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Effect of water stress and subsequent re-watering on K^+ and water flows in sunflower roots. A possible mechanism to tolerate water stress

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ABSTRACT

Little is known about the physiological mechanisms underlying plant responses and their ability to recover from water stress, especially responses that concern the root system. The effect of water stress on K^+ and water flows in excised roots as well as the relationship of these flows with water stress tolerance was studied in six sunflower (*Helianthus annuus* L.) cultivars. Plants growing in a growth chamber were subjected to water stress by deficit irrigation for 15 days. After this period, the plants were re-irrigated and the exudates from the excised roots were collected. Water stress reduced the shoot growth of all plants, although differences were observed among the cultivars. While water stress stimulated the water flow in the excised roots of all cultivars, K^+ (Rb^+) uptake by the root and its discharge into the xylem was promoted only in those cultivars that were more susceptible to water stress. The same effect was observed when plants were subjected to water stress using polyethylene glycol (PEG) 6000. The promotion of both K^+ and water flows could be considered as a mechanism to tolerate water stress, through which the plant restores cell turgidity, shoot water status and plant growth after a water stress period.

1. Introduction

One of the most important environmental constraints to plant survival in arid climates and dry-land agricultural ecosystems is water stress. The effect of water stress on growth, photosynthesis and other physiological processes has been extensively studied, while little is known about the physiological mechanisms underlying plant responses and their ability to recover from water stress, especially the responses that concern the root system (Blackman et al., 2009; Chaves and Davies, 2010; Lechner et al., 2008; Xu et al., 2009).

Osmotic adjustment through the accumulation of sugars (Wang et al., 1995), proline (Sarker et al., 2005), organic acids (Handa et al., 1983) and ions such as sodium and potassium (Turner, 1979) is a common metabolic response of higher plants to maintain water balance and avoid water stress. The role of K^+ in the regulation of plant water status is well known. Potassium is the main solute involved in cell osmotic water absorption (Hsiao and Läuchli, 1986), the maintenance of cell turgor pressure and cell elongation, and the regulation of stomatal opening (Fischer and Hsiao, 1968). It is widely accepted that K^+ deficiency increases the plant's susceptibility to water stress and that plants with adequate internal K^+ levels have better hydrated tissues than those with K^+ deficiency (Mengel and Kirkby, 2001). In fact, the symptoms of dehydration that are often observed in plants grown in arid climates and dry-land agricultural ecosystems have been associated with low potassium contents in these plants (Fernández-Escobar et al., 1994). In this context, it has been observed that potassium fertilization improves the water relations of many crops (Premachandra et al., 1993; Sangakkara et al., 2000).

Another strategy to maintain plant water balance under water-limiting conditions is the control of stomatal movement. Plants reduce stomatal conductance in response to water stress (Siemens and Zwiazek, 2004; Wilkinson et al., 1998) and abscisic acid (ABA) (Cummins et al., 1971; Hartung et al., 1999). 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). Therefore, under K^+ starvation conditions, it is logical to believe that it is difficult for the stomata to remain open. However, it has been observed that K^+ starvation inhibits water stress-induced stomatal closure, leading to plant dehydration (Benlloch-González et al., 2008). This effect has been related to the synthesis of ethylene induced by K^+ starvation (Benlloch-González

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et al., 2010). In combination with these factors, the pH of the xylem sap also appears to be involved in stomatal closure. Drought favours the alkalization of the xylem sap and this generally enhances ABA effectiveness in stomatal closure (Wilkinson and Davies, 1997; Sauter et al., 2001). Conversely, it has been observed that K^+ starvation lowers the pH of the xylem sap (Fournier et al., 2005) and the apoplast of the substomatal cavity (Felle and Hanstein, 2002). This acidification could impair ABA action on stomatal closure.

The water transport capacity of the root may also be crucial to overcome water-limiting conditions. However, many processes that affect root water uptake during and after a water stress period remain to be clarified. It is well known that water deficit increases the concentration of ABA in the root cells (Davies and Zhang, 1991) and that ABA modifies root hydraulic properties. There are numerous references of the effect of exogenous ABA added to the root growth medium on root hydraulic conductivity, although contradictory results have been observed. Most authors report a positive effect (Freundl et al., 1998; Hose et al., 2000; Lee et al., 2004; Quintero et al., 1999; Sauter et al., 2002; Schraut et al., 2005), whereas others have noted a negative effect (Davies et al., 1982; Fiscus, 1981). Root hydraulic conductivity has been shown to be regulated by the functioning of water channels (aquaporins), especially plasma membrane intrinsic proteins (PIPs) (Javot et al., 2003; Postaire et al., 2010). There have been some attempts to link the activity of plant aquaporins with drought tolerance, but there is not enough evidence to show that over-expression of the genes that encode aquaporins improves drought resistance (Aharon et al., 2003; Jang et al., 2007; Katsuhara et al., 2003). Some authors have suggested that the effect of water stress on the expression of aquaporins is dependent on the duration and intensity of the stress (Galmes et al., 2007) as well as the degree of adaptation of the different crop varieties to water deficit (Lian et al., 2004).

Despite the fact that K^+ plays an important role in plant water relations, very little research has been carried out to elucidate how the transport of K^+ in the root is affected by water stress and the consequent implication for plant re-hydration. It has been reported that Shaker K^+ channel genes are involved in K^+ release into the xylem sap and that ABA induces a marked decrease in SKOR transcript levels (Gaymard et al., 1998; Pilot et al., 2003). However, many authors have mentioned the role of ABA in promoting K^+ xylem transport (Fournier et al., 1987; Glinka, 1980; Glinka and Abir, 1983; Karmoker and Van Steveninck, 1978; Quintero et al., 1998). This suggests that water stress may prompt K^+ transport into the xylem and its accumulation in the shoot (Gholami and Rahemi, 2010). Because potassium is the main solute involved in the maintenance of cell turgor pressure and cell elongation, plants may promote the transport of K^+ from the root to the shoot as a mechanism to rapidly restore the plant water status and growth after periods of water stress. To test this hypothesis, we studied K^+ and water flows in the excised roots of six sunflower cultivars immediately after a water stress period, as well as the relationship between these flows and water stress tolerance. These cultivars are widely grown in dry-land agricultural ecosystems in the south of Spain, but there is limited information on their drought tolerance. The results obtained in this study will provide further information on how plants respond to and recover from water stress, and how root K^+ transport is related to drought tolerance.

2. Materials and methods

2.1. Plant material and growth conditions

2.1.1. Hydroponic system with perlite substrate

Six sunflower (*Helianthus annuus* L.) cultivars were used: Sanbro, Jazzy, Supersun, Alhaja, Olimpia and Sungro. Seeds were surface-sterilized in 0.5% (v/v) sodium hypochlorite for 1 min and germinated in the dark at 25 °C in Petri dishes with perlite moistened with 5 mM $CaCl_2$. On the second day, the seedlings were transferred individually to 2-L plastic pots containing perlite and placed in a plant growth chamber with a relative humidity between 60 and 80%, a day–night temperature of 25/22 °C, a photoperiod of 14 h of light and a photosynthetic photon flux density of 350 μmol m^{-2} s^{-1} (fluorescent tubes, Sylvania cool-white VHO). The plants were irrigated with a standard nutrient solution (SNS) with the following composition: 2.5 mM $Ca(NO_3)_2$; 2.5 mM KCl; 0.25 mM $Ca(H_2PO_4)_2$; 1.0 mM $MgSO_4$; 12.5 μM H_3BO_3; 1.0 μM $MnSO_4$; 1.0 μM $ZnSO_4$; 0.25 μM $CuSO_4$; 0.2 μM $(NH_4)_6Mo_7O_{24}$ and 10 μM Fe-ethylenediamine-di-*o*-hydroxy-phenylacetic acid.

During the growing period (14 days), all plants were periodically irrigated with the SNS to the point of dripping. In this way, the substrate water content was maintained at field capacity. After this period, half of the plants (4 per cultivar) were subjected to water stress by applying deficit irrigation. During the water stress period (15 days), control plants were irrigated to the point of dripping while those subjected to water stress received 70% of the dose used to water the control plants. The water dose used to irrigate the control plants was determined by measuring the difference between the water added to the pot and the drainage of excess water. This procedure was repeated daily for each cultivar.

The leaf relative water content (RWC) of fully expanded leaves was determined before the exudate collections were initiated. Fresh leaf samples were first weighed, then rehydrated over 24 h, and re-weighed according to the procedure reported by Stocker (1929).

2.1.2. Liquid hydroponic system

Seeds of the cultivars Olimpia and Jazzy were surface-sterilized in 0.5% (v/v) sodium hypochlorite for 1 min and germinated in the dark for 4 days at 25 °C in perlite moistened with 5 mM $CaCl_2$. On the fourth day, the seedlings were placed in a plant growth chamber under similar conditions to those described in Section 2.1.1. The next day, the 5-day-old seedlings were transferred individually into 720-mL glass flasks wrapped in aluminium foil that contained the SNS (see composition in Section 2.1.1). The plants were grown under these conditions for 10 days (growing period). Thereafter, the non-penetrating osmolyte polyethylene glycol (PEG 6000) was added to a freshly prepared SNS at a water potential of −0.2 MPa (53.6 g/L) (PEG plant) except for the control plants for which the SNS was renewed. In this way, the plants were subjected to water stress for 3 days. After this water stress period, each group of plants (control and PEG plants) was divided into two groups: one group for the collection of root exudates and the other to determine the relative growth rate after the removal of water stress for 24 h (recovery period). In the latter group, the nutrient solution of the water-stressed plants (PEG plants) was replaced with an identical one but without PEG, and the control plants received a renewed SNS. During this experiment, the nutrient solution was continuously aerated using an air pump and was renewed on day 7 and the day before the assay. The volume was adjusted daily to 720 mL.

In both experiments (perlite substrate and liquid system), Ca $(OH)_2$ was used to adjust the pH of the nutrient solution to 5.5.

2.2. Exudate collection

The period for collecting the exudates began 30 min after switching on the lights of the growth chamber. At the end of the water stress period, plants were de-topped 1 cm above the transition zone, and pieces of tightly fitting latex tubing were affixed to the cut stumps (Quintero et al., 1999). In the experiment in which the plants were grown in the perlite substrate, plenty of