

THE EXPORT OF AUXIN FROM TIPS AND FROM SECTIONS OF AVENA COLEOPTILES AS INFLUENCED BY RED LIGHT

B. HUISINGA

Botanisch Laboratorium, Utrecht

SUMMARY

Red light causes an increase of auxin export from subapical sections of *Avena* coleoptiles and simultaneously decreases the export from tips. Also the imposition of a physical barrier on the export of auxin from tips results in a reduced export of auxin after the barrier has been removed. This shows that red light may exert an influence by way of changes in transport properties in the coleoptile.

1. INTRODUCTION

Among the effects of red light on seedlings are known:
reduced auxin export from the tips (VAN OVERBEEK 1936, BRIGGS 1963);
reduced transport of auxin and other substances from the cotyledons or coleoptile tips through the more basal tissues (NAQUI & GORDON 1967, RAI & LALORAYA 1967, LAM & LEOPOLD 1964 and 1966, KONDO et al. 1969);
reduced growth of tissues far from the cotyledons or tips (CURRY et al. 1956, GOTO & ESASHI 1974, HUISINGA 1967);
increased growth in the tissues close to the cotyledons and tips (CURRY et al. 1956, HUISINGA 1967, GOTO & ESASHI 1974);
restriction of the phototropic and geotropic curvatures to the upper part of the seedlings (BLAAUW 1961 and 1963, WILKINS 1965, HUISINGA 1967, LION 1968).

From the fact that auxin export from *Avena* coleoptile tips is decreased by irradiation with red light the conclusion that the auxin production is decreased, is generally accepted (ZIMMERMAN & BRIGGS 1963). About the mechanism, leading to this decrease of the production, no generally accepted theory exists.

Based on the fact that auxin export from maize coleoptile tips could be decreased by red light only if the irradiation occurred before the isolation of the tips (BRIGGS 1963) and on the fact that red light causes growth (HUISINGA 1967), geotropic (HUISINGA 1964; LION 1968; BLAAUW 1961 and 1963) and phototropic curving to be more restricted to the upper part of *Avena* seedlings, HUISINGA (1964 and 1967) surmised that the decrease in auxin production might be caused by an increase in auxin concentration in the tip. This was in contrast to earlier work in which one generally started from the supposition that the decrease of auxin production causes a decrease of concentration in all parts of the seedlings (cf. ZIMMERMAN & BRIGGS 1963).

The increase in auxin concentration as supposed by Huisinga might be caused

by a decrease of auxin transport by red light which is indicated by the increase of growth of the apical parts and a simultaneous decrease in the basal parts of *Avena* seedlings (CURRY et al. 1956, HUISINGA 1967).

The mesocotyl of *Avena* appears not to be capable of transporting auxin laterally (HUISINGA 1964) yet shows geotropic curving in dark controls. It does not show growth or geotropic reaction if the plant has been irradiated with adequate amounts of red light (10^6 erg cm^{-2} or more, HUISINGA 1967). This seems sufficient to prove that red light can prevent auxin from the coleoptile to reach the mesocotyl.

If these statements and conclusions are correct, it should be possible to show that simultaneously with a decrease in export of auxin from *Avena* tips an increase in auxin concentration in subapical sections occurs. We tried to do this in the following experiments.

2. MATERIALS AND METHODS

Avena seedlings were grown and irradiated the first 24 hours with red light of an intensity of approximately 1000 erg cm^{-2} sec^{-1} (see BLAAUW & BLAAUW-JANSEN 1964). After about 90 hours the plants were used for experiments. Tips and sections were cut in darkness as described in HUISINGA (1964).

Test-plants were selected for uniformity, decapitated by 3 mm and after pulling up the primary leaf, placed in holders as described by HUISINGA (1964 and 1967). Estimates of auxin export were made either by placing tips asymmetricaly on the test-plants or by using diffusion of the substances from the tips or sections into agarblocks and placing these blocks on test-plants. Test-plants were allowed to curve for 3 hours. The resulting curvatures were measured.

To cut and place coleoptile tips on agar slabs in total darkness the apparatus, originally devised to stick sections onto glass plates as described by HUISINGA (1964), was modified by providing the lower perspex plate with a shallow basin in which a thin slab of agar could be fitted (fig. 1). The glass plate was inserted,

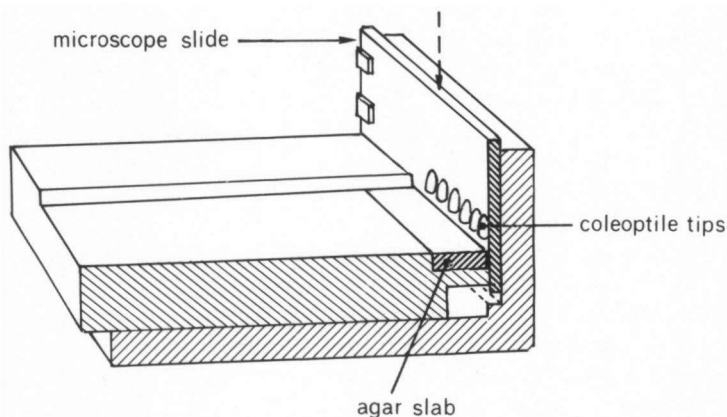


Fig. 1. Apparatus used to place the coleoptile tips on agar in total darkness.

but left at about one mm above its lowest position. It was made slightly sticky with an very thin film of gelatin. After sticking the cut tips onto the glass the plate was slid down bringing the tips into contact with the agar.

3. RESULTS

3.1 Influence of a physical barrier to auxin export from tips of coleoptiles

Tips of 1½ mm length were placed either on agar slabs or on clean glass plates, in red light.

Then they were placed in darkness, in water-saturated containers. After two hours the tips were taken out and placed on test-plants. Curvatures were allowed to develop for 3 hours.

Table 1 shows that tips placed on glass caused smaller curvatures than their counterparts on agar, indicating that less auxin may have been exported from those tips.

Desiccation of the tips can hardly be held responsible for the results as the surfaces of the glass plates got moist in the high-humidity surroundings.

3.2 Influence of red light on subsequent export of auxin from tips of coleoptiles

Seedlings received an irradiation with red light of 40 sec at 1000 erg cm⁻². Dark controls were used as reference. Between the irradiation of the plants and the decapitation (in darkness) a waiting time of 75 min elapsed. The tips were placed on test-plants and curvatures were allowed to develop for 3 hours.

Similar experiments were done using diffusion of exported substances into agar blocks. The diffusion time was 2 hours. The blocks were essayed for activity on test-plants. The irradiation time was 2 hours.

Similar experiments were done with irradiation times varying from 5 min to 120 min.

Experiments with an irradiation time of 5 min followed by a dark period to

Table 1. Mean curvature caused by tips left on glass or agar for 2 hours before being placed on test-plants. 10–12 plants per group.

	glass	agar
1	10.6	12.7
2	9.7	10.0
3	9.9	12.0
4	6.4	10.2
5	6.4	10.0
6	4.0	5.0
7	4.9	8.6
mean	7.4	9.8
ratio	1.00	1.32

Table 2. Influence of red irradiation on the export of auxin from coleoptile tips.

a. Tips directly on test plants; 10 plants per group; irradiation time 40 sec, waiting time 75 sec.		b. Tips on agar blocks; 40 blocks tested; no waiting time.		
light	dark	irradiation time	light	dark control
		5 min	4.8	
		30	4.7	
		60	4.3	
		90	3.3	6.3
		120	3.5	
		120	3.5	6.2
		120 (100 blocks)	1.3	4.7
		c. Tips on agar blocks; 30 or 40 blocks tested per figure; irradiation time 5 min.		
		waiting time after irradiation (min)	1. (30 blocks)	2. (40 blocks)
		0	6.0	6.7
		25	4.2	5.6
		55	2.9	2.8
		85	2.6	0.8
		115	3.5	2.0
		dark control	5.4	9.0
mean	7.5			
ratio	1.00			10.9 1.45

the completion of 2 hours were performed to distinguish between irradiation time and the subsequent time in which the irradiation takes effect.

As appears from *table 2*, tips from red irradiated plants caused smaller curvatures than the dark controls, indicating that less auxin may have been exported to the test-plants. The auxin export decreases with time after the irradiation up to 1½ hours. This result is in agreement with the findings of BRIGGS (1963).

3.3 Influence of red light on isolated tips

Tips of 1½ mm were cut from plants in total darkness and placed on agar in either green light or darkness, as specified in *table 3*. The tips remained on agar for 2 hours. Part of them received an irradiation with red light, as specified in *table 3*. The tips were then placed on test-plants using red light. Some tips were placed on test-plants immediately after cutting them, the curving of the plants with the tips being allowed to develop in red light or in darkness.

Table 3 shows that also isolated tips of *Avena* coleoptiles are influenced by red light because the resulting curvatures of the test-plants were consequently smaller if red light was given. This may indicate that less auxin is exported to the test-plants. BRIGGS (1963) reported, that red light did not cause an export reduction

Table 3. Influence of red light on isolated tips. Number of plants in each group 10-12.

a. Tips on test plants after 2 hours on agar; manipu- lation in green light		b. Tips on test plants after 2 hours on agar; manipu- lation in darkness	
light	dark	light	dark
15.0	16.0	5.2	7.1
9.6	16.0	9.6	11.4
7.2	9.6		
7.2	15.0		
10.2	10.7	mean	7.4
9.5	12.8	ratio	1.00
5.4	9.2		9.3
			1.25
mean	9.2	c. Tips directly on test plants curving in:	
ratio	1.00	light	darkness
	1.39		
		10.0	13.8
		7.3	9.7
		8.6	12.0
		7.9	9.6
		mean	8.5
		ratio	1.00
			1.33

in isolated maize coleoptile tips. As can be seen from *table 3* we were not able to reproduce Briggs' results with *Avena*.

Isolated tips of *Avena* of $1\frac{1}{2}$ mm are influenced by red light in a way comparable to the influence on intact plants. The possibility exists that in *Zea* tips, as used by Briggs, the production centre may be located much nearer to the cut surface of may even have been partly removed.

The effects obtained with export reduction by placing isolated tips on glass instead of on agar and the comparable effect of high evaporation (HUISINGA 1968), indicate that red light does not influence the production centre immediately. Another mode of action seems more probable viz. a reduction of auxin transport from the production centre and consequently a higher concentration of auxin. It might prove to be this increase in auxin concentration that regulates the auxin production.

This situation indicates that in the parts of the seedling immediately below the production centre the concentration of auxin might be increased by red irradiation.

3.4 The effect of red light on auxin export from tips and sections of the same coleoptiles

To see if we could find an indication of higher auxin content in parts just below the coleoptile tip, i.e. the parts, that show an increased growth after a red irradiation (HUISINGA 1967), we irradiated seedlings with 1000 erg cm^{-2} of red light for 5 min and stored them for varying times (waiting time) in darkness. Then

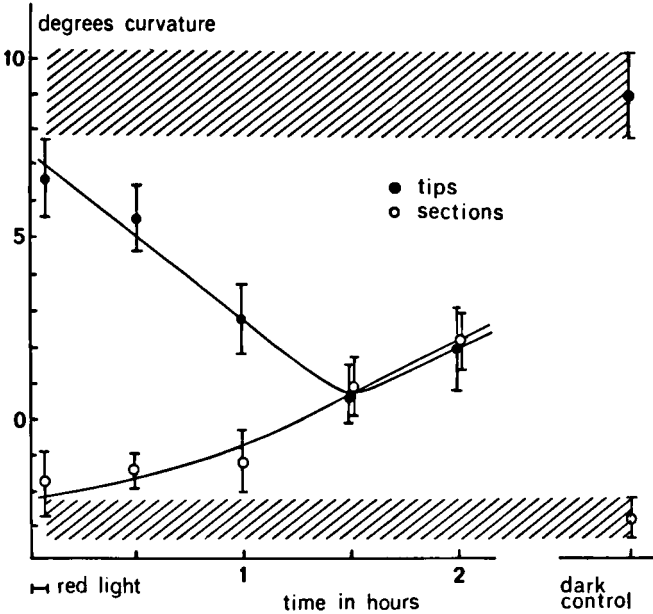


Fig. 2. The influence of red light on auxin export from tips and sections of the same coleoptiles. The shaded areas indicate the values of the dark controls.

tips of $1\frac{1}{2}$ mm and the subsequent sections of 3 mm were placed on agar slabs using red light. In each experiment dark controls served as reference. The tips and sections were allowed to export to the agar for 2 hours after which the agar blocks were placed on test-plants. Plain agar blocks and blocks, containing $\frac{1}{2} \cdot 10^{-7}$ indoleacetic acid, served as controls for the reactivity of the test-plants.

The results are given in *table 4* and in *fig. 2*, in which degrees curvature are plotted against hours waiting time.

These experiments show that irradiation with red light caused a decrease of auxin export from the tips progressing for about $1\frac{1}{2}$ hours. The matching sections show just the opposite reaction to the irradiation: the resulting curvatures increase with the waiting time.

This strongly indicates that the increase in growth, found in this part of the seedling, is correlated with this increase of exportable auxin.

Table 4. Influence of light on tips and sections of the same plants. Export of auxin into agar, agar tested; 40 plants in each group.

Irradiation time (min)	Waiting time (min)	tips	sections
0	0	9.0 ± 1.2	-2.6 ± 0.6
5	0	6.7 ± 1.1	-1.8 ± 0.9
5	25	5.6 ± 0.9	-1.5 ± 0.5
5	55	2.8 ± 1.0	-1.2 ± 0.9
5	85	0.8 ± 0.9	0.9 ± 0.9
5	115	2.0 ± 1.2	2.2 ± 0.8

4. DISCUSSION

The results presented in this paper show that by irradiating *Avena* coleoptiles with red light the amount of auxin exported from subapical sections is increased, whereas the amount exported from their tips at the same time is reduced. The influence of red light extends beyond the irradiation time, as about one hour must elapse before an appreciable amount of auxin can be detected in the sections.

By means other than red light also an increase of the growth of subapical parts and a simultaneous decrease of auxin release from the tips can be induced, as shown by NIEDERGANG-KAMIEN & SKOOG (1956), who used TIBA, and KRELLE & LIBBERT (1968), who used a morphactin. The present author reported in 1968 that the same effect could be seen in *Avena* seedlings as a result of high evaporation. DAVENPORT et al. (1974) have reported a decrease in auxin transport by water stress in *Gossypium* petioles.

In this report experiments imposing a physical barrier on the export of auxin by the tips of *Avena* coleoptiles show that this also results in a decrease of auxin exported after the barrier had been removed.

ANKER (1973) has shown that indole acetic acid is one of the most effective agents to reduce auxin synthesis; he reported effects of IAA concentrations as low as 0.005 mg/l. PLATT (1965) mentioned the same effect, without giving experimental data. This property of auxin indicates that the correlation between auxin transport, concentration and production may be causal.

An influence of red light on permeability has been reported by VIRGIN (1951) and by PECKET & HATHOUT BASSIN (1974). The mechanism by which red light exerts its influence on transport properties in plants may be a change in permeability in tissues involved in the transport of auxin.

ACKNOWLEDGEMENTS

The author thanks Mrs. A. W. M. de Groot-Knijff and Mr. T. van Gijzen for their valuable help.

REFERENCES

- ANKER, L. (1973): The auxin production of the physiological tip of the *Avena* coleoptile and the repression of tip regeneration by indoleacetic acid (not by naphthylacetic acid and 2,4-dichlorophenoxyacetic acid). *Acta Bot. Neerl.* **22**: 221–227.
- BLAAUW, O. H. (1961): The influence of blue, red and far red light on geotropism and growth of the *Avena* coleoptile. *Acta Bot. Neerl.* **10**: 397–450.
- (1963): Effects of red light on geotropism of *Avena* and their possible relations to phototropic phenomena. *Acta Bot. Neerl.* **12**: 424–432.
- & G. BLAAUW-JANSEN (1964): The influence of red light on the phototropism of *Avena* coleoptiles. *Acta Bot. Neerl.* **13**: 541–552.
- BRIGGS, W. R. (1963): Red light, auxin relationships and the phototropic responses of corn and oats coleoptiles. *Amer. J. Bot.* **50**: 196–207.

- CURRY, G. M., K. V. THIMANN & P. M. RAY (1956): The base curvature response of *Avena* seedlings to the ultraviolet. *Physiol. Plant.* **9**: 429–440.
- DAVENPORT, T. L., P. W. MORGAN & W. R. JORDAN (1974): Auxin transport: Variations due to age and water stress. *Plant Physiol.* Ann. suppl. 43.
- GOTO, N. & Y. ESASHI (1974): Differential hormone responses in different growing zones of the bean hypocotyl. *Planta (Berl.)* **116**: 225–241.
- HUISINGA, B. (1964): Influence of light on growth, geotropism and guttation of *Avena* seedlings grown in total darkness. *Acta Bot. Neerl.* **13**: 445–487.
- (1967): Influence of irradiation on the distribution of growth in dark-grown *Avena* seedlings. *Acta Bot. Neerl.* **16**: 197–201.
- (1968): On the influence of transpiration on the distribution of growth in the *Avena* mesocotyl. *Acta Bot. Neerl.* **17**: 467–474.
- KONDO, N., T. FUJII & T. YAMAKI (1969): Effect of light on auxin transport and elongation of *Avena* mesocotyl. *Development, Growth and Differentiation* **11**: 46–61.
- KRELLE, E. & E. LIBBERT (1964): Inhibition of the polar auxin transport by a morphactin. *Planta (Berl.)* **80**: 317–320.
- LAM, S. L. & A. C. LEOPOLD (1964): Effect of light on auxin transport. *Plant Physiol.* **39**: xxxviii.
- & — (1966): Role of leaves in phototropism. *Plant Physiol.* **41**: 847–851.
- LION, J. (1968): The influence of red light on geotropism of decapitated *Avena* coleoptiles. *Acta Bot. Neerl.* **17**: 416–422.
- NAQUI, S. M. & S. A. GORDON (1967): Auxin transport in *Zea mays* coleoptiles II. Influence of light on the transport of indoleacetic acid-2-¹⁴C. *Plant Physiol.* **42**: 138–143.
- NIEDERGANG-KAMIEN, E. & F. SKOOG (1956): Studies on polarity and auxin transport in plants. *Physiol. Plant.* **9**: 60–63.
- OVERBEEK, J. VAN (1936): Growth hormone and mesocotyl growth. *Rec. trav. bot. néerl.* **33**: 333–340.
- PECKET, R. C. & T. A. HATHOUT BASSIN (1974): Mechanism of phytochrome action in the control of biosynthesis of anthocyanin in *Brassica oleracea*. *Phytochemistry* **13**: 815–821.
- PLATT, R. S. JR. (1965): The control of auxin synthesis in the oat coleoptile. *Plant Physiol.* **40**: suppl. LVIII.
- RAI, V. K. & M. M. LALORAYA (1967): Correlative studies on plant growth and metabolism II. Effect of light and of gibberellic acid on the changes in protein and soluble nitrogen in lettuce seedlings. *Plant Physiol.* **42**: 440–444.
- VIRGIN, H. (1951): The effect of light on the protoplasmic viscosity. *Physiol. Plant.* **4**: 255–357.
- WILKINS, M. B. (1965): Red light and geotropic response of the *Avena* coleoptile. *Plant Physiol.* **40**: 24–34.
- ZIMMERMAN, B. K. & W. R. BRIGGS (1963): Phototropic dosage-response curves for oat coleoptiles. *Plant Physiol.* **38**: 248–253.