Gravitropism of Oat and Wheat Coleoptiles: Dependence on the Stimulation Angle and Involvement of Autotropic Straightening

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Gravitropism of oat (Avena sativa L.) and wheat (Triticum aestivum L.) coleoptiles was investigated in relation to the displacement angle or to the initially set stimulation angle (SA). We measured curvature rates at the early phase of curvature, before it was affected by the drop in SA resulting from the curvature response itself. The plot of the rates against the sines of initial SAs revealed similar curves for oats and wheat, which approached saturation as the sine increased to unity. The two species and previously analyzed rice [Iino et al. (1996) Plant Cell Environ. 19: 1160] appeared to have similar gravisensitivities. Initial SAs below and over 90° yielded comparable rates when the sine values were the same, indicating that the extent of gravitropism is determined by the gravity component perpendicular to the organ's long axis. Long-term curvature kinetics at different SAs indicated that the net curvature rate dropped sharply before the tip reached the vertical position and then the tip approached the vertical slowly, with clear oscillatory movements in the case of wheat. During this late curvature phase, the coleoptile straightened gradually, although none of its parts had yet reached the vertical. When rotated on horizontal clinostats or displaced upwards to reduce SA in the late curvature phase, coleoptiles bent in the opposite direction. These results demonstrated that autotropism counteracts gravitropism to straighten coleoptiles.

Key words: Autotropism — Coleoptile — Gravitropism — Oat (Avena sativa L.) — Stimulation angle — Wheat (Triticum aestivum L.).

In studies of gravitropism, experimental determination of the sensitivity of plants to a stimulus is limited by the constant and uniform gravity on the earth. Dependence of gravitropism on the angle of displacement, however, may be used as a measure of the sensitivity to gravity. Such angular dependence has been shown with different experimental approaches (Fitting 1905, Rutten-Pekelharing 1910, Metzner 1929, Audus 1964, Pickard 1973). One of the approaches used was to rotate plants on a horizontal clinostat after a relatively short period of displacement and to measure gravitropic curvature at a defined time (Larsen 1969, Pickard 1973). In our preceding study of gravitropism of maize and rice coleoptiles (Iino et al. 1996), the angular dependence was explored further as a measure of gravisensitivity. We measured the rate of coleoptile curvature in plants left as displaced initially. The curvature rate was determined at an early phase of curvature in which the rate was directly related to the displacement angle or the initially set stimulation angle (SA), without being influenced by the decrease in the actual SA resulting from the curvature response itself. It has been demonstrated that the angular dependence determined by this method is distinct between maize and rice, whereas it is similar in air-grown and submerged rice. In the present study, we applied the same method to coleoptiles of oats and wheat to extend the comparison of the relationship between SAs and curvature rates.

Studies of the angular dependence of gravitropism originated in the hypothesis of Sachs (1882) that the magnitude of the gravitropic response depends on the component of gravity which is perpendicular to the organ's long axis. The condition of this hypothesis that the gravitropic response is maximal at the SA of 90° was supported by Fitting (1905) and Rutten-Pekelharing (1910). However, later workers reported that the optimal SA was greater than 90° in roots and stems (Metzner 1929, Audus 1964, Larsen 1969, Iversen and Rommelhoff 1978, Legué et al. 1994, Kiss et al. 1997). Larsen (1969) conducted a detailed study using cress roots to find that the optimal angle was 90° when plants were rotated on a clinostat after a stimulation of shorter than 0.5 min. When stimulated for 1–15 min, however, the optimal angle shifted to 120–140°. To evaluate the problem of the optimal SA, we included SAs greater than 90° in the present analysis of angular dependence.

Measurements of curvature development over a long period may provide information useful in understanding the overall control of gravitropism. As shown previously (Iino et al. 1996), rice and maize coleoptiles overshot the vertical before regaining the vertical orientation and straight appearance. This overshooting was especially marked in submerged rice coleoptiles. The results appeared to support the view that straightening is achieved primarily by a gravitropic response at each locus of the coleoptile (Köhler and Daum 1979). In the present study, analyses of
long-term curvature development were extended with oat and wheat coleoptiles. We found that, unlike in maize and rice, these coleoptiles do not overshoot the vertical, and that autotropism (Simon 1912, Folkard 1936, Firn and Digby 1979) participates in the process of straightening.

Here we report the results obtained by analyzing curvatures initiated at different initial SAs in oat and wheat coleoptiles. The curvature kinetics at an early phase of gravitropism were first analyzed with respect to their relationship to SAs. The curvature kinetics during the late phase were next analyzed with respect to the response elements involved in the process of straightening.

Materials and Methods

Plant material—Plants were raised and experiments were conducted at 25°C under continuous irradiation with red light (2-3 μmol m⁻² s⁻¹) as described in lino et al. (1996). It has long been known that pretreatment of dark-adapted oat and maize seedlings with red light considerably affects the gravitropism of their coleoptiles (Blaauw 1961, Huisenga 1964, Wilkins and Goldsmith 1964, Wilkins 1965, Hild 1977). Therefore, when dark-adapted plants are gravistimulated, one has to be careful with the nature of the safelight used to handle and observe them (for general consideration, see lino and Briggs 1984, lino 1990). The red-light growth condition was adopted here to maintain plants at the steady-state with respect to the effect of red light, which is probably mediated by phytochrome, and also to ease experimental handling and photographic recording of plants.

Caryopses of oats (Avena sativa L. cv. Almighty) were purchased from Snow Brand Seed Co., Ltd., Sapporo, Japan, and those of wheat (Triticum aestivum L. cv. Shiroganekomugi) were kindly donated by Osaka Prefectural Agricultural and Forestry Research Center, Habikino-shi, Osaka, Japan.

Dehusked caryopses of oats were floated, embryo side up, on water and incubated for 40-42 h. Germinated caryopses were transplanted to acrylic cuvettes (top area: 11 x 11 mm², height: 43 mm; Elkay Products Inc., Shrewsbury, MA, U.S.A.) filled with 1% agar (Nakarai Tesque Inc., Kyoto, Japan). Wheat caryopses were surface-sterilized in 70% ethanol for 1 min, soaked in running tap water for 11-14 h, and incubated, embryo side up, on wet paper towels (Kimtowel; Jujo Kimberly Inc., Tokyo, Japan). At 22-26 h of incubation, germinated caryopses were transplanted to acrylic cuvettes filled with 1% agar.

The transplanted caryopses of oats and wheat were incubated for an additional day. Seedlings were then selected for uniform, straight coleoptiles (oats, 18-22 mm; wheat, 16-20 mm) and used for experiments. After transplantation, plants were kept in a box made of red plate acrylic (No. 102, 3 mm thick; Mitsubishi Rayon, Tokyo) until the end of gravitropism experiments, except when handled for selection and experimental treatments.

Gravitropic stimulation and curvature measurement—Coleoptiles were stimulated for gravitropism by displacing the plants by a selected angle from the vertical. Plants were oriented for gravistimulation in such a way that the direction of gravity was parallel to the plane passing through the two vascular bundles of the coleoptile. The displacement angle (the initial SA) was determined to the plane passing through the two vascular bundles of the coleoptile. The displacement angle (the initial SA) was determined to the plane passing through the two vascular bundles of the coleoptile.

Photographs of coleoptiles were taken, usually at 5 min intervals after the onset of gravistimulation, to monitor their curvature (lino 1996, lino et al. 1996). Curvature was quantified by drawing a line tangent at the tip to the long axis of each coleoptile and measuring the angular deviation of the tangent from the one obtained at the onset of gravistimulation (lino et al. 1996). In one set of experiments (Fig. 6), the angle of the tangent from the horizontal was determined. (When the initial SA is 90°, this angle is equivalent to the angle of curvature.)

In some experiments, plants were rotated on horizontal clinostats at 2.4 rpm. For clinostat treatment, the original growth direction of the coleoptile was set parallel to the rotation axis. The distance between plants and the rotation axis did not exceed 20 mm.

Results

The relationship between SAs and curvature rates—According to the method described in lino et al. (1996), we first analyzed the period of curvature development in which curvature rates are directly related to initial SAs. Figure 1 shows the results of experiments planned for this purpose. In these experiments, the curvature responses were compared at initial SAs of 10, 30 and 90° between plants kept...
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as initially displaced and those displaced for 25 min and returned to the original vertical position.

As is apparent in Fig. 1, the angle of curvature induced in oats and wheat during the 25-min stimulation was less than one-tenth of each displacement angle. Therefore, the change in the SA which resulted from curvature of the coleoptile could be regarded as negligible in this period (see lino et al. 1996 for detailed considerations). The oat and wheat coleoptiles stimulated for only 25 min returned gradually to the original vertical position after showing a curvature similar to that in the coleoptiles stimulated continuously. In both oats and wheat, the curvature response up to a period of at least 45 min after the onset of stimulation was nearly identical between the coleoptiles stimulated for 25 min and those stimulated continuously. Therefore, it was concluded that the curvature which had developed during the 45 min period in continuously stimulated coleoptiles was determined by the SA set initially and was not affected by the decrease in SA resulting from the curvature of coleoptiles.

The relationship between SAs and curvature rates were analyzed in light of the results described. For oat coleoptiles, curvature rates were measured in the period from 25 to 40 min of gravistimulation. It is preferable to measure curvature rates at a phase of linear curvature development (lino et al. 1996). This condition was met for oats, since oat coleoptiles showed a nearly linear increase of curvature from about 25 min (Fig. 1A–C, and many other time-course data not shown). However, in wheat coleoptiles, the curvature rate increased gradually over a longer period and a linear phase of curvature could not be established before 35–45 min of gravistimulation (Fig. 1D–F, and many other time-course data not shown). Since it was more critical to determine the curvature rate before it was influenced by the decrease in SA, the rate for wheat coleoptiles was determined in the period from 30 to 45 min.

Figure 2 shows the relationship between initial SAs (0–180°) and curvature rates measured for oats (A) and wheat (B) using the conditions described above. The rates were plotted against the sines of SAs (sin θ). Non-linear scales of SAs are also indicated. The function y = A(1 - e^{-Bθ}) was fitted to the data for 90° and smaller SAs with the least squares method to obtain the solid curves (y, curvature rate; θ, sin θ; A and B, constants).

Long-term time courses of gravitropism—Gravitropic curvatures were monitored over long periods. Figure 3 shows examples of such time courses obtained at three initial SAs. Oat coleoptiles showed a rapid upward curvature from about 20 min to 2.5 h (Fig. 3A). After this period, the curvature rate decreased sharply; the coleoptile then continued to bend at a much reduced rate, approaching the vertical slowly.

Wheat coleoptiles showed a rapid upward curvature from about 20 min to 2 h and ceased to bend abruptly.
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Fig. 3 Long-term time courses of gravitropic curvature in oat (A) and wheat (B) coleoptiles. Gravitropic stimulation was initiated at time 0. The initial SAs were 30° (●), 60° (●) and 90° (●). The coleoptile tip regains vertical orientation when the net curvature reaches these angles (indicated by dashed lines). Mean measurements (±SE) obtained from 12 plants are shown. The SE was smaller than the symbol size in many cases.

(Fig. 3B). After this cessation, the coleoptile showed oscillatory movements. When stimulated at 30°, the coleoptile tip reached the vertical position at the conclusion of the initial rapid curvature and then showed slight oscillation around the vertical. When stimulated at 60° and 90°, the coleoptile concluded its rapid curvature before reaching the vertical and then oscillated while gradually approaching the vertical. These oscillatory movements were highly synchronized among plants (note that the data in Fig. 3 represent the mean curvatures obtained from 12 plants).

It appeared that oat and wheat coleoptiles develop gravitropism through early and late phases. The early phase is characterized by a rapid curvature, and the late phase, by a slow curvature. In wheat, the late phase is additionally characterized by marked oscillatory movements. Such oscillatory movements were not observed in oats, but the data obtained at 90° (Fig. 3A) suggested that weak oscillation might occur in oats.

When the initial SA was 90°, the curvature rate dropped when the coleoptile tip was about 30° from the vertical (Fig. 3A, B). (Because the coleoptile was arc-shaped at this time, lower parts of the coleoptile had even greater angles from the vertical.) It was noted that the rate of the ensuing slow curvature was much smaller than the rate of the curvature induced at an initial SA of 30° (Fig. 3A, B). Therefore, the decrease in SA resulting from upward curvature of the coleoptile could not explain the observed drop in curvature rate.

Coleoptile straightening in the late phase of gravitropism—During the late phase of gravitropism, coleoptiles of oats and wheat straightened gradually. This is depicted in the photographs of Fig. 4. Following gravistimulation at 90°, an upward curvature developed along the length of the

Fig. 4 Development of gravitropic curvature in oat (A) and wheat (B) coleoptiles after 90° displacement. The photographs shown are those of the same plants obtained at the indicated times after displacement. The arrow indicates the direction of gravity.
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coeleoptile, resulting in an arc shape (1–3 h). After this early phase of gravitropism, the base of the coleoptile continued to bend upwards, but the apical to middle parts straightened gradually. Evidently, this straightening response took place before the coleoptile tip, or any part of the coleoptile, gained the vertical orientation. Therefore, the gravity perceived at any part of the coleoptile cannot be the cause of the straightening response.

It is possible that the coleoptile straightened because it reached the final stage of growth with the same maximal length being attained in its two sides. To investigate this possibility, oat and wheat coleoptiles were displaced by 90°, and, when the upper half of the coleoptile became straight at 6 h after showing curvature (see Fig. 4), they were displaced downwards again in such a way that the straight part was nearly horizontal. This part showed clear upward curvature in response to the second displacement (data not shown), demonstrating that the part which had straightened had enough growth capacity for curvature.

We conclude that the straightening of the coleoptile is an autonomic curvature response that does not directly depend on gravity but results somehow as a consequence of preceding gravitropic curvature. We choose the term autotropism (Simon 1912, Dolk 1936) to describe this autonomic curvature response.

The process of coleoptile straightening was investigated further using oat coleoptiles. The data in Fig. 5 were obtained by stimulating oat coleoptiles at 90° and rotating them on horizontal clinostats at 2.4 rpm (o). Control plants received no clinostat treatment (●). Each point is the mean (±SE) obtained from 16 independent plants.

downward curvature. The results demonstrated that when the gravitropic stimulus for an upward curvature is terminated by rotation on a clinostat, the coleoptile actually develops a curvature in the opposite direction. It appeared that autotropic response actively counteracts the gravitropic response to straighten the coleoptile in the late curvature phase.

In the next experiments, oat coleoptiles were stimulated similarly at 90°, and when the coleoptiles had just reached the late curvature phase, they were displaced upwards in such a way that the tip of the coleoptile became nearly vertical. As shown in Fig. 6, the displaced coleoptiles began to bend downwards, and the direction of these coleoptiles approached that of the non-displaced coleoptiles. Thus, when the gravitropic stimulus for upward curvature is weakened by an upward displacement, the coleoptile develops a curvature in the opposite direction. The results substantiated the conclusion that the autotropic response actively counteracts the gravitropic response in the late curvature phase.

Discussion

Angular dependence of gravitropism—The plot of curvature rates against the sines of SAs yielded a straight line for maize and a non-linear saturation curve for rice (Lino et al. 1996). The corresponding plots for oats and wheat were similar to the one obtained for rice (Fig. 2), indicating that the non-linear relationship is more common in Gramineae coleoptiles and that the coleoptiles of rice, oats, and wheat...
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Lupinus albus reported, for example, in roots of 
ing at a SA of 120-140° (see Introduction). Such a shift of 
the optimal SA to an angle greater than 90° has been 
90° and those larger than 90°, the maximal response occurr-
that the relationship is different between SAs smaller than 
tween the sines of SAs and curvature responses for oat and 
1929), cress (Larsen 1969, Iversen and Rommelhoff 1978)

tropism is determined by the component of gravity 
that is perpendicular to the organ's long axis (Sachs 1882). 
Our results appear to represent a simple and straightforward 
relationship between SAs and gravitropic responses. 
As discussed below, however, further study is necessary to 
clarify whether or not such a relationship is a general prop-
erty of plant gravitropism.

Pickard (1973) previously investigated the angular depen-
dence in oat coleoptiles. She subjected coleoptiles to 
different SAs for 25 min and subsequently rotated them on 
horizontal clinostats to obtain curvature responses. At SAs 
smaller than 90°, a linear relationship was found between 
the sines of SAs and curvatures. Our method yielded such a 
linear relationship for maize (Iino et al. 1996), but not for 
oats (Fig. 2A). Therefore, the results disagree for the same 
material. Pickard (1973) found, however, that the relation-
ship between the sines of SAs and curvatures was non-
linear at stimulation angles greater than 90°; the curvature 
response was nearly constant at SAs from 90° to about 
120°. This part of her results is similar to ours. More recent-
ly, the relationship between accelerations and gravitropism 
in oat coleoptiles was investigated in the microgravity en-
vironment of a space laboratory (Brown et al. 1995). In this 
study, the coleoptile was exposed at the right angle to its 
long axis to three different accelerations for different peri-
ods. For a given stimulation period, the extent of the gravit-
ropic response was similar at 0.4 and 0.6 g, whereas 0.4 g 
produced a greater response than 0.2 g. These results indi-
cate, in agreement with ours, that the gravitropic re-
ponse is nearly saturated at 0.4 g or at a sine SA value of 
0.4 under 1 g. Although it is not clear why the SA-response curve obtained by Pickard (1973) in the 0-90° range was 
different from ours, it is perhaps worthwhile to consider 
the possibility that the clinostat treatment practiced by 
Pickard had an additional effect on the angular depend-
ence, as reported for other aspects of gravitropism (Chap-
al. 1996).

Although we found a straightforward relationship be-
tween the sines of SAs and curvature responses for oat and 
and wheat coleoptiles, it has been concluded more commonly 
that the relationship is different between SAs smaller than 
90° and those larger than 90°, the maximal response occurr-
ing at a SA of 120-140° (see Introduction). Such a shift of 
the optimal SA to an angle greater than 90° has been 
reported, for example, in roots of Lupinus albus (Metzner 
1929), cress (Larsen 1969, Iversen and Rommelhoff 1978) 
and rapeseed (Legué 1994) and in hypocotyls of Arabi-
dopsis thaliana (Kiss et al. 1997) (for other organs and 
materials, see Metzner 1929 and Audus 1964). It is possible 
that the clinostat treatment used in the experiments had 
some uncontrolled effect on the SA-response relationship 
(e.g., Metzner 1929, Larsen 1969), but this cannot be 
the sole reason for the shift of the optimal SA, because 
the shift was also identified without clinostat treatment 
(Iversen and Rommelhoff 1978, Legué 1994, Kiss et al. 
1997). When clinostats were not used, however, the greater 
gravitropic response at a SA greater than 90° was demon-
strated by measuring curvature response over a relatively 
long period of gravistimulation (Iversen and Rommelhoff 
1978, Legué 1994, Kiss et al. 1997). Therefore, the early cur-
vature kinetics studied in the present study remain to be re-
solved in other materials. For further clarification of the 
SA-response relationship in those materials in which the op-
timal SA could not be identified at 90°, it is necessary to in-
vestigate whether or not the optimal SA is modified by the 
clinostat treatment and to evaluate the SA-response rela-
tionship by measuring the early curvature kinetics in continu-
ously stimulated plants.

The method used in the present study allows determi-
nation of the relationship between SAs and gravitropism 
without involving classical clinostat treatment. The rela-
tionship can be easily applied as an experimental criterion 
in studies of the components or the reactions that underlie 
the mechanisms of gravity perception. 

Autotropic straightening—Plant organs usually regain 
their straight appearance after showing gravitropic cur-
vature. Each part of the organ changes angular position as 
it develops gravitropism. This change in angular position 
appeared to be a cause of the straightening, especially when an 
organ bent until its upper part overshot the vertical 
(Köhler and Daum 1979, Iino et al. 1996). However, there 
have been experimental results which could not be explain-
ed simply by the change in angular position and the re-

donate to gravity. Dolk (1936) showed that oat coleoptiles 
straightened even while rotated on a horizontal clinostat. 
Firn and Digby (1979) showed that sunflower hypocotyls 
and maize coleoptiles began to straighten in the parts which 
had not reached the vertical position. Pickard (1973) pro-
vided shadowgraphs of an oat coleoptile indicating that 
straightening progressed before any part of the coleoptile 
reached the vertical position. More recently, it was shown 
that oat coleoptiles straightened even in the microgravity 
environment of a space laboratory (Chapman et al. 1994). 
These results indicate that the straightening is achieved by 
autotropism.

The present study has further substantiated the occur-
rence of an autotropic straightening response. As shown by 
Pickard (1973), oat coleoptiles began to straighten before 
any part of the coleoptile reached the vertical position 
(Fig. 4A). A similar result could also be obtained with
wheat coleoptiles (Fig. 4B). As described in the Results, the straightening occurred in the part which had the capacity to develop a further gravitropic curvature. More importantly, we were able to demonstrate that autotropism actively counteracts gravitropism to straighten the coleoptile (Fig. 5, 6).

Coleoptiles of oats and wheat sharply reduced the rate of their gravitropic curvature before the tip reached the vertical position (Fig. 3). Thereafter they developed net curvature slowly and the tip gradually approached the vertical position. The wheat coleoptiles subjected to small initial SAs seemed to be somewhat exceptional (Fig. 3B), but their curvature still dropped sharply when the tip reached the vertical position. In light of the results described in the preceding paragraph, it is most probable that the observed sharp decrease in curvature rate reflects the initiation of autotropism. In fact, the time-lapse photographs (Fig. 4, and many other photographs obtained at shorter time intervals) indicated that the straightening of the coleoptile in its upper part began at the same time as the measured sharp drop in net curvature rate.

Reported results have often indicated that gravitropic curvature progresses continuously until the apical part of a responding organ overshoots the vertical position. Such overshooting responses were recorded in sunflower hypocotyls (Köhler 1978, Firn and Digby 1979), maize coleoptiles (Firn and Digby 1979, lino et al. 1996), rice coleoptiles (lino et al. 1996), and maize roots (Ishikawa et al. 1991, Barlow et al. 1993). Autotropism is less apparent in these cases, but it may still be an important component of the process of organ straightening (Firn and Digby 1979).

During the late curvature phase, wheat coleoptiles showed marked oscillatory movements. This oscillation was probably caused by the interaction between gravitropism and autotropism. Oscillatory movements during gravitropism were also observed in rice and maize coleoptiles (Iino et al. 1996) and maize roots (Ishikawa et al. 1991, Barlow et al. 1993). Rice coleoptiles oscillated after overshooting the vertical, and the response to gravity appeared to explain this oscillation (lino et al. 1996). The oscillation of maize coleoptiles also occurred after overshooting the vertical, although it could not be explained only in terms of the response to gravity (lino et al. 1996). The oscillation of maize roots accompanied nutational movements that could also be observed without gravistimulation (Barlow et al. 1993). No such nutational movement was apparent in non-gravistimulated wheat coleoptiles (our observation). Perhaps the wheat coleoptile provides the first clear case in which oscillation is purely based on autotropism, free from complications due to overshooting and nutational responses.

The mechanism of autotropism is not clear. Gravitropic organs may detect the mechanical distortion of tissues caused by curvature. However, autotropic straightening did not take place during phototropism of oat coleoptiles (Dolk 1936), suggesting that autotropism is a specific attribute of gravitropism. During the straightening process, the tip orientation is finely adjusted so that it slowly approaches the vertical (Fig. 3, 4). This observation suggests that the plant somehow uses gravity information to regulate the expression of autotropism and to accomplish the final straightening response.

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References

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