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Light interacts with auxin during leaf elongation and leaf angle development in young corn seedlings

Abstract Modern corn (*Zea mays* L.) varieties have been selected for their ability to maintain productivity in dense plantings. We have tested the possibility that the physiological consequence of the selection of the modern hybrid, 3394, for increased crop yield includes changes in responsiveness to auxin and light. Etiolated seedlings in the modern line are shorter than in an older hybrid, 307, since they produce shorter coleoptile, mesocotyl, and leaves (blade as well as sheath). Etiolated 3394 seedlings, as well as isolated mesocotyl and sheath segments, were less responsive to auxin and an inhibitor of polar auxin transport, N-1-naphthylphthalamic acid (NPA). Reduced response of 3394 to auxin was associated with less reduction of elongation growth by light (white, red, far-red, blue) than in 307, whereas the activity of polar auxin transport (PAT) and its reduction by red or far-red light was similar in both genotypes. NPA reduced PAT in etiolated 3394 seedlings much less than in 307. A characteristic feature of 3394 plants is more erect leaves. In both hybrids, light (white, red, blue) increases leaf declination from the vertical, whereas NPA reduces leaf declination in 307, but not in 3394. Our results support findings that auxin and PAT are involved in elongation growth of corn seedlings, and we show that light interacts with auxin or PAT in regulation of leaf declination. We hypothesize that, relative to 307, more erect leaves in the modern hybrid may be primarily a consequence of a reduced amount of auxin receptor(s) and reduced responsiveness to light in etiolated 3394 plants. The more erect leaves in 3394 may contribute to the tolerance of the modern corn hybrid to dense planting.

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Introduction

Plants are able to detect neighbor proximity using sensitivity and response systems based on the quality of ambient light, and growth regulation. A mechanism for how plants indicate and respond to plant density has been proposed based on the ability of plants to detect changes in the ratio of red to far-red light (R:FR) within the canopy (Smith 1982, 1995). As plants grow and expand their leaves, light is increasingly filtered by chlorophyll, reducing R:FR. To avoid shade, plants respond to close neighbor proximity with morphological changes such as stimulation of elongation growth, reduced branching, and a redistribution of leaves to the top of the canopy (Morgan and Smith 1979).

The molecular mechanisms by which R/FR signals are perceived by different phytochromes and transduced to trigger morphological changes in plants are receiving some attention. For example, the expression of the recently cloned *Athb-2* gene is reversibly regulated by changes in R:FR (Carabelli et al. 1996). Steindler et al. (1999) have shown that overexpression of *Athb-2* inhibits primary root growth, lateral root formation, and secondary vascular growth, processes known to be regulated by auxin. The authors further showed that exogenous auxin can induce a lateral root phenotype even at high expression of *Athb-2*, and that auxin transport is needed for plant responses to shade. Consequently, the authors suggest that an FR-rich light regime produces a reorientation of the auxin transport stream, by spatial redistribution of a specific auxin efflux carrier protein, or the activation of a regulatory protein(s) controlling a specific

auxin efflux carrier protein(s) or both (Morelli and Ruberti 2000). The roles of auxin, light, and molecular links between light and auxin signaling pathways in regulation of shoot and leaf growth were recently reviewed (Chen 2001; Dengler and Kang 2001; Tian and Reed 2001).

Modern corn varieties have been selected for their ability to maintain production in dense planting, i.e. where there are likely to be low R:FR conditions. One of the phenotypic characters of modern hybrids is their more upright leaf in comparison to the older, density-sensitive varieties. We tested the hypothesis, derived from the work of Morelli and Ruberti (2000), that differential sensitivity of a modern, 3394, and an older, 307, hybrid to crowded conditions may be due to differential responses to R and/ or FR, which in turn affect distribution of, or sensitivity to, auxin. This could result in differential morphological changes in 3394 and 307 and, therefore, in their ability to respond to neighbor proximity and tolerate plant density. The objective of this study was to examine cross-sensitivity of 3394 and 307 seedlings to light, auxin, and inhibitors of polar auxin transport (PAT) with respect to elongation and the development of leaf angle.

Materials and methods

Plant material and growth conditions

Kernels from *Zea mays* L. lines 307 (a double-cross hybrid) and 3394 (a single-cross hybrid), commercially released in the 1930s and in the early 1990s, respectively, were provided by Pioneer Hi Bred Intl. (Des Moines, Iowa, USA). Seeds from a chlorophyll-free mutant in corn (*Z. mays* L.) (lemon white, *lw1*) and its isogenic wild type were purchased from Carolina Biological Supply Co. (Burlington, N.C., USA). Plants were grown in soil (Sunshine no. 4 soil mix; Sun Gro Horticulture, Bellevue, Wash., USA) in small pots (95 mm high, 105 mm diameter; one seed per pot; 1 cm deep) placed in a tray (10 pots per tray) and regularly watered. Once per week, plants were watered with nutrient solution according to the producer's directions (Plant Food; Miracle-Gro, Port Washington, N.Y., USA). Seedlings developed in growth chambers either in the dark (D), or in continuous red light (R), far-red light (FR), or blue light (B) at 23 °C. In white light (W), plants grew at high R:FR in a 16-h photoperiod and a temperature regime of 23 °C-light/21 °C-dark. Illumination was provided by cool-white fluorescent tubes (F72T12CW/VHO; Sylvania, Danvers, Mass., USA) and incandescent bulbs (100 W; Philips Lighting Company, Somerset, N.J., USA). Light intensity of fluorescent tubes and incandescent bulbs was regulated to provide W of high R:FR (2.9). R:FR was defined as photon fluence rate in a 10-nm band centered on 660 nm divided by photon fluence rate in 10-nm band centered on 730 nm. Total photon fluence rate was 200 μmol m^{-2} s^{-1} at plant level. Corn seeds were also incubated under either continuous R with maximum irradiance at 660 nm, B with maximum irradiance at 430 nm, or FR with spectral irradiance from 720 to 800 nm, at continuous 23 °C. R was provided by white fluorescent tubes wrapped with red Roscolux filter no. 27 (Rosco, Hollywood Light Inc.). Total photon fluence rate was 13.5 μmol m^{-2} s^{-1}. B was provided by white fluorescent tubes wrapped with blue Roscolux filter no. 83. Total photon fluence rate was 9 μmol m^{-2} s^{-1}. FR was provided by incandescent bulbs (PS-52 1000, 1000 W; Philips Lighting Company) filtered through Plexiglas FR filter FRF 700 (Westlake Plastic Company, Lenni, Pa., USA). Total photon fluence rate was 53 μmol m^{-2} s^{-1}. Fluence rates were measured with a portable spectroradiometer (model LI-1800; Li-Cor; Lincoln, Neb., USA) calibrated by the company at the start of experiments.

Elongation of sheath and mesocotyl segments

For study of elongation of sheath segments, seeds were sown into soil 20 per pot and grown for 7 days in the dark as described above. Six- to 10-mm-long segments were excised from the sheath of the first leaf. The segments were placed into Petri dishes (60 mm diameter, 15 mm deep) containing 10 mM KCl supplemented with or without auxin (NAA: 1-naphthalene acetic acid) or an inhibitor of polar auxin transport (NPA: N-1-naphthylphthalamic acid), and incubated in D, R, or FR for 24 h.

For study of elongation of mesocotyl segments, etiolated seedlings grown in vitro were used as a source of mesocotyl. Kernels were soaked in 50% (v/v) Reliance solution (Sysco Co., Houston, Tex., USA) (approx. 3% sodium hypochlorite) for 25 min, and then rinsed extensively with sterile distilled water. Seeds were germinated on 0.7% (w/v) agar medium in Magenta GA7 boxes (77× 77×196 mm; Sigma, St. Louis, Mo., USA) (nine seeds per box). The basal medium (BM) contained Murashige and Skoog (1962) salts, 1% (w/v) sucrose and 1 mM Mes (pH adjusted to 6.1 by KOH before autoclaving). Seeds in the Magenta boxes were placed in a growth chamber and incubated in the dark at 23 °C. Mesocotyl segments (10 mm) were excised just below the coleoptilar node in etiolated 5-day-old seedlings. Segments were placed into Petri dishes (60 mm diameter, 15 mm deep) containing liquid BM supplemented or not with NAA (0.01–100 μM). Ten segments were used per genotype and condition in each experiment. Petri dishes were sealed with Parafilm and incubated for 24 h in the dark at 23 °C, rotating slowly (50 rpm).

After the incubation time, lengths of sheath or mesocotyl segments were measured with a ruler to the nearest millimeter and new growth of the segment was calculated. Segments were prepared and measured under green light.

PAT assay

Auxin transport measurements were conducted on segments excised from elongated coleoptiles and mesocotyls of etiolated 6-day-old seedlings grown in soil as described above. Coleoptile and mesocotyl segments (20 mm) were excised just above and below the coleoptilar node. The segments were held vertical by solidified (0.7% agar) sucrose-free BM. A 5-μl drop of the same, but tepid and still liquid, medium was applied to the opposite end of the segment. The agar contained 0.04 or 0.4 μM radiolabelled IAA ([^{3}H]IAA; Amersham Pharmacia Biotech, Buckinghamshire, UK; specific activity 962 GBq mmol^{-1} or 26.0 Ci mmol^{-1}) and 1 μM unlabelled IAA. For study of auxin movement in the basipetal direction, the segment was placed basal side down and the agar drop was applied to the apical end of the segment. For acropetal auxin movement, the segment was placed upside down, i.e. apical end down, and the agar drop applied to the basal end of the segment. The samples were incubated for 4 h in D, R, or FR at 23 °C. After this time, 5 mm was excised from the basal (or apical) end of the segment and placed in a scintillation vial containing 0.5 ml 95% ethanol. After 15 min of extraction, 4.5 ml of scintillation cocktail (EcoLite; ICN Costa Mesa, Calif., USA) was added, and samples were counted in a liquid scintillation analyzer (model 1500 Tri-Carb; Packard Instruments Co., Downers Grove, Ill., USA). To study the effect of NPA on polar auxin transport, segments were first pre-incubated for 1 h in the liquid sucrose-free BM containing or not NPA (0.1–1 μM). Afterwards, segments were rinsed with distilled water and placed in the agar plate as described above. In addition to the labelled and unlabelled IAA, the agar drop applied on the opposite side of the segment contained NPA (0.1–1 μM). Segments were then incubated for 4 h and radioactivity measured as described above.

Measurement of growth of intact seedlings in vivo

Kernels were sown and seedlings grown on soil as described above. From germination, seedlings were sprayed daily either with distilled water or an aqueous solution of NPA (75 μM). Various parameters in 6–10 intact seedlings per treatment were measured each day or

every other day from the 6th to 22nd day after seed sowing. Plant height was measured from the coleoptilar node to the top of the plant with leaves extended. Sheath and blade lengths of the first and second leaves were also measured. Leaf angle, measured as a declination from vertical (Fig. 1), was determined by placing a protractor upside down along the midrib of the 30-mm segment of the leaf blade closest to the vertical axis, and measuring the angle indicated by a freely swinging rod. Finally, auricle growth was measured at the edge of the leaf blade (the auricle is the tissue creating a "joint" between blade and sheath; Fig. 1). Lengths were measured with a ruler to the nearest millimeter.

Histological analysis

Analysis of leaf vascular tissues was carried out using 19-day-old plants grown in soil as described above. Sections (10 mm) were cut from leaf blades and leaf sheaths, and the sections were located 20 mm away from the ligule. Samples were dehydrated in a graded butanol/ethanol series: 50% (50 ml water:10 ml butanol:40 ml ethanol), 70% (30:20:50), 85% (15:50:35), 95% (0:55:45), 100% (0:75:25), and 3-times 100% butanol. After dehydrating, tissue was embedded in paraffin (Paraplast-X; melting temperature 50–54 °C). Paraffin chips were added progressively to the sample in 100% butanol until the total volume of the solution was 3-times the original volume. The sections were then transferred to pure paraffin, and the step was repeated twice. Cross-sections (35 μm) were cut with a microtome and melted onto a slide. For sample staining, the slides with cross-sections were heated overnight at 35–40 °C, immersed into a solution series consisting of toluene, ethanol, safranin, or fast green as listed below. Pure toluene (5–10 min), ethanol: 100%, 95%, 70%, 50% (each 1–2 min), safranin (1% in 50% ethanol; 30 min), ethanol: 50%, 70%, 95% (each 1–2 min), fast green (0.2% in 95% ethanol; 30–60 s), ethanol: 95%, 100% (twice), pure toluene (each 1–2 min). The sections were observed under a light microscope at 400×.
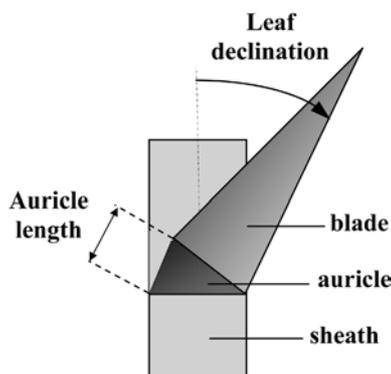
Statistical analysis

Where necessary, statistical significance of the treatment differences was assessed using Student's t-test.

Results

Elongation growth of corn seedlings in dark or light

Kernels of both genotypes sown in soil germinated in 4–5 days in all light conditions. As found for seedlings

grown in vitro (data not shown) etiolated coleoptiles of 3394 were distinctly shorter than those of 307, and the two genotypes were differentially inhibited by light. Coleoptile growth in 307 was strongly reduced by R, W, or B (Fig. 2a), whereas coleoptiles in 3394 exhibited less growth reduction in light. Similar results were observed for mesocotyls, where the inhibition of growth in 307 and 3394 reached about 75% and 65%, respectively (data not shown).

After 1–2 weeks, etiolated 3394 seedlings were about 20% shorter than 307 plants, due to distinctly shorter leaf blades (data not shown) and sheaths in the first (Fig. 2b) and second leaves (Table 1). The effects of light quality on sheath elongation (Fig. 2b) were similar to those on coleoptile growth. In 307, R and W inhibited sheath growth by 15 and 50%, respectively. In 3394, R essentially did not reduce sheath elongation, while W did reduce sheath growth, but less (35%) than in 307. In both genotypes, B had no inhibitory effect on sheath elongation in 15-day-old seedlings (Fig. 2b).

Sheath elongation in the second leaf (Table 1) was also inhibited by W and, as in the first leaf (Fig. 2), there was relatively less inhibition in 3394 compared to 307. In both
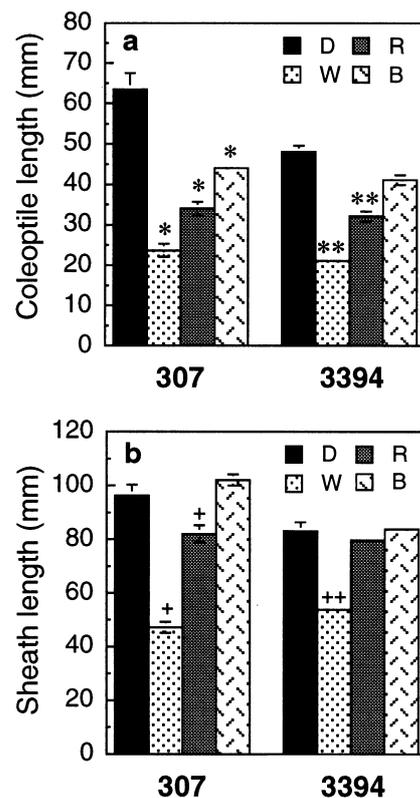

Fig. 1 Diagram showing leaf declination (angle) and auricle length measured in corn (*Zea mays*) seedlings. Leaf angle, measured as a declination from vertical, was determined by protractor. Auricle growth was measured with a ruler at the edge of the leaf blade

Fig. 2a, b Comparison of seedling growth in two corn hybrids, 307 and 3394, in response to dark and light. **a** Effect of R, W, or B on elongation of etiolated coleoptiles in 8-day-old seedlings; **b** Effect of R, W, or B on sheath elongation from the first leaf of 15-day-old seedlings. The results are the mean ± SE of 6–10 seedlings from one representative experiment. Similar results were observed in three (D, R, W) or two (B) independent experiments. *, ** Significantly different ($P \leq 0.05$) from 307 in D or 3394 in D, respectively; +, ++ significantly different ($P \leq 0.05$) from 307 in D or 3394 in D, respectively

Table 1 Effect of light and NPA (75 µM) on sheath growth in the second leaf of 11-day-old seedlings of corn (*Zea mays*) hybrids 307 and 3394. The seedlings were sprayed every day with NPA or with distilled water (control). Values show mean ± SE of 6–10 seedlings from one representative experiment. Similar results were observed in three (D, R, W) or two (B) independent experiments

Genotype	Light conditions	Sheath length (mm)	
		Control	+ NPA
307	D	94.6 ± 2.8	79.1 ± 2.2
	W	63.0 ± 4.1	44.6 ± 2.3
	R	91.0 ± 1.0	78.4 ± 2.0
	B	129.8 ± 5.6	122.0 ± 7.0
3394	D	86.0 ± 1.0	91.8 ± 5.7
	W	64.0 ± 5.5	65.0 ± 2.5
	R	91.4 ± 3.8	86.7 ± 4.4
	B	106.6 ± 3.8	109.1 ± 3.1

genotypes, R had no effect on elongation of the second leaf and, interestingly, B stimulated sheath growth in the second leaf to a similar extent in 307 and 3394 (Table 1).

Effect of NPA on growth of intact corn seedlings

As early as was measured (8 days), NPA (75 µM) distinctly inhibited elongation of the first leaf sheath in etiolated 307 seedlings (Fig. 3a). In contrast, NPA did not reduce sheath elongation until plants were 10 days old in 3394, and the relative effect was less than in 307 (Fig. 3a). In R and W, NPA significantly inhibited sheath growth in 307 but had essentially no effect on 3394 (Fig. 3b, c). In B, NPA did not inhibit sheath growth in either genotype (Fig. 3d).

In 307, but not in 3394, sheath elongation in the second leaf of D-, R- and W-grown seedlings was inhibited by NPA. The inhibitory effect was seen only in the early stage of elongation, i.e. in 11-day-old plants (Table 1), but not in 18-day-old plants (data not shown). NPA had no effect on sheath elongation in the second leaf in either genotype in B (Table 1).

Consistent with the NPA-induced inhibition of sheath growth, NPA caused deformation and twisting of leaf blades, and agravitropic roots in 307 seedlings developed in D, R, or W, whereas NPA had no or negligible effect on blade development and root growth in 3394 (data not shown). In B-grown plants, NPA-induced leaf twisting was not observed in either genotype (data not shown).

Histological examination indicated that NPA affected development of vascular bundles in leaves from plants grown in W. NPA caused vascular hypertrophy (phloem as well as xylem) in macroveins of second-leaf blades in 307, whereas no changes in vascular morphology were seen in 3394 leaves (Fig. 4).

Interaction of light with NAA or NPA in elongation of excised sheath segments

Sheath segments isolated from etiolated first leaves, and incubated in the dark in control solution (10 mM KCl),
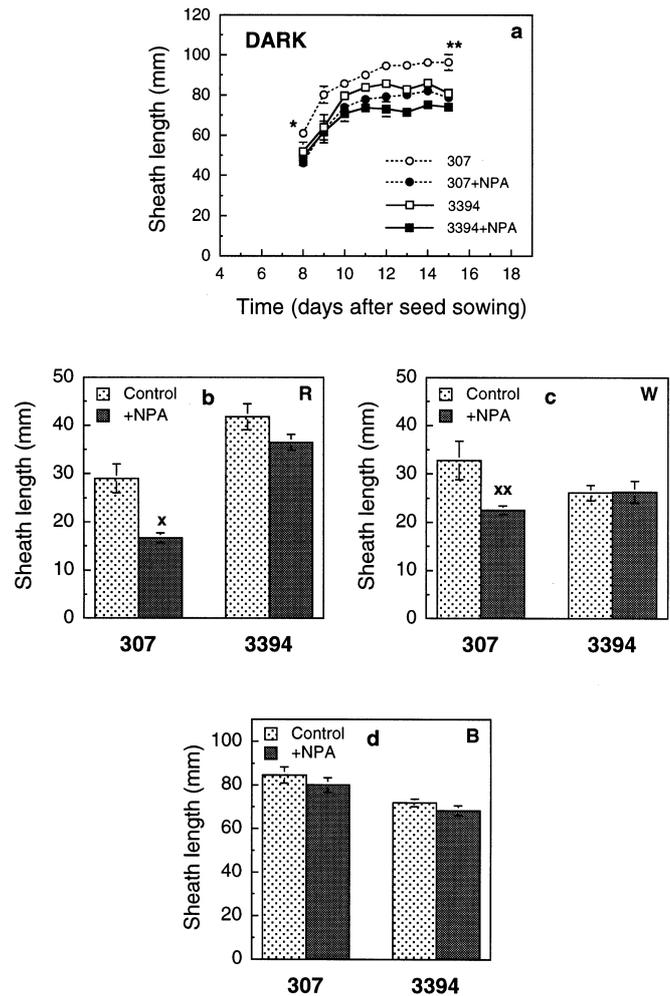
Fig. 3a–d Effect of NPA, an inhibitor of PAT, on corn seedling growth in dark or light. Kinetics of sheath elongation in etiolated plants (**a**) and sheath growth in seedlings grown under R (**b**), W (**c**), or B (**d**). Sheath length was measured on the first leaf of 307 and 3394 seedlings treated with NPA (75 µM) or distilled water (control). Lengths are shown for 7-day-old (R, W) or 8-day-old (B) seedlings. Values show mean ± SE of 6–10 seedlings from one representative experiment. Similar results were observed in three (D, R, W) or two (B) independent experiments. *, ** Significantly different ($P \leq 0.05$) from 307 + NPA at 8 days and 307 + NPA at 15 days, respectively; ^{x}, ^{xx} significantly different ($P \leq 0.01$) from 307 control

elongated less in 3394 than in 307 (Fig. 5a). Under R, growth of 307 segments was only slightly less than in D and, in 3394 segments, growth was similar to that in D. FR strongly reduced segment growth in 307 (by approx. 70%), but had no inhibitory effect in 3394 (Fig. 5a). These results are similar to those reported for coleoptile and sheath elongation in intact plants grown in D, R, and W (Fig. 2a, b) although elongation of 3394 sheath segments was much reduced in D compared to 307.

In D and R, NAA (10 µM) inhibited segment growth in 307 by 65%, whereas it had no effect on segment growth in FR (Fig. 5b in comparison with Fig. 5a). In contrast to 307, NAA stimulated rather than inhibited

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Fig. 4 Vascular morphology of macroveins in untreated 307 and 3394 corn seedlings (*left panel*) or NPA-treated plants (*right panel*) grown under W. *X* Xylem, *P* phloem. Bar = 135 μm
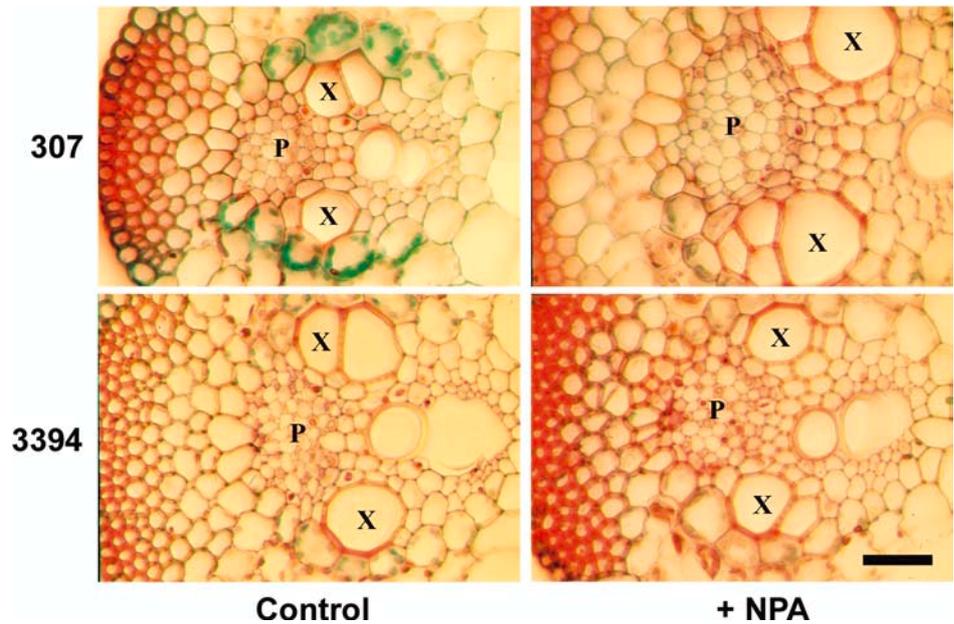
sheath elongation in 3394 in D, R, or FR (Fig. 5b). Like NAA, NPA (50 μM) reduced growth of 307 segments incubated in D or R by approx. 60%, whereas in FR, NPA had no effect on segment elongation (Fig. 5, compare c with a). In all light treatments, 3394 sheaths were insensitive to NPA (Fig. 5c, a).

Effect of exogenous NAA on growth of excised, etiolated mesocotyl segments

When segments excised from etiolated mesocotyl were incubated in D in the presence of exogenous NAA, their growth was stimulated, relative to the segments incubated without auxin. In 307, NAA promoted maximum elongation of mesocotyl segments at 1–100 μM, and the stimulation reached 70–80% (Fig. 6). As in 307, 3394 mesocotyls were most responsive to NAA at concentrations 1 μM and higher. However, the amplitude of the mesocotyl growth stimulation in 3394 was about half of that in 307, with stimulation reaching approx. 40% (Fig. 6).

Light-dependent changes in leaf declination

In etiolated 11-day-old seedlings of the older hybrid 307, the declination of the second leaf blade from the vertical was about 9°. As the plants grew declination increased, i.e. the leaf became less vertical and reached about 30° in 18-day-old seedlings (Fig. 7). In 3394, leaf declination in etiolated 11-day-old plants was approx. 5°. The kinetics of leaf angle development in 3394 were distinct from the kinetics in 307. In 307, declination increased linearly with time, whereas leaf declination in 3394 reached its maximum (approx. 13°) during the first 4 days, and afterwards it did not change (Fig. 7).

In both genotypes, W distinctly increased leaf declination, i.e. made leaves more horizontal. In 307, leaf declination reached a plateau during the first 14 days with a maximum at about 30° (Fig. 7). One of the characteristic features of 3394 plants grown in the field is an erect leaf. In growth chambers, 3394 seedlings grown under W also developed more erect leaves than 307 with a maximum angle of about 20° (Fig. 7).

To determine which part of the W spectrum increased leaf declination, we measured its development in R and B. Under R, 307 as well as 3394 plants developed more horizontal leaves than in W (Table 2), and in both genotypes, declination of the second leaf in approx. 3-week-old plants reached about 40° (data not shown). Interestingly, in both genotypes B also increased declination (Table 2).

Effect of an inhibitor of PAT on development of leaf declination

Similarly to its effects on sheath elongation (Table 1), NPA (75 μM) strongly reduced leaf declination in etiolated seedlings of 307, i.e. NPA-treated leaves were almost fully vertical (Table 2). In contrast, NPA had essentially no effect in etiolated 3394 plants (Table 2). NPA also made 307 leaves more erect when plants grew under W, R, or B, i.e. NPA abolished light-induced leaf declination. However, unlike in D, the effect of NPA on second-leaf declination was evident only in very young seedlings (Table 2), but not in older, i.e. 18-day-old plants (data not shown). Table 2 also shows that NPA had no effect on leaf declination in 3394 seedlings grown in W, R, or B.
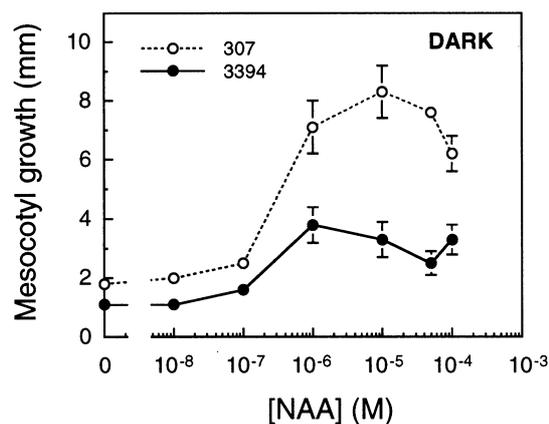
Growth of the auricle, a specialized structure at the boundary of sheath and blade allowing the leaf to bend (Fig. 1), was also measured. In both genotypes, the
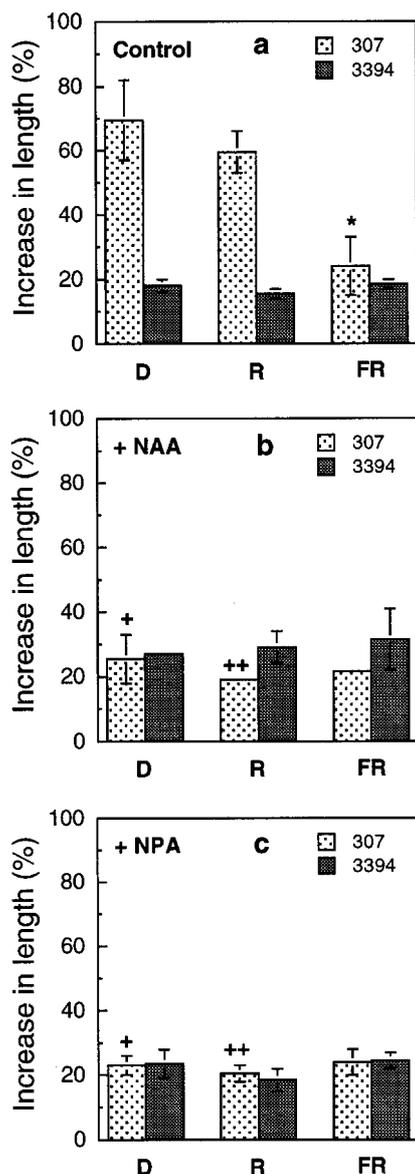
Fig. 5 Effect of NAA and NPA on growth of isolated corn sheath segments incubated for 24 h in KCl (10 mM; **a**), supplemented with NAA (10 µM; **b**), or with NPA (50 µM; **c**), in D, R, or FR. The segments were isolated from the first leaf of etiolated 7-day-old 307 and 3394 seedlings. Initial lengths of the segments were 6 mm for 307 and 10 mm for 3394. Data show mean ± SE of 5–10 segments from two independent experiments. ** Significantly different ($P \leq 0.05$) from 307 in D; $^{+}$, $^{++}$ significantly different ($P \leq 0.05$) from 307 control in D or 307 control in R, respectively

Fig. 6 Growth of corn mesocotyl segments incubated for 24 h in liquid basal medium (BM) supplemented or not with NAA in the dark. The segments were isolated from etiolated 5-day-old seedlings grown on BM in conditions in vitro. Initial length of the segments was 10 mm. Data show increase in length (mean ± SE of 20 segments) from one representative experiment. Similar results were observed in three independent experiments
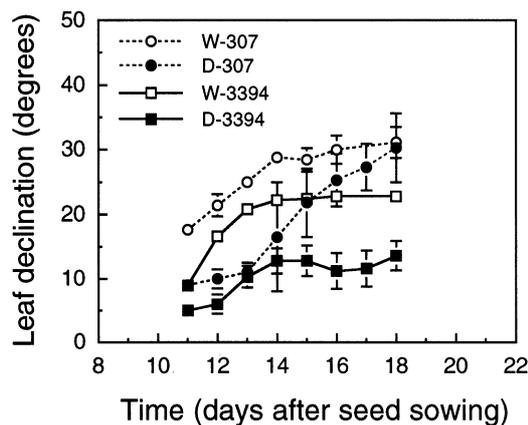
Fig. 7 Kinetics of leaf angle development in D- (etiolated) and W-grown corn seedlings. Leaf angle was measured for the second leaf. Values show mean ± SE of 6–10 seedlings from one representative experiment. Similar results were observed in three independent experiments

light-induced increase in auricle growth was more or less associated with greater leaf declination (Table 3). Similarly, in D- or light-grown young, 11-day-old, plants of 307, the inhibition of auricle elongation by NPA was associated with reduced leaf declination (Table 3), whereas, consistent with results on leaf declination, inhibition of auricle growth by NPA was not observed in the older plants (data not shown).

Very interestingly, NPA has a similar inhibitory effect on development of leaf angle in light-grown albino, chlorophyll-free, seedlings of the recessive mutant *lw1*

and green seedlings of the corresponding control *LW1* (Fig. 8). This suggests that photosynthesis does not affect auxin-mediated changes in leaf angle development, i.e. that auxin- or PAT-related changes in leaf angle are photomorphogenic responses.

Polar auxin transport in corn seedlings and its regulation by light

Measurements of PAT revealed no clear difference between 307 and 3394 in transport intensity in etiolated coleoptile segments incubated in D (Fig. 9a). NPA, an inhibitor of PAT, did not affect the intensity of IAA movement in either hybrid when applied at 0.1 µM. However, in 307, NPA at 1 µM reduced PAT by

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Table 2 Effect of light and NPA (75 μM) on angle development in the second leaf of 11-day-old seedlings of corn hybrids 307 and 3394. The seedlings were sprayed every day with NPA or with distilled water. Values show mean ± SE of 6–10 seedlings from one representative experiment. Similar results were observed in three (D, R, W) or two (B) independent experiments

Genotype	Light conditions	Leaf declination (degrees)	
		Control	+ NPA
307	D	9.0 ± 2.2	3.5 ± 0.5
	W	17.6 ± 1.1	9.3 ± 1.5
	R	20.0 ± 1.0	10.8 ± 1.7
	B	24.4 ± 5.6	14.0 ± 1.5
3394	D	5.0 ± 0.0	5.2 ± 2.6
	W	8.9 ± 0.4	9.4 ± 3.2
	R	14.4 ± 1.7	11.3 ± 0.4
	B	15.6 ± 1.3	13.5 ± 1.2

Table 3 Effect of light and NPA (75 μM) on auricle elongation in the second leaf of 11-day-old seedlings of corn hybrids 307 and 3394. The seedlings were sprayed every day with NPA or with distilled water. Values show mean ± SE of 6–10 seedlings from one representative experiment. Similar results were observed in three (D, R, W) or two (B) independent experiments

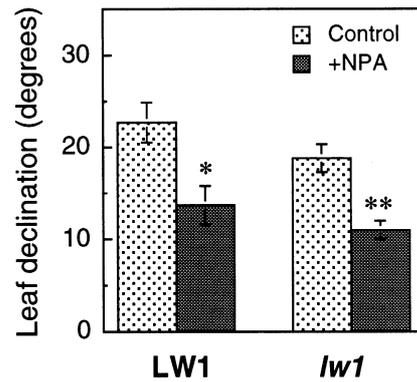| Genotype	Light conditions	Auricle length (mm)	
		Control	+ NPA
307	D	0.8 ± 0.2	0.5 ± 0.0
	W	1.0 ± 0.0	0.5 ± 0.1
	R	1.3 ± 0.8	0.5 ± 0.1
	B	1.5 ± 0.2	0.9 ± 0.3
3394	D	0.5 ± 0.0	0.5 ± 0.0
	W	0.9 ± 0.1	1.0 ± 0.2
	R	0.9 ± 0.2	0.6 ± 0.1
	B	1.1 ± 0.1	0.9 ± 0.1

Fig. 8 Effect of NPA on leaf declination in the albino, chlorophyll-free, mutant *lw1*, and corresponding, chlorophyll-containing, wild-type corn plants LW1. Seedlings were grown in R and sprayed every day with NPA (75 μM) or with distilled water (control). Leaf angle was measured for the second leaf in 14-day-old seedlings. Values show mean ± SE of 6 seedlings from one experiment. *, ** Significantly different (P ≤ 0.05) from LW1 and *lw1* controls, respectively

approx. 70%, but the same concentration had essentially no effect on PAT in 3394 coleoptile segments (Fig. 9a). As a control, movement of labelled IAA was also studied in the acropetal direction. In neither genotype was there any measurable polar movement of labelled IAA through the segment from the basal to the apical side of the segment (Fig. 9a). As in coleoptiles, the intensity of PAT in D-incubated mesocotyl segments was similar in both genotypes (Fig. 9b). Also in mesocotyls, NPA inhibited IAA transport in a concentration-dependent manner. However, in this case, NPA at 0.1 μM and 1 μM reduced PAT in mesocotyls of 307 by about 65% and almost completely, respectively. In contrast to 307, the inhibitory effect of NPA (0.1 μM) on PAT was not seen in mesocotyls of 3394 but, as in 307, PAT in 3394 was fully inhibited by NPA at 1 μM (Fig. 9b).

To investigate the effect of light on PAT in etiolated coleoptiles and mesocotyls, the segments were incubated under R or FR. In coleoptile segments of the older hybrid 307, R or FR had essentially no effect on PAT. In 3394, R did not significantly reduce the intensity of PAT, whereas FR inhibited IAA movement by approx.

30% (Fig. 9c). Unlike in coleoptiles, both R and FR reduced PAT in mesocotyls by approx. 50%, and the effect was similar in both hybrids (Fig. 9d).

We also studied the velocity of PAT by a pulse-chase experimental procedure. We found that in etiolated coleoptile and mesocotyl segments incubated in D, the velocity of PAT was similar in both genotypes, and that R or FR did not change the velocity of labelled IAA distribution in mesocotyl or in coleoptile segments of either genotype (data not shown).

Discussion

Role of auxin in growth of etiolated corn seedlings

Here we report that in the dark, an inhibitor of PAT, NPA (75 μM), inhibits coleoptile and mesocotyl growth, sheath elongation and overall growth of young corn seedlings grown in soil. In addition, NPA or exogenous auxin at high concentration inhibits growth of etiolated sheath segments. There are reports in the literature indicating that auxin and PAT play a role in normal leaf development (Jones et al. 1998; Tsiantis et al. 1999; Van Volkenburgh 1999). It has been shown that two *Arabidopsis* mutants, *lop1* (Carland and McHale 1996) and *pin1* (Bennett et al. 1995) with deficiencies in PAT both have twisted leaves. Leaf twisting was also observed in the etiolated *rs2* mutant in corn and was associated with decreased PAT in the shoot (Tsiantis et al. 1999). The *rs2* phenotype could be phenocopied by treatment of wild-type plants with NPA. Auxin is produced in young emerging leaves and transported basipetally through the shoot (Sachs 1991). The authors therefore concluded that in *rs2*, block of basipetal auxin transport results in disruption of the auxin gradient and accumulation of auxin in the leaf blade, resulting in leaf twisting (Tsiantis
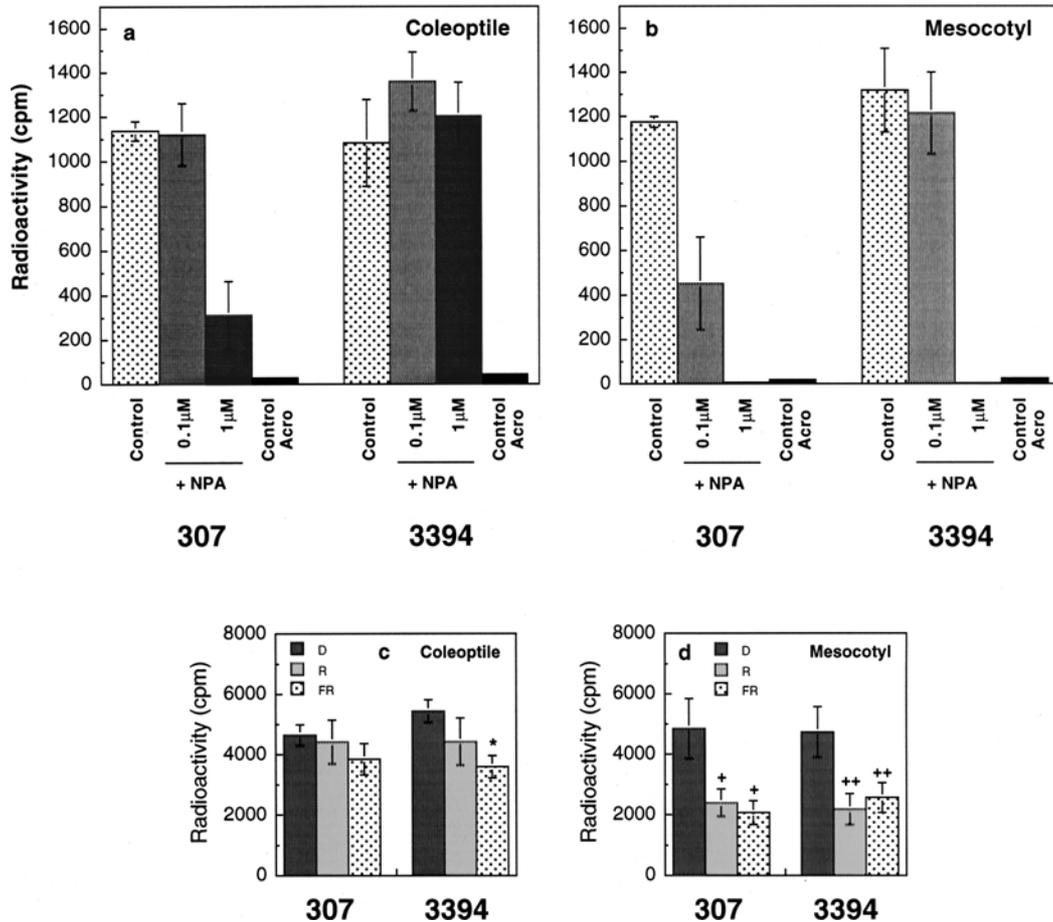
Fig. 9a–d Effect of NPA or light on PAT in coleoptiles and mesocotyls of corn hybrids 307 and 3394. For the experiments, 20-mm-long segments from coleoptiles and mesocotyls of etiolated 6-day-old seedlings grown in soil were used. **a, b** PAT of [³H]IAA in control or NPA-treated coleoptile (**a**) and mesocotyl (**b**) segments incubated in the dark. Values represent mean ± SE of seven segments from one representative experiment. Similar results were obtained in two independent experiments. **c, d** PAT in coleoptile (**c**) and mesocotyl (**d**) segments incubated in D, R, or FR. Values show mean ± SE of 12 segments from three representative experiments (**c**) and seven segments from two independent experiments (**d**). * Significantly different ($P \leq 0.05$) from 3394 in the dark; [+], [++] significantly different ($P \leq 0.05$) from 307 in the dark or 3394 in the dark, respectively

et al. 1999). Most likely, due to the unequal auxin accumulation, differential cell division and/or expansion causes the twisting of the leaf blade (Chen 2001; Chen et al. 2001). It is very likely that in our experiments, leaf twisting and inhibition of sheath growth in NPA-treated 307 plants are also caused by disruption of the auxin gradient within the leaf. Our results suggest that auxin and its polar movement may be positively involved in growth of etiolated corn seedlings.

In this study, we have compared the growth of a modern (3394) and an older (307) corn hybrid. Young etiolated 3394 seedlings are shorter than 307 plants. Along with shorter coleoptile, mesocotyl, and seminal roots, 3394 developed shorter leaves than 307, with the

length of both sheath and blade reduced. NPA applied to intact etiolated 307 seedlings phenocopied the elongation of leaf sheath in etiolated control 3394 plants. Similarly, NPA applied to 307 sheath segments mimicked the reduced elongation of untreated segments excised from 3394. This would suggest that reduced growth in etiolated 3394 plants is a consequence of reduced PAT in 3394 compared to 307. However, etiolated 3394 plants and sheath segments were resistant to the inhibitory effect of NPA or NAA. Also, unlike 307 plants, etiolated 3394 blades treated with NPA did not twist, and etiolated 3394 seedlings grown in vitro showed reduced growth responses to NAA and the anti-auxin *p*-chlorophenoxyisobutyric acid (PCIB), relative to 307 (data not shown). Most importantly, the intensity as well as velocity of PAT in etiolated seedlings is similar in both hybrids and, consistent with the results above, etiolated 3394 seedlings showed distinct resistance to the inhibitory effect of NPA on PAT, in comparison with 307. Therefore, we consider that, relative to 307, the reduced growth of etiolated 3394 seedlings and lower responsiveness of 3394 plants to NPA may rather reflect reduced responsiveness to auxin. This conclusion is strongly supported by our results revealing that, in the system of etiolated mesocotyl segments where exogenous auxin promotes elongation, the stimulatory effect of NAA is much less in 3394 than in 307 (Fig. 6). The fact

that the dose–response curves for 307 and 3394 peak at a similar concentration range, i.e. at 1–10 μM NAA, but they differ in their slope, suggests that the two hybrids do not differ in their affinity for auxin (meaning receptor affinity; Firn 1986), but rather in receptivity (meaning number of receptors; Firn 1986). That is, fewer hormone receptors are expected in growing regions of 3394 than in 307.

Interaction of light and auxin during elongation

We report here that R, FR, and especially W, inhibit growth of young corn seedlings grown in soil. Exogenous auxin or NPA inhibited elongation of intact sheaths or isolated sheath segments, and this effect was dependent on plant age and light quality. In our experiments, FR had a stronger inhibitory effect than R on elongation of sheath segments, suggesting that strong inhibition of sheath growth under W may be due to the additive effects of R and FR. This is supported by the fact that B did not inhibit leaf elongation. Our results suggest that R, FR, and thus W interact with auxin in regulation of seedling elongation growth. The interaction may involve light-reduced activity of PAT and/or light-reduced responsiveness to auxin (Jones et al. 1991, 1998). Jones et al. (1991) hypothesize that R-induced inhibition of PAT causes a decrease in the level of auxin in epidermal cells of mesocotyls, which results in growth inhibition. Similarly, we found that in both genotypes R or FR strongly inhibit the intensity, but not the velocity, of PAT in etiolated mesocotyls. However, the similar extent of the reduction of PAT by R or FR in etiolated 307 and 3394 seedlings does not correlate with the observation that elongation of etiolated 307 seedlings is inhibited by light much more than in 3394. Thus, some factor(s) in addition to PAT is involved in regulating elongation of corn seedlings in light. It is quite possible that light also reduces auxin receptivity. Jones et al. (1991) showed that R reduces abundance of an auxin-binding protein 1 (ABP1), considered to be an auxin receptor that controls cell expansion (Jones et al. 1998), and that expression of *ABP1* in maize coleoptiles is much less in light than in the dark (Im et al. 2000). The negative effect of light on auxin receptivity is strongly supported by our results in vitro, where the anti-auxin PCIB altered growth of etiolated corn seedlings, but was ineffective in R- or FR-grown seedlings (data not shown). So, it is possible that the relatively smaller effect of light on growth of 3394 than 307 reflects less reduction of ABP1 by light in 3394 than 307. This would suggest that 3394 seedlings are less sensitive to light with respect to its effect on ABP1 amount. In addition or alternatively, it is possible that a smaller number of auxin receptors in etiolated 3394 seedlings results not only in a reduced growth of 3394 plants in the dark, but causes somehow an inability of light to inhibit normally the growth of etiolated 3394 seedlings while 3394 plants may sense light normally.

Inhibition of elongation growth in young corn seedlings by light is a complex process involving changes in PAT, auxin content, changes in sensitivity to auxin, and auxin-independent processes. We hypothesize that etiolated 3394 tissues have primarily fewer auxin receptors than 307, while the possibility that 3394 seedlings are less sensitive than 307 to light, as well, cannot be overruled. Either results in less light-induced growth inhibition in 3394 than in 307 seedlings. The hypothesis is currently under investigation.

Role of auxin and light in development of leaf angle

As in field conditions, plants of the older hybrid developed in the growth chamber carried less vertical leaves than 3394 plants. Here we report that in dark-grown 307 plants, leaf declination can be reduced by NPA. The size of the auricle, a specialized structure at the boundary of sheath and blade was also reduced by NPA, proportionally associated with the size of leaf declination, which is to say, the smaller leaf declination occurred for leaves with shorter auricle dimension at the blade margin. This suggests that PAT may regulate auricle growth and that basipetal auxin movement is positively involved in development of leaf declination in dark-grown seedlings. This is quite consistent with results in the literature indicating that PAT plays a role in normal leaf development (Tsiantis et al. 1999), and with our observation that NPA strongly inhibits PAT in etiolated corn seedlings, and that NPA or NAA alter elongation growth of intact sheath or sheath segments.

We propose a mechanism for how auricle growth and leaf declination are regulated by auxin. It is believed that there is a strong auxin gradient over the etiolated leaf blade towards the sheath (Sachs 1991; Tsiantis et al. 1999). Therefore, relative to the tip, auxin accumulates at high concentration at the leaf base, where the auricle is developing in young leaves. Since auxin can also be transported in polar fashion from leaf margins to the leaf center (Berleth et al. 2000), auxin would accumulate at the midrib region relative to blade margins. Both cell expansion and division drive leaf morphogenesis; cell elongation is caused by auxin at low concentrations and mediated by ABP1, whereas auxin at high levels stimulates cell division via an unidentified receptor (Chen 2001; Chen et al. 2001). Consequently, there may be differential growth of auricle tissue at the midrib (cell division) and at the blade margin (cell elongation). Thus the auricle becomes triangular in shape, which may contribute to development of leaf declination. It follows that the inhibitory effect of NPA on auricle growth and leaf declination may be simply explained by strong inhibition of PAT from the blade resulting in auxin deficiency in the auricle.

We showed that in 307, R or W distinctly increases leaf declination, i.e. makes leaves more horizontal. B also is strongly involved in development of leaf declination, while the effect of R or B on leaf declination was

greater than the effect of W. This could suggest that another part of the light spectrum, likely FR, counter-acts the effect of R or B in leaf development. This is supported by preliminary results that FR enhances leaf declination approximately half as well as R or B (data not shown). However, additional experiments on the effect of FR on leaf declination development must be performed. Alternatively, since W was applied during a 16-h photoperiod, it is very likely that the reduction of leaf declination in W, relative to R or B, reduced during the dark period, since in etiolated seedlings leaf declination is less developed than in light-grown plants. These data strongly suggest differential responsiveness of sheath and auricle tissues to light.

Light decreases the amount of auxin transported through etiolated corn shoots, and the decrease in transport is correlated with the decrease in growth (Jones et al. 1991, 1998; see also references in *Introduction*). It is therefore interesting that, in contrast to NPA, light promotes auricle elongation and increases leaf declination. However, the fact that, at least in 307, NPA can abolish the light-induced elongation of the auricle and proportionally reduce leaf declination suggests that light regulates auricle growth and leaf declination by controlling PAT. In spite of the differential responses of leaf declination to light and NPA, the promotion effect of light can still be explained by light-reduced polar distribution of auxin through the leaf blade. It is believed that in leaves, auxin flow from a source (leaf tip and/or margins) to a sink (already developed vasculature) would create a preferred pathway for polar movement of auxin (Berleth et al. 2000; Dengler and Kang 2001). Under normal conditions, an auxin gradient from tip to base is strong and vascular differentiation remains restricted to relatively few narrow zones. Under conditions of reduced auxin transport, the auxin gradient may be reduced, i.e. relatively less auxin is available at the base of the leaf, and vasculature is restricted in the center and along the leaf margin (Berleth et al. 2000). Therefore, under conditions of reduced PAT, such as in light, relative to dark, the auricle at the blade margin may elongate more, producing a more horizontal leaf. The inhibitory effect of NPA on auricle growth and leaf declination may be caused by the absence of auxin in the auricle, resulting in reduced auricle growth and less leaf angle than in untreated plants.

In dark or light (R, W), leaf declination directly correlated with sheath elongation, i.e. when NPA has an inhibitory effect, leaf declination and sheath elongation are simultaneously affected. Thus, in young seedlings, sheath elongation and declination development may be coupled and controlled by light-induced changes in PAT or responsiveness to auxin.

Except for the highly light-sensitive mesocotyls, the inhibitory effect of B on elongation growth was none (sheath) or weak (coleoptile), in comparison with the effect of R or FR. This could be simply explained by the lower photon fluence rate of the B treatment in comparison with R or FR. In contrast, B had a strong effect on development of leaf declination, equal to R. This suggests that, relative to sheath growth, development of leaf angle is a highly light-sensitive process. The absence or presence of tissue sensitivity to B is nicely associated with the absence or presence of the inhibitory effect of NPA, respectively, which again supports the existence of cross-talk between auxin and/or PAT and light in growth of corn seedlings. However, more experiments are needed to determine if the mechanism(s) of the interaction with B may be similar to that with R or W.

Interestingly, in etiolated 307 seedlings, NPA reduced leaf declination not only in young but also in older 18-day-old seedlings (data not shown). In contrast, in light-grown 307 seedlings, the effectiveness of NPA in inhibiting leaf declination decreased with seedling age, so the inhibition was not evident in 18-day-old seedlings (data not shown). It seems that with plant aging, the activity of PAT increases, and therefore the effectiveness of NPA in inhibiting auxin distribution decreases, or possibly an auxin-independent regulation of leaf declination predominates in older light-grown plants.

Development of leaf angle in 3394

We found that in etiolated 3394 seedlings, elongation of the auricle or increase in leaf declination is much less than in 307. Like elongation responses, auricle growth and increase in leaf declination in etiolated 3394 seedlings were not affected by NPA at all. These results support our hypothesis that, relative to 307, less development of leaf declination in etiolated 3394 plants is a consequence of reduced responsiveness of leaf tissues to auxin, likely due to fewer auxin receptors.

As in 307, light increased leaf declination in 3394. Although we found that light inhibits PAT similarly in etiolated 307 and 3394 seedlings, leaves in the modern hybrid are more erect than in the older line. This could reflect reduced responsiveness of etiolated 3394 seedlings to auxin, in comparison with 307, which is consistent with our hypothesis that 3394 may have fewer auxin receptors than 307. This is supported by the fact that, as in the dark, leaf angle in light-grown 3394 seedlings is not inhibited by NPA.

Our results support findings showing that in darkness as well as in light auxin is involved in growth of corn seedlings. In addition, we show that light interacts with auxin and PAT in regulating leaf angle development. We further report that, relative to 307, etiolated 3394 seedlings elongate less in the dark, they are less responsive to auxin and to an inhibitor of PAT, and less reduced in growth by light. We hypothesize that etiolated 3394 and 307 plants differ in the amount of auxin receptor(s), while we cannot exclude the possibility that the 3394 hybrid is less sensitive than 307 to light with respect to its negative effect on tissue responsiveness to auxin. It is possible that a factor(s) controlling the number of auxin receptors and/or sensitivity to light was modified over the years, resulting in selection of the 3394 hybrid with

reduced responsiveness to auxin and/or light and, thus, with modified leaf morphology, i.e. with more vertical leaves. More vertical leaves in 3394 than in 307 may provide tolerance of the modern hybrid to neighbors, and higher yield at dense planting.

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