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# Calorimetric studies of quinoa (*Chenopodium quinoa* Willd.) seed germination under saline stress conditions

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**Abstract**

Most crops in saline environments are negatively affected in their rate of growth. This effect is attributed either to osmotic causes or to ion toxicity depending on the plant species, salt composition and salt concentration. Species of the *Chenopodiaceae* family are considered to be resistant to this type of stress. Two cultivars of quinoa (*Chenopodium quinoa* Willd.), an ancestral crop from the Andes of South America with a high nutritional value are evaluated for tolerance to saline stress by calorimetric experiments of seed germination carried out at 24.7 °C in NaCl, KCl, $Na_2SO_4$, $K_2SO_4$ and $Na_2CO_3$ solutions. Also, 0.1 mM $HgCl_2$ was used in combination with the salts to evaluate the possible existence of channels blocked by the mercurial reagent involved in the transport of ions. Results indicate that seeds of cv. Robura are less tolerant to saline stress than are seeds of cv. Sajama with a tolerance limit for seeds of the former cultivar of 100 mM NaCl. Above this concentration there is an apparent expression of proteins bearing –SH groups that block influx of NaCl, which are inhibited by 0.1 mM $HgCl_2$.


*Keywords:* Isothermal microcalorimetry; *Chenopodium quinoa*; Seed germination; Saline stress

## 1. Introduction

Most plants in saline environments are negatively affected in their rate of growth and among them are the majority of crops. This effect is associated with osmotic causes (i.e. low osmotic potential in the soil), nutritional imbalance and specific ion effect or a combination of these three factors depending on the plant species, salt composition and salt concentration [1,2]. A main problem affecting arable land nowadays is their increasing salinity and still there are not well defined plant indicators that could be used by plant breeders to improve agricultural crops for their tolerance to saline environments [2]. Therefore, it is important to investigate plants known for their salt tolerance in order to understand the mechanisms involved. The *Chenopodiaceae* family with 321 species has the highest number of genera that are halophytic and among them is quinoa (*Chenopodium quinoa* Willd.) which is an ancestral crop from the Andes of South America [3]. The high nutritional value of this crop due to its high protein content, vitamins (A, $B_2$, E) and minerals (Ca, Fe, Cu, Mg, Zn) makes it very suitable as food [3]. There are several evidences about the extraordinary adaptation of quinoa to low and high temperatures, poor rainfall seasons, excessive salinity of soils, among other stress factors [4,5]. Quinoa is able to accumulate salt ions in its tissues to control leaf water potential and thus, to avoid physiological damages [4]. The effect of salt on seed yield in two cultivars of quinoa was studied and highly significant differences were found between cultivars and between cultivars and salinity levels [4]. The highest seeds yield was obtained at 15 mS $cm^{-1}$ NaCl for both cultivars but cv. Utusaya had significantly higher yield than cv. 03-26-0036. It has also been observed that seeds of cv. Kcancolla germinate up to 75% at a concentration of 57 mS $cm^{-1}$ after 7 days [4]. In this case they found better

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responses under moderate salinity than under lower electrical conductance (10–20 mS $cm^{-1}$). Other authors reported some alterations in the levels of some primary metabolites and of certain enzymatic activities during the primary stages of seeds cv. Sajama germination under high salinity conditions [6].

In view of this background we considered of importance to study the effect of saline stress on the germination of two cultivars of quinoa to understand the possible mechanisms involved in their salt tolerance. In this sense, calorimetric experiments at the optimum temperature for germination, 24.7 °C, [7] in increasing concentrations of NaCl were performed. Common cations and anions associated with salinity are $Ca^{2+}$, $Mg^{2+}$, $Na^+$, $Cl^-$, $SO_4{}^{2-}$, and $HCO_3{}^-$. In some instances $K^+$ and $NO_3{}^-$ may contribute to salinity, and when pH is greater than 9, $CO_3{}^{2-}$ becomes an important anion [8]. Therefore, experiments were also carried out where $Na_2SO_4$ and $Na_2CO_3$ solutions were used. As potassium is the more common cation among plants and plays an important role in processes such as cellular enlargement, metabolic homeostasis, germination, stomatal opening, osmoregulation, and soidicity avoidance, among others [9], germination experiments in KCl and $K_2SO_4$ solutions were also performed to compare with results obtained in the corresponding sodium salts. To evaluate if transport of $Na^+$ might be through channels blocked by mercurial reagents, experiments were performed with $Na^+$ or $K^+$ salts in the presence of 0.1 mM $HgCl_2$.

## 2. Experimental

### 2.1. Plant material

Seeds of quinoa (*Chenopodium quinoa* Willd. cv. Robura and cv. Sajama), obtained from the Experimental Station of Patacamaya, Aroma Province (3789 m altitude, 68° long, 17° latitude), Bolivia were used. Seeds were stored at 33% relative humidity (RH) and 5 °C and were continuously tested for their ability to germinate in water during the length of this work (1 year). Seeds in all experiments were pre-sorted by hand and excessively small, large and damaged seeds were discarded.

### 2.2. Calorimetric measurements

A twin calorimeter of the heat conduction type with an amplifier (100–0.0001 mV sensitivity) designed and built at Lund University, Sweden was used, and a Kipp & Zonen BD40 recorder. Calorimetric experiments of quinoa seeds germination were carried out at 24.7 °C in increasing concentrations of NaCl, KCl, $Na_2SO_4$, $K_2SO_4$ and $Na_2CO_3$ solutions. In all calorimetric experiments five seeds ($20.0 \pm 2.0$ mg) were placed in the bottom of the calorimetric ampoule on a Whatman N° 1 filter paper disk wetted with 0.05 ml distilled water or the desired salt solution. Voltage ($V$)–time (t) curves of germination were recorded after a system equilibration period of 30 min.

### 2.3. Specific thermal power–time curves analysis

After each experiment, the $V$–$t$ curves were converted to specific thermal power ($p$)–time ($t$) curves of germination by means of a calibration constant obtained electrically and the seeds oven dry weight. A Microcal Origin program version 4.0 (Microcal Software Inc., 1991–1995) was used to average multiple curves of a given experiment and to determine specific enthalpy values of imbibition and germination, $h_i$ and $h_g$, respectively as the area under each curve between 30 min and the corresponding time value ($t_i$ or $t_g$) multiplied by 60 s $min^{-1}$. Values of $t_g$ were determined for each individual seed that germinated at the end of the corresponding endothermic peak [10]. To determine differences between treatments and between concentrations for a particular treatment, a one way ANOVA and multiple comparison (Tukey and Bonferroni) tests were performed with the SPSS 9.0 for windows program. Results reported ($\Delta h_i$, $\Delta t_i$, $\Delta h_g$ and $\Delta t_g$) are the mean ± S.D. of at least three replicates per treatment and thus, fifteen seeds to calculate the germination parameters.

### 2.4. Imbibition

Five seeds were weighed and placed in the bottom of the calorimeter ampoules under the same conditions as calorimetric measurements. After different periods of imbibition during 420 min, time at which control seeds were 100% germinated, seeds were removed, blotted dry with tissue paper and weighed to determine the percentage of water uptake. Results are reported as the mean of four replicates (±S.D.) as g $g^{-1}$ over initial air dry weight.

### 2.5. Determination of pH

A digital thermo/pHmeter with automatic temperature compensation Altronix model TPA-IV and a flat pH electrode (Broadley James Corp.) was used. Seeds (100) were placed to germinate in Petri dishes (Ø 10 cm) over a filter paper disk wetted with the desired test solution (1.0 $cm^3$) in a germination chamber at 25 °C. Measurements of pH were performed on the wetted filter paper disk prior to placement of seeds ($t = 0$) and every 30 min during 240 min after the seeds were set to imbibe. Results reported are the mean of three replicates.

## 3. Results and discussion

Heat of imbibition of quinoa seeds mainly arises from the physicochemical interactions that occur between the seed storage reserves (44.55% carbohydrates) and water [10]. When salt solutions are used, rate of imbibition (reflected

in the $\Delta t_i$ values) should be influenced by the osmotic effect caused by the salt whereas the specific enthalpy ($\Delta_i h$) involved, should include water–seed storage reserves interactions and the effects, if any, caused by the ions. It is important to stress, that the time considered as the end of imbibition here is when the *p*–*t* curve of quinoa seed germination reaches its minimum value thus, when *p* due to imbibition reaches a steady state and *p* due to metabolism starts to increase [7,10]; thus, when all the active sites for water have interacted.

Fig. 1 shows $\Delta t_i$ and $\Delta_i h$ values as obtained for the different salt treatments plotted as a function of salt concentration for seeds of cvs. Robura and Sajama. Differences between the means of the different treatments in both imbibition parameters are observed for seeds of cv. Robura ($p < 0.05$) whereas no differences are observed for seeds of cv. Sajama. Within a particular salt treatment, $\Delta t_i$ values as determined at concentrations above 75–100 mM are significantly higher than for control seeds of cv. Robura. Note the $\Delta t_i$ values in 100 mM NaCl and KCl and 75 mM $Na_2SO_4$ (curves a–c, Fig. 1A). Thus, $\Delta t_i$ values only reflect the imbibition rate as stated before which slightly decreases (higher $\Delta t_i$) with increasing salt concentration. Values of $\Delta_i h$ do not show significant differences with respect to control with the exception of the determined value in 75 mM $Na_2SO_4$ which is higher (curve c, Fig. 1C). Striking is to observe the value of $\Delta_i h$ as determined in 50 mM KCl and $K_2SO_4$ (curves b and d, respectively Fig. 1C) for seeds of cv. Robura significantly lower than the determined values in the corresponding sodium salts (curves a and c, respectively Fig. 1C). A similar but not significantly different effect is observed for seeds of cv. Sajama and 100 mM KCl with respect to 100 mM NaCl (curves b and a, respectively in Fig. 1D) with $p = 0.06$. Therefore, this effect might be related to the $K^+$ cation. Influx of $K^+$ normally occurs through high affinity uptake systems [11]. To this respect, we thought that measurements of pH could give as a clue but quinoa seeds loose viability after the years of storage [12] and in fact seeds of cv. Robura completely lost their ability to germinate after 2 years. As Patacamaya Agricultural Experimental Station does not exist any longer, we used a different seed collection to perform the pH measurements during imbibition in distilled water, 100 mM NaCl and KCl. In parallel, we run calorimetric experiments of imbibition.

Fig. 2 summarizes the results obtained. Values of pH after 2 h of imbibition are represented in Fig. 2B as the percentage below 100% from initial pH value (ΔpH) as a function of salt concentration. Note again lower $\Delta_i h$ in 100 mM KCl than in 100 mM NaCl (Fig. 2A) which is probably related to the higher decrease of pH value in KCl than in NaCl after 2 h of imbibition. These results indicate a higher efflux of $H^+$ which is probably related to the high affinity $K^+$ transport mechanism.

Table 1 shows values of specific enthalpy ($\Delta_g h$), time ($\Delta t_g$), and percentage ($G$) of germination as determined for quinoa seeds of cvs. Robura and Sajama in the different treatments. The difference from 100% germination of the $G$ values for control seeds of cv. Sajama are due to both: abort and lack of germination as well as in 100 mM $Na_2SO_4$ and $Na_2CO_3$ whereas all other values reported are only due to abortion. Also, the S.D. values as determined for $\Delta t_g$ are an indication of the uniformity of germination as was previously reported [10]. Note an improved uniformity and $G$ with respect to control when quinoa seeds cvs. Robura are germinated in 50 and Sajama in 100 mM NaCl. Values of $\Delta_g h$ as determined in 50 mM NaCl, 33 mM $Na_2SO_4$, 50 and 100 mM $Na_2CO_3$ for seeds cv. Robura and 100 mM NaCl and $Na_2CO_3$ for seeds cv. Sajama are identical among them and with control values. This might imply that at these salt concentrations the metabolic reactions involved either with ion transport or with a salt attenuating effect do not exist as was already observed in the imbibition parameters. It is now generally accepted that for moderate to high external $Na^+$ concentrations the electrochemical gradient created by $H^+$-ATPase favors passive $Na^+$ entry [11,13]. Striking are the determined $\Delta_g h$ values in KCl for both cultivars that independently of concentration

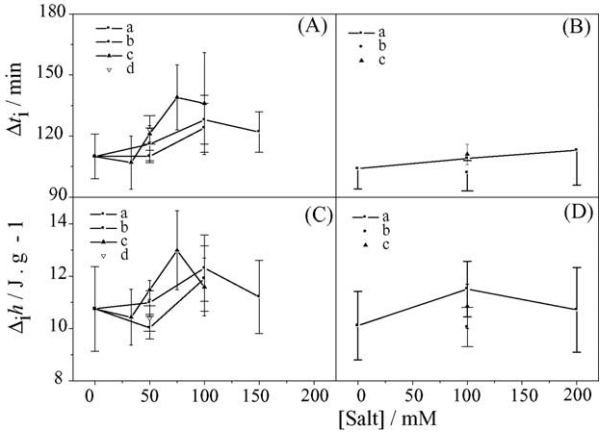


Fig. 1. Imbibition parameters plotted as a function of salt concentration for seeds of cv. Robura: (A) time, $\Delta t_i$ and (C) specific enthalpy, $\Delta_i h$ and for seeds of cv. Sajama: (B) time, $\Delta t_i$ and (D) specific enthalpy, $\Delta_i h$ in (a) NaCl, (b) KCl, (c) $Na_2SO_4$, and (d) $K_2SO_4$.

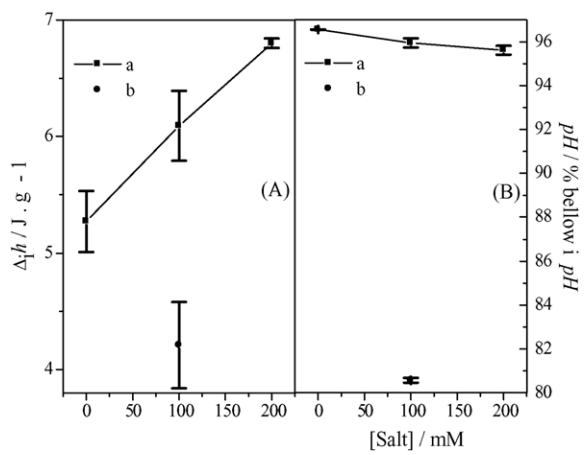


Fig. 2. (A) Specific enthalpy, $\Delta_i h$, and (B) pH after 2 h of imbibition, ΔpH, represented as percentage bellow initial value for commercial seeds in: (a) NaCl and (b) KCl.

Table 1
Average specific enthalpy ($\Delta_i h$), time ($\Delta t_g$) and percentage ($G$) of germination as determined for quinoa seeds of cvs. Robura and Sajama in the different salts and salt concentrations

| [Salt] (mM) | cv. Robura | | | cv. Sajama | | |
|---|---|---|---|---|---|---|
| | $-\Delta_i h$ (J g$^{-1}$) | $\Delta t_g$ (min) | $G$ (%) | $-\Delta_i h$ (J g$^{-1}$) | $\Delta t_g$ (min) | $G$ (%) |
| $H_2O$ | 52.98 ± 9.91 | 393 ± 47 | 97 | 48.38 ± 10.35 | 347 ± 41 | 88 |
| NaCl/50 | 53.91 ± 7.14 | 412 ± 32 | 100 | – | – | – |
| KCl/50 | 67.50 ± 13.38 | 464 ± 39 | 95 | – | – | – |
| $Na_2SO_4$/33 | 53.48 ± 12.28 | 408 ± 52 | 88 | – | – | – |
| NaCl/100 | 78.41 ± 20.69 | 543 ± 92 | 93 | 53.11 ± 6.01 | 391 ± 28 | 90 |
| KCl/100 | 68.74 ± 19.27 | 495 ± 77 | 91 | 67.25 ± 16.37 | 447 ± 69 | 90 |
| $Na_2SO_4$/50 | 63.38 ± 13.81 | 475 ± 66 | 87 | – | – | – |
| $K_2SO_4$/50 | 63.65 ± 9.31 | 491 ± 42 | 100 | – | – | – |
| $Na_2CO_3$/50 | 51.53 ± 11.31 | 393 ± 30 | 100 | – | – | – |
| NaCl/150 | 82.53 ± 14.10 | 555 ± 138 | 93 | – | – | – |
| $Na_2SO_4$/75 | 63.94 ± 15.66 | 512 ± 96 | 90 | – | – | – |
| NaCl/200 | – | – | – | 66.55 ± 12.34 | 493 ± 63 | 93 |
| $Na_2SO_4$/100 | 74.85 ± 24.06 | 620 ± 136 | 93 | 72.16 ± 9.75 | 470 ± 38 | 83 |
| $Na_2CO_3$/100 | 50.37 ± 11.74 | 395 ± 79 | 93 | 53.60 ± 8.65 | 378 ± 33 | 80 |
| $HgCl_2$/0.1 | 70.11 ± 25.95 | 458 ± 78 | 93 | 55.54 ± 8.07 | 388 ± 48 | 83 |
| NaCl–$HgCl_2$/100:0.1 | 91.10 ± 29.68 | 559 ± 101 | 89 | 52.64 ± 9.21 | 394 ± 14 | 73 |
| KCl–$HgCl_2$/100:0.1 | 75.10 ± 25.08 | 545 ± 117 | 87 | – | – | – |
| $Na_2SO_4$–$HgCl_2$/50:0.1 | 73.03 ± 14.58 | 518 ± 48 | 90 | – | – | – |

are similar among them and with the determined value in 200 mM NaCl for seeds of cv. Sajama and 100 mM NaCl for seeds of cv. Robura. Determined imbibition parameters predicted that a high affinity $K^+$ transport mechanism might be present. Apparently, seeds of cv. Sajama activate an equivalent system, in terms of energy, for the influx of $Na^+$ and $Cl^-$ at 200 mM NaCl and seeds of cv. Robura at 100 mM. Anion uptake across the plasma membrane is normally an active process requiring co-transport with protons [13] and therefore $Cl^-$ should be also considered in the energy term. These results indicate that seeds of cvs. Robura and Sajama have different behaviour in NaCl but similar in KCl, $Na_2CO_3$ and probably $Na_2SO_4$. Note the same $\Delta_g h$ values as determined in 100 mM $Na_2SO_4$ for seeds of both cultivars. Also note decreased $G$ values in $Na_2SO_4$ for seeds of both cultivars with respect to control as well as above 100 mM NaCl for seeds of cv. Robura whereas an increased $G$ value is observed for seeds of cv. Sajama in 200 mM NaCl. Probably, above 100 mM NaCl seeds of cv. Robura, suffer an internal osmotic stress caused by the salt that requires a regulation of water flux. In this sense, mobility of aquaporines inside the cells and the differential regulation in the expression of these proteins have been reported when plants are subjected to saline stress [14]. Also, other authors working with roots of two cultivars of wheat under saline stress (100 mM NaCl) demonstrated that the proteins bearing –SH groups content of the plasmatic membrane (PM P-SH) were lower with respect to control for one cultivar and higher with respect to the other [15]. These observations were associated with lipid peroxidation and reduced $H^+$-ATPase activity in the former and just with higher $H^+$-ATPase activity in the latter cultivar. Previously, we have reported the existence of aquaporines during quinoa seeds germination [16] after studying the calorimetric effect of a 0.1 mM $HgCl_2$ solution. Activity of aquaporines is associated with the –SH groups of cysteine which can be inhibited by the mercurial reagent. Therefore, we decided to carry out experiments by using 0.1 mM $HgCl_2$ in combination with 100 mM NaCl, KCl, and 50 mM $Na_2SO_4$ to evaluate the possible existence of channels blocked by mercury involved in the transport of ions.

Table 1 also shows the values of $\Delta t_g$ and $\Delta_g h$ in the salt-mercurial mixtures. Values of $\Delta t_g$ are similar among them and not significantly different from the values obtained in the corresponding salts for seeds of both cultivars. Values of $\Delta_g h$ as determined in 100:0.1 mM KCl–$HgCl_2$ and $Na_2SO_4$–$HgCl_2$ mixtures for seeds of cv. Robura and in the 100:0.1 mM NaCl–$HgCl_2$ for seeds of cv. Sajama are similar to the determined value in 0.1 mM $HgCl_2$ and not significantly different from those determined in the corresponding salts. The value of $\Delta_g h$ as determined in the 100:0.1 mM NaCl–$HgCl_2$ mixture for seeds of cv. Robura is similar to the determined value in the corresponding NaCl solution. To better understand these results it is important to understand the kinetics of the processes involved.

Fig. 3 shows average $\Delta p$–$t$ curves of germination for quinoa seeds of cvs. Robura and Sajama obtained by subtracting from the average $p$–$t$ curves of germination control those in either $HgCl_2$ (curve a, effect of $HgCl_2$) or the corresponding salt (curves b, effect of the salt) and from the average germination $p$–$t$ curves as obtained in the salt–mercurial mixtures those in either 0.1 mM $HgCl_2$ (curve c, effect of the salt when $HgCl_2$ is present) or the corresponding salt (curve d, effect of $HgCl_2$ when the salt is present). Note the similar $\Delta p$ values for seeds cv. Sajama in $HgCl_2$ and NaCl either when compared with control seeds (curves a and b, respectively, Fig. 3A) or with the mercurial mixture (curves d and c, respectively, Fig. 3A). This is consistent with the same $\Delta_g h$ and $\Delta t_g$ values as determined in the three treatments for seeds

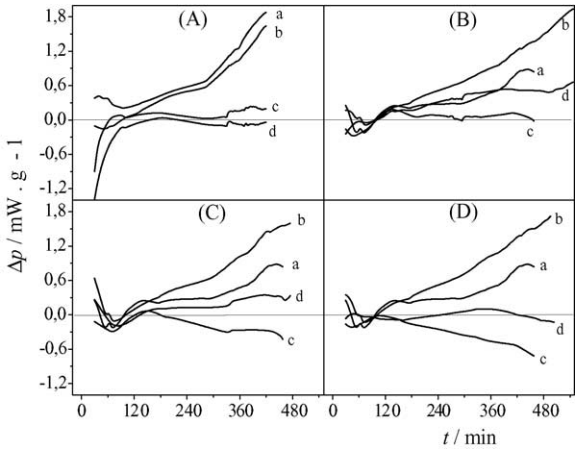


Fig. 3. Differences between average specific thermal power ($\Delta p$)–time ($t$) curves of germination of quinoa seeds as follows: (a) control and 0.1 mM $HgCl_2$, (b) control and the corresponding salt, (c) salt–mercurial mixtures and 0.1 mM $HgCl_2$ and (d) salt–mercurial mixtures and the corresponding salt for: (A) cv. Sajama in 100 mM NaCl and cv. Robura in (B) 100 mM NaCl (C) 50 mM $Na_2SO_4$ and (D) 100 mM KCl.

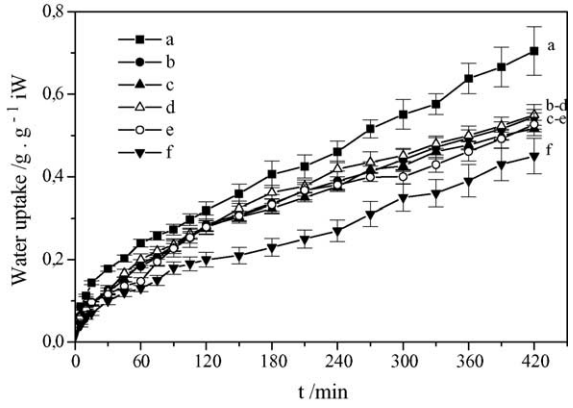


Fig. 4. imbibition curves for seeds of cv. Robura in (a) $H_2O$, (b) 100 mM KCl, (c) 100:0.1 mM KCl–$HgCl_2$, (d) 0.1 mM $HgCl_2$, (e) 100:0.1 mM NaCl–$HgCl_2$ and (f) 100 mM NaCl.

of this cultivar as shown in Table 1. For seeds of cv. Robura, the curves of $\Delta p$–$t$ that represent the effects of $HgCl_2$ either alone or in combination with NaCl (curves a and d, respectively in Fig. 3B) reach a steady state after imbibition which last until 290 min for $HgCl_2$ itself. At 180 min, values of $\Delta p$ for $HgCl_2$ when NaCl is present increases until a new steady state is attained at 300 min which last until 500 min of imbibition. The steady state for the effect of $HgCl_2$ (curve a, Fig. 3B) was previously correlated with differences in rate of water uptake of seeds in water and in $HgCl_2$ [16]. To evaluate if the steady state observed in curve d, Fig. 3B was due to a similar phenomena, imbibition curves were performed.

Fig. 4 shows imbibition curves for seeds of cv. Robura in NaCl and KCl and the corresponding salt–mercurial mixtures. A slower rate of water uptake is observed in 100 mM NaCl (curve f, Fig. 4) after 90 min of imbibition than in the 100:0.1 mM NaCl–$HgCl_2$ mixture (curve e, Fig. 4); in the latter case rate of water uptake resembles that of the mercurial solution. This difference is not observed when 100 mM KCl is involved in the imbibition of seeds cv. Robura therefore, the $\Delta p$ values due to the effect of $HgCl_2$ when NaCl is present (curve d, Fig. 3B) must also reflect differences in rate of water uptake of seeds between the salt–mercurial mixture and NaCl. Thus, the second steady state must be due to a higher number of proteins bearing –SH groups as previously stated for one of the wheat cultivars [15] which is consistent with the higher value of $\Delta_g h$ as determined in the salt–mercurial mixture. Values of $\Delta p$ in NaCl when $HgCl_2$ is present for seeds cv. Robura (curve c, Fig. 3B) indicate that this salt has no heat effect on quinoa seeds during 458 min time, at which seeds in $HgCl_2$ germinate. This is probably because $HgCl_2$ blocks some influx of NaCl and thus, reducing the seeds internal osmotic effect that might be the cause of the reduced water uptake at 100 mM NaCl.

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