Role of Petiole in Protein Metabolism of Senescing Betel (Piper betle L.) Leaves

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ABSTRACT

Effects of depetiolation on protein metabolism during senescence of detached betel (Piper betle L.) leaves have been studied. In normal petiolated leaves, the level of chlorophyll and proteins and extent of protein synthesis declined, while the protease activity registered manifold increase with the advancement of senescence. All of these changes were delayed by depetiolation/de-midribbing treatments, though without affecting the general pattern of senescence. Thus, the presence of petiole seems to expedite protein degradation, probably due to earlier attainment of optimal concentration of proposed senescence factor(s) (Mishra and Gaur 1970 Science 167: 387).

The deferment of senescence by mechanical means such as decapitation (28), defloration (10), and defruiting (16) seems to be one of the oldest and simplest practices brought into use by man. The role of the petiole has apparently been known for about a century (29), but such work received considerable impetus only in the early 40s when Chinnall (5) demonstrated that induced rooting at the petiolar end delayed the onset of senescence in detached condition. Within a few years after this monumental work, much research was done by the Mothes (15) and Osborne (17) groups. We felt the need to study further the role of this tissue in the regulation of various physiological processes during senescence of the detached leaves.

In our earlier studies we found that removal of petiole (depetiolation) or of both petiole and midrib (de-midribbing) from detached betel leaves delayed the onset of senescence considerably (13, 14). The present study deals with the changes taking place in protein metabolism.

MATERIALS AND METHODS

Betel (Piper betle L. cv. Gauchi) leaves of approximately uniform size and age (sixth from the terminal apex) were collected locally from the field. While leaves with intact petiole (20 mm length) served as petiolated control, those devoid of petiole or of both petiole and midrib served as two different treatments termed "depetiolation" and "de-midribbing," respectively. The leaves were wrapped in Hessian cloth (which was kept wet) for maintaining high humidity around the leaves throughout the experimental period and stored at 25 ± 1°C. Senescence rate was measured in terms of Chl disappearance (HMSb), which normally begins at the petiolar end and progresses distally along the midrib. To minimize the variation from one lot to another, all of the estimations were made from the same set of plant material.

Discs of 6 mm diameter were punched out from the interveinal portion of the leaves. Such selection for discing was made periodically from day zero until HMSb of each treatment. At every stage, 300 to 400 discs/treatment were selected and sterilized in 5% sodium hypochlorite solution for 10 min. From these, 50 discs in quadruplicate were finally selected for maximum uniformity in size and predominant color.

Tracer Studies. In order to study the protein-synthesizing ability of the senescing leaves, discs were incubated at 25 ± 1°C in the dark for 2 hr in radioactive solution (1dl-[1-14C]leucine, 50.75 mCi/mmol) containing streptomycin sulfate IP (20 μg/ml). The adsorbed radioactivity was removed by serially rinsing the discs in cold leucine (10 μg/ml) in distilled H2O. These discs were then kept frozen until required for different estimations.

Chemical Estimations. In the frozen discs, Chl was determined by Bruinsma's method (4) and expressed as μg/disc. AS and AIS proteins were estimated from the residue (left after Chl extraction) by method of Lowry et al. (11) with some modification to suit the betel leaf. In the case of AIS fraction, if the usual procedure for mixing various reagents was followed, the color disappeared within a few min. This problem was overcome by acidifying the AIS fraction with an equal volume of PCA (10%) and immediately after the addition of reagent C. Protein fractions have been expressed as μg/disc, using pancreatic RNase-A (Sigma Chemical Co.) as standard.

Radioassay. Radioactivity measurements were carried out by taking 0.5-ml samples in duplicate in counting vials containing 15 ml of scintillation medium (containing 4 g of BBOT in 500 ml methanol and 500 ml toluene). Vials were counted twice in a computarized Beckman LS-100 liquid scintillation counter. To avoid variation from one set of determinations to another, the cpm values were converted into dpm values. Activity is expressed as dpm/disc.

Protease Activity. In order to study the protein degradatory ability of leaves, protease activity was measured using the Penn-ner and Ashton method (20). Two g of leaves were analyzed in triplicate. The enzyme activity was calculated and expressed in terms of mg of tyrosine liberated/g of fresh weight hr of incubation.

RESULTS

The general effect of depetiolation and de-midribbing on senescence behavior of betel leaf is presented in Figure 1A. There was a change in the shape of senescence curves, which tended to be more sigmoidal in depetiolted and de-midribbed leaves compared to those in petiolated ones. The initial lag period prior to onset of appreciable senescence was considerably prolonged in the absence of petiole or midrib. The HMSb values (indicated by
Fig. 1. Changes during senescence, in petiolated (○), depetiolated (□), and de-midribbed (△) leaves in terms of number of leaves senesced (A), Chl (B), AIS protein (C), AS protein (D), label incorporation in AIS protein (E), and protease activity (F).
the interception of the dotted perpendiculars on the abscissa for the respective treatments) varied significantly from 33 days in petiolated leaves to 44 days in depetiolated and 65 days in de-midribbed leaves. Senescence span (period from senescence of first leaf to that of all of the leaves) was found to be 14 days in petiolated, 40 days in depetiolated, and 60 days in de-midribbed leaves.

**Chlorophyll Level.** Chlorophyll level declined with advancing age (Fig. 1B). The rate of this decrease was faster in petiolated than in treated leaves. There was a lag period of 10 days in petiolated compared to 20 days in depetiolated and de-midribbed leaves before the obvious reduction in this pigment level. Quantitative differences between the Chl content of treated leaves and that of petiolated leaves at various stages of senescence varied from 4 to 103%. The differences between the two treatments were significant beyond 30th day. The Chl content seemed to attain a definite minimal level in each group of treated leaves before the latter reach their respective HMGs stage.

**Protein Metabolism.** AIS protein diminished with the advance of senescence (Fig. 1C). Only 50% of original AIS fraction was left by the HMSs stage in both control and treated leaves. Rate of decrease, however, was faster in petiolated leaves than in treated ones. The difference between treated and control and also between depetiolated and de-midribbed leaves became more prominent with the passage of time in general.

Contrary to a decrease in AIS protein content, there was a concomitant increase in AS protein fraction with passage of time (Fig. 1D). A lag of 10 days was observed for both control and treated leaves before initiation of any change. Thereafter, a clear-cut difference between treated and control leaves was maintained except at the HMGs stage when the AS level was about the same in all cases. At all other times, this fraction was smaller in treated than in control leaves. There did not appear to be a marked difference between the two treatments at most of the measurement stages.

Synthetic ability of the leaves was measured in terms of [14C]leucine incorporation in AIS protein. The data in Figure 1E on specific radio-activity showed that the latter was always higher in fast senescing tissues. When treatment values at various stages were compared with control, it was found that they remained significantly low throughout the experimental period. Similarly, the curve for de-midribbed leaves was always higher than that of depetiolated leaves except at the 40th day.

As a measure of protein degradation, protease activity was measured simultaneously (Fig. 1F). Although there was a general trend of increase in enzyme activity with the passage of time, it remained comparatively slow up to the 20th day in treated leaves. The increase was faster in petiolated control, accounting for almost a 50% increase over the initial values. The activity of enzyme, however, reached its maximum in the final phases of senescence in both control and treated leaves.

**DISCUSSION**

That Chl disappearance is an event closely associated with the process of aging and senescence (3, 21, 24) is supported by the present studies (Fig. 1B). This has been attributed to the structural and functional degeneration of chloroplasts (29). Even though we do not have direct evidence, such changes in chloroplast might have been slowed down in depetiolated and de-midribbed leaves. An isolated leaf was found to show a quick emigration of the protein decomposition products into the petiole (15, 19, 22). Our work shows that if the petiole was removed, the protein degradation was slowed down (Fig. 1C), and the leaves retained Chl for a longer period. This effect may be because of the soluble nitrogenous compounds remaining available for the newer synthesis. This is also supported by our earlier finding that in de-midribbed leaves there was greening on and along the midrib and veins as compared to petiolated controls (14). This confirms the observation of Mothes and Engelbrecht (15) who showed that the degradatory products of protein moved to midrib and finally to the petiole. Simon (26) found that in Cucumis cotyledons in detached conditions these tissues retained their power of protein synthesis and explained that due to short petiole there may not be a very marked basipetal translocation of nitrogenous compounds.

The delay in senescence in betel leaves due to depetiolation/de-midribbing in the present work was accompanied by a slower decrease in AIS fractions (Fig. 1C) and also a retarded increase in AS fractions (Fig. 1D). Parthier (19) found that the depetiolated leaves of Nicotiana did not lose their methionine incorporation capacity even after 13 days of storage, whereas petiolated leaves lost one-third of their incorporative capacity during the same period. On the other hand, our finding from label incorporation studies showed higher specific activity in fast senescing tissues (Fig. 1E). Such an apparent discrepancy is explainable in terms of less total protein (AIS) available because of faster degradation and also higher label in the newly synthesized protease protein (Fig. 1, C and F). In regard to enzymic proteolysis, the most pertinent question to be answered is about the source of such enzyme. There may be two sources; one is lysosomal and/or other similar entities (12, 23), and the other may be due to de novo synthesis (25, 27). However, the explanation in terms of lysosomal liberation of proteases did not hold true because the membrane-disruptive detergents like Triton X-100 and sodium laurel sulfate did not exert any effect either on the rate or extent of senescence of oat leaves (27). De novo synthesis is supported by the fact that an inhibitor of protein synthesis like CH actually delayed the senescence (27).

Proteolysis has been also visualized as under the control of endogenous hormonal status during aging (3). It is proposed that senescence of detached leaves or leaf discs may be a function of hormonal level, and thus any manipulation which alters this property of the tissue may also change the rate of senescence. Such a role of endogenous hormone has been amply documented in the literature (6, 8, 9). Back and Richardson (2) suggested that the seasonal variations in the ability of cytokinins or gibberellin to retard senescence of the detached leaves reflect changes in the endogenous quantities of ABA. This suggestion fits well with the present findings wherein depetiolation/de-midribbing delayed the onset of the senescence probably by changing the locale of the synthesis/accumulation of the proposed SF(s) (14). As the nature of this factor is not yet established, it may not be logical to associate it with ABA or some such inhibitor or senescence-inducer like ethylene (18). The latter is also known to affect protein metabolism during senescence (1, 7).

We inferred that the absence of petiole may be interfering in the optimum accumulation of the proposed SF(s). Any reduction in the latter would, in turn, influence the protein metabolism in the lamina and consequently result into delayed expression of senescence.

**LITERATURE CITED**

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