GEOTROPIC CURVATURE OF AVENA COLEOPTILES IN SOLUTIONS OF VARIOUS OSMOTIC VALUES

G. BLAAUW-JANSEN and O. H. BLAAUW

Botanisch Laboratorium, Utrecht

SUMMARY

Isolated Avena coleoptiles are placed with their bases in mannitol solutions of various osmotic values. In this way the water uptake is inhibited. The coleoptiles are irradiated with red light and turned through 90° . Considerable geotropic curvatures are developed in solutions which prevent growth to a large extent. Red light enhances the geotropic response also under these conditions, but a red, far-red reversal of the red effect does not take place. The tips of the horizontal coleoptiles appear not to reach the vertical position, but end up growing at a constant angle to the vertical.

1. INTRODUCTION

The geotropic responsiveness of *Avena* coleoptiles is increased by a short-term red irradiation (BLAAUW 1961, 1963; WILKINS 1965). WILKINS (1965) has reported this red-induced increase in geotropic responsiveness to be reversed by far-red irradiation; BLAAUW (1961 and to be published), however, found a far-red effect in the same direction as the effect of red light.

In another context a relationship has been demonstrated between the effects of auxin and red light on plasticity and turgor pressure of the cells (CLELAND 1959; BLAAUW-JANSEN & BLAAUW 1966). Moreover, HUISINGA (1964) has related the effect of red light on the shape of geotropically curved coleoptiles to the transport of water. Therefore the discrepancy in the results of Wilkins and Blaauw might be due to a difference in the rate of water supply, since Wilkins put the isolated coleoptiles in agar, Blaauw in water. For this reason in the experiments to be described the rate of water supply was controlled by supplying solutions of various osmotic values to isolated coleoptiles, and the subsequent geotropic response was studied.

2. MATERIAL AND METHODS

Avena seedlings (var. Victory C.I. 2020) were raised as described by BLAAUW & BLAAUW-JANSEN (1964). The seeds were grown in glass trays on glass slides, wrapped in filter paper, at 23°C; after 21 hours of red light, the plants were kept in total darkness. When the seedlings were 93 hours old the coleoptiles were excised in very dim green light (intensity at the level of the plants approximately 0.002 μ W cm⁻²) of extreme spectral purity¹) and were placed upon pins after removal of their primary leaves (BLAAUW 1963). The green light ($\lambda = 560$ nm)

¹) The green safelight was the same as used in our former experiments, but more accurate measurements of the light intensity indicated a much lower intensity than reported before.

Acta Bot. Neerl. 17(4), August 1968

was obtained from an incandescent lamp passing three interference filters, namely a Calflex filter (Balzer, Liechtenstein), a 560 nm Filtraflex -B line filter (Balzer), and a 560 nm "DEPAL" double band filter (Schott u. Gen., Mainz), mounted without lenses in such a way that the rays incident on the filter did not diverge more than 5° from the normal.

The pins were placed in holders, containing twelve pins each. Each pin was fixed in a cavity, filled with a mannitol solution. The coleoptiles of each slide were distributed over all holders used.

Four hours after having been placed with their bases in the mannitol solutions the coleoptiles were irradiated from above during 6 minutes with red light from a 40 Watt Philips red fluorescent tube filtered by red Plexiglas (Röhm u. Haas no 501, Darmstadt), the intensity at the level of the plants being approximately $40 \,\mu\text{W cm}^{-2}$. During the irradiation a glass hair was attached to each coleoptile $10-12 \,\text{mm}$ from the top.

Thirty minutes after the irradiation the coleoptiles were shadowgraphed and put in a horizontal position in such a way that geotropic curvatures would develop in the plane of the two vascular bundles. During the development of geotropic curvatures shadowgraphs were made at intervals of 45 minutes during $5\frac{1}{2}$ hours, starting 90 minutes after the coleoptiles had been put horizontal. Controls were kept in a vertical position and shadowgraphed at the same intervals. For the shadowgraphs safelight of 560 nm was used (approximately 0.001 μ W sec cm⁻² a time).¹)

Straight growth was determined with the help of a photographic enlarger by measuring the increase in length from hair to top, as growth appeared to be restricted to the uppermost 10 mm of the coleoptile. Curvatures were measured by means of a protractor. We projected the shadowgraphs of the coleoptiles made before geotropic induction onto the shadowgraphs of the same coleoptiles made after curvatures had developed. Thus the length of the zone to be curved could be determined to the nearest 0.5 mm. In the projections the lengths of the concave and convex sides of the curved coleoptiles were measured by means of a curvimeter.

3. RESULTS AND DISCUSSION

3.1. Straight growth

The straight growth of vertical coleoptiles supplied with mannitol solutions of various osmotic values is shown in *fig.* 1. In water and in 0.05 M mannitol the growth rate of vertical coleoptiles is linear with time up to $5\frac{1}{2}$ hours after the first photograph was made. *Fig.* 2 shows the relation between increase in length and molarity of the mannitol solutions after $2\frac{1}{2}$ hours. The curve levels off at approximately 0.20 M mannitol.

The increase in length of horizontal coleoptiles in water is also continued throughout these $5\frac{1}{2}$ hours though it remained behind that of the vertical coleoptiles (*fig. 1*).

¹) See note on p. 273.

GEOTROPIC CURVATURE IN SOLUTIONS OF VARIOUS OSMOTIC VALUES



Fig. 1. The effect of the osmotic value of the supplied solution on the increase in length of vertical coleoptiles.



The relation between the increase in length and the osmotic value of the supplied solution after $2\frac{1}{2}$ hours.

3.2. Curvature

In contrast to the rate of increase in length the rate of curvature decreases after about 3 hours to almost zero (fig. 3). The curve representing the relation between the degree of curvature and the osmotic value of the mannitol solutions shows that the reduction of curvature is linear with the molarity of the mannitol solutions from 0.05 up to 0.40 M. (fig. 4). Clearly the development of curvature is less dependent on the osmotic value of the solution in which the coleoptiles are placed than the increase in length is. In order to elucidate this phenomenon we measured the increase in length of the concave and the convex side of coleoptiles in 0.30 M and in 0.40 M mannitol 135 and 340 minutes after geotropic induction. In the first 2 hours the concave side keeps about its initial length, but



Fig. 3.

The effect of the osmotic value of the supplied solution on the geotropic curvature.

Acta Bot. Neerl. 17(4), August 1968





The geotropic curvature and the length of the apical part to be curved versus the osmotic value after an experimental period of $2\frac{1}{2}$ hours.

between the second and the fifth hour the length of the concave side decreases with about 0.15 mm. The cells of the convex side appear to distract water from the cells of the concave side which results in shrinkage of the concave side (cf. BLAAUW (1963).

3.3. Maximum of the curvature

The geotropic curvature of the red-irradiated coleoptiles placed horizontal with their bases in water (fig. 3) develops to a maximum of about 70°, although they are kept in the horizontal position until the end of the experiment, in some cases up to 20 hours. This phenomenon has also been observed by WILKINS (1965). Ultimately the tips are growing in a constant oblique direction. Wilkins has considered depletion of nutrients or other essential factors for growth as a possible cause for the limited curvatures of the detached coleoptiles as used in his and in our experiments. However, as growth is continued after the maximum curvature is attained his explanation cannot be correct in this simple form.

The coleoptiles after having reached their maximum curvature are still capable of geotropic perception and reaction, as can be demonstrated by turning them through another 45° to an angle of 135° from the vertical. In that case a new wave of curvature passes through the coleoptile and the curvature is increased to a new maximum of about 100°. Evidently the extent of curvature is not limited by the growth capacity of the cells of the lower side.

The occurrence of a maximum geotropic curvature can be more readily understood when the shape of the curving coleoptiles is taken into consideration. In the red-irradiated coleoptiles the apical zone straightens after some time. The curvature is transmitted to lower zones of the coleoptile to the effect of a curved zone between two straight parts. The curving of the basal zones nearly compensates the straightening of the top zones, so the degree of curvature even shows a slight decrease.

Coleoptiles that are not irradiated with red light develop a maximum curvature as large as that of the irradiated ones. *Fig. 5* shows the development of curvature in isolated coleoptiles half of which were irradiated with red light. At intervals one sample of irradiated and one sample of dark coleoptiles were photographed and discarded. Other samples of irradiated coleoptiles and dark controls were maintained and photographed throughout the experimental



Fig. 5. Development of the geotropic curvature of red-irradiated coleoptiles and dark controls. 276 Acta Bot. Neerl. 17(4), August 1968

GEOTROPIC CURVATURE IN SOLUTIONS OF VARIOUS OSMOTIC VALUES

Fig. 6. The shape of geotropically curved coleoptiles after a period of 20 hours. Upper row: redirradiated coleoptiles. Lower row: dark controls.



period of 20 hours. The light used to make the shadowgraphs does not influence the development of the geotropic curvatures. Clearly the dark controls have a lower rate of curvature than the irradiated coleoptiles, but eventually they develop the same curvature. Yet in the dark controls the curvature extends over the full length of the coleoptile, whereas in irradiated coleoptiles the curvature is restricted to a small zone (*fig. 6*). So when the cells of the lower side of the dark controls stop growing they have grown significantly less than the cells in the curving zone of the irradiated coleoptiles. In contradiction to this findings WILKINS (1965) has reported a large difference between the maximum curvatures of irradiated and non-irradiated coleoptiles, this difference being maintained for at least 12 hours.

3.4. Migration of the curving zone

According to BLAAUW (1963) the geotropic response of the topmost centimeter of the coleoptile is enhanced and the geotropic response of the base is diminished by an irradiation with about 2×10^2 W sec cm⁻² of red light. When, however, the shape of the coleoptiles is observed after longer periods of time than the experimental period of $3\frac{1}{2}$ hours adopted in his experiments, it appears that the influence of red light can be more accurately described as resulting in an enhancement of the rate of curvature and the confinement of the curvature to a small zone. This zone proceeds in basal direction with a velocity of about 1 mm/hr. (auxin is transported in the coleoptiles at an average rate of about 10 mm/hr. as determined by VAN DER WEY 1932 and GOLDSMITH 1962). In dark controls the apical zones do not straighten so that curvatures remain extended up to the tip of the coleoptile. No migrating zone can be observed. DOLK (1930) explained the migration of the geotropic curvatures down the coleoptile and the autonomic straightening of the curved zones in terms of the food factor. If an unequal distribution of auxin between the two sides of the plant is brought about by gravity, then the side with the more auxin will grow rapidly (convex side) and will thus temporarily reduce the supply of food factor. When the auxin distribution becomes equal again on the clinostat, the side which has grown less (concave side) is better supplied with food factor and thus becomes slightly convex (DOLK 1930).

This theory may need a modification since straightening of the curved zones will occur also in coleoptiles that remain in the horizontal position. In this case auxin distribution may remain equal throughout the experimental period. It is conceivable that the straightening of the curved upper zones is the direct consequence of the basipetal movement of curvature along the coleoptile. When the coleoptiles are curving the lower half of the basal zones would intercept the food supply of the lower half of the more apical zones, and prevent any further growth of the lower half. The upper half would, in contrast, continue growing as a consequence of the straightening of the coleoptile. The cause of the migrating of the curvature is not known. As the velocity of the downward movement of the curvature is probably not connected with longitudinal auxin transport. The migration might be determined by the relatively slow lateral transport of auxin in the more basal parts (ANKER 1956), which might cause the basal zones to curve subsequent to the apical zones.

3.5. Effect of mannitol on the shape of the curved coleoptiles

The higher the osmotic value of the mannitol solution from which the coleoptiles absorb water the more the curvatures are limited to the upper zones of the coleoptiles (fig. 4). This might be the consequence of the reduction in the degree of curvature by the high osmotic values of the solutions, as smaller curvatures are more limited to the top zones than large curvatures are. In a 0.20 M mannitol solution for example a curvature of 17° extends over a length of 5.4 mm from the top, but a curvature of 47° extends over a length of 8.4 mm from the top (table 1). But table 1 shows also that for any curvature of a definite extent the curving zone is nearer to the top according as the concentration of the mannitol solution is increased. For example a curvature of 35° may be localized in the topmost 6.7 mm of the coleoptiles (in 0.25 M) or it may extend over a length of 8.8 mm (in water). Thus limitation of the water supply implies a shift of the curving zone towards the top (cf. Huisinga, to be published). Straightening of the apical zones and a migration of curvature were not observed in coleoptiles placed in mannitol solutions, perhaps because of the rather short experimental period (5 hours).

3.6. Red, far-red antagonism

Red light has two different effects on the geotropic curvature of *Avena* coleoptiles: it enhances the rate of curvature and it transforms the shape of the curved coleoptile. The second effect may be related to the increase in water transport by red irradiation (HUISINGA 1964). It might be possible to counteract this effect of red light by supplying mannitol solutions instead of water to the curving coleoptiles. In that case one could study the effect of red and far-red irradiation on the rate of curvature apart from the effect on the shape of the curved coleoptiles.

It appears, however, that supplying mannitol solutions does not counteract the effect of red light on the shape of the curved coleoptiles. Huisinga will discuss this phenomenon (to be published). Yet we performed an experiment about the effect of red and far-red irradiation on the geotropic response of coleoptiles.

	GEOTROPIC	CURVATURE	IN SOLUTIONS	OF VARIOUS	OSMOTIC V/	ALUES
--	-----------	-----------	--------------	------------	------------	-------

				molarit	y manni	tol				
curvature	0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45
8								28		
10										
12							44	45		
14								51		
16						47	44	47		
18					54		46	54		
20			55				63	53		
22						60		56		
24		79								
26		75	65		61 65	58 57	49 47			
28										
30	73									
32			77		70	62	56			
34										
36	88	83	78	64	70	67				
38		81			72					
40	92	87	86	67	78	72				
42										
44										
46	100	92	85		86					
48		83	83		84					
50	104									
52	104									
54										
56	108	108	99							
00										
36			102							
60	110									

Table 1. Length of the apical part to be curved (in 0.1 mm) in relation to the degree of curvature and the concentration of the supplied mannitol solution.

Acta Bot. Neerl. 17(4), August 1968

A 0.20 M solution was chosen because a mannitol solution of this molarity has a maximal inhibiting effect on growth and thus on water uptake, but permits a considerable curvature. *Table 2* shows the result: there is no red, far-red antagonism under these conditions. The far-red light was supplied by an incandescent lamp (iodine) with an appropriate system of lenses and a 5 cm layer of water in combination with a precision double band filter ("DEPAL" Schott u. Gen., Mainz, 735 nm) and an infrared mirror. The intensity of the far-red light was approximately $45 \,\mu W \, \text{cm}^{-2}$. The same quantity of this far-red light reverses completely one of the components of the effect of red light on the growth of the *Avena* mesocotyl (BLAAUW *c.s.* 1968).

Table 2.	Effect of red and far-red	l light on the geotropic curv	ature of Avena coleoptiles in w	/ater
	and in 0.20 M mannitol	. (means of the curvatures of	of 36 coleoptiles)	

	curvature in degrees		
, ,,,,,,,,	water	0.20 M mannitol	
dark	13.9	6.9	
red (c. 10 ⁴ μW sec cm ⁻²)	27.2	15.6	
red + far-red (ca. $3 \times 10^4 \mu\text{W sec}^{-2}$)	28.4	14.5	

REFERENCES

- ANKER, L. (1956): The auxin concentration rule for the geotropism of Avena coleoptiles. Acta Bot. Neerl. 5: 335-341.
- BLAAUW, O. H. (1961): The influence of blue, red and far-red light on geotropism and growth of the Avena seedling. *Acta Bot. Neerl.* 10: 397-450.
- BLAAUW, O. H. (1963): Effects of red light on the geotropism of Avena and their possible relations to phototropic phenomena. Acta Bot. Neerl. 12: 424–432.
- & BLAAUW-JANSEN, G. (1964): The influence of red light on the phototropism of Avena coleoptiles. Acta Bot. Neerl. 13: 541-552.
- --, -- & W. J. VAN LEEUWEN (1968): An irreversible Red-Light Induced Growth Response in Avena. *Planta* 82: 87-104.
- BLAAUW-JANSEN, G. & O. H. BLAAUW (1966): Effect of red light on irreversible and reversible expansion of Avena coleoptile sections. *Planta (Berl.)* 71: 291–304.
- CLELAND, R. (1959): Effect of osmotic concentration on auxin-action and on irreversible and reversible expansion of the Avena coleoptile. *Physiol. Plant.* 12: 809–825.
- DOLK, H. E. (1930): Geotropie en groeistof. Thesis, Utrecht; English transl. by F. Dolk-Hoek and K. V. Thimann, 1936. Rec. Trav. Botan. Neerl. 33: 509-585.
- GOLDSMITH M. H. M. & K. V. THIMANN (1962): Some characteristics of movement of indoleacetic acid in coleoptiles of Avena. I. Uptake, destruction, immobilization and distribution of IAA during basipetal translocation. *Plant Physiol*. 37: 492–505.
- HUISINGA, B. (1964): Influence of light on growth, geotropism and guttation of Avena seedlings grown in total darkness. *Acta Bot. Neerl.* 13: 445–487.
- WEY, H. G. VAN DER (1932): Der Mechanismus des Wuchsstofftransportes. Rec. Trav. Bot. Néerl. 29: 380–496.
- WILKINS, M. B. (1965): Red light and the geotropic response of the Avena coleoptile. *Plant Physiol.* 40: 24-34.

ERRATUM

Neerl. 17(4), 1968) the sentence "The upper half would, in contrast, continue ous osmotic values" by G. BLAAUW-JANSEN and O. H. BLAAUW (Acta Bot. as a consequence straightening of the coleoptile". growing as a consequence of the straightening of the coleoptile", p. 278, 6th In the article "Geotropic curvature of Avena coleoptiles in solutions of variline, should read: "The upper half would, in contrast, continue growing with