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Seedling development in maize cv. B73 and blue light-mediated proteomic changes in the tip vs. stem of the coleoptile

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Abstract

In 2009, the draft genome of the reference inbred line of maize (*Zea mays* L. spp. *mays* cv. B73) was published so that, using this specific corn variety, molecular analyses of physiological processes became possible. However, the morphology and developmental patterns of B73 maize, compared with that of the more frequently used hybrid varieties, have not yet been analyzed. Here, we describe organ development in seedlings of B73 maize and in those of 6 other hybrid cultivars, and document significant morphological as well as quantitative differences between these varieties of *Z. mays*. In a second set of experiments we used etiolated seedlings of B73 maize to analyze the effect of blue light (BL) on the patterns of proteins in the tip vs. growing region of this sheath-like organ. By using two-dimensional difference gel electrophoresis (2-D DIGE), coupled with tandem mass spectrometry, we detected, in the microsomal fraction of maize coleoptile tips, rapid changes in the abundance of protein spots of maize phototropin 1 and several metabolic enzymes. In the sub-apical (growing) region of the coleoptile, proteomic changes were less pronounced. These results suggest that the tip of the coleoptile of B73 maize may serve as a unique model system for dissecting BL-responses in a light-sensitive plant organ of known function.

Keywords

Blue light; Coleoptile; Seedling development; Maize; Proteomics

Introduction

According to the U.S.-USDA National Agricultural Statistics Service (Crop Production March-2013), the United States is the largest producer of maize (corn, *Zea mays* L.), with a harvested area of about 84 million acres. However, maize, a large domesticated grass of tropical Mexican origin, is not only used to produce grain and fodder, but also as a model system for the analysis of organ development. Although, after the discovery of the phytohormone auxin in 1928, seedlings of oats (*Avena sativa*) became the favourite monocot in developmental plant biology, etiolated *Z. mays*-seedlings have also been used, over the

past five decades, in numerous pertinent studies (Went and Thimann 1937, Briggs 1963, 2014, Kutschera and Wang 2016).

In a pioneering study, Briggs et al. (1957) have shown that, in etiolated maize coleoptiles, lateral blue light (BL)-illumination causes an a-symmetrical auxin-redistribution in the tip of the organ, so that the sub-apical (growing) region bends towards the light source. Recent studies revealed that not only the tip, but also the lower region of the maize coleoptile may respond to some extent to BL (Matsuda et al. 2011, Suzuki et al. 2014).

In these investigations, different hybrid varieties (i.e., maize cultivars) were used for experimentation. However, the genome of only one maize inbred line, *Zea mays* L. spp. *mays* cv. B73, is available since 2009, so that, analogous to the situation in *Arabidopsis* research, molecular analyses of physiological processes can be carried out (Feuillet and Eversole 2009). The B73-variety may differ with respect to its developmental patterns from the hybrid cultivars used by numerous previous investigators.

Hence, the aims of this study were first, to compare the development of B73 maize under controlled laboratory conditions with that of 6 hybrid varieties, one of which was used previously in our laboratory for photosynthesis- and auxin research (Kutschera et al. 2010, Kutschera and Wang 2016), and, second, to re-investigate whether or not the tip of the etiolated maize coleoptile is the most BL-sensitive part of the axial organ (Went and Thimann 1937, Hager and Brich 1993, Briggs 2014). To address this question, we used the proteomics-technology employed in our related studies with seedlings of rye (*Secale cereale*) and *Arabidopsis thaliana* (Kutschera et al. 2010, Deng et al. 2012, 2014).

Material and methods

Plant material and growth measurements

Seven varieties of maize (*Zea mays* L.) were used in this investigation:

1. *Zea mays* L. spp. *mays* cv. B73, obtained from Mr. Mark J. Millard, USDA (ARS), North Central Regional Plant Introduction Station, Ames, Iowa (USA).
2. Hybrid maize Liberal (H-L), purchased from Schmitz & Laux, Hilden, Germany,
3. Hybrid maize Frenetic (FR), obtained from Euralis (Carignan, Canada). Varieties 4., 5., 6. and 7., i.e., Hybrid maize Kalvin-Mes. (K-M), MK Famous (MK), NK-Gitago (N-G), and NK-Jasmic (N-J) were obtained from Syngenta (Half Moon Bay, California, USA).

About 30 kernels each were soaked overnight and sown in vermiculite moistened with distilled water (pH ~ 7.0). Growth took place in closed, transparent plastic boxes (relative humidity of the air ca. 100 %; darkness, 25 ± 0.5 °C). Five days after sowing, the etiolated seedlings were harvested under green safelight, and representative individuals were photographed. The lengths of the coleoptile, and closed primary leaf, mesocotyl and primary root, respectively, were measured with a ruler (accuracy ± 0.5 mm). The phototropic bending response of pre-selected (straight) coleoptiles was analyzed as described by Iino and Briggs (1984).

Blue light-irradiation and sample preparation

For photobiological experiments, 5-day-old dark-grown B73-seedlings, raised as described above, were used. Batches of etiolated seedlings (ca. 20 per tray) were irradiated for 20 minutes from one side with continuous BL ($20 \mu\text{mol m}^{-2}$), or left in darkness as control. The tip (upper 2 mm) and a sub-apical 5 mm-segment (cut ca. 8 – 13 mm below the tip) were excised and the enclosed primary leaf removed (ca. 30 preparation per experiment). Sample preparations were performed in green safe light, and the excised segments were immediately frozen in liquid nitrogen.

Protein extraction, electrophoresis, and spot identification

Microsomal proteins were extracted, quantified, and analyzed by two-dimensional difference gel electrophoresis (2-D DIGE) as described in previous reports, using Cy3- or Cy5-dyes for BL-treated/dark controls, respectively (Kutschera et al. 2010, Deng et al. 2012, 2014). The resulting DIGE-images were analyzed using DeCyder 6.5 software (GE Health care). Spot detection was performed with a differential in-gel analysis module (estimated spot number ca. 5000), and a variation analysis module served to detect protein spots that were differentially regulated by BL. Protein identifications of excised spots from 2-D DIGE-gels were performed as described by Deng et al. (2014).

Results

In a first set of experiments, we studied the morphology of a population of 5-day-old dark-grown seedlings of *Zea mays* L. spp. *mays* cv. B73 ($n = 100$ individuals; three replicates). In 95 % of the etiolated B73-seedlings, the mesocotyl was curled (i.e., not straight), as shown in Figure 1. Although, in the majority of etiolated seedlings, the coleoptile was straight, in ca. 20 % of the samples investigated, this sheath-like organ displayed some curvature. The coleoptile (length ca. 20 mm) encloses the primary leaf; in B73-seedlings, this enfolded organ is relatively short, so that the upper 50 % of this protective sheath of the developing shoot is empty.

The morphology of B73-seedlings differs from that of the 6 hybrid varieties investigated here for comparison (Fig. 2). The Hybrid maize Liberal (H-L), used before in our laboratory, is characterized by a straight mesocotyl (in ca. 99 % of the individuals). A quantitative comparison revealed that the mesocotyl in 5-day-old H-L-individuals is about twice as long as that in the B73-variety (Fig. 2b), although the lengths of the coleoptiles were not significantly different (ca. 19 – 22 mm) (Fig. 2a). Similar results were obtained for the other 5 hybrids of *Zea mays* investigated. However, in contrast to B73-maize, in all other varieties investigated the primary leaf, enclosed by the coleoptile, is elongated and reaches the tip of the sheath-like organ. A comparison of the lengths of the primary roots revealed significant differences between the 7 maize cultivars studied here (Fig. 2c).

Figure 3a shows the upper part of a representative B73-seedling, with the excised tip of the coleoptile and a 5 mm-segment cut from the growing region of the organ (with enclosed leaf-segment). A longitudinal section of the same coleoptile reveals the relatively short

primary leaf, so that, as mentioned above, the upper half of the organ is an empty, tapering sheath (Fig. 3b).

Numerous studies have shown that the growing etiolated maize coleoptile is a highly light-sensitive plant organ (Briggs 2014), and that, upon unilateral exposure of etiolated seedlings to BL, followed by a dark period, phototropic bending occurs (Fig. 4a) (Iino and Briggs 1984). Using our proteomics-technology, we analyzed possible BL-mediated changes in this organ, compared to dark controls. In order to distinguish between the light-sensitive tip and the growing region of the organ, we performed a series of experiments as detailed in Figure 4b. Etiolated seedlings were irradiated with BL for 20 min and thereafter separated into the tip (empty) and a sub-apical region (with enclosed primary leaf segment). After the removal of the leaf-fragment, the coleoptile samples were frozen in liquid nitrogen and investigated, using our proteomics technology. A comparative 2-D DIGE-analysis of microsomal proteins extracted from these BL-samples revealed, compared to the corresponding dark-controls, striking differences between the tip and the basal region of the organ. One representative experiment is shown in Fig. 5a, b. Protein spots responsive to BL-treatment were marked by arrows, and those labeled with numbers were characterized by tandem mass-spectrometry. Our results show that the tip is more light-sensitive than the basal region, although some consistent changes induced by BL were also detected in the elongating part of the coleoptile.

Quantitative data concerning protein identification and abundance ratios are summarized in Table 1, based on 4 independent experiments. The results indicate that spots 28 and 29 (proteins homologous to phototropin 1 in *Arabidopsis* hypocotyls) are down-regulated, whereas the enzyme Lipoxygenase is up-regulated. Spots 28 and 29 are in the basic region of the gel, corresponding to the hypo-phosphorylated form of phototropin 1, and decreased in their abundances. This finding is in agreement with the fact that phot1 is phosphorylated upon BL-treatment. The increase of spot abundance of hyper-phosphorylated phot1 was not observed in the gel. We suggest that these protein spots were masked by more abundant spots in acidic regions. We also observed the up- and down-regulation of the enzymes Lipoxygenase and Sucrose Synthases 1 plus 2, respectively, indicating that lipid- and sucrose metabolism are rapidly affected upon exposure of the tip to BL (Table 1).

Discussion

Maize is a gigantic, photosynthetically efficient, domesticated grass species with a tremendous genetic diversity (Feuillet and Eversole 2009). The tall, annual monocot with large, narrow, opposing leaves is used by humans to produce grain and fodder. These primary products are the basis for numerous food, feed, industrial as well as pharmaceutical manufacture. In addition to its use as a major crop plant, maize has been employed as a “green model organism” for basic plant research (Baluska et al. 1996, 2010; Edelmann et al. 1995, Edelmann and Roth 2006, Markelz et al. 2003, Dubois et al. 2010, Borucka and Fellner 2012). In previous studies, we used hybrid maize Liberal (H-L) for the analysis of C4-photosynthesis in the developing primary leaf and auxin action in the coleoptile, respectively (Kutschera et al. 2010, Kutschera and Wang 2016). Unfortunately, only the draft genome of *Zea mays* L. spp. *mays* cv. B73, but not that of any other variety, is available.

To the best of our knowledge, no comparative analysis of organ development in H-L and related hybrid varieties, inclusive of the B73-cultivar, has yet been published. Our results document that, in all 7 maize varieties studied here, grown for 5 days under identical environmental conditions (darkness), the coleoptile is ca. 20 mm long (Fig. 2). However, in B73, the mesocotyl as well as the primary root is much shorter than in the other varieties investigated. The most striking differences are specific aspects of organ morphology: in B73-seedlings, but not in the other 6 varieties, the primary leaf is relatively short and does not fill out the entire space of the coleoptile. Moreover, in 95 % of the etiolated B73 seedlings, but not in the 6 other cultivars, the mesocotyl is curled (Figs. 1, 3). The reason for this unique behavior of the developing mesocotyl is not known, but we suspect that imbalances in hormone levels (auxin, brassinosteroids) may in part be responsible for this phenomenon (Kutschera and Briggs 2013, Kutschera and Wang 2012, 2016, Zhu et al. 2013, Wang et al. 2014).

Due to the short primary leaf in B73-seedlings, it was possible to perform the experiment shown in Fig. 4b, i.e. to study light-sensitivity of the tip (empty) vs. the lower region of the etiolated organ (after the surgical removal of the enclosed primary leaf fragment). In 1937, Went and Thimann proposed that only the upper part of the coleoptile, but not the sub-apical region, represents the light-sensitive area of the organ. However, due to technical limitations, this classical hypothesis has not been corroborated by these pioneers of auxin research.

About three decades later, Briggs (1963) summarized evidence documenting that the top 3 mm of etiolated coleoptiles (oats, maize) is the most light-sensitive region of the grass shoot (see also Hager and Brich 1993); in a recent update on this issue, a similar conclusion was reached (Briggs 2014). However, in more detailed analyses, using biochemical techniques, this view of the “tip as the only BL-responding region” of the grass coleoptile has been challenged. In two independent studies, it has been documented that BL exerts a strong biochemical effect in the tip of the maize coleoptile, whereas the lower part displays a minor response (Nishimura et al. 2011, Suzuki et al. 2014). Our data are in agreement with these findings, and add a more detailed picture as to those proteins that are up- or down-regulated in response to BL (Fig. 5 a, b; Table 1). The fact that the auxin-producing tip of the coleoptile is the most light-sensitive part of the organ is in agreement with our current view of IAA-mediated regulation of cell elongation in grass seedlings (Kriechbaumer et al. 2006, Nishimura et al. 2011, Ljung 2013) and other model organisms (Kutschera and Briggs 2016).

However, the finding that the sub-apical (elongating) region of the maize coleoptile likewise displays sensitivity towards light documents that the classical “segment-test” for the study of IAA-action (anisotropic wall expansion, Baskin 2005) should be performed in darkness or green safe light. It has long been known that, under different experimental conditions, the IAA-mediated growth responses are not identical (see, for instance, Edelmann et al. 1995, Kutschera 2003, 2006, Kutschera and Wang 2016, Schopfer 2006, Niklas and Kutschera 2012, Burdach et al. 2014). We suggest that, due to the high BL-sensitivity of the growing regions of the coleoptile, at least part of this variability in the IAA-response is attributable to light effects. However, the functional significance of light perception in the elongating part

of the etiolated maize coleoptile, which may be due to the presence of a set of proteins differentially responding along a vertical gradient is unknown.

In the tip of the coleoptile, two key enzymes of plant metabolism were found to be up- and down-regulated by BL, respectively (Table 1). Lipoxygenases, a nonheme iron-containing group of dioxygenases involved in vegetative growth, defense responses and anti-microbial actions are enhanced in their abundance. In contrast, sucrose synthases 1 and 2, enzymes involved in the breakdown of the disaccharide sucrose, are down-regulated upon exposure to BL (Porta and Rocha-Sosa 2002, Kutschera and Niklas 2013, Schmolzer et al. 2016). These data document that lipid- and sucrose metabolism are both rapidly affected by BL. We suggest that these light-induced changes at the proteomic level are involved in the regulation of photomorphogenic coleoptile development, which is initiated as a result of the phototropic bending response of the organ (Briggs 2014).

In summary, our results show that the tip of the maize coleoptile cv. B73 may serve as a model system for a detailed analysis of BL action in an organ of known function (Went and Thimann 1937, Hager and Brich 1993, Briggs 1963, 2014). Plant photoreceptors, such as phototropins and phytochromes, interact in light-sensitive cells. Hence, the maize coleoptile may be used to study irradiation-dependent developmental patterns during the transition from skoto- to photomorphogenesis. In adult plants of *Arabidopsis thaliana*, key processes, such as biomass production or cell metabolism are regulated by light (Yang et al. 2016). However, in this dicot model system, these photoreceptor-effects have not yet been elucidated at the molecular level. We conclude that etiolated seedlings of B73 maize may be a useful system to further analyze these complex processes.

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References

- Baluska F, Hauskrecht M, Barlow PM, Sievers A. Gravitropism in the primary root of maize: a complex pattern of differential cellular growth in the cortex independent of the microtubular cytoskeleton. *Planta*. 1996; 198:310–318. [PubMed: 11540727]
- Baluska F, Mancuso S, Volkmann D, Barlow PM. Root apex transition zone: a signalling-response nexus in the root. *Trends Plant Sci*. 2010; 15:402–408. [PubMed: 20621671]
- Baskin TI. Anisotropic expansion of the plant cell wall. *Annu Rev Cell Dev Biol*. 2005; 21:203–222. [PubMed: 16212493]
- Borucka J, Fellner M. Auxin binding proteins ABP1 and ABP4 are involved in the light- and auxin-induced down-regulation of phytochrome gene *PHYB* in maize (*Zea mays* L.) mesocotyl. *Plant Growth Regul*. 2012; 68:503–509.
- Briggs WR. Mediation of phototropic responses of corn coleoptiles by lateral transport of auxin. *Plant Physiol*. 1963; 38:237–247. [PubMed: 16655779]
- Briggs WR, Tocher RD, Wilson JF. Phototropic auxin redistribution in corn coleoptiles. *Science*. 1957; 126:210–212. [PubMed: 13454802]
- Briggs WR. Phototropism: Some history, some puzzles, and a look ahead. *Plant Physiol*. 2014; 164:13–23. [PubMed: 24399823]

- Burdach Z, Kurtyka R, Siemieniuk A, Karcz W. Role of chloride ions in the promotion of auxin-induced growth of maize coleoptiles. *Ann Bot.* 2014; 114:1023–1034. [PubMed: 25129632]
- Deng Z, Xu S, Chalkley RJ, Osés-Prieto JA, Burlingame AL, Wang ZY, Kutschera U. Rapid auxin-mediated changes in the proteome of the epidermal cells in rye coleoptiles: Implications for the initiation of growth. *Plant Biol.* 2012; 14:420–427. [PubMed: 22117532]
- Deng Z, Osés-Prieto JA, Kutschera U, Tseng TS, Hao L, Burlingame AL, Wang ZY, Briggs WR. Blue light-induced proteomic changes in etiolated *Arabidopsis* seedlings. *J Proteome Res.* 2014; 13:2524–2523. [PubMed: 24712693]
- Dubois PG, Olsefski GT, Flint-Garcia S, Setter TL, Hoekenga OA, Brutnell TP. Physiological and genetic characterization of end-of-day far-red light response in maize seedlings. *Plant Physiol.* 2010; 154:173–186. [PubMed: 20668057]
- Edelmann HG, Bergfeld R, Schopfer P. Effect of inhibition of protein glycosylation on auxin-induced growth and the occurrence of osmiophilic particles in maize (*Zea mays* L.) coleoptiles. *J Exp Bot.* 1995; 46:1745–1752.
- Edelmann HG, Roth U. Gravitropic plant growth regulation and ethylene: an unsought cardinal coordinate for a disused model. *Protoplasma.* 2006; 229:183–191. [PubMed: 17180500]
- Feuillet C, Eversole K. Solving the maze. *Science.* 2009; 326:1071–1072. [PubMed: 19965417]
- Hager A, Brich M. Blue-light-induced phosphorylation of a plasma-membrane protein from phototropically sensitive tips of maize coleoptiles. *Planta.* 1993; 189:567–575.
- Iino M, Briggs WR. Growth distribution during the first positive phototropic curvature of maize coleoptiles. *Plant Cell Environm.* 1984; 7:97–104.
- Kriechbaumer V, Park WJ, Gierl A, Glawischnig E. Auxin biosynthesis in maize. *Plant Biol.* 2006; 8:334–339. [PubMed: 16807825]
- Kutschera U. Auxin-induced cell elongation in grass coleoptiles: a phytohormone in action. *Curr Topics Plant Biol.* 2003; 4:27–46.
- Kutschera U. Acid growth and plant development. *Science.* 2006; 311:952–953.
- Kutschera U, Briggs WR. Seedling development in buckwheat and the discovery of the photomorphogenic shade-avoidance response. *Plant Biol.* 2013; 15:931–940. [PubMed: 24112603]
- Kutschera U, Briggs WR. Phototropic solar tracking in sunflower plants: an integrative perspective. *Ann Bot.* 2016; 117:1–8. [PubMed: 26420201]
- Kutschera U, Deng Z, Osés-Prieto JA, Burlingame AL, Wang ZY. Cessation of coleoptile elongation and loss of auxin sensitivity in developing rye seedlings. A quantitative proteomic analysis. *Plant Signal Behav.* 2010; 5:509–517. [PubMed: 20234181]
- Kutschera U, Niklas KJ. Cell division and turgor-driven stem elongation in juvenile plants: a synthesis. *Plant Sci.* 2013; 207:45–56. [PubMed: 23602098]
- Kutschera U, Pieruschka R, Berry JA. Leaf development, gas exchange characteristics and photorespiratory activity in maize seedlings. *Photosynthetica.* 2010; 48:617–622.
- Kutschera U, Wang ZY. Brassinosteroid action in flowering plants: a Darwinian perspective. *J Exp Bot.* 2012; 63:3511–3522. [PubMed: 22547659]
- Kutschera U, Wang ZY. Growth-Limiting Proteins in maize coleoptiles and the auxin-brassinosteroid hypothesis of mesocotyl elongation. *Protoplasma.* 2016; 253:3–14. [PubMed: 25772679]
- Ljung K. Auxin metabolism and homeostasis during plant development. *Development.* 2013; 140:943–950. [PubMed: 23404103]
- Markelz NH, Costich DE, Brutnell TP. Photomorphogenic responses in maize seedling development. *Plant Physiol.* 2003; 133:1574–1591.
- Matsuda S, Kajizuka T, Kadota A, Nishimura T, Koshiba T. NPH3- and PGP-like genes are exclusively expressed in the apical tip region essential for blue-light perception and lateral auxin transport in maize coleoptiles. *J Exp Bot.* 2011; 62:3459–3466. [PubMed: 21459767]
- Niklas KJ, Kutschera U. Plant development, auxin, and the subsystem incompleteness theorem. *Front Plant Sci.* 2012; 3/37:1–11.
- Nishimura T, Toyooka K, Sato M, Matsumoto S, Lucas MM, Stonad M, Baluska F, Koshiba T. Immunohistochemical observation of indole-3-acetic acid at the IAA synthetic maize coleoptile tip. *Plant Signal Behav.* 2011; 6:2013–2022. [PubMed: 22112455]

- Porta H, Rocha-Sosa M. Plant lipoxygenases: physiological and molecular features. *Plant Physiol.* 2002; 130:15–21. [PubMed: 12226483]
- Schmolzer K, Gutmann A, Diricks M, Desmet T, Nidetzky B. Sucrose synthase: a unique glycosyltransferase for biocatalytic glycosylation process development. *Biotech Advances.* 2016; 34:88–111.
- Schopfer P. Biomechanics of plant growth. *Amer J Bot.* 2006; 93:1415–1425. [PubMed: 21642088]
- Suzuki H, Okamoto A, Kojima A, Nishimura T, Takano M, Kagawa T, Kadota A, Kanegae T, Koshiba T. Blue-light regulation of *ZmPOT1* and *ZmPHOT2* gene expression and the possible involvement of Zmphot1 in phototropism in maize coleoptiles. *Planta.* 2014; 240:251–261. [PubMed: 24817587]
- Wang W, Bai MY, Wang ZY. The brassinosteroid signaling network - a paradigm of signal integration. *Curr Opin Plant Biol.* 2014; 21:147–153. [PubMed: 25139830]
- Went, FW., Thimann, KV. *Phytohormones.* The MacMillan Company; New York: 1937.
- Yang D, Seaton DD, Krahmer J, Halliday KJ. Photoreceptor effects on plant biomass, resource allocation, and metabolic state. *Proc Natl Acad Sci USA.* 2016; 113:7667–7672. [PubMed: 27330114]
- Zhu JY, Sae-Seaw J, Wang ZY. Brassinosteroid signalling. *Development.* 2013; 140:1615–1620. [PubMed: 23533170]

Fig. 1. Morphology of representative 5-day-old etiolated maize seedlings (reference inbred line cv. B73). Note that the mesocotyl (Meso) is curled, whereas the coleoptile, with enclosed primary leaf, is more or less straight. The node that separates the mesocotyl from the coleoptile is indicated by an *arrow*

Fig. 2.

Comparative analysis of 5-day-old etiolated maize (*Zea mays*) seedlings that were raised under identical laboratory conditions (darkness, 100 % rel. humidity, 25 °C). Lengths of the coleoptile (a), the mesocotyl (b) and the primary root (c) (see Fig. 1) are shown for the following varieties: Inbred line B73, Hybridmaize Liberal (H-L), Calvin-Mes (K-M), MK Famous (MK), NK-Gitago (N-G), NK-Jasmonic (N-J) and Frenetic (FR). Data represent means $\pm$ s.e.m. of 40 measurements each

Fig. 3. Morphology of the coleoptile of a representative 5-day-old dark-grown seedling of *Zea mays* B73 (a). In a longitudinal median section (b), the relatively short, enclosed primary leaf can be seen. Note that the upper half of the coleoptile is an empty sheath

Fig. 4.

Effect of blue light (BL)-treatment on subsequent phototropic bending in darkness of a representative straight maize coleoptile (a). Morphology of a 5-day-old dark-grown seedling of *Zea mays* B73 and design of experiments for the analysis of blue light (BL)-effects on the tip vs. growing region in the coleoptile (b). Upon exposure to BL (20 min, 20 $\mu\text{mol photons m}^{-2}$), the entire organ was separated into two parts as indicated. For proteomic analyses, samples 1 and 2 were analyzed independently

Fig. 5.

Representative 2-D DIGE analysis of the BL-response in the microsomal fraction of the tip (a) vs. basal region (b) in maize coleoptiles. Five-day-old B73-seedlings were irradiated with BL for 20 min, and, after separation of tip vs. stem region (Fig. 4b), microsomal proteins from both dark-controls (un-irradiated, proteins labeled with Cy3) and BL-irradiated samples (proteins labeled with Cy5) analyzed separately by 2-D DIGE. Proteins induced or enhanced by BL-treatment appear as red spots, whereas those that decreased in abundance as a result of BL appear in green. Protein spots that remain unchanged are yellow. Spots that responded positively to BL are marked by arrows and those with numbers (28 to 38) were characterized by mass spectrometry (see Table 1)

Blue light (BL)-responsive proteins identified in the microsomal fraction of maize coleoptile tips (*Zea mays* cv. B73, see Fig. 4b). Quantitative data represent means of 4 experiments each

Table 1

Spot	Accession Number	Protein Name	Abundance Ratio	p-value (t-test)	Number of Unique peptides	% of Coverage	Best Expectancy Value
28	O48547	Nonphototropic hypocotyl 1	-2.87	1.0e-5	14	17.9	1.20E-06
29	O48547	Nonphototropic hypocotyl 1	-2.22	0.0056	17	22.1	5.40E-08
32	A1XC15	Lipoxygenase	1.35	0.016	40	34.0	2.80E-08
33	A1XC15	Lipoxygenase	1.45	0.00012	51	47.3	8.30E-09
34	B6U1D7	Sucrose synthase 1	-1.40	0.0040	59	45.6	7.30E-10
35	C0P6F8	Sucrose synthase1	-1.47	0.0056	69	58.3	2.30E-10
38	Q5EUE1	Protein disulfide isomerase	1.36	0.00064	52	75.9	1.50E-08