Cell maturity gradient determines light regulated accumulation of proteins in pearl millet leaves

M. Tamil Selvi and Rameshwar Sharma

School of Life Sciences, University of Hyderabad, Hyderabad - 500 046, India

ABSTRACT

Developing pearl millet leaves possess cells of increasing maturity from the leaf base to the tip with oldest cells at the leaf tip. This natural developmental gradient was used to analyze the photoregulation of enzymes located in cytosolic, peroxisomal, and plastidic compartments of the leaf in relation to the cell age. In dark-grown leaves, the level of plastidic protochlorophyllide oxidoreductase A (PORA) (EC 1.3.1.33) protein increased from the leaf base to tip. Exposure to light reduced the level of PORA protein and stimulated accumulation of PORB protein with increasing level from the base to the leaf tip. Light induced formation of cytosolic PEP carboxylase (EC 4.1.1.31) in the leaf with induction being maximal in the leaf tip. The levels of peroxisomal protein catalase (EC 1.11.1.6) showed gradual increase from the base to leaf tip in dark and light-grown leaves. By contrast, the distribution profile of cytosolic enzyme peroxidase (EC 1.11.1.7) followed reverse pattern in the dark-and light-grown leaves. In dark-grown leaves, peroxidase level showed increase towards the leaf tip, whereas light exposure lowered peroxidase level near the leaf tip and stimulated near the leaf base. These results indicate that the cell maturity gradient in cooperation with plastids modulates in a dual fashion the magnitude and pattern of photoregulation of protein levels in pearl millet leaves. It promotes the level of proteins functionally related to plastids towards the leaf tip and at the same time suppresses the level of cytosolic proteins, restricting them to the leaf base. [*Physiol. Mol. Biol. Plants* 2008; 14(1&2) : 1-8] *E-mail* : rameshwar.sharma@gmail.com

Key words : Protochlorophyllide oxidoreductase; Peroxidase; Catalase; *Pennisetum americanum*; Cell maturity gradient; PEP carboxylase

Abbreviations : WL, white light; RL, red light; D, darkness; Chl, chlorophyll; Car, Carotenoids; POR, protochlorophyllide oxidoreductase; PEP, phosphoenolpyruvate.

The monocot leaves are polarized in both cell division and cell expansion. While both dicot and monocot leaf primordia are initiated alike below the shoot apical meristem, after attainment of certain size, the growth of monocot leaf primordia stops. Thereafter, in monocot leaf only the cells in a small zone at the base of the leaf further divide leading to generation of files of cells that remain aligned from the base to the tip of the leaf (Poethig, 1984). The cells at the leaf tip are therefore most mature and leaf consists of cells at progressively younger stages of differentiation from the tip to the base (Nelson and Langdale, 1992). Unlike dicot leaves, which need light for leaf expansion, the monocot leaves continue to

grow in size even under the darkness. However, the plastids remain undifferentiated as etioplasts and greening of leaves occurs only after the exposure to light. The developing young monocot leaf thus provides an excellent and highly reproducible tissue for studying interaction between the cell maturity gradient and the photoregulation of differentiation of leaf cells.

Many studies have exploited this natural developmental gradient in monocot leaves to examine various aspects of photoregulated chloroplast development, such as synthesis of ribulose 1, 5-bisphosphate carboxylase in barley (Viro and Kloppstech, 1980), chloroplast number in wheat (Dean and Leech, 1982) and photosystem I and II in maize (Baker and Leech, 1977). These studies revealed that the basal leaf cells contain small, immature chloroplasts, are nonphotosynthetic and heterotrophic, and depend on its nutrition from either seed or mature leaf tissue. The cells

Correspondence and Reprint requests : Prof. R.P. Sharma
School of Life Sciences, University of Hyderabad, Hyderabad - 500 046, India. Phone : 0091-40-23010514, Fax : 0091-40-23010120

towards the leaf tip have increasing maturity, as evident by enlargement of the mesophyll cells, increase in the plastid size, and the coordinated development of key photosynthetic enzymes within chloroplasts.

By contrast, only few studies have examined the effect of this developmental gradient of cell maturity and plastids on photoregulation of processes in the cellular compartments other than the plastids. These isolated studies have shown that the cell maturity gradient also affects the processes like mitochondrial protein biogenesis in barley (Thompson *et al.*, 1998) and photorespiration in wheat leaves (Tobin *et al.*, 1988) that are located in the cellular compartments other than plastids. The examination of photoregulation of a range of enzymes in pearl millet and maize revealed that the magnitude of photoregulation of β -amylase (Datta *et al.*, 1999), nitrate and nitrite reductase (Datta and Sharma, 1999) and phosphorylase (Vally *et al.*, 1995) is determined by the cell maturity gradient. Importantly these studies also indicated that though cell maturity gradient in cooperation with plastid gradient strongly influences distribution of proteins along the length of leaf, but the pattern of distributions are different for cytosolic enzymes and plastidic enzymes.

In present study, we examined the pattern of influence of cell position on the level of photoregulated proteins in different cellular compartments in a quantitative and qualitative fashion in relation to cell age. We specifically examined whether the cell age uniformly influences the patterns of photoregulation of enzymes in leaves or shows pattern dependent on location and function of the enzymes. One of the important plastidic proteins is protochlorophyllide oxidoreductase (POR), primarily responsible for catalyzing reduction of Pchl_{id} to Chl_{id} on exposure to light (Griffiths, 1991). The higher plants have two distinct POR proteins; namely PORA and PORB (Holtorf *et al.*, 1995), which show opposite photoregulation response. The PORA that is present in etioplasts shows a rapid decline in its level on exposure to light, whereas PORB is present in chloroplasts and appears on exposure to light. The prominent peroxisomal enzyme catalase also shows a light stimulated increase in maize leaves. In maize leaves out of three *CAT* genes, the exposure of light stimulates the expression of *CAT2* and *CAT3* mRNA (Acevedo *et al.*, 1991). The cytosolic enzyme phosphoenolpyruvate carboxylase (PEP-carboxylase) plays a key role in C₄ photosynthesis by performing the primary carboxylating reaction in cytosol of plants such as pearl millet. The level of PEP carboxylase shows strong induction by light (Chollet *et al.*, 1996). Light also increase the level of peroxidase that

is located in cytosolic compartment in maize leaves (Sharma *et al.*, 1979). Using these representative proteins, we show that the cell maturity gradient in cooperation with plastids modulates in a dual fashion the photoregulation of protein levels in pearl millet leaves. The level of proteins functionally related to plastids increases towards the leaf tip whereas the level of cytosolic protein peroxidase is suppressed in more mature tissues restricting it to the leaf base.

MATERIAL AND METHODS

Plant material

Pearl millet seeds (*Pennisetum americanum* WGC-75) were obtained from Andhra Pradesh State Seed Corporation, Hyderabad, India. The seedlings were grown either under continuous WL (100 μ mol m⁻²) or under RL (1 max 650 nm, 3 μ mol m⁻²). The seeds were first soaked in distilled water at room temperature (25°C) for 2 hours and then sown on 4 layers of moist germination papers (Jayashree Traders, New Delhi) in plastic trays. The seedlings were watered daily with distilled water. To estimate the distribution of pigments, POR, peroxidase and catalase the leaf was excised into 1-cm-long segments successively from the base to the tip of the leaf. The segments were numbered from base to the tip of the leaf. Chlorophyll and carotenoid content was estimated using procedures of Vernon (1960) and Liaaen-Jensen and Jensen (1971) respectively.

Protochlorophyllide oxidoreductase extraction

The leaves were frozen in liquid nitrogen and homogenized in an extraction buffer (50% ethyleneglycol, 100 mM Tris- HCl, (pH 8.3), 140 mM ammonium sulfate, 10 mM Na₄-EDTA, 20 mM sodium bisulfite) in a pre-chilled mortar and pestle at 4°C. The homogenate was centrifuged at 10,000g for 30 min at 4°C. Protein was estimated according to Lowry *et al.* (1951). The SDS-PAGE was carried out according to Laemmli (1970). Western blotting was done according to Towbin *et al.* (1979). The polyclonal antibodies used in this study were raised against a mixture of PORA and PORB of barley, which also cross-reacted with pearl millet POR.

Extraction of PEP carboxylase

Leaves were extracted with 100 mM HEPES-KOH pH 7.3, w/v (1:4) containing 10 mM MgCl₂, 2 mM K₂HPO₄, 1 mM EDTA, 20 % (v/v) glycerol, 2 mM PMSF and 10 mM 2-mercaptoethanol. The extract was centrifuged at 15,000g for 5 min. The level of PEP carboxylase was determined

by Western blotting using antibodies raised against PEP carboxylase of *Amaranthus hypochondriacus*.

Peroxidase assay

Leaves were homogenized (1:3 ratio, w/v) in a pre-chilled mortar and pestle by using Tris-HCl buffer pH 7.2 followed by centrifugation for 30 min at 8,000g at 4°C. A 100 µl aliquot of supernatant was immediately added to the assay mixture, which contained 0.32 ml of 5 % (w/v) pyrogallol, 0.16 ml 0.147 M H₂O₂, 0.32 ml of 0.50 M phosphate buffer pH 7.2 and 2.0 ml distilled water. Increase in absorbance at 440 nm was monitored at every 15 s for 1.5 min in spectrophotometer and peroxidase activity was calculated as mmoles/min. The aliquots were also subjected to electrophoresis for visualization of isozymes by native PAGE (Davis, 1964). After completion of the electrophoresis the gel was stained first by submerging in a benzidine and acetic acid mixture containing 2.4 mM benzidine and 7 mM acetic acid for 10 min and then with 3 mM H₂O₂ for 2 min. Intense blue peroxidase bands were immediately visible and were photographed.

Catalase assay

Leaves were homogenized in a pre-chilled mortar and pestle (1:3 ratio, w/v) in 50 mM Tris-HCl buffer pH 7.2 and centrifuged for 30 min at 7,500g at 4 °C. A 100 µl aliquot of the supernatant was mixed with 50 mM potassium phosphate buffer pH 7.0. The enzyme assay was initiated by adding 10 µl of substrate, 20 mM of H₂O₂. Enzyme activity was estimated by measuring absorbance at 240 nm at every 5 sec interval for 30 s. An aliquot of the sample was used to examine the catalase isozymes by Native PAGE. After completion of electrophoresis the gels were incubated in 0.003 % (v/v) H₂O₂ with constant shaking for 10 min. Later the gel was washed with distilled water for 10 min and transferred to freshly prepared solution of 10 % (w/v) ferric chloride and 10 % (w/v) potassium ferricyanide III. Yellow colored catalase bands were visible and were photographed.

RESULTS

In both RL-grown and WL-grown leaves, the amount of chlorophyll continually increases with increase in the age of the seedlings (*Figure 1A*). The accumulation of carotenoid also followed a profile similar to chlorophyll accumulation (*Figure 1B*). Since the pearl millet leaves grow in size from cell divisions at the leaf base, we examined the distribution of chlorophyll and carotenoid along the length of pearl millet leaves. For both pigments the level showed continual increase towards the leaf tip

indicating that the observed accumulation profile is determined by the gradient of cell maturity (*Figure 1CD*).

Since accumulation of the chlorophyll along the length of leaf shows increased levels towards the leaf tip, it is reasonable to expect that this profile should also reflect the distribution of protochlorophyllide oxidoreductase, the enzyme responsible for conversion of protochlorophyllide to chlorophyllide. Since in higher plants, POR has two species PORA and PORB, present respectively in dark- and light-grown plants, we examined distribution of both of these proteins. Immunoblotting of POR proteins from dark- and light-grown leaves showed presence of bands, which could be distinguished as PORA and PORB based on their relative mobility on gel. The PORA that is present in dark-grown leaves has lesser mobility than the PORB present in light-grown leaves, as PORA (36 kD) has a two kD higher molecular weight than PORB. We could not detect accumulation of PORB protein in dark-grown leaves of pearl millet (*Figure 2A*). Examination of distribution profile of POR

Fig. 1. Time course of chlorophyll (**A**) and carotenoid (**B**) accumulation in the first leaf of pearl millet. Seedlings were grown under WL or RL or in darkness from sowing. At the time point (days) indicated, the first leaf was excised at the mesocotyl junction, and the pigment amount was estimated. For analysis of distribution first leaves of 7-d-old seedlings were excised at the mesocotyl junction. The leaves were then excised into 1 cm long segments from leaf base to tip, and used for estimation of chlorophyll (**C**) and carotenoids (**D**). Segments are numbered from base to the leaf tip.

revealed that in D-grown leaves the level of PORA protein increases from leaf base to the tip (Figure 2B). By contrast, the light grown leaf, which has only PORB protein due to light-mediated degradation of PORA protein, shows a similar distribution profile of PORB protein (Figure 2C).

Pearl millet is a C_4 plant and the primary fixation of CO_2 in leaves is carried out by the activity of enzyme

Fig. 2. (A). Quantification of POR protein in 7-d old WL-grown or dark-grown pearl millet leaves. The POR level was determined by Western blotting (Inset). Note the difference in mobility of POR in WL-grown leaf, which is light stable PORB. (B). Distribution of PORA protein in 7-d old dark-grown pearl millet leaves. The leaf was dissected into 1 cm long segments which were numbered from base to the tip. PORA level was determined by Western blotting (Inset). The relative amount of PORA was calculated using the intensity of the band present in leaf tip as 100%. (C). Distribution of PORB protein in 7-d old light-grown pearl millet leaves. The leaf was dissected into 1 cm long segments which were numbered from base to the tip. PORB level was determined by Western blotting (Inset). The relative amount of PORB was calculated using the intensity of the band present in leaf tip as 100%.

PEP-carboxylase. Since PEP-carboxylase activity is localized in cytosol of mesophyll cells, this enzyme was used as a marker for cytosolic enzyme in leaves. Comparison of the relative level of PEP-carboxylase in dark-and light-grown leaves by immunoblotting showed that in dark-grown leaves PEP-carboxylase level was below the level of detectability (Figure 3A). In comparison, light stimulated accumulation of PEP carboxylase protein as evident by presence a distinct band in leaf. In light-grown leaves the distribution of PEP-carboxylase was similar to that observed for PORA and PORB showing increase in levels towards leaf tip (Figure 3B).

We examined regulation of catalase activity in leaves as a marker enzyme for peroxisomes. Examination of the time course of catalase activity during the period of leaf elongation in pearl millet seedlings showed that the exposure of light had no effect on catalase activity during initial phase of leaf elongation till 6 day (Figure 4A). The inductive light effect of light on catalase activity becomes apparent only after 6 day from sowing. Since the effect of light on catalase activity was noticed only after 6-d, the effect of light on catalase activity was examined after transferring dark-grown seedlings to RL

Fig. 3. (A) Quantification of PEP carboxylase protein in 7-d old WL-grown or dark-grown pearl millet leaves. The protein level was determined by Western blotting (Inset). (B). Distribution of PEP carboxylase protein in 9-d old white light-grown pearl millet leaves. The leaf was dissected into 2 cm long segments and numbered from base to the tip. Protein level was determined by Western blotting (Inset). The relative amount of PEP carboxylase was calculated using the intensity of the band present in leaf tip as 100%.

for 12 h. In seedlings exposed to RL the level of catalase activity increased along the length of the leaf compared to dark-grown leaves and showed a pattern similar to light-grown leaves (Figure 4B). The analysis of catalase isozymes by native PAGE along the length of light-grown leaf showed a distinct gradient of catalase level from leaf base to tip (Figure 4B inset). In contrast, dark-grown leaf did not show a distinct gradient of catalase accumulation. It had nearly the same catalase activity through out the length of the leaf except in the leaf tip region (Figure 4B inset).

In plants most peroxidase activity is localized in the cytosol, therefore we used it as a marker enzyme to examine the effect of cell maturity on the level of a typical cytosolic enzyme. Analysis of peroxidase activity in first leaf of pearl millet seedlings during the course of leaf elongation revealed that in young leaf the level of peroxidase is significantly higher in light-grown leaves compared to dark-grown leaves. With the increase in the leaf length, peroxidase activity declined in the light-grown leaves. In contrast, the dark-grown leaves had low level of peroxidase activity and the peroxidase activity increased with the age. Interestingly, 9-d old light-grown leaf had peroxidase activity equivalent to 3-d old dark-grown leaves (Figure 5A). The 7-d-old light-grown leaves of pearl millet possess nearly similar activity of

peroxidase in dark-grown leaves, but shows opposite distribution of peroxidase activity along the length of leaf (Figure 5B). In dark-grown leaf, peroxidase activity increases from leaf base to tip, and in contrast, RL-grown leaf peroxidase activity declines from leaf base to tip (Figure 5B). Analysis of isozyme profile of peroxidase showed that in dark-grown leaves, the basal segment possesses low activity, and it increases towards tip of the leaf (Figure 5B inset). On the contrary, in the light-grown leaves, the maximal peroxidase activity was observed in the base of the leaf and then it gradually declined towards the leaf tip (Figure 5B inset).

DISCUSSION

In the present study, we used natural developmental gradient of cell maturity to examine the interaction between cell maturity and light mediated induction/inhibition of proteins located in plastidic, cytosolic and peroxisomal compartments of pearl millet leaf. The distinct accumulation profiles of chlorophyll and carotenoids along the length of pearl millet leaf highlight that though the signal to induce chlorophyll and carotenoid synthesis (i.e. light) was equally available to all cells of leaf, nevertheless the age of leaf cells is primarily responsible for quantitative differences in levels of these pigments along the length of leaf. Our study show that the above gradient which determines the quantitative formation of pigments also similarly determines the level

Fig. 4. (A) Time course of catalase activity in first leaf of pearl millet (WGC-75). Seedlings were grown under continuous RL or complete darkness from the time of sowing. At the time point indicated (days), the first leaf was excised at the base and used for estimating the catalase activity. (B) Distribution of catalase isozymes along the length of pearl millet leaf. Seedlings were grown under continuous darkness up to 7-d from the time of sowing and then transferred to continuous RL for 12 h. The leaves were excised from both dark control and red light-grown pearl millet seedlings. The leaf was excised at the base, cut into 1 cm long segments, which were numbered from base to the leaf tip. The amount of enzyme activity was assayed in the supernatants from each segment. For electrophoresis (inset) equal amounts of proteins were loaded onto each lane and after completion of native PAGE, stained for catalase activity.

Fig. 5. (A) Time course of peroxidase activity in first leaf of pearl millet. Seedlings were grown under continuous RL or darkness from the time of sowing. At the time point indicated (days) first leaf was excised at the base and the peroxidase activity was determined. (B) Distribution of peroxidase isozymes along the length of pearl millet (WGC-75) leaves. The leaf was excised from 7-d old dark-grown or light-grown pearl millet seedlings. The leaf was excised at the base, cut into 1 cm long segments, which were numbered from base to the leaf tip. The amount of enzyme activity was assayed in the supernatants. For electrophoresis (inset) equal amounts of proteins were loaded onto each lane and after completion of native PAGE, stained for peroxidase activity.

of plastidic protein POR, and also of proteins which are metabolically related to chloroplast function such as peroxisomal enzyme catalase and cytosolic enzyme PEP carboxylase.

The higher plants have two specific species of POR that characteristically accumulate in dark- and light-grown tissues of plants. In dark while only PORA is present, during greening of tissue PORA level is rapidly downregulated by proteolysis by a light-induced protease (Reinbothe *et al.*, 1995). At the same time, chloroplast replaces PORA with PORB and its level increases during the process of greening. The distribution pattern of PORA in dark-grown leaves with increasing level towards the leaf tip is in conformity with distribution of etioplasts that also shows increasing level and maturity towards the leaf tip. In light-grown leaves the PORA is distinctly replaced by PORB but the pattern of its accumulation remains alike to PORA with increasing level towards the leaf tip. The similarity in accumulation pattern for both PORA and PORB indicates that this pattern is likely determined by the gradient of cell age and plastid development.

In leaf cells, the plastid does not exist as a 'stand alone' organelle. Its efficient functioning requires a close coordination with activities of other cellular compartments. In many plants, the organelle peroxisome is intimately associated with chloroplast. It is believed that such an association is necessitated for exchange of metabolites between two organelles. Examination of catalase distribution, a peroxisomal marker enzyme is indicative of operation of such coordination. The profile of catalase accumulation in the light-grown pearl millet leaf indicates it to be intimately coupled with plastid development. Interestingly, the profile of catalase accumulation was also somewhat similar to that observed for plastidic phosphorylase distribution pattern in bundle sheath cells (Vally *et al.*, 1995). The examination of expression pattern of *CAT 2* gene in maize and in its chlorophyll and carotenoid deficient mutants brought forth a similar observation, that in addition to light, an other factor such as chloroplast development, also regulates *CAT* gene expression in maize leaf (Acevedo *et al.*, 1991). A similar linkage between peroxisome and chloroplast development was seen in mustard where herbicide-induced photooxidation of chloroplasts or inhibition of plastidic protein synthesis by antibiotics significantly reduced the development of peroxisomal enzymes glycollate oxidase and hydroxypyruvate reductase (Bajracharya *et al.*, 1987).

Since pearl millet is a C_4 plant, it fixes CO_2 required for photosynthesis in cytosol using PEP-carboxylase

enzyme and fixed product is then translocated to the chloroplasts. The profile of PEP-carboxylase distribution in pearl millet leaves showed a strong semblance to that of PORB distribution with levels increasing towards the leaf tip. Evidently, this resemblance indicates a possible influence of chloroplast development on accumulation profile of cytosolic PEP-carboxylase, suggesting a close interdependence of these two processes. Little information is available regarding signaling process involved in interaction of chloroplast with other cellular compartments. Though the molecular mechanism underlying interaction of plastids with other cellular compartments is not well understood, light appears to be one of the elements that may act in this interactive process.

A distinctive feature of plant cell is that the nuclear genes for chloroplast development do not express when the plastids are in etioplast stage. The light-induced differentiation of etioplast to chloroplast in turn induces expression of these nuclear genes. This observation has lead to a hypothesis that plastid sends a 'plastid signal' to nucleus to stimulate the expression of these genes. In fact, both plastid signal and light together control of expression of nuclear genes and cell differentiation. Genetic and biochemical studies now indicate that several factors such as the level of chlorophyll precursor protoporphyrin IX, photosynthetic electron transport chain and phosphoenolpyruvate/phosphate translocator protein may act as putative plastid signals for regulation of nuclear genes (Rodermeil, 2001). It is likely that the above regulation by plastid is not restricted only to the proteins destined to plastids and may possibly to some extent influence expression of proteins in other cellular compartments as observed for catalase and PEP carboxylase.

Another representative enzyme of cytosolic compartment peroxidase shows an altogether different pattern. The analysis of peroxidase activity during leaf elongation revealed that while peroxidase activity in dark-grown leaves declined with age, an opposite pattern of increase in peroxidase activity was observed for light-grown seedlings. The distribution of peroxidase activity and isozyme levels along the leaf length highlighted a drastic different pattern of distribution. In dark-grown leaves, the peroxidase activity was distributed in a fashion similar to that of POR or PEP-carboxylase, with maximal activity towards the leaf tip. However, exposure to light changed this profile of distribution, with maximal activity present in the leaf base. A similar relocation of distribution pattern has been also observed for other cytosolic proteins in pearl millet. In pearl millet and in

maize leaves the exposure of light alters the β -amylase distribution pattern with maximal activity of β -amylase located near the leaf base (Datta *et al.*, 1999). Similarly light shifted the distribution profile of cytosolic phosphorylase with maximal activity near the leaf base (Vally *et al.*, 1995). The above similarity in the distribution profile of these different enzymes in light- and dark-grown leaves is indicative of a common regulatory mechanism. The analysis of distribution profiles indicates that cytosolic enzyme follows two divergent distribution profiles. First one is specific for dark-grown plants with increasing level of proteins towards the leaf tip. This pattern is also similar to that of plastidic proteins in light-grown seedlings. The second profile is typical of light-grown seedling, where the maximal amount of enzyme is localized in cells near the leaf base.

The reason for the above specific distribution and mechanism initiating the process is not known but can only be speculated. One possibility is that the level of these enzyme peroxidase and β -amylase may be negatively determined by the presence of chloroplast in a leaf. Since in dark-grown leaves, only etioplasts are present the level of β -amylase and peroxidase follows a normal profile with increasing level towards the leaf tip. However, in light-grown leaves, the formation of chloroplast specifically suppresses the β -amylase and peroxidase induction, but at the same time, light promotes the induction of these proteins in leaf. Consequently, the effect of light on level of proteins is only visualized in younger leaf cells, which has under-developed chloroplasts. Analysis of β -amylase activity between 5-10-d old pearl millet seedling shows that during this period highest β -amylase activity is invariably localized near the leaf base (Datta *et al.*, 1999). Since in monocot leaves, the basal region contains young cells undergoing division and differentiation (Kemp, 1980; Sylvester *et al.*, 1990), the observed cytosolic enzyme distribution may be linked to the gradient of cell maturity. For example, in wheat leaves, the distribution of cell cycle control protein, p34^{cdc2} shows a pattern similar to that of peroxidase (John *et al.*, 1990). A similar selective response of cells has been observed with respect to hormone-promoted cell division in wheat leaf. In wheat, the cells that are distant from the basal meristem are unable to resume cell division, even after the administration of auxins, although all cells of the leaf possess equal rates of uptake and metabolism of auxins (Wernicke and Milkovits, 1987a,b).

In summary, our study indicates that the gradient of cell maturity and plastid development regulates the

distribution pattern of protein in pearl millet leaves in dual fashion. The mechanism governing observed distribution profile of proteins may be complex and indicates interaction between different cellular compartments of developing leaf.

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