Phytochemistry, 1970, Vol. 9, pp. 2091 to 2095. Pergamon Press. Printed in England.

EFFECTS OF END-OF-DAY RED AND FAR-RED RADIATION ON FREE SUGARS, ORGANIC ACIDS AND AMINO ACIDS OF TOBACCO

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(*Received* 9 *December* 1969, *in revised form* 7 *February* 1970)

Abstract—Tobacco plants (*Nicotiana tabacum* L.) were given 5 min of red (R) or far-red (FR) radiation at the end of each day, following full intensity long (16-hr) and short (8-hr) photoperiods. Contents of free sugars, organic acids and amino acids in the plants were examined along with photo-induced differences in morphological development. Plants under long photoperiods grew more than those under short photoperiods. Within each photoperiod, plants irradiated with R developed shorter stems and darker green leaves than did those irradiated with FR. Long photoperiods resulted in higher concentrations of free sugars and lower concentrations of organic acids than did short photoperiods. Within each photoperiod, plants receiving terminal FR radiation had higher concentrations of free sugars and organic acids than did those receiving terminal R radiation. Plants grown under short photoperiods had a higher amino acid concentration than did those under long photoperiods. Concentration of amino acids in plants grown under long photoperiods was not affected by terminal R or FR. However, under short photoperiods plants irradiated with R had higher amino acid concentrations than did those irradiated with FR.

INTRODUCTION

MANY aspects of phytochrome control of plant growth and development have been reported.[1,2] The duration and intensity of photoperiod prior to, and the duration of uninterrupted darkness following red (R) and far-red (FR) irradiations influence the morphological response of plants so treated.[3] Brief irradiations with R and FR, acting through the phytochrome system, at the end of short days markedly influence morphological development of tobacco.[4] Plants grown under long, full intensity photoperiods usually grow more rapidly and exhibit less response to terminal phytochrome manipulation. In addition to effects on morphological development, photoperiod and terminal R and FR irradiations influence alkaloid and phenolic content.[5] The objective of the research reported in this paper was to determine whether levels of free sugars, organic acids and amino acids of tobacco are influenced by length of photoperiod and by end-of-day manipulation of phytochrome with R and FR.

[1] H. A. BORTHWICK and S. B. HENDRICKS, *Science* **132**, 1223 (1960).
[2] M. FURUYA, *Progress in Phytochemistry*, Vol. 1, p. 347, Interscience, New York (1968).
[3] H. A. BORTHWICK and R. J. DOWNS, *Botan. Gaz.* **125**, 227 (1964).
[4] M. J. KASPERBAUER and A. J. HIATT, *Tobacco Sci.* **10**, 29 (1966).
[5] T. C. TSO, M. J. KASPERBAUER and T. P. SOROKIN, *Plant Physiol.* **45**, 330 (1970.

RESULTS AND DISCUSSION

Dry weight per plant varied in response to photoperiod and end-of-day light quality treatments (Table 1). More dry matter was produced under 16-hr than under 8-hr photoperiods. FR-irradiated plants developed lighter-colored leaves and longer, heavier stems than did the R-irradiated ones. The morphological differences associated with R and FR irradiations

TABLE 1. FREE SUGAR CONTENT OF TOBACCO GROWN UNDER 8- AND 16-hr PHOTOPERIODS FOLLOWED BY 5 min OF R OR FR AT THE END OF EACH DAY

Photo-period (hr)	End-of-day radiation (5 min/day)	Plant portion	Dry matter per plant (g)	Sugar content (mg/g of dry matter)			
				Sucrose	Glucose	Fructose	Total
16	R	Lamina	3·30	8·4	6·3	3·0	17·7
		Mid-rib	0·85	11·3	11·3	5·1	27·7
		Stem	0·28	30·0	30·0	22·6	82·6
	FR	Lamina	3·76	8·1	8·0	4·7	20·8
		Mid-rib	1·02	12·0	23·8	8·3	44·1
		Stem	0·44	34·0	35·0	35·0	104·0
8	R	Lamina	1·82	6·3	1·5	2·0	9·8
		Mid-rib	0·49	7·5	2·7	1·3	11·5
		Stem	0·16	17·5	10·0	10·1	37·6
	FR	Lamina	1·46	7·5	3·0	2·8	13·3
		Mid-rib	0·57	8·3	19·2	10·3	37·8
		Stem	0·47	12·5	45·0	40·0	97·5
Statistical significance*							
Photoperiods			†	†	NS	NS	†
End-of-day radiation			NS′	NS	†	†	†
Plant portion			†	†	†	†′	†

* † = Sig. at $p = 0{\cdot}05$; †′ = lamina and mid-rib portions differed from stem, but not from each other at $p = 0{\cdot}05$; NS = not sig. at $p = 0{\cdot}05$; NS′ = not significant at $p = 0{\cdot}05$ on whole plant basis, but dry matter distribution between plant portions differed between R and FR with 8-hr photoperiods.

were more pronounced when the treatments were given after 8-hr rather than after 16-hr photoperiods. Our results are consistent with those of Downs *et al.*[6] who found that the duration of uninterrupted darkness following R and FR irradiations influenced the intensity of response in several plant species to such treatments. They reasoned that R or FR radiation converted phytochrome to the biologically active or inactive forms, respectively, and that the morphological response to phytochrome depended on its form and the duration of time during which it retained that form. Differences in morphological development and chlorophyll content of tobacco, irradiated with R and FR at the end of 8-hr photoperiods, are discussed in a previous report.[4]

Sucrose, glucose and fructose contents of the various plant portions are shown in Table 1. Within each of the treatments, the concentration of these sugars was highest in the stems, intermediate in midribs, and lowest in the lamina. Plants grown under 16-hr photoperiods terminated with FR had a higher total free sugar content than those that received 8-hr photoperiods terminated with FR. The same relationship held between 16-hr and 8-hr photoperiods when both were terminated with R. Within each photoperiod, FR radiation resulted in higher concentrations of glucose, fructose and total free sugars in each of the various plant

[6] R. J. DOWNS, S. B. HENDRICKS and H. A. BORTHWICK, *Botan. Gaz.* **118**, 199 (1957).

portions. The effects of R and FR on sucrose content were inconsistent, and differences were not statistically significant.

Literature on organic acids in tobacco deals mostly with organic metabolism in the presence or absence of light. Relatively little information is available on the affects of light quality. The principal organic acid in tobacco is malic acid and it is usually considered a key compound in organic acid metabolism.[7] In darkness there is a disappearance of malic and an increase in citric acid in excised leaves of some but not all varieties of tobacco.[8] Vickery observed the loss of malic and an increase in succinic acid when excised leaves were cultured in darkness.[9] The effect of light on incorporation of C^{14} from various labelled metabolites in short-term experiments was investigated by Graham and Walker[10] who found that the C^{14} incorporation into malate was high in light but low in darkness.

Organic acid concentrations were determined in tobacco plants grown under long or short photoperiods terminated with R or FR irradiations (Table 2). Lamina and mid-rib

TABLE 2. FREE ORGANIC ACIDS IN TOBACCO GROWN UNDER 8- AND 16-hr PHOTOPERIODS FOLLOWED BY 5 min OF R OR FR AT THE END OF EACH DAY

Photo-period (hr)	End-of-day radiation (5 min/day)	Plant portion	Organic acid content (mg/g dry matter)					
			Malic	Citric	Succinic	Fumaric	Ascorbic	Total
16	R	Lamina	10·0	2·0	1·3	0·5	3·8	17·6
		Mid-rib	25·0	1·3	2·0	1·3	7·5	37·1
		Stem	12·0	<0·5	3·5	<0·5	3·0	19·5
	FR	Lamina	12·5	2·5	1·3	0·5	4·3	21·1
		Mid-rib	30·1	1·5	2·5	2·5	8·0	44·6
		Stem	12·5	<0·5	3·9	<0·5	3·0	20·4
8	R	Lamina	12·5	2·5	2·5	0·5	1·5	19·5
		Mid-rib	50·1	1·4	3·0	2·5	6·3	63·3
		Stem	12·5	<0·5	3·8	<0·5	2·8	20·1
	FR	Lamina	16·3	2·7	3·0	1·3	1·8	25·1
		Mid-rib	50·1	1·5	3·0	2·5	6·3	63·4
		Stem	13·0	<0·5	4·0	<0·5	3·0	21·0
Statistical significance*								
Photoperiod			†	NS	†	NS	†	†
End-of-day radiation			†	NS	†	†	NS	†
Plant portion			†′	†	†	†′	†′	†′

* † = Sig. at $p = 0{\cdot}05$; †′ = lamina and stem portions differed from mid-rib, but not from each other at $p = 0{\cdot}05$. NS = not sig. at $p = 0{\cdot}05$.

portions of plants grown under 8-hr photoperiods had higher total organic acid concentrations, especially of malic acid, than did such tissues from plants grown under 16-hr photoperiods. Within each photoperiod, FR radiation resulted in higher organic acid content of lamina. The same response to R and FR was obtained from mid-rib portions of plants grown under 16-hr, but not from those grown under 8-hr photoperiods. Considering the experimental plant as a whole, concentrations of malic acid associated with various treatments were 13·0, 15·9. 19·9 and 23·4 mg/g dry matter for 16-hr + R, 16-hr + FR, 8-hr + R, and 8-hr + FR, respectively. These results clearly show a response pattern to both photoperiod and end-of-day radiation.

[7] R. E. STUTZ and R. H. BURRIS, *Plant Physiol.* **26**, 266 (1951).
[8] S. RANJAN and M. M. LALORAYA, *Plant Physiol.* **35**, 714 (1960).
[9] H. B. VICKERY, *J. Biol. Chem.* **234**, 1363 (1959).
[10] D. GRAHAM and D. A. WALKER, *J. Biochem.* **82**, 554 (1962).

Lamina tissue from plants grown under short photoperiods had higher concentrations of amino acids than those grown under long photoperiods (Table 3). This result agrees with the finding of Parker and Borthwick[11] that total nitrogen and soluble non-protein nitrogen were higher in soybean plants receiving 8-hr than in those receiving 16-hr photoperiods. In addition, we found higher amino acid concentrations in lamina from plants that received brief R than in those that received FR irradiations at the end of each 8-hr day (Table 3). End-of-day R or FR irradiations given after 16-hr photoperiods did not result in different levels of total amino acids. Our data on morphological development (Table 1) and total amino acid content (Table 3) are consistent in that there were differences associated with photoperiods, and the effects of end-of-day R and FR were pronounced when given after 8-hr, but not when given after 16-hr photoperiods.

TABLE 3. TOTAL FREE AMINO ACID CONTENT μM/g dry wt. OF LEAF LAMINA FROM TOBACCO GROWN UNDER 8- OR 16-hr PHOTOPERIODS FOLLOWED BY 5 min OF R OR FR AT THE END OF EACH DAY

Photoperiod (hr)	End-of-day radiation	
	R	FR
16	39·2*	38·3
8	66·4	44·2

* Differences in total amino acid concentrations between 16- and 8-hr photoperiods are significant at $p = 0{\cdot}1$. Differences associated with R and FR are significant ($p = 0{\cdot}05$) within the 8-hr but are not significant within the 16-hr photoperiod.

EXPERIMENTAL

Tobacco (*Nicotiana tabacum* L. cv. TI-204-E) seedlings were started and grown for 6 weeks at 28° and 14-hr days with a light intensity of 17,000 lx from cool-white fluorescent lamps. They had attained a height of about 5 cm before being placed in controlled-environment chambers for various experimental treatments. In preparation for light treatments, the lower leaves were removed and the stems marked to insure that leaves and stems included in the samples would include only those portions that developed during the treatment period. One group of thirty plants was placed under 8-hr and another group under 16-hr photoperiods, both at 25° and 22,000 lx from cool-white fluorescent lamps. All plants were grown in an aerated nutrient solution.[12] Each day, at the end of their respective photoperiods, fifteen plants from each group were exposed to 5 min of red (R) radiation of 3·6 μW cm^{-2} nm^{-1} at 660 nm, while the other fifteen plants were exposed to 5 min of far-red (FR) radiation of 3·7 μW cm^{-2} nm^{-1} at 740 nm. The light sources and filters were described in an earlier report.[4] Plants from the 16-hr photoperiod were harvested after 18 days of treatment; those from the 8-hr photoperiod were harvested after 20 days. This was done to accommodate the facilities available. Plants from both photoperiods were harvested 1 hr after their final end-of-day irradiations. Plant materials were separated into lamina, mid-rib, and stem portions. The various plant portions from the fifteen plants within each treatment were freeze-dried, and pulverized for chemical analysis.

The pulverized tobacco tissue was extracted 3 times with hot 70% EtOH. Extracts were combined and concentrated under reduced pressure to 0·4 g/ml to be used for paper and column chromatography. Sugars

[11] M. W. PARKER and H. A. BORTHWICK, *Botan. Gaz.* **100**, 651 (1939).
[12] D. R. HOAGLAND and D. I. ARNON, *Calif. Agri. Exptl. Sta. Cir.* 347 (rev.) (1950).

were separated by descending chromatograms on Whatman* No. 1 paper developed in *n*-BuOH–EtOH–H_2O (4:1:5, v/v) for 64 hr.[13] The spots were developed by spraying the chromatograms with *p*-anisidine,[14] and also with aniline hydrogen phthalate.[15] For organic acid determination we used 1-pentanol–5 M formic acid (1:1, v/v) as a solvent.[16] The color reagents were 0·04% bromocresol green or 0·04% bromophenol blue in EtOH,[16] or ammonical $AgNO_3$,[17] or a combination of these reagents.[18] Amino acids were determined in a Technicon* amino acid analyzer.[19]

Acknowledgement—The authors are grateful to Dr. J. F. Chaplin for advice and assistance on statistical evaluation of the data.

* Mention of trade names is as part of the exact experimental conditions and not as endorsement by the U.S. Department of Agriculture or the University of Kentucky.

[13] A. E. Brandfield and A. E. Flood, *Nature* **166**, 264 (1950).
[14] S. Mukherjee and H. C. Stravastava, *Nature* **169**, 330 (1952).
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[16] M. L. Buch, R. Montgomery and W. L. Porter, *Anal. Chem.* **24**, 489 (1951).
[17] J. B. Stark, A. E. Goodban and H. S. Oven, *Anal. Chem.* **23**, 413 (1951).
[18] J. Paskova and V. Munk, *J. Chromatog.* **4**, 241 (1960).
[19] Technicon, Amino Acid Auto-analyzer, Instrument Manual AA-1, 6-hr column (1967).