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Full Length Article

Effect of Potassium Carbonate on Water Permeability of Peach (*Prunus laurocerasus*) Cuticles at Different Air Humidity

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ABSTRACT

Cuticles of peach (*Prunus laurocerasus* L.) were enzymiticaly isolated and the effect of K_2CO_3 on water permeability of adaxial surfaces of these cuticles at different relative air humidity was tested by using tritiated water. The results showed that water permeability of isolated cuticles of peach increased when the cuticle was exposed to different levels of relative air humidity and their surfaces were treated with 0.2 mol.m-2 of K_2CO_3. The effect of K_2CO_3 was well evident, when relative humidity was increased from 2% to 60% and removing salt debries from the cuticle lowered the effect.

Key Words: Cuticle; Water permeability; Salt effect; Salt penetration

INTRODUCTION

The cuticle covers all primary above-ground parts of the plants, such as leaves and fruits but not woody stems and wounds. The plant cuticle is a hydrophobic, continuous, flexible and thin 0, 1 to 10 µm layer consisting of two lipid fractions; the polymer matrix and cuticular waxes, which are deposited on the outer surface and embedded in the matrix (Luque *et al.,* 1995; Vogg *et al.,* 2004). The plant cuticle forms the interface between the aerial environment and the living cells of the plant. Therefore, the cuticle has to perform multiple physiological and ecological functions. It acts as an effective barrier to the transport of solutes and gases in and out of the leaf (White *et al.,* 2002). Water permeability of cuticles increases with increasing air humidity (Schönherr & Schmidt, 1979; Schönherr & Mérida, 1981; Schreiber *et al.*, 2001). Permeation of some kind of cations in cuticular layer increases water permeability. With increasing humidity, penetration rates increase, due to dissolution of salt residues on the surface of the cuticle (Schönherr, 2000 & 01). In this study, we tested, to which extant, the relative humidity can increase the effect of K_2CO_3 on water permeability of isolated cuticles of peach (*Prunus laurocerasus* L.).

MATERIALS AND METHODS

Plant material. Fully expanded healthy leaves of peach (*Prunus laurocerasus* L.) were sampled from mature plants grown in the Botanical Garden of Würzburg University. The leaves were visually investigated to exclude any damages or infections by microorganisms.

Isolation of cuticles. The isolation of cuticles was carried out according to the method described by Schönherr and Riederer (1986). Disks of 20 mm diameter were punched out from the leaves and incubated in an aqueous solution containing 2% (v/v) cellulase (Celluclast, Novo Nordisk, Bagsvared, Denmark) and 2% pectinase (Trenolin, Erbslöh, Geisenheim, Germany) in 0.01 M citric buffer (Merk, Germany; pH 3.0 adjusted with KOH). In order to prevent microbial growth, 1 mL of 1 M Sodium azide (NaN3, Fluka, Neu-Ulm, Germany) was added to 1 L of the enzyme solution. Cuticles from the adaxial leaf sides were separated from the cellular debris and incubated in 0.01 M borax buffer (Fluka, Germany) adjusted to pH 9 for about one week. Subsequently, the cuticles were incubated again for about 10 days in deionized water. The cuticles were removed from the solution and dried under a stream of pressurised air that helped to flatten them. They were stored in Petri dishes at room temperature until they were used.

The effect of K_2CO_3 on water permeability of peach isolated cuticles at different air humidity. Cuticular transpiration of peach cuticle was measured at different relative humidity as described by Schreiber *et al*. (2001). After adding 900 µL of donor solution mixed with traces of ^3H_2O (Hartmann Analytika, Braunschweig, Germany, specific activity: 925 MBq g^{-1}) into stainless steel transpiration chambers, the cuticle was mounted on the transpiration chambers with their morphological outer surface facing outwards. Subsequently, covered lids were carefully fixed to the chambers using vacuum grease. Finally, chambers were turned upside down and the grease-covered outer surfaces of the lids were placed on the top of scintillation vials containing dry silica gel.

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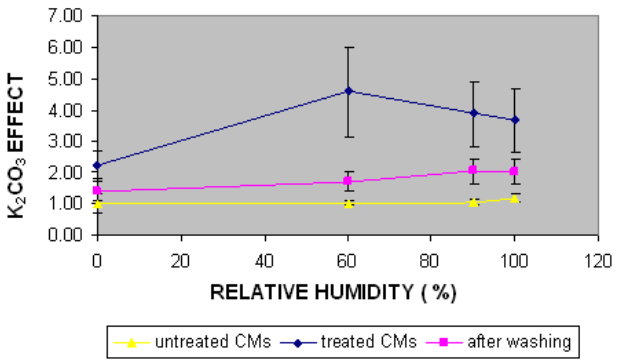
Table I. The water permeability (m s^{-1}) of *Prunus laurocerasus* L. isolated cuticles treated with 0.2 mol.m^{-2} K_2CO_3 at different relative air humidity (RH), Water permaebility are means of 13 cuticles with 95% confidence intervals (ci)

RH (%)	Untreated cuticles P (m·s^{-1}) ± ci	Treated cuticles P (m·s^{-1}) ± ci	after washing P (m·s^{-1}) ± ci
2	$3.34 \cdot 10^{-10} \pm 1.0 \cdot 10^{-10}$	$6.83 \cdot 10^{-10} \pm 1.80 \cdot 10^{-10}$	$4.43 \cdot 10^{-10} \pm 1.39 \cdot 10^{-10}$
60	$3.38 \cdot 10^{-10} \pm 1.08 \cdot 10^{-10}$	$1.31 \cdot 10^{-09} \pm 3.30 \cdot 10^{-10}$	$5.41 \cdot 10^{-10} \pm 1.63 \cdot 10^{-10}$
90	$3.52 \cdot 10^{-10} \pm 1.29 \cdot 10^{-10}$	$1.16 \cdot 10^{-09} \pm 2.83 \cdot 10^{-10}$	$6.65 \cdot 10^{-10} \pm 1.93 \cdot 10^{-10}$
100	$4.01 \cdot 10^{-10} \pm 1.45 \cdot 10^{-10}$	$1.26 \cdot 10^{-09} \pm 3.17 \cdot 10^{-10}$	$7.66 \cdot 10^{-10} \pm 2.45 \cdot 10^{-10}$

Table II. Effect of 0.2 mol.m^{-2} K_2CO_3 on water permeability of *Prunus laurocerasus* L. isolated cuticles at different relative air humidity (RH). The mean values are given with 95% confidence intervals (CI). The highest effect was observed at 60% RH, while there was no significant difference of mean values between the effect at 90% and 100% RH respectively

RH (%)	Untreated cuticles effect ± ci	Treated cuticles effect ± ci	After washing effect ± ci
2	1.00 ± 0.30	2.24 ± 0.45	1.39 ± 0.30
60	1.02 ± 0.06	4.57 ± 1.40	1.69 ± 0.31
90	1.04 ± 0.09	3.87 ± 1.02	2.05 ± 0.42
100	1.20 ± 0.13	3.67 ± 1.01	2.03 ± 0.43

Fig. 1. The relative effect on water permeability of *Prunus laurocerasus* L. before and after treatment with 0.2 mol.m^{-2} K_2CO_3 at different air humidity, Results are means of 13 cuticles with 95% confidence intervals



The chambers prepared in this way were incubated at 25±0.5°C for equilibration. Further polyethylene scintillation vials (Canberra Packard, Dreieich, Germany) were prepared containing either 100 µL of glycerol, glycerol-water mixtures or pure water. Thus, different air humidity (RH) were adjusted: pure glycerol = 2% RH, 60 µL glycerol and 40 µL water = 60% RH, 30 µL glycerol and 70 µL water = 90% RH, pure water = 100% RH. At the same time these reservoirs at the bottom of the scintillation vials served as the receiver for the radioactive water. Before the experiment was started the atmosphere in the vials was equilibrated overnight at 25±0.5°C. During the experiment, the transpiration chambers were removed carefully from the scintillation vials containing silica gel and put on top of the scintillation vials containing glycerol (2% relative humidity) and they were incubated again. After defined time intervals (30, 60 & 90 min) scintillations these vials with the same RH were replaced by new ones for 3 times. Then transpiration chambers were put on scintillation vials having a higher humidity. This was repeated with all 4 humidity between 2% and 100% RH. The amount of ^3H_2O, which had diffused across the cuticle into the vials was counted using a scintillation counter (model 1600 CA, Canberra Packard, Dreieich, Germany) after adding scintillation cocktail (Permafluor, Canberra Packard). Amounts of radioactive water, which had diffused across the cuticles at each air humidity was plotted against time, which gave good linear transpiration kinetics (r^2 was better than 0.99 in all cases). The water permeability was determined using equation

$$P = F/(A \cdot \Delta c)$$

Where the flow rate F given as dpm·s^{-1}, the donor activity Δc given as dpm·m^{-3} and the area of the cuticle given as 1.13 cm-2. After measuring the transpiration of at all 4 different air humidity, the cuticle was treated with 0.2 mol.m^{-2} K_2CO_3. Chambers were left at room temperature in the fume hood until the water was evaporated. Then they were stored again on scintillation polyethylene vials containing silica gel at 25±0.5°C for equilibration. Finally, a new set of scintillation vials with the same 4 different relative air humidity was used to determine water permeability after K_2CO_3 treatment. The effect of the salt on water permeability was calculated for each single cuticle by dividing the permeation after treatment by that measured before the treatment. To test if the salt effect is reversible or whether there was irreversible damage of the cuticles, the salt residues were washed off from the cuticles again and the experiment was continued in order to determine the permeation after washing off the salt.

Sample size and statistical analysis. Regression equations were fit to transpiration kinetics and means of water permeability of 10 to 20 cuticles were calculated. Results are given as means with 95% confidence intervals (ci).

RESULTS

Varying air humidity between 2 and 100%, cuticular water permeability of *P. laurocerasus* slightly increased by a factor of 1.2 (Table I). When the cuticles were treated with 0.2 mol.m^{-2} K_2CO_3, water permeability of *P. laurocerasus* increased and decreased again after washing with deionized water. The effect of K_2CO_3 was highest, when relative humidity was increased from 2% to 60% (Table II; Fig. 1). Increasing relative humidity up to 90% and 100% did not further increased the water permeability (Table I). Washing the cuticles again decreased the cuticular water permeability (Fig. 1).

DISCUSSION

From these findings it is evident that polar inorganic

ions are sorbed to the lipophilic cuticles. As a consequence polarity of the cuticle is increased and increasing amounts of water, which sorbed to the cuticle. This leads to a swelling of the cuticle and finally to an increased cuticular transpiration. In order to be able to sorb to the cutin polymer, the salts deposited to the cuticle surface have to be in a liquid state (Schönherr, 2001; Schlegel & Schönherr, 2002). If they dry out they will crystallize and this renders them completely immobile. Hydration and dissolution of salts is determined by their point of deliquescence "POD" (Schönherr, 2001). This point refers to the humidity over a salt solution containing solid salts. When the humidity is above the POD, the salt residue on the cuticle sorbs water from the atmosphere (Schlegel & Schönherr, 2002), dissolves and the ions of the salt are mobile and can diffuse into the cutin polymer, while below the POD this process stops. In another expirement, the effect of KCl and KNO_3 on cuticular water permeability of peach was lower than that of K_2CO_3 (data not shown). These differences can partially be explained by the POD of these salts, which was found to be 44% for K_2CO_3, while 86% for KCl and 95% for KNO_3. This means that K_2CO_3 is in a liquid state at much lower humidity than KCl and KNO_3 and thus it is more efficient in changing cuticular transport properties for water. This corresponds to reports that rate constants of potassium salt penetration through plant cuticles were increased by increasing humidity (Schönherr & Luber, 2001).

In order to analyse to what extent increasing humidity could interact with the salt effects, cuticular water permeability was measured before and after K_2CO_3 deposition to the outer surface of the cuticle of peach at increasing humidity (Table I). It is evident that much higher water permeability was measurable already at much lower humidity with salt treated compared to untreated cuticles (Table I). This observation must be explained by the fact that the presence of water is pivotal for the salts to become mobile and effective. If there is more water available due to high humidity, compared to the small amounts of salts diffusing through the cuticle from the inside, the effects of the salts on cuticular permeability will be more pronounced (Fig. 1).

In crux, in addition to the observation that cuticular permeability is increased by increasing humidity, this effect can significantly be enhanced by adding salts to the leaf surface. These results provide good evidence on polar domains in the cuticle where water molecules can absorb and induce swelling, thus leading to an increased cuticular transpiration. This effect is enhanced in the presence of salts.

REFFRENCES

Luque, P., S. Bruque and A. Heredia, 1995. Water permeability of isolated cuticular membranes: a structural analysis. *Arch. Biochem. Biophys*., 317: 412–422

Schlegel, T. and J. Schönherr, 2002. Stage of development affects penetration of calcium chloride into apple fruits. *J. Plant Nutr. Soil Sci.,* 165: 738–745

Schönherr, J., 2000. Calcium chloride penetrates cuticles via aqueous pores. *Planta*, 212: 112–118

Schönherr, J., 2001. Cuticular penetration of calcium salts: effects of humidity, anions, and adjuvants. *J. Plant Nutr. Soil Sci.*, 164: 225–231

Schönherr, J. and H. Schmidt, 1979. Water permeability of plant cuticles: dependence of permeability coefficients of cuticular transpiration on vapour pressure saturation deficit. *Planta*, 144: 391–400

Schönherr, J. and K. Lendzian, 1981. A simple and inexpensive method of measuring water permeability of isolated plant cuticular membranes. *J. Plant Physiol.*, 102: 321–327

Schönherr, J. and M. Luber, 2001. Cuticular penetration of potassium salts: effects of humidity, anions and temperature. *Plant Soil*, 236: 117–122

Schönherr, J. and T. Mérida, 1981. Water permeability of plant cuticular membranes: the effects of humidity and temperature on the permeability of non-isolated cuticles of onion bulb scales. *Plant Cell Environ.,* 4: 349–354

Schönherr, J. and M. Riederer, 1986. Plant cuticles sorb lipophilic compounds during enzymatic isolation. *Plant Cell Environ.*, 9: 459–466

Schreiber, L., M. Skrabs, K. Hartmann, P. Diamantopoulos, E. Simanova and J. Santrucek, 2001. Effect of humidity on cuticular water permeability of isolated cuticular membranes and leaf disks. *Planta*, 214: 274–282

Vogg, G., S. Fisher, J. Leide, E. Emmanuel, R. Jetter, A. Levy and M. Riederer, 2004. Tomato fruit cuticular waxes and their effects on transpiration barrier properties: functional characterization of a mutant deficient in a very-long-chain fatty acid ß-ketoacyl- CoA synthase. *J. Exp. Bot.,* 55: 1401–1410

White, A., C. Heaverlo and M. Owen, 2002. Evaluation of methods to quantify herbicide penetration in leaves. *J. Weed Technol.,* 16: 37–42

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