

Evaluation of Photosynthetic Capacity and Grain Yield of the Sea Level Quinoa Variety Titicaca Grown in a Highland Region of Northwest Argentina

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ABSTRACT

Photosynthetic characterization of the quinoa cultivar Titicaca grown at the Encalilla site (1995 m asl), a high mountain valley of the Argentinean Northwest, is described in this study. Titicaca cultivar, bred in Denmark from Chilean and Peruvian parenteral lines, is a promising short cycle cultivar and daylength neutral photoperiod. Results showed that maximal photosynthetic CO₂ assimilation (A_{max}) and stomatal conductance (g_s) were similar to other quinoa varieties. However, carboxylation capacity and leaf transpiration (E) were significantly higher in Titicaca cultivar compared with other quinoa cultivars grown in the same place. Assimilation of CO₂ and stomatal conductance exhibited a strong correlation, like that occurs between (E) and (g_s). Light saturation point (LSP) and light compensation point (LCP) were higher in relation to other quinoa cultivars. Grain yield of 2.35 and 2.51 g/plant was recorded and indicating a well adaptation to arid climatic conditions of the Argentinean Northwest region. The highest value of UV protective pigments found in Titicaca will be explained by solar irradiance in the grown area in relation to Denmark conditions. Grain yield, harvest index and some physiological parameters suggested a good adaptation of the Titicaca quinoa cultivar to high mountain valleys of the Argentina Northwest. This means that Titicaca may be considered as a good alternative for farmers in order to get similar production in less time.

Keywords: *Chenopodium quinoa*, Adaptation, Grain yield, Transpiration, Carboxylation, Pigments.

Introduction

Quinoa was originated in a Titikaka lake area (between Bolivia and Perú at 3,800 m asl) (Rojas *et al.*, 2015). Owing to quinoa is highly tolerant to weather harsh conditions and for its high adaptability to different geographical diversity, it was able to spread to lowland and high mountain of Ecuador, Perú, Bolivia and Northwest Argentine. Agriculture in highland is characterized by a high degree of risk due to drought, frost, wind, hail, soil salinity (Jacobsen, 2003) and high radiation (Hilal *et al.*, 2004; González *et al.*, 2009). Among problems for plant food production in the Andes mountains those related to water shortage are stand out. In effect, low rainfall, high evapotranspiration rate and low soil capacity retention are prevalent edaphological traits in highland of Andean regions. Among scarce crops able to develop in extreme dry conditions, the quinoa can tolerate all these constraints and also produce a highly nutritious grain with all essential amino acids, compare to our current cereals (González *et al.*, 2011). Because the quinoa grain meets and surpasses recommended nutritional requirements by the World Health Organization (WHO) (Repo-Carrasco *et al.*, 2003), quinoa is well known now as a new promising crop species for food security (Mujica *et al.*, 2001; Jacobsen *et al.*, 2013) around the world (Bazile and Baudron, 2015) and for different climatic change scenarios (González *et al.*, 2015; Jacobsen *et al.*, 2015). Due to the higher nutritional values and tolerance to stressful conditions, quinoa was

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revived as a new food crop and are being cultivated outside the Andean region (eg. USA, Europe, Africa, India and China) (Bazile and Baudron, 2015). According to recent data (Quinoa International Congress, Puno, Perú, 2017) at least 110 countries has quinoa trials and some of them are trying and getting new varieties adapted to their prevailing climatic and soil conditions. In Argentina, the first area for quinoa cultivation is located in the Northwest mountain area (Salta, Jujuy, Catamarca and Tucumán provinces). The first attempt to re-introduce quinoa in these areas was in 1984 and the second ones in 1996 (American and European Test of Quinoa) in which at least 25 varieties were used. The majority of the used varieties belong to long growth cycles (up to 140 days) group. Presently farmers use long growth cycles varieties (up to 140 days) and specially CICA variety from Perú. Of course, the long growth cycles varieties need more water for irrigation and different farming practices. All of these were cost and time effective. In Argentina mountain area quinoa sowing takes place in November and is harvested in April or May. In this period there is a wet time (summer, from December to March) and a dry one (from April to September). One problem with CICA cultivation, or other long cycle ones, is the concurrence of the dry period (autumn) with the grain filling stage. In this sense, and to preserve soil and save water, that is scarce in all the high mountain places, it is desirable to get a short growth cycle variety. Therefore, the use of short growth cycle variety has a desirable advantage. In this context, the Titicaca variety, bred in Denmark from Chilean and Peruvian parental lines, is a short growth cycle cultivar, matures early and neutral photoperiod response (Christiansen *et al.*, 2010) should be a good alternative for Argentine's highland. Presently Titicaca is cultivated in Europe (Denmark, Italy) and some countries of the Middle East and North Africa (MENA), but not in South America. In order to get new possibilities for a food crop in the Northwest mountainous region of the Argentina, we performed an ecophysiological evaluation in the field of the Titicaca variety. There are large differences in the yields of crops between and within various countries and world's regions. These differences are caused by a variety of factors including the natural environment, management practices, cultivars and/or varieties, access to water, irrigation, fertilizers, pesticides, and other inputs (Van Wart *et al.*, 2013). So, the aim of this study was to evaluate Titicaca in field conditions in term of gas exchange parameters, carboxylation capacity, water use efficiency, photosynthetic pigments concentration and yield components grown in a semiarid mountainous region of Northwest Argentina. Data can represent an important tool to evaluate new cultivars and especially the control of H₂O/CO₂ exchange that is crucial for plant growth and biomass production under stressful conditions (Gulzar *et al.*, 2005).

Materials and Methods

Plant material and sowing

Titicaca seeds were obtained from the University of Copenhagen (Taastrup, 55° 40' N; 12° 18' E, 28 m asl, Copenhagen, Denmark). Titicaca is a pure line bred in Denmark, characterized for an early maturity and neutral photoperiod response (Christiansen *et al.*, 2010). Field trials were conducted during November to February of 2014 - 2015 and 2015 - 2016 in an arid mountain region of the Argentina Northwest (Encalilla, Amaicha del Valle, 22° 31' S, 65° 59' W, 1995 m asl, Tucumán, Argentina). The soil of Encalilla site is sandy loam clay classified as Xeric Torriorthent type according to the FAO/UNESCO soil taxonomy (Batjes, 1997). Physicochemical parameters of Encalilla soil at 0.5 m depth were: pH 8.8, organic matter content 0.6 %, total nitrogen 0.55 %, and EC 2 dS m⁻¹ (González *et al.*, 2010). Climatic characteristics of the Encalilla site are typical of the high mountain valleys in the Argentina Northwest, which is characterized by a low rainfall with an annual mean value of 160 mm for the last 32 years. Near 90 % of the total rainfall occurred during the growing season (September to February). Soil evapotranspiration calculated with the Penman-Monteith equation was 420 mm. In general, Encalilla climate has been classified as an arid environment with cold winters and hot summers. Meteorological data are summarized in table below and represent mean of November to February of 2014 - 2015 and 2015 - 2016

Meteorological data for the grown period (Encalilla site, Tucumán, Argentina)

Maximal Temperature (°C)	30.5 ± 1.7	Minimal Temperature (°C)	9.7 ± 1.3°
Wind velocity (km/h)	8 to 16	RH (%)	58.1 ± 3.1
PAR (μmol m ⁻² s ⁻¹)	1.893 ± 46		

Minimum and maximum air temperatures, relative humidity and wind velocity were recorded using different sensors coupled to an automatic weather station (Pegasus EP1000, Argentina). PAR radiation was recorded over 1:30 pm during the growing season. PAR radiation was measured with a quantum sensor (LI-190S coupled to LI-1400 data logger, LI-COR, U.S.A.).

Seeds were sown on the middle of November 2014 and 2015. Each plot (10 x 5 m) had five rows (5 m long), 10 cm interplant spacing and 50 cm between rows with N–S direction. Seedbed preparation was carried out just prior to sowing by harrowing with a hand garden plow machine. Plots were irrigated by direct irrigation equivalent to 10 mm rainfall one day prior to sowing the seeds. Seeds were sown at 2 - 3 cm depth in 10 cm spaced holes (5 - 10 seeds for each hole). When seedlings had four leaves, a hand-thinning was carried out to get a plant density of 80,000 plants ha⁻¹. Along the cropping cycle, furrows were irrigated weekly in the morning to get a soil water profile of about 200 mm/year for the whole cultivation period at the end of the experiment. No additional fertilization and no control of fungal diseases and pests were carried out during the cropping cycle. During the growing cycle, inter-row weed control was made by hand.

Gas exchange measurements

Gas exchange measurements were performed during January month in sunny days only using an open infrared gas-exchange system (IRGA) equipped with a fluorometer chamber (LI-COR 6400 XT, LI-COR, Lincoln, NE, USA). Measurements were carried out at 65 - 70 DAYS (days after sowing) just to beginning of flowering stage (between 20 – 25 January). Twelve hours prior to gas exchange measurements all plots were irrigated. Photosynthetic light response curves (A_n /PPFD curves) were performed on the third uppermost fully expanded leaf exposed to light source (10 % blue and 90 % red) provided by the LI-COR equipment. Photosynthetic photon flux densities (PPFD) ranged between 0 and 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were used. Net CO₂ assimilation rate (A_n), transpiration rate (E), and stomatal to conductance (g_s) were measured at 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ after a 3-5 min acclimation period. All measurements were made at a relative humidity of 50 - 60 %, leaf temperature of 25 ± 1 °C, air flow rate of 300 $\mu\text{mol s}^{-1}$ and 400 $\mu\text{mol mol}^{-1}$ CO₂ concentration inside the leaf chamber. Each measurement was repeated until obtain at least three stable values. All measurements were made from 09:00 to 13:00 am). The carboxylation capacity and maximum carboxylation capacity were expressed as A_n/C_i and A_{max}/C_i ratios whereas the instantaneous water use efficiency ($i\text{WUE}$) was calculated as A_{max}/g_s ratio (Rawson *et al.*, 1977). Photosynthetic light response curves were used to calculate theoretical maximal photosynthetic rate (A_{max}), light compensation point (LCP), light saturation point (LSP), dark respiration rate (R_d) and apparent quantum yield of photosynthetic CO₂ assimilation (Φ_{CO_2}) (Schulte *et al.*, 2003). Chlorophyll fluorescence measurements were carried out to quantify the light-adapted quantum efficiency of photosystem II (Φ_{PSII}) (Genty *et al.*, 1989). Electron transport rate (ETR) was calculated according to Schreiber *et al.* (1986) by using the formula:

$$\text{ETR} = \Phi_{\text{PSII}} * \text{PPFD} * 0.5 * 0.84$$

Photosynthesis responses to internal leaf CO₂ concentrations (A_n/C_i curves) were determined at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of PPFD. We set the CO₂ response curve autoprogram with minimum and maximum wait times of 120 and 180 s, respectively. The IRGA equipment was matched at every CO₂ concentration. Values of CO₂ reference between 50 and 2,000 $\mu\text{mol mol}^{-1}$ and airflow rate of 500 $\mu\text{mol s}^{-1}$ were used. A_n/C_i curves were used to calculate the carboxylation efficiency according to Farquhar and Sharkey (1982). Both CO₂ saturation point (SP) and CO₂ compensation point (CP) were calculated using exponential functions described by Schulte *et al.* (2003).

Pigments content

Plants used for gas exchange measurements were also used to determine both photosynthetic and UV-B absorbing pigments. Chlorophyll and carotenoids were extracted using dimethyl sulfoxide during 12 h in darkness at 45 °C, as described by Chapelle *et al.* (1992). Chlorophyll *a*, *b* and carotenoids contents were calculated from absorbances at 649, 665 and 480 nm according to Wellburn's procedure (1994) and expressed as mg/g DW. UV-B absorbing compounds were extracted using acidified metanol/water/HCl, 79:20:1 according to the procedure of Mirecki and Teramura (1984) determined spectrophotometrically at 305 nm and expressed as $A_{305} \text{ mg}^{-1} \text{ DW}$.

Biomass production and grain yield

At physiological maturity, defined as the date when seeds from the main panicle become resistant when pressed, plants from the five central rows were hand-harvested and the height and stem diameter were measured. Grain panicles (main and secondary panicles) were cut and sun-dried to obtain seeds and bracts. The rest of plant was divided into roots and stems to determine dry weight (DW). To determine DW, plant parts were oven dried at 80 °C for 60 h till constant weight. Grain yield and 1000 seed weight were also determined. Harvest index (HI) was calculated as the ratio between grain yield and total above-ground biomass.

Statistical analysis

Data were analyzed using a one-way ANOVA and statistical package SPSS, version 11.0. The treatment mean values were compared by Duncan's Multiple Range Test at $p \leq 0.05$ level of probability (Duncan, 1955).

Results and Discussion

Because there were no large differences in environmental conditions data of gas exchange parameters reported in this study correspond to the average of the two years.

Titicaca development and cropping cycle

Titicaca completed their development cycle 96 and 99 DAS for 2015 and 2016, respectively. These cycles were a few days shorter than those reported by Jacobsen *et al.* (2010) and Pulvento *et al.* (2012) for quinoa trials in European countries. The use of short growth cycle quinoa cultivars is not a common practice among South American farmers. In Perú Gómez-Pando *et al.* (2010) reported that 15 accessions with life cycles between 150 and 200 DAS are currently used by local farmers, while Rojas *et al.* (2015) reported a mean of 177 DAS for the life cycle of the Bolivian quinoa germplasm. In Argentinean Northwest the quinoa's farmers used long growth cycle cultivars (approximately 150 DAS) such as CICA, Amarilla de Maranganí or Relagona Baer. Shorter cropping cycles imply less soil occupation, less agricultural practices and less water consumption, which could be an interesting economic advantage for peoples of the mountainous region of Argentina Northwest. Moreover, the use of short cycle cultivars opens the possibility to perform two crops per year (September - December and December - March) not only in the Northwestern Argentina but also in many other South American places. This hypothesis should be supported with future trials.

Grain yield and growth parameters

In the two years, there were no significant differences in grain yield, panicle weight and stem diameter. Grain yield of 2.35 and 2.51 g/plant were recorded in both cropping cycles (Table 1). For the Titicaca cultivar, Yang *et al.* (2016) reported a value of 13 g/plant in pot experiment, while Shams (2012) reported a value between 0.783 and 0.867 g/plant in sandy soil. It is interesting to point out that the Titicaca grain yield obtained in Encalilla was close to those reported for long cycles varieties presently cultivated in Northwest Argentina (1.8 to 3.5 g/plant) (Curti *et al.*, 2014). On the other hand, Gómez-Pando *et al.* (2010) reported a range between 1-10 g/plant for 15 different genotypes from Puno (Perú) while Delgado *et al.* (2009) reported values of 2.5 to 3.4 g/plant for 16 quinoa genotypes cultivated in the region of Nariño (Colombia). Average grain yield obtained in Encalilla will be indicating a well adaptation to arid climatic conditions of the Argentinean Northwest region.

Value of the 1000 seed weight found at Encalilla site were 2.41 and 2.59 g (2014-2015 and 2015-2016, respectively), but there no significant difference between years was realized (Table 1). These values were similar to others reported previously in Italy (Pulvento *et al.*, 2012), Denmark (Yang *et al.*, 2016) and Turkey (Kir and Temel, 2016). But were significant different to values of 0.28 to 1.10 g recently reported for the Titicaca cultivar grown in and altitudinal trials in Malawi (Maliro *et al.*, 2017). According to the findings of Maliro *et al.* (2017) the 1000 seed weight parameter of Titicaca cultivar seems to be directly correlated with temperature and precipitation when grows in altitudinal gradients.

Plant height is another important growth parameter because an ideal quinoa plant must not be very tall to avoid the wind influence and if it is possible unbranched to facilitate the harvest process (handmade or mechanical). Plant height character achieved in Encalilla (Table 1) was located among

others reported for Titicaca in other places in a world. Values ranging between 49.3 and 140.6 cm have been communicated for the Titicaca cultivar growing under both controlled and field conditions (Adolf *et al.*, 2012; Yang *et al.*, 2016; Yazar *et al.*, 2016). Regarding stem diameter character, it showed no significant differences in both crop cycles.

Our results obtained in Encalilla site were agreed with the current knowledge for grain yield and growth parameters of different quinoa cultivars, or varieties, however, those were quite variable among different countries and even among regions of the same country (Lavini *et al.*, 2014).

Table 1: Yield components of the Titicaca cultivar corresponding to 2014-2015 and 2015-2016 cropping cycles. Data are means \pm SE of 15 different plants. Means followed by the same letter within a column are not significantly different at $p \leq 0.05$.

Cropping Cycle	Grain yield (g/plant)	Panicle weight (g/plant*)	1000 grain weight (g)	Plant height (cm)	Stem diameter (mm)
2014-2015	2.35 \pm 0.6 a	4.54 \pm 0.3 a	2.41 \pm 0.1 a	72 \pm 5.1 a	5.50 \pm 2.6 a
2015-2016	2.51 \pm 0.2 a	5.40 \pm 0.7 a	2.59 \pm 0.1 a	61 \pm 4.2 a	5.62 \pm 1.7 a

(* Naked panicle)

Biomass partition and harvest index (HI)

Aerial biomass accumulation during both cropping cycles was nearly 90 %. No statistical differences ($p \leq 0.05$) in seeds, stems and roots biomass in both cycles were detected. However, a significant difference in naked panicle (panicle without seeds) weights was noticed (Table 2). Naked panicle comprises the central axis and also secondary and tertiary branches with pedicels. It is interesting to point out that seed and naked panicle weights were similar and then the investments of biomass by the plant becomes similar to produce both usefull seeds and useless panicle waste. In this sense, panicle waste probably could be used as renewable fuel. The harvest index (HI) is another important biomass parameter to assess the dry matter partitioning and efficiency of the plant to mobilizate photoassimilates (Maliro *et al.*, 2017). In both cropping cycles, HI values of the Titicaca cultivar were close to 0.3 (Table 2). Values of HI reported for this cultivar grown in other places were: 0.40 to 0.41 in Italy, 0.38 in Turkey and 0.32 to 0.56 in Malawi (Pulvento *et al.*, 2012; Kir and Temel, 2016; Maliro *et al.*, 2017). Similar values of HI were also communicated for other long cycles quinoa varieties. For example, 0.30 and 0.34 for Faro (sea level cultivar) and Cochabamba (highland cultivar) (Erley *et al.*, 2005). However, Bertero and Ruiz (2008) studying four sea level varieties (NL-6, RU-5, CO-407 and Faro) demonstrated that HI is a function of plant density. They reported values of 0.40 and 0.30 for the variety NL-6 growing at a density of 33 and 66 plant/m², respectively. These authors also reported a HI mean of 0.26 for all varieties assayed at above mentioned plant densities. However, a wide range of variation of HI values (from 0.23 to 0.37) has been reported for wild quinoa genotypes from dry valleys and highlands growing in the Argentina Northwest (Curti *et al.*, 2014). According with HI values of the Titicaca cultivar grown at Encalilla site can be concluded that it has a high efficiency in the ability of dry matter partition.

Table 2. Biomass and harvest index (HI) of the Titicaca cultivar corresponding to 2014-2015 and 2015-2016 cropping cycles. Data are means \pm SE of 15 different plants. Means followed by the same letter within a column are not significantly different at $p \leq 0.05$.

Cropping cycle	Seeds (%)	Bracts (%)	Roots (%)	Stems (%)	HI
2014 – 2015	27.7 \pm 2.6 a	25.9 \pm 3.6 a	11.1 \pm 0.6 a	35.3 \pm 0.6 a	0.31 \pm 0.01 a
2015 - 2016	25.2 \pm 1.4 a	30.2 \pm 2.7 b	9.5 \pm 1.1 a	35.2 \pm 1.6 a	0.30 \pm 0.01 a

Gas exchange parameters

Under light and CO₂ saturating conditions (1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 400 $\mu\text{mol mol}^{-1}$) the maximal photosynthetic rate (A_{max}) recorded for Titicaca was 31.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ while stomatal conductance (g_s) was 0.53 $\text{mol m}^{-2} \text{s}^{-1}$. No data of gas exchange parameters for Titicaca cultivar grown in South America are available and this is the first report concerning gas exchange parameters of Titicaca cultivar, for this we compared the previously obtained results for other quinoa cultivars grown at the Encalilla site (González *et al.*, 2010, 2014). The A_{max} obtained was high if we consider the value for some long growth

cycles varieties as CICA, Ratuqui and Robura (see Table 3). In addition, Titicaca A_{max} was higher than those other varieties such as CO-407 and Samaranti ($15.3 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Table 3). The g_s data for Titicaca was higher than the value obtained in field in Denmark ($0.23 \text{ mol m}^{-2} \text{s}^{-1}$) (Razzaghi *et al.*, 2015) but close to CICA cultivar but different to those of Robura, Ratuqui and CO-407 cultivars grown (Table 3). For trials under controlled conditions values of g_s ranging between 0.24 to $0.85 \text{ mol m}^{-2} \text{s}^{-1}$ were also communicated (Adolf *et al.*, 2012; Yang *et al.*, 2016). The C_i value was $255.1 \mu\text{mol CO}_2 \text{ mol}^{-1}$ which was similar to mean value of $273 \mu\text{mol CO}_2 \text{ mol}^{-1}$ reported for ten quinoa varieties (González *et al.*, 2010) while the maximum carboxylation capacity (A_{max}/C_i) of Titicaca variety was $122.7 \text{ mmol m}^{-2} \text{s}^{-1}$, higher than the value of $108.1 \text{ mmol m}^{-2} \text{s}^{-1}$ reported for the same cultivar grown in Denmark (Razzaghi *et al.*, 2015) and close to highland quinoa varieties (González *et al.*, 2010, 2014). The transpiration rate (E) measured in Encalilla site was near 2-fold higher than Robura Ratuqui and CICA values (Table 3), and near 3-fold higher compared with CO-407 value. Intrinsic water-use efficiency (iWUE) value of the Titicaca cultivar was intermediate regarding to values of comparative cultivars (Table 3).

Table 3. Maximum assimilation rate (A_{max}), stomatal conductance (g_s), internal CO_2 concentration (C_i), transpiration rate (E), maximum carboxylation capacity (A_{max}/C_i) and intrinsic water-use efficiency (iWUE), of the Titicaca cultivar. Data are means \pm SE of 5 different plants.

	A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	C_i ($\mu\text{mol CO}_2 \text{ mol}^{-1}$)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	A_{max}/C_i ($\text{mmol m}^{-2} \text{s}^{-1}$)	iWUA ($\mu\text{mol mol}^{-1}$)
Titicaca	31.3 \pm 3.3	0.53 \pm 0.08	255.1 \pm 8.3	10.7 \pm 1.4	122.7 \pm 1.5	59.1 \pm 5.9
Robura*	35.1 \pm 2.3	0.42 \pm 0.04	280.0 \pm 15	5.8 \pm 0.5	125.4 \pm 7.1	83.6 \pm 7.2
Ratuqui*	30.4 \pm 2.6	0.75 \pm 0.05	270.0 \pm 17	6.5 \pm 0.4	126.3 \pm 9.6	45.5 \pm 3.7
CICA*	30.9 \pm 3.2	0.63 \pm 0.05	265.0 \pm 14	6.0 \pm 0.5	116.6 \pm 3.8	49.0 \pm 4.1
Samaranti*	15.3 \pm 1.1	0.18 \pm 0.03	270.0 \pm 16	3.0 \pm 0.6	54.81 \pm 5.1	87.1 \pm 6.3
CO-407**	19.0 \pm 2.5	0.17 \pm 0.04	188.1 \pm 18	3.7 \pm 0.9	101.0 \pm 6.1	111.8 \pm 3.5

(*) Data from González *et al.* (2010);

(**) Data from González *et al.* (2014).

A_n and g_s exhibited a significant correlation ($r^2 = 0.95$, $p \leq 0.01$) with a typical hyperbolic relationship which indicated that A_n was mainly limited by g_s decline (Fig. 1A). Similar relationships between A_n and g_s were also observed for other quinoa varieties (González *et al.*, 2010) and other field-grown plant species (Centritto *et al.*, 2009). Significant correlations were also observed for the pairs C_i and g_s ($r^2 = 0.95$, $p \leq 0.01$), and E with g_s ($r^2 = 0.98$, $p \leq 0.01$) (Fig. 1B and C). These results could indicate that the stomatal play a key role in $\text{CO}_2/\text{H}_2\text{O}$ gas exchange in the Titicaca variety; however, we did not determine the mesophyll conductance (g_m) and then we can not fully confirm this assumption. All data presented herein are the first time for Titicaca not only for Argentine country but also for S. América.

Ligh-response curve

The light saturation point (LSP) of the Titicaca cultivar was found to be $1099.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 4, Fig. 2). A close similar value for LSP ($1,149.4 \mu\text{mol m}^{-2} \text{s}^{-1}$) was found for the Hualhuas variety (Eisa *et al.*, 2012), while values lower than $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ were also reported for the same *C. quinoa* cultivar (Geissler *et al.*, 2015). In relation to the light compensation point (LCP) the Titicaca cultivar exhibited a value of $31.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ whereas, for the Hualhuas variety values of 20.8 and $28.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ were reported (Geissler *et al.*, 2015; Eisa *et al.*, 2012). It is interesting to point out that LCP values o quina cultivars seem to be higher than those reported for other sun leaves, often ranging between 10 and $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Lemos Filho and Duarte, 1998). The quantum yield of photosynthetic CO_2 assimilation (Φ_{CO_2}), which represents the efficiency of absorbed light to fix CO_2 , was $0.054 \mu\text{mol CO}_2 \mu\text{mol photons}^{-1}$. This value was higher than those reported for 10 quinoa cultivars grown at the Encalilla site (González *et al.*, 2010), but lower than that reported for the Hualhuas variety (Geissler *et al.*, 2015). The (Φ_{CO_2}) value found for the Titicaca cultivar was slightly lower than that mean value reported for C_4 species and slightly higher for the mean value reported for C_3 species (Skillman, 2008). Although $0.125 \mu\text{mol CO}_2 \mu\text{mol photons}^{-1}$ corresponds to the theoric value of 8 photons required to reduce 1 mole of CO_2 in absence of the photorespiration, values higher than 0.07 are rarely observed in

natural conditions (Skillman, 2008). On the other hand, dark respiration rate (R_d) for the Titicaca cultivar was $-2.03 \mu\text{mol m}^{-2} \text{s}^{-1}$ (negative sign indicates a negative balance between fixed and released CO_2). Despite that R_d value reported here was significantly higher than the value of $-4.57 \mu\text{mol m}^{-2} \text{s}^{-1}$ communicated for the Hualhuas variety (Eisa *et al.*, 2012), it was slightly higher than those reported for sun leaves (Lombardini *et al.*, 2009).

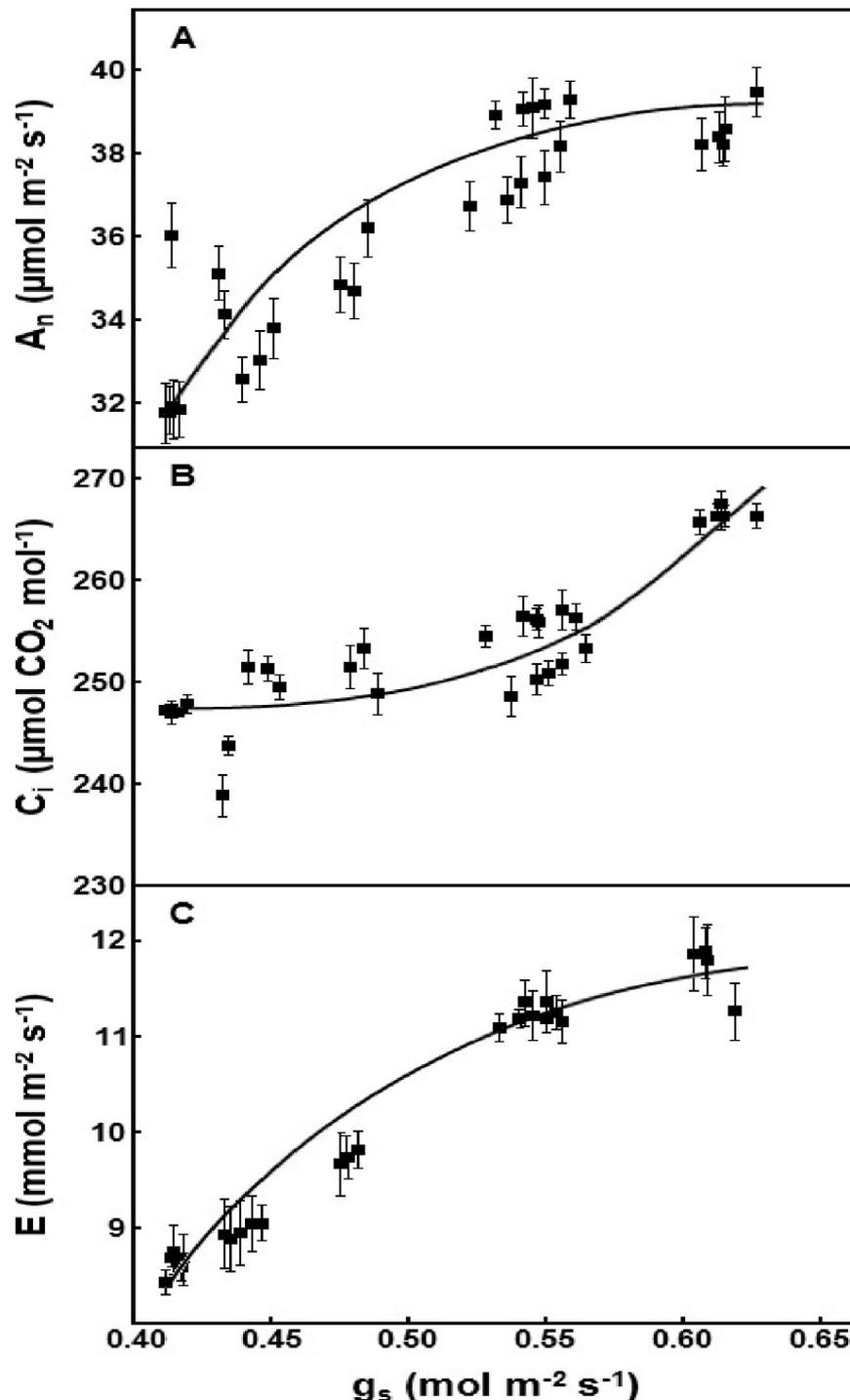


Fig. 1. Relationships between A_n and g_s (A); C_i and g_s (B); E and g_s (C) of the Titicaca cultivar. Each point is the mean \pm SE of 4 independent measurements. Vertical bars indicate SE.

$$A_n = 39.54 - (\exp(-14.31 * g_s)) * 2876.60; r^2 = 0.94 \quad p \leq 0.01$$

$$C_i = 368.21 - 551.73 * g_s + 626.9 * g_s^2; r^2 = 0.95 \quad p \leq 0.01$$

$$E = -18.32 + 98.81 * g_s - 81.59 * g_s^2; r^2 = 0.98 \quad p \leq 0.01$$

Table 4: Quantum yield of photosynthetic CO₂ assimilation (Φ_{CO_2}), dark respiration (R_d), light saturation point (LSP) and light compensation point (LCP). Data are means \pm SE of 5 different plants.

Φ_{CO_2} ($\mu\text{mol CO}_2 \mu\text{mol}^{-1}$)	R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	LSP ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	LCP ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
0.054 \pm 0.015	- 2.0 \pm 0.6	1099.3 \pm 170.4	31.3 \pm 3.1

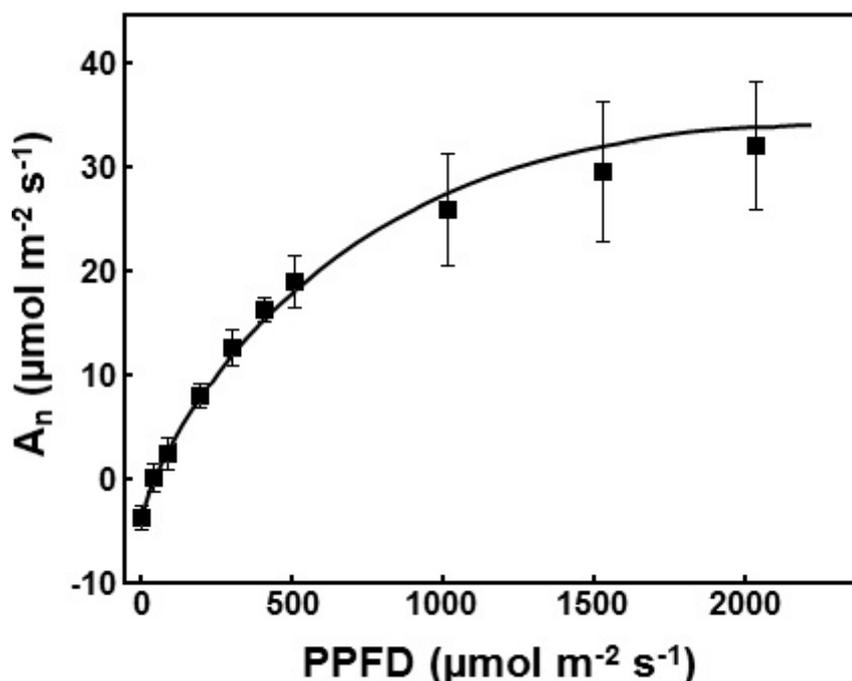


Fig. 2: Net CO₂ assimilation rate (A_n) as a function of the photosynthetic photon flux density (PPFD). Each point is the mean \pm SE of 4 independent measurements. Vertical bars indicate SE. $A_n = 35.39 - (\exp(-0.0015 * \text{PPFD})) * 37.61$; $r^2 = 0.98$ $p \leq 0.01$

A/C_i curve

The responses of A_n and ETR to C_i in the leaves of Titicaca cultivar showed typical photosynthetic characteristics of C₃ plants; initially increased and then leveled off at saturating CO₂ concentration (Fig. 3). Net photosynthesis (A_n) was linearly correlated with internal CO₂ concentration (C_i) ranging from 62 to 210 $\mu\text{mol CO}_2 \text{mol}^{-1}$. The slope of the A_n/C_i curve that corresponds to the carboxylation efficiency (CE) was 0.241 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The compensation point (CP) was 68.8 $\mu\text{mol CO}_2 \text{mol}^{-1}$. In a similar way, ETR was linearly correlated with C_i ranging from 150 to 250 $\mu\text{mol CO}_2 \text{mol}^{-1}$. Both A_n and ETR were saturated beyond 482.4 $\mu\text{mol CO}_2 \text{mol}^{-1}$ ($A_n = 43.03 \mu\text{mol m}^{-2} \text{s}^{-1}$) and at 250 $\mu\text{mol CO}_2 \text{mol}^{-1}$ (ETR = 205.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$), respectively. Similarly, A_n and ETR were curvilinearly correlated with internal CO₂ concentration (C_i). Under saturating PPFD (1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) the stomatal conductance (g_s) was sensitive to (C_i) with a non-linear decline of near 42 % when C_i was increased (Fig. 4). Although non-linear responses of g_s to C_i have been frequently reported in C₃ species, linear responses were also communicated (Düring, 2003). In fact, differences in the response of g_s to C_i in species with similar photosynthetic pathway indicate different stomatal responses to ambient level of CO₂.

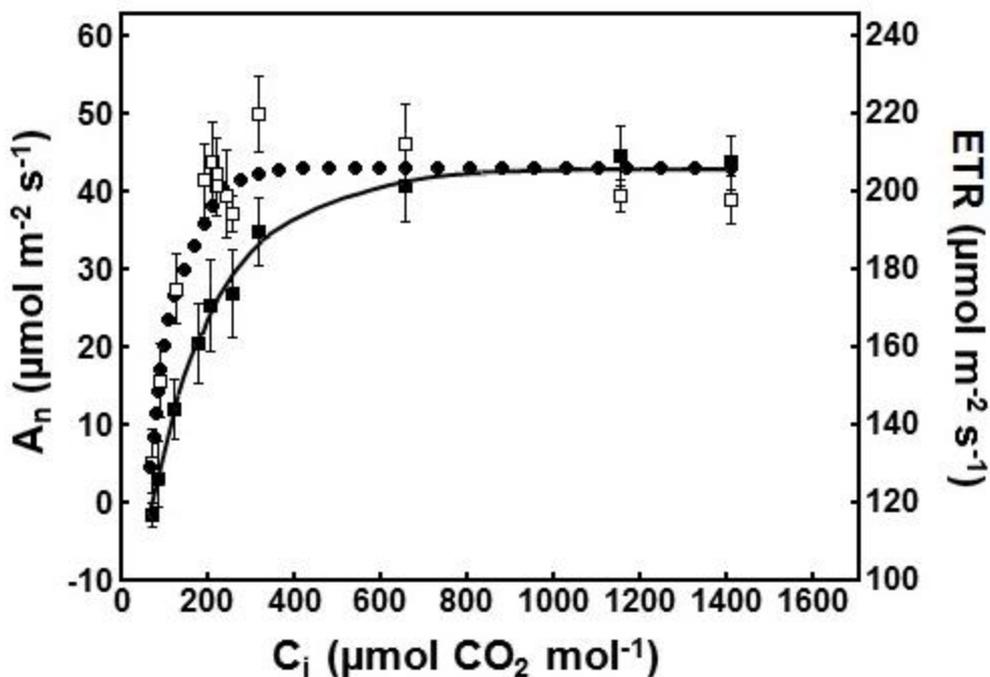


Fig. 3: Net CO₂ assimilation rate (A_n) and electron transport rate (ETR) as a function of intercellular CO₂ concentration (C_i) in leaves of the *Titicaca* cultivar at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. Each point is the mean \pm SE of 4 independent measurements. Vertical bars indicate SE.

(●●●) $A_n = 43.03 - (\exp(-0.0057 * C_i)) * 62.90; r^2 = 0.98 p \leq 0.01$
 (●●) $ETR = 205.49 - (\exp(-0.0179 * C_i)) * 229.95; r^2 = 0.96 p \leq 0.01$

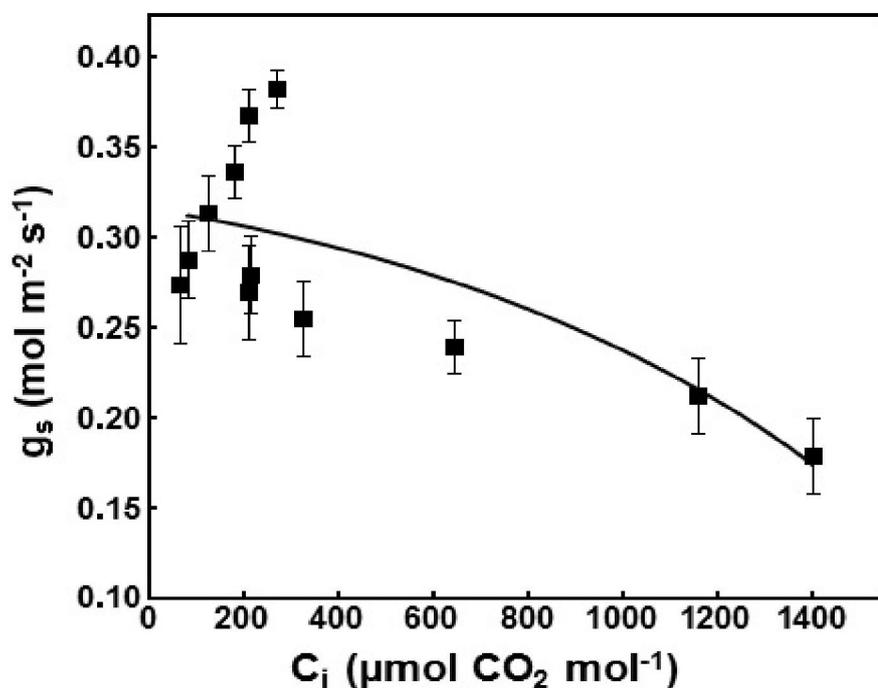


Fig. 4. Stomatal conductance (g_s) in response to intercellular CO₂ concentration (C_i) in leaves of the *Titicaca* cultivar at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. Each point is the mean \pm SE of 4 independent measurements. Vertical bars indicate SE.

$g_s = 0.3121 - 3.28 \times 10^{-5} * C_i - 4.67 \times 10^{-8} C_i^2; r^2 = 0.73 p \leq 0.01$

Photosynthetic and UV-B absorbing pigments

Chlorophyll content, carotenoids and UV-B absorbing pigments in Titicaca leaves is shown in Table 5. Chlorophyll concentration was 3.25 mg g⁻¹ DW which was either similar or lower than reported for other quinoa cultivars. Thus, González *et al.* (2010, 2014) reported that total chlorophyll contents ranged from 2.98 to 11 mg g⁻¹ DW for 12 quinoa cultivars from the Peruvian and Bolivian Altiplano grown at the Encalilla site. Similarly, carotenoid concentration in the Titicaca cultivar (0.36 mg g⁻¹ DW) was lower than those reported by other quinoa cultivars (González *et al.*, 2010, 2014). It is notwithstanding that Titicaca leaves exhibited a high content of UV-B absorbing pigments (0.46 A₃₀₅ g⁻¹ DW). It is noteworthy that available data on the concentration of photosynthetic and UV protective pigments in quinoa varieties are very variable (Gonzalez *et al.*, 2010, 2014). It is not known if this trait can be related to environmental differences of the origin sites of cultivars (Fuentes *et al.*, 2009). According to this hypothesis low values of both chlorophyll and carotenoid pigments measured in Titicaca leaves may be related to an adaptation process of this cultivar to lower value of solar irradiance in Denmark. Whilst, the highest concentration of UV-B absorbing pigments found in the Titicaca cultivar growing in the Encalilla site probably can be related to early gene expression related to the synthesis of phenolic compounds (e.g. flavonoids, lignin) to protect the photosynthetic machinery (Hilal *et al.*, 2004). Supporting our assumption, it has been well demonstrated that quinoa cultivars exhibit higher adaptation plasticity to different light environments (González *et al.*, 2009).

Table 5: Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), Total chlorophyll [Chl (*a* + *b*)], Chl *a*/Chl *b* ratio, carotenoids (Car.), and UV-B absorbing pigments in leaves of Titicaca cultivar. Values are mean ± SE of 5 different plants.

Chl <i>a</i> (mg/g DW)	Chl <i>b</i> (mg/g DW)	Chl (<i>a</i> + <i>b</i>) (mg/g DW)	Chl <i>a</i> /Chl <i>b</i>	Car. (mg/g DW)	A ₃₀₅ /g DW
2.21 ±0.3	1.05 ±0.06	3.25 ±0.4	2.10 ±0.23	0.36 ±0.03	0.46 ±0.05

Conclusion

This article reports the main results of the research activities carried out for the first time for evaluation of a promising new cultivar of quinoa developed in Denmark (Titicaca variety). The study presents the grain yield and photosynthetic characterization of the Titicaca cultivar in the Argentinean Northwest region of South America. Crop cycles in 2015 and 2016 years were 95-100 days. Grain yield, harvest index and physiological parameters suggest a good adaptation of the Titicaca cultivar to high mountain valleys of the Argentina Northwest.

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