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Phytochrome inhibits the effectiveness of gibberellins to induce cell elongation in rice

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Abstract. Red light controls cell elongation in seedlings of rice (*Oryza sativa* L.) in a far-red-reversible manner (Nick and Furuya, 1993, Plant Growth Regul. **12**, 195–206). The role of gibberellins and microtubules in the transduction of this response was investigated in the rice cultivars Nihon Masari (*japonica* type) and Kasarath (*indica* type). The dose dependence of mesocotyl elongation on applied gibberellic acid (GA_3) was shifted by red light, and this shift was reversed by far-red light. In contrast, coleoptile elongation was found to be independent of exogenous GA_3. Nevertheless, it was inhibited by red light, and this inhibition was reversed by far-red light. The content of the active gibberellin species GA_1 and GA_4 was estimated by radio-immunoassay. In the mesocotyl, the gibberellin content per cell was found to increase after irradiation with red light, and this increase was far-red reversible. Conversely, the cellular gibberellin content in *japonica*-type coleoptiles did not exhibit any significant light response. Microtubules reoriented from transverse to longitudinal arrays in response to red light and this reorientation could be reversed by subsequent far-red light in both the coleoptile and the mesocotyl. This movement was accompanied by changes in cell-wall birefringence, indicating parallel reorientations of cellulose deposition. The data indicate that phytochrome regulates the sensitivity of the tissue towards gibberellins, that gibberellin synthesis is controlled in a negative-feedback loop dependent on gibberellin effectiveness, and that at least two hormone-triggered signal chains are linked to the cytoskeleton in rice.

Key words: Cell elongation – Gibberellin sensitivity – Microtubule – *Oryza* –Phytochrome

Abbreviations: D = darkness; FR = far-red light; GA_3 = gibberellic acid; GC-SIM = gas chromatography-selected ion monitoring; R = red light

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Introduction

Important aspects of plant development appear to be regulated by the interplay of a few hormones. Changes of hormone content induced by environmental signals have been frequently invoked to explain the control of plant morphogenesis by exogenous stimuli. For instance, alterations of auxin content, following stimulation by asymmetric light or gravity, seem to mediate tropistic growth responses in grass coleoptiles (Cholodny 1927; Went 1933; Pickard 1985). Similarily, light inhibition of elongation of pea internodes was interpreted in terms of a phytochrome-induced block of late gibberellin synthesis (Campell and Bonner 1986), although this has been questioned on the base of genetic evidence (Behringer et al. 1989).

A second mode of hormone-mediated morphogenetic control has attracted less attention: it might be that the stimulus affects the response of the tissue to a given hormone rather than the content of the active hormone itself (Trewavas 1981; Firn 1986). This could involve the abundance of hormone receptors ("receptivity" in sensu Firn 1986), the interaction between hormone and receptor ("affinity") or the signal chain initiated by this hormone-receptor interaction ("response capacity"). Fluctuations of hormone content, usually ranging in the order of less than fivefold, do not suffice to explain the frequently dramatic growth responses, often spanning more than two orders of magnitude (Trewavas 1981). Many hormone mutants are in the first place affected in their sensitivity to hormones and only secondarily in their hormone content (Reid 1990). Morever, hormone sensitivity can change conspicuously during development, as shown for ethylene-dependent maturation processes in flowers (Whitehead et al. 1984) or fruits (Biale and Young 1981). This emphasizes that changes in hormone content alone cannot account for the transduction of signal-dependent morphogenetic events.

Developmental responses to hormonally mediated environmental signals are usually complex and this complexity hampers a causal analysis considerably. There are

two main reasons for that: (i) many plant hormones interact with the metabolism of other hormones (Imaseki and Pjon 1970; Hoffmann-Benning and Kende 1992) or affect the sensitivity of the tissue to other hormones (Sauter and Kende 1992); (ii) in most organs, the growth response to a hormone comprises both cell division and cell expansion, which can differ with respect to time course (Sauter and Kende 1992) and location (Martìnez-Garcìa and Garcìa-Martìnez 1992).

Cell elongation in rice seedlings provides a useful model for studying the role of hormones in signal-dependent morphogenesis. It responds readily to light, perceived by phytochrome (Pjon and Furuya 1967, coleoptiles; Nick and Furuya 1993, mesocotyls) and this response is under the control of auxins (Furuya et al. 1969, coleoptiles) and gibberellins (Nick and Furuya 1993, mesocotyls). Moreover, the response is almost completely brought about by a light-induced inhibition of cell elongation rather than by interference with cell division (Furuya et al. 1969, coleoptiles; Nick and Furuya 1993, mesocotyls). In the mesocotyl, the chain of events elicited by the light stimulus involves a shift in the dose-dependence of elongation upon exogenous gibberellins and reorientation of cortical microtubules in cells of the outer epidermis (Nick and Furuya 1993).

To investigate the role of light-induced changes in the response to gibberellins during the phytochrome-dependent inhibition of mesocotyl elongation, the endogenous content of the active gibberellin species GA_1 and GA_4 was estimated by radio-immunoassay for different light regimes. The response of the coleoptile, which is not under control of gibberellins, but regulated by auxin (Furuya et al. 1969) served as an internal control. A further level of control was secured by use of two rice cultivars with different gibberellin sensitivities (Nick and Furuya 1993). These data are discussed in relation to the dose-dependence of growth upon GA_3 and microtubular responses.

Materials and methods

Plant material and growth conditions. Caryopses of two rice (*Oryza sativa* L.) cultivars were used for this study (the seeds were a kind gift from Dr. Osamu Yatou, Institute for Radiation Breeding, National Institute of Agricultural Resources, Hitachi-Ohmiya, Japan). The *japonica*-type cultivar Nihon Masari is characterized by stunted mesocotyls, which exhibit a weak response of elongation growth to GA_3 (Nick and Furuya 1993). In contrast, the *indica*-type cultivar Kasarath is characterized by long mesocotyls and high gibberellin responsiveness (Nick and Furuya 1993). Plants were grown on floating plastic mesh in darkness for 3.5 d at 25°C and preselected for the experiments as described previously (Nick and Furuya 1993).

Light conditions and light treatments. After preselection, plants were irradiated 3.5 d after sowing with a pulse of red light (R; 10 μmol photons·m^{-2}, 3 min) and then returned to darkness (D) until response evaluation. In a second set of experiments the R pulse was followed by 10 min of far-red light (FR; 0.1 W·m^{-2}). A set of plants designated as the dark control was returned to the dark without irradiation. Red light and FR were obtained from fluorescent tubes behind acrylic filters as described in detail by Mohr et al. (1964).

Table 1. Determination of recovery yield for the radio-immunoassay and identification of endogenous GA_1 and GA_4. Endogenous gibberellins were identified in etiolated rice coleoptiles 5.5 d after sowing by GC-SIM and radio-immunoassay (RIA). Cross-detection of GA_3 and GA_7 in the radio-immunoassay was excluded by preceding $N(CH_3)_2$-HPLC. The values obtained with both methods were compared to determine the recovery yield of the radio-immunoassay. The data in Figs. 1c–d and 2c–d are corrected with respect to this recovery yield. KRI=Kovats retention index (Kovats 1958)

GA-species	KRI	Monitored ions and relative abundance (% base peak)	Content $pg \cdot (g\ FW)^{-1}$		Recovery yield
			GC-SIM	RIA	(%)
GA_1	2661	506 (M^+, 100) 491 (9) 448 (15)	384	250	65
GA_4	2506	418 (M^+, 38) 328 (34) 284 (100)	552	270	49

Estimation of the recovery yield of the radio-immunoassay and estimation of endogenous gibberellin content. Shoots of dark-grown Nihon Masari seedlings were excised 5.5 d after sowing under dim FR (less than 0.01 W·m^{-2}) and collected in cold methanol (−20°C). The material (100 g fresh weight) was then homogenized and extracted three times in a total of 2 l of methanol. Of this extract 96% was subjected to gas chromatography-selected ion monitoring (GC-SIM) analysis, and an aliquot of 4% was used for the analysis by radio-immunoassay. An aliquot of 50 ng of $[^2H_5]GA_1$ (Endo et al. 1989) and $[^2H_4]GA_4$ (Takayama et al. 1993) was added to each methanol extract as internal standards. Each extract was concentrated in vacuo and acidic ethyl-acetate fractions obtained as described by Toyomasu et al. (1992). These fractions were pre-purified by a column of 1 g polyvinylpolypyrrolidone, a Sep-Pak octadecylsilane cartridge (Waters Associates, Inc., Milford, Mass., USA) and a column with 0.5 g diethylamino-Sepralyte preceding the analysis by GC-SIM, by a Sep-Pak octadecylsilane cartridge for the analysis by radio-immunoassay. In both cases, purified samples were subjected to octadecylsilane-HPLC on a Senshu-Pak 4253D column (10 mm in diameter, height 250 mm) as described by Endo et al. (1989). The fractions corresponding to a retention time of 10–16 min (for the analysis of GA_1) and 23–27 min (for the analysis of GA_4) were separately purified on a Senshu-Pak $N(CH_3)_2$ 3151N column (8 mm in diameter, height 150 mm), and eluted with 0.05% acetic acid in methanol at a flow rate of 3 ml·min^{-1} at 50°C. The retention time for GA_1 was 11–13 min, that for GA_4 10–12 min.

For GC-SIM the samples were converted to the corresponding methyl-ester trimethylsilyl ether (Me) and analyzed by a gas-chromatography mass-spectrometer (JEOL DX-303, JEOL, Akishima, Japan) fitted with a fused silica capillary column (DB-1, 258 μm in diameter, length 15 m; J and W Scientific Inc., Forsom, Calif., USA). Details of GC-SIM are given in Toyomasu et al. (1993). For native GA_1, ions of m/z 506, 491 and 448 were monitored; for native GA_4, ions m/z 418, 328, and 284 were monitored. The concentrations were estimated from the ratios of the peak area at m/z 506 (GA_1) by 511 ($[^2H_5]GA_1$) and 284 (GA_4) by 288 ($[^2H_4]GA_4$, respectively.

The assays for quantification of GA_1 and GA_4 by radio-immunoassay were performed in triplicate using anti-GA_1-Me-antiserum as described by Yamaguchi et al. (1990). Calibration curves for quantification of GA_1 and GA_4 were measured with GA_1-Me and GA_4-Me as standards, respectively. The recovery yield of GA_1 and GA_4 in the radio-immunoassay was calculated based on the quantitative data obtained by GC-SIM (Table 1).

For the various light treatments, seedlings of both cultivars were excised under dim safelight (less than 0.01 W·m^{-2}) 6 d after sowing,

and coleoptile and mesocotyls collected separately in cold methanol (–20°C). The pooled material (about 1 g fresh weight) from at least seven independent experiments was homogenized and extracted three times with a total of 200 ml of methanol. The whole study was performed twice with seed batches from two different years. Each sample was then pretreated for the radio-immunoassay as described above. Unless explicitly stated otherwise, the quantitative data give the values corrected for the recovery yield of the radio-immunoassay (Table 1). For the calculation of the gibberellin content per cell, the relation between cell number and final length had to be estimated. Since cell length varies over the length of the seedling, it was measured in different positions for at least 30 cells in each measuring spot (Figs. 1b and 2b, upper right-hand panel). The averages of cell length thus obtained represent the data from at least five individual seedlings. They were used to present growth and gibberellin content on a cell basis (Table 2).

Evaluation of microtubule responses. Coleoptiles and mesocotyls were excised 6 d after sowing, and then fixed for 1 h at room temperature in fixation buffer (50 mM 1,4-piperazine-diethanesulfonic acid, 1 mM $MgSO_4$, 1% w/v glycerol, pH 6.8) containing 3.7% paraformaldehyde and 0.2% v/v Triton X-100). Thin tangential sections were cut with a sharp razor blade and postfixed in the same solution for a further 30 min. After three washings in fixation buffer without paraformaldehyde, sections were treated with 5% sheep normal serum (Nordic Immunology, Tilburg, The Netherlands) for 20 min at room temperature, and then incubated for 1 h at 37°C with a mouse monoclonal antiserum directed against β-tubulin (Amersham, Little Chalfont, UK) diluted 500-fold in Tris-buffered saline (150 mM NaCl, 20 mM Tris, 1 mg $MgSO_4$, pH 7.4) containing 0.2% v/v Triton X-100. After three washings in this buffer without antiserum, sections were re-incubated overnight at 4°C with a secondary antibody from sheep directed against mouse IgG, and labeled with fluorescein isothiocyanate (Amersham) diluted 25-fold in Tris-buffered saline supplemented with 0.2% v/v Triton X-100. After three further washings in the same buffer without antiserum, sections were mounted outside-up in antifading solution (Citifluor, London, UK) and then viewed under an epifluorescence microscope (Leitz, Wetzlar, Germany). The frequency of transverse microtubules (whose direction deviated less than 30° from the transverse cell axis) and longitudinal microtubules (whose direction deviated more than 60° from the transverse cell axis) was recorded. In parallel, cell-wall birefringence was assessed under a polarisation microscope (Zeiss, Oberkochen, Germany) in additive and subtractive orientation as described by Ziegenspeck (1948), and quantitated by determining the frequency of cells with longitudinal walls displaying blue or yellow Newtonian colours, respectively. Each experiment comprised 35 individual plants collected from at least two independent sets of experiments.

Results

Red light lowers the effectiveness of exogenous gibberellic acid (GA_3) in the induction of elongation growth in japonica-type seedlings. The mesocotyl in the *japonica*-type cv. Nihon Masari is stunted compared to *indica*-type cultivars (cf. Fig. 3a). It can be induced to elongate by incubation with exogenous GA_3, with the threshold of this response at about 10^{-7} M GA_3. This threshold is shifted to

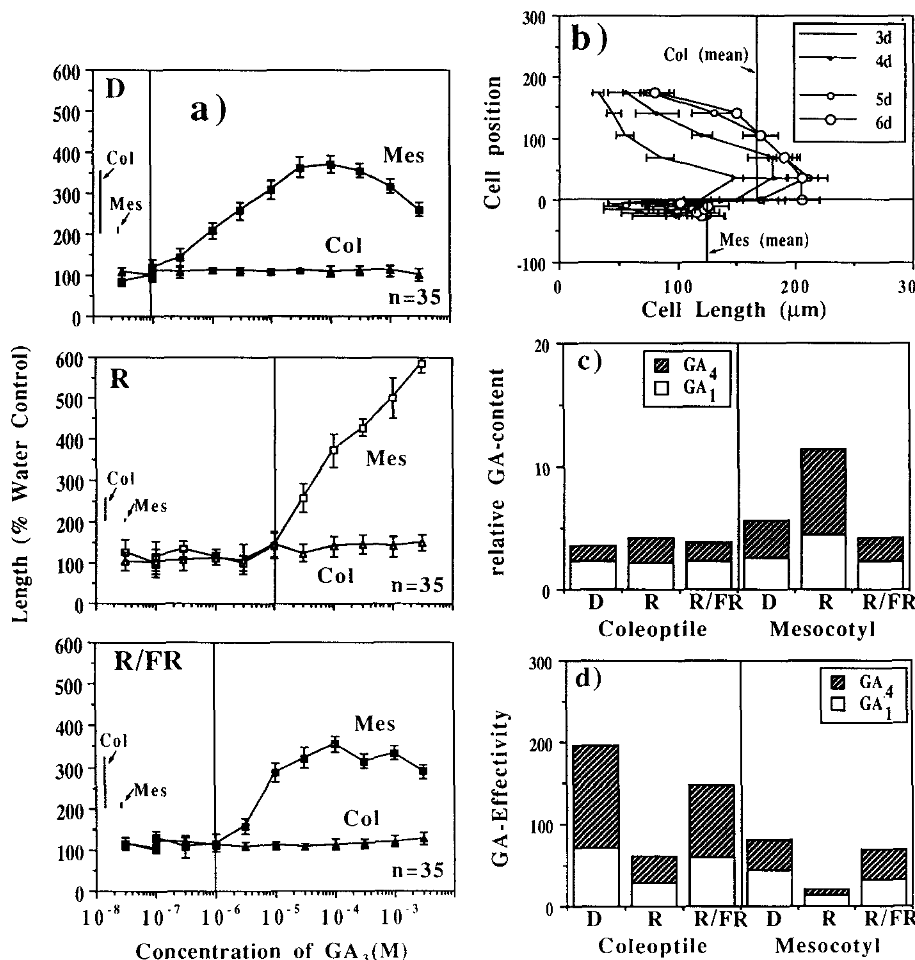

Fig. 1a–d. Effects of light upon gibberellin content and sensitivity in seedlings of the *japonica* rice type cv. Nihon Masari. **a** Dose-response dependence of final length upon exogenous GA_3 (*vertical bars* indicate the response of the water-treated controls in mm, *vertical lines* the threshold of the response to GA_3. **b** Time course of cell elongation in dark-grown seedlings. The vertical axis gives the position of a cell along from the node (position 1) to the tip of the coleoptile (positive values) or to the base of the mesocotyl (negative values). **c** Relative cellular contents of endogenous gibberellins. **d** Ratio of final cell length and cellular content of endogenous gibberellins

Table 2. Effect of various light regimes on the cell length, total length, fresh weight and endogenous gibberellin contents of rice seedling coleoptiles (Col.) and mesocotyls (Mes.). Gibberellin contents were estimated by radio-immunoassay. D, darkness; R, red light: R/FR, red light followed by far-red light

Cultivar	Organ	Average cell length (μm), organ length (mm), and organ FW (mg)					
		D		R		R/FR	
		Cell	Organ	Cell	Organ	Cell	Organ
Nihon Masari	Col. length	164 ±12	30.2±2.5	66 ±6	12.0±1.3	138 ±13	25.2±2.8
	Col. FW	3.0±0.2		7.1±0.8		3.5±0.5	
	Mes. length	112 ±9	3.2±0.4	57 ±4	1.0±0.1	74 ±4	2.1±0.3
	Mes. FW	0.6±0.1		0.9±0.2		0.6±0.2	
Kasarath	Col. length	183 ±10	48.0±3	87 ±3	12.8±1.2	133 ±6	30.4±1.8
	Col. FW	3.0±0.6		4.9±0.1		4.0±0.2	
	Mes. length	294 ±19	90.0±9.1	27 ±2	1.1±0.1	79 ±5	24.3±3.2
	Mes. FW	4.0±0.8		3.7±0.2		4.1±0.6	

Cultivar	Organ	Content of endogenous gibberellins (pg·(g FW)$^{-1}$)					
		D		R		R/FR	
		GA_1	GA_4	GA_1	GA_4	GA_1	GA_4
Nihon Masari	Coleoptile	141±19	81±10	57±9	52±6	120±18	81±10
	Mesocotyl	121±18	152±18	90±14	137±16	107±16	91±11
Kasarath	Coleoptile	490±74	204±25	145±22	9±1	244±37	577±69
	Mesocotyl	133±17	261±31	112±17	134±16	67±11	166±20

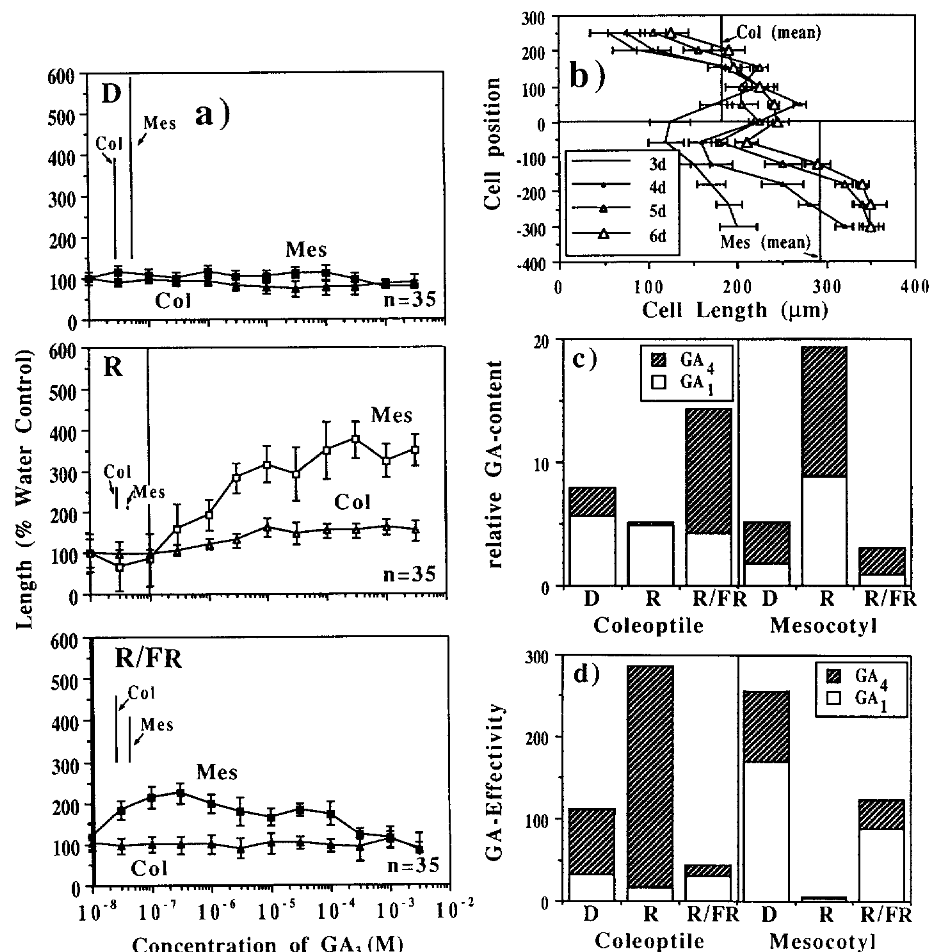

Fig. 2a–d. Effects of light upon gibberellin content and sensitivity in seedlings of the *indica* rice type cv. Kasarath. For details refer to the legend of Fig. 1

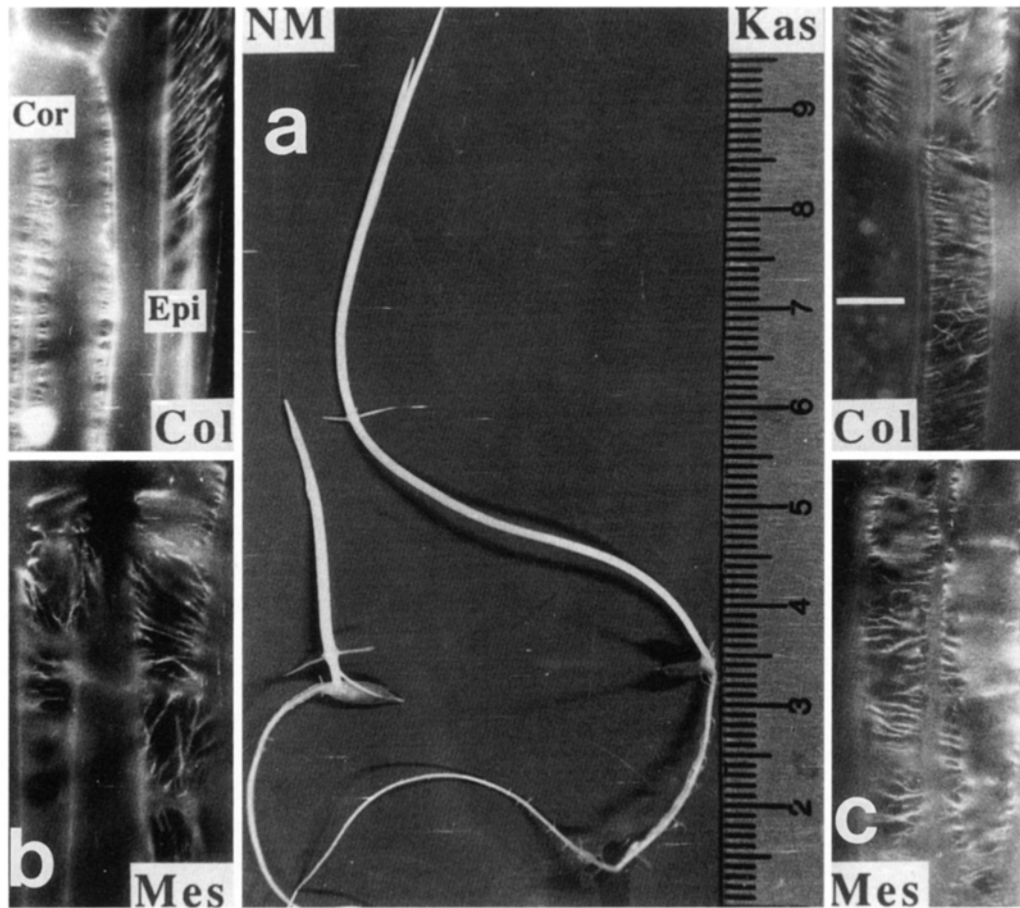

Fig. 3a–c. Phenotypes of etiolated *japonica*-type and *indica*-type rice seedlings. **a** Etiolated seedlings of cv. Nihon Masari (*NM*) and cv. Ksarath (*Kas*). The node separating the coleoptile and the mesocotyl is indicated by the emerging crown roots. **b, c** Immunofluorescence images of cortical microtubules in the coleoptile (*Col*) and mesocotyl (*Mes*). **b** Transverse microtubules in cells of the cortex (*Cor*) contrast with the oblique microtubules in the epidermis (*Epi*) of the coleoptile. **c** Cortical microtubules in epidermal cells of indicatype seedlings. Note that the microtubular arrays are more transverse than in the *japonica*-type seedlings

higher concentrations by about two orders of magnitude if plants are subjected to irradiation with a pulse of R, and saturation is not reached even for unphysiologically high concentrations of GA_3 (Fig. 1a). In addition, the final length of the mesocotyl is conspicuously decreased (Fig. 1a, vertical bars). Both effects were partially reversed if R was followed by FR. Thus, the effectiveness of GA_3 in stimulating mesocotyl elongation is decreased by R and this decrease can be partially reversed by subsequent FR. In contrast, no elongation in response to applied GA_3 could be detected in coleoptiles (Fig. 1a), although the elongation of coleoptiles was sensitive to R in a manner similar to the growth of mesocotyls (Fig. 1a, vertical bars; Table 2), and this decrease of coleoptile elongation was reversible by subsequent irradiation with FR (Table 2).

Determination of endogenous gibberellins in japonica-type seedlings. The analysis focussed on GA_1 and GA_4, the active gibberellin species in rice (Takahashi and Kobayashi 1990). If related to fresh weight, the contents of GA_1 and GA_4 decreased after irradiation with R (Table 2) both in the coleoptile and in the mesocotyl. This decrease was found to be reversed somewhat, if the R pulse was followed by FR, except in the mesocotyl where an even further decrease was observed for GA_4. Since the observed growth response of the coleoptile apparently does not involve vast changes of cell number, it was felt that fresh weight is not the appropriate standard. In order to evaluate the physiological role of gibberellins in the control of cell elongation, the relative changes of cellular gibberellin content were estimated (Fig. 1c). The ratio of average cell length and the relative gibberellin content per cell (Fig. 1d) can give a measure for the response of the tissue to endogenous gibberellin. When interpreting these data, however, one must bear in mind that the state of activity of a gibberellin species, its intracellular partitioning, its metabolic dynamics and the influence of other growth factors are ignored by this ratio. In case of the mesocotyl, the effectivity of GA_1 and GA_4 in promoting cell elongation was found to decrease after irradiation with red light (Fig. 1c, d). This decrease was reversed by subsequent irradiation with far-red light. A similar light response of gibberellin effectiveness could be detected in the coleoptile. These data are consistent with the dose-response curves obtained under the same irradiation regimes (Fig. 1a).

Effectiveness of applied GA_3 and estimation of endogenous gibberellins in indica-type seedlings. The mesocotyls of etiolated seedlings of the *indica* type cv. Kasarath are extremely long and additional elongation of neither the mesocotyl nor the coleoptile could be achieved by application of GA_3 to plants grown in darkness (Fig. 2a). Experiments involving inhibitors of gibberellin synthesis (Nick and Furuya 1993) suggest that endogenous gib-

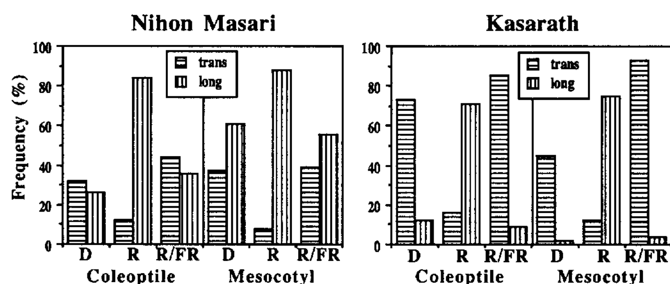

Fig. 4. Orientation of cortical microtubules in epidermal cells of rice cv. Nihon Masari and cv. Kasarath for various light treatments. The frequency of transverse and longitudinal microtubule arrays is given for light treatments identical to those in Figs. 1 and 2

Table 3. Preferential direction of cell wall anisotropy as observed by birefringence in polarized light. Epidermal strips from coleoptiles (Col) and mesocotyls (Mes) of 35 individual rice plants were collected from at least two independent sets of experiments and viewed under a polarisation microscope in additive and subtractive orientation. The data show the percentage of cells with clear transverse (T) or clear longitudinal (L) anisotropy in the cell walls parallel to the long cell axis; patches of 20 cells were counted for each strip

Light treatment		D		R		R/FR	
Cultivar	Organ	T	L	T	L	T	L
Nihon Masari	Col	85	11	2	88	45	22
	Mes	15	56	3	91	16	72
Kasarath	Col	93	0	4	69	82	15
	Mes	99	1	9	77	69	17

berellins are already saturating the response in the etiolated mesocotyls. A pulse of R caused a severe reduction of mesocotyl elongation (Fig. 2a, vertical bars), which could be somewhat alleviated by GA_3 applied at concentrations higher than 10^{-7} M. When the R pulse was followed by FR, this threshold fell by one order of magnitude (Fig. 2a). As in *japonica*-type seedlings, the coleoptile exhibited only weak, if any, responses to applied GA_3. Analysis of endogenous gibberellins revealed a pattern similar to that of *japonica*-type seedlings for the mesocotyl (Fig. 2c, d). In coleoptiles, the effectiveness of endogenous GA_4 was increased by R, but it fell by a factor of about 40 in mesocotyls subjected to R. However, the effectiveness of both GA_1 and GA_4 again approached the dark level when R was followed by FR. Thus, in mesocotyls of *indica* type seedlings, an R/FR reversible decrease of gibberellin effectiveness could be demonstrated, confirming the results obtained with *japonica*-type seedlings. This is noteworthy, since both cultivars differ considerably with respect to their response to exogenous GA_3 (Nick and Furuya 1993).

Responses of microtubules to R. The orientation of cortical microtubules was recorded in cells of the outer epidermis for the light regimes described above. In etiolated seedlings of both cultivars, transverse microtubules were more frequent than longitudinal microtubules in both coleoptile and mesocotyl (Fig. 3b, c; Fig. 4). Generally, this tendency was more clearly expressed in *indica*-type than *japonica*-type seedlings. In *japonica*-type seedlings, many epidermal cells had oblique or even longitudinal microtubular arrays (Fig. 3b; Fig. 4). However, microtubule orientation in the subtending cortex cells was found to be transverse (Fig. 3b). When the seedlings were irradiated by R, the proportion of cells with transverse microtubule arrays fell conspicuously in both cultivars, and in coleoptiles as well as in mesocotyls (Fig. 4). Irradiation by FR, subsequent to the R pulse, restored the transverse orientation characteristic of etiolated seedlings (Fig. 4). Anisotropy of epidermal cell walls was assayed by analysis of birefringence in polarized light, as described by Ziegenspeck (1948). In etiolated seedlings most longitudinal cell walls appeared yellow in the additive position and blue in the subtractive position (Table 3). This can be interpreted in terms of the preferential transverse orientation of cellulose (Ziegenspeck 1948). In contrast, in R-treated plants, many longitudinal cell walls appeared blue in the additive position and yellow in the subtractive position, suggesting that cellulose fibers were preferentially oriented in longitudinal arrays (Table 3). When the pulse of R was followed by FR, the situation resembled that observed in etiolated plants (Table 3).

Discussion

Does R inhibit growth by blocking gibberellin synthesis? It has been claimed in the past that phytochrome exerts its effects upon stem elongation by blocking a late step of gibberellin synthesis, namely, the 3β-hydroxylation of GA_{20} to active GA_1 (Campell and Bonner 1986; Sponsel 1986, internode elongation in pea; Martìnez-Garcìa and Garcìa-Martìnez 1992, epicotyl elongation in cowpea, *Vigna sinensis* L.). Alternatively, for the same phenomenon, phytochrome was suggested to act, additionally, by affecting the response of the tissue to gibberellins (Behringer et al. 1989, internode elongation in pea; Martìnez-Garcìa and Garcìa-Martìnez 1992, epicotyl elongation in cowpea). In order to distinguish between the two possibilities, we assayed the light-dependent effectiveness of applied GA_3 in stimulating cell elongation, and correlated the endogenous gibberellin content per cell with the resulting elongation growth under various light regimes (Figs. 1, 2, Table 2). Two main results should be emphasized:

(1) The dose-response dependence of mesocotyl elongation upon application of GA_3 is shifted to higher concentrations after irradiation by a pulse of R (Figs. 1, 2), and this shift can be partially reversed by subsequent irradiation with FR. This is also true for the *indica*-type cultivar, if one takes into account that in etiolated seedlings mesocotyl elongation is already saturated by endogenous gibberellins, as shown in experiments involving inhibitors of gibberellin synthesis (Nick and Furuya 1993). The growth induced by saturating concentrations of GA_3 was dramatically reduced in R from 10.4 mm to 6.1 mm in the *japonica*-type cultivar, and, even more dramatically, from 89 mm to 3.5 mm in the *indica*-type culti-

var. This indicates a second R effect upon the growth capacity of the tissue.

(2) In the mesocotyl, the cellular content of the active endogenous gibberellin species GA_1 and GA_4 increases in response to a pulse of R, but remains at the dark level when the R pulse is followed by FR (Figs. 1, 2, Table 2). Thus, the cellular gibberellin content has increased after irradiation with R.

It can be concluded that phytochrome causes a decrease of sensitivity to gibberellin in the mesocotyl of both cultivars, but it increases the cellular content of endogenous gibberellins. This means that there is a promotion rather than an inhibition of gibberellin synthesis in the response to R.

Microtubules are involved in phytochrome-dependent signal transduction. Cortical microtubules can reorient in response to various stimuli such as blue light (Nick et al. 1990), gravity (Nick et al. 1991a), and auxin (Nick et al. 1992). Moreover, they can respond to R (Nick et al. 1991b; Nick and Furuya 1993) and to gibberellins (Ishida and Katsumi 1991; Nick and Furuya 1993). These reorientations are accompanied by corresponding growth responses. According to the so called microtubule-microfibril hypothesis, cortical microtubules serve as guiding tracks for the cellulose-synthetizing enzyme complexes residing in the plasma membrane, and cellulose microfibrils are therefore deposited parallel to cortical microtubules (Robinson and Quader 1982). Transverse arrays of microtubules in conjunction with transversely deposited cellulose microfibrils provide a reinforcement mechanism ensuring longitudinal cell extension and preventing lateral swelling of cylindrical cells (Green 1969). Elimination of cortical microtubules by colchicine abolishes the growth responses of cowpea epicotyls to gibberellin and R, suggesting that microtubules are essential for transduction of the light-growth response (Garcìa-Martìnez and Martìnez-Garcìa 1992). In rice, experiments with the antimicrotubular drug ethyl-N-phenylcarbamate and the inhibitor of gibberellin synthesis uniconazol (Nick and Furuya 1993) support the importance of microtubules for the manifestation of the gibberellin-dependent growth response rather than for early signal transduction. Here, we show that cortical microtubules respond to R in an FR-reversible manner, in good correlation with the light-dependent changes of gibberellin sensitivity (Fig. 3b, c; Fig. 4). As indicated by the observed changes of cell-wall birefringence (Table 3), these reorientations of cortical microtubules are accompanied by corresponding rearrangements in the cell wall. Since the older, outermost wall layers increasingly assume a random orientation due to distorting mechanical strains (Green and King 1966), cell-wall birefringence is expected to be determined mainly by the anisotropic, newly deposited layers of the cell wall (Ziegenspeck 1948).

Hormonal growth regulation is not a simple process. The data infer a signal-transduction chain that leads from active phytochrome, via decreased effectiveness of gibberellins and reorientation of cortical microtubules from transverse to longitudinal arrays, to reorientation of cellulose deposition and corresponding mechanical changes in the epidermal cell wall. It should be emphasized, however, that the reality of hormonal growth regulation is not as simple and straightforward as this model might indicate. Two observations call for a more complex view:

(i) Far-red-reversible reorientations of microtubules and microfibrils in response to R have been found in both the mesocotyl and the coleoptile (Fig. 4, Table 3). This was correlated with an FR-reversible inhibition of growth. In contrast, the elongation response to applied GA_3 was rather weak or even lacking in coleoptiles compared to mesocotyls (Figs. 1, 2; Table 2). In the case of *indica*-type seedlings, the observed microtubular and growth responses exhibited by the coleoptile were even contrary to the predictions one would make from the changes in gibberellin responsiveness (Fig. 2; Table 2). This means that, in coleoptiles, microtubule and microfibril responses to R are not as closely linked to changes of gibberellin effectiveness as found for the mesocotyl. Classical experiments (Furuya et al. 1969) favour the view that, in rice coleoptiles, growth inhibition by R is mediated by an inhibition of basipetal auxin transport. Thus, in the same plant, in different organs, R might act via different hormonal signal chains, and it might be that the confluence of both signal chains occurs as late as the reorientation of cortical microtubules. In addition, both signal chains can mutually interact at the level of hormone synthesis (Law and Hamilton 1985). However, the observed light effects upon coleoptile elongation cannot be explained in terms of changes in cellular hormone content. This strengthens the idea of a complex light regulation, based upon complex interactions of hormone-triggered signal chains with the cytoskeleton.

(ii) In situations of low gibberellin effectiveness, cellular gibberellin contents had a tendency to be high (Figs. 1, 2). This was true for the 13-OH gibberellin GA_1 as well as for the 13-H gibberellin GA_4. The inverse correlation of hormone content with hormone sensitivity is a typical example of a gibberellin paradox. Such paradoxa have been described for the gibberellin-insensitive maize mutant *D8* (Fujioka et al. 1988) or, inversely, for the gibberellin-oversensitive barley mutant *slender* (Croker et al. 1990). This indicates a negative-feedback loop of gibberellin effectiveness upon gibberellin synthesis. Since both gibberellin species exhibit this gibberellin paradox, the feedback control must act at a step preceding the separation of the two synthesis chains.

Conclusions. Phytochrome-dependent cell elongation in rice implies changes in gibberellin sensitivity triggering reorientation of cortical microtubules. The transduction chain mediating this response contains at least two steps, where complex interactions with other signal chains occur: (1) negative feedback of gibberellin biosynthesis depending on the effectiveness of gibberellins to induce growth (producing a gibberellin paradox) and, (2) a complex response of the cytoskeleton to various hormone-dependent signal chains. These complexities have to be considered if one wants to understand signal transduction at the molecular level. They have special impact upon the analysis of hormonal or photomorphogenetic mutants.

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