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Selection of the maize hybrid tolerant to a high dense planting altered cross-talk between blue light and auxin signaling pathways

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ABSTRACT

Modern agricultural practices strive for the high density planting. Therefore, maize hybrids developed and released by Pioneer Hi-Bred International from 1930 to 2001 were primarily selected based on their higher yield in such conditions. However, this selection did not only result in higher yield but also in changes in the morphological traits, such as smaller and more erect leaves and enhanced tolerance to abiotic stress generated by dense planting. Few years ago, auxin and light were proposed to play an important role in this process. In the present study, we investigated the changes in the interaction of light and auxin signaling between four old maize hybrids (307, 317, 3306 and 3366) and the modern hybrid 3394. Etiolated seedlings of the modern hybrid displayed shorter stature and reduced sensitivity to exogenous auxin than the old hybrids, however no differences in the seedlings stature were observed when grown in continuous blue or red light. Nevertheless, the auxin-related responses of the modern hybrid were greatly affected under blue light, suggesting a modification of the interaction between light and auxin signaling pathways that has happened during the breeding process. The role of ABP1 in this interaction was investigated and discussed.

KEYWORDS: maize, growth, light, auxin, ABP1

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INTRODUCTION

With a world production of 840 millions of tonnes, maize (*Zea mays* subsp. *mays*) is the second most important crop in the world (source: FAO, 2010; http://faostat.fao.org). Consequently, several breeding programs have been developed, making the grain yield one of the targets of the selection. Hybrids commercially released by Pioneer Hi-Bred International from the 1930s to the 1990s were characterized by a gradual increase in the grain yield even under high plant density conditions. Indeed, the yield plateau of modern hybrids was reached at higher plant density than for the old hybrids [1, 2]. When grown in high density (HD) planting in the field, even if all tested hybrids displayed longer leaves, decreased leaf area and reduced leaf curvature than in low density (LD) planting, the modern hybrid had

more erect and smaller leaves than the old hybrids [3]. The reduction of leaf size can be caused by a reduction of assimilate availability per plant in HD planting condition [2]. Nevertheless, because the modern hybrid had also smaller and more erect leaves than the old hybrids when grown in individual pots under controlled fertilization [4], it is obvious that these traits were acquired during the process of hybrid selection.

Fellner *et al.* [4, 5] hypothesized that the differences in grain yield between the old and modern hybrids in HD planting (characterized by low red light/far red light ratio) may be explained by different responses to red and/or far-red light, which in turn affect auxin distribution and/or auxin sensitivity, and consequently affect leaf declination. Indeed, an exposure to light increased the leaf declination and the inhibitor of polar auxin transport, 1-N-naphthylphthalamic acid (NPA), was found to abolish the light-induced increase of leaf declination in the old hybrids but not in the modern one [4]. The proposed interaction between light and auxin signaling in the control of leaf angle establishment was also supported by the observation that the cells of modern hybrid were insensitive to auxin- and light-induced hyperpolarization of the plasma membrane driving the cell elongation and consequently the leaf declination [5].

Auxin is an important plant hormone involved in numerous growth and developmental processes [6]. Two significant auxin receptors have been identified in plants so far: the F-box protein TIR1 (TRANSPORT INHIBITOR RESPONSE 1) and the ABP1 (AUXIN-BINDING PROTEIN 1) [7]. ABP1 was found to be ubiquitous in the vascular plants, and is known to be a mediator of auxin responses associated with elongation growth [8, 9]. ABP1 is essential for the organized cell elongation and division during embryogenesis in *Arabidopsis* [10], and is also required for a normal postembryonic development. Specifically, ABP1 plays an essential role during the early leaf initiation, leaf morphogenesis [11], and root growth [12], and participates in the control of cell cycle [13]. Recent studies demonstrated that ABP1 mediates the auxin-induced inhibition of endocytosis and is important for the spatial coordination of cell expansion in *Arabidopsis* [14, 15]. Contrary to *Arabidopsis*, at least five members of ABP family are known in maize [16], but their functions in plant growth and development remain unclear except for ABP1. Whereas Im *et al.* [17] did not find any difference in the phenotype of the two maize loss-of-function mutants, *ABP1* and *ABP4*, Fellner *et al.* [5] revealed that these mutants essentially differ in their leaf angle development. Interestingly, the expression of *ABP4* gene is down regulated in the modern hybrid in comparison with the old hybrid [5], which seems to correlate with the reduced sensitivity of modern hybrid seedlings to exogenous auxin [4]. Taken all together, these data suggested that the leaf angle establishment is under the control of the ABP-mediated auxin signaling pathway and that this pathway evolved during the process of maize hybrid selection.

Interactions between light and auxin signaling pathways are intensively studied but still not fully understood since these two pathways are integrated at many different levels [18]. For instance, light affects auxin biosynthesis [19, 20, 21], homeostasis [22], transport [23, 24] and signaling/response [25, 26, 27]. Interestingly, these interactions may include a light-reduced responsiveness to auxin since red light was reported to reduce the abundance of ABP1 [22].

The objective of this work was to examine cross-sensitivity of young seedlings of the old and modern Pioneer hybrids to auxin and light and test the hypothesis that the erect leaves-developing modern hybrid 3394 and the old hybrids differ basically in auxin-related properties, which involve ABP and/or light signaling pathway(s). For this purpose, we analyzed in more details four old hybrids (307 and 317 - from 1930s, 3306 and 3366 - from 1960s) and the modern hybrid 3394, in regards to their leaf angle development, levels of endogenous auxin, responsiveness to various types of light, exogenous auxin and inhibitor of polar auxin transport, expression of *ABP1* gene and accumulation of the respective protein.

MATERIAL AND METHODS

Plant material and growth conditions

Kernels of five Pioneer hybrids of maize (*Zea mays* L.): 307, 317, 3306, 3366, 3394 (all provided by

Pioneer HiBred, Intl., Des Moines, Iowa, USA) were used for experiments. The hybrids were commercially released in 1936 (307, double cross), 1937 (317, double cross), 1963 (3306, single cross), 1972 (3366, single cross), and 1991 (3394, single cross). The hybrids 307, 317, 3306 and 3366 are referred to as old hybrids whereas the hybrid 3394 is referred to as a modern hybrid. The pedigree information is available in Smith *et al.* [28].

For sterile cultures, kernels were rinsed in 60% (v/v) commercial Savo solution (Bochemie, Czech Republic) (~3% sodium hypochlorite) supplemented with a drop of Tween 20 (Sigma-Aldrich, Czech Republic) for 30 min, and then extensively rinsed with sterile distilled water. Kernels germinated on 0.7% (w/v) agar medium in Magenta GA7 boxes, 77x77x196 mm (Sigma, USA), 9 seeds per box. The basal medium (BM) contained Murashige and Skoog salts (Sigma-Aldrich, Czech Republic) [29], 1% (w/v) sucrose and 1 mM Mes (2-(N-morpholino)-ethanesulfonic acid) (pH adjusted to 6.1 by KOH before autoclaving). For particular experiments the BM was further supplemented with various concentrations of auxin (NAA: 1-naphthalene acetic acid) or 1-N-naphthylphthalamic acid (NPA), the inhibitor of polar auxin transport. Kernels in the Magenta boxes were placed in a growth chamber (Microclima 1000E; Snijders Scientific B.V., The Netherlands), and incubated at 23°C under continuous blue light (BL) with maximum irradiance at 460 nm, continuous red light (RL) with maximum irradiance at 660 nm, or in dark (D). BL and RL were provided by blue (Philips TLD-36W/18-Blue, Phillips, USA) and red fluorescent tubes (Philips TLD-36W/15-Red, Phillips, USA), respectively. The total photon fluence rates of BL and RL were identical, i.e. 10 $\mu mol.m^{-2}.s^{-1}$. The fluence rate was measured with a portable spectroradiometer (model LI-1800; Li-Cor, USA) calibrated at the Department of Biophysics at Palacký University in Olomouc.

For the greenhouse experiments, plants were grown in soil (Potground H, Klasmann Deilmann, Germany) in small pots (190x190 mm; one seed per pot; 1 cm deep sowing) and regularly watered. In summer, plants grew in natural light conditions. In winter, plants grew under natural light extended with light from high-pressure sodium lamps PlantaStar E40/ES 400 W (Osram, Germany) to create 16-hour photoperiod. The temperature in a greenhouse was controlled in the range 15 - 27°C.

Measurement of leaf declination, leaf length and width, and determination of leaf area

For the study of a leaf angle development, plants were grown in a greenhouse in soil as described above. The leaf angle that is characterized as the declination of the leaf from the vertical axis was measured at the leaf base, as described in Fellner *et al.* [4]. Measurements were done 4 days after the appearance of well-developed leaves (indicated by visible leaf collars), i.e. 2nd leaf was measured approx. 25 days and 3rd leaf approx. 28 days after the seed germination (leaves were counted from the base of the plant). The leaf length was measured from the auricle termination to the tip of the leaf blade. The leaf width was measured in a widest part of the leaf blade. Lengths were measured with a ruler to the nearest millimeter. The leaf area was calculated using the following formula: *leaf length* x *maximum leaf width* x α, where the coefficient α, determined by Stewart and Dwyer [30], has a value 0.743. For each hybrid, 21 to 24 plants from four independent experiments were measured.

Measurement of seedling growth

The size of various organs was measured with a ruler to the nearest mm in 4-day-old intact seedlings developed in sterile conditions on the BM in D, continuous BL or RL. The mesocotyl length was measured from the scutellar to the coleoptilar node, and the coleoptile length was measured from the coleoptilar node to the tip of the coleoptile. Only the seedlings that germinated on the same day were measured. Five independent experiments per treatment were performed and results represent average of a total number of 20 to 37 measured plants.

Extraction and quantification of endogenous auxin

For the analysis of endogenous free indole-3-acetic acid (IAA) accumulation in coleoptiles and mesocotyls, 4-day-old maize seedlings grown in

sterile conditions on the BM in D, continuous BL or RL were used. Coleoptiles and mesocotyls were excised from several seedlings, placed into prechilled aluminum foil envelopes, immediately frozen in liquid nitrogen and then stored at -80°C. Endogenous auxin was extracted, purified from 10 mg sample by immunoaffinity extraction and quantified by high performance liquid chromatography coupled to tandem mass detection as described in Pěnčík *et al.* [31].

Gene expression analysis

The expression of *ABP1* gene (acc. no. L08425) was studied in coleoptiles and mesocotyls of the 4-day-old seedlings grown in sterile conditions on the BM in D, continuous BL or RL. Total RNA was extracted from both organs using RNeasy Plant Minikit (Qiagen, Germany) according to the manufacturer's instructions. Genomic DNA was removed by 30 min DNaseI treatment (Sigma-Aldrich, Czech Republic) at 37°C and total RNA was purified using phenol/chloroform/ iso-amylalcohol (25:24:1). Synthesis of first-strand cDNA was performed from 1 μg of total RNA primed with random primers using SuperScript™ III Reverse Transcriptase (Invitrogen, USA). Quantitative RT-PCR analyses were made on Mx3000P™ Real-Time PCR System (Stratagene, USA). The reaction mixture contained 50 nM of each primer, 12.5 μl of Absolute™ QPCR SYBR Green ROX Mix (Thermo Scientific, USA) and 2 μl of 50x diluted cDNA in a total volume of 25 μl. For amplification of the *ABP1* gene, the following primers were used: F: 5'-AGGTGGAAGTGTGGCTTCAG-3', and R: 5'-ATCCCATCAAGAGCGTACCC-3'. The *18S rRNA* gene of maize (gene ID: 4055912) was used as the reference gene and amplified using the following primers: F: 5'-ACGAACAACTGCGAAAGC-3', R: 5'-CGGCATCGTTTATGGTTG-3'. Amplifications were carried out as follows: 95°C for 10 min and then 50 cycles of 95°C for 30s and 60°C for 1 min. A melting curve analysis was performed at the end of each PCR reaction to confirm the product quality. All data were normalized with respect to the *18S* amplicon. The $\Delta\Delta C_T$ method and differences in the cycle numbers during linear amplification phase between samples were used for the determination of relative gene expression. The *ABP1* gene expression in the hybrids was expressed relative to that estimated for the oldest hybrid 307 grown in the D. Data represents averages of two biological repeats done in triplicates.

Analysis of amount of protein ABP1

The content of ABP1 was analyzed in coleoptiles and mesocotyls of the 4-day-old seedlings grown in sterile conditions on the BM in D, continuous BL or RL. Tissues were grounded in liquid nitrogen with a mortar and pestle and stored in -80°C before protein extraction. Proteins were extracted from the powdered tissues with extraction solution (9 M urea, 4.5% SDS (w/v), pH was adjusted to 6.8 using HCl) containing 7.5% (v/v) β-mercaptoethanol. The samples were incubated 5 min at 60°C, then vortexed, incubated again 5 min at 60°C, and centrifuged 10 min at 10000x*g*. The supernatant was transferred to a new eppendorf tube. The concentration of proteins was determined from absorption at 280 nm in 20 x diluted samples by NanoDrop 2000 (Thermo Scientific, USA). Aliquots of 200 μg were loaded and electrophoresed on a 12% SDS-PAGE gel. Proteins were transferred on a nitrocellulose membrane (Trans-Blot® Transfer Medium, Bio-Rad, Czech Republic) using OWL semi-dry electroblotter (Thermo Scientific, USA). The rabbit polyclonal antibody raised against ABP1 [32] diluted 1:2000 and Anti-Rabbit IgG - Peroxidase (Sigma-Aldrich, Czech Republic) diluted 1:5000 were used as primary and secondary antibody, respectively. Nonspecific binding sites were blocked by washing the membranes in phosphate buffered saline containing 0.1% (v/v) Tween 20 (Sigma-Aldrich, Czech Republic) (PBST) supplemented with 5% non-fat dry milk (w/v) for 1 h. Afterwards, the membranes were washed 2 times for 10 min in PBST. Then the membranes were incubated with primary and secondary antibody in the blocking solution (3% non-fat milk (w/v) in PBS), each antibody 1 hour, always followed by washing the membranes 2 times in PBST for 10 min. The peroxidase activity was detected using the Amersham ECL™ Western Blotting Detection Reagents on the Amersham Hyperfilm™ ECL (GE Healhtcare, UK). Films were scanned (CanonScan 8600F) and analyzed by ImageJ [33]. Data represents two biological repeats done in duplicates and show the abundance

of ABP1 in hybrids grown in the assigned light conditions or the effect of light on the ABP1 accumulation.

Statistical analysis

When necessary, a statistical significance of the treatment differences was assessed using the Student's *t*-test (MS Office Excel), or ANOVA (NCSS 2007).

RESULTS

Modern hybrid 3394 develops smaller and more erect leaves than old hybrids

The leaf angle and leaf surface were determined for the five Pioneer hybrids (307, 317, 3306, 3366 and 3394) grown in a greenhouse until the 3rd leaf was fully developed. These parameters were measured on the 2nd and 3rd leaves (counted from the base of the plant). Whatever the rank of the leaf considered, a decrease in the leaf angle was observed from the oldest (307) towards the youngest hybrid (3394) (Fig. 1A). The blade surface of the modern hybrid was significantly smaller than in the all old hybrids, which did not greatly differ from each other (Fig. 1B).

Modern hybrid diverges in dark- and light-regulated growth

Because the studies *in planta* are time and space consuming, the model for the study of the hybrids responsiveness to light was reduced to seedlings grown in D or in continuous BL or RL. Etiolated seedlings of all old hybrids developed significantly longer coleoptiles (Fig. 2A) and mesocotyls (Fig. 2B) than seedlings of the modern hybrid 3394. Except for the oldest hybrid 307, light stimulated the growth of the coleoptiles, while no significant difference could be observed between all hybrids (Fig. 2A). Moreover, the response was found to be independent of the light quality as BL and RL stimulated coleoptile growth to the same extent. Because the coleoptiles of the etiolated 3394 hybrid were shorter than those of the other hybrids, a greater stimulation of coleoptile growth by the light in this hybrid was observed (BL: 50%, RL: 60% in 3394, in contrast to BL: 20% and RL: 13% in 317).

The elongation of mesocotyls was strongly reduced by light, with RL being more efficient than BL to inhibit growth (Fig. 2B). Nevertheless, BL and RL were less efficient to inhibit the mesocotyl growth in the modern hybrid 3394 (39% and 59%, respectively) compared to other hybrids (average value for BL: 67%; average value for RL: 77%). Consequently, when grown in light, the modern hybrid 3394 developed longer mesocotyls than the old hybrids.

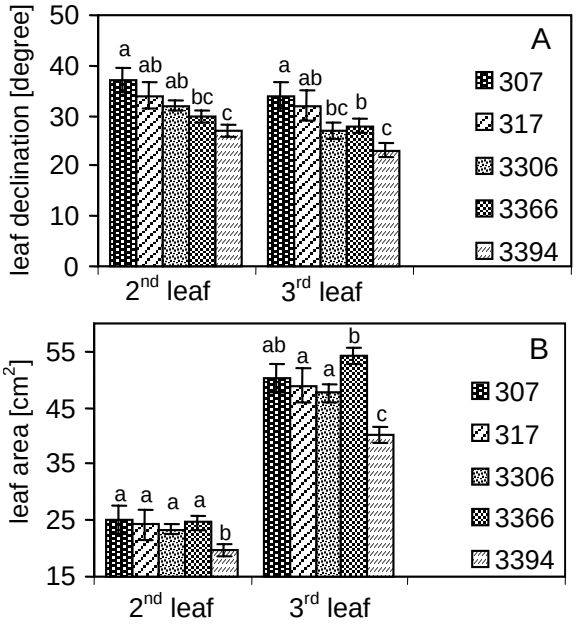

Fig. 1. Declinations (A) and leaf areas (B) of 2nd and 3rd leaf of maize hybrids grown in a greenhouse. Measurements were done 4 days after the appearance of well-developed leaves in the all hybrids examined (2nd leaf approx. 25 days and 3rd leaf approx. 28 days after germination). Data represent averages ± SE obtained 21-24 samples in 4 independent experiments. Letters a, b, c indicate significantly different groups (ANOVA, $P<0.05$).

BL does not decrease free IAA content in coleoptile of modern hybrid

Hormone auxin is known to be involved in the light-induced growth responses. The quantification of the endogenous free IAA was therefore performed in an effort to find an explanation for the altered photomorphogenic responses of the modern hybrid 3394. The analyses were done on coleoptiles and mesocotyls of seedlings grown in D, continuous BL or RL. The accumulation of the free IAA in etiolated coleoptiles was the highest in the oldest hybrid 307 and gradually decreased

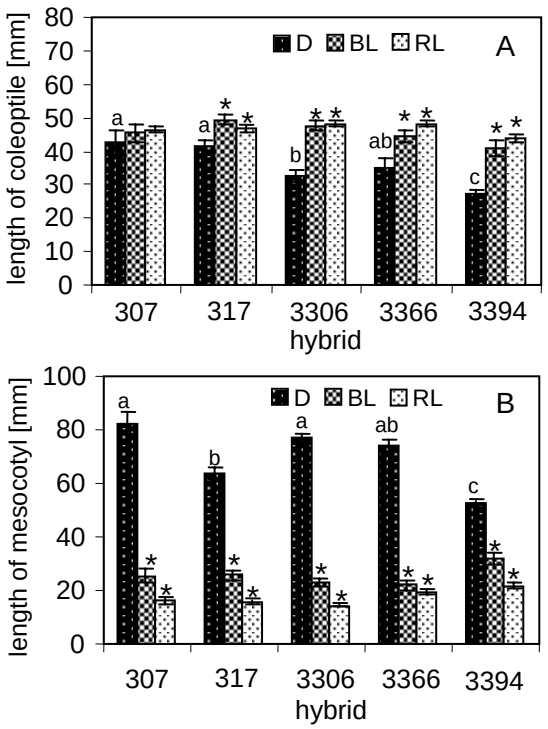

Fig. 2. The effect of light on the coleoptile (A) and mesocotyl (B) elongation in the 4-day-old maize hybrids developed in D, BL or RL. Data represent averages ± SE obtained from 20-37 samples in 5 independent experiments. Letters a, b, c indicate significantly different groups (ANOVA, P<0.05) between the D-grown plants. (*) indicates significant differences (t-test, P<0.05) between the light and dark-grown plants. ANOVA results for BL and RL-grown plants were similar, and did not show difference between the coleoptiles of all hybrids, but mesocotyls of the modern hybrid 3394 were significantly longer than mesocotyls of all old hybrids (not shown in graphs).

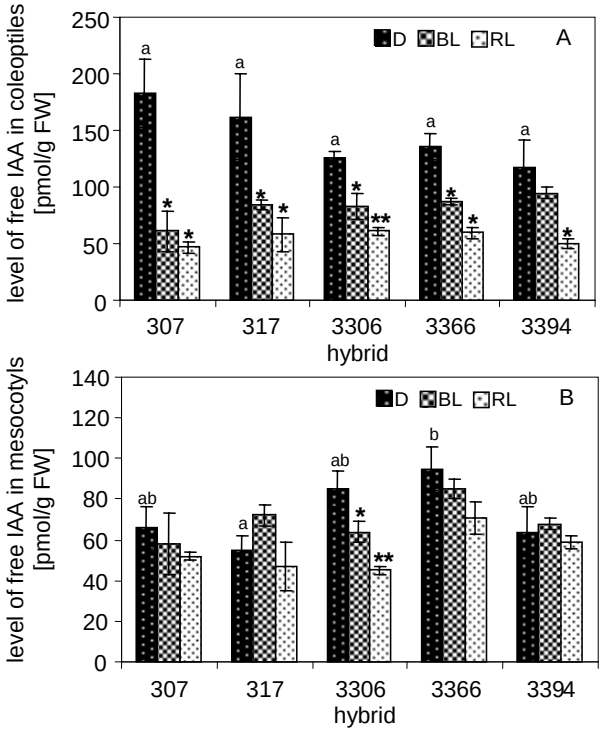

Fig. 3. The amount of endogenous free IAA in coleoptiles (A) and mesocotyls (B) of the 4-day-old maize hybrids growing in D, BL and RL. Data represent averages ± SE obtained from three independent samples harvested at the same time. Letters a, b indicate significantly different groups (ANOVA, P<0.05) between the D-grown plants. (*) indicates significantly different (t-test, P<0.05) from the D-grown plants; (**) indicates significantly different (t-test, P<0.05) from the values marked by (*). ANOVA results for the BL and RL-grown plants (coleoptiles as well as mesocotyls) were similar and showed no differences or certain differences between the old hybrids, but the modern hybrid 3394 did not differ from them (not shown in graphs).

towards the modern hybrid 3394, though no statistically significant difference was found (Fig. 3A). Coleoptiles of all hybrids grown under BL contained a comparable amount of the free IAA, but in comparison to the ones grown in D, the free IAA content was significantly decreased in the old hybrids, but not in the modern hybrid (Fig. 3A). The RL-grown coleoptiles of all hybrids contained a significantly less free IAA than the etiolated organs, and no differences were found between the hybrids (Fig. 3A).

The accumulation of free IAA in the etiolated mesocotyls of the modern hybrid was similar to that observed in all old hybrids. Light (BL and RL) significantly lowered the IAA contents only in the mesocotyls of the old hybrid 3306 (Fig. 3B), but not in other hybrids.

Modern hybrid shows altered effect of light on responsiveness to exogenous auxin

The effect of exogenous auxin on the growth of the five hybrids was examined on seedlings grown in D, continuous BL or RL. In an attempt to simplify the results, data for one representative hybrid of each group (307 - from 1930s, 3306 - from 1960s and the modern one 3394) are presented in the Fig. 4. When seedlings were grown in D, the

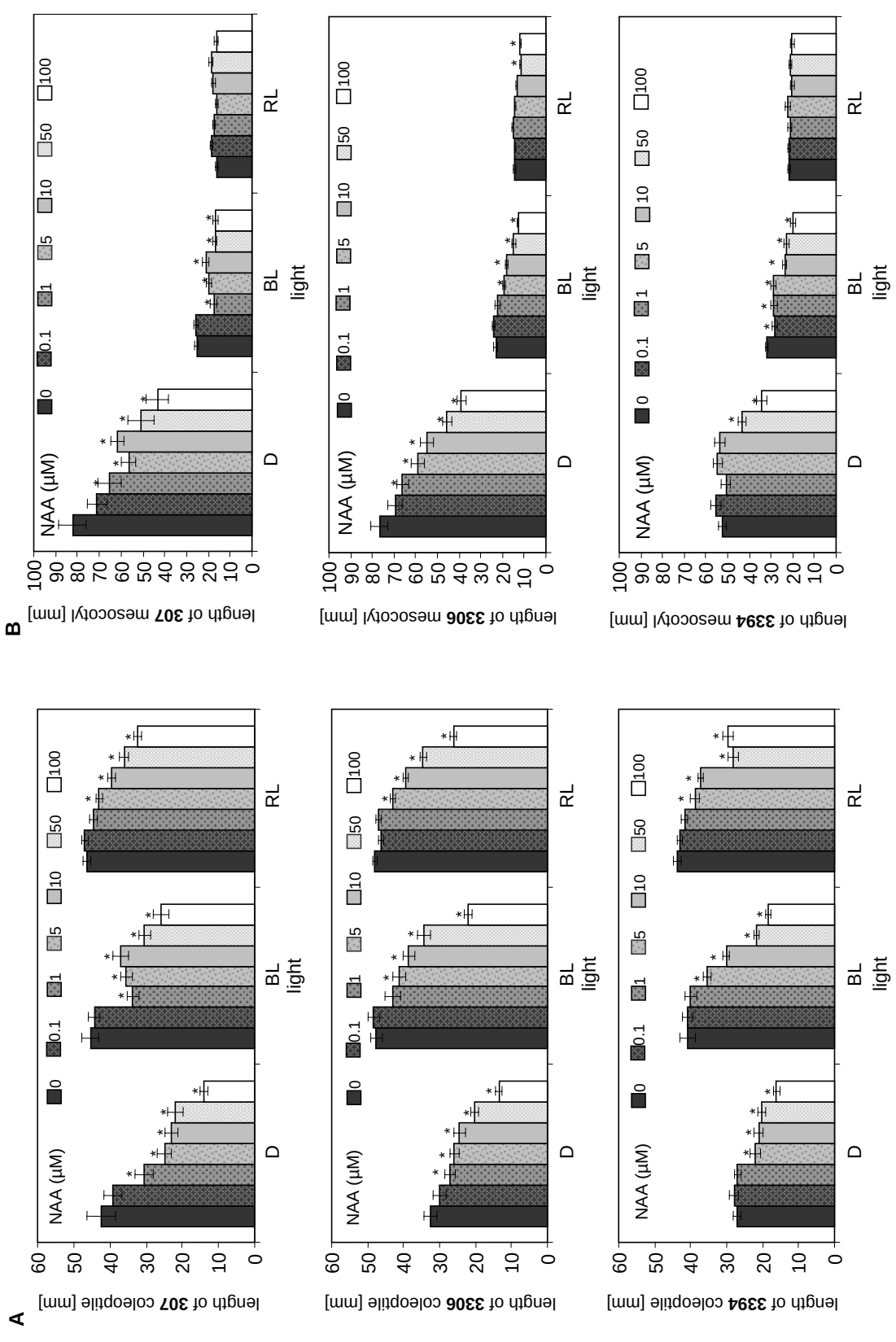

Fig. 4. The effect of exogenous auxin (NAA) on the elongation of coleoptile (A) and mesocotyl (B) in the 4-day-old maize hybrids developed in D, BL or RL. Data represent averages ± SE obtained from 20-37 seedlings in 5 independent experiments. Each graph shows data obtained for the one representative hybrid developed in 1930´s – hybrid 307, 1960´s – hybrid 3306 and the modern hybrid 3394. (*) indicates significant difference (t-test, $P<0.05$) from plants grown in the absence of NAA.

coleoptile growth of all old hybrids was inhibited by 1 μM NAA. On the contrary, the coleoptile growth of the modern hybrid 3394 was inhibited only at higher concentrations of NAA (5 μM, 19%; Fig. 4A). When seedlings of the old hybrids were grown in BL, the growth of coleoptiles was also inhibited by NAA but to a less extent than in plants grown in the D (Fig. 4A). Surprisingly, the 3394 seedlings grown in BL were more sensitive to NAA than the old hybrids. Indeed, at the concentration of 50 μM, NAA inhibited the coleoptile growth of 3394 by 47%, whereas 307 and 3306 were inhibited by 33 and 27%, respectively (Fig. 4A). Moreover, BL increased the sensitivity of 3394 hybrid to NAA. Like in BL, continuous RL also significantly decreased the inhibitory effect of exogenous auxin on the coleoptile elongation. However, this effect was found to be more striking in the hybrids selected in 1930s (307, 317), whereas the hybrid´s from 1960s (3306, 3366) and the modern hybrid 3394 were less affected in this response (Fig. 4A; for 317 and 3366 data not shown).

The effect of exogenous auxin on the mesocotyl growth was similar to that observed for coleoptiles, but the differences between the old and modern hybrids in responsiveness to auxin were more pronounced than in coleoptiles. Interestingly, in the old hybrids grown in D a 1 μM concentration of NAA was sufficient to inhibit the growth mesocotyls whereas in the 3394 hybrid a 50 μM concentrations of NAA was necessary to inhibit the growth (Fig. 4B). BL slightly decreased the responsiveness of the old hybrid mesocotyls to NAA, but greatly increased responsiveness of the modern hybrid 3394, as the lowest concentration of NAA applied (0.1 μM) was sufficient to induce the inhibition of the mesocotyl growth (Fig. 4B). The most surprising results were observed for the seedlings grown in continuous RL, where mesocotyls of all hybrids were almost or completely irresponsive to the inhibitory effect of NAA over the whole range of concentrations used (Fig. 4B).

BL affects the responsiveness to NPA

In parallel to the study of the interaction between auxin and BL during the development of hybrid seedlings, the role or the polar auxin transport (PAT) was investigated. For this purpose, seedlings of all hybrids were grown in D or continuous BL on medium containing different concentration of NPA, a specific inhibitor of PAT (Fig. 5).

When hybrids were grown in D, NPA induced the inhibition of coleoptiles growth of the two oldest hybrids (307, 317) in concentration-dependent manner, but not in the three other hybrids (3306, 3366, and 3394) (Fig. 5, for 317 and 3366 data not shown). The effect of NPA on the growth of coleoptile was markedly reduced by BL for 307 (Fig. 5) or suppressed for 317 (data not shown). On the contrary, the hybrids weakly sensitive to NPA in D showed an essentially increased response in BL (Fig. 5).

The effect of NPA on the elongation of mesocotyls was similar to that observed for coleoptiles, except for the hybrid 3306. Hybrids, of whose mesocotyl growth was greatly inhibited by NPA in D (307, 317, and 3306), showed a suppressed and reduced response in BL, and the hybrids with weak response in D showed no response (3366) or an increased response (3394) in BL (data not shown).

Auxin-binding protein 1 does not seem to be involved in differential auxin and light-induced growth responses of modern hybrid

It was hypothesized that differential light and auxin-induced responses of the modern hybrid 3394 could be mediated through the ABP1, a putative auxin receptor. Indeed the loss-of-function mutation in the maize *ABP1* gene resulted in a decrease of the leaf angle declination [5]. Hence, the expression of *ABP1* and the accumulation of the corresponding protein were investigated in this study.

The relative amounts of *ABP1* transcripts in coleoptiles and mesocotyls of all hybrids in all light conditions were considerably variable and did not show distinct differences between the old hybrids and the modern hybrid (supplemental Fig. 1).

The amount of ABP1 detected in the coleoptiles grown in D was similar for all hybrids (Fig. 6A). In coleoptiles of the seedlings developed in BL, the ABP1 content was higher in the two youngest

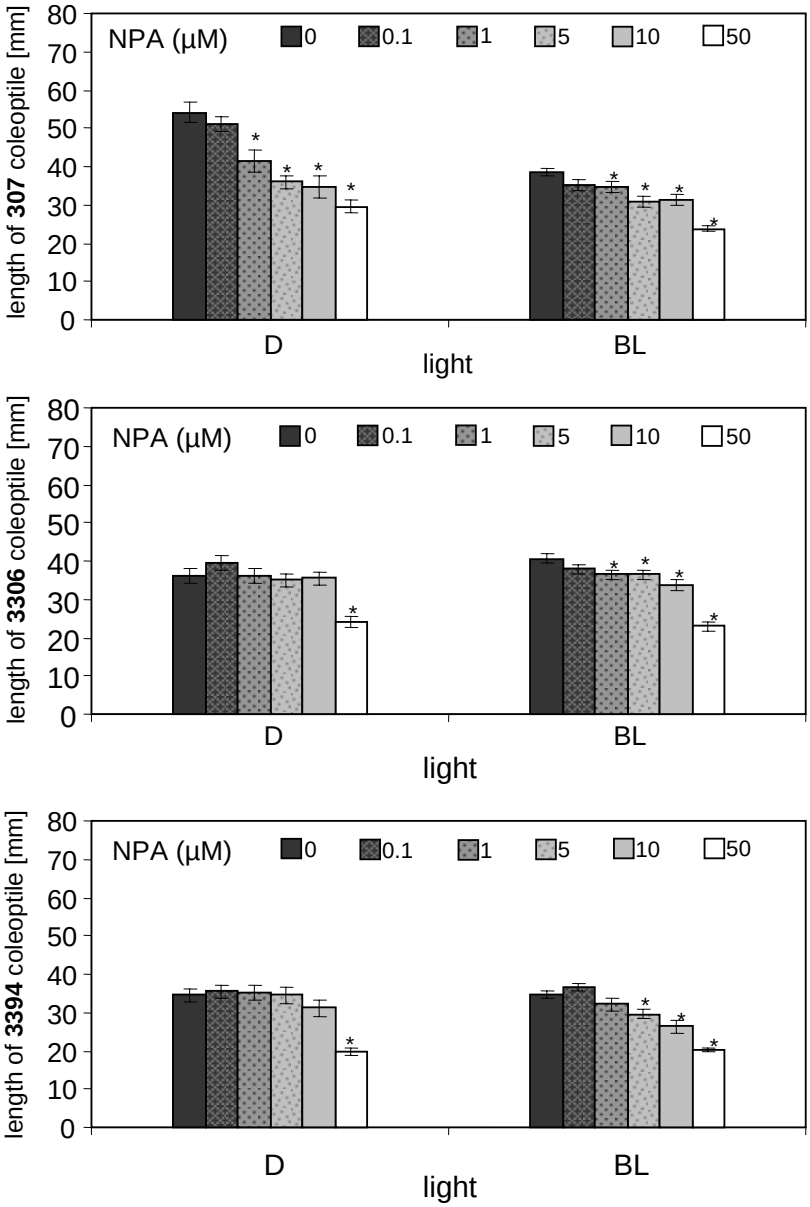

Fig. 5. The effect of NPA on the elongation of coleoptile in the 4-day-old maize hybrids developed in D and BL. Data represent averages ± SE obtained from 30 seedlings in 5 independent experiments. Each graph contains data obtained for one representative hybrid developed in 1930's - hybrid 307, 1960's - hybrid 3306 and the modern hybrid 3394. (*) indicates significant difference (t-test, $P<0.05$) from plants grown in the absence of NPA.

hybrids (3366 and 3394) and the old hybrid 317, than in the two old hybrids (307 and 3306). Similarly to D, the coleoptiles grown in RL contained a similar amount of the ABP1 protein (Fig. 6A). BL did not affect the content of ABP1 in coleoptiles of all old hybrids (307, 317, 3306, 3366), whereas slightly decreased the amount of ABP1 in the modern hybrid 3394 (Fig. 6B). Also RL had variable effect on the ABP1 content in coleoptiles. The hybrids 307, 3366 and 3394 contained approximately the same amount of ABP1 protein as in D, whereas RL stimulated the accumulation of ABP1 in coleoptiles of the old hybrids 317 and 3306 (Fig. 6B).

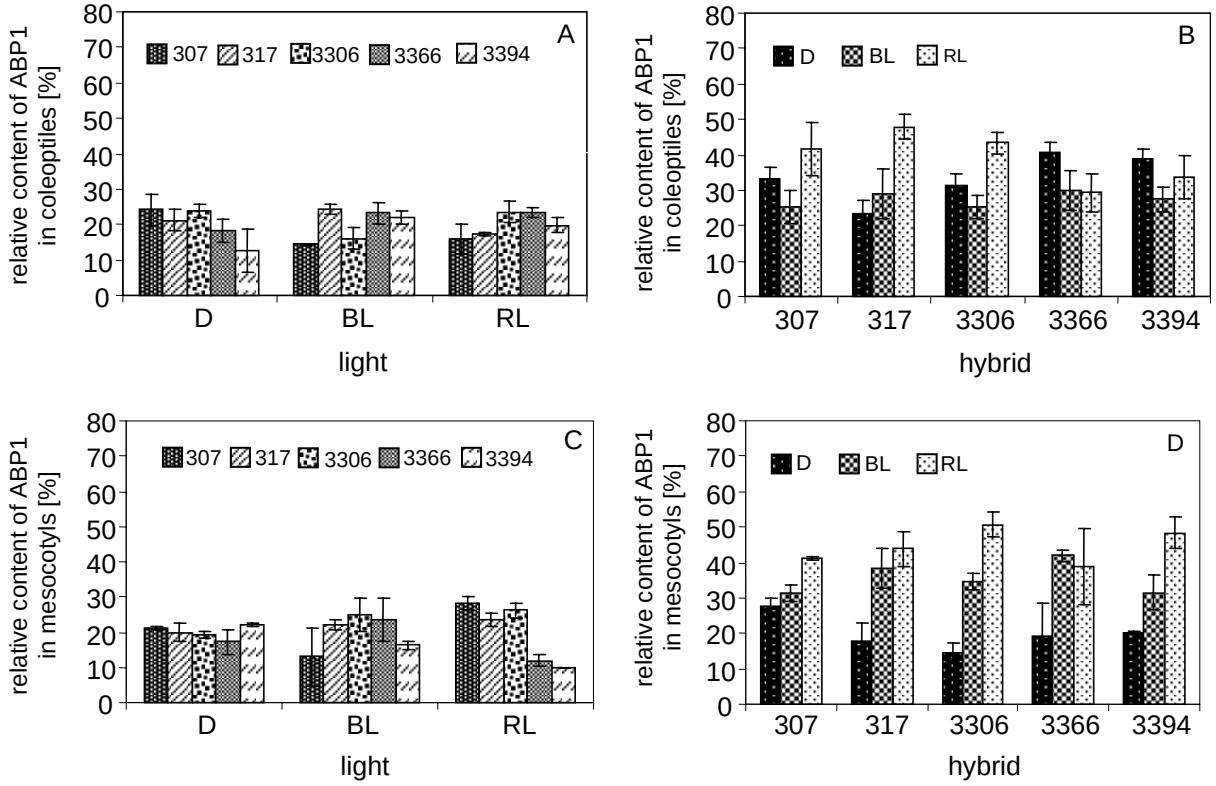

Fig. 6. The amount of ABP1 protein in coleoptiles (A, B) and mesocotyls (C, D) of the 4-day-old maize hybrids grown in D, BL and RL. The amount of protein is compared between the hybrids in certain light condition (A, C), and the effect of light on the ABP1 accumulation is shown separately (B, D). Data are expressed as a percentage of the total area of all signals present on a gel and signal area was determined by ImageJ software. Results represent averages ± SE obtained from two independent experiments done in duplicates.

In mesocotyls of the etiolated and plants grown in BL, the amounts of ABP1 were comparable for all hybrids (Fig. 6C). In the plants grown in RL, a lower content of ABP1 was found in mesocotyls of the two youngest hybrids - 3366 and 3394 (Fig. 6C). BL and RL induced the accumulation of ABP1 in mesocotyls of all hybrids with the exception of 307, for which the content in ABP1 was not significantly increased by BL (Fig. 6D).

DISCUSSION

In the present study, light and auxin associated properties of the old and modern Pioneer hybrids were investigated, since it was previously suggested that selection of the modern hybrid 3394 for increased yield could have affected the responsiveness to light and auxin [4, 5].

Analyses of leaf angle development of juvenile plants confirmed that all older hybrids tested grown in the greenhouse have leaves less erect than modern hybrid 3394. It seems to be apparent that during the breeding process, which was focused on increasing the productivity, the leaf declination was affected. Relationship between productivity and leaf angle was suggested more than forty years ago. Indeed, it was observed that backcross-derived isogenic single cross hybrid carrying the Ig_2 gene responsible for erect leaves produces 40% more grain than control plants with horizontally oriented leaves [34]. Moreover, a mechanical manipulation of leaf positioning of Pioneer hybrid 3306 (used also in this study) into more upright greatly affected the yield - yield of plants with all leaves tied was 6.5% higher, and yield of plants with upright leaves only above the ear was 14.2% greater than yield of non manipulated control plants [34]. This kind of leaves arrangement allows distribution of light energy to the lower layer of the canopy and apparently

contributes to the grain yield [34, 35, 36]. Concomitantly, we have determined that juvenile leaves of modern hybrid are characterized by smaller leaf area than leaves of all older hybrids examined, what is in compliance with results of Ford *et al.* [3] obtained from adult plants measurements.

The analysis of the seedling growth responses revealed several differences between the old and modern maize hybrids. When compared to all old hybrids, the modern hybrid 3394 showed a reduced growth and a reduced sensitivity to NAA in D condition, which is in agreement with Fellner *et al.* [4, 5]. Since no significant difference was found in the accumulation of free IAA among the studied hybrids, we assume that the short stature of the 3394 seedlings is the result of a decreased responsiveness to auxin.

The effect of light on the elongation of coleoptile is not comprehensive and depends on the stage of development of the particular organ. Indeed, its elongation may be stimulated by light at the early stage of development and inhibited in the later stage [37]. In the light conditions used in this work, light stimulated the elongation of coleoptiles, more markedly in the modern hybrid 3394. Concomitantly, light (BL and RL) reduced an accumulation of free IAA in coleoptiles. Whereas it is known that RL inhibits free IAA accumulation through the inhibition of the *de novo* synthesis from the tryptophan [19, 21], the way how BL regulates free IAA content remains unclear. Nevertheless, we can hypothesize that BL reduced the IAA content in coleoptiles by similar machinery in all old maize hybrids, but not in the modern hybrid 3394. Moreover, BL enhanced the sensitivity of coleoptile to exogenous auxin in the modern hybrid, whereas the opposite effect was observed in the old hybrids. A similar effect was observed in mesocotyls. Similarly to BL, RL reduced the auxin sensitivity in coleoptiles of all old hybrids, but not in the coleoptiles of the modern hybrid. Mesocotyls of the 1930s hybrids as well as mesocotyls of the 3394 hybrid completely lost their sensitivity to the exogenous auxin when grown in RL, while it was partially retained in the hybrids coming from 1960s. From our data, it appears that the responsiveness of maize seedlings to the exogenous auxin is a light-dependent process, like it was described for *Physcomitrella patens* [27], *Avena* mesocotyl sections [26] and pea plants [25]. Both BL and RL share signaling components (HY5, HYH), which regulate the process of de-etiolation along with the regulation of sensitivity to auxin through the regulation of auxin signaling pathways [18, 38]. An additional evidence for the alteration of the interaction between BL and auxin during the selection of 3394 hybrid comes from the experiments with NPA. In fact, during the breeding process the auxin transport lost its importance for the elongation of etiolated seedlings, but gained its importance for the elongation of plants grown in BL.

ABP1 is the major and the most studied auxin-binding protein present in maize. Since it was shown that the nonfunctional *ABP1* gene confers decreased leaf declination in maize juvenile seedlings [5], the role of the ABP1 protein during the light-driven development of maize hybrids was investigated. The content of ABP1 protein in the hybrids did not correlate with their differential light or auxin-induced growth responses; therefore we have no evidence for a pivotal function of ABP1 in mediating the light-induced auxin-dependent processes during the maize seedling development. Interestingly, ABP1 protein content seems to be regulated by an unknown posttranscriptional mechanism, which is probably under the control of other auxin-binding protein of maize, especially ABP4 [17]. Though, we cannot exclude the possibility, that post-transcriptional regulations may take part also in the regulation of an ABP4 protein content or that ABP4 controls the ABP1 function, for example through their direct interaction. Because no clear correlation was found between the ABP1 protein content, free IAA content and growth responses influenced by BL, RL or NAA, we just can conclude that the role of ABP1 in intact maize seedlings is not unambiguous and hardly declarative due to the presence of other ABPs whose actions and mutual interactions are not known. Moreover, because it was found that the light-induced suppression of the sensitivity to auxin in rice is mediated through the jasmonate signaling pathway [39], we cannot exclude that similar regulatory mechanism occurs in maize.

CONCLUSIONS

In maize, the interaction of auxin and light is known to affect the production of vegetative tillers, and the suppression of the vegetative tillers production during the maize domestication provides striking evidence about an artificial manipulation of the auxin-light interaction [40, 41, 42]. Furthermore, it is known that light signaling pathways represent a promising target for the crop improvement [43, 44], thus it is tempting to speculate that the breeding strategy used for Pioneer hybrids caused alterations of photomorphogenic responses, which contributed to the increasing of yield. We believe that our results support this hypothesis. Our results show that auxin responsiveness is a light-regulated process, and is different in the old and modern hybrids. We suppose that the breeding process that led to the selection of the modern hybrid 3394 altered sensitivity to auxin and the interaction of BL and auxin signaling pathways. However, a direct role of a putative auxin receptor - ABP1 in this mechanism was not confirmed.

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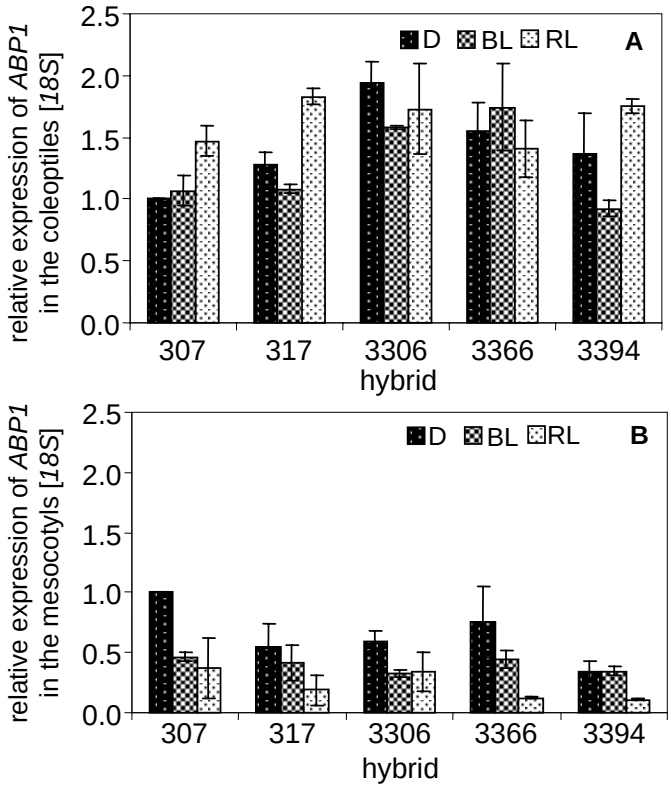

Supplemental Fig. 1. The effect of light on the relative expression of *ABP1* gene in coleoptiles (A) and mesocotyls (B) of the 4-day-old maize hybrids growing in D, BL and RL estimated by qRT-PCR. Expression data are related to the expression value obtained from etiolated plants of 307 hybrid. Data represent averages ± SE obtained from two independent experiments.

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