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Peroxidase isoenzymes in germinating barley seeds and in seminal roots

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Abstract

Roots and germinating seeds of summer barley of the cv. Alsa, Antałek, Cebeco 7161, Lubuski, Skrzeszowicki and Union were found to differ in the number of peroxidase isoenzymes. In the germinating seeds from 5 to 8 isoenzymes were found whereas in the two-week-old roots — from 10 to 14 isoenzymes. Four isoenzymes in germinating seeds and eight isoenzymes in seminal roots appeared in all the cultivars tested. The cultivars differed also in the relative activity of the isoenzymes in the tested organs.

INTRODUCTION

In recent years a large number of studies were conducted on the presence of peroxidase (PO) isoenzymes in a variety of plants. However, these isoenzymes have not been studied in more detail in barley plants (Scandalios 1974). In growing embryos of barley PO synthesis *de novo* has been studied by Anstine et al. (1970). The PO isoenzymes have been found in varying numbers in young roots, crowns, internodes and nodes in three barley cultivars (Stroiński et al. 1976).

Some of the PO isoenzymes also have the activity of the indoleacetic acid oxidase (IAA-O). Galston and Dalberg (1954) have implied that the activity of the IAA-O system is inversely correlated with growth. There seems to be a direct relation as evident from the data of Stroiński et al. (1976) and Krzywański et al. (1976), between IAA-O and PO activities and the size of barley roots. The PO isoenzymes, appearing in plant tissues in response to various growth regulators (Gaspar et al. 1973; Mendt and Stecher 1972), are bound to ribosomes (Raa, 1973) and regulate their activities (Pennon et al. 1970; Raa 1973). Thus, in view of the wide differences in root size

between barley cultivars (Krzywański et al. 1976), the heritable of types and activities of PO (Tyson and Blomberg 1971) and suggested regulatory function in respect to growth regulators (Moll et al. 1969; Levings et al. 1971), extension of the investigations on PO isoenzymes to a number of barley cultivars seemed justified.

MATERIAL AND METHODS

Seeds and two-week-old roots of the following cultivars of summer barley were analysed: Alsa, Antałek, Cebeco 7161, Lubuski, Skrzeszowicki, Union. Seeds, after soaking for 8-10 hours in water, were placed on moist gypsum plates in plastic boxes for 48 hours. Only those seedlings which showed uniform growth were taken for analysis.

In order to obtain two-week-old roots, the seedlings selected from pregerminated seeds were grown in a carpet — type water culture according to the procedure applied by Krzywański et al. (1976a). The sampled plant material was frozen with dry ice, ground with it and put into cold acetone (5 g/50 ml). The homogenate was washed with cold acetone (200 ml). Acetone powders were stored in a vacuum dessiccator at −10°C. Acetone powder (100 mg) was extracted for 1 hour at 5°C with 5 ml of 0,01 M phosphate buffer, pH 6.3, containing 1% sucrose, 0,1% cysteine, and 0,1% ascorbic acid. The suspension was centrifuged for 30 minutes at 30,000 x g. The supernatant was used for gel electrophoresis. Disc gel eletrophoresis was carried out as described by Davis (1964) with running pH 9.0. Enzymatically active bands were identified by flooding the gels for 30 minutes with a saturated benzidine solution and then developing for 1.5 minute in 0.015% H_2O_2. The colour intensity of the developed bands was measured at 576 nm in a gel scanner. The protein content of the extract was determined by the method of Mejbaum-Katzenellenbogen at al. (1955).

RESULTS

Varietal differences as regards PO isoenzymes were first discovered after electrophoresis of extracts from whole germinating seeds (Fig. 1). These seeds, depending upon the cultivar, produced 5-8 bands, four of which (nos. 1, 3, 4, and 8) were common to all cultivars. The activity of bands 1 and 8 was quite high, while that of band 4 was low. The lowest number of PO isoenzymes was found in germinating seeds of cv. Alsa (5), while in the cv. Cebeco 8 isoenzymes were present.

In germinating seeds (Fig. 1) three groups of PO isoenzymes may be found. One group of low electrophoretic mobility with bands 1, 2, 3, and 4 with R_f values ranging from 0,01 to 0.2 and, the second group having

bands 7 and 8 with R_f values above 0.8. The third group is represented by only two isoenzymes — bands 5 and 6. Both bands were present only in the cv. Cebeco. These two isoenzymes showed a rather low activity. The germinating seeds of the tested cultivars showed an increasing number of PO isoenzymes in the following order: Alsa<Lubuski, Skrzeszowicki<Antałek, Union<Cebeco.

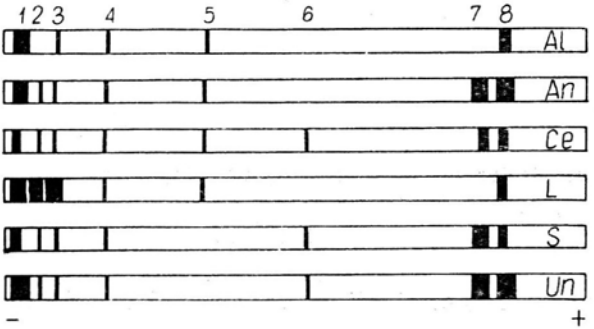

Fig. 1. Peroxidase electrophoreograms (5 μg protein/gel) of germinated barley seeds cvs. Alsa (*Al*), Antałek (*An*), Cebeco 7161 (*Ce*), Lubuski (*L*), Skrzeszowicki (*S*). Union (*Un*)

Differences in the number and relative activities of PO isoenzymes were also discovered in extracts from young, two-week-old seminal roots (Fig. 2). Their roots, depending upon the cultivar, gave 10-14 bands eight of which nos. 2, 5, 6, 9, 10, 12, 13, and 14 were common to all cultivars. Only in the case of cv. Alsa bands 13 and 14, both of high electrophoretic mobility, showed a very low activity. The bands 1, 3 and 11 of the cv. Alsa exhibited a very high activity as compared with the remaining cultivars.

In the case of young seminal roots, that is in the more homogenous material as compared with the germinating seeds, seven PO isoenzymes were present with R_f values ranging from 0.2 to 0.8. These enzymes sometimes showed very high activity. The total number of PO isoenzymes present in seminal roots varied between the cultivars. The seminal roots of cv. Antałek and Lubuski contained 10 PO isoenzymes, while the roots of cvs. Alsa and Skrzeszowicki 10 PO isoenzymes. In the seminal roots of the tested cultivars the number of PO isoenzymes increased in the following order: Antałek, Lubuski<Cebeco<Union<Alsa, Skrzeszowicki.

Comparing the isoenzymes from germinating seeds and young seminal roots one can see that in all cultivars only two PO isoenzymes of R_f value 0.17 and 0.86 were always present. These coresponded to isoenzyme

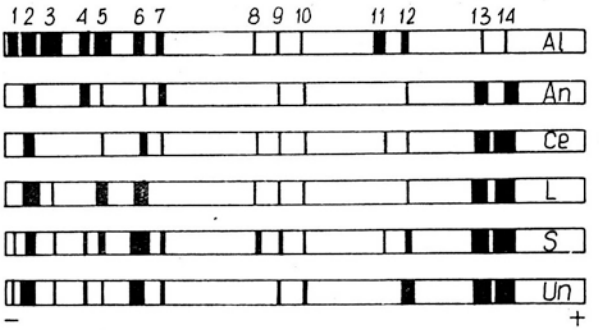

Fig. 2. Peroxidase electrophoreograms (5 μg protein/gel) of barley seminal roots of six cvs. Alsa (*Al*), Antałek (*An*), Cebeco 7161 (*Ce*), Lubuski (*L*), Skrzeszowicki (*S*), Union (*Un*).

bands nos 4 and 8 in germinating seeds and 5 and 14 in seminal roots. Generally, the PO isoenzymes were more numerous in seminal roots than in germinating seeds.

DISCUSSION

Data on total PO as well as IAA oxidase activities from previous experimentes (K r z y w a ń s k i et al. 1976) revealed significant varietal differences. These differences were visible in the activities of PO and IAA oxidase in the roots, crowns, shoots and ears of six barley cultivars tested at the ear emergence stage. Cultivar and tissue-specific patterns of PO — isoenzymes appeared (S t r o i ń s k i et al. 1976).

However, to obtain this information we have to grow the plants for a few weeks in nutrient solutions in the greenhouse. To avoid this inconvenience, without loss in the number of isoenzymes, we tried to use germinating whole seeds and roots of two-week-old seedlings grown in water culture. It is important to shorten the growth period, in order to test the transfer of PO isoenzyme patterns of pairs of parents to their progeny. The PO isoenzymes pattern of whole germinating seeds and roots of two-week-old seedlings seems to be specific to the cultivars tested, and what is more important, the two week-old seminal roots contain more PO isoenzymes than any of the plant parts tested until now (cf. S t r o i ń s k i et al. 1976). Therefore, such roots seem to be suitable organ for testing the transfer of the enzyme-protein pattern from parents to their progeny. The isoenzyme pattern might be used as a genetic marker for the growth character of roots. Such a possibility exists (S k o c z e k 1977). However, it has to be proved, whether there is any

relation between the PO isoenzymes and the growth characteristic of roots.

The lower number and relative activity of PO isoenzymes of cv. Lubuski, as compared with cv. Skrzeszowicki (Fig. 2) is evident. In a previous paper (Krzywański et al. 1976b) we reported that the size of roots of the cv. Lubuski was smaller as compared with those of cv. Antałek and Skrzeszowicki. Such a relation has not been found between cv. Antałek and Lubuski.

A lack of direct relationship between the size of roots and the number of isoenzymes is possible. It is known, that the PO isoenzymes composition may be affected by various growth regulators. Lee (1974) found that the synthesis of fast migrating PO cytoplasmic isoenzymes is strongly inhibited by external supply of cytokinin. Gaspar et al. (1973) reports that a large spectrum of PO isoenzymes appeared in response to various growth regulators supplied externally. The endogenous level of cytokinins in barley roots and crowns depends upon the cultivar and seems to be related to the size of its roots (Jeske et al. 1977).

The low number and activity of PO isoenzymes in germinating seeds, as compared with those in the vigorously growing young roots, may be the result of a lower level of growth regulators, upon which the isoenzymes number depends. Such a possibility exists as indicated by the results of Gaspar et al. (1973) in the case of the lentil embryonic axis. This problem as well as the relation between PO activity, substrate specificity of isoenzymes and the content of cytokinins in barley plants are the subject of current research.

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Izoenzymy peroksydazy kiełkujących nasion i młodych korzeni jęczmienia *

Streszczenie

Kiełkujące nasiona i młode korzenie sześciu odmian jęczmienia (cv. Alsa, Antałek, Cebeco 7161, Lubuski, Skrzeszowicki i Union) mają różny skład izoenzymów peroksydazy.

W kiełkowanych nasionach wykrywano 5 do 8 izoenzymów, natomiast w korzeniach dwutygodniowych siewek — od 10 do 14.

We wszystkich badanych odmianach stwierdzono cztery izoenzymy w kiełkujących nasionach i osiem w korzeniach.

Dodatkowo badane materiały różniły się aktywnością występujących izoenzymów.

* Praca wykonana w ramach problemu węzłowego 09.3.1. — 2.1. 7a.