Abscission as a Mobilization Phenomenon[1,2]

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Summary. The possibility that leaf abscission might involve a mobilization phenomenon leading to a localized cellular senescence was examined by following dry weight changes, chlorophyll content, and protein levels in proximal and distal tissue of excised pieces of bean petioles from *Phaseolus vulgaris* L. var. Red Kidney. Nucleic acid levels were determined as were phosphate pool sizes in conjunction with ^{32}P labeling patterns. Each of these parameters indicated a mobilization into the tissues proximal to the zone of cellular separation at the expense of the distal tissues, suggesting that mobilization and the consequent development of cellular senescence provide a component in the process of bean leaf abscission.

The suggestion has been made by Osborne and Moss (19) that leaf abscission may be regarded as a localized cellular senescence. That mobilization centers can lead to senescence has been well documented (11, 12, 14, 19). The experiments described in this paper are an attempt to relate abscission to a localized cellular senescence resulting from a mobilization effect by tissue on the proximal side of the zone of cellular separation.

Methods and Materials

The experimental plant material was grown in the manner of Rubinstein and Leopold (20). Seeds of *Phaseolus vulgaris* L. var. Red Kidney were sown in vermiculite in controlled environment chambers and the plants maintained under uniform conditions (16-hr photoperiod, 2000 ft-c and $22 \pm 2°$). Plants were approximately 15 days old and first trifoliate leaves starting to expand when the primary leaves were excised and the blades trimmed off. A single section was cut from each petiole with a 1 cm cutter so that the upper abscission zone was in the center of the explant. Unless otherwise stated, 10 explants were then placed proximal ends down in 1 % agar 4 mm deep in a plastic petri dish. The dishes were returned to the growth chamber at a light intensity of 400 ft-c. Under these experimental conditions, abscission was achieved in 50 % of the explants at about 100 hours.

The chlorophyll content for 25 explants was measured at different times by extracting the distal and proximal halves of the explants with 5 ml of methyl alcohol and reading absorbance at 660 mμ with a Beckman B spectrophotometer. The dry weight of 10 distal and proximal halves was determined. Soluble protein changes (precipitable with TCA) in distal and proximal halves of 25 explants were measured using the Lowry method (13). Total RNA content of distal and proximal tissue of 25 additional explants was measured by Method II of Cherry (3).

The total amounts of phosphate in the distal and proximal tissue of 10 explant samples were measured by the method of Fiske and Subbarow (5). Oven-dried samples were ashed at 600° and the ash taken up in 0.4 ml 10 N H_2SO_4. Since pH is a critical factor for this method no further acid was added to the sample and the colorimetric determination was made using the complete sample. The final volume was 10 ml. Total P_i in the distal and proximal tissue of 10 explants was measured by the same colorimetric method described above. The tissue was ground with 3 ml H_2O and the homogenizing flask washed with 1 ml water. Trichloroacetic acid, 3 ml of 10 % (w/v), was added to precipitate protein and the tubes were placed in the refrigerator overnight. The precipitate was removed by centrifugation and P_i measured in a 1 ml aliquot. In this case, 0.4 ml 10 N H_2SO_4 was added before color development. Final sample volume was again 10 ml.

For ^{32}P uptake experiments, explants were floated on radioactive solutions and the amount of label in a phenol-Tris buffer extract was measured. Ten explants were floated for 1 and one-half hours on 10 ml buffered ^{32}P solution (5 μc/ml in 0.01 M K_2HPO_4) and refloated on unlabeled phosphate buffer for 45 minutes. The explants were then divided into distal and proximal portions and homogenized with 3 ml phenol saturated with 0.01 M Tris buffer; 0.01 M Tris-HCl, pH 7.6, 0.06 M KCl and 0.01 M $MgCl_2$; 0.1 ml bentonite (4 mg) and 0.5 ml 5.5 % Dupanol (sodium lauryl sulfate). The flask was rinsed with 1 ml of buffer and the total homogenate placed in test tubes.

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Tris buffer (4 ml) was added to each tube and thoroughly mixed, and a 1 ml aliquot of the aqueous layer was plated onto an aluminum planchet and dried for counting.

To measure ^{32}P incorporation into total nucleic acids, 25 explants were floated for 3 hours on 30 ml buffered ^{32}P solution (20 μc/ml in 0.01 M K_2HPO_4) and washed for 2 minutes in 5×10^{-2} M H_3PO_4. The explants were then divided into distal and proximal portions and ground with 5 ml phenol saturated with 0.01 M Tris buffer; 3 ml 0.01 M Tris-HCl, pH 7.6, 0.06 M KCl and 0.01 M $MgCl_2$; 0.1 ml bentonite (4 mg) and 0.5 ml 5.5 % Dupanol (sodium lauryl sulfate). The flask was rinsed with 1 ml of buffer. The aqueous solution was removed and treated once with an equal volume of cold phenol in presence of bentonite followed by a half-volume cold phenol-bentonite treatment. Nucleic acids were precipitated by adding 2 volumes of cold ethanol. The precipitate was dialyzed for 2 and one-half days against 0.05 M phosphate buffer, pH 6.7. The dialyzing solution was changed 3 times. Final sample volume was 5 ml. Two 0.5 ml aliquots from each sample were plated onto aluminum planchets and dried for counting.

The ^{32}P mobilization experiments with excised leaves were conducted by placing excised 15-day old primary bean leaves with the entire petiole in 50° H_2O for 20 seconds to hasten the development of senescence (4). A 0.1 ml aliquot of a ^{32}P solution (5 μc/ml) was then pipetted onto the leaf blade. The leaves were placed in a dark room until the time for abscission drew near. At that time the pulvinus and petiole were cut into 2 mm sections, dried and counted. During the development of senescence the petiole bases were trimmed to retard root initiation.

All experiments reported were repeated 3 or more times with comparable results.

Results

The most obvious change that occurs with senescence in green organs is the loss of green color. Therefore, as a beginning point in studying the possible involvement of senescence phenomena in abscission, the extent of chlorophyll changes on each side of the separation zone were measured. Figure 1A shows that with the approach of abscission, the chlorophyll content declines gradually on the distal side; on the proximal side, however, there is an actual increase in chlorophyll content. This reciprocal change suggests that the cells on the proximal side may mobilize materials out of the cells distal to the abscission zone

Dry weight changes have been usefully employed in the past as a measure of mobilization phenomena (1). Measurements of the dry weight changes in explants (fig 1B) reveal that during the development of abscission a decline of about 30 % in the distal tissue is matched by a similar gain in dry weight of the proximal tissue.

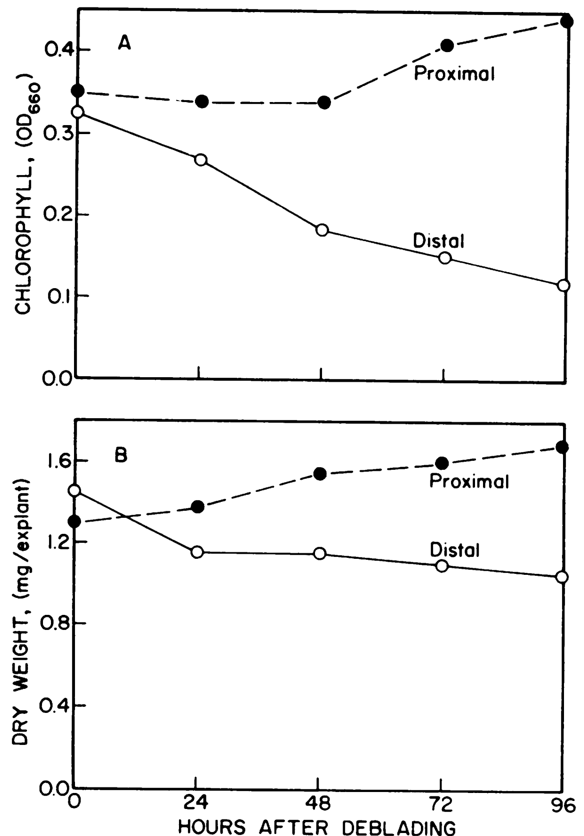

FIG. 1. A, Changes in chlorophyll content in proximal and distal halves of bean petiole explants. 50 % Abscission occurs at about 100 hours after deblading in all experiments reported. B. Changes in dry weights in explants.

A degradation of proteins in leaves during senescence has been well documented (15, 24). That mobilization activities can stimulate protein degradation from source regions of leaves has been shown more recently (16, 18). Measurements of the total soluble protein in the distal and proximal halves of explants were carried out at various stages during the development of abscission (fig 2A), and a decrease in protein in the distal tissue was found to be coincident with a rise in protein in the proximal tissue. Again this would suggest a mobilization of substances out of the distal cells.

A decline in nucleic acid during leaf senescence is well known (2, 22, 23). Figure 2B shows the pattern of changes in RNA levels in the distal and proximal tissue of the explant during abscission development. There is a decline in the RNA of the distal tissue and an increase during the first 48 hours in the proximal side.

The mobilization of materials out of leaves is associated with a dramatic loss of orthophosphate (17). Determinations of the phosphate concentrations in explants during abscission are shown in figure 3A. From the data on total phosphate contents (fig 3A) one can see a depletion of the distal tissue

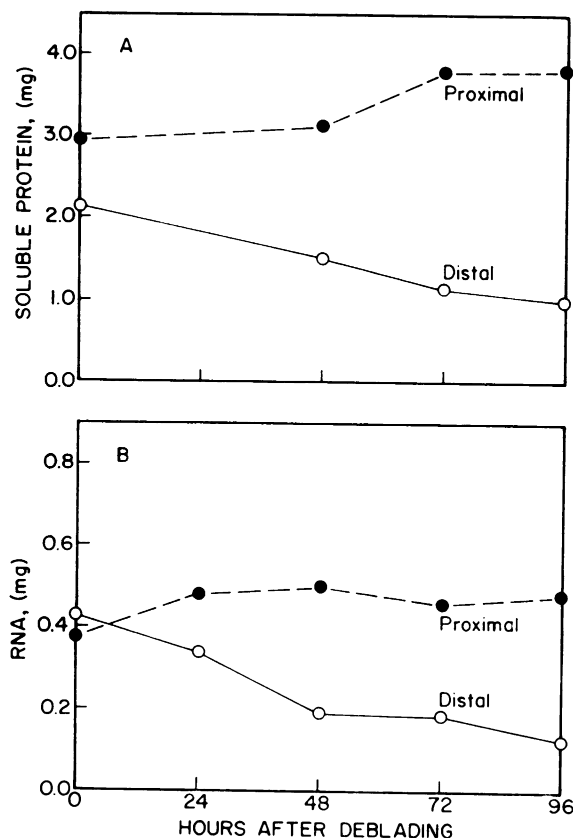

FIG. 2. A, Changes in soluble protein content in proximal and distal explant tissue. B, Changes in RNA patterns in explant tissue.

and an accumulation in the proximal tissue. Comparative data for orthophosphate and organic phosphate levels during abscission are shown in table I, the organic value being derived by difference between orthophosphate and total phosphate. These data reflect a greater change in organic phosphate than in the orthophosphate fraction, but both fractions indicate the accumulation of phosphate in the proximal tissue at the expense of the distal tissue.

Table I. *Concentration of Orthophosphate and Organic Phosphate in Excised Bean Petioles*

System: Phosphate in a 1 ml aliquot (3 ml homogenate) using 10 bean explants per sample. Color developed by method of Fiske and Subbarow using alpha amino naphthol sulfonic acid. All values are M × 10^{-4}.

	Tissue	Time after deblading		
		0	48	96 hrs.
Ortho-Phosphate	Distal	1.6	1.3	1.4
	Proximal	1.4	1.8	3.0
Organic Phosphate	Distal	1.9	1.5	0.6
	Proximal	1.3	1.6	2.0

As another approach to the changes in phosphate concentrations during the development of abscission, we floated explants on ^{32}P solutions and examined the amount of radioactivity appearing in phenol-Tris extracts of the tissue. Figure 3B shows the results of a representative experiment. The counts were corrected for the isotopic dilution by the varying P_i pool sizes as reported in table I. The data in figure 3B represent ^{32}P incorporation per mmole orthophosphate in the tissue.

The incorporation of label into both distal and proximal tissues at time zero is low. As the experiment proceeds, however, the incorporation of label into the proximal tissue rises steadily up to the time of abscission (96 hours) whereas incorporation into the distal tissue first rises at 24 hours and then decreases until the termination of the experiment. These results would again suggest that the proximal tissue develops an assimilative advantage over the distal tissue.

A more exact indication of an assimilative advantage developing in the proximal tissue would be the examination of ^{32}P incorporation into total nucleic acids. Results of such experiments are shown in table II. The data indicate that there is an increased incorporation of ^{32}P into the nucleic acids of the proximal tissue 48 hours after labeling, and this advantage is maintained after 96 hours. P^{32} incorpora-

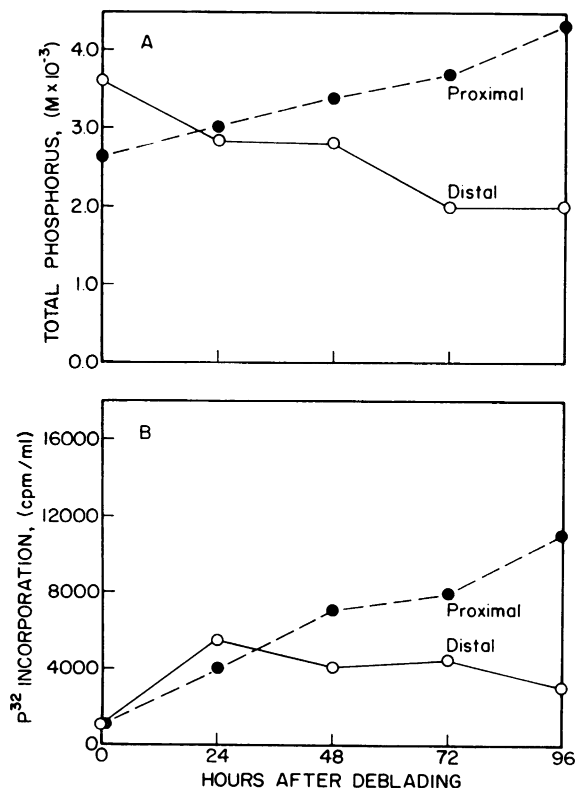

FIG. 3. A, Total phosphate distribution in proximal and distal tissue. B, phosphate incorporation for 3 hours at various times after deblading.

Table II. *^{32}P Incorporation into Total Nucleic Acids During Abscission Development*
Counts expressed as cpm/mmole phosphate.

Tissue	Time after deblading 0	48	96 hrs.
Distal	854 ± 57	700 ± 13	420 ± 16
Proximal	1080 ± 44	2040 ± 73	1950 ± 187

tion into nucleic acids of distal tissue declines steadily with time. These data are consistent with total RNA content as shown in figure 2B.

In the preceding experiments we have been examining the changes during the abscission development in explants ; to examine the involvement of a mobilization phenomenon in a more natural situation, experiments were carried out on the redistribution of ^{32}P along the petiole of excised whole bean leaves during abscission development. Excised leaves were dipped into warm water to hasten senescence (4) and radioactive phosphate was then applied to the leaf blade. At the time of abscission the distribution of ^{32}P along the pulvinus and petiole was determined. Figure 4 shows the results of 4 representative experiments. In every case, a distinct accumulation of ^{32}P (30 %) occurred in the tissue immediately proximal to the abscission zone. These data further support the concept that the tissue proximal to the abscission zone is acting as a mobilization center.

If mobilization can serve to trigger abscission development, then a treatment which suppressed abscission might be expected to suppress mobilization.

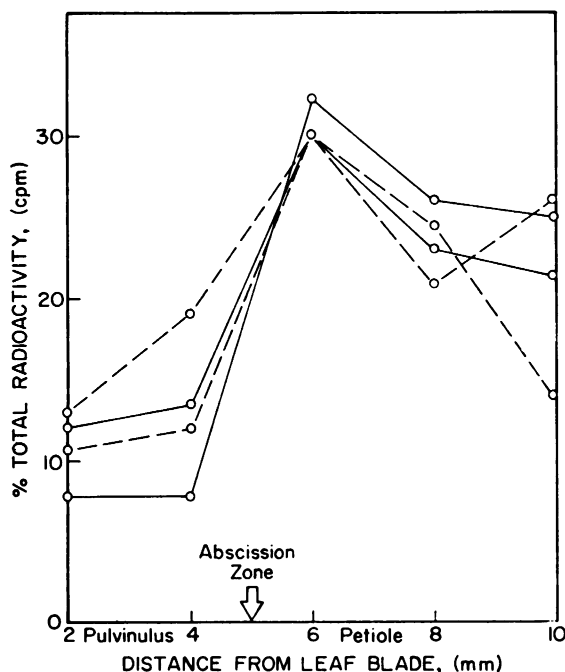

FIG. 4. Redistribution of label along the petiole at the time of abscission following application of ^{32}P to the leaf blade.

A series of explant experiments were conducted in which abscission was inhibited by proximal application of 5×10^{-4} alpha naphthaleneacetic acid (20). Under our experimental conditions none of the explants had abscised 96 hours after deblading. The results of experiments, in which 4 different mobilization parameters were followed in explants, are shown in table III. Chlorophyll contents declined similarly in distal and proximal tissues. The dry weight remained approximately constant in the distal tissue. Protein and phosphate contents declined in the distal tissue, but the extent of the change was smaller than in tissues which were undergoing abscission (cf fig 2,3). The data indicate that the extent of mobilization of materials out of the distal tissue is markedly less in explants in which abscission has been inhibited.

Table III. *Mobilization in Auxin Treated Explants: Abscission Inhibited with 5×10^{-4} M NAA*

	Tissue	Time after deblading 0	48	96 hrs.
Chlorophyll (OD_{660})	Distal	0.32	0.16	0.10
	Proximal	0.25	0.17	0.09
Dry wt (mg/explant)	Distal	1.58	1.67	1.56
	Proximal	1.32	1.50	1.54
Protein (mg)	Distal	2.12	2.12	1.56
	Proximal	1.86	1.79	1.96
Total PO_4 ($M \times 10^{-3}$)	Distal	3.42	2.85	2.05
	Proximal	2.41	3.06	3.66

Discussion

The association of mobilization with leaf senescence has been conclusively shown by Mothes (16), Leopold (11), and Leopold and Kawase (12). Osborne and Moss (19) suggested that the abscission zone is particularly sensitive to the withdrawal of metabolites and that promotion of such a withdrawal by auxins, kinins, or gibberellins could lead to a localized cellular senescence and hence abscission.

The present experiments describe losses in dry weight, chlorophyll, protein, nucleic acid, inorganic and organic phosphate from the distal tissue of explants during the development of abscission. The concomitant increase of these components in the proximal tissue implies that this area of greater synthetic activity is provoking a mobilization of materials out of the distal tissue and contributing to the senescence of cells at the separation zone.

The time sequence of mobilization events into the proximal tissue provides for an area of interesting speculation. Phosphate, dry weight and RNA increases are evident 24 hours after deblading, followed by increases in protein and chlorophyll contents. Rubinstein and Leopold (20) have described a 2 stage abscission development in bean explants using identical experimental procedures to those employed in this study. In their study stage 1 was completed about

18 hours after deblading. Our data suggest that stage 1 might involve the establishment of a metabolic advantage in the cells on the proximal side of the separation zone, evidenced, for example, as an increased nucleic acid synthesis in the first 24 hours. Stage 2 might involve the development of this advantage as an enhanced protein synthesis and dry weight accumulation.

The role of growth in the regulation of abscission processes was noted long ago by Laibach (9). He observed that debladed petioles of *Coleus* in which abscission was retarded by application of orchid pollen exhibited swelling and elongation. He implied that growth (of distal tissues) is associated with abscission inhibition, a suggestion for which Jacobs et al. (8) have recently provided experimental evidence.

Gorter (7) noted that the abscission of *Coleus* explants was hastened if larger amounts of stem tissue were present proximal to the abscission zone. Our data suggest as an explanation that the larger stem pieces provided more effective mobilizing centers, thus leading to a more rapid cellular senescence in the abscission zone.

Recently we have shown (21) that the morphological changes which take place during the development of abscission in the bean petiole are suggestive of a possible involvement of senescence in a localized layer of cells. We found that the xylem vessels in the abscission zone become plugged with tyloses which could result in a water stress, and furthermore that the phloem callose in the abscission zone underwent hydrolysis when abscission was promoted by auxin or ethylene applications. This dissolution of callose could result in facilitated movement of nutrients from the distal tissue into the proximal tissue adjacent to the abscission zone.

The frequent occurrence of local regions of active cell division just proximal to the point of abscission has led to the suggestion that cell division activity might help stimulate abscission (10). The role of cell division was later denied when cases of abscission were observed without any apparent cell division activity (6). The experiments reported here suggest that while cell division may in fact not be necessary for abscission, the presence of a localized region of such active metabolic activity could certainly accentuate a tendency for the mobilization of materials out of the separation zone into the more proximal tissues, and hence contribute to the development of cellular senescence and abscission.

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