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Control of Cellular Differentiation in Maize Leaves

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Control of cellular differentiation in maize leaves

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SUMMARY

Mature maize leaves exhibit a series of parallel veins that are surrounded by concentric rings of bundle sheath and mesophyll cells. To identify genes that control cellular differentiation patterns in the leaf, we have isolated a group of mutations that specifically disrupt the differentiation of a single cell-type. In *bundle sheath defective* (*bsd*) mutant plants, bundle sheath cells fail to differentiate yet mesophyll and all other leaf cell-types develop normally. Morphological and functional characterization of specific *bsd* mutants (*bsd1*, *bsd2*, *bsd3*, *pg14* and *g2*) reveals that they differ in the degree to which bundle sheath cell differentiation is perturbed. Mutant analysis predicts roles for BSD gene products in normal development.

1. INTRODUCTION

The development of vegetative leaves is an indispensable feature of the life cycle of non-succulent vascular plants. Despite this fact, our understanding of events that control differentiation in the leaf is limited. The maize leaf is an excellent system for the study of cellular differentiation events because the final differentiated state is well defined both morphologically and functionally. The mature leaf is composed of sheath and blade regions which are delimited by an epidermal fringe known as the ligule. As a consequence of cell division patterns, a developmental gradient exists with the oldest cells at the tip of the blade and the youngest at the base of the sheath (Sharman 1942; Sylvester *et al*. 1990). Both sheath and blade contain a series of parallel veins running the length of the leaf (Esau 1942; Russell & Evert 1985). Surrounding these veins are concentric circles of two distinct photosynthetic cell-types. Bundle sheath (BS) cells, which are situated immediately adjacent to the vein, develop coordinately with neighbouring mesophyll (M) cells to interact in the fixation of CO_2 in the C4 photosynthetic cycle (reviewed in Langdale & Nelson (1991) and Nelson & Langdale (1992)).

At maturity, BS and M cells are structurally and biochemically distinct. BS cells have agranal chloroplasts that are arranged centrifugally within the cell whereas M cells have granal chloroplasts that are randomly arranged (Brown 1975). Each of the two cell-types accumulates a distinct complement of C4 photosynthetic enzymes. Ribulose bisphosphate carboxylase (RuBPCase) and NADP-malic enzyme (NADP-ME) function in the BS cells whereas phosphoenolpyruvate carboxylase (PEPCase), pyruvate phosphate dikinase (PPdK) and NADP-malate dehydrogenease (NADP-MDH) function in the M cells (Edwards & Huber 1979). Current evidence suggests that this cell-specific accumulation of photosynthetic enzymes is regulated by cell position and light (Langdale *et al*. 1988).

BS and M cells differentiate in concert with the vascular system. Cells around the midvein mature first, followed by cells surrounding lateral veins and finally by those surrounding intermediate veins (Langdale *et al*. 1987). In light-grown plants, M cells positioned adjacent to a vascular bundle accumulate the appropriate complement of C4 enzymes. M cells located further than two cells away from the vein, however, accumulate RuBPCase instead of M cell-specific enzymes and photosynthesize using the C3 pathway (Langdale *et al*. 1988). In dark grown tissues, both BS and M cells accumulate RuBPCase although the leaves are non-photosynthetic (Sheen & Bogorad 1986; Langdale *et al*. 1988). These results suggest that maize develops a C3 pattern of cell-type differentiation (RuBPCase in all photosynthetic cells) by default and that C4 specialization is achieved through the interpretation of a light-induced signal that emanates from the leaf vasculature (Langdale *et al*. 1988). M cells at a distance from a vein either do not receive the signal or cannot interpret it. The concept of a positional control of photosynthetic cell-type differentiation has been supported by cell lineage analysis. M cells in the central layer of the leaf blade are more closely related to BS cells than to other M cells (Langdale *et al*. 1989). Therefore, as all M cells differentiate in the same way, cell position must play a greater role than cell lineage in directing cell-type differentiation.

Current models to explain mechanisms of C4 development imply that photosynthetic differentiation in BS and M cells results from a light-induced interaction between the two cell-types (Langdale & Nelson 1991; Nelson & Dengler 1992; Nelson & Langdale 1992). Because the differentiation of BS and M cells is temporally co-ordinate, however, it has not been possible to dissect this signalling process. To address this problem, we have isolated and characterized

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mutations that disrupt the differentiation of a single cell-type in light-grown leaves.

2. IDENTIFICATION OF DIFFERENTIATION-DEFECTIVE MUTANTS

We have screened transposon- and EMS- mutagenized lines of maize for mutations that disrupt photosynthetic cell-type differentiation. On the basis that differentiation-defective photosynthetic cell-types would probably contain aberrant chloroplasts, mutants were initially scored for pale green leaf phenotypes. Pale green mutants were further screened by microscopic examination of leaf sections and by Western blot analysis of cell-specific photosynthetic enzyme accumulation patterns. In a study of 34 pale green mutations (see table 1), five have been identified in which BS cell chloroplasts are absent from mutant tissue – *bundle sheath defective 1-mutable 1* (*bsd1-m1*), *bsd2-m1*, *bsd3*, *golden 2* (*g2*) and *pale green 14* (*pg14*). In all cases, Western blot analysis has shown that levels of BS cell-specific photosynthetic enzymes are reduced in mutant tissue whereas levels of M cell-specific enzymes appear to be unaffected (figure 1). Therefore, these five mutations specifically disrupt BS cell differentiation in light-grown leaves. Allelism tests have shown that *bsd1-m1*, *bsd2-m1* and *bsd3* represent three complementation groups. *g2* (Jenkins 1926) and *pg14* (Peterson 1960), however, are both allelic to *bsd1*. Through the characterization of single and double mutant phenotypes and through the isolation of mutated genes, we are starting to understand the regulation of BS cell development in maize. In this paper, we discuss our work on *bsd1* and *bsd2*.

Table 1. *Characterization of pale green mutants*

(BS – bundle sheath, M – mesophyll, MGSC – maize genetics stock centre. J.A.L. – J. A. Langdale, University of Oxford; W.F.S. – W. F. Sheridan, University of North Dakota; M.G.N. – M. G. Neuffer, University of Missouri.)

mutation	cell-type affected	source
bsd1-m1	BS	J.A.L.
bsd2-m1	BS	W.F.S.
bsd3	BS	J.A.L.
g2	BS	MGSC
pg14	BS	MGSC
pg123C	BS + M	M.G.N.
pg (yg)127	BS + M	M.G.N.
pg146A	BS + M	M.G.N.
pg213	BS + M	M.G.N.
pg219	BS + M	M.G.N
pg222	BS + M	M.G.N.
pg257B	BS + M	M.G.N.
pg298	BS + M	M.G.N.
pg526C	BS + M	M.G.N.
pg596	BS + M	M.G.N.
pg638	BS + M	M.G.N.
pg639	BS + M	M.G.N.
faint pg668	BS + M	M.G.N.
pg812C	BS + M	M.G.N.
pg1814	BS + M	M.G.N.
pg-m1822	BS + M	M.G.N.
pg1824	BS + M	M.G.N.
pg1881	BS + M	M.G.N.
pg-m1885	BS + M	M.G.N.
pg2383A	BS + M	M.G.N.
pg2406	BS + M	M.G.N.
oy	BS + M	MGSC
v4	BS + M	MGSC
v8	BS + M	MGSC
v13	BS + M	MGSC
v16	BS + M	MGSC
v17	some BS	MGSC
v18	BS + M	MGSC
v21	BS + M	MGSC

(*a*) *bsd1-m1*

bsd1-m1 is an unstable allele that was isolated in a transposon mutagenesis program that used *Spm* as an insertional mutagen. Somatic instability at the *bsd1-m1* locus leads to restoration of *Bsd1* function such that revertant sectors are phenotypically indistinguishable from wild-type. Mutant leaves have normal cellular anatomy with BS and M cells arranged in concentric circles around vascular centres. However, the *bsd1-m1* mutation leads to both morphological and biochemical disruptions in BS cells such that BS cell chloroplasts develop aberrantly and BS cell-specific C4 photosynthetic gene products fail to accumulate. M cell differentiation proceeds as normal in mutant leaves, although it is unknown whether M cell-specific enzymes are active in mutant tissue.

We have shown that in light grown plants, the *Bsd1* gene functions within a limited cell range, at or before plastochron four (Langdale & Kidner 1994). Because BS cells are not fully differentiated until plastochron eight (Nelson & Dengler 1992), *Bsd1* must influence early differentiation events. However, as *Bsd1* sectors (dark green) in *bsd1-m1* plants can be large (early) or small (late), cells must be able to respond to *Bsd1* at any stage in development. One of the characteristics of *bsd1-m1* plants is the ability of mutant cells at the tip of mature leaves to overcome the defect imposed by the mutation. In wild-type mature leaves, BS cell chloroplasts are fully differentiated 30 cm above the ligule (Kirchanski 1975). An identical situation is seen in revertant sectors of *bsd1-m1* plants. In mutant tissue, however, BS cell chloroplasts undergo most differentiation between 30 cm and 60 cm above the ligule. A similar retardation is observed with respect to the accumulation of photosynthetic gene products. BS cell-specific photosynthetic enzyme levels increase towards the tip of both wild-type and *bsd1-m1* leaves, but peak levels in mutant leaves are not reached until further up the leaf. Although differences between cells at the tip and the base of a maize leaf presumably reflect the combined action of spatial and temporal mechanisms, it is generally accepted that the maize leaf presents a developmental gradient with the oldest cells at the tip. As such, the observed differences between wild-type and mutant leaves invoke two possible explanations. First, *bsd1-m1* may be a leaky mutation such that only the most mature cells (at the tip of the leaf) accumulate enough BSD gene product to suppress the mutant phenotype. Alternatively, the *Bsd1* gene may function in conjunction with a second gene product that is

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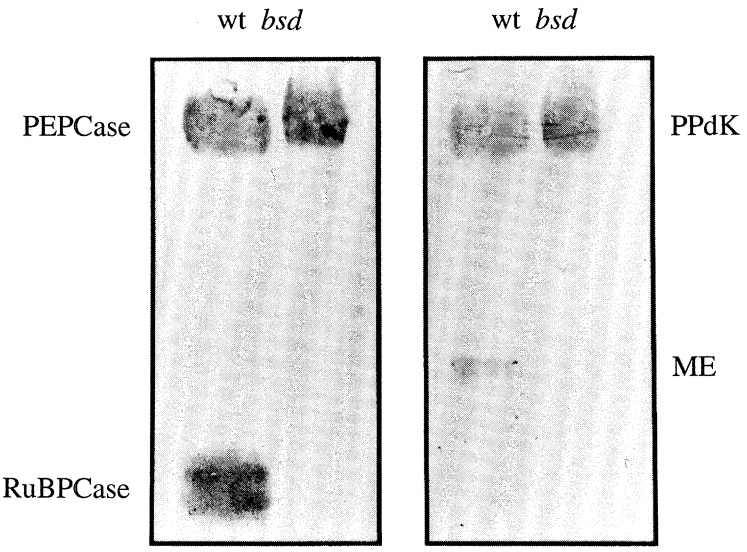

Figure 1. Western blot analysis comparing third leaves of mutant and wild-type siblings. M cell-specific enzymes (PEPCase and PPdK) accumulate to normal levels in mutant plants whereas BS cell-specific enzymes (RuBPCase and ME) are absent.

unaltered by the *bsd1-m1* mutation. For example, *Bsd1* could act early in development whereas the second gene acts at later stages. The activity of the second gene must be somewhat impaired in *bsd1-m1* leaves, however, as mutant tissue can be distinguished from revertant sectors at the leaf tip. Both of the above explanations predict that BS cells at the base of the leaf eventually recover to the same extent as those at the tip. Consistent with this prediction the phenotypic gradient in mutant tissue diminishes as the leaves age. As the gradient does not disappear completely, however, spatial mechanisms must also play a role.

Based on the *bsd1-m1* mutant phenotype, we propose that during normal development in both the light and the dark, the *Bsd1* gene influences the morphological differentiation of BS cell chloroplasts. The *Bsd1* gene product plays no role in the differentiation of M cell chloroplasts. In addition to directing morphological differentiation in BS cells, the *Bsd1* gene also affects photosynthetic enzyme accumulation patterns. However, *Bsd1* gene action on photosynthetic gene expression is only BS cell-specific in the presence of light (Langdale & Kidner 1994). This apparent requirement for light may correspond to a requirement for the induction of C4 photosynthesis. Wild-type etiolated leaves develop a C3 pattern of photosynthetic differentiation in that both BS and M cells accumulate RuBPCase (Sheen & Bogorad 1986; Langdale *et al.* 1988). A similar C3 pattern is observed in light-grown leaf-like organs such as husk leaves, where M cells are positioned at a distance from a vein (Langdale *et al.* 1988). In *bsd1-m1* etiolated leaves neither cell-type accumulates RuBPCase. Furthermore, preliminary evidence suggests that *RbcS* transcripts cannot be detected in mutant husk leaf tissue of *bsd1-m1* plants. We suggest that during normal development one function of the *Bsd1* gene product is to either activate *RbcS* gene expression or stabilize *RbcS* transcripts. In tissues that normally display a C3 pattern of photosynthetic gene expression, *Bsd1* gene action is cell-type independent such that RuBPCase accumulates in both BS and M cells. In contrast, *Bsd1* gene action in C4 photosynthetic tissues is BS cell-specific.

Current models hypothesize that in maize leaves, the switch from a C3 to a C4 photosynthetic state occurs through a light-induced interaction between BS and M cells, whereby RuBPCase becomes repressed in M cells close to a vein (Langdale & Nelson 1991; Nelson & Langdale 1992). Cell-specific C4 differentiation proceeds coincident with this repression. As M cells differentiate appropriately in light-grown *bsd1-m1* plants, the switch to C4 development is unaffected by the *bsd1-m1* mutation. We therefore propose that during normal development in the light, the *Bsd1* gene functions downstream of genes that induce the C4 state. After C4 induction, the *Bsd1* gene product becomes restricted to BS cells where it acts to direct both morphological and biochemical differentiation events. This hypothesis ramifies and extends existing models of C4 development.

(*b*) *bsd2-m1*

bsd2-m1 was isolated from a line containing active *Mutator* (*Mu*) transposable elements by Dr W. Sheridan (University of North Dakota, U.S.A.). *bsd2-m1* leaves are pale green with dark green revertant sectors suggesting that the mutation is transposon-induced. Examination of *bsd2-m1* leaves suggests that the chloroplast defect associated with the *bsd2-m1* mutation is more severe than that associated with *bsd1-m1*. This difference is particularly apparent at the ultrastructural level (see figure 2). *bsd1* mutant chloroplasts appear to be blocked at an early stage of differentiation

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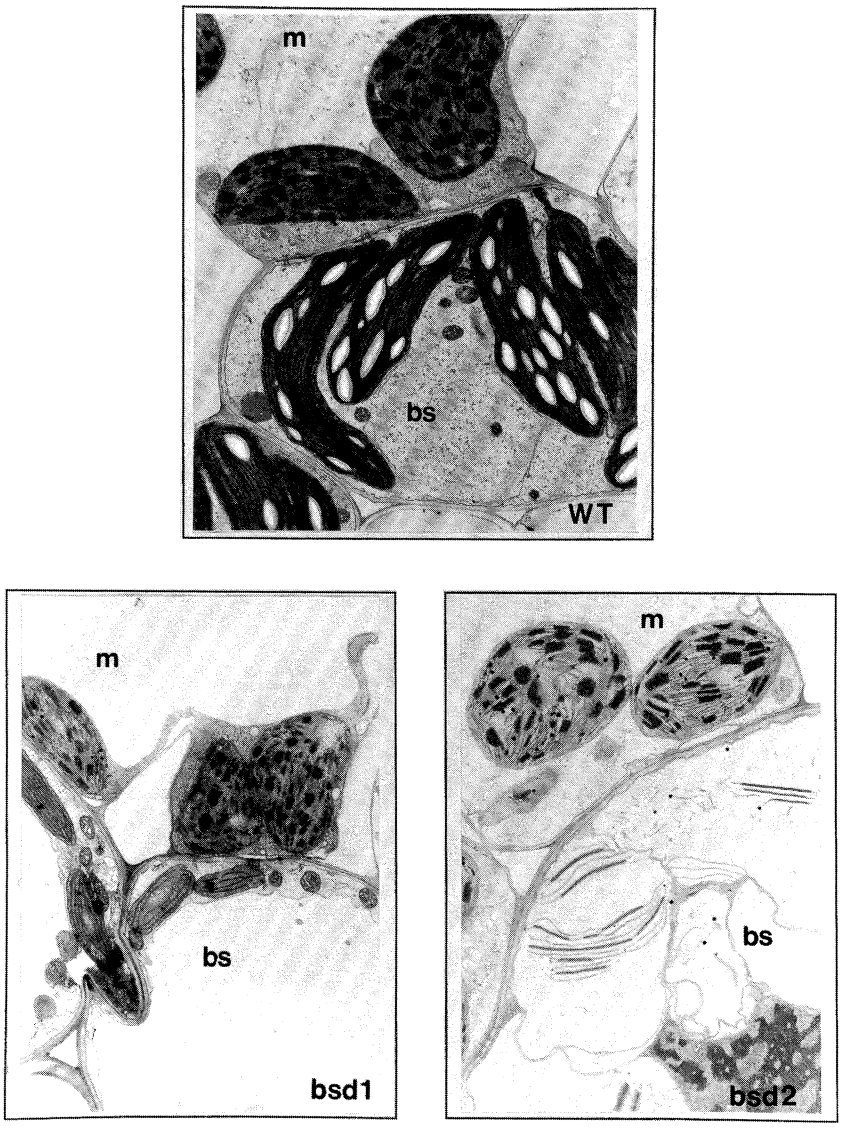

Figure 2. Electron micrographs of wild-type (wt), *bsd1* and *bsd2* mutant leaves. BS = bundle sheath cell, M = mesophyll cell.

insofar as they are smaller and less membranous than normal. In contrast, *bsd2* chloroplasts appear to disintegrate; the outer chloroplast membrane swells and the inner membranes break down. The significance of this observation has yet to be determined. However, we are currently carrying out an extensive characterization of *bsd2-m1* mutants in an attempt to understand more fully the role of *Bsd2* in BS cell differentiation.

3. PERSPECTIVES

It is clear that *Bsd1* and *Bsd2* genes play a role in BS cell differentiation in maize, however, as yet we do not know how. To address this question, we have cloned *Bsd1* and work is currently underway to clone *Bsd2*. In addition, we are investigating genetic interactions between genes through double mutant analyses. It is noteworthy that our current screens have not identified any M cell-specific mutants. Does this suggest that M cells induce differentiation in BS cells such that a mutation that causes aberrant M cell differentiation will necessarily affect BS cells as well? Alternatively, does our primary screen (scoring pale green phenotypes) exclude M cell-specific mutants? Further mutant screens should shed light in this area.

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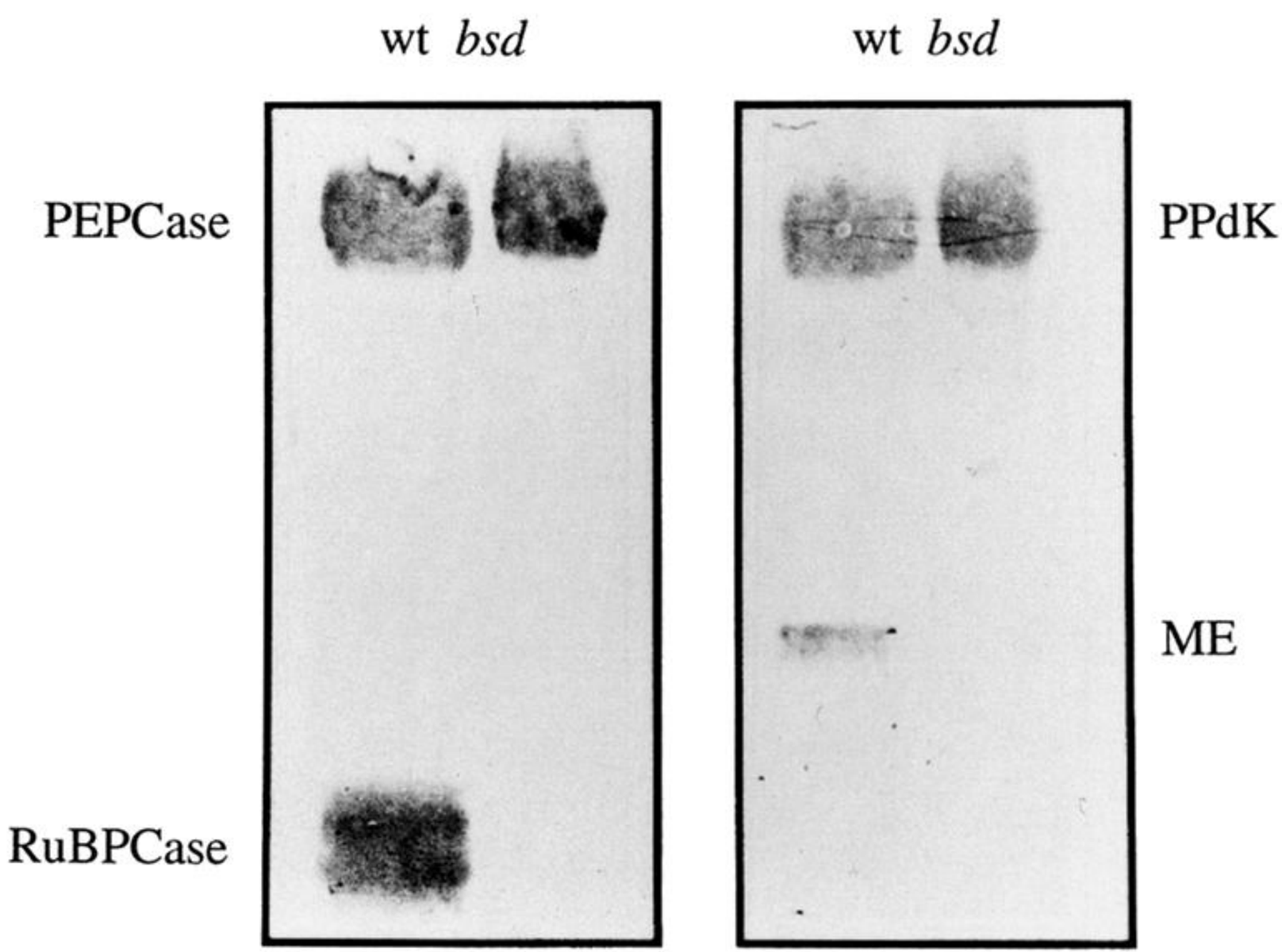

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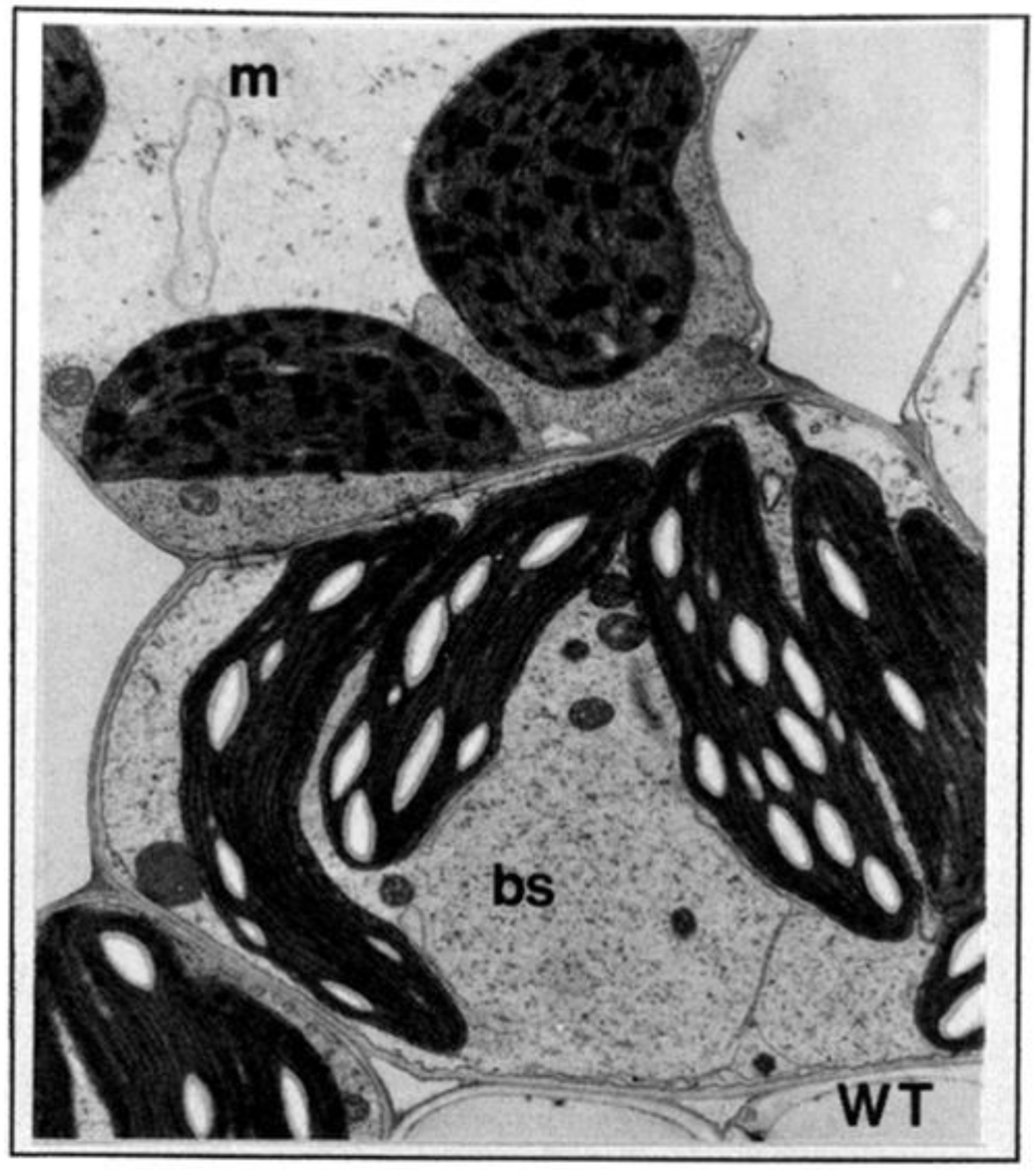

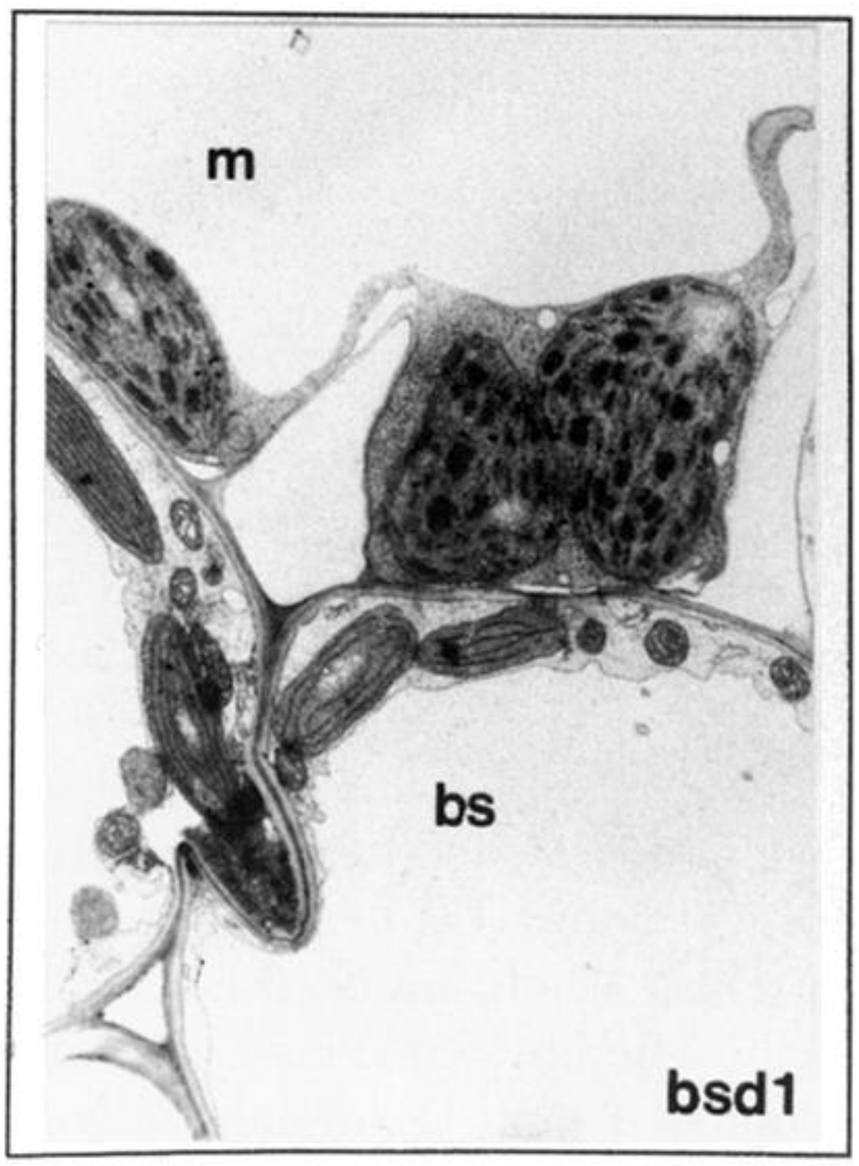

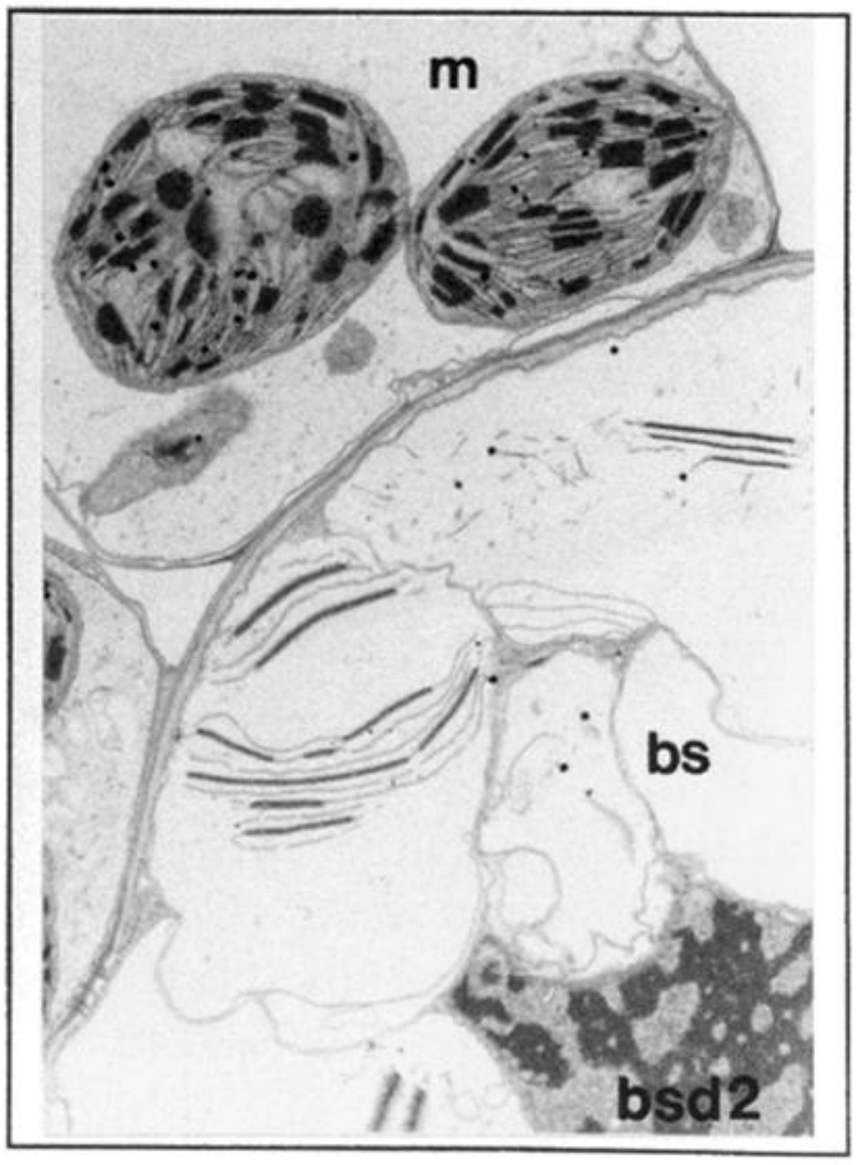

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