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Genetic variation in soybean photosynthetic electron transport capacity is related to plastocyanin concentration in the chloroplast*

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Abstract

Fifteen ancestral genotypes of United States soybean cultivars were screened for differences in photosynthetic electron transport capacity using isolated thylakoid membranes. Plants were grown in controlled environment chambers under high or low irradiance conditions. Thylakoid membranes were isolated from mature leaves. Photosynthetic electron transport was assayed as uncoupled Hill activity using 2,6-dichlorophenolindophenol (DCIP). Soybean electron transport activity was dependent on genotype and growth irradiance and ranged from 6 to 91 mmol DCIP reduced [mol chlorophyll]$^{-1}$ s^{-1}. Soybean plastocyanin pool size ranged from 0.1 to 1.3 mol plastocyanin [mol Photosystem I]$^{-1}$. In contrast, barley and spinach electron transport activities were 140 and 170 mmol DCIP reduced [mol chlorophyll]$^{-1}$ s^{-1}, respectively, with plastocyanin pool sizes of 3 to 4 mol plastocyanin [mol Photosystem I]$^{-1}$. No significant differences in the concentrations of Photosystem II, plastoquinone, cytochrome b_6f complexes, or Photosystem I were observed. Thus, genetic differences in electron transport activity were correlated with plastocyanin pool size. The results suggested that plastocyanin pool size can vary significantly and may limit photosynthetic electron transport capacity in certain species such as soybean. Soybean plastocyanin consisted of two isoforms with apparent molecular masses of 14 and 11 kDa, whereas barley and spinach plastocyanins each consisted of single polypeptides of 8 and 12 kDa, respectively.

Abbreviations: DAP – days after planting; DCIP – 2,6-dichlorophenolindophenol; LiDS – lithium dodecyl sulfate; PPFD – photosynthetic photon flux density (μmol photons m^{-2} s^{-1}); PS I – Photosystem I; PS II – Photosystem II; P700 – reaction center of Photosystem I

Introduction

Genetics provides a number of approaches for the study of higher plant photosynthesis. One approach is to screen normal genotypes for differences in photosynthesis to identify rate-limiting steps in a fully functional photosynthetic apparatus. Screening of germplasm has revealed variation in photosynthesis (Wells et al. 1982; Carver et al. 1989), and breeding for enhanced photosynthesis has been correlated with improved yield (Ashley and Boerma, 1989). Genetic variation in photosynthetic electron transport has been demonstrated in barley (Burkey, 1994), fescue (Krueger and Miles, 1981) and wheat (Zelenskii et al. 1978) and has been linked to differences in plastocyanin content in barley (Burkey, 1994) and fescue (Krueger et al. 1984). The objectives of this study were to determine if genetic variation in photosynthetic electron transport exists in soybean and identify biochemical factors that limit electron transport activity.

Materials and methods

Plant growth and harvesting

The initial screening of fifteen soybean genotypes was conducted in the phytotron facilities at the Southeastern Plant Environment Laboratories. Seeds were germinated in moist paper towels for 2 d and then transplanted into pots of soil. Standard phytotron protocols were used for soil preparation, watering and fertilization (Downs and Bonaminio, 1976). Plants were grown at temperatures of 26 °C and 22 °C for light and dark periods, respectively, with a photoperiod of 17 h to maintain vegetative growth. Fluorescent and incandescent lamps provided a PPFD of 200 to 300 μmol photons m^{-2} s^{-1} measured at leaf level. The youngest fully developed trifoliolate leaf (third or fourth leaf) was harvested and analyzed at 21 to 24 DAP.

Additional comparisons were made between selected soybean genotypes grown under moderate or high irradiance. Seeds were germinated in moist paper towels for 2 d and then transplanted into pots of soil consisting of Redi Earth Peat-Lite Mix,* Perlite, sand, and Osmocote slow release fertilizer. Plants were watered as needed and fertilized weekly with a complete nutrient solution. The photoperiod was 17 h and the day/night temperature was 26 °C/22 °C. The moderate irradiance chamber utilized fluorescent and incandescent lamps that provided an average PPFD of 430 μmol photons m^{-2} s^{-1}. The high irradiance chamber utilized a microwave powered fusion lamp that provided an average PPFD of 1500 μmol photons m^{-2} s^{-1} for 6 h during the middle of the photoperiod and 480 μmol photons m^{-2} s^{-1} for the remainder of the 17 h light period. The third trifoliolate leaf was harvested and analyzed at 22 to 25 DAP.

Barley and spinach were grown in the soil mixture described above, watered as needed and fertilized weekly with a complete nutrient solution. Barley was grown at 21 °C with a 16 h photoperiod in the fusion lamp chamber with an average PPFD of 1000 μmol photons m^{-2} s^{-1}. The third leaf of barley was harvested and analyzed at 17 DAP. Spinach was grown at 21 °C with a 10 h photoperiod using fluorescent and incandescent lamps that provided an average PPFD of 600 μmol photons m^{-2} s^{-1}. The first true leaf of spinach was harvested and analyzed at 21 DAP.

Thylakoid membrane isolation

Thylakoid membranes were isolated as described previously (Burkey and Wells 1991). The final thylakoid pellets were resuspended in buffer [0.4 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 0.2% (w/v) BSA, and 50 mM Tricine-NaOH (pH 7.8)], stored on ice during electron transport measurements and then frozen with liquid nitrogen before long term storage at –75 °C.

Activity measurements

Isolated thylakoid membranes were assayed for uncoupled photosynthetic electron transport as DCIP reduction at 580 nm (ϵ_{580} = 18.9 mM^{-1} cm^{-1}) with water as the electron donor (Izawa, 1980). The assay contained 0.1 M sorbitol, 40 mM Tricine-NaOH (pH = 8.0), 1 mM NH_4Cl, 30 μM DCIP, and 2 μg Chl ml^{-1}. Saturating actinic light was passed through a red filter (Corning 2–58) and the detector was protected with a blue filter (Corning 4-96).

Chl determination

The Chl concentration of thylakoid membrane preparations was determined spectrophotometrically with dimethylformamide (Moran 1982).

Gel electrophoresis

Analytical or preparative denaturing PAGE was conducted according to Laemmli (1970) with a gel concentration of 15% (w/v) acrylamide. LiDS was substituted for SDS. Samples were denatured by boiling for 5 min in electrophoresis sample buffer with the mercaptoethanol concentration increased to 10% (v/v). Electrophoresis was conducted overnight at 8 °C. Gels were either stained with Coomassie Blue R250 for photography or electroblotted to nitrocellulose for immunochemical analysis.

Plastocyanin isolation and antibody production

Plastocyanin was purified from barley, soybean, and spinach using a procedure published for spinach plastocyanin (Davis and San Pietro 1979). The concentra-

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tion of plastocyanin was determined from the 597 nm absorbance of the oxidized protein using an extinction coefficient of 4.9 mM^{-1} cm^{-1} (Davis and San Pietro 1979). Purified plastocyanin from barley, soybean, and spinach had oxidized A_{275nm}/A_{597nm} absorbance ratios of 1.6, 2.0, and 1.2, respectively.

Rabbit antiserum for soybean plastocyanin was produced using the 14 kDa isoform of soybean plastocyanin as the antigen. The 14 kDa isoform was purified from the final soybean plastocyanin preparation using LiDS-PAGE in 15% (w/v) gels where the 14 and 11 kDa isoforms are separated. The 14 kDa isoform was electroeluted from Coomassie stained preparative gels, precipitated with acetone, and dissolved in 10 mM sodium phosphate pH 7.0 containing 150 mM NaCl and 0.4% (w/v) SDS. Custom rabbit antiserum for soybean plastocyanin was prepared by Berkeley Antibody Company (Richmond, California). Affinity purified soybean plastocyanin antibodies were prepared using a protocol described previously (Burkey 1994). Rabbit antiserum for barley plastocyanin was prepared and characterized previously (Burkey 1993).

Quantitation of thylakoid membrane complexes

The concentration of PS II reaction centers was determined by measuring the specific binding of [^{14}C]-atrazine to the high affinity site on thylakoid membranes (Tisher and Strotmann 1977). Atrazine binding was conducted in a reaction mixture that contained 0.02 to 0.2 μM [^{14}C]-atrazine (specific activity = 62 μCi mg^{-1}), 25 mM Tricine-NaOH pH 8.0, 50 mM NaCl, 5 mM $MgCl_2$, and thylakoid membranes equivalent to 25 μg Chl ml^{-1}.

Cyt f content was determined from reduced (hydroquinone) minus oxidized (potassium ferricyanide) difference spectra. Thylakoid membranes (100 μg Chl ml^{-1} final concentration) were solubilized in 50 mM Tricine-NaOH pH 7.8, 5 mM $MgCl_2$, 10 mM KCl, and 0.5% (w/v) Triton X-100 and non pigmented particulates removed by centrifugation. Cyt f was determined as previously described (De la Torre and Burkey 1990) using an extinction coefficient of 18 mM^{-1} cm^{-1} (Hurt and Hauska 1981).

The concentration of PS I reaction centers was determined from the reversible light-induced P700 absorbance change at 697 nm using an extinction coefficient of 64 mM^{-1} cm^{-1} (Hiyama and Ke 1972). Thylakoid membranes (25 μg Chl ml^{-1} final concentration) were solubilized in 50 mM Tricine-NaOH pH 7.8, 5 mM $MgCl_2$, 10 mM KCl, and 0.2% (w/v) Triton X-100 and non pigmented particulates removed by centrifugation. P700 was determined as previously described (De la Torre and Burkey 1990) in the presence of 5 mM sodium ascorbate and 50 μM methyl viologen.

Quantitation of plastoquinone

Plastoquinone pool size was determined from measurements of the area over the Chl fluorescence induction curve in the absence and presence of the electron transport inhibitor DCMU (McCauley and Melis, 1986) using a published protocol (Burkey 1994).

Quantitation of plastocyanin

Plastocyanin content was determined using quantitative western blots as described by Burkey (1993). Thylakoid membranes were thawed and aliquots containing a known amount of Chl extracted with four volumes of ice cold acetone. The precipitated proteins were collected by centrifugation at 12 000 × *g* for 5 min. Samples were prepared for electrophoresis as described above. Gels were loaded on the basis of Chl equivalents in the sample prior to acetone precipitation. For analysis of plastocyanin in leaf tissue, leaf disks were extracted directly in electrophoresis sample buffer (0.5 ml cm^{-2}) with a glass tissue homogenizer. The whole leaf extract was heated to 55 °C for 10 min and insoluble material removed by centrifugation at 12 000 × *g* for 5 min. Gels were loaded with supernatant equivalent to a known leaf area. For quantitation, known amounts of purified plastocyanin from the appropriate plant species were analyzed in separate lanes on each gel for comparison with unknowns. Soybean and spinach plastocyanin standards (0.5 to 10 pmol) and unknowns were detected on immunoblots using affinity purified soybean plastocyanin antibodies. Barley plastocyanin standards (10 to 100 pmol) and unknowns were detected with a 500-fold dilution of barley plastocyanin antiserum. Developed blots were scanned with an LKB laser densitometer.

Results and discussion

Plastocyanin characterization

Purified plastocyanin from barley and spinach migrated as single bands of 8 kDa and 12 kDa, respectively, in denaturing LiDS-PAGE gels (Figure 1). Purified soy-

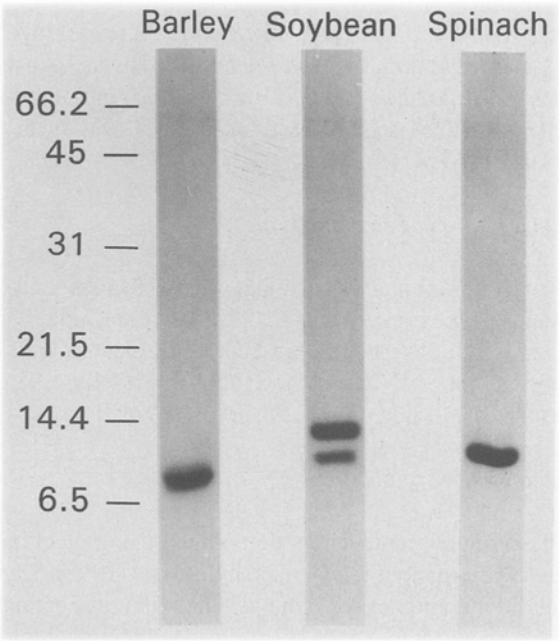

Figure 1. LiDS-PAGE analysis of purified plastocyanins from barley, soybean, and spinach. Denatured plastocyanins (1000 pmol per lane) were separated in 15% (w/v) gels and stained with Coomassie Blue R250. Apparent molecular masses are indicated in kDa.

bean plastocyanin consisted of two bands at 14 and 11 kDa (Figure 1, lane 2 in Figure 2). Antibodies produced using the 14 kDa isoform as the antigen detected both isoforms in thylakoids (Figure 2, lanes 6 and 7) and purified protein (Figure 2, lane 8). Both isoforms were observed on immunoblots when soybean leaf tissue was extracted directly into electrophoresis sample buffer (data not shown), providing evidence that the 11 kDa isoform was not a degradation product generated during isolation. The 14 and 11 kDa plastocyanin isoforms were observed in each of the nine soybean genotypes examined to date (data not shown).

Multiple plastocyanin isoforms have been observed in several plant species (Dimitrov et al. 1993), but these dimorphisms involve amino acid substitutions that do not alter the length of the polypeptide chain. The 3 kDa difference in apparent molecular mass between the two soybean isoforms suggested a different mechanism. Potential explanations include the existence of two plastocyanin genes in soybean or the differential processing of a common plastocyanin precursor during import of the polypeptide from the cytoplasm into the chloroplast lumen. At present, the molecular basis for the two isoforms is not known.

Immunochemistry

Rabbit antiserum produced using the 14 kDa soybean plastocyanin isoform was specific for plastocyanin, but had a relatively low titer. No signal was detected in soybean thylakoid membrane proteins by the preimmune serum (Figure 2, lane 3). The antiserum produced a specific signal on immunoblots of soybean thylakoids (Figure 2, lanes 4–5) that corresponded with purified plastocyanin (Figure 2, lanes 2 and 8). Sensitivity was increased and non-specific signals at high mol wt were reduced by using affinity purified soybean plastocyanin antibodies (Figure 2, lanes 6–8). The increased sensitivity was required for quantitative work with certain soybean genotype/growth irradiance combinations where plastocyanin levels were extremely low. The affinity purified antibodies allowed detection of plastocyanin in the 0.5 to 10 pmol range (Figure 3).

Genotype and growth irradiance effects on soybean photosynthetic electron transport capacity

Significant genetic variation in photosynthetic electron transport capacity was observed in soybean, but the expression of genotypic differences was dependent on growth irradiance. Electron transport activity varied between 6 and 51 mmol DCIP [mol Chl]$^{-1}$ s^{-1} for fifteen soybean genotypes, an 8-fold difference in activity, when plants were grown in the phytotron at a PPFD of 200 to 300 (Table 1). Activity increased for all genotypes when grown at a PPFD of 430, but the genotypic variation was only 1.8-fold (Table 2). Further increases in activity were observed at the highest growth irradiance (PPFD = 1500) where genotypic variation was less than 20% (Table 2). Thus, genetic differences in soybean were greatest when plants were grown at low irradiance. This point is illustrated more clearly by comparing the genotypes CNS and A.K. (Harrow) which represent the high and low activity extremes, respectively. CNS exhibited much higher activity in the phytotron relative to other genotypes and activity increased by a factor of 1.8 at the highest growth irradiance. In contrast, A.K. (Harrow) activity was extremely low in the phytotron and exhibited a 13-fold increase at high irradiance.

The fifteen soybean ancestors in our study contribute approximately 60% of the genes found in modern United States cultivars (Gizlice et al. 1994). Thus, genetic diversity in this group was likely passed on to modern cultivars. The presence of diversity for photosynthetic electron transport capacity in applied

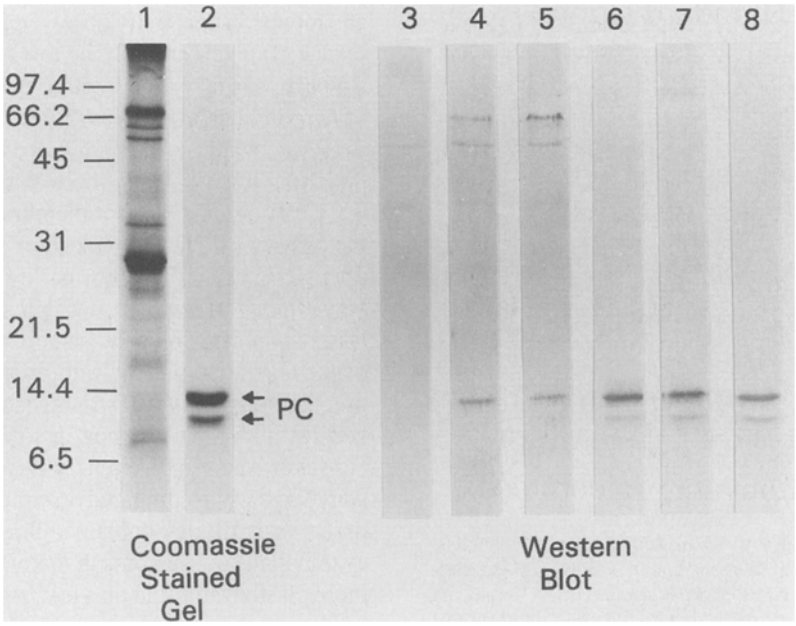

Figure 2. Immunochemical analysis. Soybean thylakoid membrane proteins (equivalent to 15 μg Chl) or purified soybean plastocyanin were separated in 15% (w/v) LiDS-PAGE gels and either stained with Coomassie Blue R250 (lanes 1–2) or subjected to immunoblot analysis (lanes 3–8). Lane 1: Stained gel of acetone precipitated soybean thylakoid membrane proteins. Lane 2: Stained gel of purified soybean plastocyanin (1000 pmol). Lane 3: Immunoblot of acetone precipitated soybean thylakoid membrane proteins developed with a 1/500 dilution of preimmune serum. Lane 4: Immunoblot of soybean thylakoid membrane proteins developed with a 1/500 dilution of soybean plastocyanin antiserum. Lane 5: Immunoblot of acetone precipitated soybean thylakoid membrane proteins developed with a 1/500 dilution of soybean plastocyanin antiserum. Lane 6: Immunoblot of soybean thylakoid membrane proteins developed with affinity-purified soybean plastocyanin antibodies. Lane 7: Immunoblot of acetone precipitated soybean thylakoid membrane proteins developed with affinity-purified soybean plastocyanin antibodies. Lane 8: Immunoblot blot of soybean plastocyanin (25 pmol) developed with affinity-purified soybean plastocyanin antibodies.

breeding would suggest that enhanced electron transport activity may have commercial importance that should be explored.

CNS, the ancestor with the highest electron transport activity, appears in the pedigree of more than 100 cultivars developed over 50 years (Carter et al. 1993). Study of cultivars derived from this ancestor through consecutive eras of soybean breeding would afford an opportunity to determine the selective advantage of enhanced electron transport activity on soybean improvement. An increased frequency of high electron transport capacity in recently developed cultivars as compared to older types would argue that genes controlling this trait are important in agriculture.

Biochemical basis for genetic variation in soybean photosynthetic electron transport

Genetic effects on the concentration of the major electron transport complexes did not explain the differences in activity. The concentrations of PS II reaction centers, Cyt b_6f complexes, and PS I reaction centers varied by 32%, 39%, and 23%, respectively, within the genotypes examined (Table 1). This variability was small compared to the 8-fold differences in activity. In fact, examples were found where differences in the concentration of the major complexes were clearly not related to activity. Electron transport capacity was four-fold greater in CNS than in S-100, yet S-100 contained the same or greater quantities of each thylakoid complex (Table 1).

Alternatively, electron transport activity could be related to differences in the concentrations of plastoquinone and plastocyanin, the mobile carriers that transfer electrons between PS II and the Cyt b_6f complex and the Cyt b_6f complex and PS I, respectively. Electron transport capacity varied between 6 and 91 mmol DCIP [mol Chl]$^{-1}$ s^{-1} when four genotypes were grown at three levels of PPFD (Table 2). Plastoquinone levels were similar for all combinations of genotype and growth irradiance (Table 2), evidence that plastoquinone was not a limiting factor. In contrast, plastocyanin levels varied between 0.06 and 3.2 mmol plastocyanin [mol Chl]$^{-1}$ which corresponded to

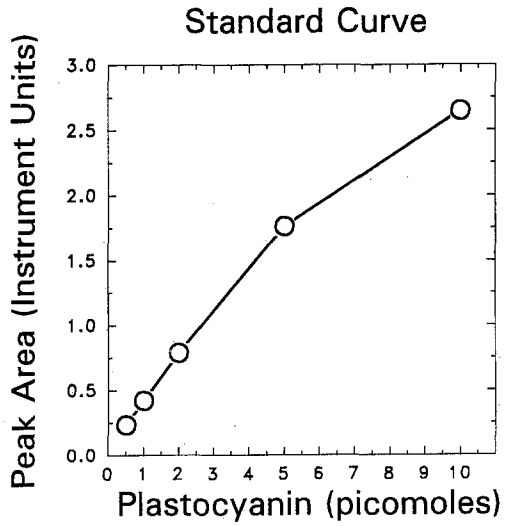

Figure 3. Quantitation of soybean plastocyanin on immunoblots. Standards were prepared by dilution of purified native soybean plastocyanin with denaturing electrophoresis sample buffer. The amount of each standard was based on the oxidized copper absorbance of the native plastocyanin stock solution. Known amounts (0.5, 1.0, 2.0, 5.0, and 10.0 pmol) of plastocyanin were analyzed in separate lanes of a 15% (w/v) gel, electroblotted to nitrocellulose and the signal detected as described previously (Burkey 1993) using affinity purified soybean plastocyanin antibodies. After color development, the signal in each lane was integrated by scanning densitometry. The combined signal from the 14 and 11 kDa isoforms were used to generate the standard curve.

a plastocyanin pool size of 0.02 to 1.3 defined as mol plastocyanin [mol P700]$^{-1}$ (Table 2). Thus, genetic variation in soybean electron transport capacity was associated with differences in plastocyanin concentration. Previous studies of fescue (Krueger and Miles 1981; Krueger et al. 1984) and barley (Burkey 1994) indicated that genotypes with high electron transport activity also contained more plastocyanin.

Species comparisons

Species differences in electron transport capacity and plastocyanin pool size were observed when soybean was compared with barley and spinach. Barley and spinach electron transport rates were greater than 100 mmol DCIP [mol Chl]$^{-1}$ s^{-1}. Plastocyanin pool sizes for barley and spinach were in the range of three to four (Table 3), similar to values reported in the literature for fescue (Krueger et al. 1984), spinach (Haehnel et al. 1989) and high activity genotypes of barley (Burkey, 1994). Soybean electron transport activity was lower with maximum rates in the range of 80 to 90 mmol DCIP [mol Chl]$^{-1}$ s^{-1} for plants grown at high PPFD and much lower activity observed at lower growth irradiance (Tables 2 and 3). The low activity observed for soybean was associated with a plastocyanin pool size of approximately 1 or less (Tables 2 and 3).

Plastocyanin is a soluble protein localized in the thylakoid lumen. Thus, the loss of plastocyanin during isolation of thylakoid membranes is an important factor to consider, particularly if the loss is different for each species. For both barley and soybean, plastocyanin levels were greater in leaf tissue than in thylakoids isolated from the same leaves (Table 3), evidence that some plastocyanin is lost during thylakoid preparation. The data also suggested that plastocyanin was lost to a greater extent in soybean than in barley. However, species differences in plastocyanin content were large when compared on either a thylakoid or leaf tissue basis. Barley contained three-fold more plastocyanin than soybean on a thylakoid basis and two-fold more plastocyanin and on a leaf basis when plants were grown at high irradiance (Table 3). A further decline in soybean plastocyanin content was observed in both thylakoids and leaf tissue at lower growth irradiance (Table 3).

These results suggest that plastocyanin pool size can be very different depending on the species studied. Certain species such as barley and spinach probably contain adequate levels of plastocyanin, but other species such as soybean contain low levels of plastocyanin that appear to limit photosynthetic electron transport capacity. These correlative observations do not prove that plastocyanin limits electron transport, but certainly suggest that plastocyanin is an important factor.

Plastocyanin as a potential limiting factor

Plastocyanin was the only thylakoid component that correlated with the observed pattern of photosynthetic electron transport. A lower steady-state concentration of plastocyanin resulted in lower activity. From a kinetic perspective, plastoquinone should limit electron transport because plastoquinol oxidation by the Cyt b_6f complex (Stiehl and Witt 1969) is at least an order of magnitude slower than Cyt f oxidation and $P700^+$ reduction by plastocyanin (Haehnel et al. 1980). Therefore, plastocyanin should not limit electron transport based on the reaction kinetics. However, artificially lowering the plastocyanin concentration has been shown to limit electron transport. Spinach thylakoids contain plastocyanin in excess of the amount required to support rapid turnover of all the P700 (Haehnel et al.

Table 1. Genetic variation in soybean photosynthetic electron transport. Plants were grown in the phytotron at a PPFD of 200 to 300 μmol photons m^{-2} s^{-1}. Each number is the mean ± S.E. for thylakoids from three or four independent plants

Genotype	Electron transport activity[a]	PS II reaction centers[b]	Cyt b_6f Complexes[b]	PS I reaction centers[b]
CNS	51 ± 6	3.7 ± 1.0	1.9 ± 0.1	2.4 ± 0.1
Tokyo	24 ± 3	n.d.	n.d.	n.d.
Palmetto	26 ± 5	3.0 ± 0.2	2.0 ± 0.1	2.2 ± 0.1
Roanoke	17 ± 3	n.d.	n.d.	n.d.
Arksoy	16 ± 4	2.8 ± 0.2	1.8 ± 0.1	2.3 ± 0.2
Mukden	16 ± 2	n.d.	n.d.	n.d.
Peking	16 ± 2	n.d.	n.d.	n.d.
Perry	16 ± 2	n.d.	n.d.	n.d.
Manchu	15 ± 1	n.d.	n.d.	n.d.
Richland	15 ± 3	n.d.	n.d.	n.d.
Dunfield	14 ± 2	n.d.	n.d.	n.d.
S-100	11 ± 1	3.7 ± 0.3	2.5 ± 0.2	2.4 ± 0.1
Mandarin (Ottowa)	8 ± 1	3.4 ± 0.1	2.3 ± 0.1	2.7 ± 0.6
Mandarin (Illinois)	7 ± 1	3.2 ± 0.6	2.1 ± 0.1	2.5 ± 0.1
A.K. (Harrow)	6 ± 1	n.d.	n.d.	n.d.

[a] mmol DCIP reduced [mol Chl^{-1}] s^{-1}.
[b] mmol component [mol Chl^{-1}].
n.d. = not determined.

1989). Treatment of spinach thylakoids with chemical inhibitors to reduce the number of functional plastocyanin molecules resulted in a decline in both whole chain and PS I electron transport activities (Haehnel 1982). While the total P700 signal was not affected by the inhibitors, the slow kinetic component of $P700^+$ reduction was greatly enhanced in treated thylakoids (Haehnel et al. 1980; Haehnel 1982). The slow kinetic component was attributed to PS I reaction centers that were separated from the remaining active plastocyanin (Haehnel 1982), suggesting the presence of multiple domains that were differentially affected by the inhibitors. Separate domains within chloroplast thylakoids have been identified where Cyt b_6f complexes and PS I reaction centers are localized (Albertsson et al. 1991; Svensson et al. 1991) and where plastocyanin must also be localized to participate in rapid electron transfer reactions. Clearly, the *in vitro* loss of functional plastocyanin had a negative effect on electron transport.

By analogy, factors that limit plastocyanin accumulation in the chloroplast could have a negative effect on photosynthetic electron transport capacity similar to the treatment of thylakoids with a plastocyanin inhibitor. Genetics is one factor. Genotypic variation in electron transport has been linked to plastocyanin concentration in barley (Burkey 1994), fescue (Krueger and Miles 1981; Krueger et al. 1984), and soybean (Table 2). Species differences in activity were also linked to plastocyanin pool size in this study (Tables 2 and 3). Growth irradiance is a second factor. Increased electron transport capacity at high growth irradiance has been linked to increased plastocyanin pool size in barley (Burkey 1993) and soybean (Table 2).

The molecular mechanism that limits plastocyanin accumulation is not known. Effects on gene expression, mRNA accumulation, and translation are obvious factors to consider, but other possibilities also exist. Plastocyanin is synthesized as a cytoplasmic precursor that must be processed into a functional polypeptide during import into the chloroplast lumen. The copper atom that serves as the redox cofactor must also be incorporated as the functional protein is formed. Thus, accumulation could be limited by effects on chloroplast import or copper metabolism. It should be noted that the mechanism that limits plastocyanin accumulation at low irradiance could be different than the mechanism responsible for genetic variation.

Table 2. Soybean photosynthetic electron transport in relation to plastoquinone and plastocyanin concentration. Plants were grown in controlled environments at the indicated PPFD. Each number is the mean ± S.E. of thylakoids from three or four independent plants

Genotype	Growth environment	Electron transport activity[a]	PQ content[b]	Plastocyanin content[c]	Plastocyanin pool size[d]
CNS	phytotron	51 ± 6	n.d.	n.d.	n.d.
	PPFD = 430	68 ± 3	33 ± 2	2.0 ± 0.04	0.8 ± 0.02
	PPFD = 1500	91 ± 1	53 ± 7	3.0 ± 0.4	1.3 ± 0.2
S100	phytotron	11 ± 1	n.d.	n.d.	n.d.
	PPFD = 430	37 ± 6	48 ± 5	2.0 ± 0.4	0.8 ± 0.2
	PPFD = 1500	82 ± 12	45 ± 3	2.5 ± 0.3	1.0 ± 0.1
Mandarin (Illinois)	phytotron	7 ± 1	n.d.	n.d.	n.d.
	PPFD = 430	47 ± 7	52 ± 4	1.5 ± 0.1	0.6 ± 0.04
	PPFD = 1500	80 ± 6	46 ± 2	3.2 ± 0.6	1.3 ± 0.2
A.K. (Harrow)	phytotron	6 ± 1	46 ± 6	0.06 ± 0.01	0.02 ± 0.003
	PPFD = 430	37 ± 7	58 ± 6	0.9 ± 0.09	0.4 ± 0.04
	PPFD = 1500	76 ± 9	50 ± 1	2.8 ± 0.6	1.1 ± 0.1

[a] mmol DCIP reduced [mol Chl]$^{-1}$ s^{-1}.
[b] Plastoquinone (PQ) was estimated from the area over the Chl fluorescence induction curve of isolated thylakoid membranes ± DCMU. $Area_{-DCMU}/Area_{+DCMU}$.
[c] mmol plastocyanin [mol Chl]$^{-1}$.
[d] mol plastocyanin [mol P700]$^{-1}$.
n.d. = not determined.

Table 3. Photosynthetic electron transport and plastocyanin pool size in barley and spinach compared with soybean. Plants were grown in controlled environment chambers at the indicated PPFD. Each number is the mean ± S.E. of thylakoids from three or four independent plants

Species (genotype)	Growth PPFD	Electron transport activity[a]	Thylakoid plastocyanin content[b]	Thylakoid P700 content[b]	Plastocyanin pool size[c]	Leaf plastocyanin content[b]
spinach (Melody)	600	169 ± 14	7.6 ± 0.4	2.0 ± 0.2	3.9 ± 0.2	n.d.
barley (Boone)	1000	136 ± 10	8.3 ± 0.1	2.4 ± 0.1	3.4 ± 0.1	12.4 ± 0.5
soybean (S100)	1500	82 ± 12	2.5 ± 0.3	2.5 ± 0.1	1.0 ± 0.1	6.2 ± 0.5
	420	42 ± 4	1.5 ± 0.1	2.6 ± 0.1	0.6 ± 0.05	3.3 ± 0.4

[a] mmol DCIP reduced [mol Chl]$^{-1}$ s^{-1}.
[b] mmol component [mol Chl]$^{-1}$.
[c] mol plastocyanin [mol P700]$^{-1}$, calculated from thylakoid membrane data.
n.d. = not determined.

Estimation of the optimum plastocyanin pool size

The above discussion suggests that a specific amount of plastocyanin is required to achieve maximum rates of electron transport. An estimate of the optimum plastocyanin pool size can be made from the wide range of activity and plastocyanin concentration observed in this study. In soybean, electron transport capacity increased from 6 to 91 mmol DCIP reduced [mol Chl]$^{-1}$ s^{-1} as the plastocyanin pool size increased from 0.1 to 1.3 (Table 2). Plastocyanin pool size increased an additional 3-fold to values of 3.4 to 3.9 in barley and spinach as electron transport capacity increased by less than a factor of two to approximately 150 mmol DCIP reduced [mol Chl]$^{-1}$ s^{-1} (Table 3). Thus, an optimum plastocyanin pool size appears to be greater than one, but less than four. A value greater than one would seem logical given that plastocyanin must interact with two complexes, Cyt b_6f and PS I, to efficiently transfer electrons at maximum rates. The evidence suggests that photosynthetic energy conversion could be improved in species with low levels of plastocyanin if the plastocyanin pool size was increased to a value greater than two.

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