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Chloroplast pigments and polypeptide profiles in nitrogen-deficient wheat seedlings

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ABSTRACT

Chlorophylls, carotenoids, and polypeptide profiles were studied in chloroplasts from wheat (Triticum aestivum L.) seedlings grown with adequate and inadequate soil nitrogen. Concentrations of chlorophyll-a, chlorophyll-b, carotene, lutein, neoxanthin and violaxanthin were all lower in seedlings grown in nitrogen-deficient soil. However, the percentage decrease in chlorophyll-a was less than the percentage decrease in chlorophyll-b and the carotenoids. Molar ratios of chlorophyll-a/all others were .434 and .614 for plants grown in the nitrogen-adequate and nitrogen-deficient soils, respectively. Of 30 polypeptide bands detected, six relatively low mol wt polypeptides (15.3, 15.9, 16.9, 17.3, 22.3, and 27.0 kD) were found only in plants grown in nitrogen-deficient soil, and one larger (43.2 kD) polypeptide was present only in plants grown in the nitrogen-adequate soil. Although present in both, plants grown with adequate soil nitrogen had a higher quantity of a polypeptide with a mol wt of approximately 74.3 kD. The relationship between nutrient stress during plant growth and chlorophyll, carotenoid and polypeptide concentrations in chloroplasts warrants further investigation of environmental regulation of gene expression in developing leaves.

INTRODUCTION

Many soils throughout the world are deficient in nitrogen, and plants growing in nitrogen-deficient soils generally have a light-green, chlorotic appearance. Nitrogen-deficiency symptoms occur frequently in plants growing on sandy soils as prevail in the Southeastern Coastal Plains of the USA. Numerous studies have demonstrated effects of nitrogen on yields of various crops; however, little information exists on effects of soil nitrogen level on the photosynthetic pigments and polypeptide profiles in field-grown plants. In addition to their role in photosynthesis, chloroplast pigments and proteins (and their degradation products) are involved in animal and human food chains. Therefore, better understanding of the relationship between soil nitrogen availability and these compounds is needed. The objectives of the present study were to: (a) determine chlorophyll and carotenoid concentrations and ratios in leaves from wheat seedlings grown with adequate and inadequate soil nitrogen, and (b) to determine chloroplast polypeptide profiles in the same samples.

MATERIALS AND METHODS

Plant Materials.

Wheat (Triticum aestivum L. cv. Coker 797) was sown on Norfolk loamy sand (Typic Paleudults) near Florence, South Carolina, in November, 1983. Plots with adequate nitrogen were fertilized according to Clemson University recommendations (1982). With the exception of nitrogen, the nitrogen-deficient plots received the same fertilizers and amounts as the nitrogen-adequate plots. Both nitrogen-adequate and nitrogen-deficient plots were irrigated as needed to avoid moisture stress.

Leaf blades were collected on April 12, 1984, before the plants began to joint. This is the stage frequently used to graze livestock. Samples were quick-frozen, freeze-dried, and ground to pass a 40-mesh screen. The ground samples were stored in darkness at -50°C until analyzed.

Chloroplast Pigments.

Chlorophyll and carotenoid pigments were quantitated according to the HPLC method of Eskins and Dutton (1979) as revised by Burton and Kasperbauer (1985). Each 250 mg sample of freeze-dried tissue was placed in a 10 x 40 mm extraction thimble and extracted with acetone (20 mL) in a microsoxhlet apparatus for 1 h. The extract was filtered under vacuum through a Buchner medium porosity filter and a Sep-Pak reverse phase cartridge. The cartridge was rinsed with 3 mL of acetone, and the filtrate was transferred to a 25 mL actinic volumetric flask and diluted to volume with acetone. For HPLC analyses a 50 µL aliquot was injected on a C_{18} reverse phase column using methanol-water (90:10, v/v) and ethyl acetate as the elution solvents. The conditions for elution were as described by Eskins and Dutton (1979). Order of elution was neoxanthin, violaxanthin, lutein, chlorophyll-b, chlorophyll-a and carotene. Quantifications of the pigments were determined at 436 nm. Calibration curves for chlorophyll-a and chlorophyll-b were obtained from chlorophyll standards obtained from Sigma Chemical. Carotene calibration curves were obtained from carotene (Eastman Chemicals). Lutein, neoxanthin

and violaxanthin curves were obtained by extrapolation of their extinction coefficients at 436 nm from the literature to the response of the carotene standard at 436 nm.

Chloroplast Polypeptides.

Chloroplasts were isolated from 1-g samples of freeze-dried leaves by the method of Morgenthaler et al. (1974) employing G-R medium (0.33 M sorbitol, 2 mM Na_2EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM $Na_2P_2O_7$, 5 mM araboascorbic acid, 50 mM HEPES, brought to pH 6.8 with 6 N NaOH). The chloroplasts were separated from other subcellular particles using centrifugation in discontinuous density gradients using Ludox AM Colloidal Sol (E.I. duPont de Nemours and Co.) according to the method of Morgenthaler et al. (1975). The density gradients ranged from 10% to 70% (v/v) with respect to Ludox mixture [70 ml of Ludox with 2.1 g PEG 6000, 0.70 g BSA, and 0.70 g Ficoll (Sigma Type 400)] in G-R medium. Chloroplasts, removed from the gradient and diluted with G-R medium, were pelleted using centrifugation, rewashed in G-R medium, and repelleted.

Lysis of Chloroplasts and Protein Preparation.

Chloroplasts were suspended in distilled water to facilitate lysis. The resulting solution was spun at 33,000 g for 50 min to sediment the chloroplast membranes, fragments, or intact chloroplasts. Proteins were precipitated by making the clear supernatant 5% (w/v) with TCA, pelleted by centrifugation at 33,000 g for 30 min, washed in distilled water and repelleted. The protein pellet was dissolved in a minimal volume (300 to 600 µL) of SDS sample buffer (Morgenthaler et al. 1975) and heated in a 90°C water bath for 10 min.

Electrophoresis and Staining.

Vertical slab SDS polyacrylamide gel electrophoresis was employed using the method of Laemmli (1970). The gels (3 mm and 0.75 mm thick) consisted of a 10% (w/v) acrylamide running gel (24 cm) and a 3% (w/v) acrylamide stacking gel (4 cm). About 25 µL of sample was applied per lane. The stacking current was 10 mamp, and the running current 20 mamp. Bromphenol blue served as the tracking dye.

Gels were stained using a modification of the Silver Staining Protocol outlined in Bio-Rad Bulletin 1089 with reagents from Bio-Rad. The procedure was derived from the method reported by Merrill et al. (1981). Gels were soaked overnight in 40% (v/v) methanol - 10% (v/v) acetic acid, and the two subsequent treatments with 10% (v/v) ethanol - 5% (v/v) acetic acid were increased to 3 h each. Other procedural modifications were a 5-min wash in distilled water before adding the oxidizer and three 10-min washes in distilled water before adding the Silver Reagent. Stained gels were stored in distilled water. Some permanent gel records were made using GAF black diazo color film exposed to the gels and developed in household ammonia as described by Terranova (1981).

Bands were measured from gels themselves and/or the diazo transparencies. The transparencies were also scanned with an LKB 2202 UltraScan Laser Densitometer attached to a LKB 2220 Recording Integrator. Profiles obtained using low mol wt standards (Bio-Rad) were used with a computer program to determine molecular weights for a wide range of R_f values from gels of 10% acrylamide.

For comparative purposes similarity coefficients (SC) were calculated by doubling the number of polypeptides in common between two profiles and dividing this number by the total number of polypeptides in both profiles (1976).

RESULTS AND DISCUSSION

Pigment Concentrations and Ratios.

Plants that received adequate nitrogen were dark-green whereas those grown on nitrogen-deficient soil were lighter green in color. Leaf color differences suggested possible differences in chloroplast pigment quantities. These are shown in Table 1. Molar ratios among various pigments (Table 2) were determined to gain a better understanding of nitrogen nutrition effects on photosynthetic pigments, which might be related to photosynthetic efficiency.

Table 1. Chlorophyll and carotenoid pigments in leaves from wheat seedlings grown in nitrogen-adequate or nitrogen-deficient soil in a field

Pigments	N Availability During Growth		
	Adequate	Deficient	Adeq./Def.[a]
	(mg/g dry wt.)		(ratios)
Chlorophyll-a	2.170	1.925	1.13
Chlorophyll-b	1.284	.749	1.71
Carotene	.704	.390	1.81
Lutein	.896	.620	1.45
Neoxanthin	.224	.149	1.50
Violaxanthin	.541	.367	1.47
Total	5.819	4.200	1.386

[a] Adeq./Def. = Adequate/Deficient

Table 2. Chloroplast pigment molar ratios in field-grown wheat seedlings

Pigments	N Availability During Growth	
	Adequate	Deficient
	(Molar ratios)	
Chl-a/Chl-b	1.716	2.610
Chl-a/Carotene	1.854	2.970
Chl-a/Lutein	1.543	1.978
Chl-a/Neoxanthin	6.183	8.261
Chl-a/Violaxanthin	2.700	3.529
Chl a+b/carotenoids	.920	1.109
Chl-a/all others	.434	.614

Leaves of seedlings that were grown in nitrogen-deficient soil had lower concentrations of chlorophyll-a, chlorophyll-b, carotene, lutein, neoxanthin and violaxanthin (Tables 1 and 2). The decreased pigment concentrations were consistent with the light-green color of the plants grown in nitrogen-deficient soil. Nevertheless, the decrease was less pronounced for chlorophyll-a than for the other pigments. That is, the chlorophyll-a/-b ratio was higher for plants grown in nitrogen-deficient soil. Other reports (Kasperbauer and Peaslee 1973, Kasperbauer and Hamilton 1984) have shown similar trends in leaves of plants that received a higher ratio of far-red relative to red light during development. They had altered chloroplast ultrastructure and a higher chlorophyll-a/-b ratio. The higher far-red relative to red light ratio apparently acts through phytochrome and is involved in regulation of gene expression in developing leaves to favor plant survival in shade from other plants (Jones and Kasperbauer 1985, Kasperbauer and Hamilton 1984, Kasperbauer and Peaslee 1973). Thus,

the similarity of change in chlorophyll-a/-b ratios in response to both nitrogen-deficiency and shade adaptation indicate environment-initiated adjustment of the photosynthetic apparatus to improve the probability of survival under the indicated stress conditions.

Chloroplast Polypeptides.

In a preliminary study of fresh versus freeze-dried tissue, electrophoretic profiles for chloroplast polypeptides yielded a similarity coefficient (SC) of 100% with all of the polypeptides detected in one source being found in the other. No quantitative differences were apparent between the comparable polypeptides of these profiles. Freeze-dried samples were, therefore, used in subsequent studies.

Electrophoretic profiles for chloroplast polypeptides from wheat seedlings grown in nitrogen-adequate versus those from seedlings grown in nitrogen-deficient soil yielded a SC value of 87%. Only 23 of the 30 polypeptides were detected in both sources (Table 3). Of the seven polypeptides that were absent in one set of samples, six were found only in leaves from plants grown in nitrogen-deficient soil, and one was found only in leaves grown with adequate nitrogen. Similarly, other researchers (Nishio et al. 1985) studying effects of iron deficiency have noted a greater number of polypeptides in chlorotic plants. It is possible that the additional polypeptides found in chlorotic plants might be precursors or breakdown products. All six of those detected exclusively in nitrogen-deficient plants (15.3, 15.9, 16.9, 17.3, 22.3, and 27.0 kD) are of relatively low mol wt, while the one solely from the nitrogen-adequate plants was larger (43.2 kD). There was also a significantly greater quantity of a 74.3 kD polypeptide in the nitrogen-adequate sample.

Table 3. R_f and molecular weight values for polypeptides found in wheat chloroplasts from seedlings grown in nitrogen-adequate and nitrogen-deficient soils in a field

R_f	MW(kD)	N Availability During Growth: Adequate	Deficient
.004	>100.0	+[a]	+
.009	>100.0	+	+
.041	>100.0	+	+
.051	>100.0	++	++
.155	>100.0	+	+
.195	98.5	+	+
.309	74.3	++	+
.370	64.0	+++	+++
.426	55.5	++	++
.459	51.3	+	+
.502	46.3	++	++
.528	43.2	+	-
.549	38.0	++	++
.617	34.7	+	+
.622	34.0	+	+
.670	30.3	++	++
.716	27.0	-	+
.759	24.3	+	+
.781	23.0	++	++
.793	22.3	-	+
.817	21.2	+	+
.847	19.5	+	+
.869	18.4	+	+
.894	17.3	-	+
.904	16.9	-	+
.917	16.4	++	++
.930	15.9	-	+
.942	15.3	-	+
.970	14.4	++	++
.993	13.6	++	++

[a] -, +, ++, and +++ indicate relative concentrations of indicated polypeptide.

Some of the polypeptides shown in Table 3 correspond (with regard to mol wt) to previously identified polypeptides. For example, those with mol wt values of 15.3, 18.4, 19.5, and 64.0 kD correspond well to those listed by Obokata (1986) as 15, 18, 20, and 66 kD of wheat photosystem I. The discrepancy between the 64 kD value in Table 3 and the 66 kD value of Obokata (1986) may be a matter of resolution due to an overabundance of protein in this area of the gel. The 34, 46, and 51 kD polypeptides identified in corn (Zea mays L.) photosystem II (Eskins et al. 1985) also have corresponding polypeptides in wheat (Table 3).

In a study of sugar beet (Beta vulgaris L.) chloroplasts (Nishio et al. 1985) a 43.8 kD polypeptide was of special interest because it differed in quantity between chlorotic and normal green tissue, and it also varied in concentration during regreening. This might be the same as the 43.2 kD polypeptide which was detected in our wheat plants grown in nitrogen adequate but not in those grown in nitrogen-deficient soil (Table 3).

Obokata (1984) noted a difference between etiochloroplasts and mature chloroplasts of wheat in the existence of a 15 kD polypeptide. The present study indicates a difference with regard to this polypeptide (listed as 15.3 kD) according to nitrogen nutrition of the plants. Chloroplasts from nitrogen-deficient leaves (like the etiochloroplasts) had that polypeptide while chloroplasts from nitrogen-adequate leaves (like Obokata's mature chloroplasts) lacked the 15.3 kD polypeptide. If, as proposed in a later article (Obokata 1986), the polypeptide is subunit-5 of the photosystem I reaction complex, its synthesis and processing may be of significance in environmental regulation of gene expression in developing leaves. The same may become apparent for the other polypeptides that differed between leaves grown with deficient or adequate nitrogen.

The chloroplast pigment and polypeptide composition changes noted for wheat (Tables 1, 2, 3) appear to be interrelated. Similarity of chlorophyll-a/-b ratios and polypeptide profiles between far-red- treated (Jones and Kasperbauer 1985) and nitrogen-starved leaves offers some interesting comparisons of adaptation of a plant to environmental stress, presumably improving its chance of survival under the stress conditions. Also, other environment-induced alterations in gene expression may influence not only plant survival and productivity but also changes in nutrient value of the plants as food for animals and humans.

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