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Light and Nitrogen Affect Storage Protein Mobilization in Germinating Cottonseed[1]

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ABSTRACT

Laboratory germination studies to evaluate seed performance and seed quality attributes are routinely conducted in the dark with distilled water used to moisten the seed blotter paper. This study was conducted to determine the effect of light and N on storage protein mobilization during the first 5 days of germination of cottonseed (*Gossypium hirsutum* L.). Dry weight, hydrolysate amino acid composition, and free amino acid composition of cotyledons and axis were determined. Ultrastructure of cotyledons was examined. In the absence of N, light enhanced the protein body breakdown and coalescence of protein bodies into the central vacuole of cotyledonary cells. The presence of N in the nutrient solution had little effect on the protein body breakdown in the light (as revealed by electron microscopy) but slowed the mobilization process in the dark. Total free amino acid content and individual amino acid composition support this conclusion. Light increases the total free amino acid content of cotyledons at 3 and 5 days in the absence of N and at 5 days in the presence of N. Similar results are seen for the prominently affected amino acids, asparagine, arginine, γ-aminobutyric acid, and histidine. These results indicate that seed germination is sensitive to environmental factors such as light and mineral nutrients.

Additional index words: ***Gossypium hirsutum*** **L., Protein bodies, Ultrastructure.**

THE storage proteins of cottonseed (*Gossypium hirsutum* L.) are located in protein bodies (aleurone grains). Yatsu (1965) and Englemann (1966) have described the ultrastructure and ontogeny of the protein bodies in cottonseed. Globoid inclusions in the protein bodies have been isolated and their contents identified as salts of phytic acid (Lui and Altschul, 1967). The amino acid composition of cottonseed storage proteins has been determined (Ibragimov et al. 1970; Elmore and King, 1978).

Various aspects of the mobilization of amino acids from the storage proteins during germination have been studied. Capdevila and Dure (1977) reported on the free amino acid pool, and Elmore and King (1978) reported on both free and total amino acid content, with a balance sheet approach. The ultrastructural aspects of germinating cottonseed have not been investigated nor has the effect of mineral nutrients or light. Laboratory germination studies to evaluate seed performance and seed quality attributes are routinely conducted in the dark with distilled water used to moisten the seed blotter paper. This study was conducted to determine the effect of light and N on storage protein mobilization during the first 5 days of germination of cottonseed. This report describes our investigation of the effects of light and mineral nutrients on storage protein mobilization, as revealed by free and total amino acid composition, and correlates this amino acid data with the ultrastructure of the germinating cottonseed. The results indicate that cottonseed germination is sensitive to light and mineral nutrients.

MATERIALS AND METHODS

Germination Procedure. 'Stoneville Glandless 48483' cottonseed were selectively fractionated in successive sucrose density gradients of 5% increments for germination studies. The seed that floated on 25% sucrose after the lower density seed fractions had been removed was used for this study. This is a modification of the procedure described by Elmore and King (1978). Seed were incubated at 25 C in growth cabinets supplied with fluorescent and incandescent lights (200 μEm^{-2}) in trays of moistened vermiculite. A nutrient solution (Radin, 1977) with or without NO_3-N was used to moisten the vermiculite. For the dark treatment, the trays were foil-wrapped while the light treatment was saran-wrapped.[3] Seed were harvested at 1, 3, and 5 days after the start of incubation; and those harvested at 3 and 5 days were separated into axis and cotyledon. The samples were then frozen and lyophilized.

Amino Acid Composition. Dry weight, free amino acids, and hydrolysate amino acid composition were determined as described previously (Elmore and King, 1978). The free amino acids were extracted with acid alcohol (Cherry 1973), deproteinized with picric acid, and analyzed on the amino acid analyzer (Beckman Model 121[3]). Two replicates of the seedlings were used for each amino acid determination. Four replicates of 25 seedlings each were used for dry weight determination and the experiment was repeated. Due to cost and time required, amino acid determinations were not repeated. Data were subjected to analysis of variance, and significant differences among amino acids were identified with Duncan's Multiple Range Test.

Table 1. Dry weight of cottonseed and of cotyledons and axes of germinating cottonseed.

Days of incubation	Treatment†	Dry weight‡		
		Seed	Axis	Cotyledon
		mg		
0	-	67.6 ± 2.4	-	-
1	D−	60.5 ± 2.8	-	-
	D+	60.1 ± 2.3	-	-
	L−	60.0 ± 2.5	-	-
	L+	60.9 ± 3.6	-	-
3	D−	62.5 ± 1.2	9.2 ± 1.9	53.3 ± 0.7
	D+	61.9 ± 2.5	9.0 ± 1.5	52.6 ± 1.9
	L−	62.3 ± 2.2	8.5 ± 2.3	53.8 ± 2.5
	L+	61.8 ± 2.5	8.3 ± 1.9	53.5 ± 0.4
5	D−	60.6 ± 0.6	12.2 ± 1.0	48.4 ± 1.2
	D+	61.4 ± 3.0	11.9 ± 0.3	49.8 ± 3.0
	L−	62.7 ± 0.1	8.8 ± 0.2	54.0 ± 0.2
	L+	64.6 ± 0.1	9.0 ± 0.8	55.6 ± 0.8

† D = dark, L = light, − = nutrient solution without N, + = nutrient solution with N.
‡ Data are means ± standard deviation.

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[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA or the Mississippi Agric. and Forestry Exp. Stn. and does not imply its approval to the exclusion of other products that may also be suitable.

Electron Microscopy. Seedlings used for electron microscopy were produced by incubating seed in petri plates with the same light and nutrient solution treatments as described above. Dark-incubated seedlings were harvested under a dim green light as a precaution to prevent any rapid light responses by the seedlings.

The cotyledonary tissue was fixed in a glutaraldehyde-paraformaldehyde fixative (Karnovsky, 1965) buffered with cacodylate (pH 7.2) and post-fixed with 2% (w/w) OsO_4 in the same buffer. After dehydration in a graded alcohol series, the cotyledonary tissue was transferred to propylene oxide and embedded in Spurr's (1969) low-viscosity embedding medium for sectioning. Sections (0.5 μm) were mounted on slides, stained with toluidine blue, and observed and photographed with a Zeiss[8] Photomicroscope. Silver sections were stained with 2% uranyl acetate, post-stained with Reynolds' (1963) lead citrate, and observed in a Hitachi Hu-llc[9] transmission electron microscope operated with an accelerating voltage of 75 kV.

RESULTS

Dry Weight. Dry weight transfer from cotyledons to axes was affected by germination conditions (Table 1). Cotyledons were heavier and axes lighter in dark treatments that in light treatments at Day 5 but not at Day 3. Light retatded transfer of dry matter from

Table 2. Levels of free amino acids in cottonseed and in cotyledons of germinating cottonseed.

	Level in cottonseed		Level in cotyledons of cottonseed subjected to indicated treatment†							
			Day 3				Day 5			
Amino acid	Day 0	Day 1‡	D−	D+	L−	L+	D−	D+	L−	L+
	μmol/g dry wt									
Alanine	2.3	2.9	4.6^{a*}	4.0^{ab}	4.2^{a}	3.1^{b}	7.4	7.5	7.3	9.6
Arginine	9.9	10.3	17.4	17.2	25.8	13.3	43.5^{c}	47.2^{bc}	61.1^{ab}	69.4^{a}
Asparagine	4.5	6.0	41.2^{c}	35.1^{c}	81.0^{a}	63.5^{b}	100.0^{b}	97.6^{b}	244.9^{a}	234.4^{a}
Aspartate	4.0	5.7	6.8	6.5	6.4	5.8	4.9^{b}	4.7^{b}	7.8^{a}	9.0^{a}
Ethanolamine	0.2	1.3	4.6	3.8	4.5	4.2	5.3	5.5	5.4	6.5
γ-aminobutyrate	0.4	6.3	15.4^{a}	7.6^{c}	12.2^{ab}	8.8^{bc}	19.3^{b}	15.4^{b}	25.4^{a}	26.3^{a}
Glutamine	–	3.9	19.0	16.4	20.6	19.1	12.2	14.6	10.9	11.3
Glutamate	4.6	6.3	14.7	18.8	18.7	16.3	–	–	–	–
Glycine	0.9	0.8	1.9^{ab}	2.0^{ab}	2.6^{a}	1.4^{b}	5.7^{b}	6.5^{b}	7.9^{ab}	10.1^{a}
Histidine	0.2	0.7	11.5^{b}	9.8^{c}	15.9^{a}	10.9^{bc}	26.5^{b}	26.7^{b}	36.4^{a}	37.2^{a}
Isoleucine	0.1	0.4	2.7^{a}	2.7^{a}	2.2^{ab}	1.6^{b}	4.0^{b}	4.3^{b}	5.2^{ab}	6.0^{a}
Leucine	0.2	0.7	4.1	3.9	3.5	2.3	6.6	6.8	8.0	8.3
Lysine	0.2	1.2	4.6	4.3	4.4	3.3	10.0	9.6	10.1	10.0
Ammonia§	12.6	6.1	13.4	16.7	16.4	11.6	16.4^{b}	16.4^{b}	33.0^{a}	22.3^{b}
Phenylalanine	0.3	0.9	4.1	3.7	2.8	1.9	6.2^{b}	7.0^{b}	5.3^{b}	11.4^{a}
Serine	0.8	3.3	12.6^{a}	11.0^{b}	12.8^{a}	9.4^{c}	23.8	25.6	18.7	25.0
Threonine	0.4	2.1	5.5	6.7	5.6	4.5	9.6^{c}	9.8^{bc}	14.5^{ab}	15.9^{a}
Tyrosine	0.1	0.7	3.9	3.9	2.3	1.8	4.4	4.7	5.7	5.2
Valine	0.2	0.7	5.9	3.5	4.4	3.2	7.7^{b}	9.7^{ab}	10.4^{ab}	13.0^{a}
Proline	Tr	Tr	4.5	3.8	3.2	3.7	3.8^{b}	4.9^{b}	6.3^{b}	9.6^{a}
N¶	76.9	103.7	337.8^{b}	307.6^{b}	449.0^{a}	336.5^{b}	634.2^{b}	646.7^{b}	1,051.4^{a}	1,078.2^{a}

* Within each amino acid and hay means followed by the same letter or no letter are not significantly different, as determined by Duncan's Multiple Range Test (5% level). † Treatment identification is as follows: D = dark, L = Light, − = nutrient solution without N, + = nutrient solution with N. ‡ Results did not differ significantly among treatments, so only the mean is presented. § The data on ammonia which is not an amino acid is included for information. ¶ N is the sum of the N contained in the free amino acids.

Table 3. Level of free amino acids in axes of germinating cottonseed.

	Level in axes of cottonseed subjected to indicated treatment†							
	Day 3				Day 5			
Amino acid	D−	D+	L−	L+	D−	D+	L−	L+
	μmol/g dry wt							
Alanine	40.3^{a*}	43.7^{a}	22.6^{b}	11.3^{c}	30.6^{b}	41.6^{a}	3.8^{c}	5.5^{c}
Arginine	18.8^{a}	19.4^{a}	10.5^{b}	10.1^{b}	9.8	9.3	4.4	3.6
Asparagine	917.4	577.7	911.3	641.6	1,101.3	924.9	969.7	818.2
Aspartate	22.8^{ab}	23.5^{a}	17.6^{ab}	14.3^{b}	20.2^{ab}	26.4^{a}	12.4^{b}	14.4^{b}
Ethanolamine	4.6	5.0	4.6	4.4	4.0^{ab}	5.6^{a}	3.7^{ab}	2.8^{b}
γ-aminobutyrate	10.6^{a}	7.7^{ab}	4.4^{b}	5.8^{b}	4.5	3.6	4.0	2.8
Glutamine	56.1^{a}	56.7^{a}	28.6^{b}	24.4^{b}	29.2^{b}	37.3^{a}	9.0^{c}	10.1^{c}
Glycine	28.8^{a}	24.4^{a}	11.4^{b}	12.2^{b}	35.0^{a}	32.8^{a}	8.4^{b}	12.4^{b}
Histidine	24.6^{a}	18.2^{b}	13.8^{c}	15.2^{bc}	27.2^{a}	26.3^{a}	17.0^{b}	14.4^{b}
Isoleucine	12.5^{a}	7.8^{b}	3.8^{c}	3.9^{c}	14.6^{a}	13.0^{a}	5.2^{b}	5.4^{b}
Leucine	8.4^{a}	6.8^{b}	4.0^{c}	4.6^{c}	7.4^{ab}	6.2^{b}	8.3^{a}	6.1^{b}
Lysine	4.8	4.2	4.5	5.0	7.4^{b}	10.4^{a}	4.3^{c}	4.1^{c}
Ammonia‡	42.8^{a}	21.2^{b}	27.4^{ab}	27.9^{ab}	29.6	29.6	35.6	28.8
Phenylalanine	8.7^{a}	9.2^{a}	4.1^{b}	4.8^{b}	10.2^{a}	10.4^{a}	2.4^{b}	2.8^{b}
Serine	59.6^{a}	51.4^{b}	29.1^{c}	29.6^{c}	69.1^{a}	64.2^{a}	26.7^{c}	40.6^{b}
Threonine	20.6^{a}	17.4^{a}	10.7^{b}	11.0^{b}	27.1^{a}	26.0^{a}	13.6^{b}	14.3^{b}
Tyrosine	1.8^{ab}	1.9^{a}	1.3^{ab}	1.2^{b}	1.6^{ab}	1.4^{b}	2.5^{a}	2.0^{ab}
Valine	20.6^{a}	14.8^{b}	6.2^{c}	5.4^{c}	19.7^{a}	18.6^{a}	5.7^{b}	6.8^{b}
N§	2,388.2^{a}	1,644.0^{b}	2,104.0^{a}	1,581.1^{b}	2,670.0^{a}	2,340.5^{b}	2,161.2^{c}	1,864.6^{d}

* Within each amino acid and day means followed by the same letter or no letter are not significantly different as determined by Duncan's Multiple Range Test (5% level). † Treatment identification is as follows: D = dark, L = light, − = nutrient solution without N, + = nutrient solution with N. ‡ The data on ammonia, which is not an amino acid, are included for information. § N is the sum of the N contained in the free amino acids.

the cotyledons to the axes. N apparently did not affect the process.

Free Amino Acids. Free mino acids increased in concentration in cotton cotyledons during germination (Table 2). A slight increase was noted on the first day of incubation, during which germination commences, but no treatment effects were observed at this time. By Day 3, however, treatment effects were apparent. The level of free amino acid N and of asparagine and histidine was highest in the light-without-N treatment. Asparagine was also higher in the light with N treatment than in either of the dark treatments, but the N contained in the free amino acid fractions of that treatment was not different from the dark treatments. On Day 5, light-germinated cotyledons had over 1½ times as much N in free amino acids as the dark-germinated cotyledons, regardless of the nutrient-solution treatment and consequently had generally higher levels of individual amino acids as well. The most prominent amino acids which were higher in the light were asparagine, aspartate, γ-aminobutyrate and histidine.

Table 4. Hydrolysate amino acid composition of cottonseed and of cotyledons of germinating cottonseed.

	Composition of cottonseed		Composition of cotyledons subjected to indicated treatment†							
			3				5			
Amino acid	Day 0	Day 1‡	D−	D+	L−	L+	D−	D+	L−	L+
					%					
Alanine	4.0	4.1	4.1^{b*}	4.2^{a}	4.2^{a}	4.2^{a}	4.6^{a}	4.6^{a}	4.5^{b}	4.5^{b}
Arginine	12.6	12.1	11.2^{a}	10.2^{b}	10.2^{b}	10.2^{b}	9.7^{a}	9.9^{a}	9.4^{b}	9.8^{a}
Aspartate	9.4	9.3	10.6^{b}	10.4^{c}	10.9^{a}	11.8^{a}	12.2^{c}	11.7^{d}	16.3^{a}	15.7^{b}
Cysteine	1.3	1.4	1.2	1.3	1.1	1.2	0.8^{ab}	0.9^{a}	0.7^{b}	0.9^{a}
Glutamate	22.7	22.6	20.2^{a}	20.1^{a}	19.3^{b}	19.1^{b}	15.7^{b}	16.2^{a}	13.8^{c}	13.7^{c}
Glycine	4.3	4.3	4.0^{b}	4.1^{a}	4.1^{a}	4.1^{a}	4.3	4.3	4.3	4.3
Histidine	3.0	3.0	3.2^{b}	3.4^{a}	3.4^{a}	3.3^{ab}	3.4^{b}	3.4^{b}	3.7^{a}	3.6^{ab}
Isoleucine	3.1	3.1	3.5	3.4	3.4	3.5	3.9^{a}	3.9^{a}	3.7^{b}	3.7^{b}
Leucine	6.2	6.2	6.7^{b}	6.8^{a}	6.7^{b}	6.8^{a}	7.5^{a}	7.4^{a}	7.3^{b}	7.2^{c}
Lysine	4.7	4.6	5.3	5.2	5.2	5.4	6.2^{a}	6.1^{ab}	5.9^{bc}	5.8^{c}
Methionine	1.6	1.6	1.7	1.6	1.6	1.6	1.7^{a}	1.7^{a}	1.3^{b}	1.5^{ab}
Ammonia§	2.1	2.2	2.2^{b}	2.3^{ab}	2.5^{a}	2.4^{ab}	2.2^{b}	2.1^{b}	2.6^{a}	2.6^{a}
Phenylalanine	5.4	5.5	5.6^{b}	5.7^{a}	5.5^{b}	5.5^{b}	5.5^{ab}	5.6^{a}	5.2^{c}	5.4^{b}
Proline	4.1	4.2	4.1^{b}	4.3^{a}	4.3^{a}	4.2^{a}	4.2	4.2	3.9	4.0
Serine	4.7	4.8	4.8^{c}	5.0^{a}	4.8^{c}	4.9^{b}	5.0^{a}	5.1^{a}	4.6^{b}	4.6^{b}
Threonine	3.4	3.6	3.8	3.9	3.8	3.9	4.3^{a}	4.2^{b}	4.3^{a}	4.2^{b}
Tyrosine	3.1	3.1	3.3^{a}	3.3^{a}	3.2^{b}	3.2^{b}	3.5^{a}	3.5^{a}	3.4^{b}	3.4^{b}
Valine	4.4	4.4	4.8^{c}	5.0^{a}	4.9^{b}	5.0^{a}	5.3^{a}	5.3^{a}	5.1^{b}	5.1^{b}
Mg amino acid (× 100)¶	37.4	38.3	38.5	36.8	37.0	37.7	37.2	38.6	36.0	37.4

* Within each amino acid and day means followed by the same letter or no letter are not significantly different, as determined by Duncan's Multiple Range Test (5% level). † Treatment identification is as follows: D = dark, L = light, − = nutrient solution without N, + = nutrient solution with N. ‡ Results did not differ significantly among treatments so only the mean is presented. § The data on ammonia, which is not an amino acid, are included for information. ¶ Mg amino acid recovered from the analysis per g dry weight of sample.

Table 5. Hydrolysate composition of amino acids of axes of germinating cottonseed.

	Composition of axes subjected to indicated treatment†							
	Day 3				Day 5			
Amino acid	D−	D+	L−	L+	D−	D+	L−	L+
				%				
Alanine	4.2^{a*}	4.1^{b}	3.1^{d}	3.3^{c}	3.2^{ab}	3.7^{a}	2.6^{b}	3.3^{ab}
Arginine	4.8^{a}	3.7^{b}	3.8^{b}	3.7^{b}	3.3	3.0	3.1	3.3
Aspartate	35.2^{c}	41.1^{b}	43.0^{a}	43.0^{a}	46.4	43.9	50.9	42.3
Cysteine	0.4^{ab}	0.6^{a}	0.3^{b}	0.6^{ab}	0.2^{b}	1.1^{a}	0.0^{b}	1.3^{a}
Glutamate	10.8^{a}	9.3^{b}	7.8^{d}	8.1^{c}	7.8	8.3	6.2	8.2
Glycine	3.7^{a}	3.1^{bc}	3.0^{c}	3.2^{b}	3.0^{ab}	3.2^{a}	2.5^{b}	3.2^{a}
Histidine	2.2^{b}	2.3^{a}	2.1^{b}	2.1^{b}	2.0^{a}	1.8^{ab}	1.5^{b}	1.7^{b}
Isoleucine	2.9^{a}	2.7^{b}	2.6^{bc}	2.4^{c}	2.5	2.6	2.2	2.7
Leucine	5.0^{a}	4.2^{d}	4.7^{b}	4.5^{c}	3.8^{b}	4.0^{ab}	4.2^{ab}	4.8^{a}
Lysine	4.7^{a}	4.2^{b}	4.8^{a}	4.6^{ab}	3.7	4.1	3.8	4.6
Methionine	1.2^{a}	1.1^{a}	1.2^{a}	0.9^{b}	0.9	0.5	0.9	0.7
Ammonia‡	5.3^{c}	5.9^{b}	6.3^{a}	6.4^{a}	6.5^{b}	7.2^{a}	7.1^{a}	7.3^{a}
Phenylalanine	3.2^{a}	2.6^{b}	2.6^{b}	2.7^{b}	2.5	2.6	2.2	2.7
Proline	2.4	2.2	2.3	2.3	1.9^{b}	2.1^{b}	2.0^{b}	2.6^{a}
Serine	4.8^{a}	4.6^{b}	4.1^{c}	4.1^{c}	4.5^{a}	4.5^{a}	3.5^{c}	4.2^{b}
Threonine	3.4^{a}	3.3^{ab}	3.2^{b}	3.2^{b}	3.2^{a}	3.2^{a}	2.9^{b}	3.2^{a}
Tyrosine	2.0^{a}	1.7^{c}	1.9^{ab}	1.8^{b}	1.4^{ab}	1.1^{b}	1.6^{a}	1.4^{ab}
Valine	3.7^{a}	3.5^{b}	3.2^{c}	3.2^{c}	3.1	3.2	2.7	3.3
Mg amino acid (×100)§	29.2	29.0	27.8	26.2	26.4	25.5	22.6	25.0

* Within each amino acid and day means followed by the same letter or no letter are not significantly different, as determined by Duncan's Multiple Range Test (5% level). † Treatment identification is as follows: D = dark, L = light, − = nutrient solution without N, + = nutrient solution with N. ‡ The data on ammonia, which is not an amino acid, is included for information. § Mg amino acid recovered from the analysis per g dry weight of sample.

Table 6. Changes in total amino plus amide N content of cottonseed during germination.

Amino acid	Content Day 0 seed	Change by Day 5†											
		Axis				Cotyledons				Whole seed			
		D−	D+	L−	L+	D−	D+	L−	L+	D−	D+	L−	L+
	μmoles·seed⁻¹												
Alanine	11.4	1.2	1.3	0.6	0.6	−0.1	−0.3	−1.0	−0.3	+0.2	+1.0	−0.4	+0.3
Arginine	18.2	0.6	0.5	0.4	0.3	−8.2	−7.3	−7.8	−6.5	−7.6	−6.8	−7.4	−6.2
Aspartate	17.8	11.2	9.9	7.6	5.7	−1.3	−1.0	+6.0	+6.8	+9.9	+8.9	+13.6	+12.5
Cysteine	2.7	<0.1	0.3	<0.1	0.2	−1.6	−1.3	−1.6	−1.1	−1.6	−1.0	−1.6	−0.9
Glutamate	38.9	1.7	1.7	0.8	1.0	−19.7	−17.8	−20.7	−19.4	−18.7	−16.1	−20.1	−18.4
Glycine	17.9	1.3	1.3	0.7	0.8	−7.5	−6.9	−6.7	−5.9	−6.2	−5.6	−6.0	−5.1
Histidine	4.8	0.4	0.4	0.2	0.2	−0.9	−0.7	−0.3	<−0.1	−0.5	−0.3	−0.1	+0.1
Isoleucine	6.0	0.6	0.6	0.3	0.4	−0.7	−0.3	−0.5	−0.2	−0.1	+0.3	−0.2	+0.2
Leucine	11.9	0.9	0.9	0.6	0.6	−1.7	−1.0	−1.1	−0.6	−0.8	−0.1	−0.5	0
Lysine	8.1	0.8	0.8	0.5	0.6	−0.5	−0.2	−0.3	+0.1	+0.3	+0.6	+0.2	+0.7
Methionine	2.6	0.2	0.1	0.1	0.1	−0.6	−0.4	−0.9	−0.5	−0.4	−0.3	−0.8	−0.4
Ammonia‡	30.5	12.3	12.6	8.3	7.8	−7.7	−6.6	−0.4	−1.1	+4.6	+6.0	+7.9	+6.7
Phenylalanine	8.3	0.5	0.5	0.3	0.3	−2.3	−1.8	−2.2	−1.5	−1.8	−1.3	−1.9	−1.2
Proline	9.1	0.5	0.5	0.4	0.4	−2.5	−2.0	−2.4	−1.8	−2.0	−1.5	−2.0	−1.4
Serine	11.2	1.4	1.3	0.7	0.7	−2.6	−2.0	−2.8	−2.1	−1.2	−0.7	−2.1	−1.4
Threonine	7.3	0.9	0.8	0.5	0.5	−0.8	−0.5	−0.3	−0.1	+0.1	+0.3	+0.2	+0.4
Tyrosine	4.3	0.3	0.2	0.2	0.1	−0.8	−0.5	−0.6	−0.4	−0.5	−0.3	−0.4	−0.3
Valine	9.4	0.8	0.8	0.5	0.5	−1.3	−0.7	−1.0	−0.4	−0.5	+0.1	−0.5	+0.1

† Treatment identification is as follows: D = dark, L = light, − = nutrient solution without N, + = nutrient solution with N. ‡ Data on ammonia, which is not an amino acid, are included for information.

Overall the free amino acids in the axes reacted differently to the various treatments (Table 3). On Day 3, when axes were first collected, the level of free amino acid N was higher in the treatments without N than in those with N. By Day 5, the treatment effects were more pronounced; the level was higher in the dark than in the light, and higher in the treatments without N than in those with N. The concentration of asparagine, which is the most prevalent amino acid in germinating cotton axes, was similarly affected by the germination conditions.

Hydrolysate Amino Acids. Amino acid composition of cottonseed (day 0 and 1, and of germinating cotton cotyledons after 3 and 5 days of incubation) are shown in Table 4. The amino acid composition changed during germination, and treatment effects were evident in these changes. The amino acids affected most dramatically were glutamate, arginine, and aspartate.

Axes, unlike cotyledons, had more aspartate than any other amino acid (Table 5). There was little change in composition of axes during germination. Treatment effects were evident for most amino acids.

Net Changes in Amino Acids. The net changes in amino acid composition of cotyledons, axes, and whole seedlings were determined (Table 6). Whatever is contained in the axis is a positive change since this is new tissue. The axis contents must either come from the cotyledon directly by molecular transport of the amino acid, or by amino acid metabolism of storage products in the cotyledon. The cotyledons, consequently, show a net loss for each amino acid in the dark. In the light, however, the cotyledons show a net gain of aspartate and a marginal increase of lysine with N treatment.

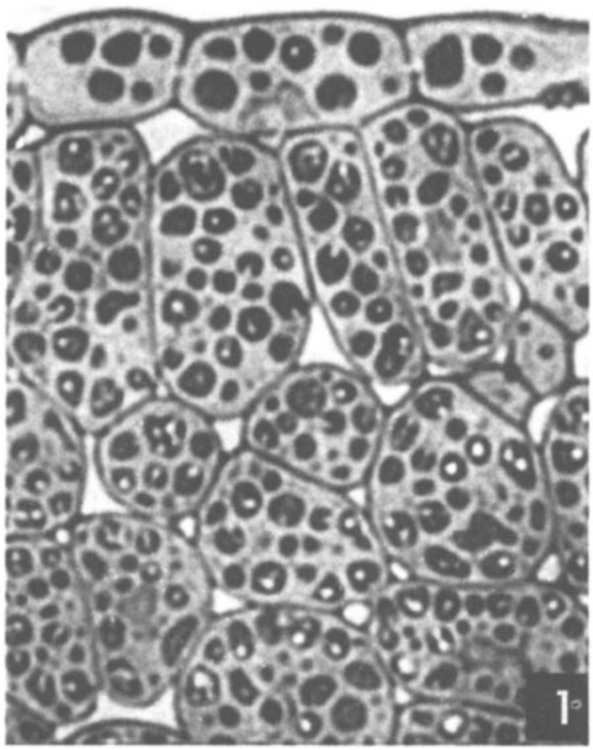
The seedlings taken as a whole show a net loss of arginine, glutamate, and glycine and marginally for cysteine, leucine, methionine, phenylalanine, proline, serine, and tyrosine. A net gain for aspartate, lysine, and ammonia is shown. Marginal increases depending upon treatment are seen for alanine, histidine, isoleucine, threonine, and valine.

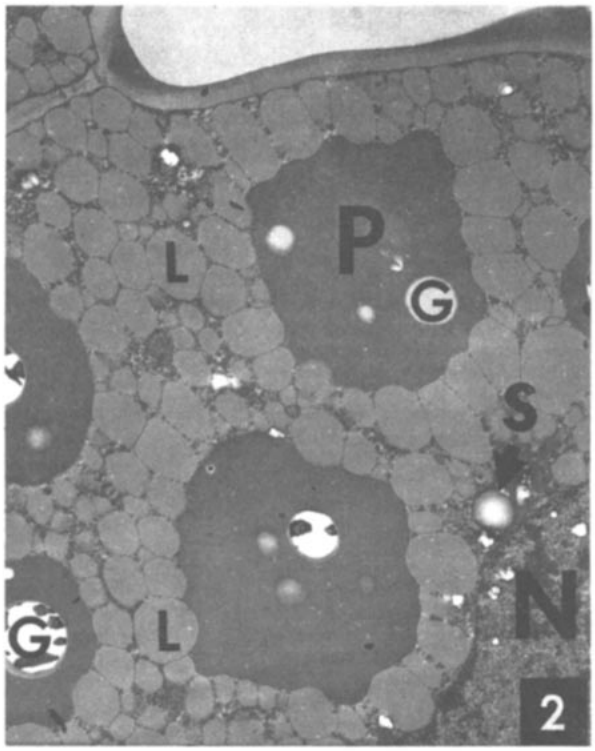
Light and Electron Microscopy. The cotyledonary cells of mature, ungerminated cottonseed were filled with storage products. As shown in Fig. 1, each cell had numerous electron-opaque protein bodies that were surrounded by smaller electron-translucent lipid bodies. The protein bodies contain globoids, which are phytin-rich mineral deposits (Fig. 2, 3). The seed also contain starch grains in the inconspicuous plastids (Fig. 2). Protein crystalloids are apparent in the protein body of Fig. 3.

After 1 day of incubation in the dark, starch was more apparent but no significant changes had occurred in the protein bodies (Fig. 4). In the light, however, with (Fig. 5) or without (Fig. 6) N, the protein bodies assumed a fibrillar appearance and seemed to shrink from the protein body membrane.

After 3 days of incubation in the dark, protein bodies showed considerable signs of dissolution or digestion and apparent coalescence (Fig. 7) if N had not been added to the nutrient medium. Globoids were not prominent at this time. Most of the contents of the the globoids had disappeared. Starch grains were more prevalent and plastids more prominent. Addition of N to the nutrient medium apparently inhibited protein body dissolution in the dark (Fig. 8). Incubation of seeds for 3 days resulted in considerable digestion of protein and coalescence of the protein body membranes to form a central vacuole (Fig. 9 and 10). Globoids were persistent and myelin-like figures were present. Addition of N did not have the striking effect that was seen in the dark-germinated seeds.

By the 5th day of incubation, protein bodies had nearly completely coalesced (Fig. 11, 12). Considerable lipid remained as well as some crystalline inclusions in the globoids. In the light starch grains enlarged (Fig. 12) as chloroplast development ensued. Little difference was apparent between N treatments.





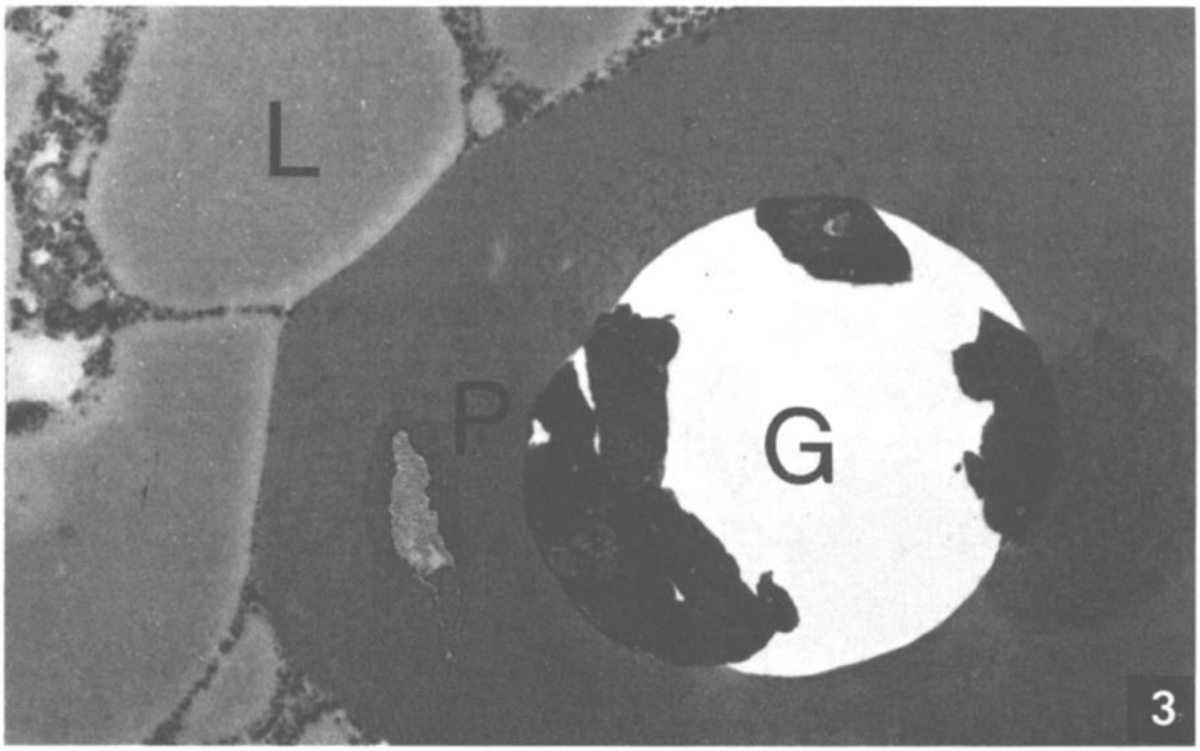
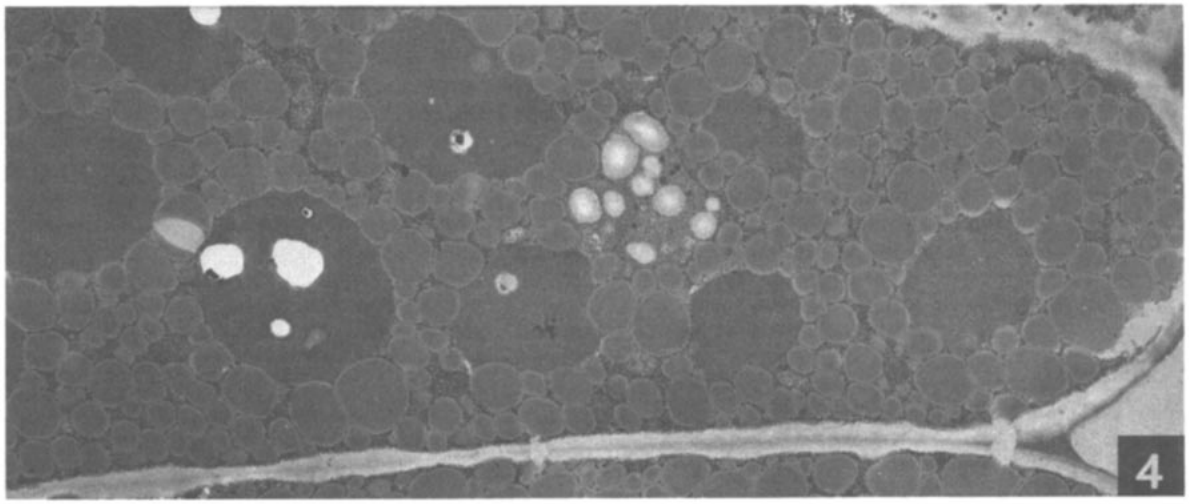
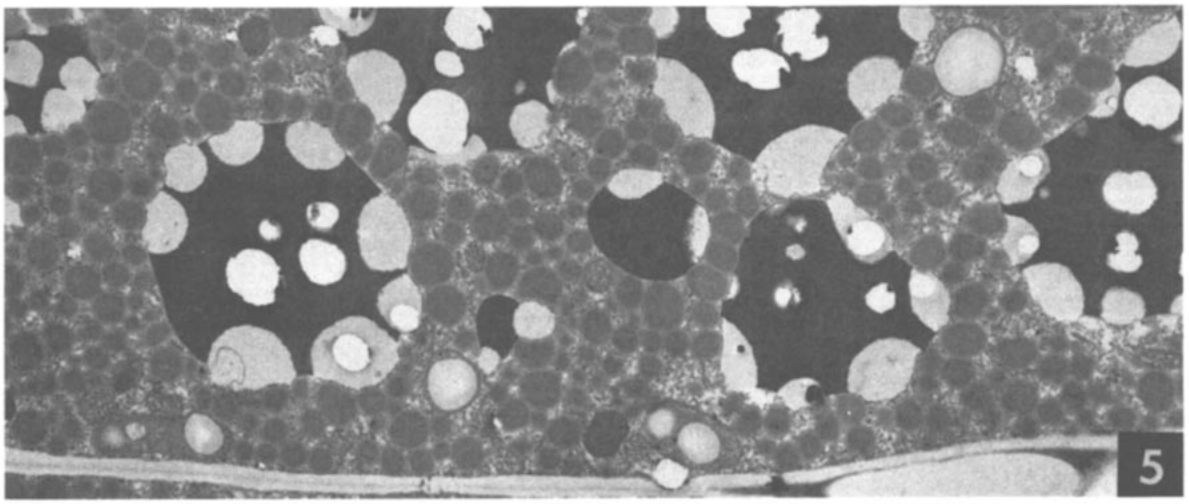


Fig. 1 to 3. ***Fig. 1.*** **Light micrograph of cotyledon from a 20-min-imbibed cottonseed (×1,000).** ***Fig. 2.*** **Portion of mesophyll cell of 20-min-imbibed cottonseed (×7,500), showing arrangement of lipid bodies (L) around protein bodies (P). G = globoids, N = nucleus, *S* = plastid starch grain.** ***Fig. 3.*** **Globoid containing protein body in mesophyll cell of 20-min-imbibed cottonseed (×50,000). G = globoid, L = Lipid body.**





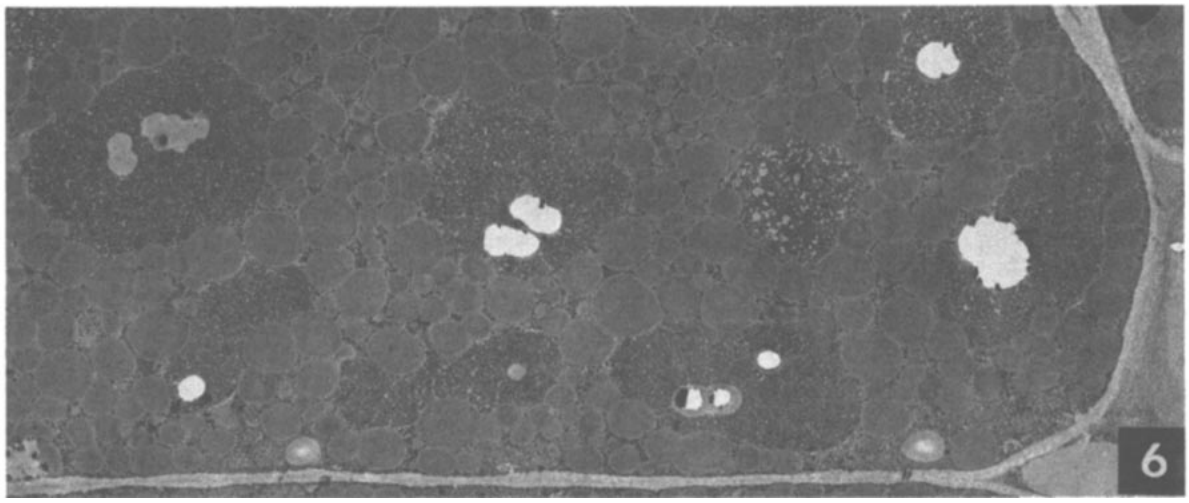


Fig. 4 to 6. ***Fig. 4.*** **Cotyledonary cell of cottonseed incubated for 1 day in the dark with no added N (×7,500).** ***Fig. 5.*** **Cotyledonary cell of cottonseed incubated for 1 day in the light with no added N (×7,500).** ***Fig. 6.*** **Cotyledonary cell of cottonseed incubated for 1 day in light with N added to medium (×7,500).**

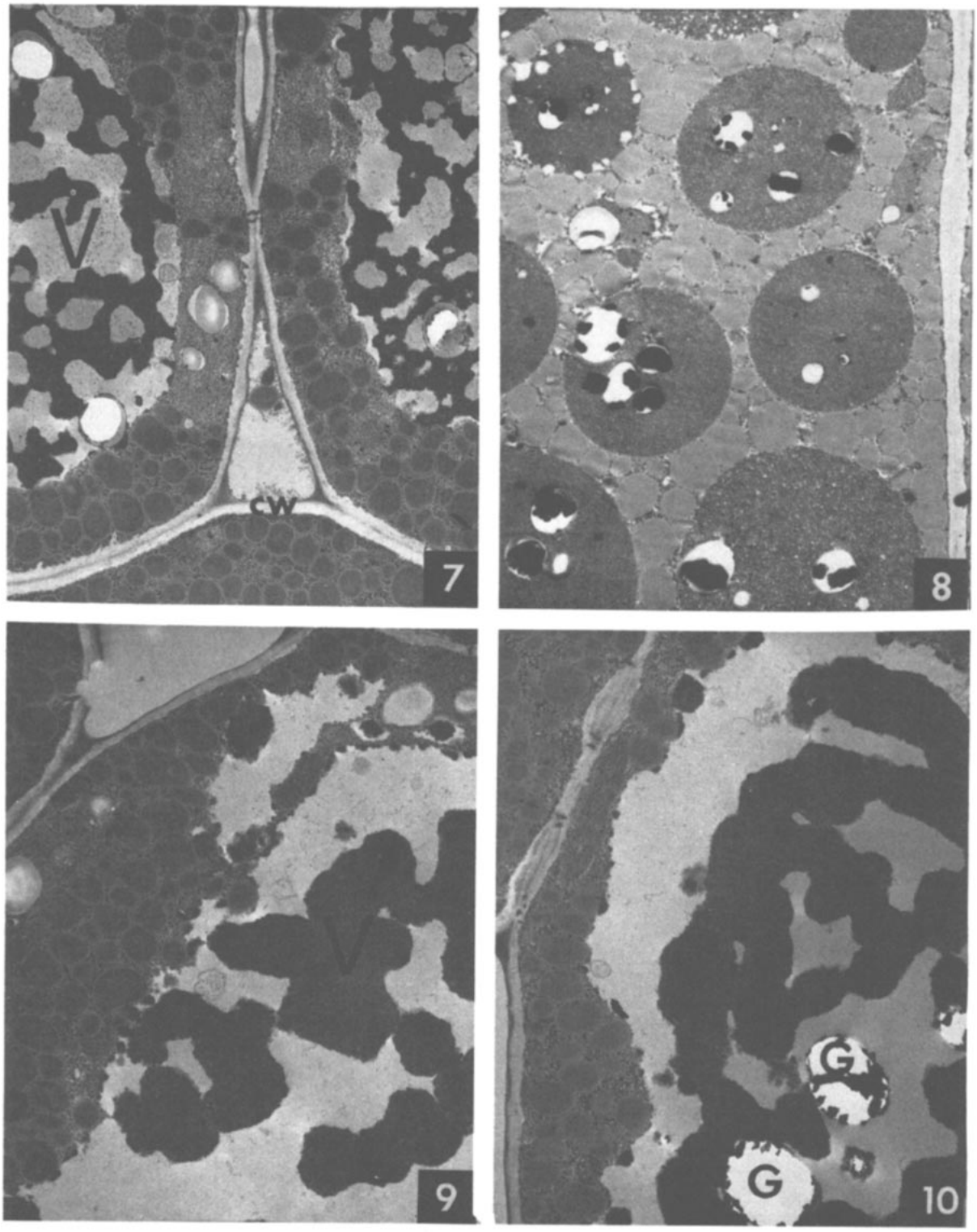
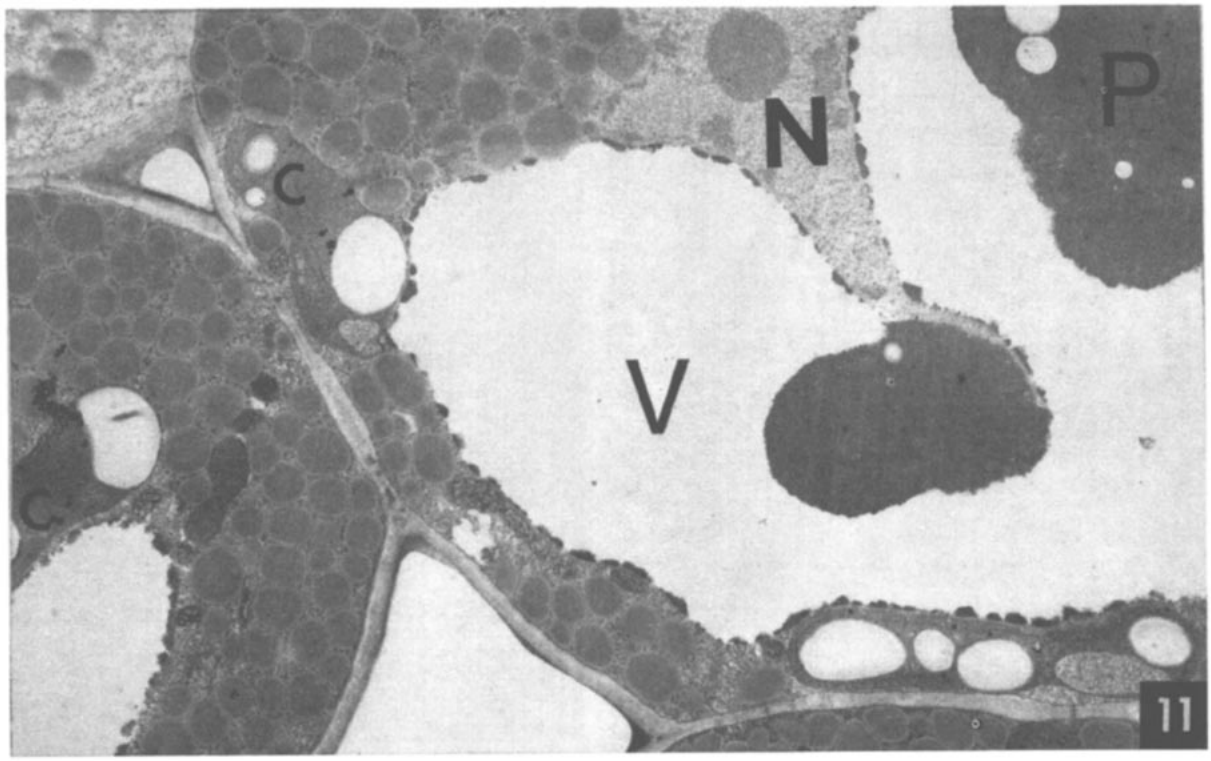


Fig. 7 to 10. ***Fig. 7.*** **Portions of three cotyledonary cells of cottonseed incubated for 3 days in dark with medium containing no added N (×7,500). Note coalescence of protein bodies into cell vacuole (V). CW = cell wall.** ***Fig. 8.*** **Portion of cotyledonary cell of cottonseed incubated for 3 days in the dark with N added to the nutrient medium. Note comparison of protein body dissolution in comparison with that of cells shown in Fig. 7, (×7,500).** ***Fig. 9.*** **Portion of cotyledonary cell of cottonseed incubated for 3 days in light with no added N (×7,500).** ***Fig. 10.*** **Portion of cotyledonary cell of cottonseed incubated for 3 days in the light with N added to the nutrient medium. Note protein body dissolution and persistence of globoids (G), (×7,500).**



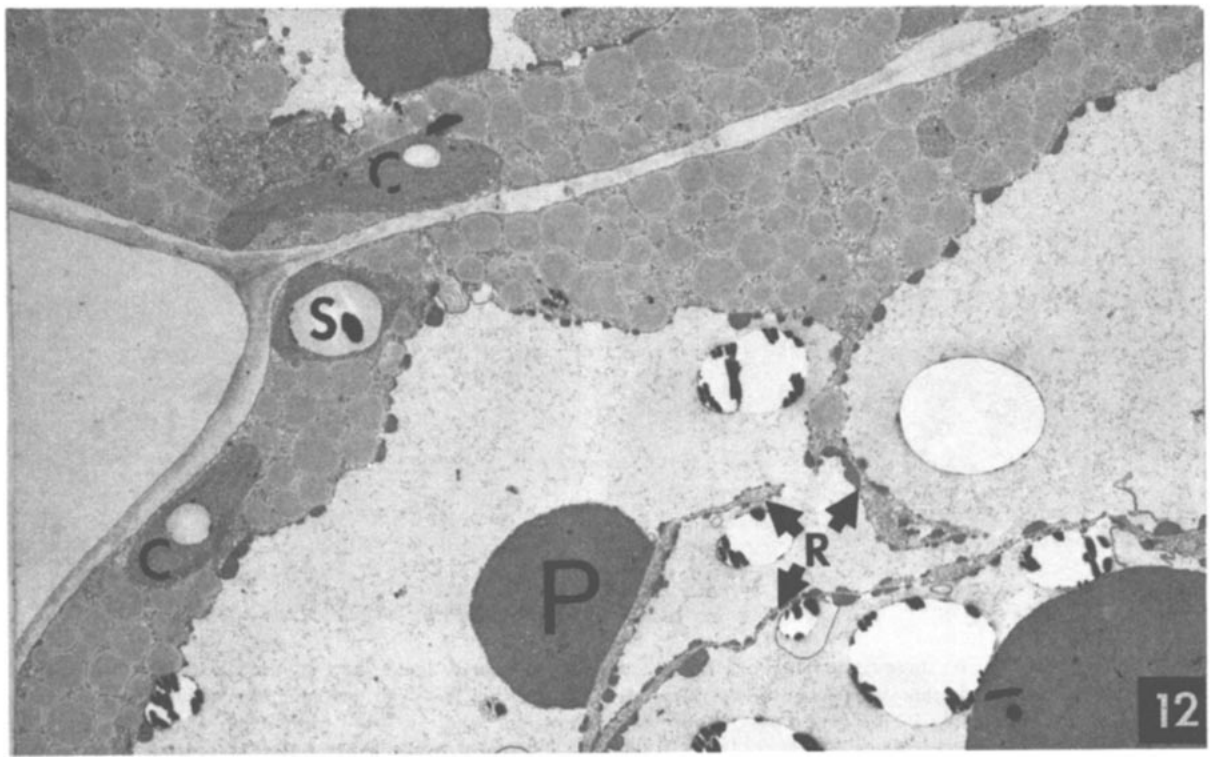


Fig. 11 to 12. ***Fig. 11.*** **Portions of cotyledonary cell of cottonseed incubated for 5 days in dark with N added to nutrient medium (×7,500). Plastids (C) have elongated but have not differentiated beyond the prolamellar body stage. N = nucleus, P = protein body remnant; V = cell vacuole.** ***Fig. 12.*** **Portions of cotyledonary cell of cottonseed incubated for 5 days in light with N added to nutrient medium (×7,500). Note remnants (R) of protein body membranes (×7,500). Plastids (C) have begun to form grana. P = protein body remnant; S = starch.**

DISCUSSION

Light appears to enhance mobilization of storage proteins in the cotton cotyledons but to retard transfer of dry matter and nutrient reserves to the developing axis. Apparent mobilization of the storage protein is shown by the readily apparent dissolution of the storage products in light incubated seeds (e.g., compare Fig. 4 with 5 and 6 for 1 day and Fig. 8 with Fig. 9 and 10). Reduced transfer of dry matter and nutrient reserves to the developing axis is shown by heavier cotyledons in light-grown tissue in Table 1, and by the increased content of aspartate in cotyledons as shown in Table 6. Nitrogen apparently retards storage protein mobilization in both light and dark (e.g., compare Fig. 8 with Fig. 7 for 3-day incubated seed). These results are, at least in part, in accord with those of Hurst and Sudia (1973). These researchers did not see any effect of light on dry weight, but they did report that free amino acids were more abundant in soybean (*Glycine max* L.) cotyledons grown in the light rather than in the dark. We found that the free amino acids were more abundant in cotton cotyledone as well.

This study, which is a followup of that of Elmore and King (1978), extends and expands their findings to include nutrient and light effects. A different cultivar was used in this study, basically to avoid the pigment gland interference with microscopy. The same balance sheet approach has been taken with similar results for the dark without N treatment. The new findings relate to the modulation of amino N transfers by N and light.

The predominance of free asparagine found in the cotyledon and especially in the axes has been noted in previous reports (Elmore and Leffler, 1976; Elmore and King, 1978) for cotton. The fact that aspartate increases in content in the cotyledon is further evidence of the predominance that aspartate and its amide, asparagine, play in nitrogen metabolism in both developing and germinating seed (Elmore and Leffler, 1976; Elmore and King, 1978). Hurst and Sudia (1973) reported similar results including the light effects on aspartate, for soybean.

Arginine concentration, which in cotyledons was significantly affected by both N and light treatments, was decreased in axes by light. In view of the high N content of arginine and the high arginine content of cottonseed, it seems likely that much of the N in the free amino acids in the axes of germinating cotton is derived from arginine. This hypothesis is in accord with the scheme presented by Dilworth and Dure (1978), in which arginine released by storage protein hydrolysis flows into asparagine. Asparagine is then transported from the cotyledons to the axis. That this scheme is correct is supported by the low, decreasing concentration of arginine in axes during germination.

Microscopy confirms the analysis presented for storage protein mobilization. Light enhances protein body dissolution and subsequent coalescence of these structures into the central vacuoles of the cotyledonary cell. These studies do not provide information on how this process occurs. Mollenhauer et al. (1978) proposed that lamellar bodies, which appear in the cytoplasm, function as intermediates in formation of endoplasmic reticulum from the stored lipid and protein reserves in the seeds of maize (*Zea mays* L.), bean (*Phaseolus vulgaris* L.), and pea (*Pisum sativum* L.). We do find occasional lamellar bodies (myelin-like figures) but have seen no evidence that they emerge from the edge of the globoids, which was one possibility suggested by Mollenhauer et al. (1978).

Cottonseed imbibed for 20 min were shown to have globoid crystals and soft globoids in accord with the terminology of Lott et al. (1971). The protein body is usually composed only of a proteinaceous matrix without the protein crystalloid found in protein bodies of other species, e.g., *Cucurbita spp.* (Lott, et al., 1971), although occasional crystalloids are seen (Fig. 3).

We have shown that light affects the ultrastructure and composition of germinating cottonseed, and that N in the nutrient solution modulates those effects. Most research on germination probably involves incubation of seed in the dark without nutrient solution, analogous to our dark without N treatment. Our research has shown that germination conditions are critical for determinates of seedling composition, and that care should, therefore, be taken to see that they are controlled.

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