Phytochemistry, 1971, Vol. 10, pp. 1011 to 1012. Pergamon Press. Printed in England.

SHORT COMMUNICATION

THE EFFECT OF ANILINE ON THE RED PIGMENTATION OF MUNG BEAN SEEDLING HYPOCOTYLS

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(*Received* 11 *May* 1970, *in revised form* 28 *July* 1970)

IN A CLASSROOM exercise in plant growth,[1] one of the more adventuresome students, Mr Paul René de Cotret, treated a mung bean seed with a few drops of aniline reagent used for the detection of sugars. The treated seed developed normally except for the absence of the normal pigmentation in the hypocotyl. Repetition and expansion of this simple test pinpointed aniline as the causative agent.

Aniline inhibited germination of mung bean seeds completely at a conc. of 0·1 M. Seedlings developed more or less normally following imbibition of solutions of 0·01 M and 0·001 M but either lacked a red colouration entirely or were markedly paler in colour. Application of gibberellic acid, an inhibitor of anthocyanin synthesis[2] maintained the colourless condition, otherwise some colouration did develop with time. Extraction of the pigments confirmed the visual observations (Table 1).

TABLE 1. THE EFFECT OF ANILINE ON RELATIVE RED COLOURATION IN HYPOCOTYLS OF MUNG BEAN, BUCKWHEAT, RADISH AND BALSAM SEEDLINGS

Species		Age (weeks)	Pigment in aniline* 0·001 M	0·01 M	0·1 M
Mung bean	imbibed seeds	1	—	20	—
		2	—	51	—
		3	56	34	0
	etiolated seedlings	1	57	0	0
		1	50	15	0
Buckwheat	imbibed seeds	3	109	161	93
	etiolated seedlings	1	127	75	41
Radish	imbibed seeds	2	66	92	—
	etiolated seedlings	1	152	41	—
Balsam	imbibed seeds	2	—	187	110

* Control = 100.

The effect of aniline on colour development in etiolated bean seedlings was tested. After germination for 1 week in darkness the hypocotyls were white with no trace of pigment. Upon excision and exposure to light the normal greening reaction occurred in the first day.

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Development of the red colour required 2 days or more. Immersion of the cut end of the hypocotyl into a 0·01 M aniline solution in the light prevented or reduced red colour development (Table 1) but had no effect on greening.

The effect of aniline on buckwheat, radish, and balsam seedlings was also tested. All showed little or no effect of treatment with 0·01 M aniline. Development of colour was either unaffected or increased (Table 1). A preliminary test of additional seedlings with red hypocotyls: flax, marigold, turnip, sorrel, egg plant and beet, also indicated no effect of aniline on colour development. Thus the effect was specific for mung bean seedlings.

Extracts of bean, buckwheat, radish and balsam were subjected to paper chromatography. There were no obvious qualitative differences as a result of aniline treatment apart from the absence of red pigments in the bean extracts. The same spots were visible in day light and ultra-violet light in chromatographs of normal and treated seedlings.

The nature of the pigments of the mung bean seedling has not been reported in the literature, although the flavonoids of mung bean leaves have been investigated.[3,4] Cyanidin glycosides alone, as in buckwheat,[5] or cyanidin and pelargonidin glycosides together as in radish[6] and balsam[7] are the pigments usually found. Work towards positive identification of the mung bean pigments is in progress.

EXPERIMENTAL

Seeds of mung bean (*Phaseolus aureus*), buckwheat (*Fagopyrum esculentum*), radish (*Raphanus sativus*), and balsam (*Impatiens balsamina*) were obtained locally and soaked in H_2O or aniline solutions for 16 hr in darkness. They were then planted in vermiculite in 3 in. styrofoam cups, 20 seeds per cup, and placed under a wide-spectrum Gro-Lux lamp on a 16 hr day for the duration of the experiment, usually 3–4 weeks at the most. The hypocotyls were excised and their fresh weight determined before extraction of the pigments. Additional batches of seeds were sown directly in vermiculite in styrofoam cups and left in darkness for 1 week. The etiolated seedlings were excised at ground level, and the cut ends placed in distilled water or aniline solutions. The seedlings were then left under the Gro-Lux lamp for 2 days before the pigments were extracted.

Pigments were extracted by placing the excised hypocotyls in cold MeOH containing 0·1% conc. HCl, 3 ml/100 mg fr.wt., and leaving at 0° overnight. Spectra of the crude extracts were taken over the range 400–700 nm and o.d. determinations made at λ_{max}. Paper chromatography on Whatman Nos 1 and 3 MM paper was carried out using the BuOH–2N HCl (1:1, upper layer) and BuOH–acetic acid–H_2O (4:1:5, upper layer).[8] Pigment areas on paper were eluted with methanolic HCl for spectral determinations.

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