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Biochemical Changes in Primary Wheat Leaves during Growth and Senescence

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Abstract. Changes in the different forms of the total activity of peroxidases (soluble, ionically and covalently bound), in the soluble isoperoxidase composition, and in the content of protein and chlorophyll during growth and senescence of the intact primary leaves of spring wheat were studied. The forms of peroxidases are in inverse relationship to the growth and increase during development and senescence, especially towards the end of senescence. The activity of peroxidases does not follow the physiological age of wheat leaves during the whole period of vegetation unlike the contents of protein and chlorophyll. The intensity of anodic and cathodic peroxidase isoenzyme bands 1 and 5 increases during senescence, but anodic bands number 2 and 4, cathodic bands 2 and 3 decrease. Changes in the activity of peroxidases and in the content of protein and chlorophyll as indicators of senescence are discussed.

Senescence is a series of natural deteriorative processes that terminate the functional life of an organism or a part of it. Senescence has been studied using intact plants, detached organs, leaf discs, and inducing senescence in different ways. Most of our knowledge of plant senescence, with special emphasis on its characterization as a genetically directed process, has been obtained on leaves.

The nature of the inducer(s) starting such a senescence programme, however, is not yet fully understood.

The first visible symptom of senescence is the yellowing of leaves caused by degradation of chlorophylls. THOMAS and STODDART (1977) note that the catabolism of chlorophylls seems to be directed by a nuclear gene, the expression of which is restricted to senescence.

Senescence in leaves is manifested by a decline in the levels of chlorophyll, protein, RNA and DNA, and in the increase in their hydrolytical enzyme activities (GARAIZABAL and RODRIGUES 1983, KUMAR and KHAN 1983, HARRIS *et al.* 1984).

The activity of peroxidase and peroxidase isoenzyme composition have also been related to leaf senescence (LAZAR and FARKAS 1970, BRABER 1980). It was demonstrated that the activity of peroxidase increased during sen-

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escence of plants. It was shown in detached leaves and in induced senescense of detached leaves during aging (KAR and MISHRA 1976, SEN *et al.* 1984).

Peroxidase has been implicated in plant senescence due to its ability to oxidize IAA and to participate in lignin formation. Rise in the peroxidase activity might be related to elimination of H_2O_2, whose production in cells increases with senescence. Thylacoid-bound peroxidase may be engaged in the catabolism of chlorophyll during leaf senescence (GASPAR *et al.* 1978, HUFF 1982, WARDLEY *et al.* 1984).

PARISH (1968) suggested that the increase in the activity of peroxidase is one of the most reliable indicators of maturity and senescence of plant. On the other hand, FORD and SIMON (1972), and PATRA and MISHRA (1979) found that the rise in peroxidase activity cannot be taken as a reliable indicator of senescence.

And so, in view of conflicting reports concerning peroxidase activity during senescence and aging of leaves, the present investigation was undertaken to study the activity of different forms (soluble, ionically and covalently bound) of peroxidases and the isoperoxidase composition of soluble protein during the natural senescence of the intact primary wheat leaves, and to determine whether the changes in peroxidase activities during normal wheat leaf senescence can be considered as an indicator of senescence.

Of the classical indicators of senescence we determined the changes in the content of chlorophyll and protein, and observed the parameters of vegetative growth.

MATERIAL AND METHODS

Laboratory experiments were performed with spring wheat *Triticum aestivum* L. cv. Leningradka. Wheat grains were soaked in distilled water for 24 h, rinsed, planted in pots (diameter 10 cm), filled with mineral soil, and thinned to 20 plants per pot. The plants were grown in phytotron under long day conditions of 16 h light (22 °C) and 8 h darkness (18 °C) with irradiances of 93 W m^{-2}. The pots were watered regularly with tap water until the soil was completely saturated. For the analyses of each index samples from 50 leaves were always used. One sample was measured and weighed.

All indices were determined from the primary leaf of wheat on the 5th, 10th, 15th, 20th, 25th and 30th days of growth after sprouting. Soluble guaiacol peroxidase activity was measured by the method described in our previous work (MIIDLA *et al.* 1985). Ionically and covalently bound cell wall peroxidases were separated from the residue by the method of RIDGE and OSBORNE (1970). Protein was assayed by the method of LOWRY *et al.* (1951), and chlorophyll after WINTERMANS and DE MOTS (1965).

The material was fixed with liquid nitrogen and homogenized samples were taken for enzymic and biochemical analyses. It is unlikely that the rise of the peroxidase activity resulted from wound effects, because we used whole leaves, not leaf sections and discs.

Soluble peroxidase anodic isoenzymes were separated on 7.0 % (pH 8.9) and cathodic isoenzymes on 7.5 % (pH 4.3) vertical polyacrylamide gel by electrophoresis according to MAURER (1968). The isoenzymes were stained by 10-min immersion of the gels into an incubation mixture containing 36 ml 0.2 M acetate buffer pH 4.0, 4 ml 0.01 M o-dianisidine-hydrochloride, 0.2 ml 0.2 M pyrocatechol and 0.2 ml 1.5 % H_2O_2 (JAASKA 1975).

The results are given as arithmetical means and were processed statistically: $n = 3$, $p = 0.05$ and $\Delta x\ \% = 2$ for protein and chlorophyll and 5 for the activity of peroxidases.

RESULTS

Primary leaf expansion (Fig. 1, A) is rapid until the 10th day, *i.e.* in the phase of growth when the laminae (length and width) reach their maximum area and volume (fresh and dry mass, Fig. 1, B). Leaf size changes a little through the mature and senescence phases.

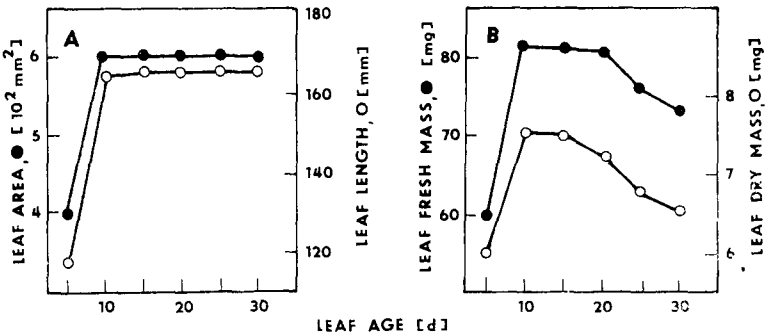

Fig. 1. Time course of wheat primary leaf growth.

Chlorophyll content and soluble protein content are maximal at the leaf age of 10 days (Fig. 2). By that time the leaf is fully developed, the leaf area and dry matter content do not increase further. At the same time the primary leaf changes its function to a material-exporting organ. At the age of 30 days the soluble protein content decreases to about 77 % and chlorophyll to 74 % of the maximum value.

The activities of all the forms of peroxidases change greatly during the growth and development of primary wheat leaves (Fig. 3). During the rapid growth of leaves the decrease in peroxidase activities is more rapid than the

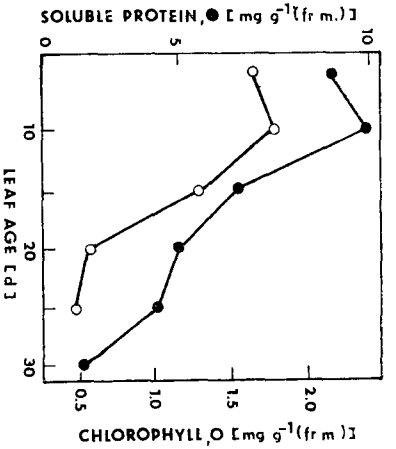

Fig. 2. Total protein and chlorophyll contents as a function of age in wheat primary leaves.

increase in protein and chlorophyll contents (Fig. 2). After the cessation of growth (from 10—20 d) the activity of total soluble peroxidase remains constant for 15 to 20 d, increasing rapidly thereafter. The specific activity of soluble peroxidase and the activity of cell-wall bound peroxidases do not reach any steady state and the increase, particularly in bound forms, is

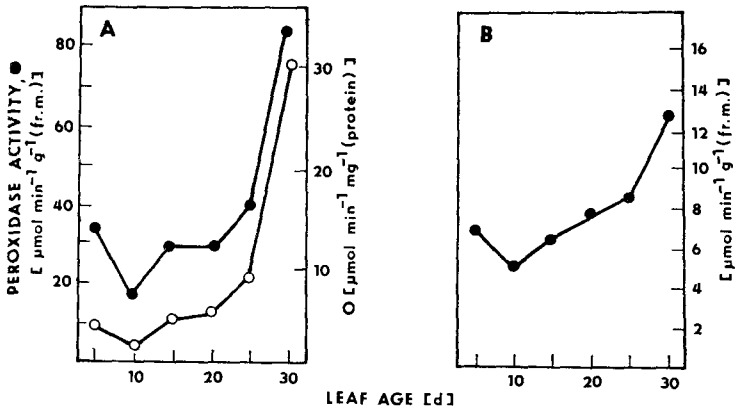

Fig. 3. Changes in peroxidases activity in wheat primary leaves during senescence. — Total (A, ●) and specific (A, ○) activity of soluble peroxidases, and total activity of ionically and covalently bound peroxidases (B).

noticeable. The activity per unit of protein and fresh matter is at its highest at the end of senescence.

Electrophoresis data show an increasing activity of anodic and cathodic peroxidase isoenzyme bands 1 and 5 (Fig. 4) during senescence. Decreasing activity is noticed in anodic bands 2 and 4 and in cathodic bands 2 and 3. The greatest changes occur after 25 days.

DISCUSSION

In general, protein and chlorophyll changes (Fig. 2) in attached primary leaves of wheat (following senescence under natural conditions) are in agreement with the results obtained by many investigators (MARTIN and THIMAN 1972, BRADY and TUNG 1975, MAKRIDES and GOLDTHWAITE 1981) in several kinds of plants. The loss of proteins and chlorophylls is certainly a dominant feature of leaf senescence.

Our results indicate that the values of protein and chlorophyll increase during the first 10 days. On the 10th d the protein as well as Chl contents start to decrease. From this point onward, the loss of protein and Chl is continuous until the end of leaf senescence (30 d). The net breakdown rate of protein from the 10th to 30th days is 3.8 % per day, and of chlorophyll 1.3 %, respectively.

These changes can be attributed to the variations in the rates of synthesis, degradation, or of both of them. Some investigators (FLETCHER and MCCULLAH 1971, LAMATTINA *et al.* 1985) have reported on the amounts of hydrolytic enzyme (protease, chlorophyllase) in bean, radish and wheat leaves. Contrary

to expectations, they found that the amounts of these degradative enzymes decreased sharply during senescence. Moreover, if senescence was reversed by the application of cytokinins, the amounts of degradative enzymes were increasing instead of going down. This means that the losses during senescence are due to decreased synthesis rather than to increased degradation. The

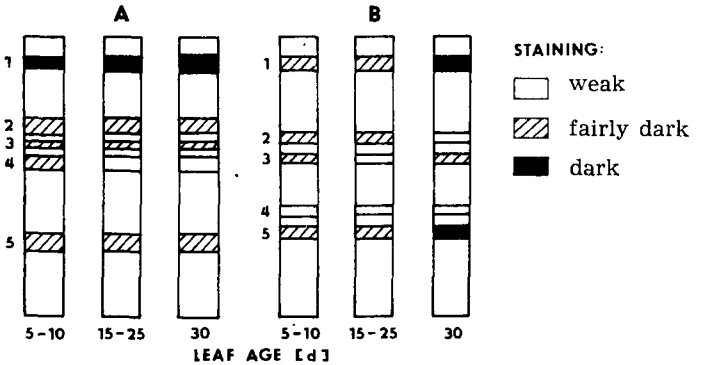

Fig. 4. Anodic (A) and cathodic (B) isoenzyme patterns of soluble peroxidase of wheat primary leaves.

implication is that the synthetic part of the turnover cycle stops or decreases faster than the degradative part, even though the latter may also decrease in rate. Studies suggest that increase of proteolytic activity in leaf extracts may have no relevance to the protein and chlorophyll loss in the whole leaf.

No direct relationship between the increasing protease activity and protein degradation in senescing leaves of various plants has been recorded by PEOPLES *et al.* (1980), RAGSTER and CHRISPEELS (1984) and WARDLEY *et al.* (1984).

MILLER and HUFFAKER (1985) determined the activity of endoproteinase during senescence of attached and detached barley leaves. Differences in proteolytic activity in the degradation of protein existed between senescing attached and detached leaves. No increase in the activity of proteolytic enzyme was detected during dark-induced senescence of attached leaves while in detached leaves it increased greatly. LEWINGTON *et al.* (1967) concluded that senescence of detached leaves is not the same process as senescence of attached leaves. However, information obtained on detached leaves must be interpreted with care.

Therefore, further work is necessary to evaluate the mechanism controlling the enhanced degradation of protein and chlorophyll associated with the onset of leaf senescence in both attached and detached leaves.

The activity of soluble and bound peroxidases changes greatly during the ontogeny of the leaves of spring wheat (Fig. 3).

The decreasing and increasing peroxidase gradient during growth and development of leaves might be related to auxin levels in the leaves (RAY 1960, BIRECKA and GALSTON 1969, BRABER 1980). GASPAR *et al.* (1978) argue that peroxidase may regulate the endogenous auxin level in the plant.

An increasing gradient of total peroxidase activity from top to base along the hypocotyl of dwarf pea stem was found by BIRECKA and GALSTON (1969)

and by DESBIEZ *et al.* (1980) in hypocotyl of *Bidens pilosus*. It means that the older the tissue, the higher is its peroxidase activity, and the lower the content of IAA.

The ontogeny of total soluble peroxidase activity in an attached primary wheat leaf is inversely related to the growth intensity (Figs. 1, 3). It is minimum on the 10th day when the growth-processes of the leaves end, starts to increase on the 15th day and remains constant till the 20th day upon cessation of growth, and from the 20th day onwards increases rapidly.

Our results agree with the data of TYSON (1970), and BIRECKA and GALSTON (1969). The former showed the inverse relationship in *Linum usitatissimum* and the latter in dwarf pea.

The activity of cell-wall bound peroxidases follows the curve of soluble peroxidase (Fig. 3A and B), but does not reach a steady state in the phase of maturity and increases rapidly. It implies that cell-wall bound peroxidases act on the last step of lignin biosynthesis, *i.e.* the polymerization of substituted cinnamyl alcohols is more intensive by bound than by soluble peroxidases (IMBERTY *et al.* 1985).

In the present study we examined changes in the intensity of five anodic and cathodic isoenzyme patterns of soluble peroxidase during wheat leaf senescence. Our results agree with the data on changes in the intensity of isoperoxidases described by BRABER (1980), who examined senescence in bean leaves. Her experiment showed that the number of anodic peroxidase isoenzymes increased from two in a young growing leaf to four in a senescent leaf. The number of different forms of peroxidase is probably species specific but the changes in the intensity of isoforms are related to lignification (MÄDER *et al.* 1980, MIIDLA *et al.* 1985) and senescence (BRABER 1980).

It is established (LESHEM *et al.* 1981, KAR and FEIERABEND 1984, MONDAL and CHOUDHURI 1984) that free radicals or oxidants (H_2O_2) accumulate during the senescence-process and this may be one of the factors involved in the regulation of senescence. It means that peroxidase in combination with superoxides (O^-_2) gives rise to hydroxyl radicals and singlet oxygen which might be implicated in aging and senescence. Our unpublished data also show that in the process of aging the content of H_2O_2 in the primary leaves of wheat increases in the period of maturity and senescence from 1.8 to 5.6 $\mu M\ g^{-1}$ fresh mass. So the onset of senescence in leaves is accompanied by an increase in the activity of peroxide-utilizing enzyme like peroxidase.

PARISH (1968) suggested that the increase in the activity of peroxidase is the most reliable indicator of plant maturity and senescence. Our results show that in attached primary wheat leaves of intact plants it is only true at the end of leaf senescence at the age of 20—30 days, but not during the whole period of leaf development. In the period of growth the activities of peroxidases are in inverse relationship to the growth and upon cessation they increase, reach a steady state and at the end of senescence they strongly increase. Thus the increase in peroxidase activity can be taken as an indicator of leaf senescence only at the end of leaf development. The activity of peroxidases does not follow physiological age of wheat leaves throughout the period of vegetation, in contrast to the contents of chlorophyll and protein. Similar data have been obtained by BREDEMEIJER (1973) with tobacco plants.

In the above-mentioned paragraphs we presented only our concepts

of indicators in the development of senescence of intact primary wheat leaves. However, our methods cannot give detailed results on the magnitude of these processes; therefore, further work is necessary to evaluate the mechanism — the cause associated with the onset of leaf senescence. We know that processes of leaf senescence are genetically controlled and programmed.

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BOOK REVIEW

IARC MONOGRAPHS ON THE EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS. VOLUME 41. SOME HALOGENATED HYDROCARBONS AND PESTICIDE EXPOSURES. — International Agency for Research on Cancer, Lyon 1986. 434 pp. Sw. Fr. 65.—.

Halogenated hydrocarbons comprise a group of chemicals which are widely used in products to which humans are exposed. They are used in the production of plastics, pesticides, solvents, paints, gasoline additives, cutting fluids *etc.* However, some marine organisms can introduce chlorine, bromine or iodine into organic molecules and the quantities of methyl chloride, methyl bromide and methyl iodide formed in the oceans exceed probably those from man-made sources. This IARC publication deals with the halogenated hydrocarbons, such as dichloromethane, 1,1,1,2-tetrachloroethane, pentachloroethane, 1,3-dichloropropene, 1,2-dichloropropane, methyl chloride, methyl bromide, polybrominated biphenyls and amitrole. Each compound covered includes: (i) Chemical and Physical data, (ii) Data on the production, use, occurrence and analysis, (iii) Biological data relevant to the evaluation of carcinogenic risks to humans, and (iv) Summary of data reported and evaluation. The publication also includes data on the risks of occupational exposures to chlorophenoxy herbicides (*e.g.* 2,4-D and 2,4,5-T). By 1974, the US Environmental Protection Agency had cancelled all registrations for chlorophenoxy herbicides, except those pertaining to uses other than on foods. Volume 41 of IARC Monographs on the genotoxic effects of some halogenated hydrocarbons represents the views and expert opinions of an IARC Working Group which met in Lyon 1986. The publication, as all IARC Mongraphs, should be of particular interest to toxicologists and to all those concerned with environmental carcinogenicity and mutagenicity.

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