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Photosynthesis in the basal growing zone of barley leaves

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Abstract

Cell proliferation, elongation, determination and differentiation mainly take place in the basal 5 mm of a barley leaf, the so-called basiplast. A considerable portion of cDNAs randomly selected from a basiplast cDNA library represented photosynthetic genes such as CP29, RUBISCO-SSU and type I-LHCP II. Therefore, we became interested in the role of the basiplast in establishing photosynthesis. (1) Northern blot analysis revealed expression of photosynthetic genes in the basiplast, although at a low level. Analysis of basiplasts at different developmental stages of the leaves revealed maximal expression of photosynthetic genes during early leaf development. The activity of these genes shows that plastid differentiation involves the development of the photosynthetic apparatus even at this early state of leaf cell expansion. (2) This conclusion was supported by the fact that chlorophylls and carotenoids are synthesized in the basiplast. The qualitative pattern of pigment composition was largely similar to that of fully differentiated green leaves. (3) The transition from proplastids to chloroplasts progressed in the basal 5 mm of the leaf, so that the number of grana lamellae per thylakoid stack increased with distance from the meristem from zero to about five. (4) Photosynthetic function was studied by chlorophyll a-fluorescence measurements. In dark-adapted 8-day-old primary leaves, the fluorescence ratio \((F_F - F_0)/F_F\) was little decreased in basiplasts as compared to leaf blades. During steady state photosynthesis, the ratio \((F_M - F_0)/F_M\) was high in the leaf blade (0.5), but low in the sheath (0.25) and in the basiplast (0.18), indicating the existence of functional, albeit low light-adapted chloroplasts in the basiplast. (5) Further on, chlorophyll a fluorescence analysis in relation to seedling age revealed efficient photosynthetic performance in the basiplast of 3- to 6-day-old seedlings which later-on differentiates into leaf blade as compared to the basiplast of 7- to 12-day-old seedlings which develops into leaf sheath and finally ceases to grow. The leaf age dependent changes in basiplast photosynthesis were reflected by changes in pigment contents and LHCP II expression both of which also revealed a maximum in the basiplast of 4-day-old seedlings.

Abbreviations: bas 1—basiplast-associated gene 1 encoding a peroxide reductase; cab—chlorophyll a/b binding protein; CP 29—29 kDa chlorophyll binding protein; DIG—digoxigenin; EMIP—epidermal major intrinsic protein; LHCP II—light harvesting complex of Photosystem II; LSU—large subunit of Rubisco; NPQ—non photochemical chlorophyll a fluorescence quenching; PS I/PS II—Photosystem I/II; PQ—photochemical chlorophyll a fluorescence quenching; Rubisco—Ribulose-1,5-bisphosphate carboxylase; SSU—small subunit of Rubisco

Introduction

As is typical for monocots, almost all cells of barley leaves derive from a meristem located at the base of the leaves. In subsequent zones the meristematic cells expand and develop into morphologically and physiologically distinct cell types such as epidermis, mesophyll and cells of the bundle sheath. Photosynthesis
is the leaf’s predominant physiological function. The development of the photosynthetic machinery depends on a dramatic specialization of cells and plastids, which is based on a complex interaction of nuclear and plastidic genes (Mullet 1993).

In barley primary leaves, differentiation of the mesophyll is accompanied by an increase in plastid number per cell from approximately 10 to 65 (Mullet 1988). The small proplastids present at the leaf base with a diameter of 1–2 μm are enlarged 4- to 6-fold and develop into disk-shaped chloroplasts (Robertson and Laetsch 1974; Mullet 1988). According to Mullet (1988), chloroplast development in barley leaves may be divided in five phases: (1) Plastid number remains low for approximately one day after cessation of cell division. All plastids are still similar to proplastids (Dannenhoffer and Evert 1994). (2) In the cell elongation zone which is 2 to 3 cm long (Baumgartner et al. 1989) the plastid number per cell increases (Baumgartner et al. 1988; Boffey et al. 1982). Plastid volume remains rather unchanged (Mullet 1988). Plastid transcription is stimulated (Baumgartner et al. 1989) and carotenoids and chlorophylls accumulate (Boffey et al. 1980). (3) Plastid transcriptional and translational activity is high (Baumgartner et al. 1989), and the bulk of the thylakoid membranes is synthesized (Dannenhoffer and Evert 1994). (4) Plastids are photosynthetically fully active in the mature leaf. (5) Degradation of plastids takes place during leaf senescence (Wardley et al. 1984).

The developmental changes in plastid morphology are accompanied by the assembly of the photosynthetic machinery (Dannenhoffer and Evert 1994; Webber et al. 1984). This involves an enhanced and coordinated expression of plastidic and nuclear genes (Mullet 1988; Rapp et al. 1992; Ougham and Davies 1990). In contrast to the model suggested for plastid development in barley (Mullet 1988), photosynthetic activity was shown to start early in leaf development of various Gramineae, for instance maize, wheat and Lolium temulentum. Measurements of gas exchange (Baker and Leech 1977; Gay and Thomas 1995), chlorophyll a fluorescence induction kinetics (Webber et al. 1984), and light induced acidification of the suspension media of thylakoids (Webber et al. 1986) demonstrated that chloroplasts are photosynthetically active even in the early phases of leaf and plastid development.

In this work we analyse gene expression, pigment composition, chloroplast ultra structure, and chlorophyll a fluorescence kinetics in order to gain additional and refined information on photosynthesis in the basal growing zone of barley leaves. We think that it is necessary to determine all the parameters simultaneously in one type of identical leaf material in order to avoid a comparison of data which are at least sometimes incompatible for reasons of species specificity or with respect to variation in growth conditions. The questions addressed in the paper are: (1) Is there a correlation between expression of various nuclear and plastome encoded photosynthetic genes and the functionality of the photosynthetic apparatus? (2) Is the composition of photosynthetic pigments subjected to only quantitative or also major qualitative changes during early plastid development? How are these changes related to photosynthetic activity? (3) During leaf development, the basiplast develops into leaf blade and later on into leaf sheath. Is this developmental transition reflected by differences in plastid development in the basiplast?

Materials and methods

Plant growth and preparation of leaf fractions

Barley (Hordeum vulgare L. var. Gerbel) seeds were germinated in vermiculite saturated with distilled water under controlled environmental conditions (14 h: 20 °C; 10 h 18 °C) for 2 days in darkness. Seedlings were transferred to hydroponic culture with a modified nutrient solution after Hoagland and Arnon (1938) containing 1.25 mM KNO₃, 1 mM KCl, 1.5 mM Ca(NO₃)₂, 32 μM Fe-EDTA, 750 μM MgSO₄, 375 μM KH₂PO₄, 68.9 μM H₃BO₃, 13.7 μM MnCl₂, 1.5 μM ZnSO₄, 0.5 μM CuCl₂, and 0.2 μM Na₂MoO₄ and illuminated under a controlled light/dark cycle (light: 14 h, 170 μmol quanta m⁻² s⁻¹, 20°C; dark: 10 h, 18 °C) (Brune et al. 1994). The residual caryopsis, the coleoptile, and the secondary leaf were gently removed from 2 to 14 day old seedlings. The basal 5 mm of the primary leaf were taken as one section and will be referred to as basiplast in the following. The upper part was cut into 1 cm sections; or fractionated into basiplast (BP), lower sheath (PC1), upper sheath (PC2), lower part of the blade (PB), middle part of the blade (PM) and leaf tip (PS).

Isolation of nucleic acids from plant material

Plant tissue was ground to a fine powder in liquid N₂ and extracted in a buffer (2.5 ml buffer / g tissue) containing 25 mM EDTA, 25 mM EGTA, 100 mM
Tris-HCl (pH 8.5), 100 mM β-mercaptoethanol and 2% (v/v) SDS in the presence of phenol (2 ml / g tissue) and chloroform (1 ml / g tissue). After repeated extraction of the aqueous phase with equal volumes of phenol/chloroform (1:1) followed by chloroform, nucleic acids were precipitated by addition of one volume isopropanol. After dissolving the pellet in water, nucleic acids were precipitated with 2.5 volumes ethanol and 1/10 volume 3 M sodium acetate and resuspended in water. Nucleic acids were quantified spectrophotometrically.

For cDNA synthesis, ribonucleic acids were purified by differential precipitation with 2-butoxyethanol as described by Manning (1991). Polyadenylated mRNA, was then enriched using the 'PolyATtract mRNA Isolation System' (Promega, Madison, USA) according to the supplier's protocol.

**Synthesis of DIG-labeled RNA and radiolabeled DNA probes**

cDNA harbouring plasmid (CP29, LHCP, SSU) equivalent to 1 µg was cut downstream of the cDNA-insert by restriction endonucleases and then used as template to synthesize digoxigenin-labeled RNA probes. Strand-specific transcription was performed in 40 mM Tris-HCl (pH 8.0), 8 mM MgCl₂, 50 mM NaCl, 2 mM spermidine, and 30 mM DTT with T3-RNA polymerase at 37 °C for 1 h. Nucleotides were added as 'DIG RNA labeling mixture' (2 µl / 25 µl) (Boehringer, Mannheim, Germany). Synthesized RNA was stabilized with 10 U RNasin (Promega, Madison, USA). Following transcription, the DNA-template was removed by digestion with RNase-free DNase. Random primed labeling was performed with DNA representing fragments of the maize plastome (D1: pZmc 427, Larrinua et al. 1983, LSU: pZmc 460, Rodermel and Bogorad 1985; cytochrome b559: 850 bp-Bam HI fragment, Larrinua et al. 1983) and with a DNA-clone of triosephosphate isomerase (Marchionni and Gilbert 1986). DNA equivalent to 200 ng was denatured at 95 °C for 10 min followed by addition of 2 µl hexanucleotide mixture (Boehringer, Mannheim, Germany), dATP, dTTP, and dGTP (Pharmacia, Heidelberg, Germany) to final concentrations of 0.5 mM each, 30 µCi [α⁵²P]-dCTP (Amersham, Braunschweig, Germany), and 3 U Klenow fragment (Boehringer, Mannheim, Germany). The labeling reaction was terminated by passage of a 'Sephacryl S-300 HR MicroSpin' column for nucleotide removal (Pharmacia, Heidelberg, Germany).

**Northern blot analysis**

After separation of 20 µg RNA/ lane in 1% formamide containing agarose gels (Sambrook et al. 1989) nucleic acids were blotted onto Nylon membranes (Hybond-N, Amersham, Braunschweig, Germany) by capillary transfer using 20 × SSC (Sambrook et al. 1989). 0.5–1 µg DIG-labeled RNA probes were hybridized to the blots in 50% (v/v) formamide, 6 × SSC, 2 × Denhardt's solution, 0.1% (w/v) SDS and 2.5 mg torula-yeast RNA (Sigma, Deisenhofen, Germany) at 60 °C for 18 h. Non-specifically bound probe was removed by three repetitive washes in 0.2 × SSC, 0.1% (v/v) SDS at 60 °C for 30 min each. Detection was performed with the 'DIG nucleic acid detection kit' (Boehringer, Mannheim, Germany) according to the supplier's protocol.

Northern blot hybridization with radiolabeled probes was performed in 4 × SSC, 3 × Denhardt's solution and 0.5% (w/v) SDS and 0.5 mg salmon sperm DNA (Boehringer, Mannheim, Germany) at 65 °C for 18 h. Following hybridization, the blots were washed in 2xSSC, 1x SSC, and 0.5x SSC containing 0.5% (w/v) SDS at 65 °C for 30 min each. Kodak XOMat AR films were developed after exposure to the membranes for 2 and 18 h.

**Isolation of cDNAs from a λUni-ZAP-library**

A λUni-ZAP-cDNA library was constructed from polyadenylated mRNA of barley basiplasts of 6–8-day-old seedlings using the 'ZAP cDNA Synthesis Kit' (Stratagene, La Jolla, USA). 100 phages were randomly selected. The cDNA harbouring pBluescript-SK plasmids were excised in vivo as described in the 'ZAP-cDNA Synthesis Kit' protocol (Stratagene, La Jolla, USA). They were amplified in E. coli TG 1 host cells and purified by alkaline lysis. Restriction digestion was performed with Eco RI and Xho I (Boehringer, Mannheim, Germany). DNA-fragments were separated in 1%; (w/v) agarose gels in Tris-acetat buffer (40 mM Tris-Acetat (pH 7.5), 1 mM EDTA) (Sambrook et al. 1988).

**Sequence analysis**

Dideoxynucleotides were synthesized by 'T7 Sequencing Kit' (Pharmacia, Uppsala Sweden) using [α⁵²S]-dATP as radiolabel (Amersham, Braunschweig, Germany) as described in the supplier's protocol. The oligonucleotides used as primers were synthesized by
Figure 1. mRNA levels of nuclear (LHCP II, CP29, SSU, triosephosphate isomerase) and plastid (D1, Cyt559, LSU) encoded photosynthetic genes in leaf blade (PM), leaf sheath (PC), basiplast (BP) and roots (R) in comparison to basl. Northern blot analysis was performed with 20 μg total RNA per lane.

Roth (Karlsruhe, Germany) or MWG (Ebersberg, Germany). Sequence comparisons were conducted using the online services offered by EBI Hinxton (UK) and the National Institute of Health (USA).

Pigment pattern

Chloroplast pigments were determined by HPLC (Bilger et al. 1995) after modification of a method published by Gilmore and Yamamoto (1991). Leaf tissue was frozen in liquid N2 and stored at −80°C until extraction. 0.5 mg of tissue was extracted by grinding in a mortar or with a potter at room temperature after addition of 0.3 ml 87% acetone. The mortar was subsequently rinsed with 100% acetone essentially as described in Thayer and Björkman (1990).

Chlorophyll a-fluorescence measurements

Chl a-fluorescence of leaves was measured using a PAM 101 (Walz, Effeltrich, Germany). To increase sensitivity and decrease fluorescence background which was not originating from leaf chlorophyll, quartz glass extension rods were placed between sample and glass fibers. The fiber for excitation light was separated from the fiber connected to the detection unit (see Figure 1 in: Schreiber et al. 1995; Schreiber 1994). Both excitation and detection were performed at an angle of 45° in respect to the plant tissue surface, and at an angle of 90° between excitation and measuring pathway. For fluorescence analysis, dark-adapted plant material was illuminated at a photon fluence rate of 1150 μmol quanta m−2 s−1 (Fp). An additional pulse of 1200 μmol quanta m−2 s−1 of 1 s duration was applied immediately after the beginning of illumination. The fluorescence yield during the pulse did not exceed the initial fluorescence yield Fp suggesting that all reaction centers had been closed at Fp. The CO2 concentration was about 400 μl l−1. The fluorescence parameters were also measured during steady state photosynthesis after illuminating the samples for 8 min with actinic light. Photosynthesis was activated at 1150 μmol quanta m−2 s−1 for 2.5 min. Measurements were then performed at 170 μmol quanta m−2 s−1 after illumination times of more than 5 min. Light pulses of an intensity of 1200 μmol quanta m−2 s−1 and of 1 s duration were applied every 60 s to transiently determine maximal fluorescence Fm′. Calculations were performed as described by Schneiber and Bilger (1993).

Electron microscopy

Plant material was fixed for one hour in 50% Karnowsky solution containing 2% formaldehyde and 2.5% glutaraldehyde in 50 mM sodium cacodylate buffer (pH 7.2) and postfixed in 50 mM sodium cacodylate buffer (pH 7.2) supplemented with 2% OsO4 for 3 h. Before and after the subsequent overnight incubation in 2% aqueous uranyl acetate the leaf sections were washed five times in water for 3 min each. Dehydration of the tissue in ethanol was followed by transfer into the intermedium propylenoxide (3 × 30 min). For embedding, the tissue was first incubated in propylenoxide / epon (1:1) overnight and then in epon (2 × 2 h + 1 h). Epon was prepared by mixing 4 volumes of solution A (97.1 g Epon 812 + 130.8 g dodecenyl succinic anhydride) with 3 volumes solution B (90 g Epon 812 + 81.37 g methylnadic anhydride) and adding 1.5–2% (v/v) dimethyl amino methyl phenol.

Ultrathin cross sections were stained in 2% uranyl acetate in methanol for about 15 min followed by 50%
Reynolds’ lead citrate solution (Reynolds 1963) for 10 min. Sections were partially destained by consecutive rinsing the grids in methanol, methanol / H2O (1:1) and H2O. Electron microscopy was performed using a Zeiss EM 900 at 80 kV.

Results

Genes involved in photosynthesis are expressed in the basiplast

To characterize gene expression in the basal growing zone of monocotyledoneous leaves, we randomly selected 100 cDNA clones of a basiplast cDNA library, 29 of which were partially sequenced. Three cDNAs were found to encode proteins involved in photosynthesis as deduced from sequence homology or identity. The results suggested that photosynthetic genes are expressed in the basiplast. A clone named HvB069 (Hordeum vulgare basiplast, clone 69) contained exons 4 to 6 of the gene for the 29 kD chlorophyll-binding protein (CP29) reported by Sorensen et al. (1992) (data not shown). HvB036 coded for a yet unknown member of the gene family of the small subunit of ribulose-1,5-bisphosphate carboxylase (data not shown). Nucleic acid and deduced amino acid sequences gave evidence that HvB055 (EMBL Ac. Nr. X89023) encodes the yet unknown barley homologue of a wheat cab gene published by Lamppa and coworkers in 1985. Based on this observation, it seemed interesting to understand the function of photosynthetic genes in the basiplast.

The expressional pattern of these and other photosynthetic genes (psbA: D1 protein; psbE: cytochrome-b559; rbcL: RUBISCO large subunit; triosephosphate isomerase) was analyzed by Northern blot hybridization. Expression in the basiplast was detected for all probes (Figure 1). However, as expected, transcript amounts were much higher in leaf blades. RNA levels were on an intermediate level in the leaf sheath and not detectable in roots. The distribution of mRNA among tissues was similar for LHCP II and CP29. As compared to leaf blade and sheath expression of SSU was particularly low in the basiplast. Expression of the plastome-encoded genes psbA, rbcL and psbF, followed the same pattern. mRNA-levels of the triosephosphate isomerase gene were maximal in roots, still high in the leaf blade and low in the sheath and basiplast. As a control we performed Northern-experiments with probes not related to photosynthesis. Bas1 encodes a 2-Cys peroxidased (Baier and Dietz 1996) and was maximally expressed in the basiplast (Figure 1). Similar results were observed for subunit E of the vacuolar H+-ATPase (Dietz et al. 1995) and for emip which encodes a plasma membrane water channel (Hollenbach and Dietz 1995) (results not shown). The latter results demonstrate that the basiplast is characterized by high transcriptional activity of non-photosynthetic genes.

The expression of photosynthesis-related genes was also studied during ageing of the basiplast (Figure 2). Highest amounts of all transcripts were found in the basiplast of 4-day-old plants. During leaf development, tissue derived from the basiplast until day 6 to 7 after germination differentiates into leaf blade. LHCP II expression or RNA stability decreased after day 6. This coincides with the transition from formation of leaf blade to formation of leaf sheath. The leaf sheath is characterized by low chlorophyll contents and low photosynthetic activity. Expression of LHCP II was low at day 2 due to etiolation of the seedlings which were germinated in the dark for two days before being transferred to the light (Figure 2). The mRNA level detected at day 2 corresponds to the light-independent background expression of cab genes which was similar to that in leaf blades of 8-day-old etiolated plants (data not shown). In contrast to the increased expression of LHCP II, mRNA amounts were high from the beginning for cyt b559, D1-protein and Rubisco subunits and only slightly lower than at day 4. It is interesting that mRNA levels of D1-protein and Rubisco-SSU remained detectable even after the primary leaf had ceased growing 10 days after sowing.

Pigment composition of the basiplast as compared to older leaf segments

Detection of gene expression of chlorophyll binding polypeptides prompted us to analyze the pigment composition of the basiplast. Chlorophylls and carotenoids were determined in various fractions of the leaf. Total pigment contents of the basiplast as related to leaf fresh weight was only 1.2% of the leaf blade (Figure 3A). However, the qualitative composition of pigments was very similar in the basiplast, leaf sheath and leaf blade (Figure 3B).

Pigment composition in basiplasts during organogenesis

As the leaf develops, the basiplast first forms cells which differentiate into leaf blade (day 1–6) and later-
on leaf sheath (day 7–12) before it ceases growing. We analyzed the pigment composition during leaf organogenesis. Chlorophyll $a$ and $b$ revealed highest levels in the basiplast of 4-day-old seedlings (Figure 4A). Of the carotenoids, only neoxanthin followed the same pattern of age-dependent change as the chlorophylls, whereas $\alpha$- and $\beta$-carotene were rather unchanged between day 2 and 4 and then decreased as the leaf developed. In contrast, lutein and the xanthophyll cycle pigments ($V+\ A+Z$) were highest in the basiplast of very young tissue and then continuously declined during aging of the seedling.

Six days after sowing, levels of all analysed pigments were low in the basiplast. From that time on, cells derived from the basiplast form the leaf sheath. Figure 4B depicts the relative pigment composition of basiplasts in dependence of seedling age. In 2-day-old seedlings carotenoids made up almost 70% of the pigments. This relative portion declined to less than 20% in the basiplast of 8-day-old plants.

The development of chloroplasts in the basiplast

The results presented in the previous sections demonstrate that at least part of the molecular basis for chloroplast development is realized in the basiplast. Therefore, we analyzed the structural differentiation of the plastids within the basiplast. Ultrathin sections of basiplasts from 5-day-old barley seedlings were examined in 1 mm intervals. Analysis was focused on plastid size and thylakoid formation (Figure 5). Within the 5 mm region defined as basiplast, longitudinal extensions of plastids increased about twofold from $0.87 \pm 0.05 \mu m$ to $1.76 \pm 0.04 \mu m$ in cross sections. Plastids initially contained up to 4 stroma thylakoids but no grana stacks. Grana stacking started 3 mm above the leaf base. At the upper end of the basiplast stacks of up to 4 grana thylakoid layers were observed. Plastid division started within the 2nd and 3rd millimeter. Thus, plastid division followed cell division in close spatial and temporal sequence. The largest number of dividing plastids was counted in the 3rd mm of the basiplast.
5 mm above leaf base, cells contained already a full set of chloroplasts (55–75 chloroplasts/cell; Klein and Mullet 1986; Robertson and Laetsch 1974; Baumgartner et al. 1989) with a very homogeneous thylakoid system. 21 plastids were distributed in a typical cross section of a subepidermal mesophyll cell at 5 mm distance from the leaf meristem (data not shown). Plastid size varied from 1.2 μm to 2.4 μm. They contained grana stacks of 2 to 4 thylakoids. This compares to fully differentiated chloroplasts in the leaf blade with a size of 6 to 8 μm (Mullet 1988;) and with complex organisation of stroma lamellae and many grana thylakoids per stack (Eriksson et al. 1961).

**Photosynthetic activity in the basiplast**

In order to complement the molecular and structural analysis we investigated photosynthetic activity in the basiplast by means of sensitive chlorophyll a fluorescence measurements and calculation of PS II quantum efficiency. For comparison measurements were repeated along the axis of complete 8-day-old primary leaves. It should be noted that fluorescence measurements in the basiplast where pigment contents were less than 2% of the leaf blade require a highly sensitive setup.
with insignificant background fluorescence. The fluorescence ratio (Fp - Fo/Fp) of dark adapted tissues did not vary from the tip to the middle of the sheath of the primary leaf being close to 0.8 (Figure 6). In fact the fluorescence ratio of 0.8 also indicates that the initial light intensity of 1150 μmol quanta/m² s was saturating or close to saturation and that Fp was close or equal to the maximal fluorescence yield Fm. Webber et al. (1984) determined the same fluorescence parameters at 77 K and found a ratio of less than 0.8. In the lower part of the sheath it decreased considerably to about 0.65 in the basiplast which is represented by the very first data point. There was a small decrease also in the leaf tip which may indicate beginning senescence (Wardley et al. 1984).

Following determination of quantum yield of dark adapted tissues, illumination was continued at 170 μmol quanta m⁻² s⁻¹ which corresponds to the light intensity incident on top of the canopy during growth. Following application of light pulses of high fluence rate, the fluorescence ratio (Fm' - Fo/Fm') was calculated which corresponds to the effective quantum yield (Genty et al. 1989). It represents PS II activity when it is limited by electron consuming reactions of photosynthesis. Light titration of the photochemical fluorescence quenching indicated that the pulse intensity of 1200 μmol quanta/m² s was sufficient to
transiently reduce most or all reaction centers. The fluorescence ratio was highest in the fully expanded part of the leaf blade (8–13.5 cm) while it declined in its youngest not completely expanded part (5.5–8 cm) as well as in the leaf sheath (0–5.5 cm). Minimal values were obtained in the basiplast (0–0.5 cm). However, it is important to note that a fluorescence ratio of 0.17 still indicates photosynthetic activity in the basiplast. Obviously the photosynthetic apparatus is at least to a certain extent functional and active. We also detected strong light induced non-photochemical fluorescence quenching which was rapidly reversible in the dark, indicating a build-up of a proton gradient across thylakoid membranes.

PS II fluorescence in the basiplast in dependence of leaf age

As last step of our analysis, the fluorescence parameters were determined in the basiplast in dependence of seedling age. The fluorescence parameter $(F_p - F_o)/F_p$ of dark-adapted segments was not much different in the basiplast of young versus old seedlings (Figure 7). The ratio of $(F_m' - F_o)/F_m'$ during steady state photosynthesis was about 0.3 in basiplasts of 3- to 6-day-old seedlings and dropped to values between 0.19 and 0.12 in the basiplast of 8- to 12-day-old seedlings. This decrease coincides with the transition of leaf blade to leaf sheath formation.

Discussion

In typical monocotyledonous plants, such as barley, leaf cells derive from a meristem at the leaf base. Therefore, monocot leaves have a well defined gradient of young tissue at the base and old tissue at the tip (Sharman 1942). Accordingly, monocot leaves have become a model system for studying leaf development (for instance: Baker and Leech 1977; Baumgartner et al. 1989; Boffey and Leech 1982; Dean and Leech 1982). However, despite a large number of detailed investigations during the last years, several aspects of plastid development in the basal zone still need clarification, some of which are addressed in this paper. In fact, the main problem seems to lie in some species and growth specific variation of plastid development. Therefore, the data compiled from different plants and laboratories are often not directly comparable and hence, do not sum up to a complete picture of plastid development in the basiplast. In this investigation we have aimed at resolving this problem using a wide range of techniques for studying the same object.
Expression of photosynthetic and nonphotosynthetic genes in the basiplast. A defined portion of the cDNAs randomly selected from our basiplast cDNA library coded for proteins involved in photosynthesis. This demonstrated that a considerable part of the transcriptional and presumably translational activity of the basiplast is already directed towards development of the plastids. Our approach to describe the developmental processes in the basal growing zone of barley leaves is not restricted to photosynthesis. We have demonstrated high levels of expression of structural and metabolic proteins, for instance a water channel (Hollenbach and Dietz 1995), a subunit of the tonoplas ATPase (Dietz et al. 1995) and a peroxide reductase (Baier and Dietz 1996). On a fresh weight basis, the basiplast had 2–3 times the nucleic acid contents of mature leaf tissue (Ougham and Davies 1990; Baier and Dietz, unpublished). This may partly be due to the smaller cell size, but also reflects the strong expression of nonphotosynthetic genes. Therefore, in the light of an enhanced overall expression, the observed expression of genes for photosynthetic components may be an underestimate of the absolute investment in the development of photosynthesis in the basal zone of leaves as compared with the expanded leaf blade. Obviously, tissue specific factors required as signals for chloroplast development were sufficiently active to initiate plastid development although the volume of the chloroplast compartment per cell was only small as compared to that in mature mesophyll cells where chloroplasts represent a large volume fraction of the cytoplasm, for instance 28% in barley (Winter et al. 1993).

Variations in photosynthetic gene expression in the basiplast among species. 3 mm apical from the base, the meristematic cells differentiate into the cells of the epidermis, the mesophyll and the leaf bundles (Dannenhoffer and Evert 1994; Baier and Dietz, unpublished). Simultaneously, the development of the photosynthetic machinery takes place. Based on the results of other groups (Webber et al. 1984; Mullet 1988; Ougham and Davies 1990; Gay and Thomas 1995) we attempted a more refined temporal and in part spatial analysis of the changes in the basiplast during the phases of leaf expansion. Up to now, usually either sections of fully expanded leaves were analyzed (Baker and Leech 1977; Ougham and Davies 1990), or two stages of leaf development were compared (Webber et al. 1984; Krupinska 1992). Already in meristematic cells generating mesophyll cells, plastid size is larger than in non-leaf meristems (Miyamura et al. 1986). Analyses of leaf sections from the base to the tip have shown expression of various photosynthetic genes beginning in the second and third section corresponding to a distance of 0.5 to 1.5 cm from the scutellum (Ougham and Davies 1990). In our system, expression of photosynthetic genes was detected even in the lowest section of expanded leaves of 8-d-old seedlings, corresponding to a distance of 0 to 0.5 cm to the scutellum. In fact all probes tested, i.e. genes for nuclear and plastome encoded proteins such as CP29, LHCP II, D1, cytochrome-b559, and RUBISCO-SSU and LSU, showed a significant transcript level in the basal five millimeters of the leaves where the transition of blade to sheath formation had already occurred. Expression of cab and rbcS genes was also observed in the basal centimeter of the expanding fourth foliage leaf of maize (Martineau and Taylor 1985). However at that stage of development the maize leaf meristem still forms leaf blade and is therefore comparable with basiplasts of 4 to 6-day-old barley seedlings. There seems to exist a species-specific variation of plastid development which is further modulated by environmental factors, particularly light. In our experiments, the photon fluence density at the top of the canopy was about 170 µmol m−2 s−1 and the seedling density high which means that the photon fluence was very low at the base of the leaves. Nevertheless, plastid development was faster than in other species such as Lolium grown at the higher irradiance of 350 µmol m−2 s−1 (Ougham and Davies 1990). The changes in expression during ageing of the basiplast were similar for the nuclear encoded Rubisco-SSU and for the plastome encoded D1 protein.

Variation in photosynthetic activity and pigment composition of the basiplast. Webber et al. (1984, 1986) reported a detailed analysis of wheat leaf development. When comparing their and our study, chlorophyll contents per unit leaf area changed similarly along the axis of an 8-day-old leaf of wheat and barley (Figure 4). In wheat, the chlorophyll a to b ratio was 3.4 at the base and 2.9 at the tip. In our experiment the corresponding data were 3.5 and 3.4, respectively, showing that both approaches were highly comparable. However there was a major difference between 4-day-old wheat and barley leaves. Usually, illumination with actinic light causes PS II-dependent chlorophyll a fluorescence to increase to an initial maximum followed by a decrease to a lower steady state level. At high light intensities, the decrease in chlorophyll a fluorescence is mainly due to non-photochemical quenching (NPQ).
Photosynthetic activity along the acropetal axis of basiplast. The leaf blade or leaf sheath is characterized by clearly distinguishable properties of the chloroplasts in the first time that the differentiation of leaf tissue to the two sets of data on chlorophyll a fluorescence, i.e. on the different tissue sections from the tip to the base is related to functionality of the photosynthetic apparatus at this early stage of leaf development. Further on, the two sets of data on chlorophyll a fluorescence, i.e. on the different tissue sections from the tip to the base on the one hand, and in the basiplast in dependence of leaf age on the other hand, clearly demonstrate for the first time that the differentiation of leaf tissue to the leaf blade or leaf sheath is characterized by clearly distinguishable properties of the chloroplasts in the basiplast.

Baker and Leech (1977) observed a delay in CO₂ fixation as compared to O₂ evolution when analysing photosynthetic activity along the acro-petal axis of maize leaves. The apparently higher relative expression in the basiplast of genes for components of the thylakoid membrane than for Rubisco SSU may fit this picture of a slightly delayed development of Calvin cycle reactions. Expression of nuclear and plastidic encoded photosynthetic genes seems not yet well coordinated at this early stage of plastid development. Additional evidence for this conclusion comes from run-on-transcription experiments (Krupinska 1992) which revealed that regulation of gene expression of proplastids and young chloroplasts is only partly regulated on the level of transcription.

Similarity between relative pigment composition in basiplast and other leaf segments. The basiplast contained the whole set of pigments required in the photosynthetic apparatus. The large quantitative difference in pigment contents between leaf blade and basiplast has frequently been described (e.g. Boffey et al. 1980; Webber et al. 1984). However, it was striking to see the similarity in relative pigment composition of basiplast and leaf blade. Assembly and stabilization of LHC II and PS II require chlorophyll a, chlorophyll b, lutein, violaxanthin and neoxanthin (Paulsen et al. 1990; Kühlbrandt et al. 1994). The chlorophyll a/chlorophyll b-ratio was constant, similarly to other studies (Baker and Leech 1977). But, in addition, we show that all pigments essential for the formation of functional antennas are present in barley basiplasts. Lutein and V+A+Z are present in relative excess in respect to chlorophyll comparable to etiolated tissues (Pfündel and Strasser 1988).

Spatial pattern of structural plastid differentiation. In many studies, the differentiation of plastids was investigated in large leaf sections of 1 cm length or more which only provides a coarse picture of the developmental processes in the basiplast (Baker and Leech 1977; Webber et al. 1984). We show that development proceeds within a well-defined program so that each millimeter is characterized by a specific structural state of plastids. In primary leaves of 5-day-old barley seedlings the zone of plastid division was restricted to the 2nd and 3rd millimeter of the leaf. In 5 mm distance from the leaf base the plastids were already very homogeneous as far as thylakoid organisation is concerned, although their size still varied by a factor of up to 2. Grana stacking is not yet completed. During further development of the leaf the average appression of the thylakoids will increase by a factor of 2 (Robertson and Laetsch 1974). Plastid size and plastid volume increase during leaf maturation, the latter by a factor of about four. Both processes contribute to the higher pigment contents of the leaf blade.

Changes in the basiplast as indication of leaf development. Chloroplast development in the basiplast changes during leaf development. Thylakoids are less stacked in the basiplast of young leaves (Webber et al. 1984). PS I- and LHC-PS II-complexes are more randomly distributed in thylakoid membranes. LHCP II transcript amounts were highest in basiplasts of 4-day-old seedlings and decreased in the basiplast of older leaves. An almost identical role was observed for the chlorophyll content. All other pigments were also present to build up functional photosystems. Chlorophyll fluorescence kinetics indicated that the chloroplasts in the basiplast of 4-day-old seedlings are capable to efficiently perform the photochemical reactions.
of photosynthesis even at elevated light intensities. According to the model proposed by Webber et al. (1984), the excitation energy is partitioned between PS I and PS II in order to protect PS II. In conclusion, three phases of plastid development in the basiplast are to be distinguished on the basis of photosynthetic parameters:

(1) day 3–6: Growth rate and expression of photosynthetic genes is highest. Effective quantum yield is between 0.25 and 0.27. The basiplast forms cells of the later photosynthetically highly active leaf blade.

(2) day 8–11: The basiplast is still active and forms leaf sheath, however at a decreasing rate. Effective quantum yield is between 0.14 and 0.19.

(3) day 12 and older: The basiplast ceases to grow and becomes part of the leaf sheath only. Effective quantum yield drops below 0.13.

In a model previously suggested by Mullet (1993), distinct zones of DNA, replication RNA transcription, photosynthetic protein synthesis and light induced transcription are distinguished. It is obvious from our and previous results that this model only gives the rough picture of plastid development in the basiplast and needs some refinement. The developmental activities attributed to distinct zones in the Mullet model take in fact place in close temporal and spatial vicinity within the basiplast. As a consequence, the basiplast contains always functional chloroplasts, even after cessation of leaf growth. In relation to the questions posed in the introduction, we conclude for barley that (i) expression of photosynthetic genes in the basiplast is directly related to photosynthetic activity, (ii) the basiplast contains the full complement of pigments required for photosynthesis in relative composition similar to the leaf blade, and (iii) chloroplast development in the basiplast indeed reflects the developmental transitions of the leaf tissue.

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