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A microcalorimetric study of *Chenopodium quinoa* Willd. seed germination

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is an ancestral crop from the Andes of South America. Due to its high nutritional value, several countries started to promote research to develop quinoa as a new crop. One of quinoa problems is the poor germinability of their seeds.

In this investigation, isothermal microcalorimetry is used as a monitor of two cultivars (cv. Robura and cv. Sajama) of quinoa seed germination. Results are compared with seed imbibition measurements. The optimum temperature of germination for seeds. cv. Robura was 25°C. A higher rate of germination is observed for seeds cv. Sajama at 25°C attributed to a higher rate of water uptake during the first 15 min. The enthalpy due to hydration of quinoa seeds was determined to be $-16.6\ J\ g^{-1}$ (seed) for both cultivars corresponding to a moisture content of 0.27. The estimated enthalpies, until the moment that the first root protrudes, are coincident for seeds of both cultivars. The same behaviour is observed with the estimated enthalpies of germination.

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

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is an ancestral crop from the Andes region of South America. It has a protein content of 14–20%, particularly rich in essential aminoacids such as lysine and methionine which are rare in most cereals [1,2]. A biological value of 82.8 has been attributed to quinoa by FAO whereas the value attributed to wheat is 59.0 [3]. Moreover, quinoa grains contain an energetic value of 1818.4 kJ/100 g, similar to that of soya bean [4]. Due to its high nutritional value, countries such as USA, England, Japan, Denmark and Canada have started to encourage research concerned with the development of quinoa as a new crop [3].

Quinoa crop presents a problem with germinability of seeds. The reason for this is still a question of debate [5].

Seed germination can be defined as a process that begins with water uptake and finishes with radicle emergence [6]. The total process consists of a series of interrelated metabolic events, such as protein hydration, subcellular structural changes, respiration, macromolecular synthesis and cell elongation [7].

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Germination can be considered as composed by two main part-processes: imbibition (water uptake) and metabolic reactions conducting the poorly defined embryo to a new plant.

Measurements of water uptake and oxygen consumption (respiration) are the most common methods used to monitor the germination process [8,9]. In both cases only one of the part-processes is being investigated. Calorimetry, being a non-specific technique measures the rate of heat flow due to all processes taking place during seed germination, either of physical or chemical nature. Microcalorimeters have proven to be useful as monitors for many types of physical, chemical and biological processes [10,11]. In several cases, the technique has been used to record the overall process of seed germination, to investigate the influence of biotic and abiotic factors on germination or the viability of seeds [12–15].

In order to better understand the process of quinoa seed germination, and to elucidate the problems involved with it, investigations were initiated by using isothermal microcalorimetry as monitor. In particular, two of the widely distributed cultivars of quinoa in Bolivia and North-western Argentina were studied to asses their differences in rate of imbibition and that of germination.

2. Experimental

2.1. Plant material

Seeds of quinoa (*Chenopodium quinoa* Willd. cv. Robura and cv. Sajama), obtained from the Experimental Station of Patacamaya, Bolivia were used. Their water content was determined by drying 50 mg of seeds at 75°C until constant weight (48 h) in a forced draft drying oven. The results gave 8.9 ± 0.8 g H_2O/100 g dry weight for seeds cv. Robura and 8.6 ± 0.43 g H_2O/100 g dry weight for seeds cv. Sajama.

2.2. Calorimetric measurements

A microcalorimeter of the thermopile heat-conduction type arranged as a twin instrument was used [16,17] with an amplifier (100 mV–0.1 μV sensitivity) designed and built at Lund University – Sweden and a Kipp and Zonen BD40 recorder. In all calorimetric experiments, five seeds (20.0 ± 2.0 mg) were placed at the bottom of the calorimetric ampoule on a Whatman No. 1 filter paper disk wetted with 40 μl distilled water or the desired test solution. Measurements were performed at 24.7°C after an equilibration period of 35 min. The curves obtained were analysed with an Origin 4.0 computer program after reading the recorded thermopile potential at different time intervals. The instrument was electrically calibrated. All curves reported are an average of at least four measurements. All reported values in the typescript are expressed as the mean ± SD. Specific thermal powers and enthalpy values are referred to dry weight of seeds.

The optimum temperature of germination was determined at Lund University, Sweden by using a TAM microcalorimeter (Thermometric, Järfälla, Sweden). This instrument contains four channels, each arranged as the instrument described above, ensembled in one thermostatic bath. Experiments were run at 20°, 25°, 28° and 30°C by placing three seeds over a filter paper disk wetted with 40 μl of distilled water for those measurements at 30° and 28°C and 30 μl for experiments at the lower temperatures.

2.3. Imbibition curve

Five seeds cv. Robura and cv. Sajama were weighed and placed at the bottom of the calorimeter sample ampoule for imbibition curves in distilled water at 24.7°C. After different periods of imbibition, seeds were removed from the calorimeter and weighed to determine the percentage of water uptake. Results are the mean of four replicates (±SD).

3. Results and discussion

A typical thermal power (=heat production rate)–time curve of germination at 24.7°C is represented in Fig. 1 for seeds cv. Robura. Seeds cv. Sajama gave similar curves. Thermal power of quinoa seeds in distilled water at 24.7°C decreases during the first 90 min. After 105–120 min, the curves show a steady increase of thermal power with time. The small endothermic peaks observed between 310 and 390 min of imbibition indicate the moment at which each root protrudes; this has been determined by

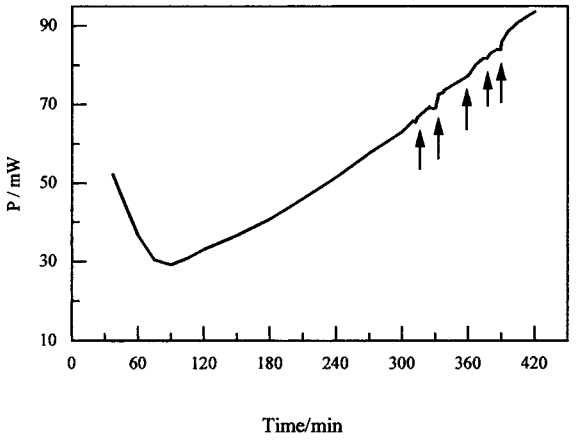

Fig. 1. Thermal power–time curve of quinoa seed germination as obtained from a single calorimetric measurement. The small endothermic peaks observed between 310 and 390 min represent the moment at which roots protrude.

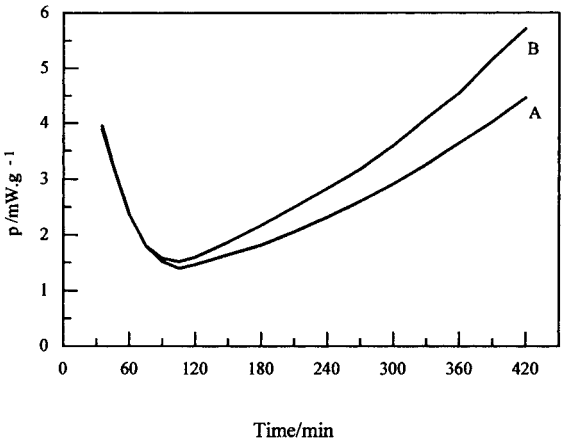

Fig. 3. Average specific thermal power–time curves of quinoa seed germination in distilled water at 24.7°C for: (A) cv. Robura (seven measurements) and (B) cv. Sajama (five measurements).

several observations of seeds before and after the peaks appear in the curves.

Fig. 2 shows a comparison between specific thermal powers for seeds cv. Robura during germination in distilled water at 20°, 25°, 28° and 30°C. It is seen that they increased with the temperature. This is in agreement with rate of germination although a higher proportion of seed abortion (abnormal germination: emergence of cotyledons instead of roots) is observed at 30°C (33%) and at 28°C (28%). At 25°C only 2% of seeds failed to germinate; at 20°C the corresponding value was 25%. Thus, the optimal germination temperature of the tested cultivar of quinoa seeds appears to be close to 25°C. The percentage of aborted seeds increases rapidly outside this narrow range, which could be one of the reasons involved with their germination problem.

The curves in Fig. 3 represent the time course of the average specific thermal power for seeds cv. Robura (Fig. 4(A), seven measurements) and seeds cv. Sajama

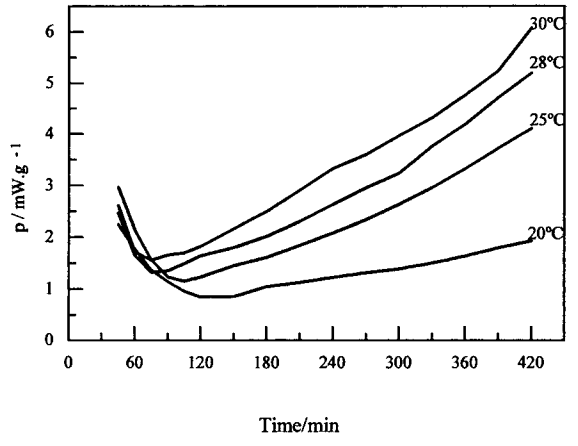

Fig. 2. Specific thermal power–time curves of quinoa seed cv. Robura during germination in distilled water at: (A) 30°C, (B) 28°C, (C) 25°C and (D) 20°C obtained between 45 and 420 min of imbibition. Each curve is the average of four measurements.

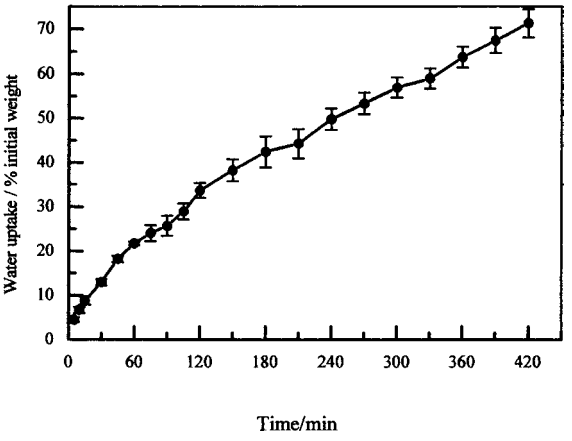

Fig. 4. Time course of water uptake of quinoa seed cv. Robura in distilled water at 24.7°C. Each point is the mean of four replicates ±SD.

(Fig. 4(B), five measurements) during germination. It is interesting to note that the endothermic peaks start to appear at different times in the different experiments, 338 ± 9 min and 279 ± 16.5 min for seeds cv. Robura and seeds cv. Sajama, respectively; thus, they are not perceptible in these curves. The energy of the process between 35 min of imbibition and the moment at which the first endothermic peak appears has been calculated as the area under each curve times 60 s min^{-1}. It is striking the consistency found between these values being -41.5 ± 2.1 J g^{-1} (54.1 ± 2.3% water uptake) and -35.2 ± 3.6 J g^{-1} (56.0 ± 2.3% water uptake) for seeds cv. Robura and seeds cv. Sajama, respectively. This consistency still holds when the energy until the end of germination was determined being -64.0 ± 0.9 J g^{-1} (438 ± 9 min) for seeds of the former and -71.5 ± 5.9 J g^{-1} (397 ± 21 min) for seeds of the latter cultivar.

Water uptake by quinoa seeds was determined at different time intervals in distilled water at 24.7°C. Fig. 4 shows the rate of water uptake for seeds cv. Robura. Seeds cv. Sajama gave a similar curve. Between 5 and 15 min of imbibition, the rate of water uptake for seeds of the former cultivar was determined to be 4.3×10^{-3} g (H_2O) g^{-1} (seed) min^{-1} whereas the value for seeds of the latter during the same period of time was 10.0×10^{-3} g (H_2O) g^{1} (seed) min^{-1}. After 15 min, seeds of both cultivars show a similar rate of water uptake.

Germination is a process characterised by a massive entrance of water accompanied by an increment in respiration [18–23]. Water uptake by seeds depends on several factors such as temperature, seed coat, salinity and nature of storage reserves [24,25]. Quinoa seeds contain mainly starch as storage reserve [1,25] and much of the thermal power released during the imbibition phase is therefore judged to be due to hydration of starch. However, all metabolic events due to germination are energy-requiring processes [26]. Mitochondria in dry and freshly imbibed seeds are functionally and structurally deficient, although it is accepted that they can conduct oxidative phosphorylation from the beginning of imbibition [19,21,26–28]. In this sense, ATP production is reported to be initiated from the first minute of imbibition, mainly in starch containing seeds, and inhibited by the presence of KCN [26,27]. Respiration experiments conducted with pea cotyledons in the presence of cyanide indicate that there is no contribution of the cyanide insensitive pathway to the oxygen consumed during tissue imbibition [28]. Heat evolved by seeds in the presence of KCN solution is thus judged to be mainly due to imbibition. Fig. 5(A) shows the specific thermal power–time curve obtained for seeds cv. Robura during imbibition with a 5 mM KCN solution. Seeds cv. Sajama show a similar curve. A decrease of specific thermal power with time is observed until 120 min where a steady state is attained which lasts for more than 10 h. This steady state indicates the non-existence of other metabolic reactions (cyanide insensitive pathway or anabolic) during imbibition in the cyanide solution. Moreover, a direct correlation is observed when specific thermal powers produced by quinoa seeds in the cyanide solution are plotted against water uptake at different time intervals. A slope of -24.1 mW g^{-1} (H_2O) ($r^2 = 0.997$) was obtained for seeds cv. Robura between 35 and 90 min of imbibition. The slope for seeds cv. Sajama was -36.3 mW g^{-1} (H_2O) ($r^2 = 1$); in this latter case between 35 and 60 min of imbibition which is consistent with the higher rate of water uptake observed for seeds of this cultivar during the first 15 min. These correlations are not observed when specific thermal powers obtained in distilled water are plotted against water uptake; indicating in the latter case the existence

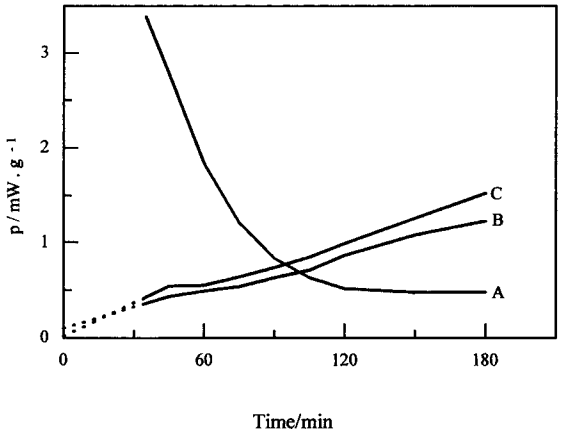

Fig. 5. Specific thermal power–time curves of quinoa seed germination: (A) in the presence of a 5 mM KCN solution, (B) estimated metabolism for seed cv. Robura as obtained when subtracting thermal power due to imbibition (KCN treated) from the total heat effect during germination and (C) idem (B) for seed cv. Sajama. Dot lines in curves B and C represent extrapolated values.

of other heat producing reactions than that of imbibition. If the assumption is made that this relation holds from the beginning of imbibition the specific thermal power extrapolated to zero time can be calculated. Thus, the value of the energy due to imbibition can be derived. The calculated values are -17.1 ± 2.0 and -16.18 ± 0.5 J g^{-1} (seed) for seeds cv. Robura and cv. Sajama, respectively. In both cases moisture content was 0.27.

Results in curves B and C of Fig. 5, are specific thermal power–time curves due to the apparent metabolic reactions of the germination process. These curves were obtained by subtracting the values of specific thermal power due to imbibition (KCN) from specific thermal power data obtained in distilled water for seeds cv. Robura (curve B) and seeds cv. Sajama (curve C) during the first 180 min. These curves, indicate that both cultivars apparently start their metabolism at the beginning of imbibition although the increase of specific thermal power is slower for seeds cv. Robura (curve B, Fig. 5), than for seeds cv. Sajama (curve C, Fig. 5) at the beginning of the process. This difference is again in agreement with the slower rate of water uptake of seeds of the former cultivar during the first 15 min of imbibition. After 180 min, the behaviour of germination for both cultivars can be followed in Fig. 3. The coincidences found with the values of enthalpies of hydration and that of germination for seeds of both cultivars indicate that they are identical, except for the rate of imbibition at the beginning of the process. This fact could be attributed to a higher content in saponins of the seed coat of seeds cv. Robura (higher persistent foam formation when shaken in hot water).

4. Conclusions

The results presented here show that germination of *Ch. quinoa* seeds can be monitored by microcalorimetry. This technique has the advantage that measurements are continuous and do not interfere with the investigated processes. Much information can be derived from calorimetric measurements. The small endothermic peaks observed in each calorimetric curve of germination indicating root protrusion as shown in Fig. 1, are an indicator of the specificity of calorimetry to monitor seed germination. The other methods used with this purpose show an increase of water uptake or of oxygen consumption when the process has ended and root elongation starts but they do not show the moment at which each root protrudes. Thus, calorimetry can serve as a tool to determine germinability of seeds. The ±SD estimated for the energies and times of germination precisely indicate seed germinability.

Calorimetry can thus serve as a 'timer' indicating the precise moment when specific analytical measurements should be carried out in order to clarify in detail the complex process of seed germination. Alternatively, it can be desirable to combine isothermal microcalorimetry with specific analytical techniques, presently a development trend in this instrument field [11].

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