

## 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, CATESSON 1980, GOLDBERG *et al.* 1983, HARCHE 1984). At the cellular level peroxidase has been localized in cell walls (CZANINSKI 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  $H_2O_2$ , *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

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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 SEIDLOVÁ 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  $\mu\text{m}$  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  $\mu\text{M}$  of  $\text{MnCl}_2$ , 50  $\mu\text{M}$  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  $\text{MnCl}_2$  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).

## DISCUSSION

Localization of IAA-oxidase activity has only rarely been studied. It has been shown to be associated with subcellular fractions representing the Golgi apparatus and endoplasmic reticulum from pea plants (WALDRUM and DAVIES 1981). IAA-oxidase activity was detected by the histochemical method of SÁGI (1972) in the epidermis, in the hypodermal layers of the cortex and in the vascular bundles of *Lupinus albus* hypocotyls (BENEŠ and SEIDLOVÁ 1978).

In the present work we always found IAA-oxidase to be localized in cell walls with prominent thickening showing a high tendency to lignification (ESAU 1977). This localization suggests that although the enzyme (isozyme) reacts as IAA-oxidase, its primary function is probably the formation of lignin which is a well-described peroxidase-catalyzed reaction (HARKIN and OBST 1973). This view is supported by the numerous findings of cell wall peroxidase activity (e.g. MÄDER and AMBERG-FISCHER 1982) and by our previous *in vitro* results on the chemical composition and reaction kinetics of pure isozyme B 1 with high IAA-oxidase activity (ZMRHAL and MACHÁČKOVÁ 1978). Its chemical composition (arabinogalactan moiety) shows its cell wall origin and its kinetics shows preferential oxidation of phenolics. As we know that the B 1 isozyme content is relatively higher than that of the other isozymes (ZMRHAL and MACHÁČKOVÁ 1978 and unpublished results), it is very probable that the greater part of the IAA-oxidase activity localized in the sclerenchyma cell walls and vessels could be ascribed to this isozyme.

The reaction was very weak or absent in thin-walled parenchyma and in phloem. CATÉSSON (1980) found a highly positive peroxidase reaction in phloem of the *Triticum* stem. It might be possible that this phloem peroxidase(s) has no IAA-oxidase activity. Some discrepancies in localization at tissue level obtained by different authors may be explained by developmental changes in peroxidase activity. It is known that peroxidase activity in the cell wall usually increases as the cells leave the meristematic region (HALL and SEXTON 1972, GOLDBERG *et al.* 1983) while during maturation and lignification the cell wall peroxidase then decreases in activity (CZANINSKI 1978, HACHE 1984). Similar results have been obtained with IAA-oxidase in the present work. If the still-growing internodes were sectioned at increasing distances from the node the cell wall staining decreased (unpublished results).

A positive reaction of the tissues and cells which are rich in cytosol and have metabolic activity — the leaf primordia and the bulbous ends of the guard cells — is also observed. It is probable that this staining is due to an isozyme(s) other than B 1 and that this cytosolic activity might be responsible for the control of IAA level. But we have to take into account the diffusion of reacting products in the case of cytosolic IAA-oxidase, which is apparent from the staining pattern (BENEŠ and SEIDLOVÁ 1978). We may exclude the leaching of the enzyme out of the cell walls where it is covalently bound (GASPAR *et al.* 1982). Thus, the differences in staining of the thick-walled sclerenchyma and the neighbouring cells of thin-walled parenchyma are not artifacts.

Some problems of interpretation of the results may be posed by the presence of natural inhibitors of IAA-oxidase, diphenols and polyphenols, the so called IAA protectors (STONIER *et al.* 1979). In the cells these phenols are localized in the vacuole and moreover, the concentration ratio of IAA given

as a substrate to IAA protectors in cells is much higher than the inhibitory one (MACHÁČKOVÁ *et al.* 1975). We therefore do not consider the possibility of interference by IAA protectors to be of any great importance.

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*Fig. 1 at the end of the issue.*