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Immunochemical localization of a willow storage protein with a poplar storage protein antibody

Rapid Communication

J. J. Sauter* and **Barbara van Cleve**

Botany Institute, University of Kiel, Kiel

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Summary. Antibodies raised from a 32 kDa storage protein of poplar wood are found to bind specifically to protein bodies in willow wood ray cells when the immunogold method is used. A polypeptide of ca. 32 kDa is also obtained from the willow wood which reacts with the poplar storage protein antibodies when the immunoblotting technique is used. The results indicate that willow possesses a storage protein which appears closely related to that of poplar.

Keywords: Immunogold labelling; *Populus*; Protein bodies; Protein storage; Ray cells; *Salix*; Xylem.

Abbreviations: kDa kiloDalton; SDS-PAGE sodium dodecyl sulfate polyacrylamide gel electrophoresis.

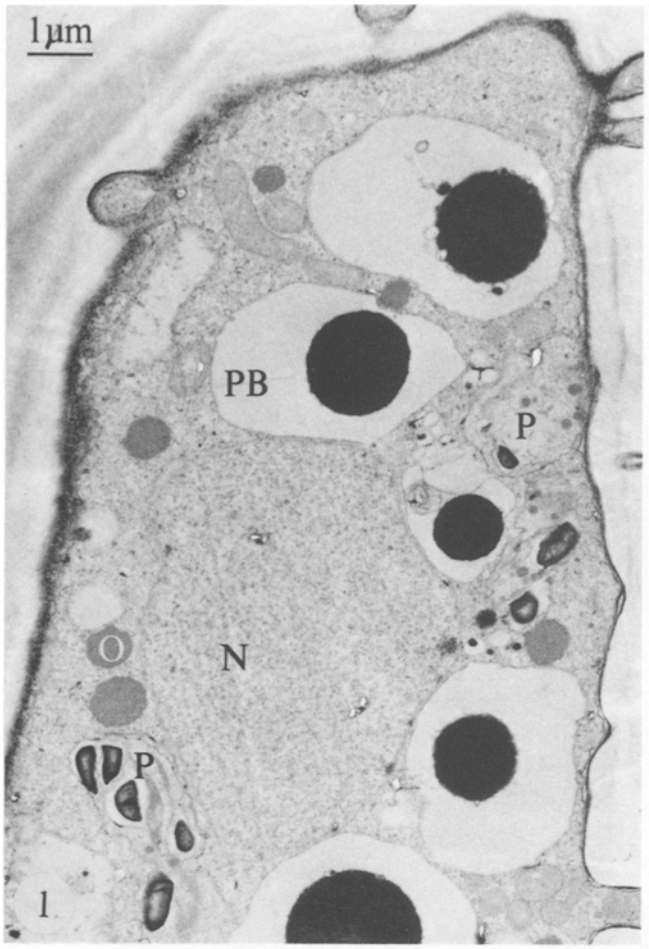
Introduction

Recently electron-dense intravacuolar bodies have been described in poplar ray cells which accumulated during fall and disappeared again during spring (Sauter and Kloth 1987, Sauter et al. 1989). Because of their structural resemblance with protein bodies in oat endosperm cells (Saigo et al. 1983), in storage parenchyma cells of cotyledons (e.g., Davey and van Staden 1978, Adler and Müntz 1983), and in phloem parenchyma cells of *Sambucus* (Greenwood et al. 1986) they were suspected to be of proteinaceous nature. In the crude protein extract of the wood a prominent polypeptide of about 32 kDa was detected which vanished during spring indicating its function in protein storage (Sauter et al. 1988). This 32 kDa wood protein of poplar was used for raising antibodies in rabbits. With the aid of the immunogold labelling technique, the suspected protein bodies were found to be the specific binding sites of these antibodies (van Cleve et al. 1988). Convincing evidence thus exists for these bodies being the particular sites of protein storage in the wood. In willow, protein bodies of similar appearance were found in ray cells of the wood (Sauter and Wellenkamp 1988). Because poplar and willow are closely related genera it was of interest to see whether the antibodies raised from the poplar storage protein do react also with the storage protein of willow, both, at the ultrastructural level and in the immunoblot.

Material and methods

At the end of winter, in late March and early April, 2- to 3-year-old twigs of willow (*Salix caprea* L.) and poplar (*Populus* × *canadensis* Moench "*robusta*") were collected from 7- to 9-year-old trees growing in the Botanical Garden of Kiel University. Radial longitudinal sections of the wood were prepared and used for the electron microscopy [fixation in glutaraldehyde (5%) — paraformaldehyde (4%), post-fixation in OsO_4 (2%)], for the immunochemical protein localization with protein A gold, for the protein extraction and separation by SDS-PAGE, and for the immunoblotting method on nitrocellulose as described elsewhere (see van Cleve et al. 1988). For both, the immunogold labelling of protein bodies at the electron microscopical level and for the immunoblotting procedure of the wood protein extracts, antibodies were taken which have been raised in white rabbits against the storage protein of poplar wood of ca. 32 kDa (Sauter et al. 1988).

* Correspondence and reprints: Botanisches Institut, Universität Kiel, Olshausenstrasse 40, D-2300 Kiel, Federal Republic of Germany.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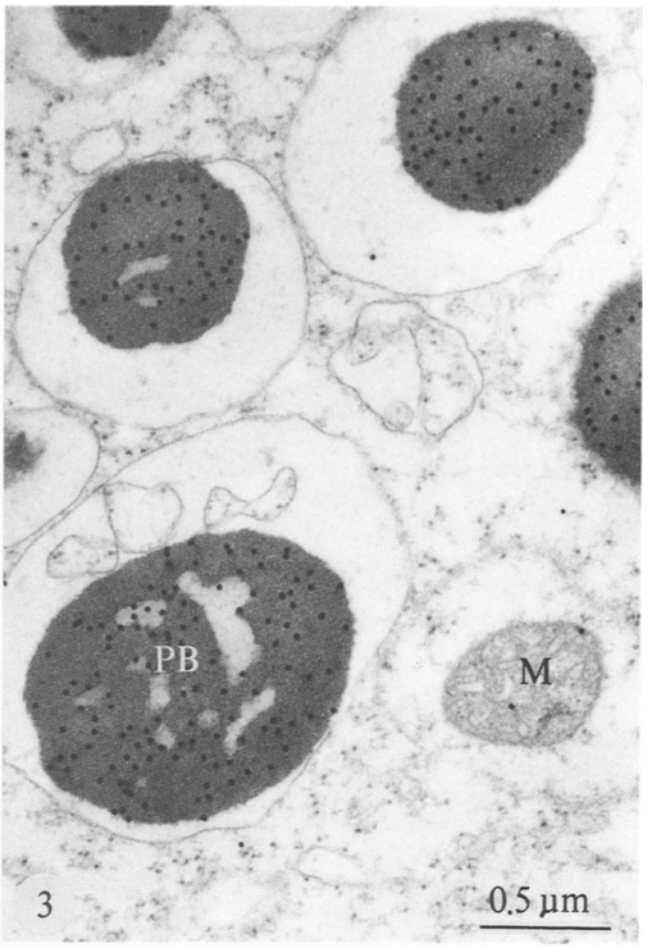
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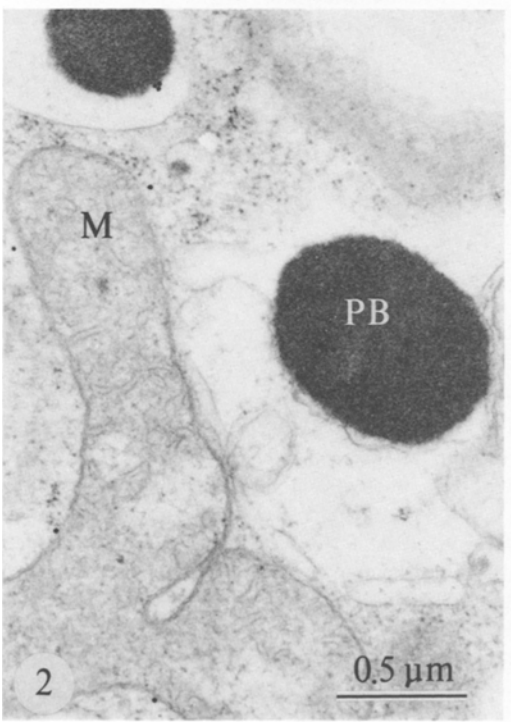
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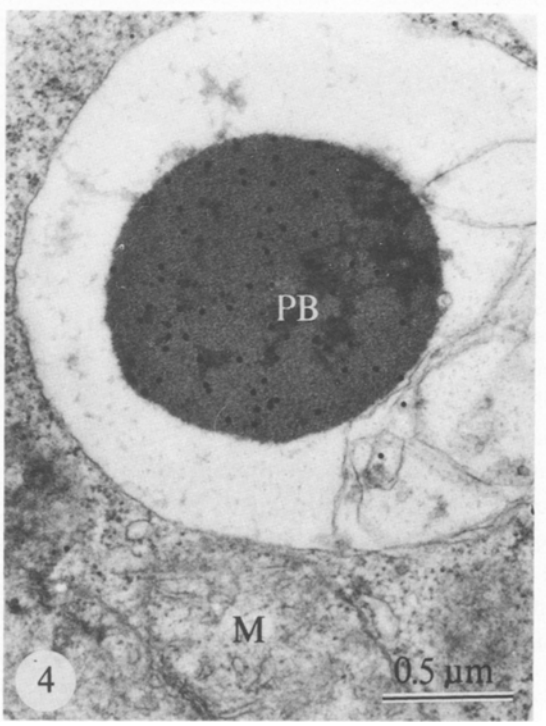
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Results and discussion

Figure 1 shows a wood ray cell of willow during late winter with the three main storage compounds, e.g. the starch, the oleosomes, and the protein bodies. Using the preimmun-serum, no labelling of protein bodies is obtained in willow (Fig. 2). However, when the serum containing the antibodies against the 32 kDa poplar storage protein is used, both the protein bodies in poplar ray cells (Fig. 3) and in willow ray cells (Fig. 4) become labelled specifically with immunogold. When the proteins extracted from poplar and from willow wood are separated by SDS-PAGE and then transferred to nitrocellulose for the immunoblot test, the 32 kDa bands become again specifically labelled in both species in response to the poplar antibody (Fig. 5).

The results show that the protein bodies which become accumulated in willow ray cells during fall (Sauter and Wellenkamp 1988) do not only resemble in structure and distribution the protein bodies described for poplar (Sauter et al. 1988, 1989) but that they contain also a polypeptide of ca. 32 kDa which binds specifically to the antibodies raised against the poplar storage protein. The storage proteins of these two genera thus appear to be closely related. This preliminary result becomes further strengthened by the fact that the same antibodies do not bind to protein bodies of other tree species, e.g., of birch (unpubl. result). It was shown recently that the youngest twigs are the sites of most intense seasonal deposition of proteins (Sauter et al. 1989). In willow, rapid mobilization of proteins from local depots in twigs and translocation via the xylem pathway was found during blossoming of catkins in early spring (Sauter 1981). The present results give additional support to the view that the protein bodies of the ray cells are the specific sites of protein storage in the wood of trees.

Acknowledgements

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Fig. 1. Ray cell in the wood of *Salix* in late March showing protein bodies (*PB*), oleosomes (*O*), and amyloplasts (*P*). Protein bodies in the stage of mobilization. *N* Nucleus. × 8 400

Fig. 2. Detail of a ray cell of *Salix* after immunogold labelling using the preimmun-serum. Protein body (*PB*) without label. *M* Mitochondrion. × 33 500

Fig. 3. Detail of a ray cell of *Populus* showing specific immunogold labelling of the protein bodies (*PB*) when the antibodies against its own 32 kDa storage protein are used. *M* Mitochondrion. × 33 500

Fig. 4. Detail of a ray cell of *Salix* after immunogold labelling with the 32 kDa storage protein antibodies of *Populus*. The willow protein bodies (*PB*) are labelled specifically. *M* Mitochondrion. × 33 500

Fig. 5. Immunoblot test of proteins extracted from the wood of *Populus* (*P*) and *Salix* (*S*) after their separation by SDS-PAGE, transfer to nitrocellulose, and immunochemical staining. The 32 kDa protein bands of both species show positive staining with the antibodies against the poplar storage protein