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A microcalorimetric analysis of quinoa seeds with different initial water content during germination at 25°C

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is an ancestral crop from the Andes of South America. Its high-energy and nutritional value content makes this crop very suitable for food. Germination of quinoa seed remains a problem. In the present work germination of quinoa seeds with different initial moisture contents were monitored by microcalorimetry at 25°C. Storage at moisture contents above 0.082 g g^{-1} drastically reduces seed germination. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Calorimetry; *Chenopodium quinoa*; Seed germination; Seed imbibition

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is an ancestral crop from the Andes of South America. The high nutritional value and energetic content of this crop makes it very suitable as-food [1]. Much research is carried out world-wide about agricultural aspects of this crop [2], but little is done on the physiological level. Quinoa seeds have the advantage of fast germination in vitro, although they germinate very poorly in soil. The reasons for this are still a matter of debate [3].

Physiologists nowadays are deeply concerned with storage conditions of seeds in which water content and temperature play a fundamental role [4]. Seeds normally goes through a period of desiccation and maturation as the final step of their development [5,6]. Desiccation of developing seeds inhibits metabolic pathways that lead to germination and growth but promotes germination on subsequent imbibition. However, mature dry seeds always contain certain amount of water which can be tightly bound to macromolecules (type 1), semi-bound having glassy characteristics (type 2) or less defined producing changes in the phase behaviour of membrane lipids (type 3) [5]. Seeds with water involved in type 1 or 2 bonding scarcely support metabolic reactions during storage, but some metabolism has been detected in seeds with water of the type 3.

Microcalorimetry has proven to be a suitable technique to monitor quinoa seed germination [1]. In the present work, we report the behaviour of quinoa seeds stored at different relative humidities during germination at 25°C, by using titration microcalorimetry. The optimum moisture content for storage of quinoa seeds at 25°C is also evaluated.

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2. Experimental

2.1. Plant material

Seeds of quinoa (*Chenopodium quinoa* Willd.) obtained from the market in Salta Province, Argentina were stored at 33% relative humidity (RH) and 5°C until used. Seeds in all experiments were pre-sorted by hand and excessively small, large and damaged seeds were discarded.

2.2. Moisture adjustment

Seeds were suspended in small aluminium foil baskets from the lids of 500 ml jars over saturated salt solutions for 8 days at 25°C. Moisture content was determined by drying seeds for 48 h at 75°C in a forced draft drying oven. All moisture contents are expressed on a g H_2O/g fresh weight basis (g g^{-1}).

2.3. Calorimetric measurements

A TAM microcalorimeter (Thermometric, Järfälla, Sweden) was used for titration experiments. Two seeds (8.0 ± 2.0 mg) with the desired initial water content (iWC) were placed in the bottom of the calorimetric ampoule on a Whatman No. 1 filter paper disk equilibrated at the same relative vapour pressure (p/p_0) as-seeds. Measurements were performed at 25°C until the end of germination. After an equilibration period of 35 min, distilled water (30 μl) thermostatted at 25°C was injected into the sample ampoule. Control experiments were also conducted with filter paper disks to correct the specific thermal power–time (p–t) curves of germination for heat effect and water evaporation from wetting of filter paper in the ampoules.

Calorimetric measurements of imbibition over a 10 mM KCN solution were conducted for 240 min as-reported elsewhere [1]. For each experiment, imbibition curves were fitted to a first exponential decay equation with offset, p_0, of the type: $p = p_0 + A_1 \exp(-t/t_1)$ ($r^2 > 0.999$) where p, and A_1 are power values for time t and 0, respectively; t_1, is the decay time constant. The fitting procedure was performed by means of the Microbial Origin program version 4.0 (Microbial Software, Inc., 1991–1995) which uses the Levenberg–Marquardt method to minimise the sum of squares. Then, with these equations, thermal power values were calculated for the time interval 10–30 min.

3. Results and Discussion

Water content values (WC) of quinoa seeds, after equilibration at different relative vapour pressures (p/p_0) between 0.113 and 0.845 at 25°C was plotted against the corresponding p/p_0. Fig. 1 shows the water

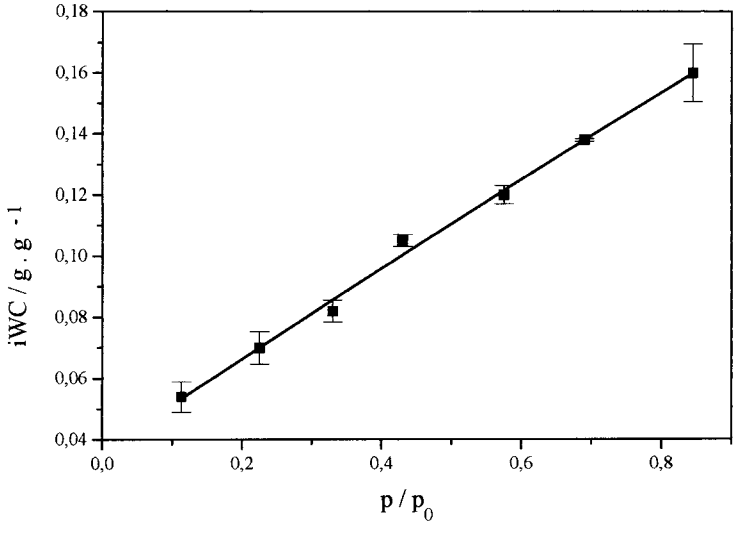

Fig. 1. Sorption isotherm of water for quinoa seeds stored at different relative vapour pressures (p/p_0).

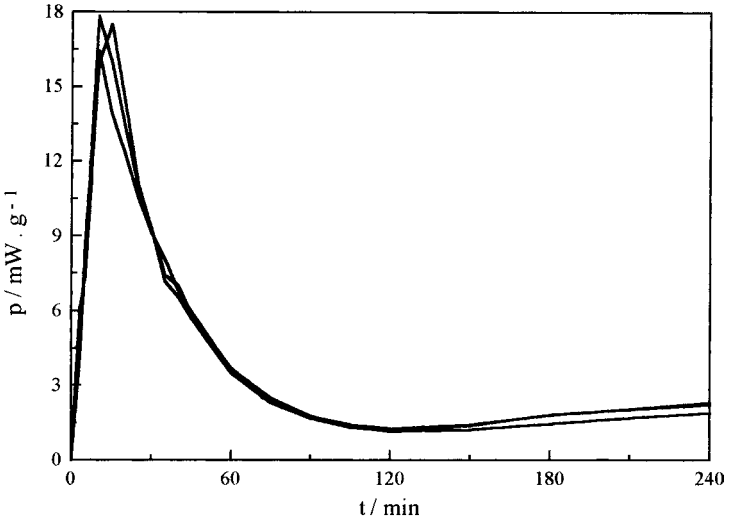

Fig. 2. Specific thermal power–time curves as-determined from three individual experiments during germination of quinoa seeds with an iWC of 0.054 g g^{-1}. Subsequent *p–t* curves reported are average of three or more of these curves.

sorption isotherm of quinoa seeds which is described with 95% confidence by the expression

$$\mathrm{iWC} = \left[\frac{-0.923}{(1+\exp 0.648p/p_0)}\right] + 0.498, \quad r^2 = 0.996 \tag{1}$$

Fig. 2 shows specific thermal power (*p*)–time (*t*) curves of germination during 240 min measured using quinoa seeds with an iWC of 0.054 g g^{-1}. Subsequent *p–t* curves shown are average of three or more curves for quinoa seeds with the different WC.

Fig. 3 shows average *p–t* curves of germination during 240 min for quinoa seeds with a WC of 0.054 (curve A), 0.070 (curve B), 0.082 (curve C), 0.105 (curve D), 0.120 (curve E), 0.138 (curve F) and 0.160 g g^{-1} (curve G). It is interesting to note that

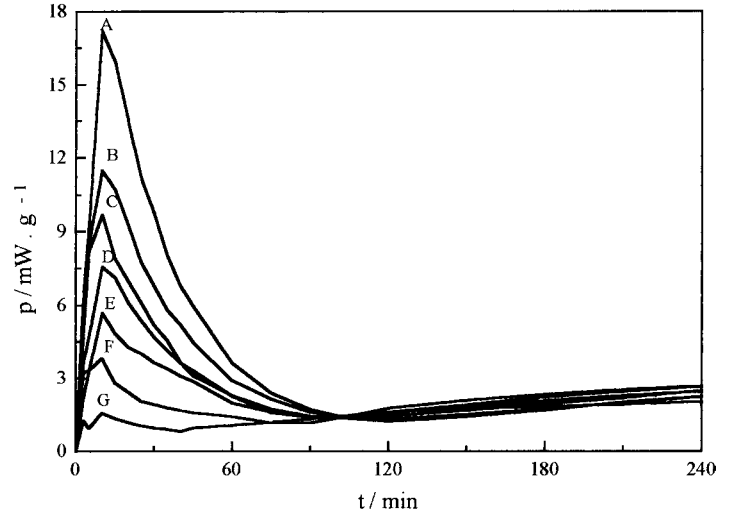

Fig. 3. Average specific thermal power–time curves of germination during 240 min of imbibition over distilled water for seeds with a WC of: (A) 0.054; (B) 0.070; (C) 0.082; (D) 0.105; (E) 0.120; (F) 0.138 and (G) 0.160 g g^{-1}.

all curves have a peak at 10 min of imbibition with an intensity that decreases with increasing seed WC. After 120 min, curves due to germination of quinoa seed with a WC of 0.054 and 0.070 g g^{-1} show a steady increase of p with time until the end of germination (not shown). The steady increase is observed for seeds with a WC of 0.082 g g^{-1} after 105 min, 0.105 and 0.120 g g^{-1} after 90 min, 0.138 g g^{-1} after 75 min and 0.160 g g^{-1} after 40 min. These thermal power–time curves are the result of two contributing processes: seed imbibition and metabolic reactions of germination [1,5]. The peaks of these curves are probably mainly due to imbibition with the subsequent ascending slopes due to metabolism. To determine, if this assumption was correct, imbibition experiments were performed in the presence of 10 mM KCN solution after 30 min of equilibration of the calorimetric system. Power values between 10 and 30 min were calculated from the equations obtained by fitting each calorimetric p–t curve (see experimental). A linear increase of thermal power with time was assumed between the onset of imbibition and 10 min.

Fig. 4 shows average thermal power–time curves of imbibition in the presence of KCN. A steady-state is attained after approximately 180 min for seeds with a WC of 0.054 (curve A), 0.070 (curve B), 0.082 (curve C) and 0.105 g g^{-1} (curve D), after 150 min for seeds with a WC of 0.120 (curve E) and 0.138 g g^{-1} (curve F) and after 120 min for seeds with a WC of 0.160 g g^{-1} (curve G). The time at which this steady-state starts indicates the end of imbibition [1]. Then, the heat evolved during the peak in those curves of Figs. 3 and 4 was determined.

Table 1 shows the specific enthalpy values determined from each curve that contributes to the average p–t curves shown in Fig. 3 ($\Delta_g h$) and in Fig. 4 ($\Delta_i h$), as the area under the peak times 60 s min^{-1}. It is interesting to note the similarity between these values. The slight differences observed are probably due to the fitting procedure used to work out p values of imbibition and to metabolic heat evolution as will be demonstrated later on in this work. Thus, with a good approximation one can evaluate the energetic contribution of imbibition to the total heat evolution due to germination from the first portion of the p–t curves shown in Fig. 3. The $\Delta_i h$ values shown in Table 1, determined for seeds with WC values between 0.054 and 0.082 g g^{-1} are linearly related to the corresponding WC through Eq. (2). Eq. (3) shows the linear relation between the same parameters for quinoa seeds with a WC between 0.082 and 0.160 g g^{-1}.

$$\Delta_i h = -74.0 + 637.8\,\mathrm{iWC}, \quad r^2 = 0.998 \tag{2}$$

$$\Delta_i h = -40.2 + 219.7\,\mathrm{iWC}, \quad r^2 = 0.996 \tag{3}$$

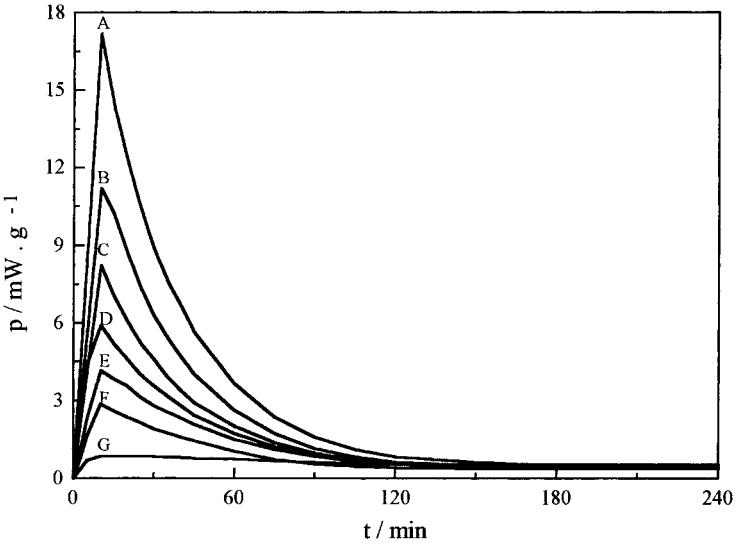

Fig. 4. Average specific thermal power–time curves of imbibition over a 10 mM KCN solution of quinoa seeds with a WC of (A) 0.054; (B) 0.070; (C) 0.082; (D) 0.105; (E) 0.120; (F) 0.138 and (G) 0.160 g g^{-1}.

Table 1
Shows values of initial water content of quinoa seeds, WC; specific enthalpy values due to imbibition as-determined from: (i) imbibition curve over distilled water $\Delta_g h$, and (ii) imbibition curve over a 10 mM KCN solution, $\Delta_i h$; the time interval during which germination occurs (Δt_g) and the percent germinability of seeds (germ.)

WC (g g^{-1})	$-\Delta_g h$ (J g^{-1})	$-\Delta_i h$ (J g^{-1})	Δt_g (min)	Germinability (%)
0.054	40.0 ± 0.6	39.7 ± 1.3	361 ± 12	83.3
0.070	30.2 ± 0.7	28.9 ± 0.3	368 ± 17	83.3
0.082	22.6 ± 1.5	21.9 ± 1.0	356 ± 26	62.5
0.105	19.2 ± 1.0	17.7 ± 1.0	312 ± 30	50.0
0.120	15.3 ± 0.5	13.7 ± 0.3	333 ± 50	50.0
0.138	8.8 ± 0.3	9.4 ± 0.9	348 ± 29	50.0
0.160	2.6 ± 0.1	5.2 ± 0.5	356 ± 55	50.0

From these equations it is clear that the nature of water–seed reserve interactions is different below and above a WC of 0.082 g g^{-1}. Probably, this is a WC value where all primary active sites for water (sites having the highest affinity for water) become saturated. Quinoa seeds contain mainly carbohydrates as storage reserve (44.55%) [7]. Thus, the heat of imbibition of quinoa seeds should reflect hydrogen bond type of interactions. This is true when the units of the slope of Eq. (2) (637.8 J g^{-1} (H_2O)) are transformed into kJ mol^{-1} and the value obtained (11.5 kJ mol^{-1}) is referred to the amount of carbohydrates of quinoa seeds. A value for the enthalpy of quinoa seed carbohydrate hydration $\Delta_{hyd} h = -25.8$ kJ mol^{-1} was calculated. The reported value for the formation of one hydrogen bond is -29 ± 4 kJ mol^{-1} [8]. This result implies the formation of one hydrogen bond per mol of water uptake by seeds until all its active sites (–OH groups from carbohydrates) have been hydrogen bonded. This occurs at a WC of 0.082 g g^{-1} obtained from the interception of the two relations expressed above. This value would be the break point in moisture content above which quinoa seeds should not be stored at 25°C ($p/p_0 = 0.33$). This is clear when one observes the germinability (germ.) and the time interval (Δt_g) during which germination occurs for seeds with the different WC shown in Table 1. Seeds with a WC of 0.054 and 0.070 g g^{-1} present the higher germinability and homogeneity ($\Delta t_g \pm$ S.D.). Germinability of seeds is reduced 25% when the WC is 0.082 g g^{-1} with respect to a lower WC. At 0.105 g g^{-1}, germinability is further reduced another 20%. Thus, from these results one can conclude that capacity and homogeneity of quinoa seed germination at 25°C is at its best when WC is below 0.082 g g^{-1}. Given the similarity between the energy values due to imbibition determined from Figs. 3 and 4, one can use the curves of germination to evaluate the moisture content of quinoa seeds above which they should not be stored at a given temperature.

With the average power–time curves due to quinoa seed germination and the corresponding ones due to imbibition, average p–t curves due to metabolism were obtained by subtracting the latter ones from the corresponding former ones.

Fig. 5 shows the time course of p due to metabolism after 10 min of imbibition for seeds with a WC of 0.054 (curve A), 0.082 (curve B) and 0.138 g g^{-1} (curve C). It is interesting to note, that metabolism apparently starts after 5 min for seeds with a WC of 0.054 g g^{-1}. Seeds with higher WC apparently start their metabolic reactions as soon as they are placed in water (i.e. 0.070 g g^{-1}, not shown) or they are already metabolising when placed to germinate. It is striking the shape of the curves. Multiple peaks are observed at the beginning of imbibition except for seeds with a higher WC than 0.120 g g^{-1}. After the occurrence of the last peak, power decreases to reach values close to zero heat effect and then, increases again until the end of germination. In order to understand this behaviour, germination experiments over a 0.1 mM $HgCl_2$ solution were performed with seeds with a WC of 0.082 g g^{-1}. Seed water uptake occurs in part through water channels called 'aquaporines' [9–13]. An inhibitor of these channels is the mercurial solution. The recorded voltage outputs after 30 min of equilibration were converted to thermal power. The resulting average specific thermal power–time curve obtained during the time interval 30–240 min was fitted to an equation of the type: $p = 9.74 - 0.23t + 2.19\times$

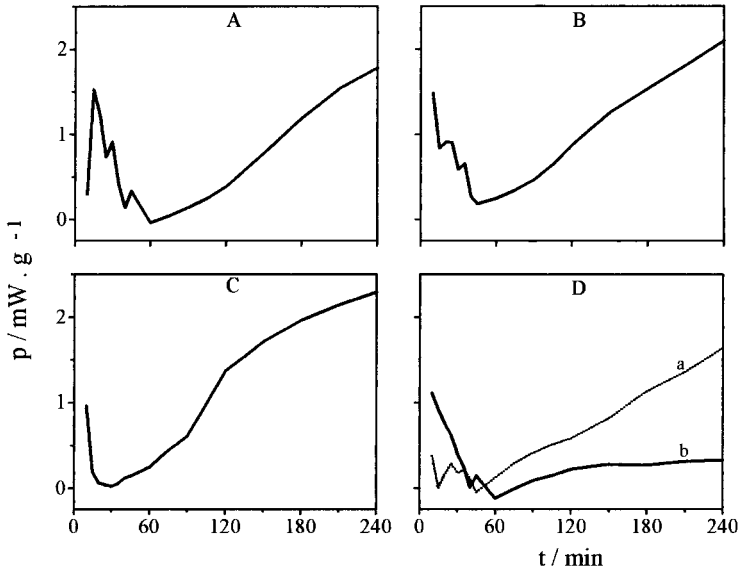

Fig. 5. Average specific thermal power–time curves of metabolism of quinoa seeds during 240 min of imbibition in distilled water with a WC of: (A) 0.057; (B) 0.082; (C) 0.138 and (D) 0.082 g g^{-1}: (a) metabolism excluding aquaporines contribution and (b) metabolism due to aquaporines, as-determined by comparing the *p–t* control curves with germination curves obtained in $HgCl_2$.

$10^{-3}t^2 - 8.93\times10^{-6}t^3 + 1.35 \times 10^{-8}t^4$ ($r^2 = 0.992$). Values of p were calculated from this equation between 10 and 30 min. To be able to compare these calculated p values with those of the control curve, the same fitting procedure was performed. The resulting equation was $p = 11.32 - 0.28t + 2.84 \times 10^{-3}t^2 - 1.19 \times 10^{-5}t^3 + 1.82 \times 10^{-8}t^4$ ($r^2 = 0.995$).

Fig. 5D (curve b) shows the metabolic contribution from aquaporines to the thermal power–time curve due to germination. Fig. 5D (curve a) shows the *p–t*

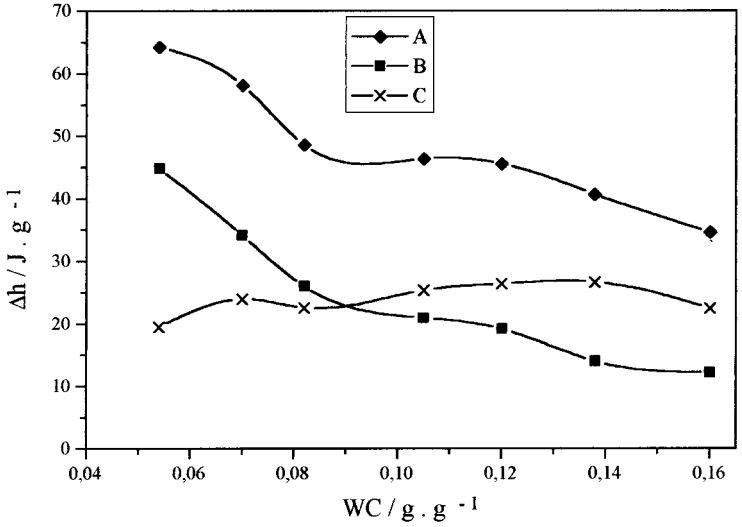

Fig. 6. Specific enthalpy values, Δh, as a function of WC of quinoa seeds due to: (A) germination; (B) imbibition and (C) metabolism.

curve due to metabolism that excludes the contribution of metabolic effects due to aquaporines. It seems that that aquaporines are expressed during the first minutes of imbibition to acquire later a structural functional stage. Hence inhibition of water uptake is observed after 120 min [13].

Fig. 6 shows curves were enthalpy values due to germination (curve A), to imbibition (curve B) and to metabolism (curve C) were plotted as a function of WC of quinoa seeds. To determine these values, germination curves were considered until the end of the process. It is interesting to note, that the trend of the curve that represents germination (curves A) follows the trend of the curve that represents imbibition (curve B). Also, it is remarkable that at a WC of 0.090 g g^{-1}, the heat evolved by seeds until the end of germination is the result of equivalent energetical contribution from both part-processes involved: imbibition and metabolism. This WC (0.090 g g^{-1}) value is acquired at $p/p_0 = 0.36$ and is slightly above the limit in moisture content at which quinoa seeds should not be stored at 25°C. At this WC value, the heat due to germination (Fig. 6A) becomes constant until a WC of 0.120 g g^{-1} where it starts to decrease.

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References

[1] E.E. Sigstad, F.E. Prado, Thermochim. Acta 326 (1999) 159.
[2] M.E. Tapia, in: M.E. Tapia (Ed.), Quinua y Kaniua-Cultivos Andinos, Serie Libros y Manuales Educativos, No. 49, Bogotá, Inter-American Institute of Agricultural Sciences, Colombia, 1979.
[3] W. Aufhammer, H.P. Kaul, M. Kruse, J.H. Lee, D. Schweisig, Eur. J. Agron. 3 (1994) 205–210.
[4] O. Leprince, C.W. Vertucci, Plant Physiol. 109 (1995) 1471–1481.
[5] J.D. Bewley, M. Black, Seeds: Physiology of Development and Germination, 2nd Edition, Plenum Press, London, 1994.
[6] W.D. Wolk, P.F. Dillon, L.C. Copeland, D.R. Dilley, Plant Physiol. 89 (1989) 805–810.
[7] J.A. Gonzalez, A. Roldán, M.- Gallardo, T. Escudero, F.E. Prado, Plant Foods Hum. Nutr. 39 (1989) 331–337.
[8] C. Fringant, J. Desbrières, M. Milas, M. Rinaud, O.C. Joly, M. Escoubes, Int. J. Biol. Macromol. 18 (1996) 281–286.
[9] E. Steudle, T. Henzler, J. Exp. Bot. 46 (1995) 1067–1076.
[10] M.J. Chrispeels, C. Maurel, Plant Physiol. 105 (1994) 9–13.
[11] T. Henzler, E. Steudle, J. Exp. Bot. 46 (1995) 199–209.
[12] D. Ludevid, H. Höfte, E. Himelblau, M.J. Chrispeels, Plant Physiol. 100 (1992) 1633–1639.
[13] E.E. Sigstad, F.I. Schabes, Thermochim. Acta 349 (2000) 95–101.