Plant Physiology and Biochemistry 49 (2011) 341–345

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

In vitro effect of different Na^+/K^+ ratios on plasma membrane H^+-ATPase activity in maize and sugar beet shoot

Abdul Wakeel [a,b,*], Ali Sümer [c], Stefan Hanstein [a], Feng Yan [d], Sven Schubert [a]

[a] Institute of Plant Nutrition, Justus Liebig University, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany
[b] Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad, Pakistan
[c] Department of Soil Science, Faculty of Agriculture, Canakkale Onsekiz Mart University, Canakkale, Turkey
[d] Institute of Crop Science and Plant Breeding I, Justus Liebig University, Ludwigstrasse 23, 35390, Giessen, Germany

ARTICLE INFO

Article history:
Received 19 August 2010
Accepted 5 November 2011
Available online 13 January 2011

Keywords:
Beta vulgaris
Na^+ toxicity
Proton pump
Salt stress
Zea mays

ABSTRACT

Plant growth is impaired primarily by osmotic stress in the first phase of salt stress, whereas Na^+ toxicity affects the plant growth mainly in the second phase. Salinity leads to increased Na^+/K^+ ratio and thus displacement of K^+ by Na^+ in the plant cell. Relatively higher cytosolic Na^+ concentrations may have an effect on the activity of plasma membrane (PM) H^+-ATPase. A decreased PM-H^+-ATPase activity could increase the apoplastic pH. This process could limit the cell-wall extensibility and thus reduce growth according to the acid growth theory. To compare the effect of Na^+ on PM H^+-ATPase activity in salt-sensitive maize (*Zea mays* L.) and salt-resistant sugar beet (*Beta vulgaris* L.) shoot, PM vesicles were isolated from growing shoots of both species and ATPase activity was determined by assaying the P_i released by hydrolysis of ATP. The H^+ pumping activity was measured as the quenching of acridine-orange absorbance. An increased Na^+/K^+ ratio decreased the PM H^+-ATPase activity in vesicles of maize as well as of sugar beet shoots. Nevertheless, the detrimental effect of increased Na^+/K^+ ratio was more severe in salt-sensitive maize compared to salt-resistant sugar beet. At 25 mM Na^+ concentration, hydrolytic activity was not affected in sugar beet. However, a significant decrease in hydrolytic activity was observed in maize at pH 7. In maize and sugar beet, reduction in active H^+ flux was 20% and 5% at 25 mM Na^+ concentration in the assay, respectively. The active H^+ flux was decreased to 80% and 60%, when 100 mM K^+ were substituted by 100 mM Na^+. We conclude that PM H^+-ATPases of salt-resistant sugar beet and maize shoot are sensitive to higher concentration of Na^+. However, sugar beet PM-H^+-ATPases are relatively efficient and may have constitutive resistance against lower concentration (25 mM) of Na^+ as compared to that of salt-sensitive maize.

1. Introduction

Environmental stress caused by salinity is a serious factor limiting the productivity of agricultural crops. Although drainage and the supply of high-quality water can solve the problem, these measures are extremely costly and hardly applicable in extensive agriculture [1]. Besides sustainable agricultural practices and soil amelioration, the improvement in salt resistance of crops has been proposed [2,3]. Salt resistance is a multigenic character and only limited success in improving the salt resistance of crops has been achieved so far. Identification of physiological and biochemical processes in plants provides a better understanding for salt resistance [4–6].

According to the biphasic model by Munns, plant growth is impaired by osmotic stress in the first phase [7] and growth reduction is probably regulated by inhibitory hormonal signals (e.g. ABA) from the roots [8]. In the second phase, a fast increase of the Na^+ concentration in leaf tissue affects the physiological and biochemical processes [9]. However, Sümer et al. [10] reported that beside osmotic effects, Na^+ toxicity contributed to decreased maize growth in the first phase of salt stress.

Leaf growth comprises cell elongation and cell division Determinants of cell elongation are turgor and cell-wall extensibility [11]. However, under saline conditions the turgor appears not to limit cell elongation [12]. Instead, hardening of the cell wall is a limiting factor in elongation growth [13,14]. Apoplastic pH has been suggested to play an important role in cell-wall loosening and growth [15]. In maize leaves, increased rates of growth are associated with increased acidification of the cell wall [16]. Decreased cell-wall pH is generally thought to promote wall-loosening

* Corresponding author at: Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. Tel.: +92 419200716; fax: +92 41 9201221.
E-mail address: abdul.wakeel@agrar.uni-giessen.de (A. Wakeel).

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doi:10.1016/j.plaphy.2011.01.006

necessary for cell enlargement [17,18], and inhibition of cell-wall acidification may, therefore, reduce extension growth. The plasma membrane H^+-ATPase is an electrogenic pump that directly couples ATP hydrolysis with the transport of H^+ into the apoplast [19] and has an important role for cell-wall acidification. Palmgren [20] proposed that pH changes in symplast and apoplast could be due to changes in plasma membrane H^+-ATPase activity because the root hair initiation in *Arabidopsis* was associated with slight alkalinization of cytoplasm and cell-wall acidification at the site of initiation [21]. Reduced H^+ release by plasma membrane H^+-ATPase is a limiting factor for leaf growth in salt-sensitive maize genotypes but not in salt-resistant maize genotypes and sugar beet under salt stress [22–24].

Potassium stimulates the PM ATPase activity by promoting the dephosphorylation of this enzyme [25,26] and low Na^+/K^+ ratio is very important for optimum PM-ATPase activity. High cytosolic Na^+ concentrations and relatively small K^+ concentrations that may occur in the second phase of salt stress can reduce the PM H^+-ATPase activity, which ultimately could increase the apoplastic pH. We hypothesized that PM H^+-ATPases in sugar beet are resistant to higher cytosolic Na^+/K^+ ratio as compared to that of maize and may contribute to salt resistance.

2. Results

2.1. *In vitro effect of various Na^+/K^+ ratios on PM H^+-ATPase hydrolytic activity*

To determine the purity of the plasma membrane (PM) fraction isolated from maize and sugar beet shoots, the ATPase hydrolytic activity was analyzed in the presence of nitrate, azide, molybdate or vanadate, which are inhibitors of tonoplast H^+-ATPase, mitochondrial H^+-ATPase, unspecific phosphatases and PM H^+-ATPase, respectively [27]. The vanadate-inhibited H^+-ATPase activity was 91.8% in maize and 92.5% in sugar beet membrane vesicles. These data demonstrate that the PM fractions obtained from maize and sugar beet shoots were of high purity (Table 1). Nevertheless to exclude a contamination effect, the activity measurements were performed in the presence of an inhibitor complex solution (nitrate, azide and molybedate) with and without vanadate. The difference is defined as hydrolytic activity of plasma membrane H^+-ATPase [28].

The hydrolytic activity of maize PM H^+-ATPase, at biochemically optimum pH (6.5), decreased with increasing Na^+ concentration and decreasing K^+ concentration in the assay. This decrease was significant when 50% K^+ concentration was substituted by Na^+ and hydrolytic activity was decreased from 0.73 to 0.53 μmol Pi mg^{-1} min^{-1}, when 100 mM K^+ concentration was replaced by 100 mM Na^+ (Fig. 1). At the physiologically relevant (cytosolic) pH 7.0 PM H^+-ATPase was reduced compared with pH 6.5. Substitution of 25 mM K^+ by Na^+ resulted in a significant decrease in PM H^+-ATPase activity. Sugar beet PM H^+-ATPase did not show a significant decrease in hydrolytic activity even at 100/0 mM Na^+/K^+ ratio in the assay at pH 6.5 and at pH 7.0. As found for maize, a significant decrease in hydrolytic activity was observed due to increased pH (Fig. 1).

Table 1
Effect of various inhibitors on H^+-ATPase activity of isolated plasma membrane vesicles from maize and sugar beet shoots. The values represent the relative activity of H^+-ATPase in %.

	H^+-ATPase activity (%)	
	Maize	Sugar beet
Control	100	100
Nitrate (50 mM)	104	93
Azide (1 mM)	102	95
Molybdate (1 mM)	87	86
Vanadate (0.1 mM)	8	8

2.2. *In vitro effect of various Na^+/K^+ ratios on PM H^+-ATPase pumping activity*

Plasma-membrane H^+ transport was measured to investigate the effect of various Na^+/K^+ ratios on pumping activity of *inside-out* vesicles isolated from maize and sugar beet shoots. PM-H^+ transport was monitored as A_{492}-quenching of AO. Pumping reaction was initiated by adding 5 mM Mg-ATP. The proton pumping activity was characterized by two parameters i.e. (a) the initial rate of H^+ transport by PM H^+-ATPase into the vesicles and (b) the maximum quenching (pH gradient) achieved by H^+ transport into the vesicles. Initial rate of H^+ transport represents the quenching of absorbance in the first minute after activation of H^+-ATPase by addition of 5 mM Mg-ATP. It shows active H^+ influx into the plasma-membrane vesicles [28]. Assay with 0/100 mM Na^+/K^+ was considered as control for calculation of relative activity. Inside out PM vesicles isolated from maize and sugar beet shoots showed a decrease in active H^+ influx into the vesicles, when Na^+ to K^+ ratio in the assay was increased. However, maize vesicles showed a larger decrease and relative active H^+ influx was 55% at 50/50 mM Na^+/K^+ ratio and further decreased to 22% at 100/0 mM Na^+/K^+ ratio. In contrast, sugar beet showed 78% active H^+ influx at 50/50 mM Na^+/K^+ ratio and 40% at 100/0 mM Na^+/K^+ ratio in the assay (Figs. 2 and 3). Moreover, active H^+ influx at 50/50 mM Na^+/K^+ ratio was significantly decreased in maize but sugar beet showed a significant decrease at 75/25 mM Na^+/K^+ ratio. Like active H^+ influx, total pH gradient was decreased with increasing Na^+/K^+ ratio in the assay for both maize and sugar beet. Like active H^+ flux, the decrease was more pronounced in maize vesicles as compared to that of sugar beet. The maximum pH gradient decreased to 21% in maize and 60% in sugar beet, relative to their respective controls (0/100 mM Na^+/K^+ ratio), when 100 mM K^+ was substituted by 100 mM of Na^+ (Figs. 2 and 3).

3. Discussion

Potassium (K^+) stimulates the PM H^+-ATPase and the effect of monovalent cations on enzyme activity is in the order

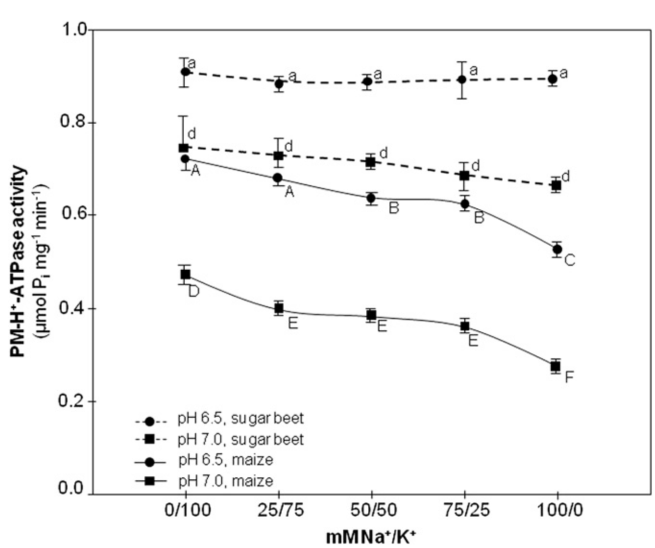

Fig. 1. *In vitro* effect of different Na^+/K^+ ratios at two pH values on the H^+-ATPase activity of *inside-out* plasma-membrane vesicles from maize and sugar beet shoots. The values represent means (±SE) of three independent isolations. Different letters indicate significant differences between the treatments ($p \leq 5\%$).