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Morphological and Biochemical Characterization of Quinoa (*Chenopodium quinoa* Willd.) - A Preliminary Study

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal grown for its edible seeds and has been the staple food crop in Andean region of South America. Global interest on cultivating quinoa grew due to its outstanding nutritional richness and resistance to abiotic and biotic stress conditions. However, quinoa has not received due recognition as a high-scope crop in Sri Lanka mainly because of lack of knowledge about the crop. The aim of the study was to investigate growth and development and morphological and biochemical characteristics of selected varieties of quinoa. A preliminary pot experiment in greenhouse conditions (27-31 °C, 65-70% RH and 16/8h light dark period) was conducted using three commonly grown commercial varieties in the world, namely, Amarillo Marangani, INIA 427 Amarillo Sacaca and Blanca de Junin (05 replicates each). Morphological characteristics (plant height, stem diameter and number of branches) were recorded across 32-weeks. Total chlorophyll and carotenoid, and proline contents of the fully opened new leaves, and total protein contents of the seeds were analyzed. Data were subjected to descriptive data analysis using Minitab software (version17.1). After 32-weeks of growth, all the varieties reached a mean height of 70-90 cm, and a mean stem diameter of 7.0 - 9.5 mm. INIA 427 Amarillo Sacaca variety showed a rapid increase in height and stem diameter. Total chlorophyll contents were in the range of 2.8-3.3 µg/g and average proline contents were 4.7- 9.9 µmoles /g of fresh weight again recording significantly higher values for INIA 427 Amarillo Sacaca variety (3.30, $p=0.05$) and (9.90, $p=0.05$) respectively. Total protein contents of the seeds were 1300-1900 ppm and seeds of INIA 427 Amarillo Sacaca variety recorded the highest protein content. Preliminary data showed enhanced growth performance in INIA 427 Amarillo Sacaca compared to the other two varieties. Further, high proline accumulation in leaves of this variety suggests better stress tolerance ability. The findings of this study will provide groundwork for comprehensive future research to initiate growing quinoa as a novel crop.

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1. Introduction

Extreme weather events such as long drought spells, rise in temperatures and floods are major challenges facing the crop productivity in Sri Lanka including rice cultivation. Crop diversification which implies cultivating a range of crops, that enhance the utilization of land, water and other resources is an effective strategy in sustainable agriculture in the country. It is crucial to introduce alternative crop sources either underutilized, improved, or new crops that are resilient to adverse environmental conditions in order to prepare for the challenges posed by climate change. Given the remarkable drought and high salinity tolerance, quinoa (*Chenopodium quiona* Willd.) is one such crop that can be cultivated in regions where agricultural and environmental settings are not conducive to traditional crop production.

Quinoa is an herbaceous annual pseudocereal plant in the family Amaranthaceae and recognized as “sacred” and “the mother of all grains” by Incas, South American Indians (FAO, 2013). Quinoa grains are rich sources of protein, dietary fibre, polyunsaturated fats, minerals such as iron, magnesium and zinc and vitamins (Jacobsen, 2003; Pathan and Siddiqui, 2022). Quantity of protein in seeds is generally higher than in most of the cereals and rich in essential amino acid, lysine (FAO, 2013).

Quinoa cultivation dates back to more than 7000 years and native to the Andes region, which includes the countries such as Chile, Peru, Colombia, Ecuador, Venezuela and Bolivia (González et al., 2015). According to the Food and Agricultural Organization (FAO), quinoa production increased globally during the years 2000 to 2009 (Jaikishun et al., 2019). Because of the outstanding ability to withstand wide range of climatic and soil conditions, cultivation of quinoa has expanded to subtropical regions in Europe and Asia, Middle East and hot arid regions of Africa (Angeli et al., 2020). In India, water scarcity due to extreme droughts is the major constrain for agricultural production in arid and semi-arid regions. Quino is one of the crops that has been selected as a potential crop under adverse climatic conditions and is destined as a crop for food security in the next century (Rane et al., 2019).

The FAO initiated a project named "Technical assistance for the introduction of Quinoa towards improved food and nutrition security in Sri Lanka and Bhutan " in 2015, with the collaboration of the Department of Agriculture, Sri Lanka. Quinoa was designated in the project as one of the primary crops for which adaption testing was conducted in selected locations in Sri Lanka, mainly to determine the adaptability and suitability for commercial cultivation in the country (FAO, 2015). However, research findings on morphological traits, physiology and biochemistry of different varieties of quinoa that are appropriate to Sri Lanka is lacking.

The present preliminary study was aimed to assess morphological, and biochemical characteristics of selected commercially important quinoa varieties. The findings of this study will aid in future research and crop diversification efforts.

2. Materials and Methods

2.1 Planting materials, growing and establishment

Three varieties of *C. quinoa* namely Amarillo Marangani, INIA 427 Amarillo Sacaca and Balnca de Junin were obtained from Leibniz Institute of Plant Genetics and Crop Plant Research, Germany. The growth medium was composed of top soil: compost: coconut husk chips (0.5 cm), coir dust and cattle manure (ratio of 1:1:1:1:1). Germination of the seeds were assessed by sowing 20 seeds from each variety on seed trays (03 replicate trays) with above substrate. Seeds were germinated and plants were grown under greenhouse conditions at 27-31ºC temperature, 65-70% RH and 16/8h light dark period (Jacobsen et al., 2005). After 3-4 weeks of establishment and growth, healthy seedlings were transplanted into pots (one plant/pot) and arranged in a completely randomized design. Five replicates of each variety were used for the study. Morphological, and biochemical parameters of the quinoa plants were measured across 32 weeks starting from October 2021 to May 2022.

2.2 Morphological parameters

As morphological parameters, plant height was measured as the main stem height from the base of the plant to the apical shoot, main stem diameter, number of primary and secondary branches, number of internodes, presence of pigmented axils, number of leaves, leaf colour, leaf shape, leaf arrangement and colour of the petiole were observed and recorded at 14-day intervals.

2.3 Biochemical parameters

Total chlorophyll and carotenoid concentration, proline content and total protein contents of quinoa leaves and seeds were determined.

2.3.1 Total chlorophyll and carotenoid contents

Fully expanded mature healthy leaves from each variety were pooled and the total chlorophyll and carotenoid contents were determined following the method of Lichtenthaler (1987).

2.3.2 Proline content

Proline concentration of fully opened fresh leaves was determined as per the method described by Bates et al. (1973).

2.3.3 Total protein

Total soluble protein from fully opened mature leaves and mature seeds (200 mg) was quantified according to the method described by Bradford (1976).

2.4 Statistical analysis

The experimental design was a completely randomized design (CRD). The MINITAB software package (version 17.1) and Excel (2019) were used for data analysis. One-way analysis of variance (one-way ANOVA) followed by the Tukey’s HSD test were used to examine significant differences. The statistical significance was defined at $P < 0.05$ for all analyses.

3. Results

3.1 Growing and establishment of plants

A variable mean percent seed germination (03 replicates each with 20 seeds) was observed in the 03 varieties after 3-4 days under the given conditions. The highest mean percent germination was shown by Amarillo Marangani amounting to 28.3%. INIA 427 Amarillo Sacaca showed a relatively low mean percent germination (2%) compared to Amarillo Marangani and Balnca de Junin showed 19.4% germination.

3.2 Morphological parameters

Table 1 shows stem and leaf morphological traits of adult plants of the three varieties. No difference in the leaf colour, leaf shape, leaf arrangement, petiole colour observed among the varieties. A notable difference in Balnca de Junin compared to the other two varieties was that the axillae were not pigmented. Stem colour of Balnca de Junin was light pink to green whereas in the other two verities stems were purple to green colour.

Figure 2a shows the height increment of main stem of the three varieties. The mean heights of three varieties were in the range 70-86 cm after 33-weeks of growth under greenhouse conditions. INIA 427 Amarillo Sacaca showed the highest mean height of 86.0 ± 4.3 cm. Figure 2b shows the increment of stem diameter of the three varieties during 33-weeks of growth. The mean stem diameter from 25 cm of soil level were in the range 8.0 – 8.6 mm. INIA 427 Amarillo Sacaca variety showed the highest mean stem diameter of 8.6 ± 0.6 mm, nevertheless, not significantly different from other two varieties. In the last 16-weeks of growing period, the INIA 427 Amarillo Sacaca variety demonstrated rapid growth in stem height and diameter although during early growth period it exhibited slower growth compared to the other two varieties.

Figure 1 shows the morphology of 32-week-old representative plants grown in greenhouse. All the three varieties demonstrated branched growth habit. None of the varieties produced panicles at this stage of growth.

Table 1**:** Morphological traits of Amarillo Marangani, INIA 427 Amarillo Sacaca, and Balnca de Junin varieties of Quinoa

Morphological trait	Variety		
	Amarillo Marangani	INIA427 Amarillo Sacaca	Balnca de Junin
Leaf colour	Green	Green	Green
Leaf shape	Lanceolate to Triangular	Lanceolate to Triangular	Lanceolate to Triangular
Leaf arrangement	Alternate	Alternate	Alternate
Presence of pigmented axils	Yes	Yes	No
Petiole colour	Green	Green	Green
Stem colour	Purple to Green	Purple to Green	Light pink to Green

Figure 1: Quinoa plants **(a)** Amarillo Marangani **(b)** INIA 427 Amarillo Sacaca, and **(c)** Balnca de Junin grown in greenhouse for a period of 32 weeks.

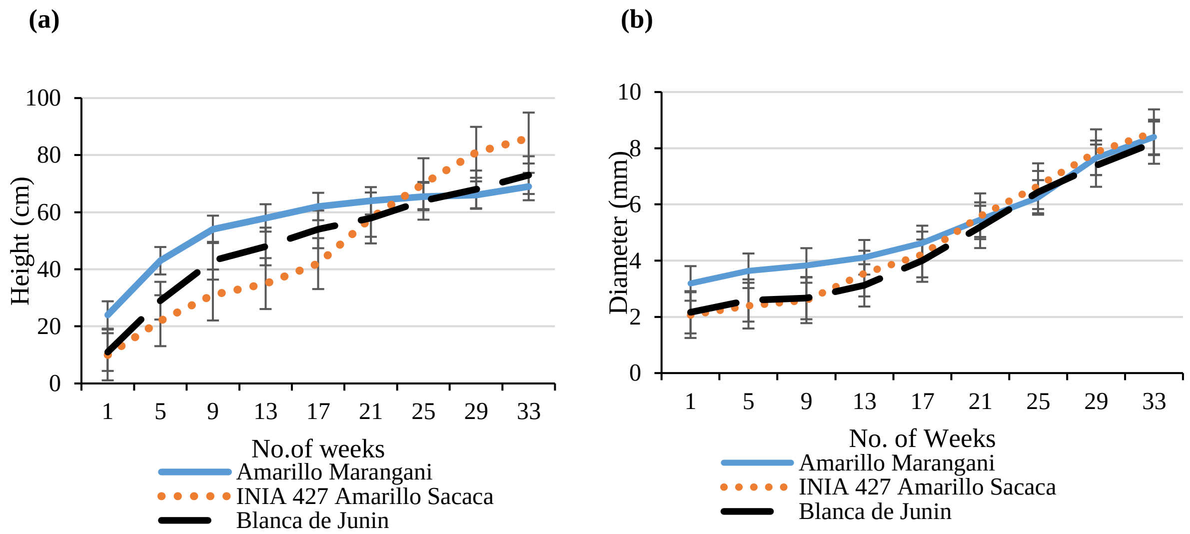

Figure 2: **a)** Increment of stem height and **b)** stem diameter of three tested varieties

3.3 Biochemical parameters

The highest mean total chlorophyll (3.30 ± 0.03 µg /ml) and carotenoid contents (0.35 ± 0.04 µg /ml) were recorded in the variety INIA 427 Amarillo Sacaca (Table 2). Out of the three quinoa varieties, Balnca de Junin showed the highest total protein content in the leaves while seeds of INIA 427 Amarillo Sacaca showed the highest total protein content (1944.25 ± 52.92 ppm). Proline contents recorded in the leaves were in the range 4.77-9.9 µmoles/g.

Table 2**:** Biochemical parameters tested in Amarillo Marangani, INIA 427 Amarillo Sacaca, and Balnca de Junin varieties of Quinoa

Parameter	**Variety**		
	Amarillo Marangani	INIA 427 Amarillo Sacaca	Balnca de Junin
Total chlorophyll (µg/ml) (Leaves)	2.80 ± 0.18	3.30 ± 0.03	3.24 ± 0.07
Total carotenoids (µg/ml) (Leaves)	0.24 ± 0.03	0.35 ± 0.04	0.32 ± 0.05
Total proteins (ppm) (Leaves)	1384.25 ± 79.00	1441.75 ± 49.75	1904.25 ± 62.10
(Seeds)	1146.75 ± 15.07	1944.25 ± 52.92	506.75 ± 37.61
Proline (µmoles/g) (Leaves)	7.42 ± 0.77	9.90 ± 0.07	4.77 ± 0.77

4. Discussion

Under the conditions provided (27-31°C, 65-70% RH and 16/8h light dark, finely structured soil medium, with adequate moisture), Amarillo Marangani variety showed the highest percent germination (28.33%) compared with the other two varieties. However, the germination of Amarillo Marangani variety was also observed to be low, and conditions of seed germination had to be optimized. The small size of quinoa seed makes it susceptible to both dehydration and waterlogging when planted too shallow, or deep and are vulnerable to germination under high soil temperature. Hydropriming for 2-3 hours and growing under 20-23°C has improved mean germination time and germination percentage of several quinoa varieties (Singh et al., 2024). The establishment phase was the most challenging phase in the cultivation of quinoa.

All the varieties showed a slow growth in terms of stem elongation and stem diameter increase in the early stages of vegetative growth up to 13-14 weeks. Thereafter, growth of plants was enhanced. Amarillo Marangani variety showed an increased increment in both stem height and diameter compared with other two varieties during 15-20 weeks of growth, and INIA 427 Amarillo Sacaca variety showed an enhanced growth in 16-32 weeks although it had the slowest germination ability among the three varieties. The results indicate that plants perform well under the given growth conditions.

Early vegetative growth and establishment of quinoa is largely depended on the varietal differences, soil substrate and irrigation frequency (Chaibva et al. 2021). Further, recent green house and field studies reported significant genotypic differences in growth and yield of quinoa across various locations and environments (Pathan et al., 2023). Although the best agronomic practices are established in Andean quinoa farming, cultivation guidelines are not widely available for the other regions. In order to recommend quinoa as a future crop in Sri Lanka, extensive studies have to be conducted to determine optimum conditions for vegetative growth and seed production. A widespread effort is also necessary to choose the best suited quinoa varieties for growing in new regions.

Analysing the physiology, biochemistry and behaviour of plants in controlled environments has a significance on investigating plant growth and adaptation. The amount of chlorophyll pigments in quinoa depends on its type, nutritional state, and accessibility to water. Higher chlorophyll and total carotenoid contents (3.30 ± 0.03 µg/ml and 0.35 ± 0.04 µg/ml respectively) in the INIA 427 Amarillo Sacaca variety suggests high vegetative production of the variety. Since proline rapidly builds up in stressed plants, and plays a crucial role in controlling osmosis, and plant's ability to withstand osmotic stress can be determined by the accumulation of proline in its leaves (Fang and Xiong, 2015). Higher level of proline concentration in the leaves of INIA 427 Amarillo Sacaca variety suggests that it could withstand the adverse environmental conditions better, compared to other two varieties.

The preliminary findings of the study provide groundwork for comprehensive studies with several varieties and varied growth conditions. Identifying suitable agronomic practices and varieties for cultivation in the different agroecological zones will help disseminate the information to initiate growing quinoa as a novel crop. Once the optimum conditions and suitable varieties for each region are established, large-scale cultivation of quinoa can be popularized both as economically as well as ecologically viable domestic crop in the country. Nevertheless, it might take a while longer until the growers and the consumers adapt to this new crop resource.

Declaration of Competing Interest

The authors declare no conflict of interest related to this article.

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