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THE EFFECT OF THE OSMOTIC POTENTIAL AND SPECIFIC ION CONCENTRATION OF THE NUTRIENT SOLUTION ON THE UPTAKE AND REDUCTION OF NITRATE BY BARLEY SEEDLINGS

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KEY WORDS

Barley Chloride *Hordeum vulgare* Nitrate reduction Nitrate uptake Osmotic potential Salinity Salt excess Transpiration

SUMMARY

Short-term absorption experiments were conducted with intact barley (*Hordeum vulgare* L.) seedlings to observe the effects of the osmotic potential (Ψ_π) and salt species on nitrate uptake and *in vivo* nitrate reduction. The experiments consisted of growing barley seedlings for 5 days in complete nutrient solutions salinized to Ψ_π levels of -0.6, -1.8, -3.0, -4.2, and -5.4 bars with NaCl, $CaCl_2$ or Na_2SO_4. After the absorption period, the seedlings were separated into shoots and roots, weighed, then analyzed for NO_3. The nutrient solutions were sampled for NO_3 analysis each day immediately before renewing the solutions. The accumulative loss of NO_3 from the solutions was considered to be uptake whereas NO_3 reduction was the difference between uptake and seedling content. Lowering the Ψ_π of the nutrient solutions resulted in decreased concentrations of NO_3 in the plant, little or no effect (except at the lowest Ψ_π level) on uptake, and increased nitrate reductase activity. Increased rates of NO_3 reduction were in particular associated with the Cl concentration of the nutrient solution.

INTRODUCTION

A general observation that we have made from conducting salt tolerance experiments with a variety of field and vegetable crops is for markedly lower concentrations of NO_3 in leaves collected from plants under salinity stress. Specifically, leaf NO_3 concentrations were found to be inversely correlated with the degree to which the root system (soil, sand, or solution culture) was salinized with Cl-salts[4,5,14,15,17]. As an example of the Cl–NO_3 relationship, leaf NO_3 concentration of wheat plants (*Triticum aestivum* L.) grown to maturity with a complete

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nutrient solution without Cl salts ranged from 0.30 to 0.70%, whereas leaf NO_3 concentrations of wheat plants grown in a NaCl salinized nutrients solution were usually below 0.20%. However, these plants did not manifest visual N deficiency symptoms nor were the total N concentrations markedly reduced. We have made similar observations of the Cl-leaf NO_3 relationship with sesame, *Sesamum indicum* L.[4,17], and tomato, *Lycopersicon esculentum* L.[5].

However, the above experiments were not designed to separate specific salt or ion effects from osmotic effects regarding NO_3–N uptake and assimilation; thus the present investigation was undertaken. This investigation consisted of a series of experiments conducted with intact barley (*Hordeum vulgare* L.) seedlings under NaCl, $CaCl_2$, and Na_2SO_4 salinity treatments in order to separate osmotic potential (Ψ_π) effects from specific ion effects on NO_3 uptake and reduction.

MATERIALS AND METHODS

Barley seedling (*Hordeum vulgare* L. var 'Numar') were produced for the nitrate absorption experiments by the technique described by Chantarotwong *et al.*[6] In brief, the technique entailed soaking seed for 24 hours in aerated distilled water, placing the seed on guaze covered screens on trays containing 0.002 *M* $CaSO_4$ and then germinating the seed in darkness over a 5 day period. On the sixth day, the germination trays were taken to an air-conditioned greenhouse preparatory to setting up an absorption experiment on the seventh day.

Three nitrate absorption experiments are conducted, each at a separate time. The first consisted of transferring 14 uniform seedlings to each of 25 solution cultures containing 400 ml of a complete nutrient solution (*meq/l*: Ca, 8.6; Mg, 3.8; K, 6.6; H_2PO_4, 0.5; SO_4, 3.5; and NO_3, 15; plus the following in mg/l: B, 0.5; Cu, 0.05; Fe, 5; Mn, 0.05; Mo, 0.01; and Zn, 0.05). These solution cultures were amended with different amounts of NaCl such that the Ψ_π of the nutrient solutions were adjusted to -0.6, -1.8, -3.0, -4.2 and 5.4 bars. Treatments were replicated fivefold. The solution cultures were weighed each day at 0900 to determine volume of solution lost, and then, a sample of the solution was collected for NO_3 analysis immediately before replacing the solution with 400 ml of the NaCl treated nutrient solution. The experiment was terminated five days later at which time the seedlings were separated into roots and shoots, rinsed approximately one minute with distilled water, blotted dry with a cotton towel, and dried at 65°C for 48 hours. The dried root and shoot samples were weighed, ground, and then analyzed for NO_3 and Cl using the chemical procedures outlined below.

The same techniques was used for the $CaCl_2$ and Na_2SO_4 experiments, *i.e.*, the complete nutrient solutions were adjusted to the same levels of Ψ_π with $CaCl_2$ or Na_2SO_4 and NO_3 uptake and assimilation observed over a 5 day period.

The nitrate content of the ground shoot and root materials was determined by extracting NO_3 from the ground sample using water and then determining the NO_3 concentration of the extract colorimetrically by a procedure that produces a colored complex upon nitration of salicylic acid[3].

The NO_3 concentration of the nutrient solutions was also determined by the above procedure. Each analysis was repeated four times.

Chloride in the ground plant samples was extracted with 0.1 *N* HNO_3 and the extract analyzed with an automatic Cl titrator[1].

The nutrient solutions were weighed without plants every 24 hours immediately before renewing the solutions to have a measure of the volume of each test solution. The NO_3–N concentration of each solution was also determined immediately before renewing the solutions. Nitrate uptake

was considered to be the total loss of NO_3–N from the solution over the 5 day absorption period.

The difference between nitrate uptake as defined above, and the sum of nitrate in the intact plant after the 5 day absorption trial was considered to be the quantity of nitrate reduced.

Weight loss from the solution culture containers corrected for the evaporation loss (obtained from containers without plants) was taken as the measure of water transpired during the 5 day absorption period.

Where results are described as significantly different, analysis of variance indicated that treatments differed at the 1% level (not shown).

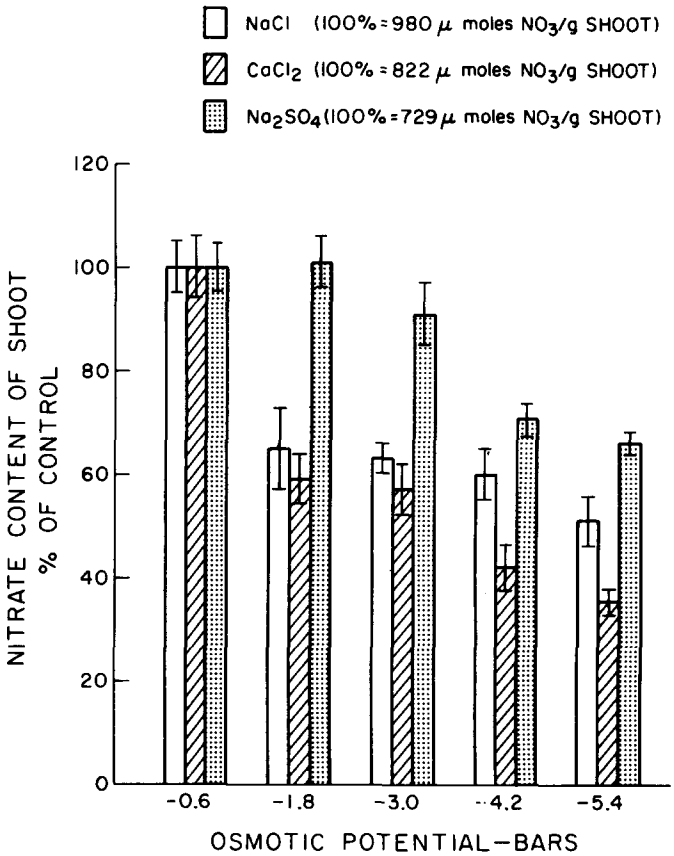

Fig. 1. Relative NO_3 concentration of barley shoots in relation to the osmotic potential of a complete nutrient solution salinized with NaCl, $CaCl_2$, or Na_2SO_4.

RESULTS

Nitrate concentration of shoots and roots

Fig. 1 illustrates the relationship between NO_3 concentration of shoots and Ψ_π of the nutrient solutions and salt used to salinize the nutrient solutions. The NO_3 concentration of shoots from the -5.4 bar treated seedlings were markedly lower than that of the -0.6 bar treatment, being respectively for the NaCl, $CaCl_2$, and Na_2SO_4 experiments 56%, 38% and 66% of concentrations found in the control plants. Compared at the same Ψ_π levels, the $CaCl_2$ treatments resulted in the lowest NO_3 shoot concentrations followed by NaCl, and then Na_2SO_4.

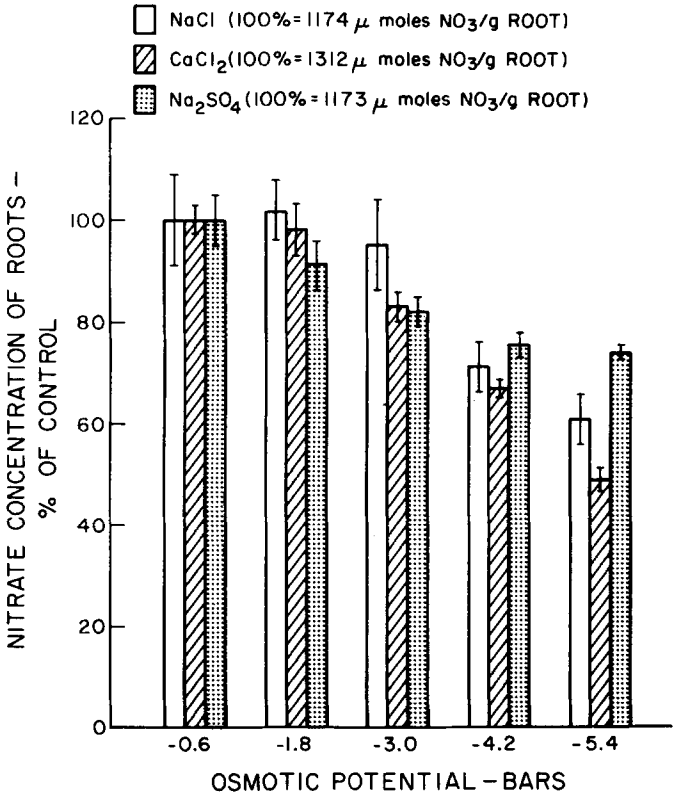

Fig. 2. Relative NO_3 concentration of barley roots in relation to the osmotic potential of a complete nutrient solution salinized with NaCl, $CaCl_2$, or Na_2SO_4.

Ψ_π is important decreasing NO_3^- content, as evidenced by Na_2SO_4 treatment, but the decrease of NO_3^- in shoots is more in relation with the Cl^- concentration than with the osmotic potential of the nutrient solution. There are significant differences between NaCl or $CaCl_2$ and Na_2SO_4 treatments, and also between NaCl and $CaCl_2$ in the last two osmotic potentials where the Cl concentrations are higher with $CaCl_2$ (135 and 180 meq/l) than with NaCl (90 and 120 meq/l).

In general different trends were exhibited by the NO_3 concentration in root (Fig. 2). The decrease of NO_3 is relatively more related with the osmotic potential than with the salt species of the nutrient solution. The NO_3 concentrations in roots were somewhat greater than those found for the shoots.

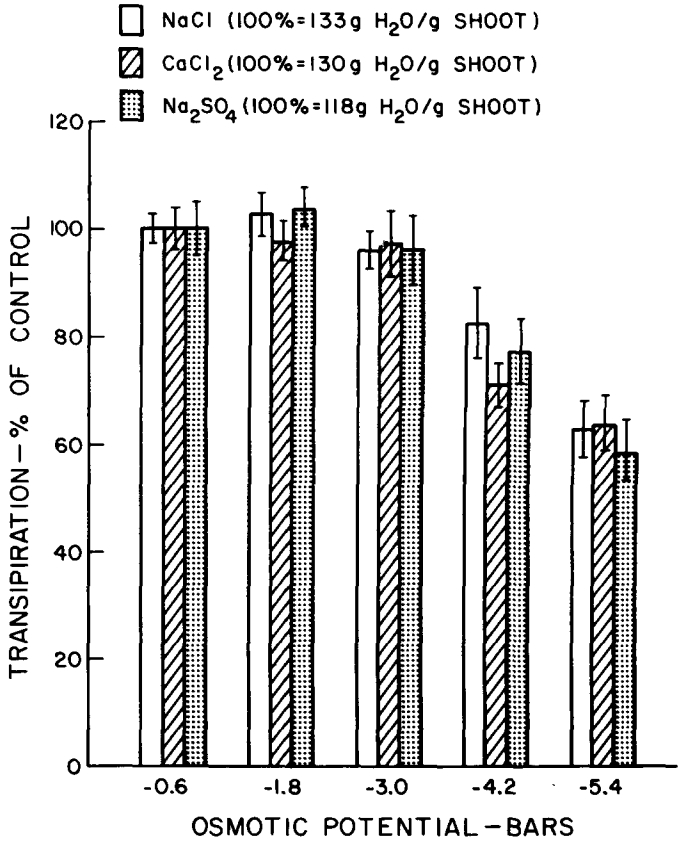

Fig. 3. Relative transpiration of barley seedlings in relation to the osmotic potential of a complete nutrient solution salinized with NaCl, $CaCl_2$, or Na_2SO_4.

Chloride concentration of shoots and roots

The Cl concentration of shoot and root tissue paralleled that of the nutrient solutions. Data are not reported.

Transpiration losses

Fig. 3 depicts transpiration loss as a function of Ψ_π of the nutrient solution salinized with each of the salts. Transpiration did not materially decrease at Ψ_π treatments of -3.0 bars or higher; however, the -5.4 treatment curtailed

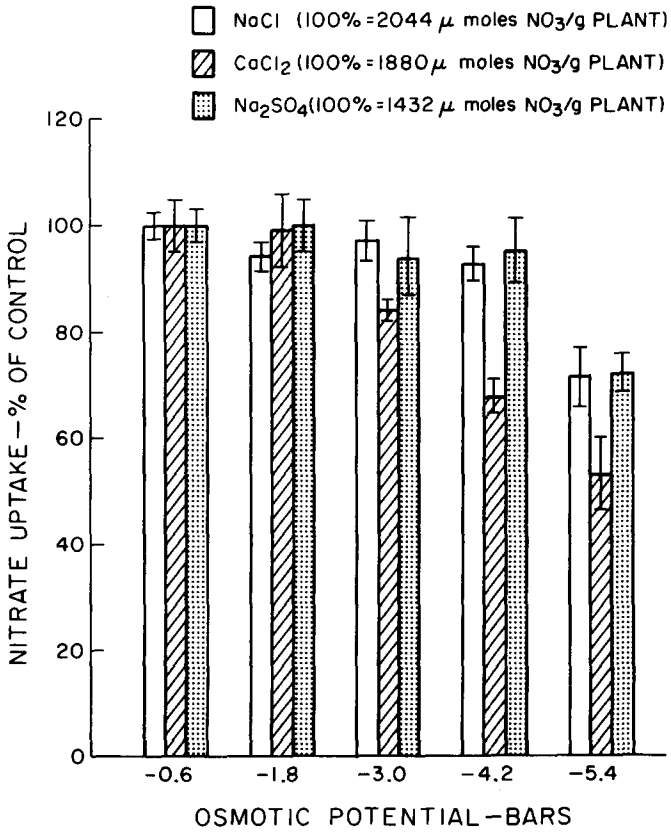

Fig. 4. Relative nitrate uptake of barley seedlings in relation to the osmotic potential of the nutrient solution salinized with NaCl, $CaCl_2$, or Na_2SO_4.

transpiration approximately 40% essentially not related to ionic species. There are significant differences between the last two points of osmotic potential and the control.

Nitrate uptake

Fig. 4 shows the relationship between the amount of NO_3 absorbed per g of seedling over a 5 day absorption period and Ψ_π of the nutrient solution amended

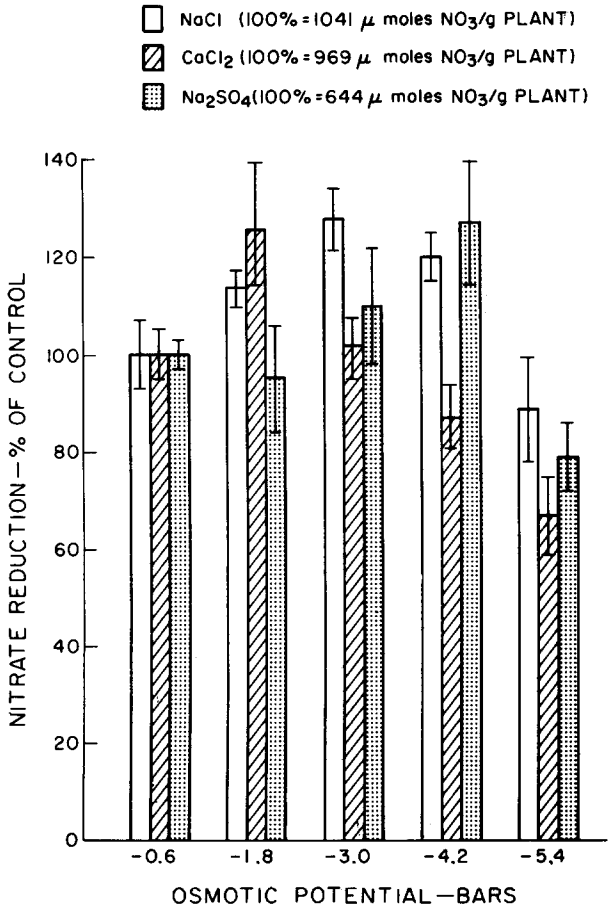

Fig. 5. Relative nitrate reduction of barley seedlings in relation to the osmotic potential of the nutrient solution salinized with NaCl, $CaCl_2$, or Na_2SO_4.

with NaCl, $CaCl_2$, or Na_2SO_4. The -0.6 bar treated seedlings absorbed 2044, 1880, and 1432 μmoles of NO_3 per g of seedling from the nutrient solutions used for the NaCl, $CaCl_2$, and Na_2SO_4 experiments. With the exception of the $CaCl_2$ treatment at the -4.2 bar level, only the -5.4 bar treatments reduced NO_3 uptake. The $CaCl_2$ treatment resulted in the lowest NO_3 uptake with significant differences in relation with the NaCl and Na_2SO_4 treatments.

Nitrate reduction

Nitrate reduced per g of seedlings over the five day absorption period is plotted in Fig. 5 as a function of Ψ_π and salt used. The NO_3 reduction rates for the control

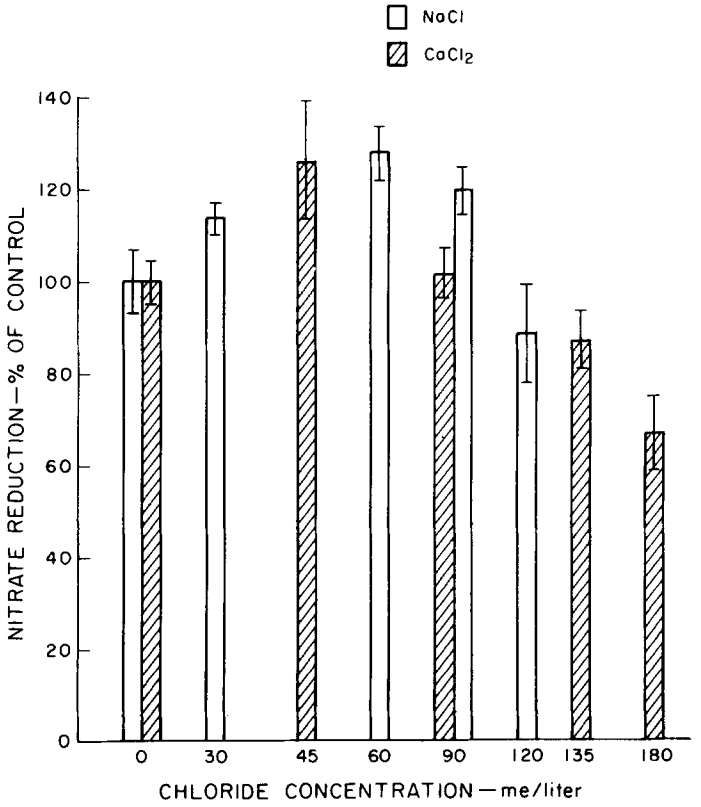

Fig. 6. Relative nitrate reduction of barley seedlings in relation to the Cl concentration of the nutrient solution salinized with NaCl or $CaCl_2$.

seedling of the NaCl, $CaCl_2$, and Na_2SO_4 experiments were respectively 1041, 969 and 644 μmoles NO_3 per g of seedling. The lower addition of salts to the nutrient solution increase nitrate reduction over control, but the highest rate led generally to decrease rates of NO_3 reduction. For example, the NO_3 reduction rates for seedlings receiving NaCl treatments at Ψ_π levels of -0.6, -1.8, -3.0, -4.2 and -5.4 bars were 1041, 1188, 1332, 1249, and 926 μmoles NO_3 per g seedlings. The experiments with Na_2SO_4 had high variations, therefore there are only significant differences at the -5.4 bars of osmotic potential in relation to the control. Fig. 6 shows the relation between NO_3 reduction rate and the Cl concentration of the nutrient solution salinized with NaCl or $CaCl_2$. The nitrate reduction rate increased as the Cl level of the nutrient was increased to 60 meq Cl/l, then the rate decreased progressively as the Cl concentration was increased beyond the 60 meq Cl/l level.

DISCUSSION

The relationship between the concentration of NO_3 in barley seedling and the osmotic potential or Cl concentration of the nutrient solution culture has been observed for a wide assortment of crop plants under treatment leading to the accumulation of Cl within the rootzone. The relative longterm experiments with bermudagrass[9], stargrass[10], potato[12], sesame[4,17], tomato[5], and wheat[5,14,15], demonstrated a negative effect of rootzone accumulation of Cl on leaf NO_3 concentrations.

When the osmotic potentials of the nutrient solutions decrease the NO_3^- concentrations in roots decrease (Fig. 2) independent of the salt specie, and similar to the effect of osmotic potential on the transpiration rates (Fig. 3).

There are reports on the effect of water stress (drought, low leaf water potential Ψ_π) on NO_3 reductase activity (NRA). Under drought treatment lowered Ψ_π levels led to curtailed NRA[8,11,14]. Shanner and Boyer[13] concluded that this curtailment of the NRA associated with drought stress was actually the result of drought restricting NO_3 flux rather than having a direct effect on the NRA system.

The sequence of effects: Salinity (rootzone) → Decrease of Ψ_π → Decrease of the transpiration → Decrease of the nitrate flux → Decrease of the nitrate contents, explains our results at the lowest osmotic potential of the nutrient solutions (Figs. 1, 2, 3 and 4), but it does not explain the decrease of NO_3 content in shoots with NaCl and $CaCl_2$ at -1.8, -3.0 and -4.2 bars and the differences between both salts (Fig. 1).

The 40% decrease in the concentration of NO_3 in barley shoots associated with

salinizing with either NaCl or $CaCl_2$ to a level of -1.8 bars (Fig. 1) is considered to be the result of a Cl-enhanced rate of NO_3 reduction. Fig. 5 and, in particular, Fig. 6 show nitrate reduction in relation to the Cl treatment rate (meq Cl/l). Salinizing the nutrient solution with Cl up to 90 meq/l led to an increase nitrate reduction rate of 10 to 30% greater than that observed for the unsalinized plants (control). We do not have a satisfactory explanation on this stimulation of NO_3 reduction rates by Cl. Helal and Mengel[7] have shown a similar result in barley plant, but considered the increase of N reduced as influenced by the Na/K relation; however our results show that the increment of NO_3 reduction is a function of Cl and not of the accompanied cations. Possibly the Cl stimulation of NRA is an indirect effect whereby Cl increases the flux of NO_3 to the RNA sites.

The different responses of nitrate contents in shoots and roots to the treatments (Figs. 1 and 2) and the differences between NaCl and $CaCl_2$ treatments on nitrate uptake and reduction (Figs. 4 and 5) are related with a specific effect of Cl on NO_3 transport.

This effect of Cl on NO_3 transport could be indirect and related with the decrease of malate by Cl-ions[16] since malate is a highly active ion in the NO_3 transport[2]. The present set of experiments were not designed to yield information on NO_3 fluxes, transport and/or distribution of NO_3 at the cellular level. It will need further physiological research.

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