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Uptake of sodium in quince, sugar beet, and wheat protoplasts determined by the fluorescent sodium-binding dye benzofuran isophthalate

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Summary

The uptake of sodium into protoplasts of quince (*Cydonia oblonga* Mill, clone BA29), sugar beet (*Beta vulgaris* L. cv. Monohill), and wheat (*Triticum aestivum* L. cv. Kadett) was determined by use of the acetoxy methyl ester of the fluorescent sodium-binding benzofuran isopthalate (SBFI-AM). In the presence of 1 mM $CaCl_2$, little sodium was taken up in the cytosol of quince mesophyll cells compared to cytosols of sugar beet and wheat. Upon addition of 40 mM NaCl, approximately the same amount of sodium was taken up in leaf and root protoplasts of wheat, but no sodium was taken up in quince. However, in calcium-free medium, obtained by addition of ethylene glycol tetra acetic acid (EGTA), quince protoplasts transiently took up sodium in the cytosol when 200–400 mM NaCl was added to the protoplast medium. Moreover, after cultivation of quince in the presence of 200 mM sodium for 4 weeks, the cytosol of isolated protoplasts did not take up any sodium at all from a calcium-free medium. The results show that protoplasts from salt tolerant quince only temporarily take up sodium in the cytosol and that they have a mechanism for fast extrusion of sodium from that compartment. These mechanisms are probably important for the high salt tolerance of quince. Calcium blocks the sodium uptake into the cytosol of both quince and wheat protoplasts.

Abbreviations: SBFI-AM, acetoxy methyl ester of sodium-binding benzofuran isopthalate; EGTA, ethylene glycol tetra acetic acid

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Introduction

Excess of sodium chloride in the soil is the principal cause of salinity and, because about 10–25% of arable lands are saline, it is one of the most important problems in agriculture (Postel, 1989). It has long been known that calcium, by competition with sodium, can attenuate the toxic effects of Na^+ salinity on plants (LaHaye and Epstein, 1971; Cramer et al., 1985; Ben-Hayyim et al., 1987; Allen et al., 1995). At the cellular level, calcium may reduce the flux of sodium that enters the cytosol (Cramer et al., 1987; Whittington and Smith, 1992; Maathuis and Amtmann, 1999) and sodium also can move calcium from cellular membranes (Cramer et al., 1985; Lynch et al., 1987) and reduce the influx of Na^+ through the low-affinity cation channel (Amtmann et al., 2001). The isolation and use of the *Arabidopsis* sos3 mutant provided further evidence supporting the role of Ca^{2+} in plant salt tolerance (Liu and Zhu, 1998, Qiu et al., 2002).

The sodium-binding benzofuran isophthalate (SBFI), introduced by Minta and Tsien (1989), was used to determine changes in intracellular sodium (Na^+). This probe has benzofuran fluorophores similar to those of Fura-2 and PBFI and, therefore, is compatible with filters used for these dyes. The specific potassium-binding dye PBFI is most similar to SBFI and was used earlier to determine concentrations of potassium in protoplasts of barley and wheat (Lindberg, 1995; Lindberg and Strid, 1997). At present, there are many investigations where SBFI is used to study Na^+ metabolism in animal and human cells, such as fibroblasts (Harootunian et al., 1989), retinal cells (Agostinho et al., 1996) and neuroblastoma cells (Kopper and Adorante. 2002). Recently, SBFI was used to detect sodium accumulation in root hairs of *Arabidopsis* (Halperin and Lynch. 2003). The main purpose of the present study was to investigate whether the acetoxy methyl ester of SBFI could also be used as a tool for detection of sodium uptake in the cytosol of plant protoplasts.

Another purpose was to compare the uptake of sodium in protoplasts of the salt-tolerant quince with the uptake in protoplasts of less tolerant wheat and sugar beet. Roots of quince are used as rootstocks for cultivation of fruit trees, especially pear trees on salty soils, but little is known about the mechanism for salt tolerance in this plant. We determined the uptake of sodium into protoplasts of quince and wheat in the absence and presence of free calcium concentrations. Such information may explain the different levels of salinity resistance in these species.

Materials and methods

Cultivation

Shoots of Quince BA29 (*Cydonia oblonga* Mill) were propagated in vitro on DKW salt medium (Driver and Kuniyuki, 1984) supplemented with 1 mg l^{-1} thiamine hydrochloride, 100 mg l^{-1} myo-inositol, 1.5 mg l^{-1} BA (6-benzylaminopurine), 1.5 mg l^{-1} BAr (6-benzylaminopurine riboside), 30 g l^{-1} sucrose, and with and without 200 mM NaCl. The cultivations were repeated four times with or without 200 mM sodium. The medium was adjusted to pH 5.2 with KOH before addition of 4 g l^{-1} of agar and 5 g l^{-1} of pectin. The medium was sterilized by autoclavation at 120 °C for 20 min.

Seeds of sugar beet (*Beta maritima* L. cv. Monohill) from Hammenhög, Sweden were developed in moist vermiculite for 1 week. The seedlings were then transferred to plastic holders in 1-L beakers containing a complete mineral nutrition as described by Lindberg (1990).

Seeds of wheat (*Triticum aestivum* L. cv. Kadett) from Svalöf Weibull AB, Sweden were treated and cultivated in a complete nutrient solution as described by Shishova and Lindberg (1999).

Cultures of quince were maintained at 22 ± 1 °C under white light (60 μmol $m^{-2} s^{-1}$) with a 16-h photoperiod and were transferred to fresh medium every third week. Sugar beet and wheat seedlings were cultivated at 20 ± 1 °C in the light and at 17 ± 1 °C during darkness in a growth chamber at the same photoperiod and light condition as for quince. The relative humidity was 50–60% during the cultivation of sugar beet and wheat.

Protoplast isolation

All protoplasts from leaves were isolated from mesophyll cells. The protoplasts from quince were prepared as described by D'Onofrio et al. (1999). The young leaves of the first three shoot apical nodes were harvested, sliced transversely into pieces smaller than 1 mm, and treated with 50 μg ml^{-1} cellulase from *Trichoderma resei* (Sigma, EC 3.2.1.) and 10 μg ml^{-1} pectinase from *Rhizopus* sp. (Sigma, EC 3.2.1.) in a CPW salt medium (Frearson et al., 1973) containing 0.45 M sucrose, 0.05% (w/v) polyvinyl pyrrolidone (PVP; Sigma), 0.05% bovine serum albumin (BSA; Sigma), 1 mM $CaCl_2$, and 5 mM Tris[hydroxymethyl] amino methane hydrochloride and Mes, 2-[*N*-morpholino] ethane sulfonic acid (Tris-Mes), pH 5.7 for 21 h at 27 °C in darkness. At the end of the incubation the suspension was filtered through a nylon net with