

The values of broad-sense heritability for DSG, MNL, RSL, MPP and MCL were 29.4%, 58.8%, 60.17%, 21.76%, and 16.27%, respectively. RSL and MNL were highly heritable in a relatively stable manner and may be improved by selection during early generations of maize breeding programs.

Table 4 - Correlation coefficients between stay-green and grain yield traits in temperate-tropical hybrids of maize.

Trait	EL	ER	RGN	HKW	PGY
DSG	0.474**	0.161	0.642**	–0.096	0.535**
MNL	–0.151	0.155	0.009	0.287	0.112
MPP	0.177	0.180	0.219	–0.382*	–0.119
RSL	–0.110	0.116	0.035	0.298	0.083
MCL	–0.249	0.118	–0.419*	–0.167	–0.609**

DSG - degree of stay-green trait; EL - ear length; ER - rows per ear; HKW - 100-kernel weight; MCL - chlorophyll content at maturity; MNL - number of visible green leaves at maturity; MPP - photosynthetic potential at maturity; PGY - plot grain yield; RGN - number of grains per row; RSL - ratio of visible source leaves at physiological maturity vs. at flowering; * 0.01 < P ≤ 0.05; ** P ≤ 0.01.

Discussion

DSG was significantly positively correlated to three stay-green traits (MNL, MPP, and RSL), one important photosynthetic parameter (Pn), and three grain-yield traits (PGY, EL, RGN). These results indicated that DSG is a suitable indicator of stay-green traits in temperate–tropical maize hybrids. Stay-green temperate–tropical maize hybrids showed considerably high photosynthetic capacity. Therefore, the stay-green trait might play an important role in grain yield by increasing the number of effective grains per ear and reducing the grain abortion rate.

Chlorophyll is the major photosynthetic pigment in plants and functions to capture and transfer light energy. Our study demonstrated that MCL was significantly positively correlated to Pn, which corroborates the fact that chlorophyll plays an important role in photosynthesis and yield. The degradation of chlorophyll marks the start of leaf senescence (Thomas and Howarth, 2000) and is controlled by the expression of chlorophyll catabolism enzymes (Barry, 2009). Abiotic stresses, such as drought and high temperatures, induce and accelerate leaf senescence. Tropical maize varieties have stay-green traits that have undergone long-term natural selection at high temperatures and humidities (Liu et al, 2000), and their chlorophyll-degrading enzymes may tolerate higher levels of stress (Barry, 2009; Shi et al, 2009). Our results were consistent with a previous report (Liu et al, 2003) in that MCL was significantly negatively correlated to both RGN and PGY. This may be a result of photo-oxidation and light damage accelerating leaf senescence and in turn decreasing yield at maturity. Our studies suggested that CHL serve as an indirect selection criterion for yield improvement in inbred maize lines and breeding varieties. The selection of plants with light green leaves, which quickly transform their color at maturity, might prove favorable to breeding new varieties.

Lee and Tollenaar (2007) demonstrated that accumulated dry matter accounts for 50% of the to-

Table 5 - Correlation coefficients between the stay-green traits and photosynthetic parameters in temperate–tropical hybrids of maize.

Trait	Pn	Gs	Tr
DSG	0.615**	0.401	0.281
MNL	0.466*	0.563**	0.498*
MPP	0.793**	–0.014	–0.076
RSL	0.491*	0.514*	0.443
MCL	0.574**	–0.122	–0.173

DSG - degree of stay-green trait; Gs - stomatal conductance; MCL - chlorophyll content at maturity; MNL - number of visible green leaves at maturity; MPP - photosynthetic potential at maturity; PGY - plot grain yield; RGN - number of grains per row; Pn - net photosynthetic rate; RSL - ratio of visible source leaves at physiological maturity vs. at flowering; Tr - transpiration coefficient; * 0.01 < P ≤ 0.05; ** P ≤ 0.01.

tal yield after maize flowering. Therefore, increased yields depend on whether the visible ear leaves remain green between flowering and physiological maturity (Supplementary Figure 2). Thus, RSL must be considered when evaluating stay-green traits. In the present study, we found that RSL was significantly correlated with DSG, MNL, MPP, Pn, and Gs. A regression equation was established between RSL and DSG. Because RSL has high heritability and is easy to observe in the field, it may be a valuable tool for maize breeding and genetic research. Overall, our results demonstrate that RSL is an important indicator of stay-green traits.

The broad-sense heritabilities of RSL and MNL were relatively high; and those of DSG, MPP, and MCL were relatively low; these traits may be influenced by the environment, which could lead to poor results during early-generation selection and evaluation. Our findings suggested that multi-environment identification should be conducted during the late generation stages of maize breeding and population improvement projects. If temperate and tropical germplasms are used to breed stay-green inbred lines, RSL and MNL should be evaluated and selected during the early generations, and DSG, MPP, and MCL should be evaluated in multiple environments during late-generation selection.

Maize originated in the tropical regions of Central and South America. The tropical maize germplasm has much more genetic diversity than the temperate germplasm (Chen and Fan, 2001). The introduction of tropical maize genes into temperate lines may be an effective way to improve stay-green traits and stress tolerance (Goodman, 1999) To date, tropical maize varieties, such as Suwan and Tuxpeno, have been important heterosis groups in high-yield and high-stress resistance breeding programs in China's mountainous southwest (Chen et al, 2010). This fact provides strong evidence that the stay-green traits of tropical germplasm can play a key role in improving yield components and stress resistance traits in temperate maize.

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