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Abscisic Acid and Osmotic Relations in *Phaseolus vulgaris* L. Shoots under Salt Stress

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Abstract. The exogenous application of abscisic acid (ABA) to well-watered plants may be of interest in imitating the effects of salinity on shoot growth. In this paper we have determined the time course of ABA accumulation in control and salt-stressed *Phaseolus vulgaris* plants and its possible relation to the accumulation of solutes and other physiologic conditions. The effect on shoot parameters of the application of exogenous ABA to the root system has also been checked. The addition of exogenous ABA to control plants caused a retardation of growth. The amount of ABA applied to the growth medium caused tissue ABA concentrations to become close to those of salinized plants. The addition of exogenous ABA to plants under control conditions resulted in a profile of proline and total sugar accumulation very similar to that observed in salinized plants. It was also found that NaCl treatment decreased the stomatal conductance and transpiration rate of leaves as well as the osmotic and turgor potentials. The addition of exogenous ABA also mimicked these responses, resulting in qualitatively and quantitatively similar results. These results, particularly those showing that the early transient rise in ABA upon exposure to NaCl coincides with the period of proline and total sugar accumulation, and that treatment of plants with exogenous ABA mimics these effects, are discussed around the idea that ABA stimulates the cellular processes of osmotic adjustment in *P. vulgaris*.

Abscisic acid (ABA) is a ubiquitous molecule in higher plants. It was originally described as a dormancy-inducing and abscission-accelerating substance, although investigators soon realized that it had many other physiologic effects in plants (Creelman 1989, Saab et al. 1990).

It has been shown that salt toxicity does not cause early growth reduction in tissues growing in saline solution (Munns et al. 1988, Ortiz et al. 1994). Thus, it has yet to be established how roots sense the low water potential. In this respect it is possible that altered levels of plant growth substances such as ABA are involved in modulating the plant responses to water deficits. Thus, it has been suggested that ABA may act as a signal for the initiation of regulatory processes involved in adaptation during growth at low-water potential (Davies et al. 1986) following the observations that ABA increases in plants under salt stress and inhibits growth when applied to plants under control conditions (Munns and Sharp 1993).

The application of salt or ABA to plants has frequently been associated with the accumulation of proline (Huber et al. 1977, Rajagopal and Andersen 1978, Pesci 1989, 1992). Nevertheless, there are few studies in which endogenous ABA, as well as the incorporation of ABA exogenously applied to the root system, have been measured (Downton and Loveys 1981, Schussler et al. 1991).

The exogenous application of ABA to well-watered plants may be of interest in imitating the effects of salinity on shoot growth. Creelman et al. (1990) showed that exogenously applied ABA caused a decrease in hypocotyl growth in soybeans. In this paper we have determined the time course of ABA accumulation in control and salt-stressed *Phaseolus vulgaris* plants and its possible relation to the accumulation of solutes and other physiologic conditions. The effect on shoot parameters of the application of exogenous ABA to the root system has been also investigated.

Abbreviations: ABA, abscisic acid; HPLC, high performance liquid chromatography; DW, dry weight; FW, fresh weight.

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Materials and Methods

Plant Growth

Seeds of *P. vulgaris* were germinated on filter paper saturated with 0.5 mM $CaSO_4$ in a germination chamber in the dark at 25 °C after rinsing and shaking in water for 2 h. After 3 days, 30 seedlings of similar size were transferred to hydroponic culture in 15-liter containers and kept in a growth chamber with controlled environment, a cycle of 16-h day/8-h night, temperatures of 25–20 °C, respectively, and a relative humidity of 60–80%. Hoagland solution (Hoagland and Arnon 1950) was used as nutrient medium. The pH was adjusted between 5.5 and 6.0 every day, and the solution was continuously aerated and renewed every 3 days to avoid ion depletion. NaCl and ABA were added at different concentrations when indicated. The treatments were initiated 2 days after the plants were transferred to nutrient solutions. Sampling and measurements were made at different times after the treatments were started. The plants were weighed and then separated into shoots and roots and harvested for different analysis.

Leaf Water Relations

Leaf discs of 0.55-cm diameter were placed in a Wescor C-52 chamber for water potential measurements. After 2 h, when equilibration was reached, the readings were taken in a Wescor HR-33 microvoltmeter. The same leaves were sealed in plastic bags and then frozen and thawed to measure osmotic potential with the Wescor apparatus. Turgor potential was calculated by difference.

Transpiration and stomatal conductance were measured in fresh leaves in the plant using a LI-COR 1600 porometer.

Ion Analysis

Lyophillized shoot samples were subjected to ion extraction by shaking with hot 100 mM acetic acid for 30 min. After the extracts were filtered, the ion concentrations were determined: Na^+, K^+, Ca^{2+}, and Mg^{2+} by atomic absorption spectrometry and Cl^- by titration.

ABA Analysis

Extraction of ABA from tissue was carried out as described by Hubick and Reid (1980) with minor modifications. One gram of lyophillized shoot material was homogenized for 10 min in 100 mL of methanol/ethyl acetate/acetic acid (50:50:1), with 0.02% butylated hydroxytoluene to avoid oxidation. The samples were kept on a shaker at 4 °C in the dark to prevent ABA isomerization. The homogenates were filtered and dried under vacuum, redissolved in 5 mL of methylene chloride, and loaded onto a silica Sep-Pak. A series of organic solvent mixtures were used to remove interfering substances. After the filtrate was dried, it was redissolved with 10 mL of 0.1 M phosphate buffer, pH 8, and the pH was adjusted to 2.5. ABA was extracted with ethyl acetate. The extracts were dried under vacuum and dissolved in 1 mL of absolute methanol, HPLC grade, and filtered through 0.45-μm filters.

ABA was determined according to the method described by Markhart (1982). A Kontron HPLC 420 system, equipped with two pumps and diode array UV detector was used. The separation was carried out on a C18 column (Spherisorb ODS 2–3 mm, Teknochroma), 10 × 0.46 cm, using an isocratic system of solvent (water/methanol/acetic acid, 40:60:1). The flow rate was 0.9 mL min^{-1}, and detection was performed at 263 nm. Identification and quantitation were done by the external standard procedure, using pure ABA (*cis,trans*-isomer, Sigma).

Organic Components

Proline contents were measured using the method described by Levy (1980). For sugar determinations, triplicate samples of 0.2 g of lyophilized shoot material from each treatment were extracted with 90% ethanol and centrifuged. The tissue was reextracted twice more with 70% ethanol. The supernatants were pooled, evaporated to dryness, and dissolved in 5 mL of deionized water. Total sugars were quantitated in the extract by the anthrone method (Jermyn 1975), using glucose as standard.

Results

Figure 1 shows the growth of *P. vulgaris* shoots at different time periods after the initiation of the various treatments. Control plants, grown in 5 mM NaCl, displayed a linear rate of growth during this phase of shoot growth. The addition of 80 mM NaCl resulted in a clear decrease in the growth rate so that after 144 h of NaCl treatment (the last experimental data point), shoots were 90% bigger than at zero time, as compared with controls, which were 140% bigger.

The addition of exogenous ABA to control plants caused a retardation of growth during the time of the experiment (Fig. 1). This growth inhibition was dependent on the concentration of ABA applied. A concentration of ABA of 50 μM or higher, used extensively in other works (Jones et al. 1987), resulted in practically a total inhibition of shoot growth (Fig. 1).

To check the levels of ABA in plant shoots, both endogenous and after external application to the root medium, we determined the level of this hormone in the shoots at the different time periods (Fig. 2). When the amount of ABA applied to the growth medium was 10 μM, tissue ABA concentrations became similar to those of salinized plants.

After the initiation of the NaCl treatment, the shoot ABA content started to increase to a maximum of about 2.9 μg g DW^{-1} at 12 h and then decreased to background levels (about 0.5 μg g DW^{-1}) and kept constant. The time course observed for ABA content in control plants was different. The maximum value appeared at 6 h and was not as high, the contents always ranging between 0.2 and 1.5 μg g DW^{-1}.

After the addition of 10 μM exogenous ABA to the root medium, the contents of the hormone in-

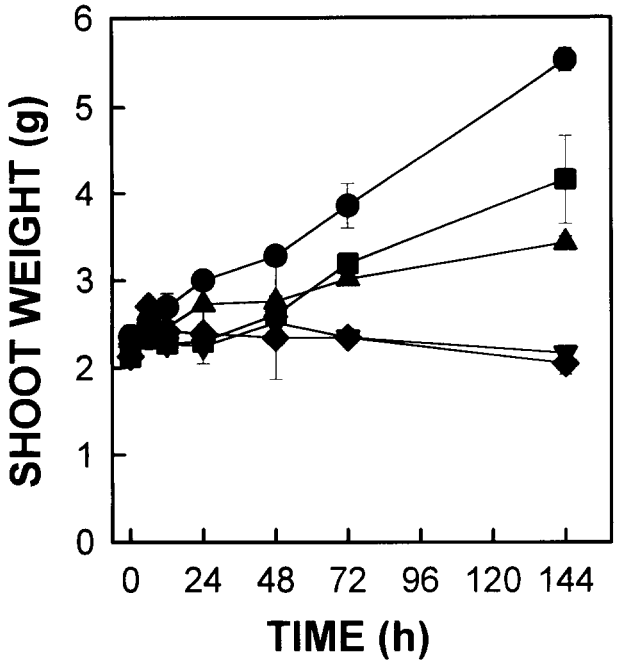

Fig. 1. Time course of *P. vulgaris* shoot weight grown under different conditions. (●) control; (■) 80 mM NaCl; (▲) 10 μM ABA; (▼) 25 μM ABA; (◆) 50 μM ABA. Data correspond to the mean of three experiments ± S.D. Error bars are shown when bigger than the symbols.

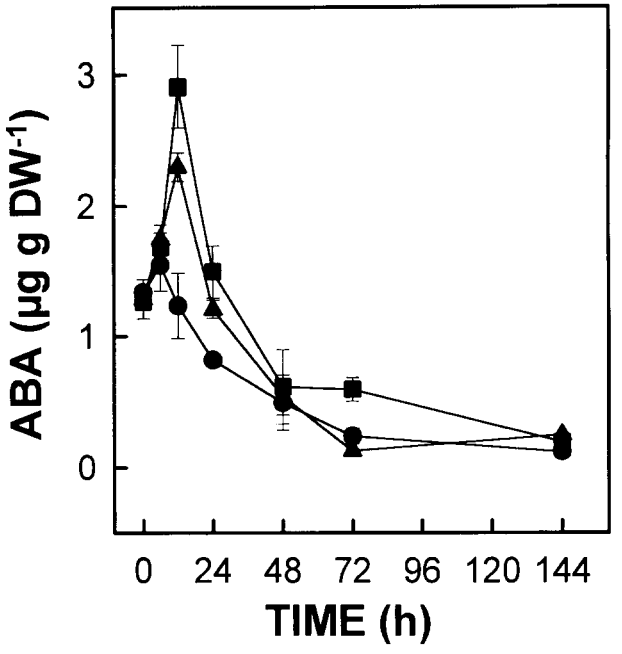

Fig. 2. Endogenous levels of ABA in *P. vulgaris* shoots grown under different conditions as a function of time. (●) control; (□) 80 mM NaCl; (▲) 10 μM external ABA. Data correspond to the mean of three experiments ± S.D. Error bars are shown when bigger than the symbols.

creased to a maximum peak of 2.3 μg g DW^{-1} at around 12 h, a level similar to that shown for NaCl-treated plants, and then decreased to background levels. Therefore, this concentration of exogenous ABA was used in the following experiments.

Figure 3 shows the effects of the addition of NaCl or ABA on the proline content of *P. vulgaris* shoots at different times. The proline content in control plants was almost unchanged or even slightly decreased with time, keeping close to a mean value of 0.25 μmol g FW^{-1} for the duration of the experiment. After salinization, the proline content increased from initial levels, around 0.3 μmol g FW^{-1} to a value of about 0.45 μmol g FW^{-1}, which practically stabilized after 48 h of treatment. The addition of exogenous ABA to plants under control conditions resulted in a profile of proline contents very similar to that observed for salinized plants, both qualitatively and quantitatively, reaching maximal values of proline of about 0.45 μmol g FW^{-1}.

The levels of sugars in *P. vulgaris* shoots at different times are shown in Figure 4. In control plants the total sugar contents showed a tendency to maintain a value of around 140 μmol g DW^{-1} or even to decrease with time. The addition of NaCl completely changed the shape of this profile, showing a maximum of about 205 μmol g DW^{-1} after 12 h and then decreasing with increasing time. The content of sugar in plants treated with 10 μM exogenous ABA, at the different times, were very close to those of salinized plants, with a 12-h maximum of 190 μmol g DW^{-1}.

Table 1 documents the changes in ionic content of *P. vulgaris* shoots during exposure to control (5 mM) or high (80 mM) NaCl levels or to 10 μM exogenous ABA. Shoots began to accumulate Cl^- within 6 h of exposure to 80 mM NaCl. Na^+ and K^+ uptake started to be more evident after 12 h. In salinized plants the K^+ uptake observed was certainly not as dramatic as that observed for Na^+, presumably because the concentration of K^+ was limiting. On the other hand, whereas ABA treatment also gave rise to a certain accumulation of Cl^- in relation to control plants, Na^+ and K^+ levels remained essentially unaffected by this treatment. Ca^{2+} and Mg^{2+} contents in shoots of control plants showed a tendency to increase with time. It was generally observed that both NaCl and 10 μM exogenous ABA treatments decreased Ca^{2+} and Mg^{2+} contents in relation to control plants.

The responses of osmotic, water, and turgor potentials, as well as stomatal conductance to treatment with 80 mM NaCl or 10 μM ABA in the root zone, after different time periods, are shown in Table 2. Leaf water potential decreased by about 0.15

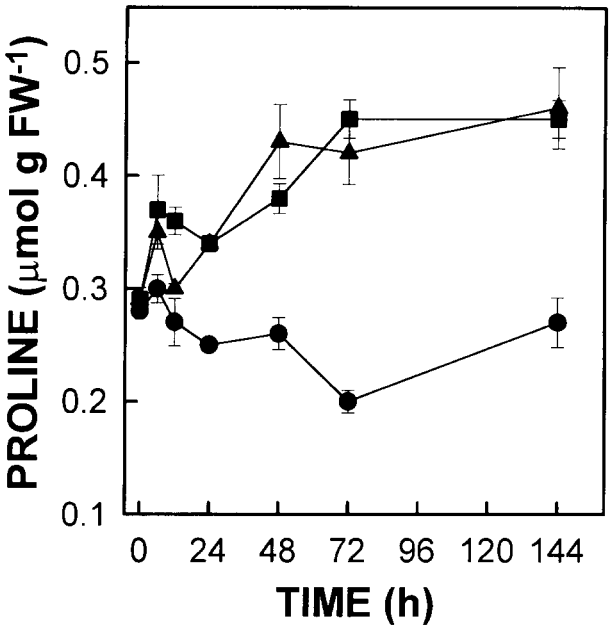

Fig. 3. Proline concentration in *P. vulgaris* shoots as a function of time and for the different treatments. (●) control; (□) 80 mM NaCl; (▲) 10 μM external ABA. Data correspond to the mean of three experiments ± S.D. Error bars are shown when bigger than the symbols.

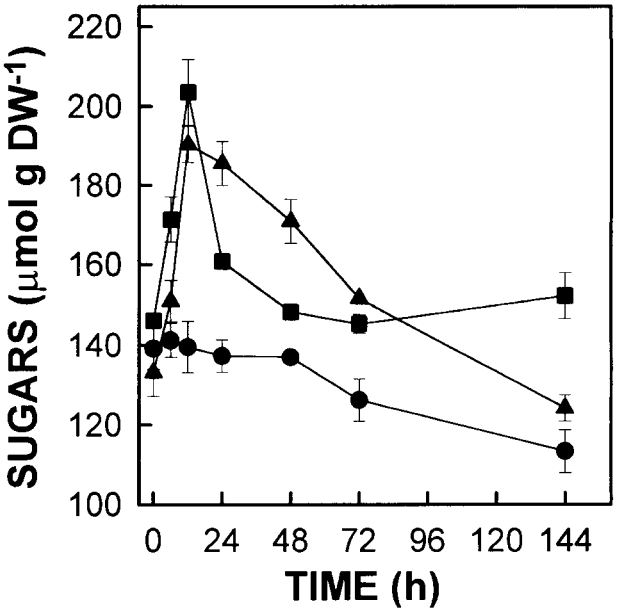

Fig. 4. Concentration of total sugars in *P. vulgaris* shoots as a function of time, obtained for the different treatments. (●) control; (□) 80 mM NaCl; (▲) 10 μM external ABA. Data correspond to the mean of three experiments ± S.D. Error bars are shown when bigger than the symbols.

MPa 1 day after the salinity treatment began and by more than 0.30 MPa after 6 days, in relation to controls. The osmotic potential decreased by about 0.25 MPa 6 days after the initiation of saline treatments. Treatment of plants with 10 μM ABA produced results that were qualitatively similar to those obtained with NaCl treatment. In this case, the water potential decreased by about 0.11 MPa after 2 days and by 0.18 MPa at the end of treatment. Osmotic potential was also decreased by about 0.15 MPa after 6 days of treatment with ABA. The turgor potential was maintained within control values for both NaCl and ABA treatments. Plants from the both treatments continued to adjust osmotically over the entire experimental period. On the other hand, salinization of plants resulted in a clearly reduced stomatal conductance at all sampling periods, with a similar effect on transpiration rates (results not shown). Treatment of plants with exogenous ABA also mimicked this effect, particularly at times longer than 6 h.

Discussion

Our results on the growth of *P. vulgaris* shoots indicate that under salt stress plant growth is retarded (Fig. 1), as has been reported before for this species (Cachorro et al. 1993). The application of exogenous ABA to control plants retarded shoot growth to varying degrees, depending on the ABA concentration. In our experiment, we found 10 μM ABA to produce growth retardation similar to that with 80 mM NaCl. This effect has been described for several species (Jones et al. 1987, Saab et al. 1990, Creelman et al. 1990, Munns and Sharp 1993), and it might point to a role for this hormone as a mediator in the response of plant growth to salinity.

The results presented in this paper showing time course changes in proline and sugars, osmotically active compounds, as well as endogenous ABA, effected by NaCl or exogenous ABA, suggest that ABA stimulates the cellular processes of osmotic adjustment. The transitory elevation of shoot ABA after an imposed NaCl stress in the root medium (Fig. 2) might be the hormonal signal triggering a chain of processes leading to elevation of osmotically active solutes, in this case proline (Fig. 3), total sugars (Fig. 4), and Na^+, K^+, and Cl^- (Table 1). When 10 μM ABA is applied exogenously, it is important that this causes realistic changes in the internal concentrations of ABA, as we have shown here (Fig. 2). It can be seen in Figure 2 that the internal concentrations of ABA measured after treatment with 80 mM NaCl or with 10 μM exogenous ABA were very close at all sampling periods.

Our results indicate that in *P. vulgaris* there is a causal relationship between ABA and proline accu-

Table 1. Ion concentration in shoots from salt- (80 mM NaCl) and ABA-treated (10 μM) *P. vulgaris* plants compared with control (5 mM), at different sampling times.

Time (h)	Ion concentration (mmol kg^{-1})														
	Cl^-			Na^+			K^+			Ca^{2+}			Mg^{2+}		
	Control	NaCl	ABA	Control	NaCl	ABA	Control	NaCl	ABA	Control	NaCl	ABA	Control	NaCl	ABA
0	57	50	45	17	12	10	993	968	1,065	286	320	297	69	72	75
6	52	101	48	12	28	10	837	1,002	1,005	332	325	344	67	69	74
12	49	150	56	10	62	11	997	1,149	1,079	345	323	331	69	66	68
24	47	215	58	15	598	11	1,045	1,305	1,007	414	336	308	75	70	60
48	41	371	54	13	787	16	1,169	1,394	1,228	475	392	419	71	64	66
72	33	617	61	14	985	11	1,024	1,435	1,148	510	460	430	73	64	68
144	35	917	68	17	978	14	1,086	1,312	1,206	793	623	575	97	64	76

Table 2. Water, osmotic, and turgor potentials and stomatal conductance of shoots of *P. vulgaris* plants at different times under control (5 mM NaCl), salinized (80 mM NaCl), and ABA (10 μM) conditions.

Time (h)	Water potential (MPa)			Osmotic potential (MPa)			Turgor potential (MPa)			Stomatal conductance (m s^{-1})		
	Control	NaCl	ABA	Control	NaCl	ABA	Control	NaCl	ABA	Control	NaCl	ABA
0	−0.06	−0.08	−0.07	−0.34	−0.38	−0.42	0.28	0.30	0.35	0.55	0.54	0.57
6	−0.12	−0.19	−0.14	−0.42	−0.52	−0.43	0.30	0.33	0.29	0.39	0.36	0.40
12	−0.17	−0.29	−0.17	−0.44	−0.55	−0.45	0.27	0.23	0.27	0.51	0.50	0.45
24	−0.13	−0.28	−0.17	−0.44	−0.65	−0.53	0.30	0.27	0.35	0.44	0.42	0.34
48	−0.12	−0.22	−0.18	−0.43	−0.62	−0.51	0.30	0.43	0.33	0.54	0.45	0.45
72	−0.17	−0.31	−0.21	−0.43	−0.63	−0.56	0.26	0.32	0.35	0.66	0.56	0.44
144	−0.17	−0.41	−0.25	−0.45	−0.65	−0.57	0.27	0.24	0.31	0.58	0.44	0.35

mulation, as has been described for other species (LaRosa et al. 1987, Ober and Sharp 1994). We found that NaCl treatment, as well as treatment with exogenous ABA to reach internal levels of the hormone otherwise within the same range as those produced by the effect of salt, produce similar elevations in the concentration of proline. ABA levels always increased prior to proline. This suggests a link among these three events: salt stress, elevation of the concentration of ABA, and accumulation of the amino acid proline. Downton and Loveys (1981) reported similar results for grapevine leaves, although they did not apply exogenous ABA to their plants. On the other hand, a direct relationship between exogenously applied ABA and the accumulation of proline has been reported in barley (Stewart 1980, Pesci 1987) and maize (Ober and Sharp 1994). Nevertheless, the fact that ABA does not induce proline accumulation in several other species has been used to argue that ABA accumulation is not involved in stress-induced proline accumulation (Aspinall 1980, Stewart and Voetberg 1985). Perhaps this is not a ubiquitous mechanism, or it could be possible that results obtained with intact plants, as in our case, are not directly comparable to those obtained with excised leaves (Stewart and Voetberg 1985, Pesci 1987). Such differences could be related to differences in the metabolic mechanisms leading to proline accumulation (Stewart and Voetberg 1985).

ABA has also been implicated in the movement and accumulation of sugars in higher plants (Schussler et al. 1991, Häuser et al. 1992), although its role remains uncertain. In our study, total sugar shoot contents were affected in an essentially similar way by NaCl stress and exogenous ABA treatments (Fig. 4), suggesting that there is a relationship between the levels of sugars and ABA in shoots. This increase may represent the accumulation of material that could normally be used during metabolism in case there was no growth inhibition by ABA or NaCl (Jones et al. 1987). We can conclude that each of these two treatments has some component that links one to the other.

There is evidence that under salt stress ABA stimulates ion flux to the transpiration stream (Downton and Loveys 1981) and controls stomatal conductance (Munns and Sharp, 1993). Our data show that 6 h after applying 80 mM NaCl a two to threefold times increase in endogenous ABA is observed which parallels an enhancement of Cl^- and Na^+ uptake, as well as of K^+ to some extent (Table

1). The increase in these three ions has been related to ABA-induced proline accumulation, whereas it has been shown that Ca^{2+} and Mg^{2+} are not involved in it (Pesci 1989). On the other hand, our results on stomatal conductance and transpiration rates (Table 2) clearly point to a role of ABA as a mediator in the effect of NaCl on these physiologic parameters.

A rise of endogenous ABA has been shown to stimulate the accumulation of Cl^- and Na^+ in salt-stressed grapevines (Downton and Loveys 1981). However, in *P. vulgaris*, a salt-sensitive species, the continuous accumulation of Cl^-, and to a lesser extent Na^+, may produce toxic effects disrupting metabolic processes.

Summarizing, in this study it is shown that 10 μM exogenous ABA, under normal conditions, retards shoot growth to an extent similar to that of 80 mM NaCl stress. All of the data reported here suggest a strong and rather specific link between salt stress and both endogenous and exogenous ABA in inducing proline and total sugar accumulation, as well as in the control of stomatal conductance of leaves.

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