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SHORT COMMUNICATION

THE ACCUMULATION OF ANTHOCYANINS IN GREEN AND RED SEEDLING STRAINS OF *SORGHUM VULGARE*

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Abstract—Cyanidin glycoside biosynthesis was completely blocked in coleoptiles and almost completely in first internodes of "green" (drsP) in contrast to "red" (dRsP) seedlings of isogenic strains of *Sorghum vulgare*. Since the green seedlings can accumulate two types of deoxyanthocyanins, the block was peculiar to the synthesis of cyanidin and not to anthocyanins in general. Mature leaves of the green strain, however, could form the cyanidin glycoside in amounts equivalent to the red strain.

INTRODUCTION, RESULTS AND DISCUSSION

THE ACCUMULATION of a red cyanidin glycoside in Sorghum has been shown to be controlled by both a high intensity blue light response and a reversible phytochrome effect.[1] As a continuation of studies of the accumulation of flavonoids in seedlings of Sorghum,[2-4] genetic variants involving "red" and "green" seedling characteristics have been compared. These seedling color patterns are controlled by a linkage group first reported in 1939 by Stephens and Quinby;[5] the contrasting phenotypes are dry (*D*) vs. juicy (*d*) stalks, red (*R*) vs. green (*r*) seedling color and purple (*P*) vs. brown (*p*) plant color. These and additional genes control the flavonoid color patterns in seeds and mature plants.[6] Isogenic strains of milo parentage differing only in this red vs. green seedling characteristic were supplied by DeKalb Agricultural Association, Lubbock, Texas. They can be designated in terms of the above linkage group as *dRsP* for the red and *drsP* for the green strain.[7]

The lack of accumulation of significant amounts of the red cyanidin in the DeKalb green mutant is only a seedling characteristic. Mature leaves of the green strain grown in the greenhouse formed the cyanidin glycoside in amounts similar to those of the red strain. Both these red pigments were chromatographically and spectrally similar to the acylated form identified in the Oklahoma Wheatland milo variety.[2] The flavone, luteolin-7-glucoside[4] was also present in comparable amounts.

The block to cyanidin synthesis in the green mutant was not complete in the first internode, but the maximum amount formed was only about 5% of that which can be formed in the red strain (Table 1). The greatest accumulation occurred when the first internode was incubated with the seed and coleoptile attached (seed-shoot), rather than as an isolated internode and after pretreatment of the intact seedling with 1 hr of low intensity red light at 2 days.

[1] R. J. DOWNS and H. W. SIEGELMAN, *Plant Physiol.* **38**, 25 (1963).
[2] H. A. STAFFORD, *Plant Physiol.* **40**, 130 (1965).
[3] H. A. STAFFORD, *Plant Physiol.* **43**, 318 (1968).
[4] H. A. STAFFORD, *Phytochem.* **8**, 743 (1969).
[5] J. C. STEPHENS and J. R. QUINBY, *J. Agri. Res.* **70**, 725 (1939).
[6] J. R. QUINBY and J. H. MARTIN, *Adv. Agron.* **6**, 305 (1954).
[7] B. MAUNDERS, private communication.

TABLE 1. ACCUMULATION OF CYANIDIN GLYCOSIDE IN FIRST INTERNODES OF RED AND GREEN STRAINS (DEKALB) AND COMPARISONS WITH WHEATLAND MILO STRAIN (OKLAHOMA)

	mμmoles/g fr. wt. First internode incubated as	
	Seed-shoot	First internode
Red—no light pretreatment*	890	290
Green—no light pretreatment*	20	Trace
Green—+ light pretreatment†	35	15
Oklahoma—no light pretreatment*	690	293

* Three days dark-grown, excised as seed-shoot or internode and incubated under bright light for 12 hr light followed by 12 hr dark.

† Intact seedlings pretreated with 1 hr of low intensity red-light (1000 erg × cm^{-2} × sec^{-1}) with a plexiglas filter No. 2423, followed by one more day in the dark before excision and incubation in bright light as in above.

The block was not due to anthocyanin synthesis in general, since two deoxyanthocyanins (apigeninidin and luteolinidin) were found in significant amounts in the green mutant in both internodes and coleoptiles (Table 2). The green variety formed more of these than the red variant even in the dark when there could be no competition with cyanidin production, but the sum of the accumulation of these two deoxyanthocyanins was still not equivalent to the production of the cyanidin in the red strain in bright light.

The block to cyanidin synthesis was complete in the case of the coleoptile. Except for patches of orange or brown due to the deoxyanthocyanins, the coleoptiles were colorless. The light treated seedling looked green because of the young leaf enclosed within the coleoptile. While both the red and green strains accumulated both deoxyanthocyanins, the Oklahoma strain did not form detectable amounts of these in the coleoptiles. Pre-treatment with red light (Table 2) to inhibit the growth of the first internode and and accelerate that of the coleoptile was necessary for this accumulation of deoxyanthocyanins; perhaps the absence of them in the coleoptile of the Oklahoma strain was just because the appropriate conditions were not found.

Growth of adventitious roots was inhibited in bright light in both red and green variants as was reported for the Oklahoma strain.[3] Although bright light inhibited the production of apigeninidin and luteolinidin in the data shown in Table 2, this effect was not always duplicated in other experiments. While there was evidence that low intensities of red light accelerated the production of luteolinidin, no far red reversibility could be demonstrated; the light effects in these tissues may be indirect and quite complex.

No major differences were observed in the accumulation of phenolic esters or in the activity of the two amino acid ammonia lyases in green and red strains. The seedling growth pattern of red and green strains were similar to each other and to the Oklahoma strain, except that the Dekalb varieties had thinner internodes. Germination of the red strain was poor, but growth was similar once initiated.

No significant increase in the amount of cyanidin formed was obtained by infiltration of green seedling internodes with varying concentrations of IAA and kinetin, both singly and in combination, nor with the addition of a variety of sugars, amino acids or extracts of endosperm.

TABLE 2. FORMATION OF ANTHOCYANINS IN EXCISED FIRST INTERNODES AND COLEOPTILES OF THE DEKALB ISOGENIC STRAINS AND COMPARISONS WITH THE OKLAHOMA VARIANT

		Apigeninidin	Luteolinidin	Cyanidin
		mμmoles/g fr.wt.		
Internodes*	Dark incubated			
	Red	36 ± 4	47 ± 17	0
	Green	51 ± 5	124 ± 19	0
	Light treated			
	Red	13 ± 3	29 ± 5	611 ± 14
	Green	26 ± 7	54 ± 16	Trace
Coleoptiles†	Dark incubated			
	Red	—	—	—
	Green	17	30	0
	Oklahoma	0	0	0
	Light treated			
	Red	25	60	180
	Green	29	58	0
	Oklahoma	0	0	190

* 3-day-old internodes (4–5 cm long) were excised in laboratory light, incubated for 2 days in the dark or in bright light (12:12 light–dark cycle). Each sample contained 10 internodes weighing about 255 mg fr. wt. and each value is the average of 3 samples ± S.E.

† Pretreated with red light as in Table 1 before excision at 3 days. Incubated and weighed as coleoptile plus leaf, but only coleoptile was extracted. Each sample contained 20 coleoptiles about 1 cm long and each value is the average of 3 samples. Coleoptile plus enclosed leaf weighed about 200 mg fr. wt./20.

In conclusion, the block to the accumulation of the cyanidin glycoside in the "green" seedling strain was complete in the coleoptile, and only trace amounts were formed in the first internode. Since the deoxyanthocyanins were produced in significant amounts in both internodes and coleoptiles, the block was not in any step common to these syntheses. Since adventitious root initiation and growth were just as great in the green and red strains, a defect in some aspect of nucleic acid or protein synthesis common to both cyanidin and root growth[3] would not be expected. Instead, the mutation must affect some step peculiar to cyanidin synthesis, i.e. one related to the hydroxylation at the C_3 position or to the high intensity blue light requirement. But this block is only in the seedling stages. The mature plant is not affected. The *rr* allele suppresses the accumulation of cyanidin in seedling stages by an unknown mechanism, possibly via a defect in some part of the synthetic sequence or in the control of the relative rates of synthesis or degradation of the cyanidin formed. Analysis of defective enzymes awaits the demonstration of flavonoid biosynthesis in cell free extracts.

GENERAL METHODS

Seedlings were grown and flavonoids extracted and analyzed as in previous studies.[2–4] Parts of seedlings were excised after 3 days of growth in the dark at 25° and were incubated for 1–2 days as seed-shoots or only first internodes in the dark or bright light (supplied by VHO cool white fluorescent lamps at about 200 lux). Flavonoids were extracted in 0·1% HCl in MeOH and were eluted for spectrophotometric analyses after two-dimensional chromatography in BuOH–HOAc–H_2O (6:1:2, v/v) and 10% HOAc (v/v).

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