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Phytochrome dependent decrease of gibberellin-sensitivity

A case study of cell extension growth in the mesocotyl of japonica and indica type rice cultivars

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Abstract

Exogenous gibberellin removes the genetical suppression of mesocotyl elongation in dark-grown seedlings of the rice cultivar 'Nihon Masari' (*japonica* type). This gibberellin effect can be cancelled by light. All light effects can be accounted for by phytochrome. Dose-response and fluence-response studies show that phytochrome induces a reduction of the sensitivity to exogenous gibberellins. A cytological analysis of cell elongation and cortical microtubules led to a model where gibberellin and red light regulate mesocotyl elongation by controlling microtubule orientation in the epidermis of the mesocotyl. This causes corresponding changes of cellular extension growth, which can account for a large part of the observed growth responses. Comparative studies involving antimicrotubular drugs and gibberellin-synthesis inhibitors in the rice cultivar 'Kasarath' (*indica* type) and a hybrid cultivar suggest that some of the differences between the cultivars are due to differences in gibberellin-sensitivity.

Introduction

In grasses, elongation growth is regulated mainly by changing the extensibility of the epidermis [13]. Loosening of epidermal cell walls relieves the compression imposed upon the subtending cortex tissue and results in longitudinal shoot extension. Biomechanical considerations indicate that the elongate epidermal cells should grow in a transverse rather than in a longitudinal direction [7]. The force exerted by the cellular turgor pressure should act mainly on the lateral cell walls, because the area around the cell poles is comparatively small. Thus, elongation of epidermal cells is supposed to rely on a reinforcement mechanism, preventing the cell from thickening. Transverse arrays of microtubules, in conjunction with transversely deposited cellulose microfibrils provide such a reinforcement mechanism [2, 6]. The importance of microtubules for the maintenance of ordered elongation growth is emphasized by the observation that treatment of maize seedlings with microtubule-eliminating drugs, such as colchicine [2] or ethyl-N-phenylcarbamate [19], produces thick and stunted coleoptiles, and seriously affects tropistic growth responses. The importance of microtubules in the regulation of growth is supported by various studies on the behaviour of the cytoskeleton in maize. In this species, growth responses such as phototropism [18], gravitropism [19], growth stimulation by auxin [2, 18], red light [20] or gibberellins [11] are correlated with conspicuous reorientations of the microtubules in the epidermal cells.

At this point the question arises as to whether all these various stimuli, such as gravity, blue light, and red light, act via the same mediator upon microtubules and cell elongation. It appears that, at least for the red-light induced promotion of coleoptile growth in maize, microtubule reorien-

tation might be triggered by more than one mediator [20]. This complexity in the behaviour of the cytoskeleton is mirrored by a concomitant complexity of growth regulation by red light in graminean seedlings: whereas elongation growth is *stimulated* by red light in coleoptiles of maize [3], it is *inhibited* in the mesocotyl of the same species [9]. In contrast to maize, rice cultivars of the *japonica* type exhibit a highly sensitive *inhibition* of coleoptile elongation by red light, which is mediated by phytochrome [21]. For this plant, the red-light effects upon coleoptile growth were shown to be correlated with an inhibition of cell elongation, whereas cell division was not affected [5]. It should be mentioned that mesocotyl elongation is genetically reduced in rice cultivars of the *japonica* type [8, 15, 25]. It might be that the differences in coleoptile growth between maize and rice have to do with the altered balance between coleoptile and mesocotyl development.

Rice provides an excellent system to study such growth regulation by light:

(i) Mesocotyl elongation is genetically inhibited in cultivars of the *japonica* type, but not in cultivars of the *indica* type [8, 15, 24, 25]. A few key genes appear to be responsible for this difference [15].
(ii) Preliminary experiments demonstrated that mesocotyl elongation can be altered in a light-dependent manner using exogenous plant hormones, such as brassinolide or gibberellins.

The aim of the study presented here, was to analyze

(a) the role of phytochrome and red light in mesocotyl elongation, and
(b) the role of microtubules in the growth regulation of rice seedlings.

Material and methods

Seed material

Caryopses of three rice (*Oryza sativa* L.) cultivars were used for this study: The cultivar 'Nihon Masari' belongs to the *japonica* type, the cultivar 'Kasarath' to the *indica* type, and the cultivar 'IR30' represents a hybrid. All seeds were a kind gift from Dr. Osamu Yatou (Institute for Radiation Breeding, Hitachi-Ohmiya, Japan).

Growth conditions and application of chemicals

Rice seedlings were raised using a floating-mesh method (Yatou, personal communication) in plant tissue-culture vessels (9.5 cm × 9.5 cm × 13.5 cm, Flow Laboratories Inc.; McLean, Virginia, U.S.A.): 30 seeds for each experiment were placed equidistantly on a light plastic mesh kept floating on the medium by small polystyrene blocks. The capillary suction of the seed coat ensured soaking of the seeds, and the floating mesh ensured that there was sufficient access of oxygen. All hormones and drugs were mixed with the medium at the time of sowing. Gibberellin (GA_3) and the microtubule-eliminating herbicide ethyl-N-phenylcarbamate were purchased from Wako Chemical (Tokyo, Japan). They were diluted from a ethanolic stock solution. To account for possible effects of the solvent, the final ethanol concentration was kept at 1% in all samples. The gibberellin biosynthesis-inhibitor Uniconazole-P (Sumiseven, Sumitomo; Osaka, Japan) was applied as described earlier [12]. Seedlings were grown in the dark at 25 °C in a growth chamber (Koito; Tokyo, Japan). Germination was checked 3.5 days after sowing under dim green safety light (1.10^{-4} Wm^{-2}) and incompletely germinated seeds were removed in order to maintain a physiologically homogenous population. About 70% of the seeds were selected for the continuation of the experiment.

Light treatments and light conditions

If not stated otherwise, plants were subjected to pulse irradiation 3.5 days after sowing (immediately after the selection). Preliminary experiments showed that at this time point the effect of a light pulse on mesocotyl growth was maximal. Red light was obtained from day-light white fluorescent tubes (Toshiba FL20SW, Toshiba; Tokyo, Japan) in combination with a red acrylic filter (Shinkolite A102, λ_{max} 660 nm, Mitsubishi Rayon; Tokyo, Japan), far-red light from fluorescent tubes with an emission maximum at 730 nm (TL20SFR-74, Toshiba; Tokyo, Japan) behind a black plastic film (IRP-1, Koto-Denki; Urawa, Japan) and a red acrylic filter (Shinkolite A102, λ_{max} 720 nm, Mitsubishi Rayon; Tokyo, Japan), and green safety-light from fluorescence tubes (FL20SW, Shibaura Electric; Tokyo, Japan) wrapped with many layers

of green plastic film (Filmolux 087, Hans Neschen; Bückeburg, FRG). Monochromatic blue light was isolated from a stereo microscope light-source (Olympus; Tokyo, Japan) behind a heat filter and an interference filter (λ_{max} 450 nm, halfband-width 32 nm, T_{max} 78%, No. 8910–1113 13 P77, Olympus; Tokyo, Japan). Filter spectra were verified using a spectrophotometer (DU-50, Beckman; Tokyo, Japan). The energy flux was measured using a radiometer (YSI Model 65-A, Yellow Springs Instrument; Yellow Springs, Ohio, U.S.A.). Fluences were varied by changing irradiation time (1 s up to 1 h) and by changing fluence rate by means of neutral filters.

Immunofluorescence staining of cortical microtubules

Microtubules were visualized using a modification of a recently described method [14]: Seedlings were excised under dim green safety-light (1×10^{-4} Wm^{-2}) immediately above the seed coat and fixed for 45 min in fixation buffer (50 mM 1,4-piperazidine-diethanolsulfonic acid, 1 mM $MgSO_4$, 1% glycerol, pH 6.8) containing 2.5% v/v formaldehyde, and 10 mg/ml pepstatin A and leupeptin. After three washings in the same buffer without formaldehyde, very thin tangential sections of the mesocotyl were cut with a sharp surgical blade. The margins of the sections consisted of the epidermal layer; occasionally the adjacent layer of cortical cells could be seen.The sections were incubated for 1 h at 37 °C with a mouse monoclonal antibody directed against tubulin from calf brain (Transformation Research Inc.; Framingham, Massachusetts, U.S.A.) diluted 50-fold in phosphate-buffered saline (140 mM NaCl, 3 mM KCl, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4, 0.1% w/v NaN_3, 1 mg/ml bovine serum albumin, pH 7.3). Then the sections were washed three times with phosphate-buffered saline and reincubated for 1 h at 37 °C with a secondary antibody, labeled with fluorescein isothiocyanate (anti-mouse IgG, Sigma; Tokyo, Japan) diluted 20-fold in phosphate-buffered saline. After three further washings in phosphate-buffered saline, sections were kept overnight at 4 °C in phosphate-buffered saline supplemented with 0.1% w/v Triton X-100 and 10 mM dithioerythritol. They were mounted the next morning in an antifading solution [14] with the outer side of the epidermis facing upwards and kept in the cold for a further day to allow for penetration of the antifading agent. The sections were viewed under a fluorescence microscope (BHX, Olympus; Tokyo, Japan) and photographed on Kodak TriX Pan 400 ASA film (Kodak; Rochester, New York, U.S.A.).

Response evaluation

Plants were harvested 7 d after sowing and the mesocotyl length was measured by means of a ruler. Each experiment was performed twice at different days. Cell length in tangential sections obtained as described above was determined as described earlier [5] and frequency distributions constructed from the data of about 20 plants (chosen from two independent experiments). Microtubule orientation at the outer edge of epidermal and cortical cells was determined as deviation from the long cell-axis. Thus, transverse microtubules were scored by an angle of 90°, longitudinal microtubules by an angle of 0°. Since 'left-turns' and 'right-turns' occurred with equal frequency, they were not distinguished for the purpose of this study. Again, frequency distributions comprise the data from 20 plants (corresponding to two experiments).

Results

Gibberellin promotes, whereas red light inhibits mesocotyl elongation

If seedlings of the *japonica* type cultivar are grown without red light and without gibberellin, the mesocotyl is extremely short (Fig. 1, upper left). It is even more stunted if the seedlings have grown under red light (Fig. 1, lower left). With 10^{-4} M GA_3 mesocotyls were long, although the coleoptiles did not show additional elongation (Fig. 1, upper right). This mesocotyl elongation was prevented by irradiation with red light (Fig. 1, lower right). The dose-response curves (Fig. 2) show that promotion of mesocotyl growth by exogenous gibberellin can be detected by 10^{-7} M GA_3 in dark-grown seedlings (Fig. 2, upper row), but only at 10^{-5} M if seedlings were irradiated with continuous red light (Fig. 2, lower row). The maximal response was reached at only 3×10^{-5} M

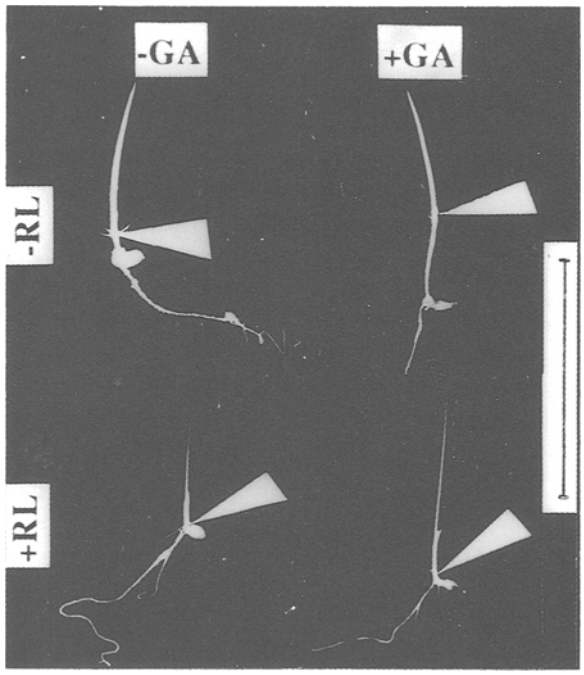

Fig. 1. Effect of red light and gibberellin on mesocotyl elongation in the cultivar 'Nihon Masari' (*japonica* type). Seedlings were grown for a week at 25 °C either in the dark (−RL) or under 0.5 W × m^{-2} continuous red light (+RL) with no (−GA) or with 10^{-4} M GA_3 (+GA). White arrows denote the position of the node, the vertical bars correspond to 5 cm.

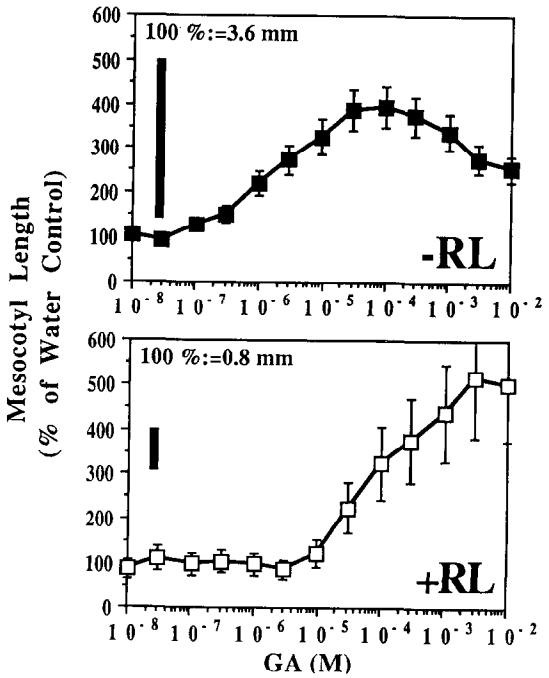

Fig. 2. Dose-response of mesocotyl elongation to applied gibberellin in the cultivar 'Nihon Masari' (*japonica* type). For details refer to Fig. 1. The thick black vertical bars represent the length of the mesocotyl; the ordinate gives the mesocotyl length relative to the water control. Error bars denote the standard *deviation* (if no error bar is shown, it is smaller than the plot symbol). Each data point represents the mean of 40 plants (comprising two independent experiments).

GA_3 in the dark, but at 3 × 10^{-3} M GA_3 in the light. Thus, red-light seems to shift the dose-response curve for gibberellin-promoted mesocotyl elongation by two orders of magnitude to higher concentrations. In other words: the light desensitizes the response to exogenous gibberellins by about 100 times. It should be noted that for very high concentrations of GA_3 the growth stimulation decreases again. This is seen only for dark-grown seedlings but not in the light. However, it would require extreme treatments to reach the conditions where such a decrease would be expected in light-grown plants. It should be noted further that the optimal effect amounts to about 4 to 5 times of the growth response observed on water independently of the light treatment. Thus, although mesocotyls are shorter in the light compared to the dark, the relative effect of exogenous gibberellin is roughly the same. This indicates a multiplicative, rather than additive interaction of red light and gibberellin with relation to mesocotyl growth.

The effect of light upon mesocotyl elongation is mediated by phytochrome

The fluence-response curve for the inhibition of gibberellin-promoted mesocotyl elongation by red light (Fig. 3, upper row) reveals a very sensitive light response, which is already saturated by 1 μmol × m^{-2} of red light. The red-light effect was reversible by irradiation with far-red light (Fig. 3, middle row). A far-red pulse without preceding red-light irradiation causes a slight reduction of mesocotyl growth compared to the dark. This suggests that the growth inhibition response is already evoked by a low phytochrome photoequilibrium. Not only red, but also blue light can cause a growth inhibition (Fig. 3, lower row). However, all effects of blue light can be cancelled by subsequent irradiation with far-red light.

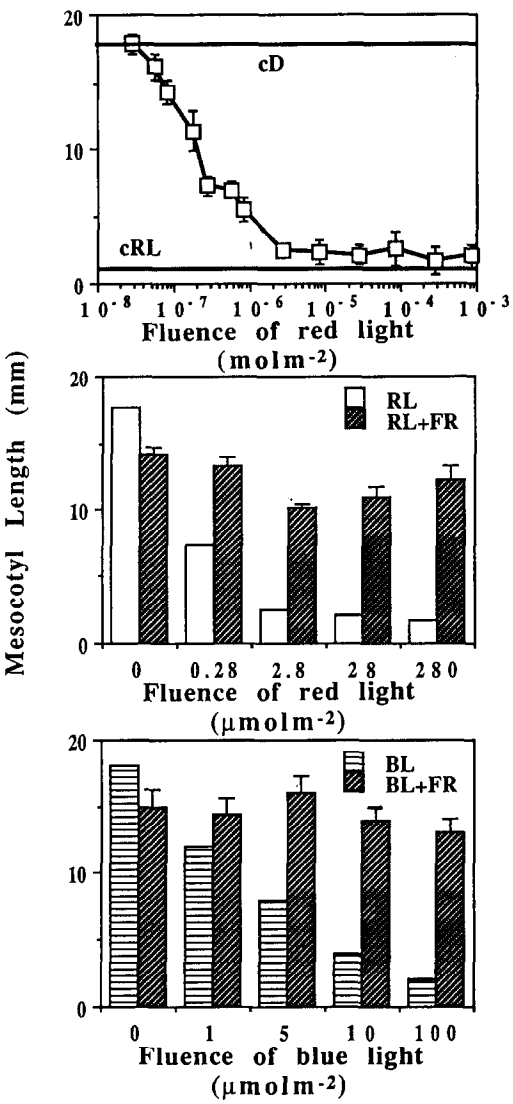

Fig. 3. Characterization of the light induced inhibition of gibberellin-promoted mesocotyl elongation in the cultivar 'Nihon Masari' (*japonica* type). The concentration of GA_3 was 10^{-4} M. Plants were irradiated at 3.5 d after sowing with pulses and returned to the dark until evaluation (7 d after sowing). Upper row: Fluence-response curve for growth inhibition induced by red light. Bold lines give the dark control (cD) and the control for continuous irradiation with red light (cRL). Middle row: Reversibility experiments with various pulses of red light followed by 10 min of far-red light (0.1 W × m^{-2}) (hatched bars). The white bars show the results for the respective controls without far-red irradiation. Lower row: reversibility experiments replacing the red-light treatment by irradiation with a pulse of blue light.

Red light and gibberellin act inversely upon cell elongation and microtubule orientation

The length of epidermal cells in the mesocotyl was measured for various combinations of light and hormone treatments. The microscopical images (Fig. 4) show clearly that epidermal cells are longer in gibberellin-treated plants and shorter in red-light irradiated plants. This impression is confirmed by frequency distributions of cell length (Fig. 5). Average cell length was reduced from 120 mm to 60 mm by red light, if no gibberellin was added, and from 280 mm to 150 mm in the presence of 10^{-4} M GA_3. Thus, red light causes a 50% reduction of cell-elongation, irrespective of the applied gibberellin treatment. This is consistent with a multiplicative, rather than additive mode of interaction between red light and gibberellin with respect to epidermal cell elongation. 10^{-4} M GA_3 causes a 2.5-fold increase in cell length, irrespective of the light treatment. The corresponding stimulation of mesocotyl elongation (Fig. 2) amounts to about three to four times that of the water controls. Thus, the promoting effect of gibberellin on mesocotyl elongation is correlated with a promotion of cell elongation in the epidermis. However, it is evident that in addition to cell elongation there must be an increase in cell number.

In the attempt to understand the effects of light and gibberellin upon cell elongation better, microtubules in cortical and epidermal cells were analyzed by immunofluorescence labelling (Fig. 6). It appeared that gibberellin treatment was associated with transverse microtubules, whereas red-light irradiation resulted in longitudinal microtubules in epidermal cells. In contrast, microtubules in cortical cells (which are more scarce than in epidermal cells) maintain a transverse array, irrespective of light or hormone treatment (Fig. 6). This difference is confirmed by the frequency distributions of microtubule orientation (Figs. 7, 8). In epidermal cells, irradiation by red light shifted the distributions towards a more longitudinal orientation, whereas gibberellins increased the proportion of transverse microtubules (Fig. 7). Microtubules in the cortex did not show any significant reorientation response (Fig. 8).

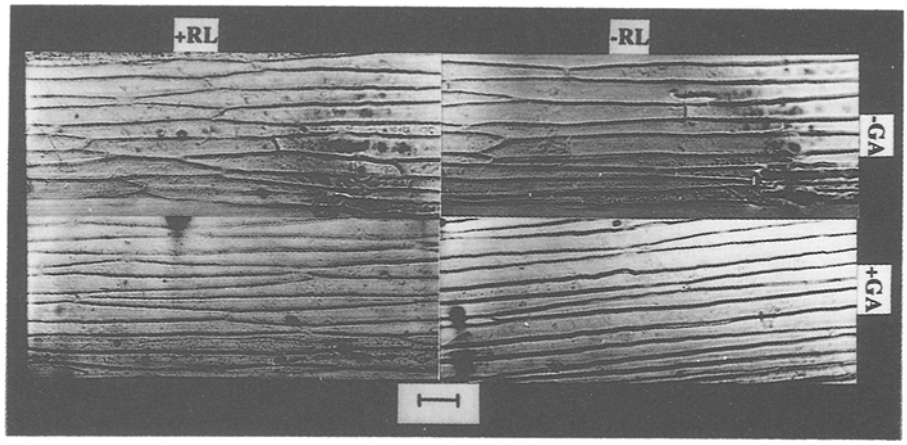

Fig. 4. Effect of red light and gibberellin on the length of epidermal cells in the mesocotyl of the cultivar 'Nihon Masari' (*japonica* type). Red-light and gibberellin treatment was as specified in Fig. 1. The bar corresponds to 10 mm.

Comparative analysis of japonica and indica rice cultivars

Seedlings of the *indica* type rice cultivar 'Kasarath' grown in the dark on water exhibit very long mesocotyls (Fig. 9, upper left). This is in sharp contrast to plants belonging to the *japonica* type (Fig. 1, upper left). However, again irradiation with red light yields short mesocotyls (Fig. 9, lower row). Exogenous gibberellin does not yield additional growth in dark-grown plants (Fig. 9, upper row), although there is a clear growth promotion of the coleoptile detectable in red-light irradiated plants (Fig. 9, lower row). Thus, under irradiation with red light, *indica* type plants have as short a mesocotyl as dark-grown *japonica* type plants. Further, after application of exogenous gibberellins dark-grown *japonica* type plants are similar to

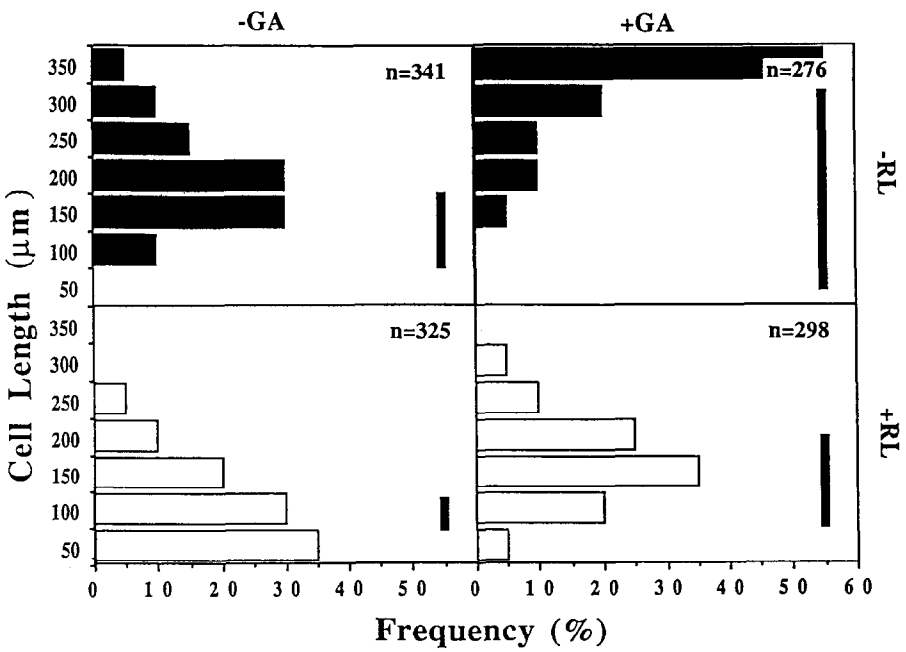

Fig. 5. Frequency distributions over cell length for different red-light and gibberellin treatments in the cultivar 'Nihon Masari' (*japonica* type). For details refer to Fig. 1. The vertical bars represent the average cell length (the mean of the distribution).

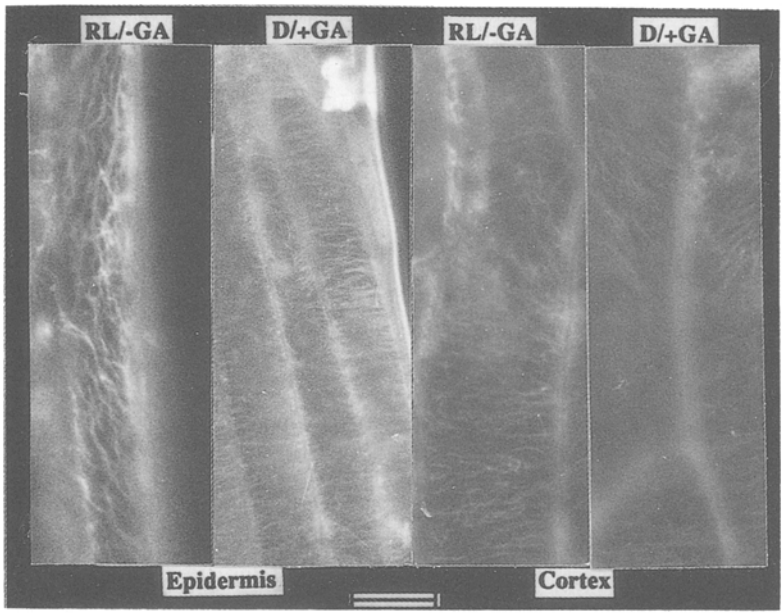

Fig. 6. Effect of red light and gibberellin on the orientation of microtubules in cortical and epidermal cells in the mesocotyl in the cultivar 'Nihon Masari' (*japonica* type). Red-light and gibberellin-treatment was as specified in Fig. 1. Immunofluorescence images of microtubules are shown. The bar corresponds to 10 mm.

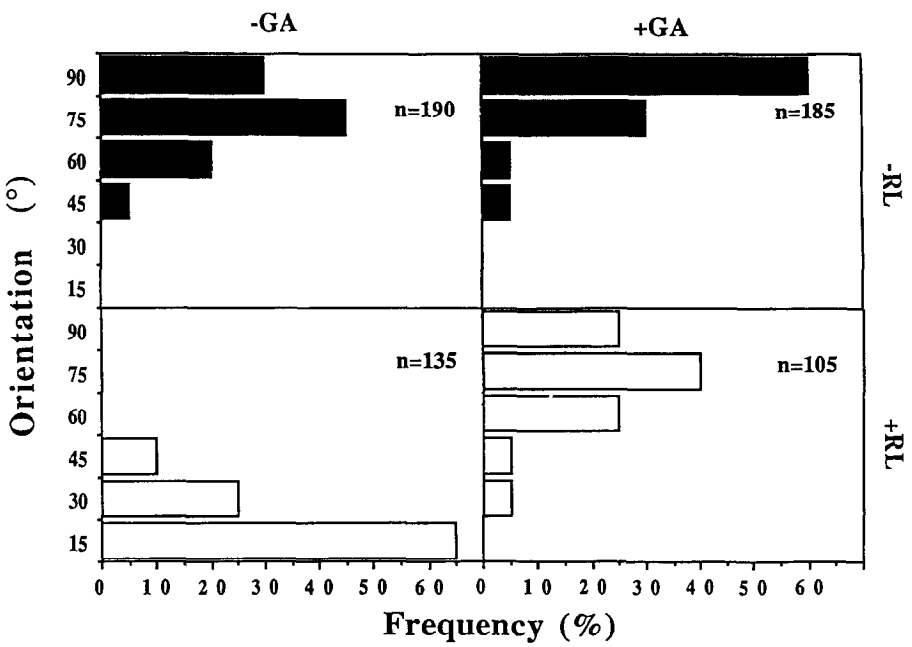

Fig. 7. Frequency distributions of microtubule orientation in epidermal cells for different red-light and gibberellin treatments in the cultivar 'Nihon Masari' (*japonica* type). For details of the treatments refer to Fig. 1. n gives the number of cells counted.

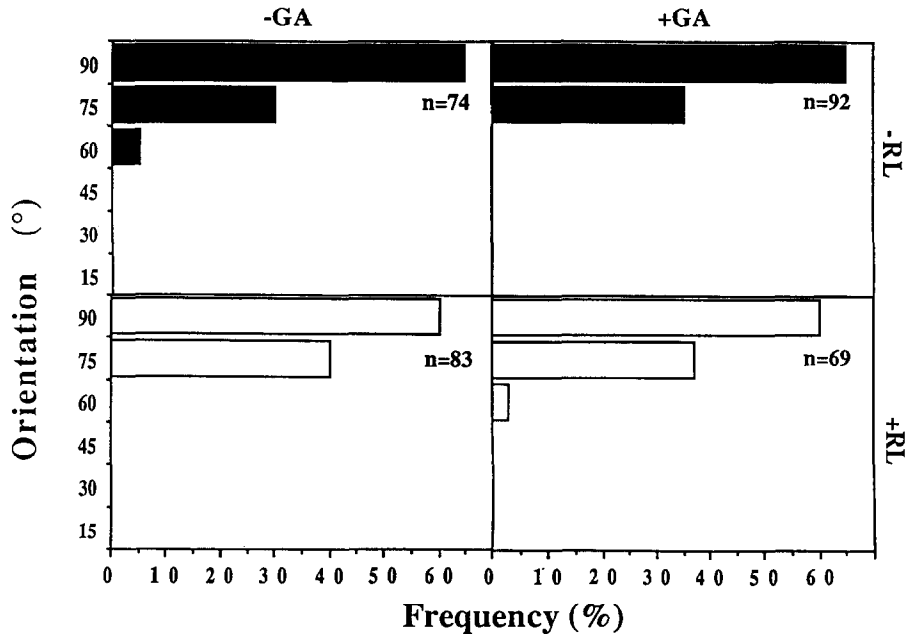

Fig. 8. Frequency distributions of microtubule orientation in cortical cells for different red-light and gibberellin treatments in the cultivar 'Nihon Masari' (*japonica* type). For details of the treatments refer to Fig. 1. n gives the number of cells counted.

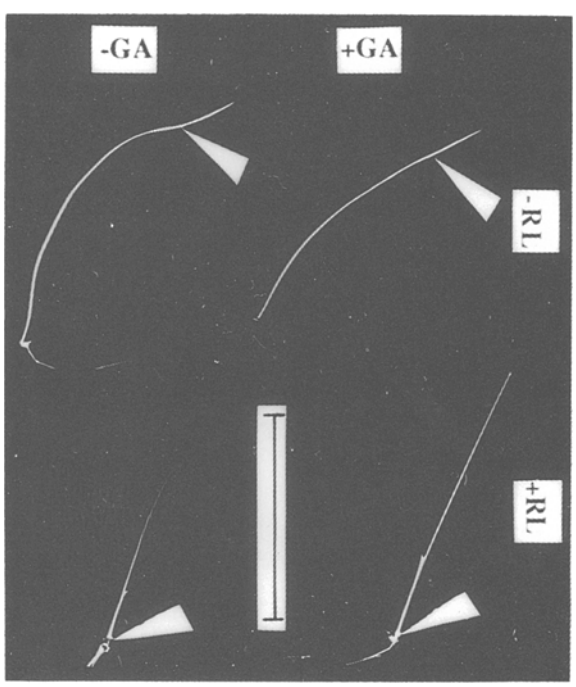

Fig. 9. Effect of red light and gibberellin on mesocotyl elongation in the cultivar 'Kasarath' (*indica* type). For details refer to Fig. 1.

dark-grown *indica* type plants. The dose-response curve of gibberellin-promoted mesocotyl elongation in red-light irradiated *indica* type plants is shifted by two orders of magnitude towards lower concentrations compared to the corresponding curve for *japonica* type plants (Fig. 10, lower row and Fig. 2, lower row). In the dark, however, the (extreme) growth of mesocotyls in *indica* type plants cannot be promoted further by exogenous gibberellin (Fig. 9, upper row and Fig. 10, upper row).

In order to understand this surprising observation two sets of experiments were undertaken: In the first type of experiment, an inhibitor of gibberellin synthesis (uniconazol-P) was applied to *indica* seedlings with the intention of lowering the level of endogenous gibberellins (Fig. 11, upper row). Under these conditions (uniconazol-P treatment), the dose-response curve for dark-grown *indica* type seedlings displayed a clear reduction of mesocotyl elongation at less than 3×10^{-7} M GA_3, with the threshold of action under those conditions at 10^{-8} M. In the other type of experiment, growth was inhibited by a microtubule-eliminating drug, ethyl-N-phenylcarbamate (Fig. 11, lower row). A treatment of dark-grown *indica* type seed-

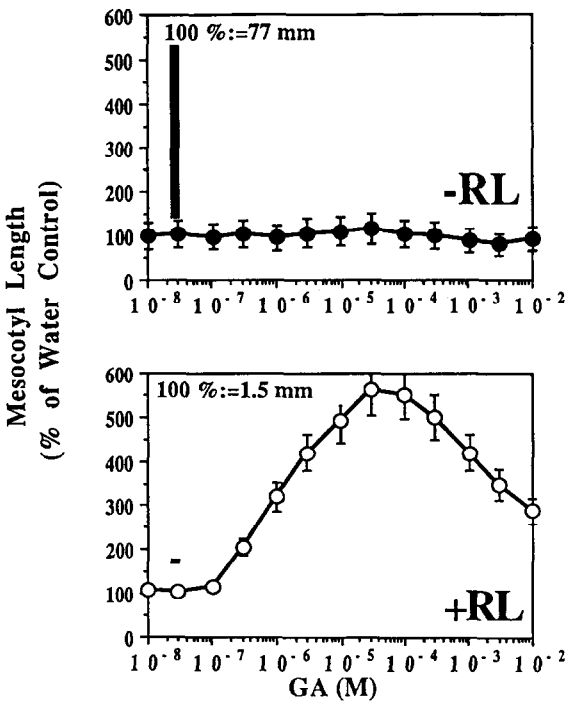

Fig. 10. Dose-response of mesocotyl elongation to applied gibberellin in the cultivar 'Kasarath' (*indica* type). For details refer to Figs. 1 and 2.

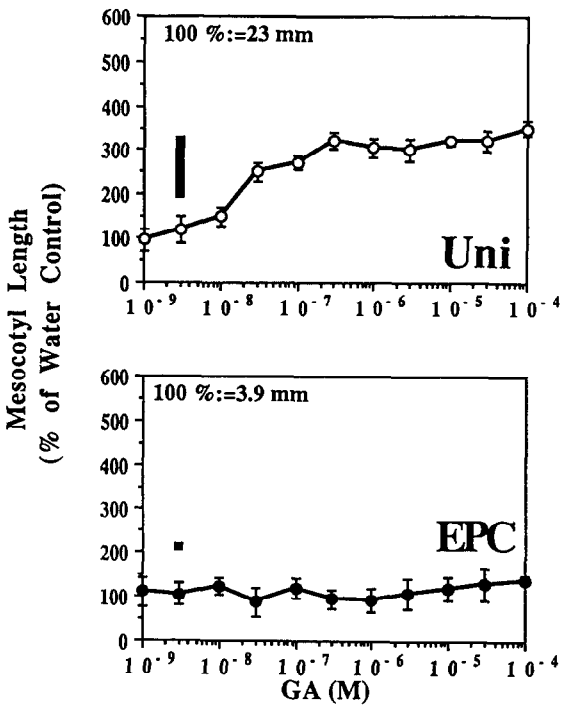

Fig. 11. Effect of growth inhibitors on the dose-response curve for gibberellin-promoted mesocotyl elongation in the cultivar 'Kasarath' (*indica* type). The conditions were identical to that of Fig. 10 except for the addition of 10 ppm uniconazol-P (Uni) (upper row) or 0.5 mM ethyl-N-phenylcarbamate (EPC)(lower row).

lings with 0.5 mM ethyl-N-phenylcarbamate produced plants whose mesocotyl was exactly as long as in dark-grown *japonica* type plants. However, although mesocotyl elongation was small under these conditions, addition of exogenous gibberellins could not induce additional mesocotyl elongation. In seedlings of the hybrid rice cultivar 'IR30' (Figs. 12, 13) the mesocotyl is much shorter than in the *indica* cultivar and promoted by gibberellin. This resembles the situation observed in the *japonica* cultivar. However, this promotion is much smaller than in the *japonica* cultivar, suggesting a combination of *japonica* and *indica* type traits. As in the other cultivars, irradiation with red light yields very short mesocotyls.

Discussion

Phytochrome decreases gibberellin-sensitivity

It has been shown that gibberellin promotes and red light inhibits mesocotyl elongation. The threshold for the gibberellin-dependent growth promotion is shifted after irradiation with red light by about two orders of magnitude to higher concentrations in all rice cultivars tested (Figs. 2, 10 and 11, 13). There is no evidence that the imbibition of dried rice seeds is different in the dark from that under red light. Thus, it is not very likely that the shift of the dose-response curves results from differential uptake of GA_3. If this holds true, one arrives at the conclusion that irradiation with red light reduces the sensitivity of mesocotyl elongation to exogenous gibberellin by about 100 times. If phytochrome and gibberellin share a common signal chain, one would predict that their interaction is multiplicative [16]. The maximum promotion of mesocotyl elongation is almost identical in relative terms, which is consistent with such a model. The photobiological analysis (Fig. 3) shows that all light effects, including those of blue light, are reversible by far-red irradiation, suggesting that phytochrome is the sole responsible photoreceptor and that the blue-light response is not involved.

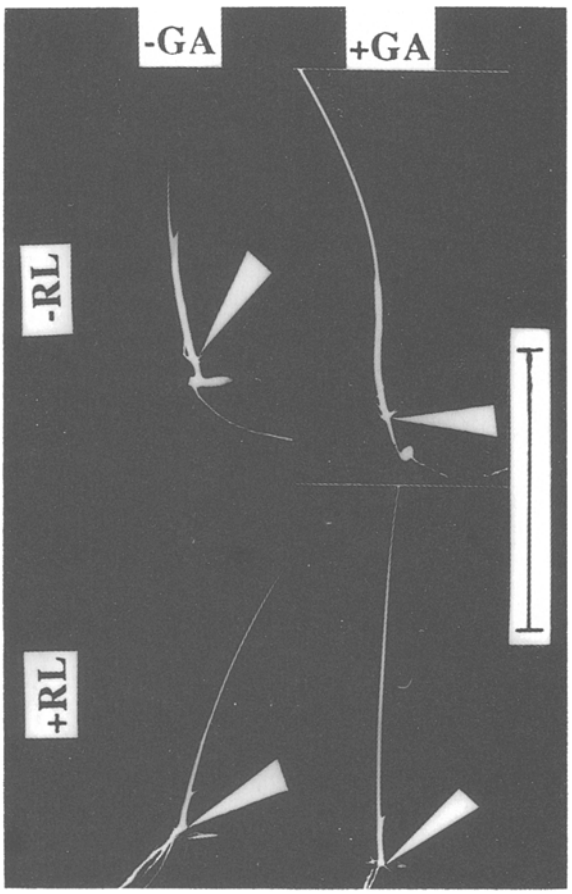

Fig. 12. Effect of red light and gibberellin on mesocotyl elongation in the hybrid cultivar 'IR30'. For details refer to Fig. 1.

Thus, these data do not provide evidence for any other photoreceptor involved in light-dependent inhibition of mesocotyl elongation. A very similar conclusion has been drawn from an analysis of light-dependent growth inhibition of rice *coleoptiles* [21].

A model for the signal chain regulating mesocotyl elongation

The analysis of cell elongation and microtubule orientation in the mesocotyl (Figs. 4–8) revealed two important correlations:

(A) Promotion of mesocotyl elongation by gibberellin appears to be correlated with stimulation of cell elongation and transverse microtubules in the epidermis.

(B) Inhibition of mesocotyl elongation by red light appears to be correlated with inhibition of cell elongation and longitudinal microtubules in the epidermis.

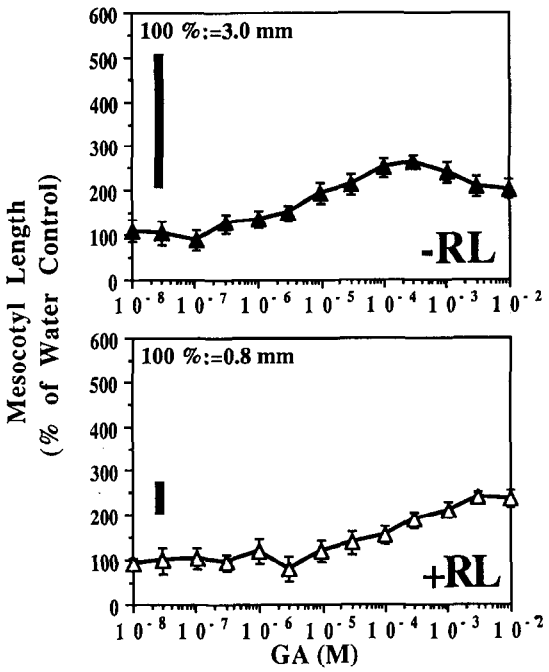

Fig. 13. Dose-response of mesocotyl elongation to applied gibberellin in the hybrid cultivar 'IR30'. For details refer to Fig. 1.

Thus, the simplest possible model for the chain of events involves the following steps:

(1) Phytochrome triggers a decrease of gibberellin-sensitivity (Fig. 2). In addition, it must inhibit growth by a further mechanism, since even saturating doses of gibberellin can not restore full elongation.

(2) The signal chain mediating gibberellin-dependent growth is down-regulated.

(3) Microtubules in the epidermis reorient from transverse to longitudinal (Figs. 6, 7).

(4) Cellulose microfibrils are deposited in parallel to the long cell axis [6] decreasing the extensibility of the cell wall in longitudinal direction.

(5) Epidermal cells become shorter and broader (Figs. 4, 5).

(6) Microtubules in the cortical tissue do not reorient (Figs. 6, 8), leading to a further extension of the inner tissue. This tissue extension is then forced by the epidermis into a transverse rather than into a longitudinal direction [2].

(7) Mesocotyl elongation is inhibited (Figs. 1, 2).

A connection between phytochrome and gibberellin in the regulation of stem elongation by light has been concluded from a number of previous studies [4, 10, 17, 22, 23]. For the system presented here, such a connection can be defined more precisely: phytochrome acts by reducing the sensitivity to gibberellins. A similar mechanism has been deduced from an analysis of the *lv* mutant in pea [17]. This does not exclude, however, that in addition light reduces the content of endogenous gibberellins as has been claimed in case of internode elongation in dwarf peas [4], but disputed by other authors [1]. Therefore, measurements of gibberellin content in dark- and red-light treated mesocotyls are warranted.

Growth regulation by gibberellin has also been correlated with microtubule reorientation in mesocotyls of dwarf mutants of maize [11]. In both species, such reorientations appear to be restricted to the epidermis (Figs. 4–8). An analogous causal chain between light, reorientation of epidermal microtubules and growth responses has been discovered for the tropistic responses of maize coleoptiles [2, 18, 20]. Thus, microtubules appear to play a general role in the growth regulation of graminean seedlings.

Are japonica type rice cultivars gibberellin-insensitive?

The differences in mesocotyl growth between the *japonica* (Figs. 1, 2) and *indica* (Figs. 9, 10) cultivars are striking. In both cultivars, it is possible to produce similar mesocotyl lengths by appropriate treatments with red light and gibberellin (Figs. 1, 9). The most puzzling observation, however, is the extremely long mesocotyl of dark-grown *indica* type seedlings, whose growth cannot be promoted by addition of exogenous gibberellin (Figs. 9, 10). There are two possibilities to explain this phenomenon:

(1) The dose-response curve for dark-grown *indica* type plants might be shifted towards lower concentrations as is observed for the respective red-light curve (Fig. 2, lower row and Fig. 10, lower row). In other words: even in water-grown plants the level of endogenous gibberellins could be saturating with respect to the signal chain mediating the gibberellin response. The curve would be shifted so far that Fig. 10 (upper row) shows only its saturated part. If the level of endogenous gibberellins is lowered by application of a gibberellin synthesis-inhibitor, uniconazol-P, the shift of the dose-response curve for dark-grown plants to lower concentrations becomes visible (Fig. 11, upper row). It is similar to the shift obtained for red-light irradiated plants – about two orders of magnitude. This suggests that in *indica* type plants the sensitivity to gibberellin is about 100 times higher than in *japonica* plants. It is so high that the endogenous level of gibberellin appears to saturate the transduction of the gibberellin response.

(2) It might be that the failure to induce additional mesocotyl elongation by exogenous gibberellin in dark-grown *indica* type plants is due to a saturation of growth as such, rather than to a saturation in the signal transduction triggered by gibberellin. In order to test this possibility, the growth response was reduced by 0.5 mM ethyl-N-phenylcarbamate to the level observed for dark-grown *japonica* type plants. This drug affects microtubules [19] and thus should act on the final steps rather than on the initial part of the transduction chain mediating the gibberellin signal. Under these conditions, exogenous gibberellin could not induce additional mesocotyl elongation (Fig. 11, lower row). This experiment suggests that, in fact, the peculiar behaviour of dark-grown *indica* type seedlings is due to the high gibberellin-sensitivity in this cultivar and not to a limitation of the growth response as such. Otherwise mesocotyls of *japonica* type seedlings, which are the same length as the EPC-treated mesocotyls in the *indica* plants, should not respond to exogenous gibberellin either. Again, this experiment emphasizes the important role of microtubules in late signal transduction.

It appears from the comparative analysis that the mesocotyl in *japonica* type cultivars is short, because the sensitivity to endogenous gibberellins is not sufficient to saturate the growth response. A similar conclusion has been drawn for ethylene-sensitivity of mesocotyl elongation in rice cultivars of the *japonica* and *indica* type [24]. Thus, *japonica* type cultivars might have arisen from *indica* cultivars by mutations reducing the sensitivity to gibberellins. A comparative analysis suggests that, in

fact, *japonica* type cultivars have evolved from *indica* type ancestors by a loss of the capacity for mesocotyl elongation [25]. Although the Mendelian analysis indicates the involvement of more than one gene, crossing experiments including eight pairs of *japonica* and *indica* cultivars [15] are compatible with the idea of a relatively simple genetic control by only a few genes. Hybrids were found to exhibit an intermediate behaviour [15], which confirms the data obtained with the cultivar 'IR30'. In order to get a deeper understanding of the observed differences in hormone sensitivity, measurements of gibberellin content in red-light treated versus dark-grown mesocotyls of both cultivars are essential.

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