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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 ± 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 (Δh_i, Δt_i, Δh_g and Δt_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