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Auxin Transport as Related to Leaf Abscission during Water Stress in Cotton[1]

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ABSTRACT

Plant water deficits reduced the basipetal transport of auxin in cotyledonary petiole sections taken from cotton (*Gossypium hirsutum* L.) seedlings. A pulse-labeling technique was employed to eliminate complications of uptake or exit of ^{14}C-indoleacetic acid from the tissue. The transport capacity or the relative amount of radioactivity in a 30-minute pulse which was basipetally translocated was approximately 30% per hour in petioles excised from well watered seedlings (plant water potentials of approximately −4 to −8 bars). No cotyledonary leaf abscission took place in well watered seedlings. Plant water potentials from −8 to −12 bars reduced the transport capacity from 30 to 15% per hour, and although the leaves were wilted, cotyledonary abscission did not increase appreciably at these levels of stress. The threshold water potential sufficient to induce leaf abscission was approximately −13 bars and abscission increased with increasing stress while the auxin transport capacity of the petioles remained relatively constant (15% per hour). The basipetal transport capacity of well watered petioles tested under anaerobic conditions and acropetal transport tested under all conditions were typically less than basipetal transport under the most severe stress conditions. Cotyledonary abscission took place during and 24 hours after relief of stress with little or no abscission taking place 48 hours after relief of stress. Although the water potential returned to −4 bars within hours after rewatering the stressed plants, partial recovery of the basipetal transport capacity of the petioles was not apparent until 48 hours after rewatering, and at least 72 hours was required to return the transport capacity to near normal values. These data support the view that decreased levels of auxin reaching the abscission zone from the leaf blade influence the abscission process and further suggest that the length of time that the auxin supply is maximally reduced is more critical than the degree of reduction.

Various lines of indirect evidence suggest that foliar abscission resulting from water deficits may be at least partly mediated by a decrease in the availability of auxin reaching the abscission zone from the leaf blade. Since the work of Laibach (14) on debladed petioles, numerous workers have demonstrated the requirement of a constant auxin supply from the leaf blade to the abscission zone for maintenance of the cellular integrity of that zone (2-4). Indoleacetic acid enhances the retardation of leaf abscission when applied to debladed petioles distal to the abscission zone (5, 15). Although it has been suggested that auxin transport in petioles may be reduced during periods of plant water stress (17), thus resulting in foliar abscission, no data bear directly on this hypothesis. We report here experiments which quantitatively relate ψ[4] to the auxin transport capacity of stressed petioles.

MATERIALS AND METHODS

Plant Culture. Cotton (*Gossypium hirsutum* L. cv. Stoneville 213) was planted in trays filled with fine meshed vermiculite, moistened with distilled H_2O, and allowed to germinate in a controlled environmental chamber (35 C and 27 C, day and night temperatures, respectively; 80% constant relative humidity, and an irradiance of 260 μeinsteins/m^2 sec [PAR, 400-700 nm] with a 15-hr photoperiod). Following emergence, the plants were watered with modified Hoagland solution (12). After 12 days from planting, those trays of seedlings destined for stress studies were transferred to a similar growth chamber programed for identical conditions but which supplied only 30% constant relative humidity. Four days in this low relative humidity chamber (without additional water) was sufficient to impose severe water stress (≤ -20 bars) on the seedlings. However, by removing trays for the transport experiments at the appropriate time, a range of ψ was obtained. Those plants destined to be controls remained in the high humidity growth chamber a total of 14 days from planting and were watered daily. Thus, all transport experiments were conducted on seedlings which were 14 ± 2 days old.

The ψ was determined immediately prior to each transport trial with a Scholander pressure bomb (18) as the average value of three or more plants in a tray. The ψ did not vary more than ±1 bar within a tray. All transport experiments were performed on plants which were approximately 5 hr into the photoperiod and which were uniform in growth and appearance.

Auxin Transport. The movement of auxin in the cotyledonary petioles was examined independent of uptake or exit from the tissue using pulse-labeling techniques similar to those of Goldsmith (8). In each experimental trial, 40 20-mm sections were cut from cotyledonary petioles of plants selected for uniformity and were placed vertically in Plexiglas holders so that the base of each section rested on a 1.5% agar block. The distal ends were placed up for basipetal transport (20 sections) and down for acropetal transport studies (20 sections). The sections were then enclosed in a ventilated plastic container lined with wet paper towels and agar blocks (14.14 mm^3, 1.5%) containing 7.5 μM 1-^{14}C-indole-3-acetic acid (Nuclear-Chicago, 33 mCi/mmol) were placed atop the sections. The label was allowed to diffuse into the tissue for 0.5 hr, after which the blocks were replaced with

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[4] Abbreviation: ψ: plant water potential; PAR: photosynthetically active radiation.

agar blocks containing 7.5 μM nonlabeled IAA. These blocks were allowed to remain on 10 sections for 0.5 hr and for 2.5 hr on the remaining 10 sections within a trial. Immediately following removal of these "chase" blocks, the 20-mm sections were cut into 10 2-mm segments with the aid of a multibladed cutter. Corresponding segments were pooled and placed in scintillation vials so that each vial contained 10 segments and each series of 10 vials represented the sum of the activity in 10 petiole sections. When appropriate, anaerobic conditions were achieved by purging an air-tight plastic container lined with moist paper towels with humidified N_2 after the 1-hr total transport sections had been removed for segmentation. Low O_2 tension was maintained for the remaining 2-hr transport period by a continuous flow of N_2 (1.0 l/min) through the container.

Into each scintillation vial was dispensed 15 ml of cocktail, made by dissolving 100 g naphthalene and 5 g PPO in enough 1,4-dioxane to make 1 liter of solution. After the vials were capped, they were shaken for 24 hr at room temperature and loaded into a Beckman model LS-200B or model LS-100C scintillation spectrometer. Each sample was counted for 20 min using the counts derived from the ^{14}C with ^{3}H iso-set module and corrected for background.

Where appropriate, results are given in cpm/section. The relative level of radioactivity in each 2-mm segment is represented by histobars indicating the per cent of the total activity within the 20-mm sections. A measure of the petioles' capacity to transport IAA was calculated by subtracting the per cent of the total activity indicated in each 1-hr histobar from the corresponding 3-hr histobar and summing the positive values. This value represents the per cent of labeled material transported between the 1st and 3rd hr of transport.

Abscission. Seedlings employed in abscission experiments were grown in pint containers using fine meshed vermiculite under conditions supplied by the high relative humidity growth chamber. Cheesecloth wicks, which were embedded in the vermiculite and suspended in modified Hoagland solution (12), facilitated a constant supply of water and mineral nutrients to the plants. The wicks were removed from the nutrient solution 12 days after planting, and the containers were transferred to the low relative humidity growth chamber. After 1-day exposure to the desiccating environment, several pots were removed from the chamber. Nonrepresentative plants in each pot were clipped at the soil surface and discarded. The ψ was determined, and the number of abscised cotyledons on the remaining six to 10 seedlings were counted. Abscission was ascertained by gently pressing on the adaxial surface of the cotyledonary petioles. The containers were then watered and returned to the high relative humidity chamber where abscission was noted daily. This harvest procedure was repeated at the same time (5 hr of photoperiod) each day so that a range of ψ values were achieved during a period of several days.

RESULTS

The ^{14}C label distribution after 1-hr and 3-hr basipetal transport in the 20-mm petiole sections under various degrees of water stress is shown in Figure 1, A–D. Figure 1A is a typical label distribution pattern found in well watered seedlings (−4 bars). The amount of label transported over a 2-hr period in petioles from such seedlings was typically about 60% (30%/hr). The velocity of transport under well watered conditions, determined by comparison of the distance traveled by the leading edges of the label distribution patterns between 1 hr and 3 hr, was 5 mm/hr. The appearance of a labeled pulse in the 3-hr distribution pattern was quite reproducible, and along with transport capacity and velocity, was notably similar to results obtained from young, true leaves taken from well watered, older plants (unpublished data).

At ψ sufficient to wilt these seedlings (−8 to −9 bars), the labeled pulse became less distinct (Fig. 1B), and the transport capacity of the petioles was reduced. Increasingly severe stress reduced the pulse to a diffusion pattern, markedly decreasing the transport capacity to approximately 15%/hr and the velocity to about 3 mm/hr (Fig. 1, C and D).

A plot of the transport capacity as a function of ψ (solid circles in Fig. 2) indicates that the former was not markedly affected

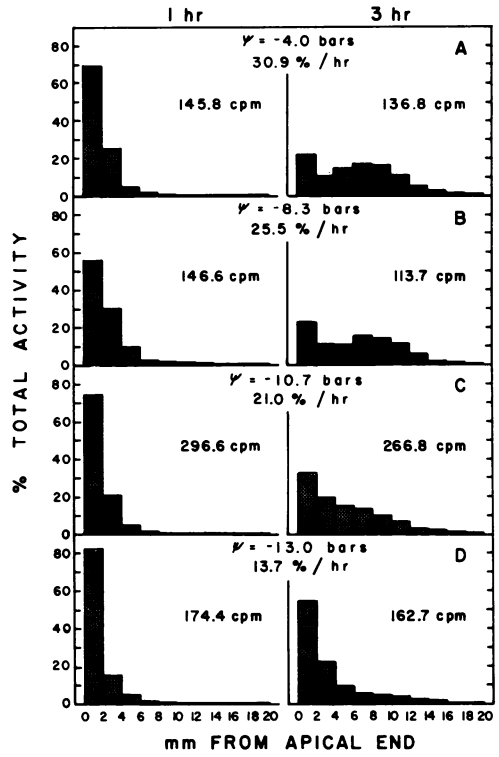

FIG. 1. Distribution of a 30-min pulse of 7.5 μM 1-^{14}C-IAA after 1-hr and 3-hr basipetal transport in 20-mm cotyledonary petiole sections taken from well watered (A), slightly wilted (B), and severely water-stressed (C, and D) cotton seedlings. Both the total activity per section (cpm), averaged from 10 sections after 1-hr and 3-hr transport, and the plant water potential (ψ) at the time of the experiment are given. Each histobar represents the average relative level of extractable radioactivity in each 2-mm segment and is expressed by a percentage of the total activity contained in the 20-mm petiole sections. The relative amount of 1-^{14}C-IAA transported/hr was calculated by subtracting the per cent of the total activity indicated in each 1-hr histobar from the corresponding 3-hr histobar and summing the positive values.

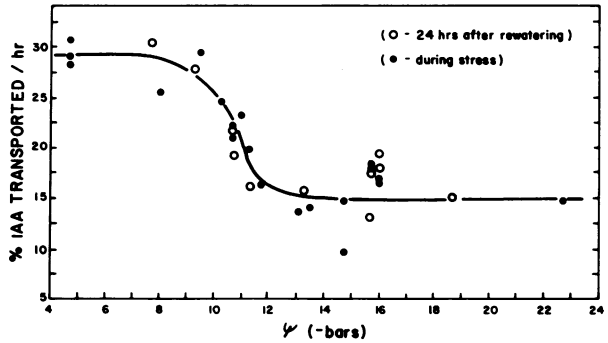

FIG. 2. Effect of ψ on the basipetal 1-^{14}C-IAA transport capacity of cotton cotyledonary petioles. The transport trials were performed at the same time the ψ were determined (●) and 24 hr after the plants were rewatered (○). The ψ of the latter were determined just prior to rewatering.

until wilting was induced (−8 to −9 bars). ψ between −8 and −12 bars, however, reduced the auxin transport capacity to one-half of that of the well watered controls. Further reduction in the ψ, surprisingly, did not cause a continued drop in the transport capacity. Rather, the transport capacity remained relatively constant even when carried to extreme water deficit levels. Interestingly, the basipetal transport capacity at such levels of stress was still higher than was commonly found for acropetal transport (4.6 ± 2.3%/hr) or basipetal transport under anaerobic conditions in well watered tissue (approximately 8%/hr). Water stress appeared to have no effect on acropetal transport.

Following the transport experiments during stress, the trays of remaining seedlings were rewatered with nutrient solution and returned to the high relative humidity chamber. Twenty-four hr was sufficient time to return the plants to full turgor with ψ approximating −4 bars. The seedlings were again examined for auxin transport capacity. The open circles of Figure 2 indicate that 24 hr after rewatering, the capacity of the petioles to transport IAA was not greatly different from that found under the stress conditions. In an effort to determine if the transport capacity eventually recovered to normal levels, seedlings were rewatered and returned to the high relative humidity chamber for up to 72 hr. As seen in the earlier experiment, there was no apparent increase in the transport capacity by 24 hr after rewatering, but 48 hr was sufficient to increase it (Fig. 3). By 72 hr, the transport capacity was approaching levels normally found under well watered conditions.

The extent of cotyledonary leaf abscission during water stress is shown in Figure 4. Although the data are scattered, −12 to −14 bars appears to be the threshold range of ψ required to induce abscission of the cotyledonary leaves. Maximum abscission of about 75% was reached at −18 bars and greater. After the stressed seedlings were rewatered, there was additional abscission the following day, but the over-all shape and level of the abscission curve were similar to those found while stress was in progress.

DISCUSSION

The movement of auxin in plant tissues has been characterized as being basipetally polar and facilitated by active transport processes (9, 11, 16, 19). The data presented here support this view and further lend support to the hypothesis that auxin transport in petioles is a controlling factor in stress-induced abscission. That the level of endogenous auxin arriving at the abscission zone from the leaf blade determines the fate of the cells adjacent to the separation layer has been clearly demonstrated in numerous studies (1-4). The petiole is the crucial link between this specialized area of cells and the sites of auxin synthesis in the lamina. Hence, the auxin transport capacity of the petioles can be a major factor in controlling the cellular activity of the abscission zone. Veen and Jacobs (20, 21) have shown that decreases in the auxin supply to the abscission zone from the lamina and induction of changes in the separation layer are correlated with reduced auxin transport capacity of petioles of increasing age. The results presented in this report are the first to provide direct evidence that stress-induced abscission of leaves is associated with a decrease in the transport of auxin.

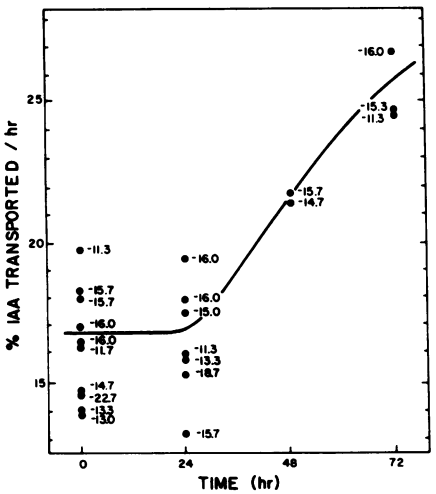

FIG. 3. Recovery of the IAA transport system after relief of stress. Data represent the transport capacity of cotyledonary petioles at various times after rewatering. ψ at the time of rewatering are noted for each datum.

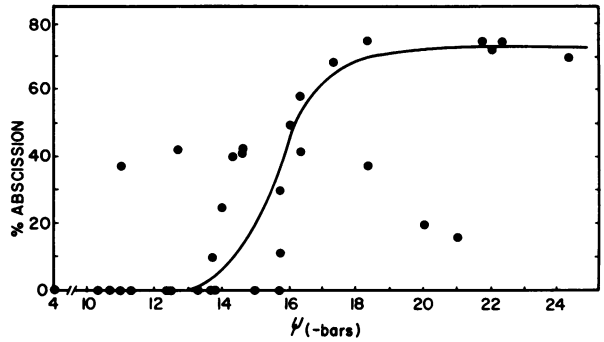

FIG. 4. Abscission of cotyledonary leaves of cotton seedlings during moisture deficit. Abscission and ψ were measured at 5 hr into photoperiod. Per cent abscission represents the relative number of abscised cotyledons from the total number of cotyledons within each pot. Each pot contained six to 10 seedlings.

After this paper was accepted for publication, we became aware of the recent work of Kaldewey *et al.* (13) demonstrating a reduction in auxin transport capacity of internodes of water-stressed pea seedlings. A water deficit was achieved with mannitol added to the nutrient solution. Agreement of the results from cotton petioles (present data) with those from pea internodes (13) suggests that inhibition of auxin transport by water stress may be general in nature.

Imposed moisture deficits in the petioles of −9 to −12 bars were shown to decrease markedly the auxin transport capacity of the petioles (Fig. 2). Maximum inhibition of transport occurred at −12 bars and further stress had little additional inhibitory effect on the system. The minimum transport capacity was about 15%/hr. Therefore, IAA transport was maximally reduced by one-half when subjected to ψ of −12 bars or lower. The apparent threshold ψ for the induction of abscission in cotton cotyledonary leaves was the same stress level, approximately −13 bars (Fig. 4). It appears that the continued or increased abscission with increasing stress (Fig. 4) could logically be a function of the length of time that auxin transport is retained at this minimum level and not simply the degree of inhibition of transport that occurs. After the transport capacity declines, the level of auxin in the abscission zone cells would begin to fall, soon setting in motion the events which culminate in separation. As stress increases, the duration of impairment of transport is also increasing as is the time for the plant to respond to the decreased auxin level. Thus, one would not expect abscission to occur immediately after the transport capacity drops, in agreement with what was observed (Figs. 2 and 4).

The recovery of the transport system was slow. After rewatering the plants, more than 24 hr was required to see any significant improvement in the transport capacity from that determined under moderate or severe stress conditions (Figs. 2 and 3). By 48 hr, the transport capacity of petioles from rewatered plants increased to an intermediate value between those ob-

tained from stressed and well watered plants. Recovery of the transport system to a level near that of well watered plants required at least 72 hr in plants which were subjected to stress resulting in water potentials greater than −11 bars (Fig. 3). Continued abscission of the cotyledons after relief of stress was evident 24 hr after rewatering. However, negligible or no additional abscission of the cotyledons took place beyond 24 hr after rewatering, further supporting the suggestion that as long as the minimum transport capacity is retained, abscission may take place.

The IAA transport system was apparently never fully inhibited at low ψ even under the most severe stress conditions because the transport capacity was never reduced to those levels found in the acropetal direction. Anaerobic conditions severely inhibited the transport capacity of the petioles to levels near that of acropetal movement. Hence, it would appear that the petiole has some potential to supply auxin to the abscission zone from the leaf even under stress conditions.

It is not clear whether the decrease in auxin transport and the associated foliar abscission response are a direct result of water deficit in the tissue or are coordinated with some intermediate event. Although endogenous IAA levels were not examined in this study, the stress-induced decrease in transport capacity of the petioles may be associated with or possibly a reflection of reduced auxin synthesis. Hartung and Witt (10) have reported reduced auxin levels in *Anastatica hierochuntica* and *Helianthus annuus* stem sections subject to water deficit. The initial decrease in the availability of auxin to the abscission zone as a function of water deficits could then be a result of both decreased auxin transport and decreased auxin synthesis in the blade. IAA-oxidase also increases in water-stressed tissue (6, 7). It is plausible that the increase in abscission associated with decreased ψ (lower than −12 bars) could be the result of further decreases in auxin supply from the leaf blades. Further study is required to answer the questions bearing on this possibility.

LITERATURE CITED

1. Abeles FB 1968 Role of RNA and protein synthesis in abscission. Plant Physiol 43: 1577–1586
2. Addicott FT 1970 Plant hormones in the control of abscission. Biol Rev Cambridge Phil Soc 45: 485–524
3. Addicott FT, RS Lynch 1955 Physiology of abscission. Annu Rev Plant Physiol 6: 211–238
4. Carns HR 1966 Abscission and its control. Annu Rev Plant Physiol 14: 295–314
5. Chatterjee SK, AC Leopold 1963 Auxin structure and abscission activity. Plant Physiol 38: 268–273
6. Darbyshire B 1971 Changes in indoleacetic acid oxidase activity associated with plant water potential. Physiol Plant 25: 80–84
7. Darbyshire B 1971 The effect of water stress on indoleacetic acid oxidase in pea plants. Plant Physiol 47: 65–67
8. Goldsmith MHM 1967 Separation of transit of auxin from uptake: average velocity and reversible inhibition by anaerobic conditions. Science 156: 661–663
9. Goldsmith MHM 1968 The transport of auxin. Annu Rev Plant Physiol 29: 347–360
10. Hartung W, J Witt 1968 On the influence of soil moisture on the auxin content of *Anastatica hierochuntica* and *Helianthus annuus*. Flora Abt B Bd 157: 603–614
11. Hertel R, ML Evans, AC Leopold, HM Sell 1969 The specificity of the auxin transport system. Planta 84: 238–249
12. Hoagland DR, DI Arnon 1938 The water culture method for growing plants without soil. Calif Agric Exp Sta Circ 347: 1–32
13. Kaldewey H, U Ginkel, G Wawczyniak 1974 Auxin transport and water stress in pea (*Pisum sativum* L.). Ber Deutsch Bot Ges 87: 563–576
14. Laibach F 1933 Wuchsstoffversuche mit lebenden Orchideenpollinien. Ber Deutsch Bot Ges 51: 336–340
15. Louie DS Jr, FT Addicott 1970 Applied auxin gradients and abscission in explants. Plant Physiol 45: 654–657
16. McCready CC 1966 Translocation of growth regulators. Annu Rev Plant Physiol 27: 283–294
17. McMichael BL, WR Jordan, RD Powell 1972 An effect of water stress on ethylene production by intact cotton petioles. Plant Physiol 49: 658–660
18. Scholander PF, HT Hammel, ED Bradstreet, EA Hemmingsen 1965 Sap pressure in vascular plants. Science 148: 339–346
19. van Overbeek J 1956 Absorption and translocation of plant regulators. Annu Rev Plant Physiol 7: 355–372
20. Veen H 1972 Relationship between transport and metabolism of α-naphthaleneacetic acid, β-naphthaleneacetic acid and α-decalylacetic acid in segments of *Coleus*. Planta 103: 35–44
21. Veen H, WP Jacobs 1969 Transport and metabolism of indole-3-acetic acid in *Coleus* petiole segments of increasing age. Plant Physiol 44: 1157–1162