Histochemical IAA-Oxidase Localization in the Shoot of Wheat

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Abstract. A histochemical method for the determination of IAA-oxidase has been used in sections of various aerial parts of winter wheat plants. High IAA-oxidase activity was localized in the cell walls of sclerenchyma near the periphery of the stem, in the vascular bundle sheath of sclerenchyma and in xylem, both in the stem and in the leaf. The cell wall-bound IAA-oxidase activity therefore appeared in lignifying tissues. The staining was very weak or absent in the cell walls of parenchyma tissues and phloem. The positive reaction of the cytosol at the bulbous ends of guard cells and in the leaf primordia is presumed to be due to cytosolic IAA-oxidase. These results are discussed in relation to peroxidase localization and to our previous in vitro studies.

Peroxidase is an enzyme with a very broad substrate specificity (Gaspar et al. 1982). The function of this enzyme and its numerous isozymes found in extracts from different plant tissues is still not known. One of the most promising approaches to gain some insight into this problem is to study the localization of peroxidase activity using cytochemical techniques.

At the tissue level, peroxidase activity was found in the epidermis, xylem and phloem, root cortex parenchyma, and leaf mesophyll (Hall and Sexton 1972, Catterson 1980, Goldberg et al. 1983, Harche 1984). At the cellular level peroxidase has been localized in cell walls (Czarninski 1978, Goldberg et al. 1983, Harche 1984) as well as in the cytosol, associated with the membrane system, in vacuoles and in nuclear structures (Gaspar et al. 1982).

It is known that most of the peroxidase isozymes possess IAA-oxidase activity and many authors believe this activity to take part in controlling IAA level (Gaspar et al. 1982). Some years ago a cytochemical method for detecting IAA-oxidase activity was developed (Sági 1972, Beneš and Seidlová 1978). The IAA activity is much more specific than the peroxidase one. IAA is oxidized without added H₂O₂, i.e. the enzyme functions as a terminal oxidase.

In our previous work we isolated a peroxidase isozyme from young wheat leaves, which was an active IAA-oxidase and we inferred from its composition and its mechanism of action that it was a cell wall-bound isozyme (Zmrhal and Macháčková 1978). Also some other peroxidase isozymes were
able to oxidize IAA (unpubl. results). To get more information about the possible role of IAA oxidase in wheat plants we decided to study its localization at the tissue level.

**MATERIAL AND METHODS**

Winter wheat (*Triticum aestivum* L., cv. Jubilar) was grown in the field. Samples were taken at the stage of one or two leaves and at the stage of ear emergence.

The histochemical method of IAA-oxidase determination according to Sági (1972 and personal communication) was used. This method was described for *Lupinus* hypocotyls (Beneš and Šeidlůvá 1978). The method uses IAA as a substrate for the enzymatic reaction and the products of auxin oxidation are detected *in situ* by means of p-dimethylaminocinnamaldehyde, DMACA (Meudt and Gaines 1967). Sections of unfixed tissue 50 to 100 μm thick were prepared with a freezing microtome; free-hand sections were used in some experiments. Free sections were placed without delay into incubation medium containing 50 μM of MnCl₂, 50 μM of p-coumaric acid and 2mM of IAA in 0.06M phosphate buffer (pH 5.6). Fresh medium was always prepared using stock solutions on MnCl₂ and p-coumaric acid stored at about 5°C for several days. The sections were incubated in this medium for two hours in light at room temperature, then washed twice in 0.06M phosphate buffer (pH 5.6) and transferred into 0.5 per cent DMACA in 2M HCl and kept in darkness until photographing. The pink-purple staining was visible in a few minutes. The photographs were taken in the reaction medium. A series of control incubations were carried out. The validity of detection was tested by heating control sections or by incubation of control sections in medium lacking IAA. No staining was observed in the controls.

**RESULTS**

In the internodes (at the stage of ear emergence) IAA-oxidase activity was detected in cell walls in the continuous cylinder of sclerenchyma close to the stem periphery and in the vascular bundles (Fig. 1A). In the vascular bundles it was localized in the cell walls of vessels, especially in metaxylem and in the bundle sheath of sclerenchyma. The outer bundles which are embedded in the peripheral sclerenchyma showed higher IAA-oxidase activity than the inner ones. A virtually negative reaction was found in parenchyma including parenchyma cells of the vascular bundles.

Similar coupling of staining to cell walls of sclerenchyma and xylem elements was found in the leaves (Fig. 1B, C). A strong reaction appeared in plates of sclerenchyma located on the abaxial side of the leaf sheath, contrasting with the non-stained neighbouring thin-walled parenchyma of mesophyll and with the phloem. The smaller bundles containing mainly xylem elements and surrounded by the sheath of sclerenchyma were also heavily stained.

The epidermis of the leaf sheath and of the node also showed considerable IAA-oxidase activity, bound to the cell walls (Fig. 1C, D).

The stomata showed more IAA-oxidase activity than the other epidermal cells of the young leaf. The purple staining appeared in the bulbous ends of the guard cells (Fig. 1E). IAA-oxidase activity was also found in leaf primordia tissue of young wheat plants (Fig. 1F).