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Synthesis and Movement of Abscisic Acid in Water-Stressed Cotton Leaves[1]

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ABSTRACT

Synthesis and movement of abscisic acid (ABA) into the apoplast of water-stressed cotton (*Gossypium hirsutum* L.) leaves were examined using pressure dehydration techniques. The exudates of leaves dehydrated in a pressure chamber contained ABA. The level of ABA in the exudates was insensitive to the leaf water potential when dehydration occurred over a 3-hour period. When leaves were rapidly dehydrated in the pressure chamber and held at a balance pressure coincident with the point of zero turgor, ABA accumulated in the leaf tissue and then in the apoplast, but only after 2 to 3 hours of zero turgor. Slow dehydration of leaves by equilibration over varying mannitol concentrations resulted in some accumulation of ABA prior to the point of zero turgor, but ABA accumulated in the tissue and apoplast most rapidly after the onset of zero turgor.

When intact plants were allowed to dehydrate, stomata of leaves attached to the plant began closing as the leaf water potential decreased to −12 bars. Leaves began accumulating ABA at about −14 bars, but accumulation of ABA in the apoplast did not occur until the leaf water potential reached −16 bars. Although the apoplastic fraction of ABA should represent an "active" pool of ABA readily accessible to the guard cells, the data suggest that there may be stomatal closure prior to accumulation of ABA in the apoplast. A role for the small amounts of apoplastic ABA not dependent upon water stress is proposed for this initial stress-induced stomatal response.

Application of exogenous abscisic acid (ABA) to leaves results in stomatal closure (4, 8, 14; for reviews, see 12, 16, 23). This fact in conjunction with data demonstrating an accumulation of ABA in most water-stressed leaf tissue (1, 3, 5, 13, 15, 25) provides reasonably strong evidence that ABA modulates stomatal aperture in leaves undergoing water stress (16).

The accumulation of ABA in leaves generally occurs in response to declining leaf water potentials (1, 3, 5, 13, 15), with an apparent threshold leaf water potential required for initiation of ABA synthesis at least in some species (15, 25). Recently, Pierce and Raschke (15) demonstrated that ABA accumulation occurred only as leaf turgor approached zero. Furthermore, leaves continued to accumulate ABA for several hours after the onset of zero turgor (15).

Although declining leaf water potentials and loss of turgor appear to initiate ABA synthesis, the manner in which ABA moves from the presumed site of synthesis, the mesophyll cell chloroplast (10, 11), to the guard cells remains unclear. ABA could move in the symplast via plasmodesmata from cell to cell until reaching the guard cells where it accumulates (24). However, plasmodesmatal connections to the guard cells are extremely rare (24, for example). Alternatively, ABA could enter the apoplast from mesophyll cells and move to the guard cells via the transpiration stream. If this were the case, water moving out of dehydrating cells could conceivably carry ABA to the stomata.

Experiments in this report were conducted to elucidate more fully the synthesis and movement of ABA in response to water stress. Previous data have indicated that ABA moves in the phloem (6, 7, 26) and is present in small quantities in xylem exudates (9). There is little evidence to indicate whether ABA moves into the apoplast during water stress. In this study, leaves were dehydrated to predetermined levels under pressure in a pressure chamber (20–22). ABA in the apoplast was studied by analyzing xylem exudate collected from the leaves as they dehydrated under pressure.

MATERIALS AND METHODS

Plant Material. Cotton (*Gossypium hirsutum* L. cv. Tamcot SP 37) was grown from seed in a controlled environment facility as previously described (1, 2). Plants were continuously fully watered, never stressed, and were supplied with a complete nutrient solution three times weekly. Leaves of two different ages were utilized since ABA accumulation under water stress is highly dependent on leaf age (1, 18). Fully expanded leaves from plants 4 to 5 weeks old are referred to as young leaves. Fully expanded mature leaves from plants 9 to 10 weeks old are referred to as old leaves.

Pressure-Volume Determinations. Pressure-volume curves were obtained for individual leaves according to the methods of Tyree *et al.* (20–22). After establishing the initial balance pressure, pressure on the leaf was increased in steps of 3 to 5 bars. Water efflux in response to the applied overpressure was complete within 15 to 45 min depending on the initial balance pressure and overpressure. The exudate resulting from each overpressure was collected for analysis of ABA.

Initially, a gas mixture of N_2 (78.97%), O_2 (21%), and CO_2 (0.03%) was used in the pressure chamber. Leaves exposed to this mixture in the chamber for less than 3 h appeared healthy. Longer exposure resulted in the development of chlorotic lesions throughout the leaves. In all the experiments reported here, an ultra-high purity gas mixture (Matheson Gas Products) containing N_2 (78.2%) and O_2 (21.8%) was used, which did not cause chlorotic lesions in leaves even after 6 h under pressure.

For clarification, the terms balancing pressure and overpressure are defined as follows. The balance pressure is the pressure required to bring the water in the leaf back to the surface of the cut petiole after the leaf is detached from the plant. As such, it approximately represents the total leaf water potential of the leaf. The overpressure is the small incremental pressure (3 to 5 bars) applied to the leaf after the balance pressure has been attained. This pressure results in the leaf water potential becoming greater

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than zero, such that water flows out of the leaf (through the petiole) in response to a water potential gradient.

Osmotic potentials of the exudates were determined using thermocouple psychometry (1, 2). Osmotic potentials ranged from −0.5 to −2.0 bars depending on the balance pressure. This indicates that large quantities of cellular constituents were not forced into the apoplast in response to pressure dehydration (20, 21).

ABA Analysis and Leaf Conductance. ABA in the leaf tissue was extracted and determined with HPLC as previously described (1). Extraction of ABA in the exudates was facilitated by adding 7 ml of distilled H_2O to each exudate prior to extraction. Recovery of ABA was determined by adding ^{14}C[ABA] (~6,000 dpm; 35 ng) to each sample prior to extraction. Recovery averaged 50 to 60% for leaf samples and 75 to 80% for exudates. After correcting all data for recovery losses, the amount of ABA added (35 ng) was subtracted to obtain the final value.

Leaf conductance was determined using a Li-Cor Li-1600 steady-state porometer.

RESULTS AND DISCUSSION

A typical pressure-volume curve for a recently expanded young cotton leaf is shown in Figure 1. In general, leaves of this age lost turgor when the leaf water potential (negative balance pressure) declined to −12.5 ± 1.0 bars. This value corresponds to data obtained by thermocouple psychrometry in previous experiments (2). Fully mature leaves from older plants had dehydration profiles similar to that presented, with the exception that loss of turgor occurred at −17 ± 2.0 bars (data not shown, see Ref. 2).

Exudates obtained from these dehydrating leaves contained ABA (Fig. 2). Exudates from old leaves had 20 to 30 ng of ABA, while those of the most recently expanded young leaves contained 40 to 60 ng of ABA (Fig. 2A). The amount of ABA in the total exudate was almost independent of the balance pressure with small increases in ABA levels occurring as the balance pressure increased and leaves became progressively dehydrated. On a concentration basis, the initial exudates of a pressure series always contained more ABA per unit of exudate (Fig. 2B). As the balance pressure increased, ABA concentrations of the exudates declined and appeared to reach a constant low value.

The reason for the initial exudates having higher concentrations of ABA than those obtained during latter stages of dehydration is unclear. If some ABA was located in the apoplast prior to dehydration, it would be carried out of the leaf early during the first stages of exudation. Additionally, any ABA residing in the petiole of the leaf may be removed during the initial dehydration steps, thus resulting in an overestimation of the true apoplastic concentration. These explanations are alternatives to the possibility that

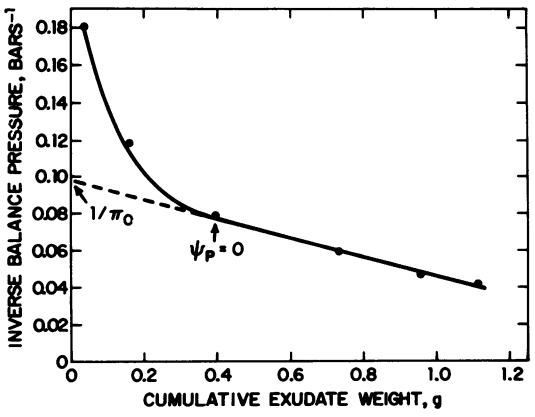

FIG. 1. Typical pressure volume curve obtained for a fully expanded young cotton leaf. ($1/\pi_0$), inverse osmotic potential at full hydration; ($\psi_p = 0$), inverse balance pressure coincident with loss of turgor.

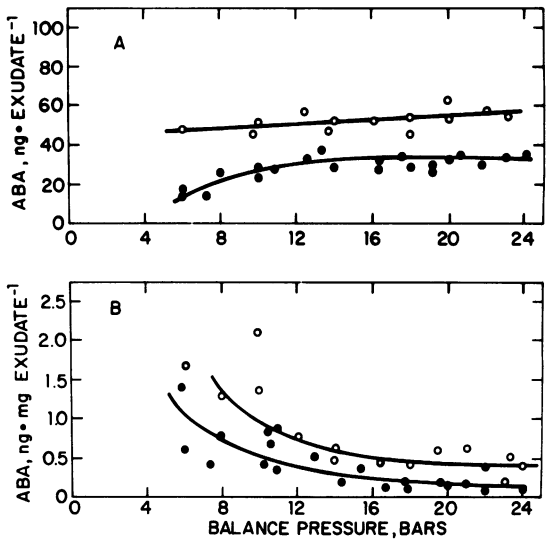

FIG. 2. ABA levels in exudates of cotton leaves subjected to pressure dehydration. Overpressures used to obtain the exudates were from 3 to 5 bars. Total ABA found in the exudates is shown in A. Concentration of ABA per mg of exudate is shown in B. (●), values typically obtained for old leaves; (○), values obtained from young leaves. Data were composited from several leaves.

ABA is being synthesized and transported to the apoplast in response to the initial dehydration steps. As will be demonstrated, this last possibility is disproved in subsequent experiments.

The experiment depicted in Fig. 2 involved a dehydration period totalling only about 3 h, with turgor reaching zero only in the later part. Recent evidence indicates that, after the onset of zero turgor pressure, maximum accumulation of ABA in water-stressed leaves may require several hours (15). Therefore, the duration of zero turgor was varied to determine its effect. This was accomplished by raising the pressure in the chamber to the balance pressure corresponding to the leaf water potential coincident with zero turgor (~−14 bars for young leaves; ~−17 bars for old leaves). The initial exudate was collected and analyzed for ABA. After exudation was complete, leaves were maintained at the balance pressure for 0 to 4 h prior to applying an overpressure of 5 bars and collecting the subsequent exudate. Accumulation of ABA in the leaf tissue began within 2 h after the onset of zero turgor (Fig. 3A). Younger leaves synthesized considerably more ABA than older leaves when water stressed, which is in agreement with previous data (1, 18). Movement of ABA into the apoplast did not occur until 3 to 4 h after the onset of zero turgor (Fig. 3B). Concentration of ABA in the final exudates of leaves that were held at zero turgor for various times increased in response to water stress and in accordance with the accumulation of ABA in the apoplast (Fig. 3C).

The data obtained from these experiments are in agreement with the results of Pierce and Raschke (15), indicating that in rapidly wilted leaves maximum accumulation of ABA occurs after the onset of zero turgor. Furthermore, the data suggest that once ABA is synthesized in the tissue it can move into the apoplast in reasonably high quantities (Fig. 3). However, ABA occurs in the exudates of leaves even in the absence of the stress-induced synthesis and accumulation that arises when leaves are held at zero turgor for several hours. This pre-existing fraction of ABA may represent ABA that is normally synthesized in the absence of water stress and is transported to the apoplast for loading into the phloem (6, 7, 26) and subsequent removal from the leaf. In this regard, ABA that accumulates in the phloem of water stressed plants that are capable of translocation (6, 7) may also move into the apoplast of detached leaves during stress.

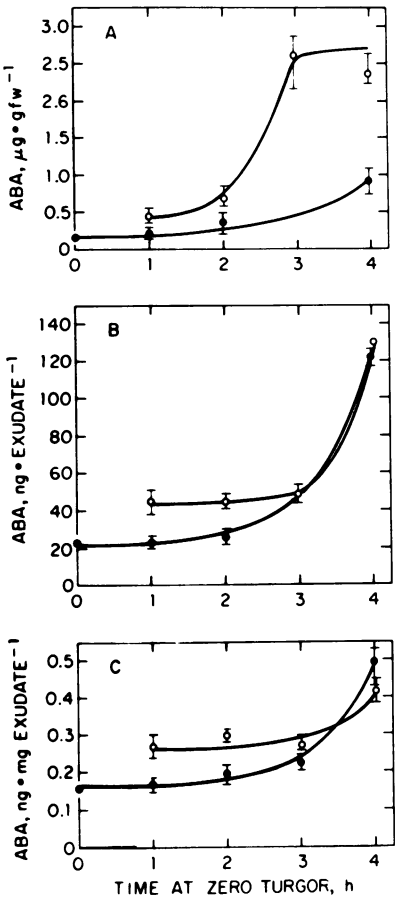

FIG. 3. ABA levels in leaf tissue (A) and exudates (B) of cotton leaves allowed to remain at zero turgor in the pressure chamber for various times. Leaves were brought to zero turgor by applying pressure in the chamber. After remaining at zero turgor for the specified time, a 5 bar overpressure was applied and sap was collected until exudation was complete. Data in (C) indicate the concentration of ABA in the exudate. The initial exudates (sap expressed to achieve zero turgor) contained the same amount of ABA as did leaves depicted in Figure 2. Data are from old (●) and young (○) leaves and represent the mean of two to three leaves.

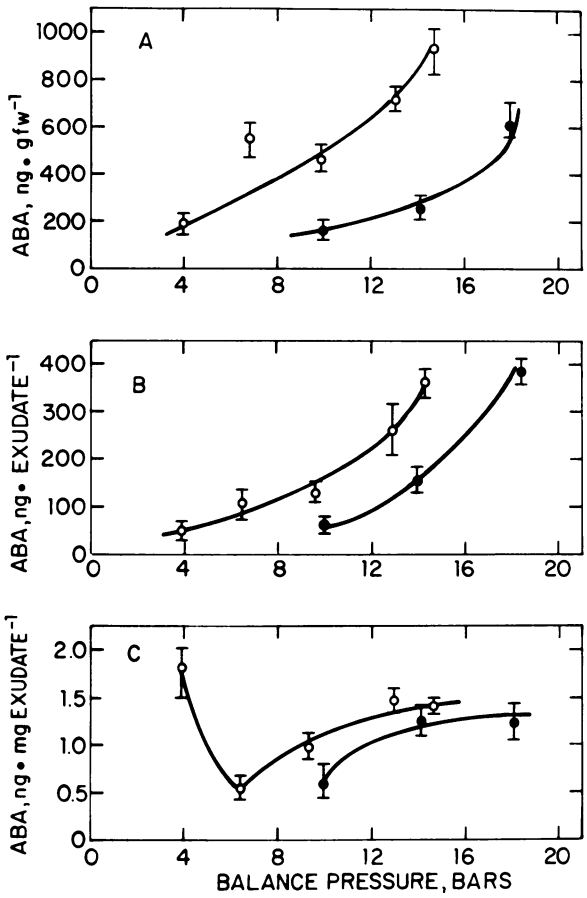

FIG. 4. ABA levels in leaf tissue (A) and exudates (B, C) of cotton leaves after equilibration over varying concentrations of mannitol for 17 bars in sealed containers. Data represent young (○) and old (●) leaves. Each data point is the mean of two leaves. The balance pressure represents the measured xylem pressure potential immediately after removal from the container. Exudates were obtained by applying a 5 bar overpressure and collecting sap until the new balance pressure was attained (15–45 min). In these leaves, zero turgor is achieved at about −12.5 bars (young leaves) and −16 to −17 bars (old leaves).

The influence of rate of dehydration on ABA synthesis and movement was further investigated by allowing leaves to equilibrate over varying concentrations (0.1 to 1.0 M) of mannitol in sealed containers for 17 h. As the leaf water potential declined, ABA levels increased within the tissue (Fig. 4A). When each leaf was then subjected to a 5 bar overpressure in the pressure chamber, the amount of ABA in the exudates also increased (Fig. 4B). Thus, ABA which accumulated in response to the imposition of slow water stress accumulated in the apoplast in quantities considerably greater than those observed during standard pressure dehydration (Fig. 2). The concentration of ABA in the exudates also increased in response to stress (Fig. 4C). Although the concentration of ABA in the first exudates seemed high, it is consistent with data presented in Figure 2.

Accumulation of ABA in cotton leaves can occur prior to complete loss of turgor when leaves are allowed to dehydrate slowly. Rapid dehydration, as in the pressure chamber, results in a 2 to 3-h time lag after the onset of zero turgor before ABA accumulates to maximum levels. It is clear from these data that ABA synthesis in cotton leaves exhibits a stress-time interaction.

In comparing the data obtained by pressure chamber dehydration and slowly stressing leaves over mannitol, it is important to note that fundamental differences exist with respect to the internal water relations of these leaves. When leaves are wilted over mannitol, the turgor pressure of the leaves is close to zero when the total water potential is −12 bars. Thus, the turgor pressure of these leaves was close to zero when they were placed in the pressure chamber. In contrast, when leaves are dehydrated in the pressure chamber to −12 bars and kept under this pressure, their total water potential was zero and the hydrostatic pressure (turgor) was equal to or greater than 12 bars. Thus, although leaves were dehydrated in the pressure chamber, this is not analogous to stress imposed by other methods. This aspect of stress-induced ABA synthesis is dealt with in detail in another paper.

It seemed important to ascertain whether the trends in ABA synthesis and movement into the apoplast observed in detached leaves were relevant with respect to stomatal closure. Cotton plants were allowed to dehydrate and stomatal conductance, tissue ABA and exudate ABA were determined during the course of dehydration. Stomata of the cotton leaves began closing when the leaf water potential approached −12 bars (Fig. 5A). ABA began accumulating in leaves at about −14 bars and maximum levels occurred at about −17.5 bars (Fig. 5A). As was the case previously (Fig. 3), ABA movement and accumulation in the apoplast did not occur until after tissue ABA had begun accumulating. Thus, during the initial stages of water stress, stomatal closure appeared to be more rapid than the build up of ABA in the tissue or apoplast, as is evident from data presented in Figure 6.

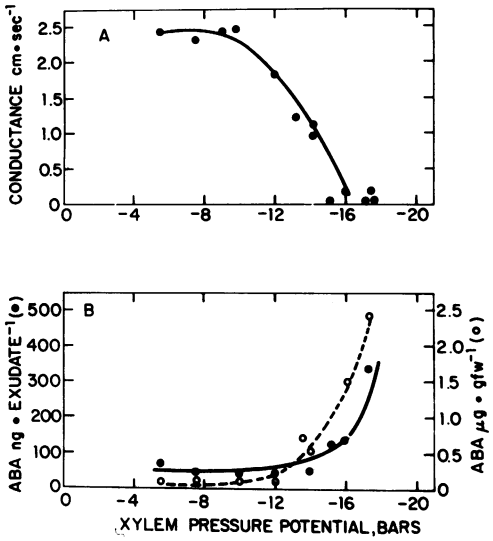

FIG. 5. Relationship between stomatal conductance and xylem pressure potential (A) of recently fully expanded cotton leaves that were attached to plants undergoing dehydration. (Plants were stressed by withholding water, and the rate of dehydration was as described in Figure legend 6). After measurement of stomatal conductance, the leaf was excised and placed in a pressure chamber, the initial balance pressure determined (xylem pressure potential in A) and then subjected to a 5 bar overpressure. Sap was collected for 30 min. The leaf was removed from the chamber, and ABA of the tissue was analyzed as described in "Materials and Methods." Exudation time was restricted so that tissue ABA levels did not change dramatically during time in the chamber. Data represent means of two to three leaves. SD for ABA determinations was as shown in Figures 3 and 4. See Table I for specific SD. The SD for leaf conductance ranged from 0.1 to 0.4 cm/s.

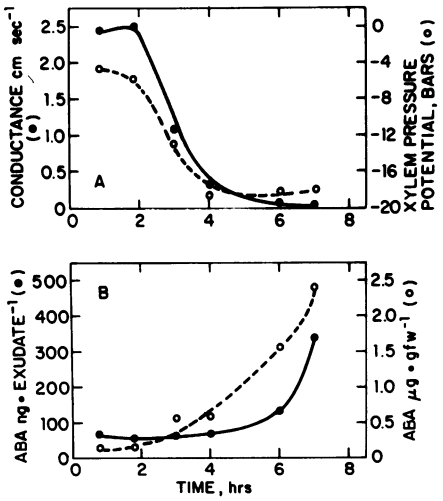

FIG. 6. Time course changes in xylem pressure potential and stomatal conductance of recently fully expanded cotton leaves during dehydration. Time refers to hours after the start of the photoperiod and beginning of the stress period. The plants had been last watered the previous day, 2 h before the dark period. The dark period was 12 h. The amount of ABA in the leaf tissue and exudates (B) was determined as described in Figure 5.

The nature of pressure-induced dehydration might suggest that increasing ABA levels in the exudates occurs only with increasing amounts of volume expressed. Data are presented to emphasize that this is not the case (Table I). ABA levels in leaves that had leaf water potential of −14.0 and −15.5 bars (leaves 4 and 5) were higher than in nonstressed leaves (leaves 1 and 2). However, the amount of ABA in the apoplast was similar in all of these leaves, even though considerably more sap was expressed from leaves 4 and 5. Thus, the amount of ABA in the exudates is not strictly dependent on the amount of sap expressed under pressure.

Table I. *Relationship between the Volume Expressed from Cotton Leaves, Tissue ABA, and Apoplastic ABA*

Data collected from leaves that were dehydrated *in situ* (Figs. 5 and 6). Each value represents the mean ± SD of two to three leaves.

Leaf No.	Volume	ψ[a]	Tissue ABA	Apoplastic ABA[b]	[ABA][c]
	mg	*bars*	*ng/g fresh wt*	*ng*	*ng/mg exudate*
1	90 ± 14	−5.5	145 ± 26	78 ± 14	0.86
2	91 ± 8	−7.0	162 ± 13	48 ± 20	0.52
3	82 ± 16	−12.0	217 ± 42	41 ± 13	0.50
4	250 ± 30	−14.0	507 ± 52	56 ± 8	0.22
5	281 ± 27	−15.5	576 ± 51	61 ± 16	0.22
6	306 ± 40	−17.5	2490 ± 247	339 ± 31	1.10

[a] ψ, Xylem pressure potential (initial balance pressure ± 1.2 bars.
[b] Total ABA in the exudate.
[c] Concentration of ABA in the exudate.

In many species the amount of exogenously supplied ABA required to elicit a specific degree of stomatal closure is only about 2-fold greater than is contained in these fully turgid leaves (8, 18). Compartmentation of ABA in sites remote from the guard cells has been invoked to account for these observations (17, 18). Similarly, leaf girdling petioles and chilling intact plants results in partial stomatal closure with only a moderate rise in tissue level ABA being apparent (17, 19). The approach and results of the present experiments indicate that ABA can accumulate in the apoplast during water stress. However, ABA is found in the apoplast of even fully turgid leaves. Consequently, changes in the total amount of ABA in the apoplast may not be necessary to initiate stomatal closure during the initial phases of water stress. In *Commelina* leaf epidermis, only 6 fmol of ABA per stomate were required to cause stomatal closure even though uptake at ABA by the guard cells continued after stomatal closure (24). Ackerson (1) demonstrated that about 0.64 fmol of ABA per stomatal complex was required to change abaxial leaf conductance from 0.4 to 0.3 cm/s. Thus, the amount of ABA in the apoplast present at the initiation of stress-induced stomatal closure may be sufficient to facilitate stomatal closure assuming that it is released to the transpiration stream. However, inasmuch as ABA present in the apoplast of fully turgid leaves does not cause stomatal closure, stress may be required to release ABA to the transpiration stream. It is important to note that there are numerous evaporative surfaces within the leaf not adjacent to the stomatal complexes, suggesting that all ABA in the apoplast may not reach the guard cells. The copious accumulation of ABA that occurs in the apoplast during the latter stages of stress (Figs. 3–5) may also be of physiological importance. Since stomata continue to accumulate ABA even after stomatal closure (23), ABA in the apoplast represents a large pool of ABA that could be taken up by the guard cells after stomatal closure has been initiated. Continued uptake of ABA from the apoplastic pool may suggest why a lag in stomatal opening occurs even after tissue has regained a favorable water status following alleviation of stress (1, 3, 16).

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