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Dark Response of Seedlings Evaluated by Chlorophyll Concentration in Maize Natural Population

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Abstract

Chlorophyll, one of the major chloroplast components for photosynthesis, has a positive relationship with the photosynthetic rate. The chlorophyll content is an important assessment parameter in agronomy and plant biology research. This study was conducted to evaluate the natural variation in the chlorophyll content and to determine the differential response of the chlorophyll concentration to dark treatment in a natural population containing 139 maize inbreds. A five-fold higher chlorophyll concentration was measured in the light compared with the dark. Meanwhile, the wide variation in the chlorophyll concentration showed the differential response of the natural maize population to dark. Finally, we identified some inbreds that were highly sensitive to the dark with more than 70% difference between the light and dark treatment, such as Dan598, Zheng29, Zheng35, DH29, and R08, as well as some inbreds that had lower sensitivity to the dark, with less than 35% difference in the chlorophyll content between the light and dark treatment, such as Chuan48-2, 4F1, 303WX, 9642, and LY042.

Keywords

Maize, Inbred Lines, Seedlings, Chlorophyll, Dark Treatment

1. Introduction

As the most important pigment in the world, chlorophyll (Chl) plays a central role in photosynthesis and is re-

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sponsible for absorbing sunlight and converting it into chemical energy [1]. Maintaining a higher Chl content for a longer period in the reproductive stage is essential for increasing crop production [2] [3]. However, excess Chl in its free state produces reactive oxygen species and results in cell death [4]; therefore, plant cells must degrade these species through self-metabolism. Green plants contain two major Chl components, chlorophyll a (Chla) and chlorophyll b (Chlb), both with the same absorption spectra [5] [6]. Chla and Chlb function as photoreceptors in photosynthesis. Chla is a unique pigment that exists in all oxygenic photosynthetic organisms [7]. Chlb functions in the light-harvesting Chla/b protein complexes (LHCs) that harvest and transfer light energy to both photosystems [8].

The Chl content is an important experimental parameter in agronomy and plant biology research [9]. The Chl content is used as an effective index to assess photosynthetic efficiency in breeding programs [10]. This parameter is fundamental to understanding the response of a plant to the adverse environment in which it resides. Because it is a quantitative trait, it is difficult to select for Chl content in breeding programs [11]. The amount of Chl varies depending on many edaphic and climatic factors, such as salt stress [12]-[14], light [15]-[19], water stress [20]-[25], air pollution [26], fertilization [27], the vegetation period [28], plant species and leaf position [29]. Therefore, the tolerance of plants for stresses such as cold and drought [30]-[33] or low temperature [34] [35] can be determined by the Chl concentration.

Maize (*Zea mays* L.) is the world's most widely grown crop. It is also an important source of biofuel, animal feed and raw material in industry [36]. In addition, maize is an important model organism for cytogenetic, genetic, genomic, and functional genomic studies based on complete whole genome sequencing [37]. Increasing the grain yield and biomass per acre is one of the most important goals of maize production [38]. Nowadays, adverse weather and environmental diversity threaten maize yield, which has resulted in a research hotpot on the maize response to adverse environmental conditions. In this present study, a natural population with 139 inbred maize lines was used to investigate the variation in the Chl content before and after dark treatment and to evaluate the response of different inbred lines to dark treatment.

2. Materials and Methods

2.1. Experimental Conditions and Treatments

The inbred maize lines were provided by the National Maize Improvement Centre of China [39]. Two seed replicates were germinated in petri dishes for 4 days and transplanted to enriched soil (light nutritional soil: vermiculite = 1:1) under the following growth conditions: light/dark: 10/14 hours; temperature: 25˚C; white light: 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. When the second leaf had emerged completely, one replicate was placed under light and the other was placed in the dark for four days. All the phenotypic data were collected after four days of dark treatment.

2.2. Phenotypic Determination and Analysis

When two leaves had fully unfolding, two types of methods were used for Chl determination before and after 4 days of dark treatment. The first method was the use of the SPAD-502 chlorophyll meter (Minolta Camera Co., Osaka, Japan) and the second was the chemical method in which Chl from the green part of three plants was extracted using 80% acetone and measured using spectrophotometer at wavelengths of 645 nm and 663 nm [40]. All data were analyzed by using SPSS v.19 software and the Microsoft Excel program, including basic statistics description, mono factor analysis of variance and Pearsoncorrelation. The relative contents of chlorophyll a, b and total chlorophyll (Tchl = Chla + Chlb) and Chla/b (Chla/Chlb) were calculated using the following equations [40].

$$\text{Chla}(\text{mg/g}) = \text{N}\frac{\left(\left(12.21\times \text{A}_{663}\right)-\left(2.81\times \text{A}_{645}\right)\right)\times \text{V}}{\left(1000\times \text{W}\right)} \quad (1)$$

$$\text{Chlb}(\text{mg/g}) = \text{N}\frac{\left(\left(20.13\times \text{A}_{645}\right)-\left(5.03\times \text{A}_{663}\right)\right)\times \text{V}}{\left(1000\times \text{W}\right)} \quad (2)$$

$$\text{TChl}(\text{mg/g}) = \text{Chla} + \text{Chlb} \quad (3)$$

$$\text{Chl a/b} = \text{Chla/Chlb} \quad (4)$$

where A_{645} = absorbance at 645 nm; A_{663} = absorbance at 663 nm; V = solvent volume, W = fresh weight of the extracted tissue, N = dilution factor.

3. Results

3.1. Phenotypic Analysis

In general, there was wide variation in the SPAD value and the Chl concentration measured in the light and dark. The average SPAD values were 38.02 and 29.83 under light and dark conditions, respectively, and the variation was double that measured in the natural population. The largest values were 48.35 from ZaC546 and 42.12 from Shen137, and the smallest SPAD values were 26.33 from NMJT and 18.32 from HSBN under light and dark conditions, respectively. This suggested that a smaller variation of 22.03 was observed in the light compared with a greater variation of 23.80 in the dark (**Table 1**).

For the Chla, Chlb, and Tchl concentrations, there was two-fold difference between the light and dark for the average values; however, a small difference was observed in Chla/b under light and dark conditions. The average Chla concentration was 0.57 mg/g (range = 1.03 mg/g) in the light, compared with 0.22 mg/g (range = 0.54 mg/g) in the dark. The variation of Chlain the population was more than ten-fold, and ranged from 0.18 - 1.21 mg/g and 0.04 - 0.58 mg/g under light and dark conditions, respectively. The highest concentration was measured in Mo17 and the lowest in K14 in the light, compared with the highest concentration in TY-2 and the lowest in Zheng-35 in the dark. The Chlb concentrations ranged from 0.26 mg/g (238) to 0.96 mg/g (Qi319) in the light, *i.e*., almost a four-fold variation, compared with the range from 0.06 mg/g (Dan598) to 0.58 mg/g (TY-2) in the dark, *i.e*., almost a ten-fold variation. The average Chlb concentrations determined in the light and dark were 0.52 mg/g and 0.26 mg/g, respectively. The Tchl concentration in the light ranged from 0.55 - 2.05 mg/g, with a high variation of 1.51 mg/g and an average value of 1.09 mg/g, compared with the range of 0.12 - 1.16 mg/g, a variation of 1.05 mg/g and an average of 0.49 mg/g measured under dark conditions. The Chla/b range was 0.44 - 1.49 and the average was 1.07 under light conditions, compared with the range of 0.09 - 1.21 and the average of 0.84 under dark conditions. When we measured the Chla and Chlb percent in Tchl under light and dark conditions, only a small difference was found between the average values. A two-fold variation in the Chla percent in Tchl (range of 30.95 - 59.92) was observed in the light, and a seven-fold variation was measured in the dark (range of 8.26 - 54.79). With respect to the Chlb percent in Tchl, there was a two-fold variation under the light and dark conditions, where ranges of 40.08 - 69.05 and 45.21 - 91.74, respectively, were measured (**Table 1**). Under light conditions, a higher Chla percent was observed compared with a lower value under dark conditions. This indicates that the seedlings required the condition of a distribution in the Chla and Chlb concentrations to adapt to changes in the environment.

3.2. Correlation Coefficients between the SPAD Readings and the Chl Concentration

To explore the relationship between the SPAD readings and the Chl concentration, Pearson correlation coefficients were calculated for the different treatments as shown in **Table 2**. For the SPAD readings, there was a highly significant relationship, with a value of 0.561, between the light and dark. However, no significant correlation was detected between the SPAD readings and the Chl concentration for the light or dark treatment. Furthermore, there was no significant correlation between the light and dark treatment for a particular Chl component, including Chla/b. Chla under light conditions was highly significantly positively correlated with Chlb (0.845), Tchl (0.973), and Chla/b (0.608), similar to the dark conditions. In addition, a highly significant positive relationship was also found between Chlb and Tchl, *i.e*., 0.944 and 0.949 under light and dark conditions, respectively. Chla/b had a highly significant positive correlation with Chla (0.608) but not with Chlb or Tchl under light conditions, and similar associations were observed in the dark. There was no significant difference in the chlorophyll components between the different treatments (**Table 2**).

3.3. Difference in Chl Concentration under Light and Dark Conditions

The relative difference in the Chl content between the light and dark treatments was also analyzed for this population, as shown in **Table S1**. It was found that the Top 10 least difference for Tchl was less than 35%, and the inbreds were Chuan48-2, 4F1, 303WX, 9642, and LY042. However, the difference between the Chla and Chlb contents was less than 40%. Therefore, we considered that these inbreds could be insensitive to dark treatment. On the other hand, it was determined that the inbreds Dan598, Zheng29, Zheng35, DH29, and R08 obtained the Top highest difference percent of more than 70% for Tchl and Chla, and a difference percent of more than 60% for Chlb. Compared with Chlb, Tchl and Chla showed a more rapid degradation under dark treatment, which suggested that these inbreds might be highly sensitive to dark treatment.

In particular, there was a miniscule increment in Chla in inbreds 4F1 and 9642, with a small decrease in Tchl and a higher decrease in Chlb. Meanwhile, there was a small increment in Chla in inbred 303WX, which exhibited a 15% decrease in Chla and a 7% decrease in Tchl. This result verified that the mode of transition between Chla and Chlb during the dark treatment differed between individual inbred lines (**Table S1**).

4. Discussion

A significant relationship between SPAD readings and the Chl concentration was determined by using chemical extraction in a previous report [41]; however, this relationship was not significant in the current study. It was possible that a different leaf part was used for the chemical extraction. In this study, which was in contrast to previous reports, only the middle section of the first leaf was measured by using the SPAD-502 meter, and all the green parts of the seedling were used for Chl determination. In other studies, however, the same part of the ear leaf was used to evaluate the Chl content.

According to differences in their Chl contents between light and dark, inbred lines were identified as sensitive or insensitive to dark. Inbreds such as Dan598, Zheng29, Zheng35, DH29, and R08 will be likely senescence rapidly under dark conditions, and Chuan48-2, 4F1, 303WX, 9642, and LY042 exhibit a stay-green phenotype with slow senescence in the dark. In addition, there were several inbred lines that exhibited a miniscule increment in one type of Chl but a decrease in another type of Chl and in Tchl. It can be concluded that the mechanism of transition between Chla and Chlb is different in different inbred lines under dark conditions.

Contrary to our expectations, Qi319 was not identified as a stay-green inbred under dark induced-senescence at the seedling stage, although it was classified as a stay-green type at the mature stage according to reference [41]. We infer that the mechanisms in the maize leaf differ under natural and induced senescence. This is consistent with the conclusion from the differential expression analysis using transcription analysis [42].

5. Conclusion

The amount of Chl in leaves varies and is affected by many factors. In this study, it was observed that the Chl concentration was five-fold higher in the light than in the dark, and inbreds that were sensitive or insensitive to dark treatment were identified according to their different response to dark. During the dark treatment, the transition between Chla and Chlb was different in the different inbreds. Moreover, a difference was observed between natural and induced leaf senescence in maize. Determination of Chl content can be used in many field studies, and its related research should be increased and performed for efficient use of Chl content.

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Author Contributions

Conceived and designed the experiments: Xue, J. Q. and Xu, S. T.; Performed the experiments: Aye, N. C., Shi, Y. Q. and Li, Y. N.; Analyzed the data: Aye, N. C. and Xu, S. T.; Contributed to modify the manuscripts: Li, Y. J., Guo, D. W. and Xue, J. Q.; Wrote the paper: Xu, S. T. and Aye, N. C.,

Competing Interests

The authors have declared that no competing interests exist.

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Appendices

Table 1. Descriptive statistics of traits for Chl content in inbred lines population.

Trait	SPAD-L	SPAD-D	Chla-L	Chla-D	Chlb-L	Chla-D	TChl-L	TChl-D	Chla/b-L	Chla/b-D	Chla%-L	Chla%-D	Chlb%-L	Chlb%-D
Max	48.35	42.12	1.21	0.58	0.96	0.58	2.05	1.16	1.49	1.21	59.92	54.79	69.05	91.74
Min	26.33	18.32	0.18	0.04	0.26	0.06	0.55	0.12	0.44	0.09	30.95	8.26	40.08	45.21
Range	22.02	23.80	1.03	0.54	0.71	0.52	1.51	1.05	1.05	1.12	28.97	46.53	28.97	46.53
Mean	38.02	29.83	0.57	0.22	0.52	0.26	1.09	0.49	1.07	0.84	51.33	45.13	48.66	54.87
SD	4.34	4.81	0.18	0.10	0.13	0.10	0.30	0.19	0.19	0.17	4.85	5.85	4.85	5.85

Note: SPAD value, Chlorophyll compounds content; Chla, Chlb: chlorophyll a and chlorophyll b content (mg/g); TChl: total chlorophyll (Chla + b) (mg/g); Chla/b: the ratio of chlorophyll a and b content; Chla%, Chlb%: chlorophyll a and b percent in total chlorophyll content; SD = standard deviation, SPAD reading and Chla/b have no unit.

Table 2. Correlation coefficients for SPAD readings and Chl concentration.

Trait	SPAD-L	SPAD-D	Chla-L	Chlb-L	TChl-L	Chla/b-L	Chla-D	Chlb-D	TChl-D	Chla/b-D
SPAD-L	1									
SPAD-D	**0.561****	1								
Chla-L	0.165	0.181	1							
Chlb-L	0.280	0.257	**0.845****	1						
TChl-L	0.215	0.213	**0.973****	**0.944****	1					
Chla/b-L	−0.091	−0.050	**0.608****	**0.229**	**0.437**	1				
Chla-D	0.068	0.186	**0.245**	0.295	0.265	0.068	1			
Chlb-D	0.075	0.207	0.274	**0.373**	0.319	−0.024	**0.722****	1		
TChl-D	0.051	0.177	0.267	0.339	**0.297**	0.029	**0.951****	**0.949****	1	
Chla/b-D	−0.144	0.016	0.153	0.014	0.088	**0.350**	**0.533****	**0.258**	**0.436**	1

Note: SPAD: SPAD readings; Chla: Chlorophyll a; Chlb: Chlorophyll b; TChl: total chlorophyll (Chl a + b); Chla/b: the ratio of chlorophyll a and b; L: light; D: dark; **Significant at the 0.01 probability level.

Supplementary Information

Table S1. Relative chlorophyll differences between selected inbreds under dark treatment.

Inbred Lines	Differential %		
	Chla	Chlb	Tchl
chuan48-2	2	5	3
4F1	−6	14	5
303WX	15	−1	7
9642	−2	21	10
LY042	−32	34	12
B77	35	14	24
526018	30	22	26
TY2	33	18	26
4019	34	19	27
04K5686	33	22	28
384-2	31	31	31
05W002	31	32	32
238	39	26	33
M97	31	36	34
B113	36	32	34
EN25	46	22	35
D863F	43	26	35
LY	41	33	36
JY01	21	46	36
MN	39	32	36
B73	49	21	36
B110	36	37	36
C8605	49	26	37
L3180	37	38	38
Zheng30	37	39	38
Dan360	36	40	38
A619	46	29	38
FCD0602	42	37	39
835a	36	43	40
3411	41	38	40
Si273	49	34	41
Chang3	43	40	42
Lx9801	42	42	42
18-599	46	38	42
K12	45	42	43
LG001	45	42	43

Continued

04K5702	42	46	44
835b	47	41	44
TY1	52	35	44
501	47	42	45
C17	59	27	45
U8112	53	36	45
TY4	49	43	46
K14	30	54	47
Qi205	52	40	47
TY3	50	43	47
Zi330	53	40	47
07KS4	59	54	56
Shen137	64	48	56
Shen5003	64	48	57
Zheng22	63	50	57
268	61	54	57
TY9	63	52	58
7884-4Ht	66	50	59
Zheng28	66	49	59
Tian77	64	51	59
05WN230	58	60	59
Ye8001	64	52	59
Liao159	65	51	59
5213	64	54	59
TT16	65	55	59
HSBN	68	49	59
JH96C	65	53	60
IRF314	57	62	60
Ji63	64	57	60
HTH-17	66	56	61
Jiao51	65	55	61
975-12	72	52	62
Si434	70	52	62
Tie7922	70	54	63
Yan414	70	56	63
Q1261	71	56	63
Xun971	77	51	64
1323	64	64	64
R15X1141	68	60	64
Dan4245	70	56	64

Continued

TX5	67	62	64
K10	68	62	65
Dan3130	67	63	65
Ye52106	73	56	65
Yu87-1	70	60	65
7327	72	58	65
Dan340	81	45	65
Ye515	70	62	67
Mo17	71	61	67
8902	69	64	67
Liao138	74	60	67
Chang7-2	75	57	67
Ye478	73	63	68
W138	69	67	68
Lv28	71	65	68
J4112	76	61	69
ZB648	72	65	69
1462	51	46	48
WH413	44	53	49
812	51	49	50
Yu374	57	43	51
LXN	60	42	51
M153	56	47	52
5311	59	45	52
Dan599	58	47	52
M165	55	50	53
B111	61	46	53
HB	61	44	53
TY10	60	47	53
Zhi41	62	46	54
B151	60	47	54
HYS	59	50	54
Ji53	62	43	54
S22	61	46	55
P178	61	50	55
Zheng58	94	4	56
K22	60	51	56
R15	73	71	72
P138	77	66	72
Liao5114	78	66	72

Continued

ES40	75	69	72
Dan9046	78	65	73
Ji846	77	69	73
Si444	75	70	73
Zheng32	77	69	74
3H-2	76	72	74
HuangC	80	69	74
Zong3	78	70	75
Qi319	77	73	75
NMJT	82	71	78
ZaC546	82	74	78
R08	86	70	78
DH29	85	72	79
Zheng35	92	80	86
Zheng29	93	83	88
Dan598	92	88	90
JH59	48	46	47
Ji853	50	45	47
Si446	84	48	69
TY7	76	64	71
7381	49	44	47
Dong46	75	63	69
Nan21-3	74	63	69

Note: Chla: Chlorophyll a; Chlb: Chlorophyll b; Tchl: Total chlorophyll (Chl a + b).