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Full Length Research Paper

Photosynthesis, chlorophyll fluorescence and non-structural carbohydrates changes in husk leaves of maize in black soils region of Northeast China

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Two varieties of maize (*Zea mays* L.) grown in fields in Black soils of Northeast China were tested to study the variation of chlorophyll (Chl) concentration, Chl fluorescence, photosynthetic parameters, and non-structural carbohydrates in husk leaves of maize. The results showed that Chl a, Chl b and total Chl concentrations increased dramatically from silking to grain filling period. Increased maximum quantum efficiency of Photosystem II primary photochemistry (Fv/Fm), potential photochemical efficiency (Fv/Fo), net photosynthetic rate (Pn), transpiration rate (E) and stomatal conductance (g_s) were observed at grain filling period. The metabolic changes revealed that patterns of total soluble sugars (TSS) and sucrose were consistent with photosynthesis. Decline or a little increase of intercellular CO_2 concentrations (Ci) was found for both varieties, suggesting the existence of a non-stomatal limitation of photosynthesis in husk leaves in maize. Our results suggest that PS II apparatus remained functional in senescent husk leaves and that there was a continuously efficient use of the light captured by the remaining PS II apparatus.

Key words: Maize, husk leaves, chlorophyll concentration, chlorophyll fluorescence, photosynthesis, photoinhibition, total soluble sugar, sucrose.

INTRODUCTION

Measuring Chl fluorescence has been proven particularly useful for assessing photosynthetic performance under natural and some stress conditions (Waldhoff et al., 2002; Mallick and Mohn, 2003). It has become a method widely used to study the functioning of photosynthesis. Chl fluorescence measurements can provide non-intrusive and rapid information on light conversion, transfer, and dissipation in photosystem II (PS II) (Rascher et al., 2000; Schreiber et al., 1994). Without some fluorescence data, the investigation into the photosynthetic performance of plants under field conditions seems incomplete (Kim et al., 2007; Horst et al., 2008). As a special kind of maize (*Zea mays* L.), husk leaves contribute to grain formation more efficiently than foliar leaves (Fujita et al., 2001), and have different photosynthesis tissue (Hahnen et al., 2003; Ewing et al., 1998) and photosynthetic pathway from foliar leaves (Yakir et al., 1991; Antonielli et al., 1981). Thus, it is called improved leaves (Cheng and Pareddy, 1993; Antonielli et al., 1979). Some studies focused on the comparison of photosynthetic rates between husk leaves and foliar leaves. Fujita et al. (2001) found insignificant difference in Pn between husk leaves and foliar leaves, but opposite results was reported by Langdale et al. (1988). Although researches on Chl fluorescence and photosynthesis in maize have been well documented, most are about foliar leaves and there are limited systematic investigations on Chl fluorescence

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Abbreviations: Chl, chlorophyll; **Fv/Fm,** Photosystem II primary photochemistry; **Fv/Fo,** potential photochemical efficiency; **Pn**, net photosynthetic rate; **E,** transpiration rate; g_s, stomatal conductance; **TSS,** total soluble sugars; **Ci,** intercellular CO_2; **Fv/Fo,** potential photochemical efficiency; **Fm,** maximal fluorescence; **Fv,** variable fluorescence; **Fo,** primary fluorescence.

and photosynthesis, and even no report on diurnal fluctuations of Chl fluorescence in husk leaves of maize. Studying the change of Chl fluorescence and its relationship with photosynthesis in husk leaves of maize would be valuable for better understanding photosynthetic mechanism in husk leaves of maize and thereby selecting and breeding maize varieties with high photosynthetic efficiency.

Sugar is a major source to provide carbohydrate for the growth of maize. Soluble sugar in maize acted as a transportable and storable form of sugar derived from photosynthesis (Chen and Burris, 1990). Sugar accumulation or translocation of photosynthate was driven by the sucrose concentration gradient from source to sink. When the sucrose concentration in the source was relatively larger than in the sink, the amount of translocation was dependent on the strength of sucrose cleavage at the unloading site. Therefore, a close examination of husk leaves non-structural carbohydrates may provide a more meaningful insight to the physiological events related to grain filling.

In order to analyze changes of Chl concentration, Chl fluorescence and photosynthesis parameters and thereby to understand the photosynthetic mechanism of husk leaves of maize in Black Soils Region of Northeast China, two maize varieties (ZD 958 and ND 364) were chosen in the present experiment. Husk leaves of maize living in the field were used to explore possible metabolic alterations through the analysis of TSS and sucrose. Three major objectives were (a) to study the diurnal variation of Chl fluorescence and its changes along with the growth of maize; (b) to analyze changes of Chl concentration and photosynthesis parameters during the whole growth period; (c) to make clear non-structural carbohydrates changes in husk leaves of maize.

MATERIALS AND METHODS

Experimental design

This study was carried out at Agricultural Experimental Station (44° 12′ N, 125° 33′ E) of Northeast Institute of Geography and Agro-ecology, Chinese Academy of Sciences, at Dehui City of Jilin Province, China. The study site was in Black soils (Udolls, US Soil Taxonomy) region with fertile soil and abundant water resource. The study site lies in Temperate Zone with a continental monsoon climate. The mean annual temperature is 4.4ºC and the mean annual precipitation is 520.2 mm with more than 70% occurring in June, July and August. Soil physical and chemical properties are given in Table 1. Two maize varieties, (ZD 958 and ND 364), which are widely cultivated in Black soils of Northeast China nowadays, were used in this experiment. The experiment was arranged in two blocks, and each variety was cultivated in a block with an area of 1500 m^2. Samples were collected in "S" shape in each block. The study was randomly conducted in five sample plots with three replicates. Planting was done on April 27, 2007 with fertilizer consisting 60 kg of each N, P and K ha^{-1}. An additional 56 kg N ha^{-1} was side dressed as ammonium nitrate was applied on June 25, 2007. The field management was the same as the local.

Physiological measurements

Chl was extracted using ethanol and acetone (1:1, V/V). Concentrations of Chl a and Chl b in the extracts were determined by the absorbance at 663 and 645 nm, respectively, with a UV/VIS spectrophotometer (Zhang and Zhang, 2006).

Photosynthetic and Chl fluorescence measurements

Pn, E, g_s and Ci were measured using a portable open flow gas exchange system LI - 6400 (Li-cor Inc., USA) between 9:00 am and 11:00 am in the field. Measurements were conducted on attached and fully expanded leaves of maize plants. The photo synthetically active radiation was 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, CO_2 concentration was 350 $\mu mol \cdot mol^{-1}$ and leaf temperature was 25ºC. Chl fluorescence was measured simultaneously using a portable Chl fluorometer (OS-30P Inc., USA). After darkening the husk leaves for 30 min, primary fluorescence (Fo), maximal fluorescence (Fm), Fv/Fm were recorded after application of a 3s saturating pulse of radiation (3000 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Subsequently, variable fluorescence (Fv) and Fv/Fo were determined.

Contents of TSS and sucrose

TTS were determined by the method of Gao et al. (2006), and sucrose estimations were performed according to the methods of Riaze et al. (1985).

Data analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan test at 0.05 significance level to compare the means using SPSS 13.0 for Windows.

RESULTS

Photosynthetic pigments

Concentration of Chl a, Chl b, Chl a+b and Chl a/ Chl b were measured from silking to dough period (Table 2). The similar tendency of concentrations of Chl a、Chl b and Chl a+b was found for both varieties, that is, increased first and then decreased with the growing of maize, and finally reached the peak value at grain filling period. The concentration of ND 364 had a distinctly faster increase and lower decrease in comparison with ZD 958, and retained significantly higher value at dough stage. Chl a/ Chl b was similar in two varieties, and a decrease of Chl a/ Chl b ratio occurred at grain filling period for both varieties (Table 2), which was caused mainly by a higher increase in Chl b concentration than Chl a.

Chl fluorescence measurements

The diurnal change of Fo in husk leaves of two maize varieties had a single peak at 12:00, The Fo in husk leaves of two maize varieties increased from minima at 8:00 and achieved the highest level at 12:00, and then

Table 1. Some soil physical and chemical properties in the study site.

Parameter	Organic matter (g/kg)	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)	Avail. N (mg/kg)	Avail. P (mg/kg)	Avail. K (mg/kg)	pH
Mean	26.90	1.20	1.06	16.87	118.8	18.00	111.0	6.60

Table 2. Chlorophyll concentration in husk leaves of maize during different growing periods.

Growing periods	Varieties	Chlorophyll concentration (mg/g·FW)			Chl a/Chl b
		Chl a	Chl b	Chl a + b	
Silking period	ND 364	2.24±0.04d	1.67±0.02d	3.91±0.04d	1.36±0.01b
	ZD 958	2.69±0.05c	1.98±0.02c	4.67±0.07c	1.36±0.01b
Grain filling period	ND 364	4.58±0.06a	3.52±0.05a	8.10±0.09a	1.30±0.01b
	ZD 958	4.68±0.09a	3.55±0.04a	8.23±0.09a	1.32±0.02b
Dough period	ND 364	3.92±0.04b	2.56±0.02b	6.48±0.07b	1.53±0.02a
	ZD 958	2.91±0.03c	1.99±0.02c	4.90±0.05c	1.46±0.02a

Means (±SD) labeled with different letters within each column are significantly different ($P < 0.05$) by Duncan's test, n = 5.

declined from their maxima and remained at the lower level at 18:00. The diurnal changes of both Fv and Fm exhibited an opposite trend to Fo. In the morning, both Fv and Fm decreased continuously until it reached its minimum at 12:00, and in the afternoon a reverse trend occurred (Figure 1).

The diurnal changes of Fv/Fm and Fv/Fo in husk leaves of two maize varieties showed the same tendencies (Figure 2). Both Fv/Fm and Fv/Fo were the highest at 8:00 am, the lowest at noon, and mostly recovered from 16:00 to 18:00. Both Fv/Fm and Fv/Fo were higher in ZD 958 than that in ND 364 at the same time of the day. The pattern of diurnal changes for Fv/Fm and Fv/Fo in husk leaves of two maize varieties showed a sharp decrease during the morning and a smooth increase during the afternoon for both maize varieties studied. The changes of both Fv/Fm and Fv/Fo in husk leaves exhibited almost the same trend with maize growing, reaching peak value at grain filling period. Compared with Fv/Fo, the change amplitude of Fv/Fm in two varieties was narrow (Table 3).

Photosynthesis parameters measurements

As shown in Figure 3, Pn of husk leaves for both maize varieties increased first and then decreased, and reached peak values at grain filling period, which was consistent with the changes of E and g_s. Some differences between two varieties in changes of Ci, with highest Ci observed at silking period and dough period for ND 364 and ZD 958, respectively. Also, Pn and E in husk leaves of ZD 958 were significantly lower than that in ND 364, but no significant difference in g_s was found between two varieties.

Non-structural carbohydrates changes

Under field condition, both TSS and sucrose in husk leaves of maize increased from silking to grain filling period. TSS build-up was mainly attributed to the accumulation of sucrose, whose concentrations increased by 2.57 and 2.39 times in two maize varieties, respectively (Figure 4). Both TSS and sucrose decreased from grain filling to dough period, especially sucrose sharply deceased by 70.38 and 88.43% in two maize varieties, respectively, while TSS decreased smoothly approximately halved for both maize varieties.

DISCUSSION

Leaf Chl concentration is one of the most important factors in determining the photosynthetic rate (Mao et al., 2007). Chl a, is concentrated around PS II, and directly involved in determining photosynthetic activity (Sestak, 1996), and Chl b is usually the main component of light harvesting chlorophyll protein (Yamasato et al., 2005). Our results showed that the concentration of Chl and its components all reached peak value in grain filling period, demonstrating the husk leaves' ability to maximize the light-harvesting capacity (Dai et al., 2009). The declines in the Chl a/ Chl b ratio from silking to grain filling period

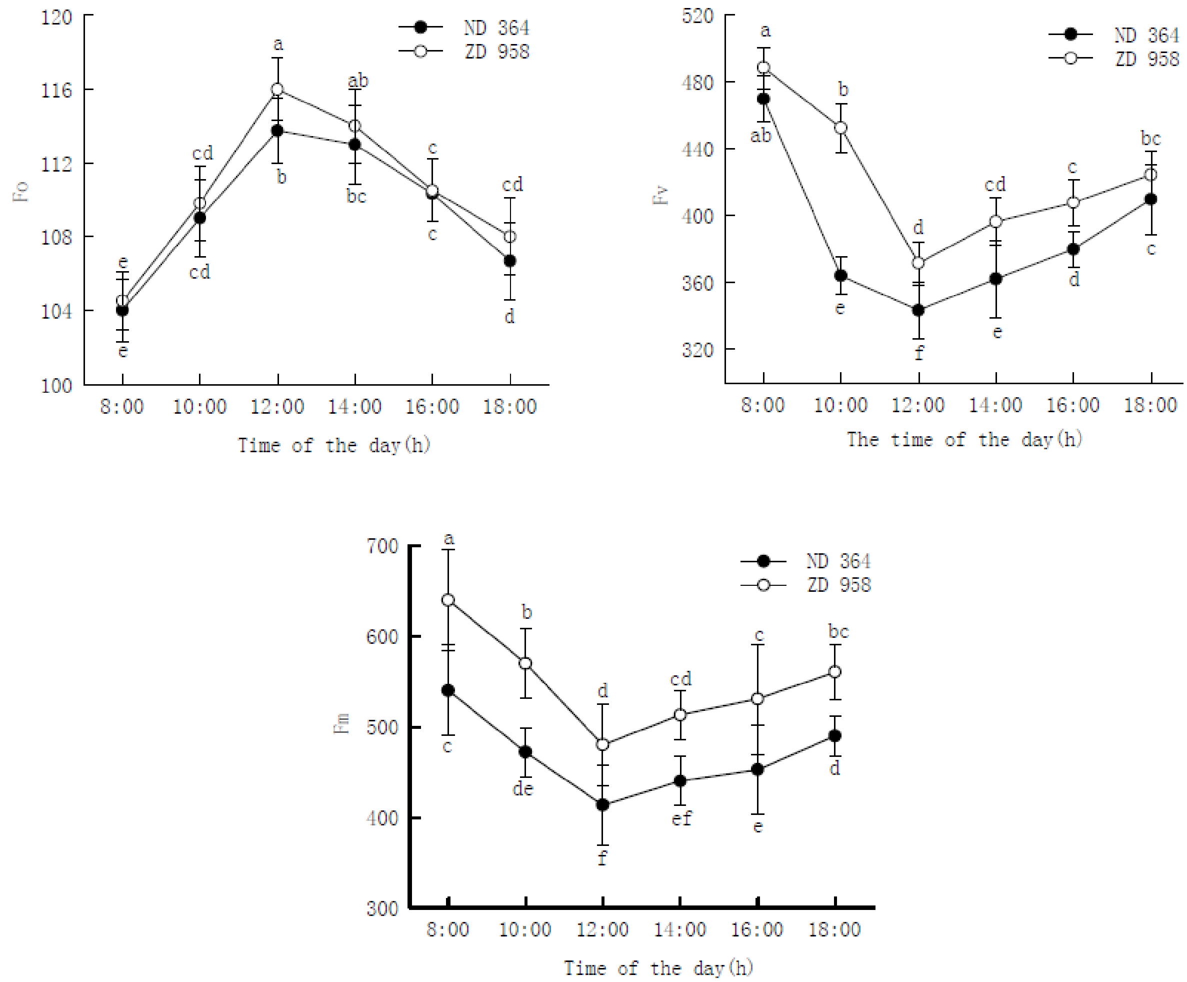

Figure 1. Diurnal changes of primary fluorescence (Fo), variable fluorescence (Fv), maximal fluorescence (Fm) in husk leaves of maize in different growing period. Means pairs followed by different letters are significantly different (p<0.5) by Duncan's test, n = 5.

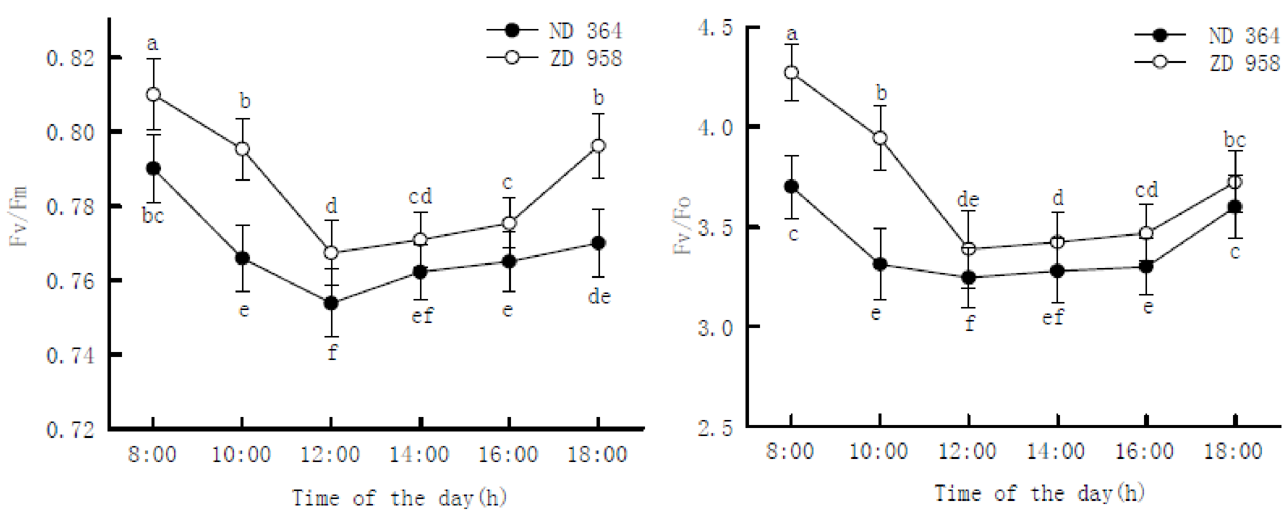

Figure 2. Diurnal change of maximum quantum efficiency of PS II primary photochemistry (Fv/Fm), and potential photochemical efficiency (Fv/Fo) in husk leaves of maize in different growing periods. Means pairs followed by different letters are significantly different (p < 0.05) by Duncan's test, n = 5.

Table 3. Changes in Fv/Fm and Fv/Fo of husk leaves of maize at different growing periods.

Growing periods	Varieties	Chl fluorescence parameters	
		Fv/Fm	**Fv/Fo**
Silking period	ND 364	0.76±0.06b	3.16±0.05e
	ZD958	0.77±0.07b	3.21±0.02e
Grain filling period	ND 364	0.80±0.07a	4.01±0.03b
	ZD958	0.82±0.06a	4.15±0.04a
Dough period	ND 364	0.77±0.08b	3.41±0.04d
	ZD958	0.78±0.07b	3.67±0.03c

Means (±SD) labeled with different letters within each column are significantly different (*P* < 0.05) by Duncan's test, n = 5.

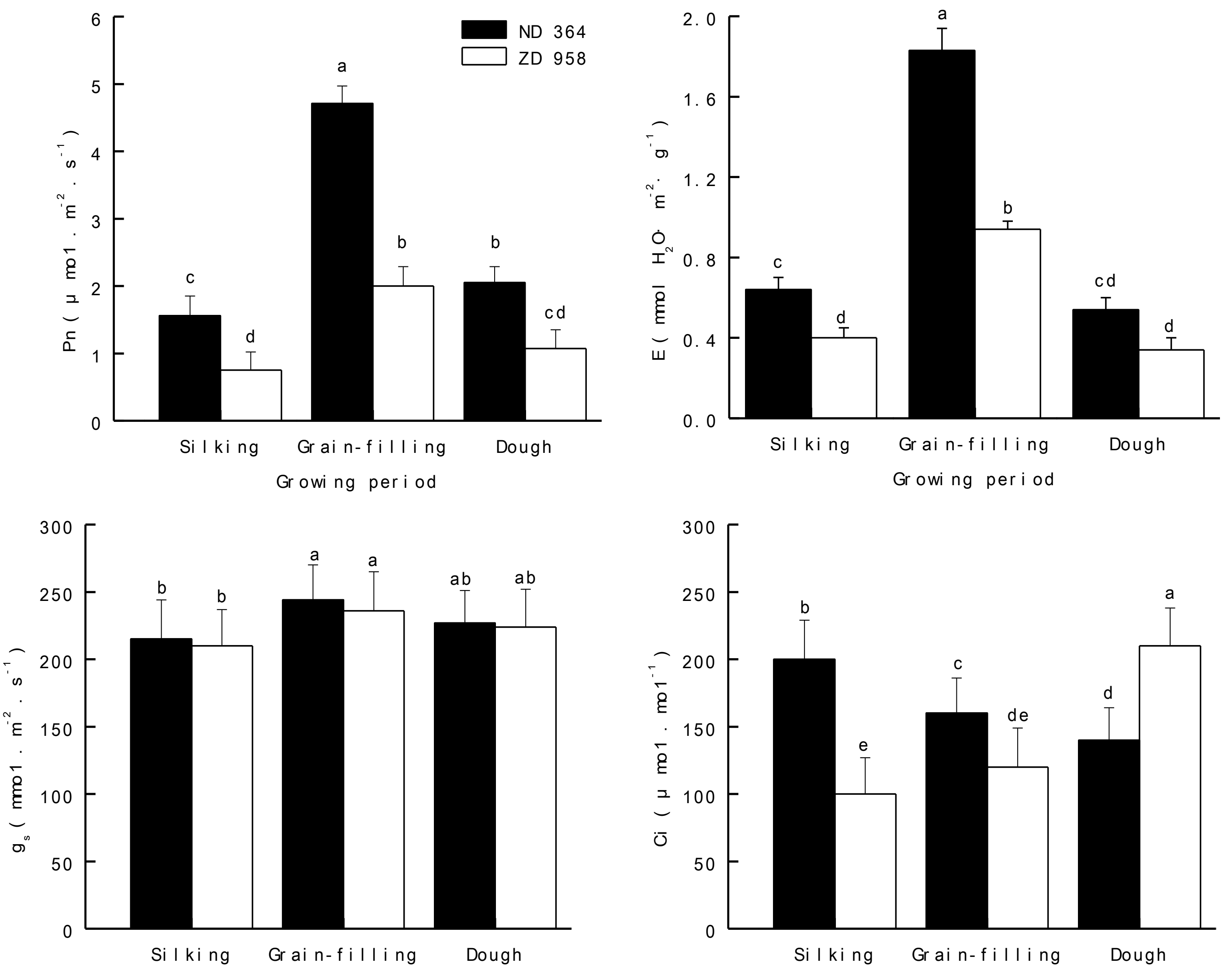

Figure 3. Changes of net photosynthetic rate (Pn), transpiration (E), stomatal conductance (g_s) and intercellular CO_2 concentration (Ci) in husk leaves of maize in different growing periods. Means pairs followed by different letters are significantly different (*p* < 0.05) by Duncan's test, n = 5.

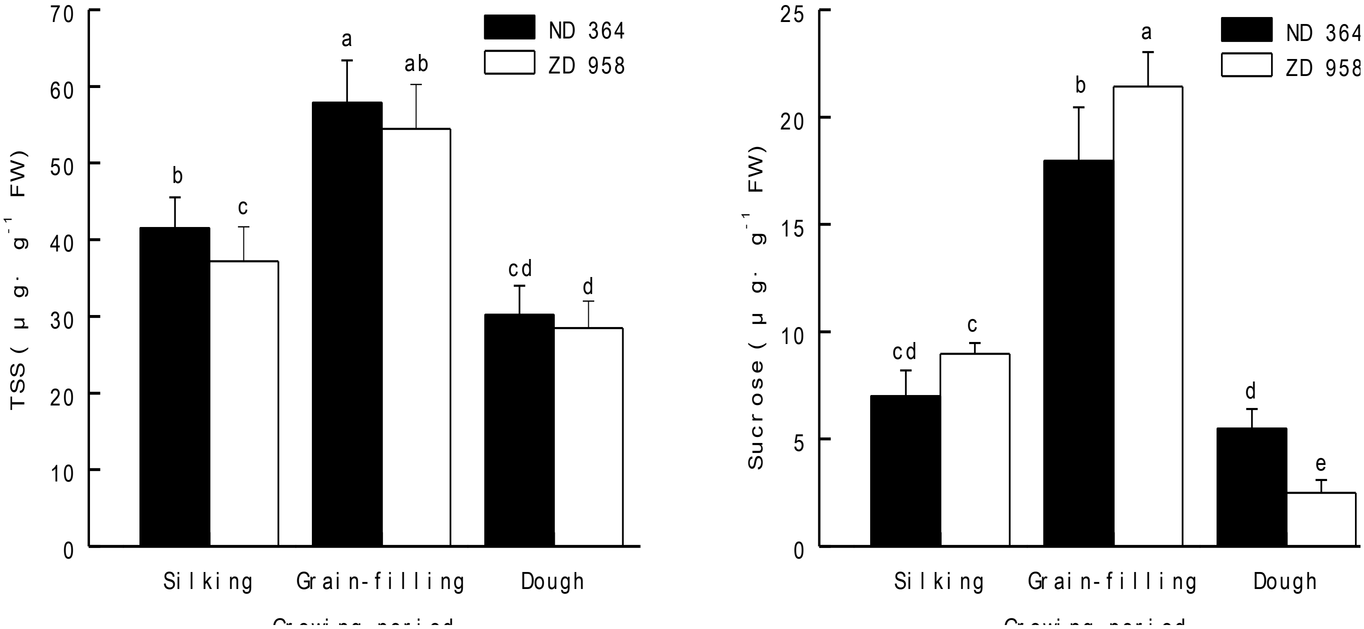

Figure 4. Concentration of total soluble sugar (TSS) and sucrose in husk leaves of maize in different growing periods. Means pairs followed by different letters are significantly different (*p*<0.05) by Duncan's test, n = 5.

might result from the faster increase for Chl b, and also might due to changes in organizations of both light-harvesting and electron transport components (Schiefthaler et al., 1997).

Decrease of Fo in the afternoon indicated that non-reversible photo inhibitory damage occurred in husk leaves. Reductions in Fv in the morning indicated that a structural and functional disorder of the photosynthetic apparatus and damage to the PS II had occurred (Pereira et al., 2000; Murkowski, 2001). It was confirmed as well by the progressive decrease in Fv/Fm observed in husk leaves grown at 12:00, and recoveries of Fv/Fm were found for both maize varieties after the mid-day, indicating that there was a protective mechanism which might aid in photosynthesis recovery when favorable conditions were restored (Wang et al., 2007). After the grain filling period, the photoinhibition resulted in the reduction of Fv/Fm ratio (Colom and vazana, 2003). In this situation, accumulation of reduced electron acceptors might boost the generation of reactive radicals and simultaneous occurrence of oxidative injuries (Souza et al., 2004). These oxidative injuries could enhance Chl degradation or the inhibition of its photosynthesis (Papadakis et al., 2004), which was confirmed by the changes of Chl concentrations and photosynthetic rates. Fv/Fm had little change during senescence, suggesting that PS II apparatus remained functional in senescent husk leaves and that there was a continuously efficient use of the light captured by the remaining PS II apparatus.

Pn increased accompanied by the increase of E and g_s at grain filling period, indicating that husk leaves could improve the gas exchange capacity through maintaining opened stomata, decreasing stomatal resistances, and increasing transpiration fluxes. Decline or slight increases of Ci were found at grain filling period, and there were significant differences between two varieties at all periods, which suggested the existence of a non-stomatal limitation of photosynthesis in husk leaves of maize. After grain filling period, the decreases of both Pn and g_s indicated that reductions in CO_2 assimilation for both maize varieties were partly attributed to stomatal limitation. The limitations to CO_2 assimilation caused by stomatal closure might push forward an imbalance between photochemical activity at PS II and electron requirement for photosynthesis, resulting in photo inhibitory damage of PS II reaction centers (Cui et al., 2006; Souza et al., 2004).

Patterns of TSS and sucrose were consistent with Pn, showing that husk leaves carbohydrates content was closely related to leaf photosynthetic capacity. Sucrose concentrations of the husk leaves were more variable than TSS, which showed that sucrose was the predominant transport and storage sugar, and complex physiological and biochemical changes might occur in the husk leaves. Sucrose was the main substance in the process of photosynthate translocation in maize. Photosynthate, in the form of sucrose was imported into developing grain, where it was converted into precursors of storage compounds. Leaf sucrose export was closely related to both source and sink strengths (Zhao and Oosterhuis, 2000). Additionally, sucrose synthase was most likely responsible for sink strength due to correlations between sucrose synthase activity with both maize growth and sucrose uptake (Zrenner et al., 1995). Our results supported a speculation that husk leaves were helpful in adjusting assimilate transport, because a high fraction of sucrose in husk leaves might be beneficial to carbohydrates translocation from husk leaves to grain. In conclusion, our results suggested higher matter translocation efficiency of husk leaves, where sucrose was the predominant transport and storage sugar. However, complex physiological and biochemical changes might occur in the husk leaves. Thus, it is necessary to conduct further studies on the way through which husk leaves affect photosynthesis in order to understand their role in metabolism of maize.

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REFERENCES

Antonielli M, Lupattelli M, Venanzi G (1981). Some characteristics of the chlorophyllous parenchyma of maize outside the leaf lamina. Plant Sci. Lett. 21: 107-119.

Antonielli M, Venanzi G (1979). Structural properties of the rachis and hypsophyll of the maize ear. Plant Sci. Lett. 15: 301-304.

Chen Y, Burris JS (1990). Role of carbohydrates in desiccation tolerance and membrane behavior in maturing maize seeds. Crop Sci. 30: 971-975.

Cheng PC, Pareddy DR (1993). Morphology and development of the tassel and ear. In The maize Handbook. (Freeling M and Walbot Veds.). New York: Springer-Verlag, Inc: pp. 37-47.

Colom MR, Vazzana CV (2003). Photosynthesis, PS II functionality of drought-resistant and drought-sensitive weeping lovegrass plants. Environ. Exp. Bot. 49: 135-144.

Cui LJ, Li JL, Fan YM, Xu T, Zhang Z (2006). High temperature effects on photosynthesis, PSII functionality and antioxidant activity of two Fescue arundinacea cultivars with different heat susceptibility. Bot. Stud. 41: 61-69.

Dai YJ, Shen ZG, Liu Y, Wang LL, Hananaway D, Lu HF (2009). Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. Environ. Exp. Bot. 65: 177-182.

Ewing RM, Jenkins, GI, Langdale JA (1998). Transcripts of maize RbcS genes accumulate differentially in C_3 and C_4 tissues. Plant Mol. Biol. 36: 593-599.

Fujita K, El-Shemy H, Sakurai1 N, Sendo S (2001). Sugar metabolism in expanding husk leaf of flint corn (*Zea mays* L.) genotypes differing in husk leaf size. Plant nutrition-food security and sustainability of agro-ecosystems. 92: 278-279.

Gao TP, Chen T, Feng HY, An LZ, Xu SJ, Wang XL (2006). Seasonal and annual variation of osmotic solute and stable carbon isotope composition in leaves of endangered desert evergreen shrub *Ammopiptanthus mongolicus*. S. Afr. J. Bot. 72: 570-578.

Hahnen S, Joeris T, Kreuzaler F, Peterhansel C (2003). Quantification of photosynthetic gene expression in maize C_3 and C_4. Photosynth Res. 75: 183-192.

Horst RJ, Engelsdorf T, Sonnewald U, Voll LM (2008). Infection of maize leaves with *Ustilago maydis* prevents establishment of C_4 photosynthesis. J. Plant Physiol. 165: 19-28.

Kim SH, Gitz DC, Sicher RC, Baker JT, Timlin DJ, Reddy VR (2007). Temperature dependence of growth, development, and photosynthesis in maize under elevated CO_2. J. Plant Physiol. 61: 224-236.

Langdale JA, Zelitch I, Miller E, Nelson T (1988). Cell position and light influence C_4 versus C_3 patterns of photosynthetic gene expression in maize. EMBO. J. 7: 3643-3651.

Mallick N, Mohn FH (2003). Use of chlorophyll fluorescence in metal-stress research: a case study with the green microalga *Scenedesmus*. Ecotox. Environ. Safe. 55: 64-69.

Mao LZ, Lu HF, Wang Q, Cai MM (2007). Comparative photosynthesis characteristics of *Calycanthus chinensi*s and *Chimonanthus praecox*. Photosynthetica 45: 601-605.

Murkowski A (2000). Heat stress and spermidine, effect on chlorophyll fluorescence in tomato plants. Biol. Plant. 44: 53-57.

Papadakis IE, Dimassi KN, Bosabalidis AM, Therios IN, Patakas A (2004). Effects of B excess on some physiological and anatomical parameters of'Na-velina'orange plants grated on two rootstocks. Environ. Exp. Bot. 51: 247-257.

Pereira WE, de Siqueira DL, Martynez CA, Puiatti M (2000). Gas exchange and chlorophyll fluorescence in four citrus rootstocks under aluminium stress. J. Plant Physiol. 157: 513-520.

Rascher U, Liebig M, Lüttge U (2000). Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. Plant Cell Environ. 23: 1397-1405.

Riaze A, Matsuda K, Arslan A (1985). Water-stress induced changes in concentrations of praline and other solute in growing regions of young barley leaves. J. Exp. Bot. 36: 1716-1725.

Schiefthaler U, Russell AW, Bolhar-Nordenkampf HR, Critchley C (1997). Photoregulation and photodamage in *Scheferaarboricola* leaves adapted to different light environments. Aust. J. Plant Physiol. 26: 485-494.

Schreiber U, Bilger W, Neubauer C (1994). Chlorophyll fluorescence as a noninstructive indicator for rapid assessment of *in vivo* photosynthesis. *In*: Schulze, E.D., Caldwell, M.M. (eds.) Ecophysiology of photosynthesis. Springer, New York, pp: 49-70.

Sestak Z (1996). Liminations for finding linear relationship between chlorophyll content and photosynthetic activity. Biol. Plant. 8: 336-346.

Souza RP, Machadoa EC, Silva JAB, Lagoa AMMA, Silveira JAG (2004). Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. Environ. Exp. Bot. 51: 45-56.

Waldhoff D, Furch B, Junk WJ (2002). Fluorescence parameters, chlorophyll concentration, and anatomical features as indicators for flood adaptation of an abundant tree species in central Amazonia: *Symmeria paniculata*. Environ. Exp. Bot. 48: 225-235.

Wang H, Wang FL, Wang G, Majourhat K (2007). The responses of photosynthetic capacity, chlorophyll fluorescence and chlorophyll content of nectarine (*Prunus persica var.Nectarina Maxim*) to green house and field conditions. Scientia Horticulture 112. 66-72.

Yakir D, Osmond B, Giles L (1991). Autotrophy in maize husk leaves. Plant Physiol. 97: 1196-1198.

Yamasato A, Nagata N, Tanaka R, Tanaka A (2005). The N-terminal domain of chlorophyllide a oxygenase confers protein instability in response to chlorophyll b accumulation in Arabidopsis. Plant Cell 17: 1585-l597.

Zhang ZA, Zhang MS (2006). Experimental guide for plant physiology. High education, Beijing.

Zhao D, Oosterhuis DM (2000). Dynamics of non-structural carbohydrates in developing leaves, bracts and floral buds of cotton. Environ. Exp. Bot. 43: 185-195.

Zrenner R, Salanoubat M, Willmitzer L, Sonnewald U (1995). Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (*Solanum tuberosum* L.). Plant J. 7: 97-107.