Changes in Abscission Processes with Aging 1, 2

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As leaves grow older they show increasing tendencies for abscission. With aging there are changes in the substances which can regulate abscission, such as a decline in the auxin level (9, 15), increases in the amino acids (17, 19, 11), and increases in extractable abscission-stimulating factors (10, 3, 4, 16). More recently the physiological processes leading to bean leaf abscission have been separated into 2 successive stages (13) and the present study aims to analyse the increasing tendencies for abscission as they may relate to the completion of the 2 successive stages.

Materials and Methods

The abscission tests were carried out in the manner described by Rubinstein and Leopold (13) and Chatterjee and Leopold (5). Seedlings of Phaseolus vulgaris cultivar red kidney were grown in vermiculite under controlled environment condition of 2000 ft-c, 23 ± 2°C and 16-hour photoperiod for different durations of time. In order to compare leaves of different ages, primary leaves were selected from seedlings 9 to 10 days old (designated as A stage), 15 to 16 days old (B stage), 21 to 22 days old (C stage), and 28 to 29 days old (D stage). As the primary leaves expand at about 7 days from sowing, the actual leaf ages are about 3, 9, 15 and 22 days for the stages A, B, C, and D respectively.

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For the explant abscission test, 1 cm petiole pieces were cut from leaves of A, B, and C stages, consisting of 5 mm of tissue on each side of the upper abscission zone. Substances to be tested were combined with 1% agar solution and poured into petri dishes to a depth of 4 mm. The proximal ends of 10 petiole explants were then inserted into the agar and the dishes returned to the controlled environment chamber at a light intensity of 400 ft-c.

For the intact petiole abscission test, stem cuttings were taken from the kidney bean plants of the 4 different ages (A, B, C, and D), the hypocotyls were trimmed to a length of 10 cm and the basal end placed in distilled water. The primary leaves were cut off leaving approximately 2.8 cm of the petiole and the lower abscission zone intact on the plant. Substances were applied to the cut surface of the petiole as lanolin pastes. The plants were kept in the controlled environment chamber (400 ft-c) during the course of the experiments.

Abscission was determined visually as the development of a ring of cell separation at the abscission zone. Readings were taken every 12 hours and the time required for abscission of 50% of the 10 pieces was taken as the experimental result. The least significant difference (LSD 5%) between treatments in the abscission test varied from 19 to 21 hours. All experiments reported here were repeated at least 3 times with consistent results.
Experimental Results

Illustrating the increasing tendency for natural abscission of bean leaves as they age, the abscission times for explants from 4 different ages of leaves have been entered in figure 1. The time for 50% abscission is greatest for explants from youngest leaves (136–138 hours) and successively lesser abscission times are shown for older explants. The increasing tendency to abscise with age suggests some progressive changes in the physiological systems controlling abscission.

Changes in abscission responses to auxin with increasing leaf age were examined. Petiole explants taken from leaves of various ages were treated with naphthaleneacetic acid (NAA) at concentrations from $10^{-8}$ to $10^{-3}$ M. From the data in figure 2, the 2-phase response curve described earlier by Biggs and Leopold (3) is evident for each of the leaf ages studied, with higher concentrations inhibiting and lower concentrations promoting abscission. The extent of the promotion was greatest for young leaves and declined with age, indicating that the sensitivity of abscission processes to auxin declines with increasing leaf age.

Changes in abscission responses to $\beta$-alanine were next examined. This compound was selected since it was one of the most stimulatory of the various amino acids tested by Rubinstein and Leopold (12). Explants were treated with alanine in the concentration range of $10^{-7}$ to $10^{-3}$ M. The data in figure 2 show that abscission by the youngest explants (A) was only slightly promoted, $10^{-2}$ M alanine causing only a 30-hour promotion of abscission. Explants from medium-aged leaves (B) were somewhat more promoted, and those from old leaves (C) were markedly more promoted (60 hours promotion at $10^{-4}$ M alanine). Thus the sensitivity of abscission processes to alanine increases with increasing leaf age.

In the abscission of bean petioles, 2 stages have been distinguished by Rubinstein and Leopold (13), and it would be interesting to know whether there may be a progressive completion of the 2 stages of abscission with leaf age. The first stage is known to be inhibited by NAA, thus making it possible to establish the completion of that stage as the time when a high concentration of NAA does not any longer inhibit abscission. To do this, explants are cut from uniform leaves of a given age, and inserted into plain agar for various lengths of time (induction periods) before transfer to an agar containing a high concentration of NAA. In this way, it is possible to determine the time required after deblading for the completion of the inhibitable first stage of abscission. Comparisons of the time required for completion of stage 1 in leaves of different ages are presented in figure 3. Comparing the induction times needed for the passage out of the inhibitory auxin response, the youngest (A) explants had passed out of the inhibitable stage after about a 16-hour induction period; this induction requirement was successively shorter for leaves of increasing age. With explants from old leaves (C), the induction period required for completion of the
To further assess the changes in the inhibitory effects of auxin with age, explants of different aged leaves were placed for different durations in agar containing NAA, after which they were transferred to plain agar. The data in figure 4 indicate that the inhibitory influence is most rapidly achieved with youngest (A) explants, reaching about 100 hours inhibition after only 2 hours of auxin treatment. The B explants required 3 times as long in the auxin treatment to reach 100 hours inhibition. And in the oldest explants (C) the extent of inhibition never surpassed 40 hours even when auxin was applied for 18 hours. These results confirm that there is a distinct decline in the ability of the abscission zone to be inhibited by auxin as the leaf ages.

Since alanine promotes only the second stage of abscission (13), the alanine promotion may be used to estimate the extent to which the second stage regulates abscission. Comparisons of the responses by explants from leaves of various ages given alanine (5 × 10⁻³ M) for different periods of time are shown in figure 4. The youngest (A) explants were significantly promoted (20 hours) when the alanine treatment was continued for about 18 hours; medium aged (B) explants were promoted with only 14 to 16 hours.

Fig. 3. The effects of induction periods of various lengths on the abscission of bean explants and petioles from leaves of different ages. After the induction period indicated, the auxin was applied as 5 × 10⁻⁴ M NAA in agar for the explant test or as 5 × 10⁻³ M NAA in lanolin for the intact petiole test. Control values for the explant test were: A, 3-day leaves 128 hours; B, 9-day leaves 98 hours; and C, 15-day leaves 72 hours. Control values for the intact petiole test were: A, 164; B, 134; C, 110; and D, 21-day leaves 90 hours.

Fig. 4. Effects of duration of treatment with auxin (NAA) or alanine on the abscission responses of explants from leaves of 3 ages. Abscission times for controls: A, 3-day leaves 129 hours; B, 9-day leaves 99 hours; and C, 15-day leaves 79 hours.
treatment, whereas explants from old leaves (C) required only about 6-hours treatment with alanine to produce a significant promotion. The extent of promotion also varied with age, being least in the A explants (about 28 hours) greater in the B explants (48 hours) and still greater in C explants (about 58 hours). It is evident that the responsiveness of the abscission zone to alanine increases with leaf age.

In a further effort to detect changes in the second stage of abscission with age, a series of explants was given an induction period of 20 hours in plain agar in order to complete stage 1, and then placed on concentration ranges of 10^{-3} to 10^{-8} M of NAA or 10^{-2} to 10^{-7} M of alanine. As seen in figure 5, the explants from the youngest leaves (A) showed the greatest promotive response to NAA (70 hours), whereas the oldest (C) were much less promoted (30 hours). Conversely, the youngest explants (A) were the least stimulated (28-30 hours) by alanine, and the oldest explants (C) showed significantly greater promotions (58 hours). These results indicate a declining auxin control of abscission with leaf age and an increasing alanine responsiveness.

The changing responsiveness of abscission with leaf age can be represented graphically as the changes in promotions achievable with NAA and with alanine, as in figure 6. In 2 separate experiments, explants were held on plain agar for induction periods of either 18 or 24 hours, and then placed on agar containing either NAA or alanine to compare the promotions achievable for explants from leaves of different ages. The results plotted in figure 6 show again the declining promotive effects of auxin on stage 2 with age, and the increasing promotive effects of alanine.

**Discussion**

These experiments present several types of evidence of a declining role of auxin in the control of abscission with increasing leaf age. And they also establish that with increase in age of the bean leaf, there is an increasing responsiveness of abscission to alanine.

The recognition of 2 successive partial processes in bean leaf abscission was made by Rubinstein and Leopold (13). Applying a high concentration of NAA to explants after various intervals of time, they found that in an initial stage the auxin inhibited abscission, whereas in a later stage the same auxin concentration would promote abscission. They deduced that there are 2 physiological stages in abscission, the first being inhibited and the second being promoted by auxins. They also established that the promotion of abscission by alanine was an action on the second stage. The data reported here indicate that the increasing tendency for abscission with leaf age is related to a completion of the first stage and an enhancement of the second stage of abscission.

In studies of the effects of defoliants on cotton, Lineweber et al. (8) observed different degrees of effectiveness for chemicals applied to leaves of different ages, advanced age classes being more responsive to defoliant action. Whatever partial processes of abscission may be affected by the defoliants, the increasing effectiveness with leaf age is consistent with the evidence for a progressive completion of stage 1 with aging.

Biggs and Leopold (2) compared auxin effects on abscission of explants from leaves of different ages and concluded that the abscission responses of bean
explants to NAA are strongly altered by the age of the leaf, promotive effects of NAA being greatest in the youngest material and decreasing with age. However, if the tests were done in darkness, the promotive effects of auxin actually increased with increasing leaf age. This inversion of auxin responses may conceivably be due either to a light requirement for the successful completion of the first stage of abscission, or to a substrate deficiency slowing the first stage in darkness as Biggs and Leopold (2) have already indicated for the overall abscission process.

As the leaf ages, the auxin content of the leaf declines. This was first established by Avery (1) and has since been observed by numerous other workers in a wide range of species (6,9,15,18). A simultaneous decline of auxin content of the leaf and responsiveness of the abscission zone to auxin appears to be a basic part of the aging process.

It may not be fortuitous that substances like amino acids which increase in leaves during aging will stimulate abscission. In this study an increase of responsiveness to alanine with age has been shown. The hydrolysis of proteins in leaves to amino acids either with aging or with removal from the plant is well known (17). The accumulation of soluble nitrogen compounds in the abscission zone (8), particularly amino acids (20) is very suggestive. Osborne (10) reported that senescent leaves of beans and of several woody species contain a diffusible material which accelerates abscission. Rubinstein and Leopold (12) have shown that a wide variety of amino acids are effective in promoting bean leaf abscission and extracts of aging leaves showed a marked rise in abscission promoting activity in the amino acid fractions.

The increasing tendency for abscission as the leaf ages may be due to the progressive completion of the first stage of abscission with age. A declining auxin content and auxin responsiveness would be expected to contribute to this tendency, and conversely the increasing amino acid content may further accentuate the readiness for abscission.

Summary

The present study is an analysis of the changes which occur in the natural controls of abscission as leaves of Phaseolus vulgaris L. increase in age. The effects of naphthaleneacetic acid and -alanine on explant abscission have been followed separately for the 2 stages of abscission as the leaf ages.

A decrease in overall sensitivity of the abscission processes towards auxin was observed with increase in leaf age. The extent of inhibition of the first stage of abscission is greatest for young leaves and declines with age; also promotive effects on the second stage of abscission gradually become lesser as the leaf increases in age.

A converse picture is seen for the promotive effects of -alanine. Younger explants appear to be weakly responsive towards alanine, increasing in responsiveness with age. The increasing promotion of abscission by alanine is interpreted as an increasing dominance of the second stage of abscission with leaf age.

The results suggest that as a bean leaf ages, the first stage of abscission, which is inhibitable with auxin, is gradually completed. The development of abscission becomes then dominated by a second set of activities which actually bring about the separation and leaf fall.

Literature Cited

Calcium Accumulation by Maize Mitochondria \(^1,2\)

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A number of laboratories have reported that animal mitochondria can actively accumulate various inorganic ions \((8)\). Energy can be supplied by either substrate oxidation or ATP. The antibiotic, oligomycin, inhibits ATP-driven Ca uptake, but not substrate-driven uptake \((6,18)\). On the basis of these findings, plus the observation that ADP also inhibits substrate-driven ion accumulation, Brierley et al. \((5)\) suggest that a high energy intermediate of oxidative phosphorylation is a common energy source for ATP formation and for ion transport.

Inasmuch as these studies may be relevant to the problem of the connection between respiratory energy and ion transport in plants, we have conducted similar studies on the accumulation of inorganic ions by plant mitochondria. Earlier attempts to demonstrate an energy dependent accumulation of ions by plant mitochondria had only limited success \((17,21)\). In general, our results are similar to those obtained with animal mitochondria, and support the view that a high energy intermediate of oxidative phosphorylation participates in Ca, Mg and phosphate uptake. However, a few differences do exist. The uptake of phosphate, Mg and Ca\(^{45}\) is dependent upon the presence of Ca. Substrate-driven Ca and phosphate uptake does not require exogenous Mg. In addition, we have been unable to find Ca plus Mg: phosphate uptake ratios suggestive of hydroxyapatite formation \((5,6,18,22)\). Preliminary communications on phases of this work have appeared elsewhere \((11,13,25)\).

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Materials and Methods

Isolation of Mitochondria. Corn seeds \((Zea mays L., W F9 \times M14)\) were germinated in the dark at 28\(^\circ\) on paper towels saturated with 10\(^–4\) m CaCl\(_2\). After 3 and one-half days, the shoots were excised, chilled, and ground in an ice-cold mortar with 0.25 m sucrose, 0.05 m KH\(_2\)PO\(_4\) and 0.005 m EDTA, adjusted to pH 7.5 with Tris (hydroxymethyl) aminomethane. The slurry was strained through cheesecloth. Mitochondria were isolated in a refrigerated centrifuge as the fraction sedimenting between 2000 \(\times\) \(g\) for 5 minutes and 12,000 \(\times\) \(g\) for 10 minutes. The mitochondria were twice washed, first in the grinding medium, next in 0.25 m sucrose and were finally suspended in 0.25 m sucrose. The mitochondria were quite active, giving QO\(_2\) (N) values of about 1500 and P/O values of about 2.5 when oxidizing a mixture of pyruvate and succinate in the absence of inhibitors or uncouplers. Procedures for determining oxidative phosphorylation have been described \((12)\).

Procedures for Measuring Ion Uptake. The experiments were carried out at 28\(^\circ\) (except for experiments where temperature was varied) in centrifuge tubes in a shaking waterbath. Unless otherwise noted, the reaction period was 10 minutes. Total volume of mitochondria plus additives was 2.5 ml. Except for the sucrose concentration of 0.25 m, buffering with Tris to pH 7.5 and 4–8 \(\times\) 10\(^4\) cpm Ca\(^{45}\) per tube the reaction mixtures varied, and are given with the experimental data. When pyruvate + succinate served as substrate, 0.1 \(\mu\) mole coenzyme A, 0.4 \(\mu\) mole thiamine pyrophosphate and 0.6 \(\mu\) mole NAD per tube were used. About 0.1 mg mitochondrial N was used per tube, except for experiments where total Mg and P were determined; here about 0.7 mg N per tube was used.