Free Amino Acid Content of Burley Tobacco Leaves Developed under Different Light and Temperature Conditions

Robert E. Wilkinson,* Michael J. Kasperbauer, and Clyde T. Young

Free amino acid contents of burley tobacco (*Nicotiana tabacum* L.) leaves differed among genetically uniform plants that were grown under different temperature and light environments. The amino acids were listed in metabolically related groups. Those derived from oxalacetate, α-ketoglutarate, and D-3-phosphoglycerate accumulated in higher concentrations in leaves that developed under short, relative to long, photoperiods. There was a photoperiod by temperature interaction. When end-of-day red or far-red followed 8- or 16-h photosynthetic periods, the highest concentrations of free amino acids accumulated in leaves of plants that received red light at the end of daily 8-h photosynthetic periods. Far-red light following 8-h photosynthetic periods resulted in lower free amino acid concentrations. The responses to brief irradiations with red or far-red light are interpreted to indicate that the phytochrome system was involved, contributing to differences in free amino acid concentrations. Thus, leaf free amino acid contents were influenced by the relative synthesis and depletion activities that existed under each particular environment.

Plant biochemical responses to differing photoperiods are varied (Wilkinson, 1966, 1970, 1974) and include alterations of free sugars, organic acids, and total amino acids (Kasperbauer et al., 1970). Total amino acid contents were greater in leaves of tobacco plants grown under 8-h days than in leaves from tobacco grown under 16-h days, and this was consistent with increased total nitrogen and soluble nonprotein nitrogen in soybeans [*Glycine max* (L.) Merr.] grown under short, relative to long, photoperiods (Parker and Borthwick, 1939).

In an effort to produce safer tobacco products, recent research has been directed toward possible removal of potentially hazardous constituents, or their precursors, during the curing process (Tso and Gori, 1976). An experimental procedure called homogenized leaf curing (HLC) involves harvesting green leaves and grinding them to form a slurry from which selected components can be removed before the product is incubated, cured, and dried (Tso et al., 1975). Protein is one of the components removed during the HLC process because pyrolytic products of protein have undesirable aroma and include hydrogen cyanide and oxides of nitrogen (DeJong and Lam, 1977). The experimental removal of protein from tobacco and the potential value of the removed protein as a byproduct have resulted in recent interest in protein extraction from green tobacco leaves (Tso and Gori, 1975). Rapidly growing tobacco had higher protein content than mature plants (DeJong and Lam, 1977); however, effects of growth environment parameters on individual free amino acid qualitative and quantitative analyses have not been reported for burley tobacco.

The objective of the present study was to determine whether concentrations of individual free amino acids in fully expanded burley tobacco leaves would be influenced by the light and temperature regimes under which the leaves developed.

METHODS AND MATERIALS

First Experiment. *Plant Material.* Burley tobacco (*Nicotiana tabacum* L. cv. Burley 21) plants were started and grown for ~6 weeks at 28 °C under 14-h photoperiods at 16 000 lx from cool-white fluorescent lamps. Uniformly sized seedlings were transplanted to a field plot or transferred to pots of soil and placed in controlled-environment chambers. Plants transferred to the controlled-environment chambers were transplanted to the same soil type as were those transplanted to the field.

Growth Environments. Temperatures within the controlled-environment chambers were 18 or 28 °C. All chambers were illuminated with cool-white fluorescent lamps (22 000 lx) for 8 h daily. Short photoperiod treatment plants remained in uninterrupted darkness for the remainder of each diurnal cycle. Long photoperiod treatment plants received a 4-h low-intensity light interruption in the middle of the night (250 lx).

Sampling Procedure. The fifth and sixth leaves from the apex (excluding all leaves shorter than 7.5 cm) were collected from each of five plants from the field and from each of the four controlled environments. The leaves included in these samples were the most recent to attain maximum expansion, and their entire growth period occurred under the respective controlled and field environments. Leaf areas were determined by planimetry. A 50-cm^2 disk of lamina was removed from each side of the midvein of each leaf, without touching the midvein. The 100-cm^2 samples from each leaf were quick-frozen in dry ice, freeze-dried, and weighed. The pair of disks from each leaf constituted a replicate for analyses.

Extraction and Analyses. Samples were ground in 50 mL of a methanol, chloroform, and water mixture (60:25:15 v/v/v) for 1 min by using a Brinkman polytron at full speed. Ground samples were centrifuged, and the clear liquid was decanted into 100-mL beakers and allowed to partially evaporate overnight under a hood to remove the methanol and chloroform. The samples were taken to dryness in a vacuum desiccator. The dried extracts were suspended in 5 mL of pH 2.2 citrate buffer, centrifuged, and analyzed. Free amino acid analyses were performed by the ion-exchange chromatography technique with a Durrum Model D-500 amino acid analyzer using a 1.75 mm (i.d.) × 48 cm length column packed with the Durram high-resolution cation exchanger; bead diameter was 8 ± 2 μm. Elution solvents were citrate buffers with sodium adjusted to 0.2 N at pH 3.15, 4.25, and 7.9 (Stackman et al., 1958). Running time was 94 min, including a 20-min regeneration of the column.

Department of Agronomy (R.E.W.) and Department of Food Science (C.T.Y.), Georgia Station, Experiment, Georgia 30212, and Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, and Department of Agronomy, University of Kentucky, Lexington, Kentucky 40546 (M.J.K.).

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Second Experiment. Seedlings were started and grown for 6 weeks as described for the first experiment. Thirty uniform seedlings were transferred to each of two controlled-environment chambers. Plants in one chamber received light for 8 h and the other for 16 h each day from cool-white fluorescent lamps (22 000 lx). Day and night temperatures in each chamber were 25 °C. All plants were grown in aerated nutrient solution (Hoagland and Arnon, 1950). At the end of the day, 15 plants from each photoperiod group were exposed to 5 min of red light of 360 $\mu W/cm^2$ between 600 and 700 nm, while the other 15 plants from each group were exposed to 5 min of far-red light of 360 $\mu W/cm^2$ between 700 and 770 nm. The red and far-red light sources and filter systems were as described previously (Kasperbauer and Peaslee, 1973). After 18 days of treatment, plants were harvested 1 h after their final end-of-day treatment. The two most recently fully expanded leaves from each plant were harvested. Midveins were removed. Lamina samples were quick-frozen in dry ice, freeze-dried, weighed, and pulverized for analysis. Entries shown in Table II are means for 15 plants.

RESULTS AND DISCUSSION

Free amino acid contents of leaves grown under various light and temperature environments were measured after the leaves had become fully expanded but before senescence started. In the first experiment, plants received 8-h photosynthetic periods with or without low-intensity light interruptions of the 16-h night to give long or short photoperiods, respectively, during leaf growth. In the second experiment, plants were grown under 8- or 16-h photosynthetic periods, followed immediately by 5 min of red or 5 min of far-red light to put phytochrome in the biologically active or inactive form, respectively, at the beginning of uninterrupted nights. Such end-of-day phytochrome manipulations influence plant morphology and chemical content (Kasperbauer et al., 1970; DeGreef and Fredericq, 1969; Kasperbauer and Peaslee, 1973).

First Experiment. Concentrations of free amino acids in leaves that developed under various photoperiod and temperature regimes are shown in Table I. The amino acids are listed in metabolically related groups according to the organic acid from which they are derived (Mahler and Cordes, 1966) and a miscellaneous group for amino acids not derived from a specific organic acid.

Concentrations of free amino acids derived from oxalacetate, α-ketoglutarate, D-3-phosphoglycerate, and miscellaneous groups were higher in leaves that developed under short photoperiods than in those that developed under long photoperiods at both 18 and 28 °C (Table I). For most of the groups compared, free amino acid concentrations were higher at 28 °C than at 18 °C for short photoperiods and lower at 28 °C than at 18 °C for long photoperiods.

Field-grown plants received fluctuating temperatures, higher light intensities, and longer photosynthetic periods. They had higher free amino acid concentrations in the α-ketoglutarate group, especially in proline content.

Although all of the growth chamber plants received the same daily photosynthetic period of high-intensity light, it is apparent that the period of low-intensity light was provided in the middle of each night to obtain long photoperiods caused dramatic differences in metabolism and growth. Thus, two responses are evident in this short-day annual: (a) an interaction of temperature and photoperiod exists in the metabolic activity of the genetically uniform leaves and (b) the free amino acid content must be a function of the individual enzymatic processes as influenced by these environmental parameters. A similar pattern was observed for a perennial plant, mint (*Mentha piperita* L.), wherein temperature and photoperiod markedly altered plant morphology and organic acid and amino acid contents (Steward, 1962).

Table I. Free Amino Acid Content of Tobacco Leaf Lamina from Plants Grown in Differing Temperatures and Photoperiods, Expressed on a Dry Weight Basis

	μM/g dry weight				
	18 °C		28 °C		
amino acid	short	long	short	long	field
	Oxalacetate Group				
Asp	4.8	2.8	5.1	2.2	5.8
Lys	2.8	2.6	3.3	2.0	2.3
Met	0.3	0.1	0.4	0.2	0.0
Ill	1.2	0.8	1.7	0.9	0.6
Thr	2.7	2.2	4.0	1.6	5.1
	(11.8)bc[a]	(8.5)d	(14.6)a	(6.9)e	(13.8)ab
	α-Ketoglutarate Group				
Glu	1.0	0.9	1.1	0.5	1.5
Pro	3.8	3.1	5.7	0.0	16.6
Arg	0.9	0.4	1.7	0.8	0.0
	(5.7)c	(4.4)c	(8.5)b	(1.3)d	(18.1)a
	Pyruvate Group				
Ala	13.3	18.1	20.1	11.5	19.2
Leu	2.3	1.3	3.2	1.4	1.0
Val	1.7	1.2	2.8	1.2	0.9
	(17.3)c	(20.6)b	(26.1)a	(14.1)d	(21.1)b
	D-3-Phosphoglycerate Group				
Ser	4.9	2.3	3.8	1.6	3.6
Gly	1.7	1.1	2.0	1.0	2.1
	(6.6)a	(3.4)b	(5.8)a	(2.6)c	(5.7)a
	Miscellaneous				
Phe	18.4	17.4	18.4	8.8	16.2
His	2.7	3.0	3.7	2.3	4.0
Tyr	3.4	3.0	5.7	1.8	6.1
	(24.5)b	(23.4)b	(27.8)a	(12.9)c	(26.3)a

[a] Values in parentheses are subtotals for each group of amino acids. Within each group, subtotals that are followed by the same letter do not differ significantly at the 5% level.

Second Experiment. Photoperiodic control of plant growth is associated with phytochrome action (Mitrakos and Shropshire, 1972) which is controlled by the light spectra. Therefore, manipulation of the phytochrome system at the beginning of the night was undertaken by exposure of the leaves to red (600–700-nm) or far-red (700–770-nm) light. In this way the phytochrome influence on utilization of the day's production of photosynthate could be studied. Previous work with burley tobacco has shown that such treatment affects morphological development (Kasperbauer and Peaslee, 1973). Morphological and chemical responses of tobacco leaves that received 5 min of red light immediately following the photosynthetic radiation from cool-white fluorescent lamps were the same as those of control plants that were transferred directly to darkness without the 5-min exposure to red light. This was expected because the ratio of red to far-red light emitted by cool-white fluorescent lamps was very similar to that emitted by our red radiation source.

End-of-day phytochrome manipulation can also be used to simulate some of the light-quality effects of shading by other plants; these effects include a shift in the spectral distribution of light received at the beginning of the night (Kasperbauer, 1971). Because of the competitive absorption of red light by chlorophyll, plants in crowded populations receive more far-red relative to red light than do

Table II. Free Amino Acid Content of Tobacco Leaves That Developed at 25 °C under 8- or 16-h Daily Light Periods That Ended with 5 min of Red or 5 min of Far-Red Light Each Day; Entries Are Means for Fifteen Plants and Expressed on a Dry Weight Basis

amino acid	μM/g dry weight at day length and end-of-day light quality			
	8-h		16-h	
	red	far-red	red	far-red
Oxalacetate Group				
Asp	8.0	2.9	2.4	4.7
Lys	1.9	1.1	0.7	0.5
Met	0.9	0.4	0.4	0.7
Ile	1.9	1.0	0.8	0.6
Thr	11.6	8.9	10.0	10.5
	(24.3)a[a]	(14.3)c	(14.3)c	(17.0)b
α-Ketoglutarate Group				
Glu	5.7	6.7	2.6	3.0
Pro	7.5	4.9	6.6	4.5
Arg	1.3	0.9	0.6	0.4
	(14.5)a	(12.5)b	(9.8)c	(7.9)d
Pyruvate Group				
Ala	7.4	6.1	5.4	4.1
Leu	2.7	1.8	1.2	0.8
Val	2.7	1.6	1.3	0.8
	(12.8)a	(9.5)b	(7.9)c	(5.7)d
D-3-Phosphoglycerate Group				
Ser	7.3	4.2	3.8	3.9
Gly	1.6	1.3	0.5	1.0
	(8.9)a	(5.5)b	(4.3)b	(4.9)b
Miscellaneous				
Phe	2.9	1.4	1.3	1.4
His	0.8	0.3	0.2	0.2
Tyr	1.9	0.9	1.1	1.0
Cys	0.3		0.4	0.4
	(5.9)a	(2.6)b	(3.0)b	(3.0)b

[a] Values in parentheses are subtotals for each group of amino acids. Within each group, subtotals that are followed by the same letter do not differ significantly at the 5% level.

plants grown in sparse populations.

Free amino acid contents in all of the groups were highest in leaves that developed under short photosynthetic periods, followed each day by the brief exposure to red light (Table II). Concentrations of free amino acids in the α-ketoglutarate and pyruvate groups were higher in the plants grown under 8-h than in those grown under 16-h day lengths, and within each day length, plants that received red light last each day had higher free amino acid concentrations than those that received far-red light last each day. Thus, the free amino acid composition of leaves of the same stage of development responds dramatically to temperature, length of the daily photosynthetic period, and the form that phytochrome is in at the end of the daily photosynthetic period.

Our experiments with uniform genetic lines of burley tobacco demonstrate that leaves grown to the same stage of development under different environments can differ markedly in chemical content. This is important in plant culture systems in which the leaf is the consumed portion of the plant. Tobacco is an example of a plant whose leaf chemistry influences the quality of the consumed products. Also, investigation into possible use of tobacco as a source of high-quality protein (Tso and Gori, 1976) points out the need to realize that the quantity and quality of that protein could be altered by the growth environment, including plant population densities and spacing arrangements that alter the spectral composition of light received by the leaves during their growth and development. The effects of light intensity and duration on the photosynthetic process are established; however, effects of the phytochrome system on regulating plant development and composition need further investigation, especially under field conditions.

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