K+ starvation inhibits water-stress-induced stomatal closure via ethylene synthesis in sunflower plants

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Abstract

The effect of water stress on stomatal closure in sunflower plants has been found to be dependent on K+ nutrient status. When plants with different internal K+ content were subjected to a water-stress period, stomatal conductance was reduced more markedly in plants with an adequate K+ supply than in K+-starved plants. K+ starvation promoted the production of ethylene by detached leaves, as well as by the shoot of whole plants. Water stress had no significant effect on this synthesis. The effect on stomatal conductance of adding 5 μM cobalt (an ethylene synthesis inhibitor) to the growing medium of plants subjected to water stress was also dependent on their K+ nutritional status: conductance was not significantly affected in normal K+ plants whereas it was reduced in K+-starved plants. Cobalt had no harmful effects on growth, and did not alter the internal K+ content in the plants. These results suggest that ethylene may play a role in the inhibiting effect of K+ starvation on stomatal closure.

Key words: Ethylene, potassium starvation, stomatal conductance, sunflower.

Introduction

In wild plants grown in arid climates and in dryland agricultural ecosystems, a syndrome of dehydration is often observed, which, in many cases, is associated with low levels of K+ in the plant. It is widely accepted that K+ deficiency increases the plant’s susceptibility to the water stress and that plants with an adequate K+ supply have better-hydrated tissues than those with K+ deficiency (Mengel and Kirkby, 2001). The role of K+ in the regulation of plant water status is also well known. In cells, it plays a crucial role in osmoregulation, contributing to osmotic water uptake and growth (Wyn Jones et al., 1979). Over the whole plant, K+ accumulation in root xylem vessels and guard cells facilitates both osmotic water absorption by the root (Läuchli, 1984) and stomatal opening and transpiration (Hsiao and Läuchli, 1986).

Different factors, including darkness (Zeiger et al., 1977; Moody and Zeiger, 1978), water-stress (Assmann and Shimazaki, 1999), and abscisic acid (ABA) (Cummins et al., 1971; Hartung et al., 1998) are involved in the stomatal closure mechanism. In all cases, stomatal closure is preceded by a rapid release of K+ from the guard cells into the apoplast (Schroeder and Hagiwara, 1989; Hetherington and Quatrano, 1991; Kearns and Assmann, 1993). It is logical to believe that under K+ starvation conditions, the stomata would find it difficult to remain open. However, numerous studies have shown that K+ starvation promotes transpiration (Brag, 1972; Bednarz et al., 1998; Sudama et al., 1998; Cabañero and Carvajal, 2007). Nevertheless, when K+ deficiency is very severe it may inhibit transpiration (Desai, 1973; Nagarajah and Ratnasuriya, 1978; Dhakal and Erdei, 1986; Smith and Stewart, 1990; Tomemori et al., 2002). In sunflower plants and in olive trees, it has recently been found that K+ starvation inhibits water-stress-induced stomatal closure. In fact, the stomata of plants grown with a low level of K+ remained open wider than those of plants with an adequate K+ supply, and this favoured...
transpiration (Arquero et al., 2006; Benlloch-González et al., 2008). It is also known that K⁺ starvation favours the accumulation of ABA in the root (Peuke et al., 2002) and its long-distance xylem transport (Schraut et al., 2005). Despite the fact that ABA promotes stomatal closure and inhibits transpiration, K⁺ starvation favours transpiration. This suggests that in K⁺-starved plants there must be some factor that modulates the action of ABA on stomatal closure. There are some results that support this hypothesis. Fournier et al. (2005) have demonstrated, in sunflower plants, that the application of exogenous ABA inhibits water movement more markedly in K⁺-sufficient plants than in the K⁺-starved ones. On the other hand, studies with Arabidopsis plants have found that K⁺ starvation favours the synthesis of ethylene (Shin and Schachtman, 2004; Jung et al., 2009) and that ethylene inhibits the action of ABA on stomata, delaying their closure (Tanaka et al., 2005). A similar effect of ethylene on stomatal response has recently been observed in Leontodon hispidus (Wilkinson and Davies, 2009). However, the mechanism by which ethylene antagonizes ABA-induced stomatal closure is still not well understood (Tanaka et al., 2006). Moreover, Desikan et al. (2006) have shown that, in the absence of ABA, ethylene can induce stomatal closure in Arabidopsis leaves but, in the presence of ABA, it has the opposite effect: it inhibits ABA-induced stomatal closure.

It is becoming increasingly evident that stomatal movement is governed via a complex network of signalling components (Hetherington and Woodward, 2003; Fan et al., 2004; Acharya and Assmann, 2008). As a result, the action of K⁺ starvation on the stomatal opening mechanism may involve the interaction of different hormonal signals. Since the synthesis of ethylene increases when plants are K⁺-deficient (Shin and Schachtman, 2004; Jung et al., 2009), the aim of this work was to study whether ethylene is involved in the inhibiting effect of K⁺ starvation on stomatal closure. In order to reach this goal, two experimental approaches were followed: (i) to determine the effect of K⁺ starvation and water stress on ethylene production both in detached leaves as well as in the shoots of whole plants, and (ii) to test whether cobalt (Co) (an inhibitor of ethylene synthesis) modifies the effect of K⁺ starvation on stomatal conductance.

Materials and methods

Plant material and growth conditions

Sunflower seeds (Helianthus annuus L. cv. Sun-Gro 393, Eurosemillas SA, Córdoba, Spain) were surface-sterilized in 0.5% (v/v) sodium hypochlorite for 1 min, and germinated at 25 °C in Petri dishes with perlite moistened with 5 mM CaCl₂. On the second day, seedlings were transferred individually to 2.0 l plastic pots containing perlite and placed in a plant growth chamber (Conviron, Model no. PGR15) with a relative humidity between 60–80%, a day/night temperature of 25/22 °C, a photoperiod of 14 h of light, and a photosynthetic photon flux density of 450 µmol m⁻² s⁻¹ (fluorescent tubes, Sylvania cool-white VHO). In some experiments, the seeds were sown directly into pots filled with perlite and placed in a greenhouse with a relative humidity between 50-80%, a day/night temperature of 20/30 °C, and a photoperiod of 16 h of light (June–July). Plants were irrigated with a K⁺-free standard nutrient solution (modified Hoagland’s nutrient solutions; Hoagland and Arnon, 1950) with the following composition: 2.5 mM Ca(NO₃)₂, 0.25 mM Ca(H₂PO₄)₂, 1.0 mM MgSO₄, 12.5 mM H₃BO₃, 0.1 µM MnSO₄, 1.0 µM ZnSO₄, 0.25 µM CuSO₄, 0.2 µM (NH₄)₂MoO₄, and 10.0 µM Fe-ethylenediamine-di-o-hydroxy-phenylacetic acid. The experiment comprised two stages. In the first growing stage, and in order to obtain plants with different K⁺ status, the plants were irrigated periodically to the point of dripping with K⁺-free standard nutrient solution supplemented with differing concentrations of KCl, either 5 (Normal K) or 1 (Low K) mM KCl. Substrate water content was kept close to field capacity at all times. This stage lasted between 15 d and 19 d (Irrigation period) depending on the assay. Thereafter, watering of 50% of plants was stopped for 4–5 d (Drought period), and the plants underwent progressive water stress. In other experiments, all plants were subjected to two cycles of water stress of 3 d and 2 d each. Both cycles were separated by watering the plants with their corresponding irrigation solutions until dripping point. In these experiments, cobalt treatments were applied by adding CoSO₄ (5 µM) to the irrigation solution previously to each drought period.

Data collection

K⁺ was determined by atomic absorption spectrophotometry (Perkin Elmer 1100 B) after extraction from the leaves by ashing at 600 °C; ashes were dissolved with 1 N HCl.

Stomatal conductance was measured at different times of the water stress period (Drought) and also at the same time of day, i.e. 5 h after switching on the lights of the growth chamber. Measurements were made in fully-developed leaves from the third pair, using a porometer LI-1600; Li-Cor, Lincoln, Nebraska.

Ethylene production measurements

In detached leaves: Leaves were rolled and enclosed in 15 ml test tubes containing 200 µl of tap water. Tubes were sealed with rubber caps and incubated in the dark at 25 °C for 2 h. Gas samples were withdrawn from the incubation tubes with a 1 ml syringe and assayed with a Hewlett Packard gas chromatograph (Model 5890A) equipped with a 50 m alumina column with an internal diameter of 0.32 mm and a flame ionization detector. The temperature of the oven was 80 °C. N₂, H₂, and O₂ flow rates were 35, 30, and 300 ml min⁻¹, respectively. Ethylene identity was based on a retention time compared to a standard. Finally, leaves were taken from the tubes and their fresh weights recorded.

In the shoots of whole plants: Ethylene production was measured with a commercial laser-based ethylene detector (type ETD-300, Sensor Sense BV, Nijmegen, the Netherlands) in line with a flow-through sampling system (type VC-6, Sensor Sense BV, Nijmegen, the Netherlands), designed for measuring up to six sampling cuvettes per experiment (Cristescus et al., 2008; Salman et al., 2009). Briefly, radiation from the laser is absorbed by ethylene molecules and subsequently converted into an acoustic wave with amplitude that is proportional to the gas concentration.

Ethylene production was measured from whole plants in glass cuvettes as previously described. Whole plants were placed in glass cuvettes, hermetically closed and fitted with inlet and outlet ports, and flushed with compressed air as the carrier gas at a flow rate of 2.0 l h⁻¹. The compressed air was passed through a humidifier replaced with a CaCl₂ scrubber to reduce the RH to 2% before entering the cuvettes. The flow from each cuvette was directed into the PA cell, where a resonator detected the acoustic signal.

Experimental design

The experiments were set up in a completely randomized design. In all experiments, four plant replications were used for each
Results

The effect of K+ starvation and water-stress on ethylene production

Sunflower plants were watered regularly with nutrient solutions containing different concentrations of KCl (5/1 mM). In this way, two types of plant were obtained: plants with normal K+ status (Normal K) and plants starved in K+ (Low K) (Table 1). The water-stress treatment was applied at the end of the growth period by ceasing irrigation for the last four days (Drought plants). The accumulation of K+ in leaves was significantly greater in irrigated plants than in those subjected to water stress (Table 1).

After 3 d without irrigation, stomatal conductance was determined in all plants, in the leaves of the third pair. Water stress treatment significantly inhibited stomatal conductance in all plants, although this effect was more marked in Normal K plants (Table 2).

The production of ethylene was measured on the following day in the same leaf in which stomatal conductance had been measured. Ethylene production in detached leaves shows that K+-starved plants (Low K) produce more ethylene than Normal K plants and that water-stress does not significantly affect the production of ethylene in any type of plant (Fig. 1). In another group of experiments, the effect of K+ starvation on the continuous production of ethylene by the shoot of whole plants subjected to water stress was studied. The results showed that K+ starvation significantly promotes the production of ethylene by the shoots of whole plants (Fig. 2). These results support those obtained with detached leaves (Fig. 1).

Table 1. Effect of K+ concentration in the irrigation water (Normal versus Low K) and water availability in the growth medium (Irrigation versus Drought) on K+ accumulation in leaves (μmol g\(^{-1}\) FW)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>K+ leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal K: Irrigated</td>
<td>133.6±7.3</td>
</tr>
<tr>
<td>Normal K: Drought</td>
<td>119.4±3.8</td>
</tr>
<tr>
<td>Low K: Irrigation</td>
<td>36.3±1.4</td>
</tr>
<tr>
<td>Low K: Drought</td>
<td>25.7±0.8</td>
</tr>
</tbody>
</table>

Table 2. Effect of K+ concentration in the irrigation water (Normal K versus Low K) and water availability in the growth medium (Irrigation versus Drought) on stomatal conductance (mmol m\(^{-2}\) s\(^{-1}\))

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stomatal conductance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal K: Irrigated</td>
<td>456±5.6</td>
</tr>
<tr>
<td>Normal K: Drought</td>
<td>281±27.9</td>
</tr>
<tr>
<td>Low K: Irrigation</td>
<td>462±4.0</td>
</tr>
<tr>
<td>Low K: Drought</td>
<td>351±15.2</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of K+ concentration in the irrigation water (Normal K versus Low K) and water availability in the growth medium (Irrigation versus Drought) on ethylene production by detached leaves. Water stress treatment was applied on 19-d-old plants, by ceasing irrigation for 4 d (Drought). Ethylene production was determined in leaves of the third pair at the end of the water-stress period. Values are the mean of four replicates ± error standard.

Fig. 2. Effect of K+ concentration in the irrigation water (Normal K versus Low K) on ethylene production by the shoot of whole plants subjected to water stress (Drought). 15-d-old plants were subjected to a 5 d water stress period by ceasing irrigation. Ethylene production was measured after this water-stress period in the shoot of intact plants. Values are the mean of four replicates ± error standard. Significant differences were found at \( P < 0.05 \) by Tukey’s test.
The effect of cobalt on stomatal conductance

In this set of experiments, the effect of cobalt treatment and plant K+ nutritional status on stomatal conductance was studied. At the end of the growing period, the plants were subjected to two correlating periods of water stress. The cobalt treatment was applied by adding CoSO4 (5 μM) to the nutrient solution in the watering prior to each cycle of water stress. The treatments with cobalt did not negatively affect the plant’s growth (Table 3), nor the K+ content, which in the leaves of Normal K and Low K plants were around 140 and 40 μmol g⁻¹ FW, respectively.

Stomatal conductance was measured after 24 h and 48 h of the second period of water stress. After 24 h, neither the K+ nutritional status nor the treatment with Co had any effect on the stomatal conductance, with values close to 350 mmol m⁻² s⁻¹ in all cases (data not shown). However, after 48 h of the second period of water stress, an interaction on stomatal conductance between K+ concentration in the irrigation solution and water availability in the growth medium was found. Stomatal conductance was significantly greater in control plants grown in a low K+ medium (Low K) than in those grown with an adequate supply of K+ (Normal K). Differences in stomatal conductance were not observed between control and cobalt-treated plants when plants were grown in a K+-rich medium (Normal K). However, in Low K plants, the cobalt treatment inhibited stomatal conductance to similar values as those of plants with an adequate K+ status (Fig. 3). These results show that cobalt partially counteracts the effect of K+ starvation on stomatal opening. In other words, the inhibiting effect of K+ starvation on the water-stress stomatal closure partially disappears after treatment with cobalt.

Discussion

The results obtained in this work suggest that ethylene may be involved in the inhibiting effect of K+ starvation on water-stress-induced stomatal closure. It is widely documented that, under water-stress situations, plants synthesize ABA and that ABA prompts stomata closure (Wilkinson and Davies, 2002; Mori and Schroeder, 2004). It is also well known that ABA promotes hydraulic conductivity in the root (Glinka, 1980; Quintero et al., 1999) and in the shoot via aquaporin gating (Parent et al., 2009). It has been suggested that this opposite action of ABA on different parts of the plant, the root and the stomata, contributes to avoiding water stress in plants (Fournier et al., 2005). This effect of ABA has been observed in plants which have an adequate K+ nutrition, but the situation may be different in K+ -starved plants. It is known that K+ starvation inhibits water-stress-induced stomatal closure (Arquero et al., 2006; Benlloch-González et al., 2008), and that the effect of ABA on the control of water flow through the plant is less effective when plant K+ status is low (Fournier et al., 2005). This suggests that K+ starvation produces some unknown factor that inhibits the action of ABA in stomata closure.

One hypothesis might be that K+ starvation inhibits the synthesis of ABA or, at least, its transport and accumulation in the shoot, and, as a result, this suppresses the response of the stomata to water stress. According to available information, this hypothesis is not correct, since the deficiency of K+ promotes the synthesis and accumulation of ABA in the root (Peuke et al., 2002), and, in some cases, its transport to the shoot (Schraut et al., 2005). According to this information, the hypothesis that K+ starvation produces a certain factor that interacts negatively with ABA in stomatal closure remains a valid one, and this factor could be ethylene. Ethylene is a hormone present in many situations of stress (Abeles et al., 1992), and it has been found that K+ starvation favours its synthesis (Shin

![Fig. 3. Effect of K+ concentration in the irrigation water (Normal K versus Low K) and cobalt treatment (Cobalt) on stomatal conductance in plants subjected to water-stress. 19-d-old plants were subjected to two correlating periods of water-stress of 3 d or 2 d each by ceasing irrigation. Between each period plants were watered until dripping point. The cobalt treatment was applied by adding CoSO4 (5 μM) to the irrigation solution in the watering prior to each cycle of water stress. Stomatal conductance was measured in the leaves of the third pair at the end of the second period of water stress. Values are the mean of four replicates ± standard error. Interaction between K+ concentration in the irrigation water and cobalt treatment was significant at \( P < 0.05 \).]

Table 3. Effect of K+ concentration in the irrigation water (Normal K versus Low K) and cobalt treatment (Control versus 5 μM Co) on the fresh weight (g) of different organs of plants subjected to water stress

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaves (g)</th>
<th>Shoot (g)</th>
<th>Root (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal K; Control</td>
<td>10.4±0.5</td>
<td>10.4±0.5</td>
<td>28.8±1.2</td>
</tr>
<tr>
<td>Normal K; Cobalt</td>
<td>10.0±0.5</td>
<td>10.6±0.4</td>
<td>34.2±1.5</td>
</tr>
<tr>
<td>Low K; Control</td>
<td>8.7±0.5</td>
<td>6.5±0.4</td>
<td>23.3±3.0</td>
</tr>
<tr>
<td>Low K; Cobalt</td>
<td>9.3±0.3</td>
<td>7.5±0.6</td>
<td>22.7±0.9</td>
</tr>
</tbody>
</table>

For all treatments, values are means of four replicates ± standard error.
K⁺ starvation inhibits stomatal closure via ethylene synthesis

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