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Light Alters Cytosolic and Plastidic Phosphorylase Distribution in Pearl Millet Leaves[1]

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In pearl millet (*Pennisetum americanum*) seedlings, although the cytosolic phosphorylase was present in all organs, the plastidic phosphorylase was restricted to the leaf. Intercellular fractionation of the leaf revealed that cytosolic and plastidic phosphorylase were localized in the mesophyll and bundle-sheath cells, respectively. In dark-grown leaves phosphorylase activity increased from the leaf base to the tip. The dark-grown leaves possessed both cytosolic and plastidic phosphorylase isoforms; however, their distribution followed different patterns along the length of the leaf. The plastidic phosphorylase level increased from the base to the tip of the leaf, and the cytosolic phosphorylase level was higher in the basal half of the leaf and declined toward the tip. In light-grown leaves phosphorylase activity was higher in a region near the leaf base and declined toward the leaf tip. Light stimulated cytosolic phosphorylase level and repressed plastidic phosphorylase level, resulting in an altered distribution of the respective phosphorylase isoforms along the length of the light-grown leaf. Since pearl millet leaf possesses a cell maturity and chloroplast development gradient from the leaf base to the tip, the inverse effect of light on cytosolic and plastidic phosphorylase levels might have been determined by its interaction with these gradients.

Among the environmental factors controlling plant growth and development, light plays a major role by regulating several morphogenic processes throughout the life cycle of plants (Kendrick and Kronenberg, 1993). One of the important processes controlled by light is the biogenesis of chloroplasts and associated enzymes, leading to the acquisition of photosynthesis in the leaves (Nelson and Langdale, 1992). Thereafter, light plays a dual role in regulating photosynthesis, first by providing energy to fuel the process of photosynthesis, and second by regulating the activity of several enzymes participating in the photosynthesis and associated metabolic pathways, such as generation and degradation of carbohydrates (Beck and Ziegler, 1989). Although it is known that the surplus carbohydrates generated during photosynthesis are stored in the form of transitory starch in chloroplasts, the regulation of enzymes participating in starch synthesis and degradation has not been fully elucidated (Beck and Ziegler, 1989). It is assumed that the mobilization of transitory starch is brought about by the action of several starch-degrading enzymes, such as amylases, α-glucosidase, phosphorylases, and debranching enzyme present in the leaf cells (Steup, 1988).

Although the activity of phosphorylase has been detected in many starch-containing tissues, only a small amount of information is available regarding its metabolic and developmental regulation, particularly in the leaf cells. Leaf phosphorylase has been purified from a few plant species, such as maize (Mateyka and Schnarrenberger, 1984, 1988), spinach (Preiss et al., 1980; Steup et al., 1980; Steup and Schächtele, 1981), and pea (Conrads et al., 1986; Berkel et al., 1991). Biochemical evidence has indicated that the phosphorylase consists of two physicochemically distinct molecular species encoded by two distinct genes (Brisson et al., 1989; Nakano et al., 1989), which are also located in two distinct cellular compartments: the cytosol and the plastid, respectively (Conrads et al., 1986; Schächtele and Steup, 1986). In potato leaf, the plastidic phosphorylase exists as a homodimeric protein with 104-kD subunits and is antigenically identical to a major phosphorylase form present in the tuber. Likewise, the minor potato tuber phosphorylase is antigenically identical with the cytosolic phosphorylase of leaves and is a monomer of 94 kD (Nakano et al., 1989). The plastidic and cytosolic phosphorylase represent two antigenically distinct enzymes, and they do not cross-react with each other (Conrads et al., 1986; Steup and Schächtele, 1986).

The regulation of phosphorylase activity in the leaves, particularly during leaf development, has not been investigated in detail. In spinach leaf, the activity of cytosolic phosphorylase increases 4- to 8-fold after 35 d from the sowing and then declines to the initial level, whereas the level of plastidic phosphorylase remains nearly the same (Hammond and Preiss, 1983). In pea cotyledons, the amount of cytosolic and plastidic phosphorylases depends on the developmental state of the organ. During seed development, the plastidic phosphorylase is a major enzyme, but during germination, although the level of plastidic enzyme remains constant, the level of cytosolic enzyme increases significantly (Steup et al., 1986; Berkel et al., 1991). Moreover, pea chloroplasts possess two types of plastidic phosphorylases: one in the etioplasts and another that appears only after photoinduction of chloroplast biogenesis (Steup et al., 1986; Berkel et al., 1991). In pea leaves, both phosphorylase isoforms coexist in the same cell, and in C_4 maize leaves, phosphorylases are located at two

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distinct cellular locations, with the cytosolic being confined to the mesophyll cells and the plastidic in the bundle-sheath chloroplasts (Mateyka and Schnarrenberger, 1984, 1988).

In the present study we investigated the effect of light on the development of phosphorylase activity in pearl millet (*Pennisetum americanum*) leaf, a C_4 plant. We show that pearl millet leaf possesses cytosolic and plastidic phosphorylase in the mesophyll and the bundle-sheath cells, respectively. We also show that light alters the distribution profiles of these phosphorylases along the length of the leaf.

MATERIALS AND METHODS

Plant Materials and General Methods

Pearl millet (*Pennisetum americanum* var WCG.75) seeds, obtained from the Andhra Pradesh State Seed Corporation (Hyderabad, India), were used in this study. Seeds were sown on germination paper moistened with distilled water, and the seedlings were grown at 25°C either under continuous red light (transmission maxima of light output 650 nm, 0.67 W m^{-2}) or in darkness. From 3 d until 10 d from sowing, one set of seedlings was harvested every day, and the first leaves (outermost leaf) were carefully removed and used for the estimation of phosphorylase activity. To estimate the distribution of phosphorylases along the length of the leaf, the first leaf was excised into 1-cm-long segments successively from the base to the tip of the leaf. The segments were numbered from the base to the leaf tip. In case of Norflurazon (Sandoz, Basel, Switzerland) treatment, seedlings were grown in the Norflurazon (0.4 mm) solution from the time of sowing in red light or in darkness.

The procedures that were used for the isolation of chloroplasts and etioplasts from leaves (Palmer, 1986), isolation of mesophyll protoplasts (Day et al., 1981), and mechanical isolation of bundle-sheath strands (Chollet and Ogren, 1973) were nearly the same as those described earlier (Vally and Sharma, 1991).

Phosphorylase Assay

Phosphorylase activity was measured using a coupled photometric enzyme assay following the protocol described by Steup and Latzko (1979) with a few modifications. One gram of tissue (seed, leaf, root, coleoptile) was homogenized in a precooled mortar and pestle on ice along with an equal amount of sea sand in 5 mL of 50 mM K-phosphate buffer, pH 7.5. The homogenate was centrifuged for 30 min at 25,000*g* at 4°C, and an aliquot from the clear supernatant was used for the phosphorylase assay. The assay mixture contained, in a final volume of 5 mL, 100 mM Hepes, pH 7, 50 mM K-phosphate buffer, pH 7, and 15 mg of amylose, and the assay was carried out for 15 min at 30°C. The aliquots were withdrawn at the beginning and at the end of the assay and were boiled at 100°C for 4 min. After cooling, the denatured protein was removed by centrifugation at 1,000*g* for 30 s. Thereafter, an aliquot was withdrawn from the supernatant to determine the amount of Glc-1-P formed by the coupled enzyme assay.

The aliquot (0.1 mL) was assayed in a 1-mL assay mixture containing 30 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 1.8 mM NADP, 1.45 units of phosphoglucomutase (Sigma), and 0.15 units of Glc-6-P dehydrogenase (Sigma). The reaction was initiated by the addition of NADP and, thereafter, the increase in A_{340} due to NADPH formation was measured. The phosphorylase activity was calculated by using the NADPH molar extinction coefficient (6.2 mm^{-1} cm^{-1}). The amount of protein in the samples were measured by the Bradford (1976) and Lowry et al. (1951) methods.

Native PAGE

Native PAGE was performed using the electrophoretic buffer system of Davis (1964) in 7.5% slab gels. Phosphorylase activity was visualized by incubating polyacrylamide gels in a mixture that favored biosynthesis of starch by providing amylose as primer. After electrophoresis, the gel was washed for 30 min with 50 mM citrate buffer, pH 6, for 30 min and incubated at 30°C in a mixture containing 28.5 mM Glc-1-P (dipotassium salt), 0.1% (w/v) amylose, and 71.5 mM Na-citrate, pH 5.5, for 3 h. The incubation medium was also supplemented with 20 mM NaF, 0.3 mM $HgCl_2$, and 0.1 mM ammonium molybdate to inhibit the amylase and phosphatase activity present in the gel. At the end of the incubation period the gel was washed with distilled water for 1 h to remove any adhering amylose. Phosphorylase bands were visualized by staining gel with iodine solution (24 mM KI and 1 mM iodine) for formation of starch. The phosphorylase isoforms were visualized as deep violet bands against the light-blue-stained gel.

RESULTS

In 7-d-old light-grown seedlings, phosphorylase activity was present in all organs, such as root, seed, coleoptile, and leaf. Maximal phosphorylase activity was detected in the leaf and coleoptile, and phosphorylase activity in the seed and root was nearly 3 times less than that in the leaf (Fig. 1). Native PAGE of phosphorylase isoforms from these organs revealed that a single phosphorylase isoform (R_F 0.24) was ubiquitously present in all organs except the leaf, which possessed an additional phosphorylase isoform with higher mobility (R_F 0.28) (Fig. 1, inset).

The possible localization of leaf-specific phosphorylase isoform in chloroplasts was investigated by growing seedlings in the presence of Norflurazon, a chlorosis-inducing herbicide (Henson, 1984). Both dark-grown and light-grown seedlings possessed two phosphorylase isoforms in the leaves (Fig. 2A). In Norflurazon-treated light-grown leaves, the fast-migrating isoform of phosphorylase was absent, and the leaves retained only the slow-migrating phosphorylase isoform (Fig. 2A, lane 2). By contrast, Norflurazon treatment did not affect the phosphorylase isoform profile of dark-grown leaves. Since Norflurazon treatment damages chloroplasts in light-grown leaves, leading

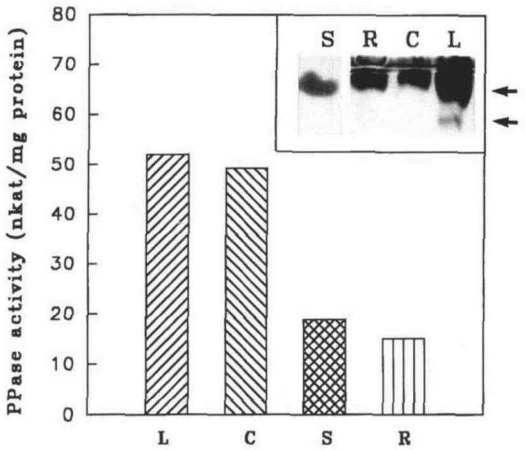

Figure 1. Profile of phosphorylase (PPase) distribution in pearl millet seedlings. Phosphorylase activity was determined in different organs of 7-d-old dark-grown pearl millet seedlings. The inset shows the phosphorylase isoforms present in different organs of pearl millet seedlings. L, Leaf; R, root; C, coleoptile; S, seed.

to the loss of the activity of several plastidic enzymes (Oelmüller, 1989), it is evident that the missing phosphorylase isoform (R_F 0.28) is localized in the chloroplast. Because the level of the ubiquitous phosphorylase isoform is unaffected by the Norflurazon treatment, it is likely to be located in the cytosol.

Pearl millet, being a C_4 plant, possesses transitory starch mainly in the bundle-sheath chloroplasts. Therefore, it is reasonable to expect a predominant localization of plastidic phosphorylase in the bundle-sheath cells. Therefore, we analyzed the intercellular distribution of phosphorylase in isolated mesophyll and bundle-sheath cells. Figure 2B shows that phosphorylase activity can be detected both in the mesophyll and bundle-sheath cells. In mesophyll cells phosphorylase appears to be solely localized in the cytosol, because the isolated mesophyll chloroplasts are totally devoid of phosphorylase activity. By contrast, in bundle-sheath cells phosphorylase seems to be located only in the chloroplasts, since more than 95% phosphorylase activity of the bundle-sheath cell homogenate is recovered in the chloroplasts. Analysis of phosphorylase isoforms from mesophyll and bundle-sheath cells confirmed the above observations. Native PAGE showed that mesophyll cells contained only the slow-migrating phosphorylase isoform. No phosphorylase isoform could be detected in the chloroplasts isolated from the mesophyll cells (Fig. 2B, inset). On the other hand, the bundle-sheath cells and the chloroplasts isolated from them possessed only the fast-migrating phosphorylase (Fig. 2B, inset). These results clearly indicate that the slow- and fast-migrating phosphorylase isoforms are located in the cytosol and the plastids of the mesophyll and the bundle-sheath cells, respectively.

The specific localization of phosphorylase activity in bundle-sheath chloroplasts is further supported by the observation that bundle-sheath cells isolated from Norflurazon-treated light-grown leaves were completely devoid of phosphorylase activity (Fig. 2C). On PAGE no plastidic phosphorylase isoform was detected in these bundle-sheath cells. However, the appearance of plastidic phosphorylase activity in bundle-sheath cells was independent of chloroplast biogenesis. Phosphorylase activity was also detected in bundle-sheath cells isolated from the dark-grown leaves (Fig. 2C) and also in the etioplasts isolated from bundle-sheath cells (data not shown).

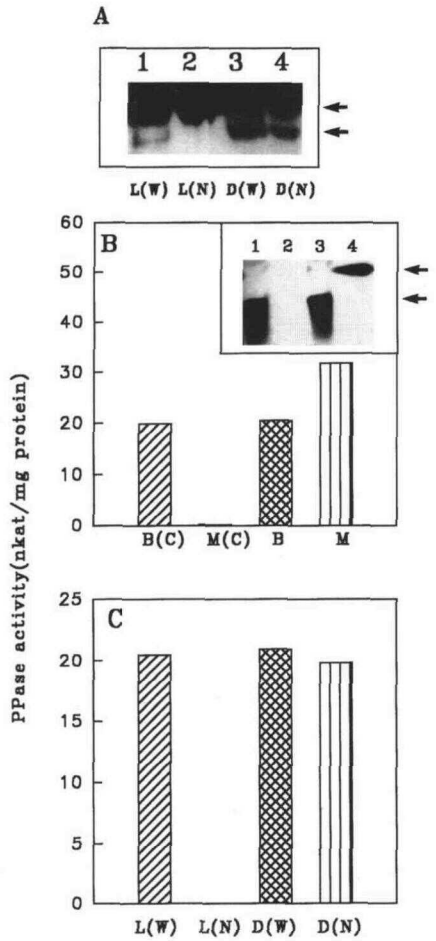

Figure 2. Phosphorylase (PPase) activity and isozymes present in leaves and in different cellular fractions isolated from them. A, Seedlings were grown for 7 d in red light (L) or darkness (D) in the presence of either distilled water (W) or 0.4 mM Norflurazon (N) solution right from sowing. The first leaf was harvested and the phosphorylase isoforms were determined by PAGE. Lane 1, L(W); lane 2, L(N); lane 3, D(W); lane 4, D(N). B, The leaves from 7-d-old light-grown seedlings were fractionated to mesophyll and bundle-sheath cells as described in "Materials and Methods." The isolated bundle-sheath cells (B) and mesophyll cells (M) were homogenized and the phosphorylase activity and the isoform profiles were determined. The chloroplasts (C) were isolated from mesophyll and bundle-sheath cells, respectively, and phosphorylase activity and isoform profiles were determined. Inset, The phosphorylase isoforms present in the respective cell fraction homogenate and chloroplasts isolated from them. Lane 1, Bundle-sheath chloroplast [B(C)]; lane 2, mesophyll chloroplast [M(C)]; lane 3, bundle-sheath cells (B); lane 4, mesophyll cells (M). C, Phosphorylase activity in bundle-sheath cells isolated from the leaves of dark- and light-grown seedlings grown in the presence and absence of Norflurazon. Seedlings were grown for 7 d in red light (L) or darkness (D) in the presence of either distilled water (W) or 0.4 mM Norflurazon (N) solution right from sowing.

The effect of light on phosphorylase activity was determined by analyzing the phosphorylase activity in leaves during 3 to 10 d of seedling growth. Figure 3 shows that phosphorylase activity in leaves increases with age, reaching a peak on the 7th d after sowing, and it then declines. Since both light-grown and dark-grown leaves show identical profiles of phosphorylase activity, there is no apparent photostimulation or photoinhibition of phosphorylase activity in the leaves. However, the observed time course of phosphorylase activity reflects a sum of activity of both plastidic and cytosolic phosphorylase, which might have responded differentially to light treatment.

The analysis of phosphorylase activity along the length of dark-grown and light-grown leaves revealed that light does regulate phosphorylase activity, but its effect is restricted to specific regions of the leaf. Figure 4A shows that in the dark-grown leaves phosphorylase activity was low in the leaf base and progressively increased toward the tip, with phosphorylase activity at the tip being nearly 2-fold higher than activity at the base. By contrast, in light-grown leaves phosphorylase activity was significantly higher in the region 1 to 3 cm away from the leaf base, beyond which it declined toward the tip (Fig. 4B). It is interesting that it was found that dark-grown and light-grown leaves possessed nearly equal phosphorylase activity, i.e. 3.02 and 3.31 nkat/mg protein, respectively, when the phosphorylase activity of all segments was pooled and represented for the whole leaf. In fact, this equivalence in total phosphorylase activity of the whole leaf masks the specific effect of light on stimulation of phosphorylase activity, which is clearly evident in the region close to the leaf base. It is self-evident from these results that the use of the whole leaf as a unit to investigate the effect of light leads to the false impression that light does not influence the phosphorylase activity.

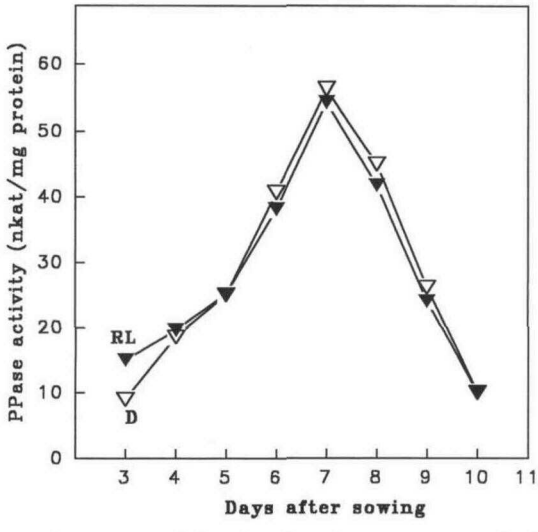

Figure 3. Time course of phosphorylase (PPase) activity in the leaves of pearl millet seedlings. Seedlings were grown in continuous red light (▼) or in continuous dark (▽) from the time of sowing. At the time points indicated phosphorylase activity was assayed in the first leaf of the seedlings.

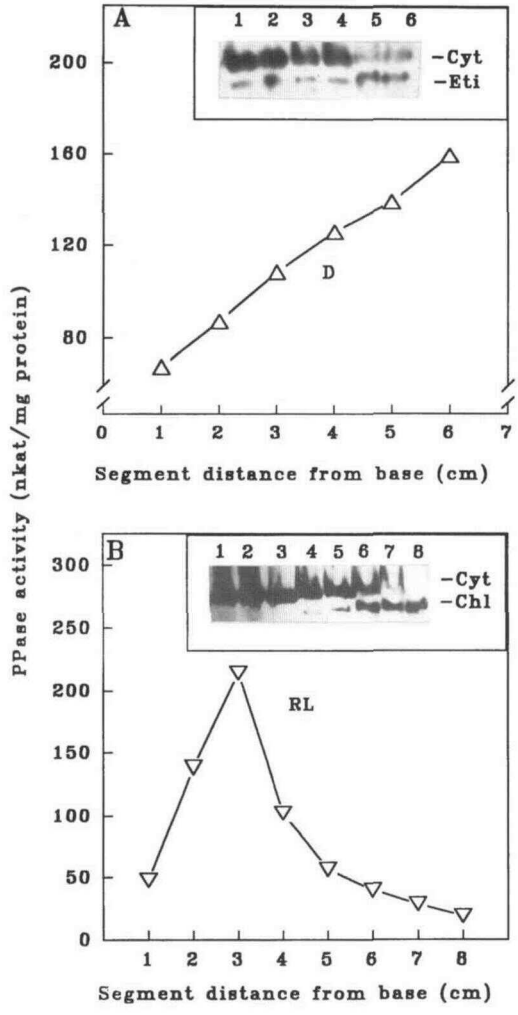

Figure 4. Distribution of phosphorylase (PPase) activity along the length of the first leaf. Seedlings grown for 7 d in continuous darkness or red light were harvested, and the first leaves from them were excised at the mesocotyl junction. The leaves were then excised into 1-cm-long segments. Segments are numbered from the base (1) to the leaf tip. The lane numbers on the top of the gel correspond to the segment number with reference to the leaf base. A, Distribution of phosphorylase activity along the length of dark-grown leaves. Inset, The profile of cytosolic (Cyt) and plastidic (Eti) isoforms along the length of dark-grown leaves. B, Distribution of phosphorylase activity along the length of light-grown leaves. Inset, The profile of cytosolic (Cyt) and plastidic (Chl) isoforms along the length of light-grown leaves.

PAGE analysis of phosphorylase isoforms revealed that light diversely influenced plastidic and cytosolic phosphorylase distribution along the leaf length. The dark-grown leaves possessed both cytosolic and plastidic phosphorylase isoforms throughout their lengths, but the distribution of these isoforms followed different patterns along the length of the leaf. The plastidic phosphorylase level increased from the base to the tip of the leaf, and cytosolic phosphorylase level was higher in the basal half of the leaf and declined toward the tip (Fig. 4A, inset). Light repressed the level of plastidic phosphorylase in the basal region of the leaf. Its level was below the detectability limit

in the region up to 3 cm from the leaf base, and thereafter its level gradually increased toward the leaf tip (Fig. 4B, inset). By contrast, light significantly stimulated cytosolic phosphorylase level in the basal half of the leaf, whereas its level declined in the upper half toward the tip of the leaf. It is interesting that in the same leaf, plastidic phosphorylase could not be detected in the lower half, and cytosolic phosphorylase was absent in the region close to the tip (Fig. 4B, inset).

The above results indicate that the effect of light on cytosolic and plastidic phosphorylase levels follows an inverse pattern and that this effect is mainly observed in the basal half of the leaf. We took advantage of spatial separation of phosphorylase isoforms in mesophyll and bundle-sheath cells in order to individually assay the activity of the respective phosphorylase isoforms. Phosphorylase activity was assayed after separating mesophyll and bundle-sheath cells from individual segments from 7-d-old light-grown pearl millet leaves (Fig. 5). The results obtained clearly show that in bundle-sheath cells of light-grown leaves, the plastidic isoform is absent in the basal half of the leaf and its level increases gradually toward the tip of the leaf. Similarly in the mesophyll cells, the level of cytosolic phosphorylase is higher in the region 1 to 3 cm from the leaf base and declines toward the leaf tip. Moreover, the profiles of phosphorylase activity are closely related to the level of the phosphorylase isoforms present in the respective group of cells. The similarity between the profile of phosphorylase activity and level of cytosolic isoform in light-grown leaves and the profile of phosphorylase activity and isoform observed for isolated mesophyll cells, clearly shows that the cytosolic phosphorylase is the predominant phosphorylase species in pearl millet leaf. These results also support the argument that light inversely influences the cytosolic and plastidic phosphorylase levels, leading to the altered phosphorylase distribution observed in the light-grown leaves (Fig. 5).

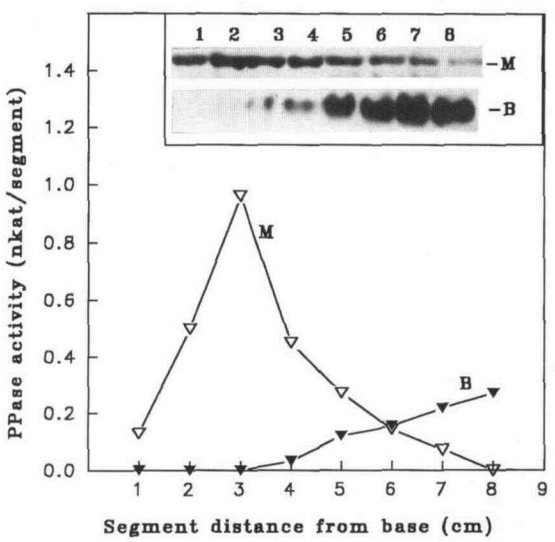

Figure 5. Distribution of phosphorylase (PPase) activity in bundle-sheath (B) and mesophyll (M) cells isolated from different segments of the leaf. Seedlings grown for 7 d in red light were harvested and the first leaves were excised at the mesocotyl junction. The leaves were then excised into 1-cm-long segments and used for the isolation of bundle-sheath and mesophyll cells. Segments are numbered from the base (1) to the leaf tip. Inset, The profile of phosphorylase isoforms present in isolated bundle-sheath and mesophyll cells. The cytosolic and plastidic phosphorylases were localized in mesophyll and bundle-sheath cells, respectively. The lane numbers on the top of the gel correspond to the segment number with reference to the leaf base.

DISCUSSION

In pearl millet seedlings, leaves were found to possess two phosphorylase isoforms, whereas only one phosphorylase isoform could be detected in other organs. The existence of a leaf-specific phosphorylase isoform indicates that this additional phosphorylase isoform is probably localized in the plastids. The notion that the leaf-specific phosphorylase isoform is localized in the chloroplasts is strongly supported by the observation that the Norflurazon-treated light-grown seedlings, which lack functional chloroplasts, also lack the additional phosphorylase isoform. Since Norflurazon treatment does not affect the level of the ubiquitous phosphorylase isoform, it is likely to be located in the cytosol. In contrast to pea, which has both cytosolic and plastidic phosphorylase isoforms in the root (Steup and Latzko, 1979) and etiolated shoots (Steup et al., 1986), in pearl millet the organs other than the leaf lack the plastidic phosphorylase isoform. It is interesting that the appearance of plastidic phosphorylase in leaves is independent of light-regulated chloroplast biogenesis, since it is present in the dark-grown leaves and the etioplasts isolated from them.

Distribution of the respective phosphorylase isoforms in pearl millet leaves seems to be determined by the positional signal associated with the development of Kranz anatomy and affiliated biochemical specialization (Nelson and Langdale, 1989, 1992), with specific localization of cytosolic and plastidic phosphorylase in mesophyll and bundle-sheath cells, respectively. Native PAGE of phosphorylase from homogenates of isolated bundle-sheath and mesophyll cells clearly shows that each of these cell types possessed a specific isoform of phosphorylase. Moreover, the plastidic phosphorylase isoform could be detected only in the chloroplasts or etioplasts isolated from the bundle-sheath cells. It could not be detected in the mesophyll chloroplasts. Likewise, the cytosolic phosphorylase isoform was restricted solely to mesophyll cells. The above intercellular distribution of phosphorylases appears to be a characteristic feature of C_4 plants, since phosphorylase isoforms are also similarly distributed in mesophyll and bundle-sheath cells in maize (Matekya and Schnarrenberger, 1984, 1988).

Although the influence of light on the regulation of several enzymes in leaves has been extensively studied (Thompson and White, 1991), information on light effect on phosphorylase regulation is sparse. In maize, continuous illumination of leaves triggered a significant increase in phosphorylase activity both in the mesophyll and bundle-sheath cells (Downton and Hawker, 1973). In the present study, pearl millet leaves isolated from dark- and light-

grown seedlings showed nearly equal phosphorylase activity during the 3 to 10 d period of seedling growth, although the same treatment stimulated amylase activity by severalfold (Vally and Sharma, 1991).

It is evident from results obtained that although the phosphorylase activity in the different segments of the leaf is diversely influenced by light along the length of the leaf, when the whole leaf is considered the activity is nearly equal. Analysis of phosphorylase activity in the segments excised from dark- and light-grown leaves clearly shows that the distribution of phosphorylase activity in these leaves is notably different. Evidently, light alters phosphorylase distribution in a leaf by specifically stimulating phosphorylase activity in the basal half.

Native PAGE of phosphorylase isoforms along the length of the light-grown leaf highlights the fact that plastidic and cytosolic isoforms of phosphorylase respond differentially to light. Basically, the distribution of the respective phosphorylases in leaves seems to be governed by a cell maturity gradient, since pearl millet leaf possesses younger cells at the leaf base and older cells at the leaf tip; this is typical of monocots (Sherman, 1942). The fact that the cell maturity gradient influences the respective phosphorylases in a differential fashion is clearly evident in the dark-grown leaves, where the level of cytosolic phosphorylase is higher in the basal half of the leaf and declines toward the leaf tip. On the other hand, the plastidic phosphorylase level increases from the base of the leaf to the leaf tip. Moreover, the influence of light on the phosphorylases is also related to the cell maturity gradient. Exposure to light drastically alters the above distribution of phosphorylase by its diametrically opposite action on cytosolic and plastidic phosphorylases. Light appears to play a dual role, particularly in the basal region of the leaf, where it suppresses the plastidic phosphorylase level and stimulates the cytosolic phosphorylase level. In the apical half of the leaf, however, light is ineffective in suppressing the plastidic phosphorylase level. These results also highlight the fact that the effect of light on phosphorylase isoform is primarily determined by their cellular location along the leaf length, and light acts as a modulating agent on this pattern of cell maturity.

In etiolated pea seedlings, which possess a plastidic phosphorylase isoform in etioplasts, exposure to light induces formation of an additional phosphorylase isoform, whose appearance is specifically associated with chloroplast development (Steup et al., 1986). Our results in pearl millet show that light suppresses the plastidic phosphorylase isoform in the leaf base, although it can be detected in the apical half of the leaf. Whether the plastidic phosphorylase isoforms present in dark-grown and light-grown pearl millet leaves are the same or different needs further investigation. In light-grown leaves the appearance of chloroplastic phosphorylase seems to be regulated by bundle-sheath differentiation, and its distribution along the length of the leaf seems to be correlated to the biochemical differentiation of bundle-sheath cells (Perchorowicz and Gibbs, 1980; Nelson and Langdale, 1989). This was further corroborated by the observed phosphorylase distribution in bundle-sheath cells isolated from light-grown leaves. This distribution is closely similar to that of other bundle-sheath-specific plastidic proteins such as Rubisco (Nelson and Langdale, 1989, 1992).

At the moment the relationship between phosphorylase distribution and its proposed function—the mobilization of transitory starch—is not known and can only be speculated. Because both α-amylase (Vally and Sharma, 1995) and phosphorylase coexist in pearl millet bundle-sheath chloroplasts, it may be assumed that these enzymes act in a cooperative fashion in mobilization of transitory starch, which is predominantly synthesized in bundle-sheath cells in C_4 plants (Echeverria and Boyer, 1986; Spilatro and Preiss, 1987). The exclusive localization of plastidic phosphorylase in bundle-sheath chloroplasts also emphasizes its likely importance in the mobilization of photosynthetic starch produced in bundle-sheath chloroplasts. At present the function of cytosolic phosphorylase and plastidic phosphorylase present in the etioplast is not obvious, but it has been suggested that the cytosolic phosphorylase may participate in the mobilization of cytosolic polysaccharides (Yang and Steup, 1990).

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