

REVISTA DE AGRICULTURA

(VERSIÓN EN INGLÉS)

SPECIAL NUMBER DEDICATED TO PROINPA FOUNDATION
IN THE FRAMEWORK OF RESEARCH WITH CHENOPODIUM QUINOA

Content

CURRENT NATIONAL AFFAIRS:

Quinoa economy: Perspectives and challenges. *Jorge Blajos; Norka Ojeda; Edson Gandarillas; Antonio Gandarillas* (pp. 3-10)

The current and potential role of *q'ilaq'ila* (*Lupinus* spp.) in sustainable production systems of quinoa. *Alejandro Bonifacio; Genaro Aroni; Milton Villca; Patricia Ramos; Miriam Alcon; Antonio Gandarillas* (pp. 11-18)

The most important diseases affecting the quinoa crop in Bolivia. *Giovanna Plata; Antonio Gandarillas* (pp. 19-28)

TECHNOLOGY DEVELOPMENT

Generating varieties of quinoa in a market context and climate change. *Alejandro Bonifacio; Amalia Vargas; Genaro Aroni* (pp. 29-35)

Parasitoid complex associated with the quinoa moth - key crop pest in the Bolivian Altiplano. *Reinaldo Quispe; Raúl Saravia; Miguel Barrantes* (pp. 36-45)

Lepidoptera associated to quinoa crop in the Bolivian Altiplano: Taxonomic updating. *Raúl Saravia; Reinaldo Quispe; Luis Crespo* (pp. 46-52)

Bacteria associated to quinoa crop in the Bolivian Altiplano and its biotechnological potential. *Noel Ortuño; Mayra Claros; Claudia Gutiérrez; Marlene Angulo; José Castillo* (pp. 53-61)

Synthesis and development of sex pheromones for two noctuidae, key pests of the quinoa crop. *Raúl Saravia; Luis Crespo; Reinaldo Quispe; Milton Villca* (pp. 62-67)

Mass diffusion of the Integrated Pest Management (IPM) strategy in quinoa. *Vladimir Lino; José Olivera; Raúl Saravia; Reinaldo Quispe, Edson Gandarillas, Luis Crespo* (pp. 68-72)

Native shrubs and their perspectives contribution to the quinoa production sustainability. *Alejandro Bonifacio; Genaro Aroni; Milton Villca; Miriam Alcon; Patricia Ramos; Liz Chambi* (pp. 73-83)

Formation of the *Chenopodium quinoa* Willd (Quinoa) core collection in Bolivia with morphological and molecular data. *Silene Veramendi; Ximena Cadima; Milton Pinto; Wilfredo Rojas* (pp. 84-91)

Potential uses of the genetic diversity of quinoa in agribusiness: Opportunities and challenges. *Wilfredo Rojas; Milton Pinto; Amalia Vargas* (pp. 92-99)



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Cover Photo:

*Royal quinoa crop
in the Southern Altiplano of Bolivia*

PROINPA Foundation

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EDITORIAL

Quinoa, a God's gift of the past, present and future

Quinoa (*Chenopodium quinoa* Willd.), an ancient Andean agricultural product, which probably went through different processes to satisfy the hunger of the Andean people, has also experimented a series of technical actions ranging from the collection and identification of germplasm to postharvest and its transformation.

Half a century ago, quinoa was a product of daily consumption for Andean populations in different forms, such as phisara, the phiri, the chaque, the kispina, the mucuna, the q'usa (chichi), chiwa and other delicacies that satisfied human nutritional needs; thus, rural families, miners and other social groups, had in their hands a golden grain of high value. In those years consumption was lower in the cities, to the point that there were generations that had not even tasted this food, to date this grain is sought by the World. This trend causes a rapid increase in the area cultivated with quinoa in Bolivia (currently more than 100,000 hectares), affecting fragile ecosystems by removing native vegetation, resulting in an imbalance of the referential trophic level, for example, the feeding of camelids and other wildlife, ignoring that everything is part of the whole, taking the risk of accelerating desertification processes.

To address this situation many institutions generate technology and disseminate knowledge. It is the case of PROINPA Foundation, which in the present edition of the "Revista de Agricultura", presents outstanding research results that should be used to inform policy making at national level and to establish the framework for a capacity building and applied research strategy, so that the cultivation of this ancient crop can be focused on three aspects: that it is accesible for a good life, that it is profitable in order not to frustrate farmers and the entire value chain, and fundamentally that it is sustainable to achieve effective food security in the country.

Fimo Alemán Daza
Chairman, Editorial Committee
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Presentation Revista de Agricultura

For the institutions that work on agriculture and technology development, quinoa is a success case, but also a concern. Indicators of production, processing and export have exceeded all expectations, generating significant resources for the country and mainly for farmers.

There is concern within all actors of the quinoa value chain, including the central government with regards to sustainability and how to pass from a temporary success known as the "quinoa boom" to a sustained success.

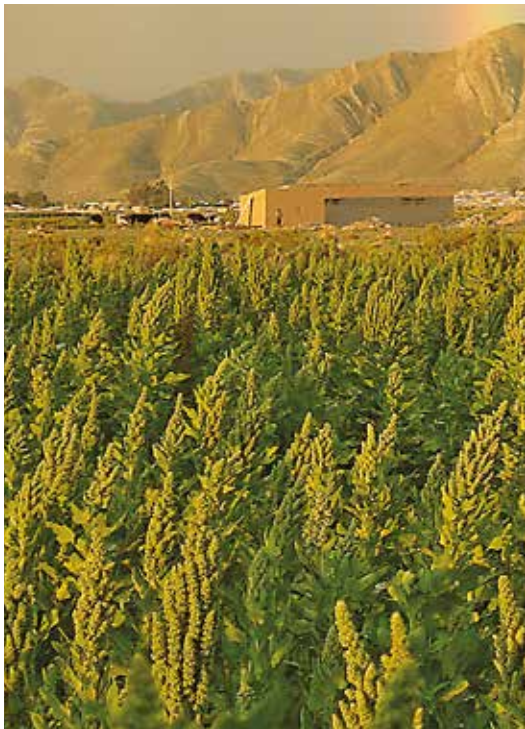
One of the key elements to achieve sustainability of production is the development of technology, in this line of thinking; the McKnight Foundation and DANIDA have invested and entrusted the contribution to this task to PROINPA.

In this issue of the magazine, several research pieces generated by PROINPA are summarized. The objective is to improve productive efficiency with a comprehensive view of the landscape and the quinoa production system as a whole, considering elements of integrated pest management, the use of varieties and seeds among others.

We hope that these articles contribute to foster knowledge about quinoa production in Bolivia and the world, and that they are useful to nurture a more sustainable and productive agriculture for farmers of the Bolivian Altiplano.

Ing. César Villagómez Villarroel PhD. (c)
Board Chairman of PROINPA Foundation

Quinoa crops in Bolivia



Economy of quinoa: Perspectives and Challenges

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Summary. Economy of quinoa: Perspectives and Challenges. Quinoa has become the main non-traditional export product from the Andean region and from Bolivia. This has enabled thousands of families to improve their income, a fundamental condition for the development of a country. The production and prices of quinoa are growing at a very fast pace, which threatens the sustainability of its production and its competitiveness. The loss of yield in quinoa is one of the clearest indicators of the situation, and of the implications on the loss of competitiveness and unsustainability of its production. This raises the urgent need for joint efforts to improve productivity, reversing the negative trend, as a major action to promote that benefits being generated by quinoa remain permanent for thousands of farmer families.

Keywords: Exports; Competitiveness; Sustainability

Resumen. Economía de la Quinua: Perspectivas y desafíos. La quinua se ha constituido en el principal rubro de exportación no tradicional de la región andina y de Bolivia. Esto ha permitido que miles de familias mejoren sus ingresos, condición fundamental para el desarrollo de un país. La producción y los precios de la quinua están creciendo a un ritmo muy acelerado, lo que pone en riesgo la propia sostenibilidad de su producción y competitividad. La pérdida en el rendimiento de la quinua, es uno de los indicadores claros de esta situación, y de las implicancias sobre la pérdida de competitividad e insostenibilidad de su producción. Esto plantea la urgencia de unir esfuerzos para mejorar la productividad, revirtiendo la tendencia negativa, esto como una de las principales acciones para promover que los beneficios que está generando la quinua para miles de familias de productores, sean duraderos.

Palabras clave: Exportaciones; Competitividad; Sostenibilidad

Background

The economic development of a country is a necessary but not sufficient condition for the existence of human development, understanding the later as a general development of the individual, in all of its dimensions (Guerra, 2012).

In order to promote economic development, countries establish policies that include actions to foster exports. As a country develops its capacity to export goods and services, it generates revenue

and strengthens its economic system. Exports drive demand for production factors, particularly labor; thus creating employment.

Usually countries classify exports in traditional and nontraditional, referring to their history and importance in the economy. In the case of Bolivia, the export of food is considered nontraditional, mainly because it is of recent inclusion in the context of the national economy.

Among the nontraditional exports, soy-bean and its byproducts stand out, with an export value of 685 million per year. The export statistics of nontraditional products show that the Andean region, and particularly the Altiplano region, both have a minor participation in exports. Nevertheless, it is important to highlight that the export of quinoa in these regions constitutes the main non-traditional export product of the Andes and of the Bolivian Altiplano, with a value of up to 153 million USD in 2013.

For a good or service to be exported, it must have comparative advantages. In the case of quinoa these advantages are manifested in the nutritional quality of the grain, the fact that it does not contain gluten and the uniqueness of the Royal Quinoa organic production.

Evolution of exports of Bolivian quinoa

According to data provided by the Vice-Ministry of Rural Development and

Agriculture (VDRA), export volumes of quinoa in 2013 have exceeded 35 thousand tons, equivalent to more than 153 million dollars. This represents a rise of 33 % in volume, in comparison to the year 2012; and an increase in over 90% of the export value of quinoa, also in comparison with the previous year. The FOB (free on board) price showed a record high in 2013 (6,000 USD/t in November) reaching an average of more than 4,300 USD/t, in comparison to 1,200 USD/t in 2006 (Figure 1).

Figure 1 highlights the growing trend of the export value of quinoa, where the primary endpoint was the price. This is generating a significant increase in the value of domestic exports of quinoa (Gandarillas *et al.*, 2014) and driving at the same time the production of this grain not only in Bolivia but also in different countries that see in quinoa a growing business opportunity because of the increasing international demand.

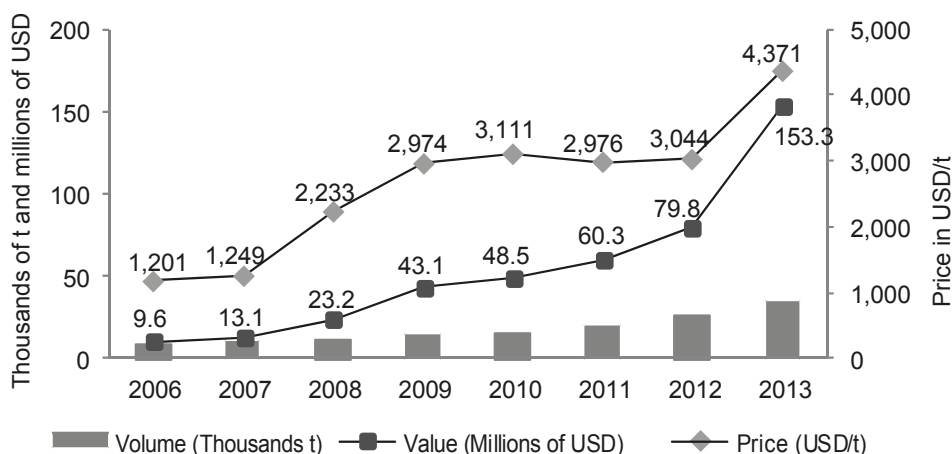


Figure 1. Price, quantity and value of quinoa exports in Bolivia

(Source: Own elaboration based on data from INE, IBCE, VDRA and CABOLQUI)

Evolution of quinoa production in Bolivia

The quinoa production in Bolivia has experienced a significant increase in the last decade, from a production of 23,000 tons in the year 2000 to above 61,000 tons in 2013 (Figure 2).

Such production volumes have also implied an increase in the cultivated area, ranging from almost 36,000 hectares in 2000 to more than 130,000 hectares by 2013 (Gandarillas et al., 2014).

This substantial increase in the cultivated area can be explained through the boom caused by the appreciation of consumers worldwide, for a food with the characteristics of quinoa. In addition to this, the declaration of the *International Year of Quinoa* by the United Nations, which after a series of national and international promotional events, has managed to position it among foods of the highest culinary standard; causing a sharp

increase in foreign demand for this product.

The quantity of quinoa produced has increased due to the expansion of the cultivated area, while yield reflects a clear downward trend (Figure 2) (Gandarillas et al., 2014).

Agricultural practices, little or no replacement of soil fertility, the intensity of the negative effects of climate change, population dynamics of pests and diseases and the use of grain as seed, are the elements that explain the negative trend in yield. This fact, in addition to the considerable increase in selling prices, has driven the generation of technology and varieties adapted to other regions of the country with benign climate for quinoa production. Nevertheless, the Southern Altiplano of Bolivia continues to be the major quinoa producing region in the country, where it is estimated that more than twenty thousand families live from the production and marketing of quinoa.

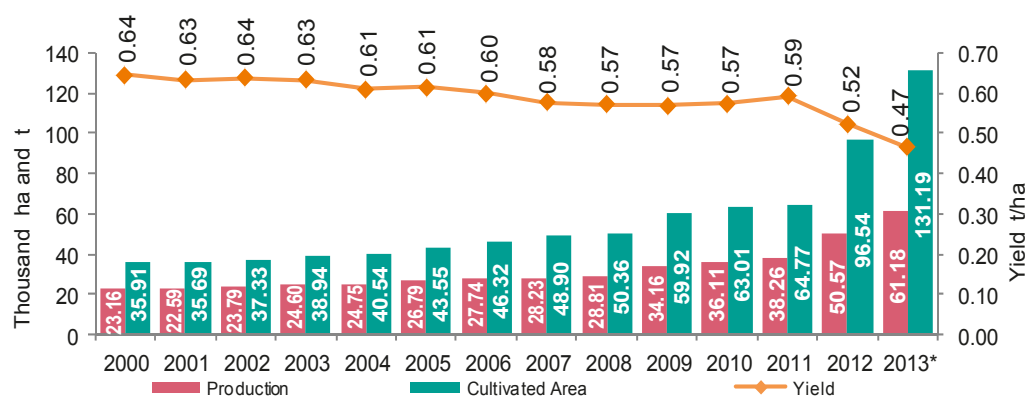


Figure 2. Quinoa production area and yield in Bolivia.

(Source: Own elaboration based on data from INE, IBCE, VDRA and CABOLQUI)

Economic effects of the quinoa boom

An important outcome of this whole situation is the economic effect that the quinoa boom has generated for every actor in the value chain. Producers, intermediaries, brokers and exporters, have benefited from price increases and from the international demand for quinoa. In the specific case of quinoa producing families, although their unitary costs of production have been increased mainly due to a downward trend in yield, sky-high prices in the export market have allowed their revenues to remain stable or even increase. This outcome has given farmer families the opportunity to implement modern technology to increase production, and at the same time to improve their quality of life (Figure 3).

Figure 3 shows an approximation of the benefits received by different actors from the quinoa value chain of, and the major costs they deal with. It can be observed that the decline in profitability of quinoa production, during the last years, has had a considerable effect on the unitary costs of production per ton, a fact that has led to a rise in prices in the domestic market as well as in the export price.

This has forced farmers to increase crop production areas, inputs (mainly chemical) and general efforts to sustain production volumes; in order to maintain their standard of living. Additionally, the expansion of cultivated area is detrimental to other sectors such as live-stock production (cameloid husbandry), threatening the balance of the local ecosystem.

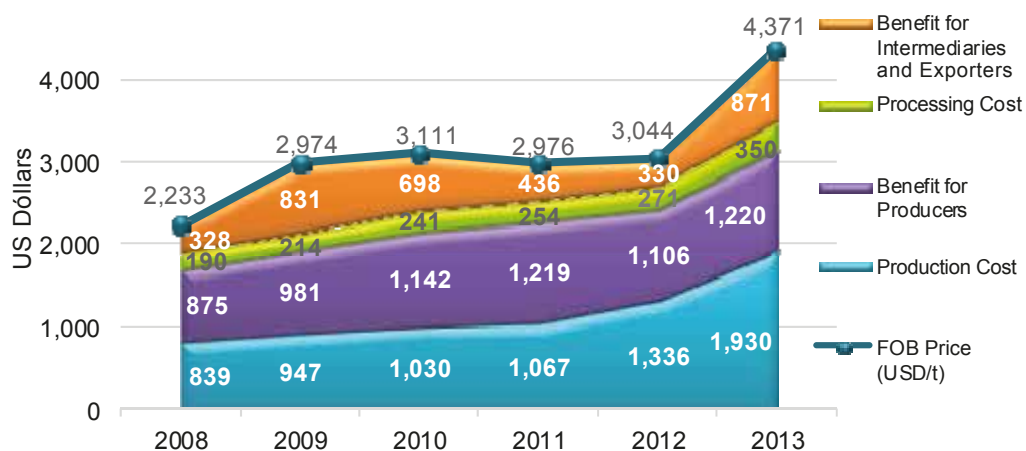


Figure 3. Cost ratio and benefit distribution USD/t
(Source: Own elaboration based on data from IBCE, VDRA and primary production costs information)

This constitutes a challenge for local authorities, institutions and quinoa producer organizations themselves; which to date are validating crop management strategies that enable them to maintain and improve farmer's living standards and the conditions of the local ecosystem.

According to Avitabile (2013), the rise in the price of quinoa has enabled farming families to improve their living standards, to have greater access to basic services, and to increase their access to education. It also indicates that while quinoa consumption has declined in the Southern Altiplano, the diet of farm families has been diversified; now having access to foods like fruits and vegetables, which was not possible before mainly due to economic and geographical problems. Despite the rise in the price of quinoa, 70% of surveyed households in the study by Avitabile mentioned that they consumed quinoa between 2-4 times a week.

Apparently farmers have also social motives that have privileged other products in their diets. In many cases, current quinoa producers, especially those that hold greater extensions of land and means of production, have experienced recent migration processes. Many of them have returned to their communities thanks to the quinoa boom, and usually do not stay to live permanently in the producing areas, but are now "resident" community members, meaning sporadic settlers. Migration processes have played an important role in changing the eating habits of "resident" producers, who now seek a diet similar to the one they had in the cities (Pacheco *et al.*, 2014).

Moreover, we must consider that quinoa was the main food in producing areas for a long time, and many producers at the ease they currently have to vary their diet, choose other foods that they previously could not access. This ease of access to a variety of foods is quite evident in the region known as "intersalar" located between the salt flatlands of Uyuni and Coipasa. People who have worked in this area for several years are aware of the number of stores, small restaurants and pensions that have opened in the region in recent years. This along with the improvement of roads and the enhanced purchasing power of the population has greatly increased food supply and its diversity (including processed foods).

An aspect related to the quinoa boom has been the creation and consolidation of the export industry. Consequently, in recent years there has been an increase in legally established enterprises that contribute to enhance grain quality (processing) and to open new and better channels of trade, facilitating the export of quinoa and improving conditions of input purchase and supply for producers. Some companies have forayed into the manufacture of products from flour and quinoa flakes, opening a new marketing channel to international markets and generating greater added value. Other actors who have been added to the value chain are the bulking intermediaries. Before, it was the producers who brought their produce directly to companies, now there is a specific segment of the population dedicated to grain bulking (both on farms and in popular markets). This has also contributed to the rise in prices and to an increase of informal trade for quinoa. This situation has forced

companies to invest more in order to keep their trade commitments, incurring in quinoa production of their own and creating new mechanisms to establish loyalty relations with primary producers.

Up to this point in the analysis, only the effects of the quinoa boom in the actors directly linked to the value chain have been mentioned. Nevertheless, changes in the national dynamics of quinoa consumption have also been observed. According to the Vice Ministry of Rural Development and Agriculture (VDRA), per capita quinoa consumption has increased from 0.35 kg in 2008 to 1.11 kilograms in 2012 (IBCE, 2013). This fact is explained by the government's social policies, such as maternity and nursing subsidies, and other policies that promote consumption of quinoa in cities and in rural areas. However, a segment of the population, who has seen how the prices of quinoa grain increased and how it moved from popular markets to luxurious city shelves, remains isolated.

Quinoa's international market trends

Quinoa has positioned itself in world markets. The trend of the international price of quinoa is reaching unimagined levels, which can be very encouraging in a short term perspective and for certain actors of the value chain. However questions emerge in terms of what can happen with the market and, until when and to what extent will prices continue to rise.

Clearly the expectation generated by a product of the characteristics of quinoa

along with its high prices, will motivate producers and governments from various regions of the world to start promoting local production. An example of this is the Anantha Project in India, which will invest millions of dollars to promote regional development and boost quinoa production (<http://www.apard.gov.in/project-anantha>).

The increase in quinoa's global supply is a matter of time (Zuckerman, 2014), therefore it is expected that as demand grows, prices will tend to stabilize. If prices stabilize at current levels Bolivian quinoa farmers, particularly organic quinoa producers, will continue to have favorable income assuming of course that efforts to stabilize crop yields are effective. In parallel, no significant increases in production costs are expected. Additionally, the organic feature of the Bolivian quinoa production would become the most important differentiation factor for Bolivia to maintain productive leadership.

The big challenge for Bolivian quinoa producers and for all actors in the value chain is to ensure the sustainability of production, particularly in the case organic quinoa.

The pressure exerted on production systems, the expansion of the agricultural frontier, the effects of climate change, and declining productivity, are the main causes that can lead Bolivia to lose its comparative advantages for quinoa production. Additionally, it should be considered that more and more countries are introducing quinoa in their production systems and therefore are improving their performance.

On the other hand, if the increase in international supply causes a significant drop in the international price of quinoa, Bolivian producers will still have profit, because production costs are relatively low. However, this situation would not be sustainable due to the factors mentioned in the preceding paragraph.

Obviously, if Bolivia intends to maintain world leadership in the production and marketing of quinoa, and to continue benefiting thousands of farmer families and other actors from the quinoa value chain, it is imperative that measures to promote sustainability and competitiveness of quinoa are adopted.

Much of these measures have to do with investment in research and technology development. Without it, it is impossible to reverse the negative trend in yields, a key indicator of the loss of competitiveness. Neither will it be possible to promote the sustainable management of production systems and landscape, nor achieve the agroecological production of quinoa. Another important group of measures has to do with the generation and enforcement of rules and regulations in relation to the sustainable production of quinoa.

While the Bolivian government is making efforts to allocate resources to the development of technology, the historical moment demands that this investment be proportional to the importance of quinoa for human development in the Altiplano region and for the national economy.

The importance of increasing quinoa yield

One factor that is making quinoa more expensive is the gradual yield reduction. To produce the same amount of quinoa there is a gradual need for more and more acreage, which means higher cost, and therefore the unitary cost tends to rise. If one also considers the growing demand, the result of this combination (yield reduction and increasing demand) is the expansion of the agricultural frontier, which brings along a number of other pro-problems, including social issues related to land tenure.

Cultivating a hectare of quinoa has an average cost of 6,044 Bs, considering the average yield of 0.45 t/ha, it produces a unitary production cost of 13,430 Bs/t (1,930 USD/t). If the negative trend of the quinoa yield could be reverted and the average yield of 0.6 t/ha restored, the unitary cost of production would drop to 10,073 Bs/t (1,447 USD/t). If additionally policies for the development of quinoa production are implemented, setting as a target to increase yield to at least 0.8 t/ha, the unitary cost would drop to 7,555 Bs/t (1,085 USD/t), which would allow a reduction in the selling price without reducing producers' profits.

Competitiveness, sustainability, affordability and accessibility of quinoa are possible as long as the production of quinoa takes an agro-ecological approach and one of continuous improvement of productivity. Improve yields of quinoa, with a focus on agro-ecological production, must be the premise of all stakeholders involved directly and

indirectly with the quinoa production complex. If this goal is not achieved, the Altiplano production systems will be in serious risk of disappearing and with them, the wellbeing achieved by thousands of quinoa farming families.

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- **Manzano** (Pie de Injerto variedad Maruba)
- **Duraznero** (Pie de injerto variedad GxN)

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The current and potential role of q'ila q'ila (*Lupinus* spp.) in quinoa sustainable production systems

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Summary. The problem of quinoa in the Southern Altiplano of Bolivia is generally described emphasizing the risks of unsustainable commercial production, due to environmental and human factors. The main factors affecting sustainability are erosion, low fertility, limited sources of organic matter (manure), reduction in the population of llamas, new land clearing for quinoa production, etc. While it is well recognized that manure is the main source of organic matter in quinoa production systems, the role of the diversity of species growing in the highlands, particularly that of wild legumes such as *Lupinus* spp. or known in Aymara as *q'ila q'ila*, must also be highlighted. These species adapted to the highlands have the particularity of germinating in the rainy season (December and January), growing in winter (dry and cold) and fruiting in the following months of January and February. This behavior of their life cycle and their ancient adaptation to the area turns these species into a priority for quinoa production systems. This study suggests that they should be considered as alternative or complementary to llama manure in quinoa production systems. This represents a much more feasible option for use in large areas, and with possibilities of playing a central role in the longed for sustainability of Bolivian quinoa production.

Keywords: Wild Legumes; Agricultural Practices; Natural Fertility

Resumen. El rol actual y potencial de las *q'ila q'ila* (*Lupinus* spp) en sistemas de producción sostenible de quinua. La problemática de la quinua en el Altiplano Sur de Bolivia generalmente se describe haciendo énfasis en los riesgos de insostenibilidad de la producción comercial, por efectos ambientales y antrópicos. Los principales factores que inciden en la sostenibilidad son la erosión, baja fertilidad, escasez de fuentes de materia orgánica (estiércol), reducción de la población de llamas, avance de la frontera agrícola de la quinua, etc. Si bien es reconocido que el estiércol es la principal fuente de materia orgánica en los sistemas productivos de quinua, también debe resaltarse el rol de la diversidad de especies que crecen en el altiplano, haciendo énfasis a las leguminosas silvestres como *Lupinus* spp. o conocidas en Aymara como *q'ila q'ila*. Estas especies adaptadas al Altiplano, presentan la particularidad de germinar en la época de lluvias (diciembre y enero), crecer en invierno (seco y frío) y fructificar en los meses siguientes de enero y febrero. Este comportamiento de su ciclo biológico y su adaptación milenaria a la zona, las convierte en especies de interés prioritario en sistemas de producción de quinua. El presente estudio plantea que deben ser consideradas como fuentes alternativas o complementarias al estiércol de llama en sistemas de producción de quinua. Esto representa una opción mucho más factible para su aplicación en grandes superficies, y con posibilidades de jugar un rol central en la ansiada sostenibilidad de la producción de quinua boliviana.

Palabras clave: Leguminosas Silvestres; Prácticas Agronómicas; Fertilidad Natural

Background

The most important area of quinoa (*Chenopodium quinoa* Willd.) production in Bolivia is the Southern Altiplano, so when the issue of organic Royal Quinoa is addressed, many authors (Jaldín, 2010; Medrano, 2010; Puschiasi, 2009) focus on the “intersalar” region (strip between the salt flats of Uyuni and Coipasa). However, quinoa production has surpassed the intersalar region and is cultivated throughout the Southern Altiplano, including the Central Altiplano, where environmental problems are already visible.

According to Soraide (2011) and Orsag *et al.* (2013), soils in the Souther Altiplano are coarse textured (sand content above 70% and gravel > 30%), especially in lower strata.

Due to the predominance of sand in the soil and the low content of organic matter (less than 1%), they do not form stable aggregates and have a weak or null degree of structuration. This situation favors their high susceptibility to wind and water erosion, particularly under excessive tillage (use of agricultural equipment).

The great international demand for quinoa, along with high prices, creates a great opportunity for thousands of families who have lived in poverty for generations. This has led to overexploitation of soils and expansion of the agricultural frontier, so that the area under cultivation has increased from 35,907 ha in the year 2000 to 131,192 ha in 2013, with a negative trend in yields (Gandarillas *et al.*, 2014).

With the quinoa boom, in the late nineties, agroecological imbalances arise (VSF / CICDA, 2009). The expansion of the agricultural frontier in the Southern Altiplano has had implications in the reduction of areas for grazing of llamas; in addition, the irregularity and reduction of rainfall results also in shortage of pasture.

It is important to note that llama's husbandry is not competitive with quinoa production. This leads to changes in the land use of the Southern Altiplano, from its livestock farming aptitude to agricultural use. In addition, grazing llamas (plots are distributed erratically) is inconsistent with quinoa production, since animals enter the fields and consume growing plants, generating serious disputes between producers. Under these conditions, grazing involves more herdsmen of the appropriate age to manage the herd.

Soil management has many deficiencies, being the most crucial poor incorporation of organic matter and lack of crop rotation options. Fallow periods for the recovery of soil fertility last several years, but due to low humidity and low average temperature in the region, the repopulation of native species is slow. Thereby, restoration of nutrients and organic matter is slow, taking in some cases over 10 years for the recovery of biomass and the replacement of soil microbial activity.

Farmers do not add organic matter or if they do it is in small quantities, because there is not enough availability in the area. The conservation and improvement of soil fertility depends on the actions of man on his environment, or more precisely on the actions on soil and plants; highlighting the

interaction plant-environment-man-society (Puschiasi, 2009).

In summary, the pressure on soils degrades them and makes them unproductive. The lack of organic matter makes them more susceptible to erosion and limits the increase in productivity.

The highest production and export volumes registered in recent years, are achieved based on the incorporation of new areas of production and not on the increase in yield per unitary area. This situation can lead to an unprofitable and uncompetitive agriculture, boosting rural-urban migration again, resulting in thousands of farm families returning to poverty; thus losing the country the unique opportunity of generating an income of more than 150 million dollars.

The solution or mitigation of the unsustainability of quinoa production is concurrently addressed by public entities, development projects and producer organizations. Among the existing alternatives is an increased use of manure through the repopulation of cameloids, protection of grazing areas, implementation barriers both physical and biological, etc. However, no proposal includes native legume species that can become part of a sustainable production system. This is the case of *q'ila q'ila* that grows in the highlands and whose importance has not been assessed in quinoa production systems.

Taking advantage of these promising native and wild species requires knowledge of their reproductive characteristics, physiology of their seeds, their ecological adaptation, the amount of organic matter they produce, the

symbiosis generated with nitrogen-fixing bacteria, etc.

Faced with the problem of sustainability of quinoa based production systems on the Southern Altiplano, researchers from PROINPA Foundation have explored the current and potential role of native species, giving priority to native and wild legumes.

For this purpose prospections and collections of several species of *Lupinus* spp. have been conducted in different localities of the Southern Altiplano, transition areas of the South-Central Altiplano, Central and Northern Altiplano. The genetic diversity of species was examined through their growth habit, ecological adaptation, seed formation, seed viability, symbiosis with *Rhizobium* and experimental planting. The adaptation of species has been studied in relation to micro-areas within the South and Central Altiplano, taking into account the topography and landscape of the areas; differentiating flatlands, hillside, foothills and steep hills.

Alternatives to increase organic matter in the soils of the Southern Altiplano

The most promoted alternative to increase the availability of manure in the Southern Altiplano is the reestablishment of the balance between the population of llamas and the acreage of quinoa. In this sense, it is proposed that for every hectare of quinoa seven llamas are kept (Anze, 2010). It is unquestionable that traditional grazing areas should be protected to raise llamas along with the management of

pasture and forages. However, in practice it is very difficult to implement and to achieve the desired result. For example, in the year 2010 a total of 63,010 ha were harvested and in 2013 the acreage increased to 131,192 ha (Gandarillas *et al.*, 2014). This would mean that the population of llamas should be increased by about 477,000 animals, which is difficult to achieve.

According to the knowledge of researchers and decision makers, the Altiplano is characterized as an altitude ecoregion, dry, cold and semiarid; where quinoa is grown and where few native species develop. However, a careful examination, can demonstrate the presence of a large diversity of native and naturalized species. To date their adaptive characteristics are not valued and neither are their potential benefits for quinoa production systems.

Among the native and naturalized species existent, there are **shrubs**: *Parastrephia lepidophylla* (Weddell) Cabrera, *P. quadrangulare* Meyen Cabrera, *P. lucida*, (Meyen) Cabrera, *Baccharis tola* Phil., *B. tricuneata* (L.f.), *Lamphapa castellani* Mold.; **pastures**: *Nassella pubiflora* (Trin & Rupr), *N. nardoides* Phil., *Festuca ortophilla* Pilg., *F. dolychophilla* J. Prsi., *Chondrosium simplex* (Lagasca Kunth); **legumes**: *Lupinus ottobutchini* C.P. Sm., *L. montanus* C.P. Sm., *L. chilensis* C.P. Sm *L. cuzcensis*, C.P. Sm., etc.; whose ancient adaptation allows its validity, but human activity is reducing their populations.

The native legume *q'ila q'ila*

Q'ila q'ila (pronounced as qela qela) is a generic name in Aymara language, which refers to several legume species used as forage in green and dry state (*Lupinus chilensis* CP Sm., *L. otto -butchini* CP Sm.), and others used as forage only when dry or cured (*Lupinus montanus* CP Sm.). These features should be complemented with research on anti-nutritional principles that some species usually contain.

In the literature studied, the scientific names do not include a detail of the collection areas, let alone mention the native names of the species. Therefore, for now, it is better to name them as plural species (*Lupinus* spp.). Species from the Altiplano are known with native names *q'ila q'ila*, *salqa* or *salqiri*. The plants usually grow in colonies, depending on the natural seed dispersal and favorable conditions for colonization, ranging from small areas of 100 m² to 200 m² or even areas of up to 1 km².

While the taxonomic classification of species is not clear, the diversity of species and the genetic diversity within each species or ecotype are evident. Jacobsen *et al.* (2006) listed 83 species for the area of the Andes. Meanwhile Barney (2011) cites 85 wild species for South America.

The adaptation of species is large; however, some ecotypes prefer specific environmental conditions such as sandy or stony soils, loamy soils, gravelly or stony soils, or otherwise flatlands, slopes, foothills and mountains.



Lupinus sp. on slope



Lupinus sp. in flatlands

The biology of the species of *Lupinus* presents several differential biologic processes in comparison to other plant species, among them seed dormancy, high tolerance to frost, abundant nodulation (N fixation), broad ecological adaptation and high capacity for diversification.

The seed of wild lupines is viable and can germinate when it reaches physiological maturity, before drying. However, as it dries, it enters a prolonged dormancy of three to four years, after that time the dormancy ends and it can germinate naturally. This means that prior to a directed sowing process seeds must be pretreated.

Q'ila q'ila seedlings emerge in late December and January, are established in the field between January and February,

grow slowly in winter, bloom in November and December, and fructify from December to January, according to the different areas and ecotypes. What stands out is their adaptation to highlands, their tolerance to winter frost (-18°C from May to September) and their resistance to prolonged droughts (from March to December). By staying alive during the winter, they provide ground cover, develop abundant green material and fix nitrogen. All of these beneficial processes take place in the period when quinoa is not being produced, that is, in the fallow period. These characteristics give guidelines for the use of these species for enhanced management of fallow periods, ground cover and atmospheric nitrogen fixation. Their ability to grow on marginal periods, their pluri-seasonal and even multi-year cycle, makes them very appealing species for their use in quinoa production systems in the Southern Altiplano.

Currently *q'ila q'ilas* are not valued for their adaptive qualities, organic matter production and nitrogen fixation. Some farmers do recognize their contributions for soil management, because when they clear land previously populated by these species, quinoa grows and yields well. The possible explanation for the lack of interest in these species is their erratic growth due to seed dormancy. In natural conditions, plants are born every three to four years at the same site. This characteristic of the species makes them difficult to manage without prior application of seed treatment techniques. Therefore, the current role of wild species from the genus *Lupinus* in the Altiplano is of little or no importance for quinoa production systems.

Perspectives of wild lupines for the sustainability of quinoa production systems in the Southern Altiplano

Wild legumes produce considerable amounts of dry matter. It is estimated that on average they can produce the equivalent of eight tons of dry matter per hectare, without considering nitrogen fixation estimated at over 100 kg/ha. The benefits of legumes in cropping systems are widely documented (Prager *et al*, 2012; Céspedes, 2005), which supports the proposal of using wild legumes in quinoa production systems.

The proposal of guided usage of these lupin species involves planting in late December and early January. Plants grow in autumn, winter and summer (cold and dry seasons) and produce seed during the rainy season. It is important to highlight that the conclusion of the life cycle of the q'ila q'ila matches the period of soil preparation in the Altiplano. It therefore becomes a valuable local source of green matter to incorporate in the soil, with the advantage of not being a woody plant, which makes its decomposition easier. In

this case, wild legumes are another source of organic matter in quinoa production systems. An alternative option for the management of *q'ila q'ila* is to harvest seed before incorporating the complete plant into the soil.

The sustainable production approach involves the management and utilization of soil to achieve high productivity per unitary area, maintaining soil health, and including legume species in rotation systems and improved fallow management practices.

In the case of the Southern Altiplano, where conditions for agriculture are so hard, wild legumes are an excellent alternative to establish rotation systems, improve fallow management practices, produce green manures and establish crop tandem systems with quinoa. These practices offer numerous benefits: reducing erosion and improving soil fertility, improved yields, reduced sediment transport and diversification in production. In this line of analysis, eight tons of dry matter incorporated by q'ila q'ila to the soil may be sufficient to achieve a yield of quinoa of twenty quintals per hectare.



Lupinus sp. in tandem in a commercial plot quinoa



Lupinus sp. in a seed plot



Flowering colony of *q'ila q'ila*
(Llavica at 4230 msnm, August 18, 2014)



Dust covered *q'ila q'ila* plant
at a time when quinoa is not produced

For crop rotation purposes, after quinoa harvest, *q'ila q'ila* should be sown; and after two growing seasons the soil will again be available to grow quinoa. For improved fallow management practices, *q'ila q'ila* can be planted in any sequence of fallow years, provided planting is made at the appropriate time (late December and early January).

For intercropping in tandem, in a field set with quinoa plants in panicle phase, *q'ila q'ila* should be planted interspersed or between rows or lines. This involves planting interlayer on deferred date (tandem or in posts).



Sowing in tandem with a
double trench seeder prototype

Due to all that is known so far about the *q'ila q'ila*, productivity, sustainability and competitiveness of Bolivian quinoa can be seen optimistically. The challenge posed by the Bolivian government of increasing the area of cultivated quinoa, may be feasible with the use of wild legumes as alternative and complementary to the use of llama dung.

Challenges and limitations in the management of wild lupine

There are several limitations that must be addressed to achieve the commercial cultivation of wild lupines, which in the field of agronomy are essentially: the gradual fruiting, dehiscence of seed and seed dormancy.

Other issues to be addressed are: seed production on a commercial scale, isolation and inoculation of seeds with nitrogen-fixing bacteria, weevil control *Apion* sp., the use of equipment and machinery for planting and harvesting, and mechanized incorporation of organic fertilizer, among others.

In addition, research programs should be established on agronomic management, multiple use (coverage, organic matter, forage), microbiology, etc. A first task to undertake, given the great diversity of species and morphotypes, is the establishment of a working collection, for the purpose of characterization, selection and targeted use.

For the efficient use of *q'ila q'ila* a vital challenge is the participation of producers in the maintenance of natural areas where these species grow, timely seed collection, directed planting, appropriate incorporation in the soil, and other practices.

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The most important diseases affecting the quinoa crop in Bolivia

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Summary. The objective of this study was to determine the diseases that affect quinoa in Bolivia from germination to harvest, in the agro-ecological regions of the Central Altiplano, Northern Altiplano, and the Inter-Andean Valleys. The most important disease in economic terms is mildew, caused by *Peronospora variabilis*. The other diseases that have been detected are of secondary importance, yet due to the expansion of areas and production systems, they can turn into problems of primary importance. These problems correspond to the death of plants (*Rhizoctonia solani*, *Fusarium* sp.) a disease that manifests itself during seedlings emergence; if plants survive this problem it subsequently manifests itself as wilting and is called "Fusarium wilt". During plant development the "green mold" (*Cladosporium* sp.) appears, almost simultaneous or subsequent to the appearance of mildew and the "leaf spot" (*Ascochyta* sp.). At senescence, when tissue is lignified, the "ogival spot" (*Phoma* sp.) appears. At any stage of development symptoms caused by viruses (yellowing, shortening of internodes, etc.) can appear with a low incidence, less than 5%.

Keywords: Mildew; Fusarium wilt; Viral Diseases; Phytopathology

Resumen. Enfermedades más importantes que afectan al cultivo de la quinua en Bolivia. El objetivo del presente estudio fue determinar las enfermedades que afectan al cultivo de quinua en Bolivia, desde la germinación hasta la cosecha en las zonas agroecológicas del Altiplano Centro, Altiplano Norte y Valles Interandinos. La enfermedad más importante a nivel económico es el mildiu, ocasionada por *Peronospora variabilis*; las otras enfermedades que se han detectado son de importancia secundaria, aunque por la ampliación de las zonas y los sistemas de producción, pueden tornarse en problemas de importancia primaria. Estas enfermedades corresponden a la muerte de plantas (*Rhizoctonia solani*, *Fusarium* sp.) enfermedad que se presenta a la emergencia; si las plantas sobreviven a este problema posteriormente se manifiesta como una marchitez y recibe el nombre de "fusariosis". Durante el desarrollo se presenta el "moho verde" (*Cladosporium* sp.) casi simultánea o posterior al mildiu y la "mancha foliar" (*Ascochyta* sp.). A la senescencia, cuando el tejido está lignificado, aparece la "mancha ojival" (*Phoma* sp.). En cualquier fase de desarrollo también pueden presentarse síntomas ocasionados por virosis (amarillamiento, acortamiento de entrenudos, etc.) que presentan incidencia baja, menor al 5%.

Palabras clave: Mildiu; Fusariosis; Virosis; Fitopatología

Introduction

The strong international demand for quinoa (*Chenopodium quinoa* Willd.) in recent years, has led to a significant

increase in cultivated area in a very short period of time (Gandarillas *et al.*, 2014). Commercial cultivation has expanded from the traditional area of the Southern Altiplano where Royal Quinoa is grown,

to new areas of "expansion" in the Central and Northern Altiplano, and the inter-Andean valleys. This situation has led to face new problems, mainly due to moisture. In the south, environmental conditions are very dry, with an annual average precipitation of 250 mm, while in the Central and Northern Altiplano there is an average precipitation of 500 mm/year, and in the valleys it reaches 800 mm/year.

This article attempts to update information on the incidence and significance of the main fungal diseases that affect quinoa, with emphasis on the "expansion" areas, in the new context of climate change, based on the publication of Tapia *et al.*, 1979.

The objectives of this work were:

- Identify and characterize the diseases affecting quinoa cultivars with emphasis on expansion areas (Central Altiplano, Northern Altiplano and inter-Andean Valleys).
- Categorize diseases according to their economic importance.

Materials and methods

This study was based primarily on visits and sampling to 50 quinoa plots in different production areas (10 to 12 plots per area).

Samples were taken of the different diseases that appeared throughout crop development, considering the weather conditions at the time of sampling. This work was carried out from November

2013 to April 2014, with a season characterized by high precipitation. Samples of seedlings and plants in various stages of development, and of different types of tissues (leaves, stem, roots and grains) were collected.

The samples were collected in plastic bags and in a refrigerated container to keep samples fresh, each one being identified according to the collection site. The samples were taken to PROINPA's laboratories in the shortest time possible, and immediately processed using routine techniques, ie direct microscope observation of signs, humid chamber and seeding in culture media (Potato Dextrose Agar). Taxonomic keys developed based on morphological characteristics of the different kinds of fungi (Barnett, 1960), were used to identify the structures.

Results and discussion

The presentation of results is made based on the appearance of diseases in the crop, according to the phenological stage at which they arose:

⇒ Diseases arising from emergence to the appearance of 6-8 true leaves:

At the beginning of the cultivation cycle samples were taken from dead seedlings in cotyledoneal phase (emergence); a constriction at the neck of the plant (Figure 1) was observed. With no movement of nutrients and water to the stem, there was a massive seedling death. Digging the ground, in places where there was no emergence of seeds, seedling death was evident.



Figure 1. Quinoa seedlings with neck constriction at ground level

This symptom of strangulation was diagnosed in the Central and Northern Altiplano, and the inter-Andean valleys. In the field it appears as an irregular emergence (Figure 2), little perceived by farmers, usually attributed to soil problems, salinity or lack of moisture.

The disease occurs in years of excessive moisture, soils with high clay content, fertilized with partially decomposed manure and poor drainage. Once the disease is present it remains in the soil attached to the roots and stems of dead plants, as conservation structures (chlamydospores in the case of *Fusarium* and sclerotia for *Rhizoctonia*).



Figure 2. Plot of quinoa with low plant emergence

Due to several factors that influence the emergence, it is estimated that the death of seedlings due to *Rhizoctonia* and/or *Fusarium*, is of up to 10%.

⇒ ***Diseases that occur in the phenological phases of branching, panicle formation, flowering, grain formation and physiological maturity***

As the plants continued to develop, in the branching phase (eight true leaves) and/or panicle formation (inflorescence begins to emerge from the apex of the plant), in some areas of production and depending on weather conditions; chlorotic spots appeared on the leaves (Figure 3). Depending on the variety these spots can be red, orange, brown or black.



Figure 3. Plants with chlorotic spots (top) and red spots (below) caused by *Peronospora variabilis*

As the disease progresses, the undersides of leaves present a grayish sporulation (Figure 4), which corresponds to *Peronospora variabilis*¹, causal agent of the quinoa mildew. Depending on the humidity, this disease can remain until harvest. The yellowing of leaves reduces the photosynthetic capacity and also causes defoliation of the plants (Figure 5), thus yields are reduced.



Figure 4. Leaf with sporulation on the underside



Figure 5. Severe defoliation caused by mildew

¹ Formerly known as *Peronospora farinosa* f.sp. *chenopodii* (Fr.) Fr, works of Choi *et al.*, 2008 and 2010 have reclassified this causal agent through molecular techniques using rDNA intergenic regions.

P. variabilis is a pathogen of easy dispersion (wind and rain). During crop development dissemination structures are mainly spores, however at senescence or absence of crop, the disease is spread by oospores (sexual reproduction structures) that may be adhered to the surface of the grain or inside the stubble that remains in the field. Therefore dissemination occurs, at close range by means of spores, and at long range through oospores.

Mildew occurs in all agro-ecological regions, with varying severity depending on environmental conditions (high relative humidity, above 80%). In the Southern Highlands it only appears when rainfall is intense and prolonged. When the rainy period stops automatically the mycelium is dried and the disease progression is interrupted. Varieties of royal quinoa are susceptible to this disease.

The losses caused by mildew depend on the phenological phase at which the plant is attacked and the degree of resistance of the variety. When susceptible varieties are grown and favorable weather conditions are given, especially high relative humidity, the effects of mildew are severe. If the attack occurs in early stages of plant development, production can be completely lost. In resistant varieties, losses fluctuate between 20% and 40% (Danielsen *et al.* 2003). Therefore, economic losses range from 2-5 quintals, which depending on the price of quinoa can be very significant. Consequently, for the year 2014 losses could be equivalent to 6,000 Bs/ha.

Coinciding with the appearance of mildew in the panicle formation and flowering stages, on the chlorotic spots, a green sporulation was observed on the top side of leaves (Figure 6). The causal agent is *Cladosporium* sp. which depending on humidity, can cover the entire surface of the spots (Figure 7) and accelerate the falling of leaves. Just like mildew this disease can occur up to harvesting stage.



Figure 6. Beginning of green sporulation



Figure 7. Leaf with abundant sporulation

When there is excessive humidity the pathogen spreads from the foliage to the panicle, and it tends to turn it black (Figure 8). Depending on the stage of the filling phase, when the attack takes place, the grains may even remain empty. If the grains are already formed, they will be slightly stained. When processing these grains in the laboratory, it was observed that *Cladosporium* conidia remain attached to the surface of the grain, being



Figure 8. Darkened panicle caused by *Cladosporium* sp

its mode of dissemination and survival for the next planting season.

Since this disease is strongly associated with mildew, when damage occurs in the panicle, economic losses due to the green mold would produce at least a 5% loss in addition to the losses caused by mildew.

At the same time, during the development of plants in places where there is poor drainage or where water accumulates, a symptom of wilting and yellowing occurs. At the beginning wilting is only apical but later it is widespread, it even causes the death of plants.

Going through the root system, lesions sunk in the neck of the plant are observed, similar to those found during the

emergence stage. Taking the full root system from the soil, necrosis of the main root and rootlets is observed (Figure 9). If winds occur during this condition, plants fall (Figure 10).

Due to excess moisture, the surface portion (skin) of the root decomposes quickly, exposing the underlying tissue to attack by other pathogens. The diagnosis of these samples shows that the symptom corresponds to *Fusarium* sp. Therefore, this pathogen appears in pre and post-emergence, and may manifest again during crop development. The way to recognize diseased plants is through their weak development in comparison to healthy plants.



Figure 9. Lesions to the neck with decomposition of the root epidermis



Figure 10. Wilting and yellowing of plants caused by *Fusarium* sp.

This disease has been observed mainly in areas close to Lake Titicaca (Northern Altiplano) and to a lesser extent in the Central Altiplano, in areas with heavy soils and poor drainage.

In the case of inter-Andean valleys, similar symptoms are observed in adult plants. Other plants with little development simply show a pink color on roots and rootlets (Figure 11). An analysis of these roots shows that symptoms can also be attributed to *Fusarium* sp.

During development, necrotic spots have also been observed on the foliage. These are roughly circular or irregular in shape, with beige centers and slightly brown edges (Figure 12), inside which pycnidia are present (black dots). The size of the lesions ranges from 5 to 10 mm in diameter. Work done in the laboratory evidenced that the causal agent of this disease is *Ascochyta* sp. and the common name of the disease is "leaf spot".

When attacks are severe, intense defoliation occurs, thus reducing photosynthetic capacity; and if the panicle is in formation, the quality of grains is affected (brown color).

Studies of the grains conducted in laboratory show that the fungus produces abundant pycnidiospores accompanied by a mild to severe necrosis in roots and/or hypocotyl. Severely affected seedlings die.

Therefore, the disease is transmitted through seeds; pycnidia remain attached to the surface of the grains and also stay in the stubble.

For a new disease cycle to occur in the next season a temperature of 18 to 24 °C and a relative humidity of 80% is required, the pycnidia germinate and give rise to pycnidiospores and the infection initiates again.



Figure 11. Pink roots caused by *Fusarium* sp. in quinoa



Figure 12. Circular spots with pycnidia inside

The geographical distribution of this disease is not exactly known and apparently it is not of major economic importance (Danielsen *et al.*, 2003).

Finally, at the stage of senescence, when the stem is lignified, ogival shaped lesions can be observed. These lesions are light gray in the center with brown edges, surrounded by an aura of glassy appearance, inside which you may notice small black spots (Figure 13) which correspond to pycnidia of the fungus *Phoma* sp. The size of these lesions ranges from 2 to 3 cm. When conditions are favorable (especially high relative humidity) a number of spots can be seen in a single stem (Figure 14). During severe attacks these spots grow and reach each other, covering the entire area of the stem.

From season to season, the fungus survives in the stubble that remains in the field. This pathogen does not require mechanical wounds to enter the plant, it enters through natural openings.

This disease has been observed in the Central and Northern Altiplano, and in the inter-Andean valleys, and basically its

occurrence depends on the presence of inoculum and high relative humidity.



Figure 13. Ogival spot in the stem with pycnidia inside



Figure 14. Several ogival spots in the stem and lateral branches

In general, in all production areas of Bolivia plants from all stages of development (from emergence until harvest), with virus infection symptoms, have been observed. The causal agents have not been determined, yet what is known is that quinoa is used as universal

plant (indicator plant) for the detection of various viruses. The most frequent symptoms observed in quinoa are: yellowing, shortening of internodes (rosetting), leathery leaves, etc. (Figure 15).



Figure 15. Quinoa plant with yellowing (left); shortening of internodes, leathery leaves, yellowing and little development (right); characteristic symptoms of the incidence of virus infections in this species

Conclusions

- The most important disease is the "quinoa mildew", caused by *Peronospora variabilis*, followed by "green mold" and "seedling death." These three diseases occur in different agro-ecological regions of Bolivia.
- The incidence of "leaf spot" is low and the symptoms have been observed in the Central and Northern Altiplano and inter-Andean valleys, without causing significant economic losses.

- The "Ogival spot" appears at senescence, affecting the stems and rachis of the panicle. It has no incidence in reducing quinoa grain yield.
- The seed is a source of inoculum for "mildew", "green mold" and "seedling death".
- Economically "mildew", "green mold" and "seedling death" can result in losses of up to 50%.

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Breeding varieties of quinoa for a market and climate change context

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Summary. The article describes the technological implications of the commercial production of quinoa in the South and Central Altiplano of Bolivia, highlighting the expansion of acreage and the flow of varieties drawn from one area to another. These issues imply a process of maladaptation of varieties, which produces susceptibility to mildew, difficulties to complete the production cycle, spontaneous variation, lack of purity, varietal heterogeneity and poor usage quality. The document presents the characteristics of the varieties generated, with specifications of their adaptation range and product quality, and concludes with the challenges faced by quinoa breeding.

Keywords: Adaptation; Variability; Breeding

Resumen. Generación de variedades de quinua en un contexto de mercado y cambio climático. El artículo describe las implicaciones tecnológicas de la producción comercial de quinua en el Altiplano Sur y Central de Bolivia, resaltando la ampliación de la superficie de cultivo y el movimiento de variedades de una zona a otra. Lo anterior implica un proceso de desadaptación de variedades que se traduce en susceptibilidad a mildiu, dificultades para completar el ciclo productivo, variación espontánea, falta de pureza, heterogeneidad varietal y baja calidad de uso. El documento presenta las características de las variedades generadas con especificación de su rango de adaptación y calidad, y concluye con los desafíos que enfrenta el mejoramiento genético de la quinua.

Palabras clave: Adaptación; Variabilidad; Mejoramiento Genético

Introduction

Quinoa has evolved in the Andes. It was domesticated by men and women from civilizations like the Tiwanaku and the Inca. Until the early eighties, the Andean people cultivated quinoa due to their advantages in adaptation to altitudes between 2800 to 4000 m.a.s.l., and semi-arid climates, and for its nutritional benefits.

The main product of the quinoa plant is the grain, highly appreciated for the preparation of traditional dishes in a way

somewhat similar to rice. The leaves can also be eaten fresh and, by products from harvesting and threshing are destined for animal feed (llamas).

Quinoa comprises a wide diversity of native varieties adapted to specific areas of the Central, Northern and Southern Altiplano, and valleys. In the highlands, quinoa is the main food crop for the inhabitants of this area. In the Southern Altiplano, quinoa is the only crop that thrives acceptably, this is why researchers describe the production system as a quinoa monoculture.

With the discovery of its nutritional properties and its promotion, quinoa has become an important export product and an important source of income generation.

Currently, quinoa is a product with high demand in the market, with encouraging prices for producers, processors and traders, creating an important economic activity for the country.

The demand and high prices of quinoa have stimulated the expansion of acreage, covering new areas of production. This expansion involves changes in the production process and new technological needs on several fronts: management of pests and diseases, plant nutrition, seed varieties and agronomic management.

Climate change, in turn, has led to changes in rainfall and new technological demands, such as earlier varieties. On the other hand the market demands various colors of quinoa and more suitable varieties for industrial processing.

In this context, PROINPA has undertaken the development of a new generation of varieties focused on the following priorities:

- Early large grain varieties for the Southern Altiplano, necessary for late planting, for re planting in case of drought losses or seedlings buried by strong winds, and for their shorter time to harvest.
- Early large grain varieties with mildew resistance for the Central Altiplano.
- Mildew resistant varieties for the Northern Altiplano and valleys.
- Bitter (with saponin) and sweet (saponin free) varieties for specific market demands and, for local and family consumption of quinoa. These varieties have the advantage of facilitating grain processing.
- Varieties for agribusiness (flour and flakes) and for nutritional objectives (nutraceuticals).
- In all cases, varieties suitable for mechanical harvesting are also sought, mainly due to their drive towards competitive, sustainable and resilient production in the occurrence of adverse climate change phenomena.

Results and discussion

With the application of classical breeding methods, a number of varieties with common and differential characteristics have been generated.

Common characteristics relate to performance, size and color of grain. The differential characteristics refer to adaptation to different agro-ecological zones, earliness, resistance to mildew, bitter or sweet quinoa, industrial quality among others.

Large grain varieties are directed to the use of quinoa in ways similar to rice (pearled quinoa), ie. for cooking in soups and boiled grain. For this purposes grain may be preferably white, but also red and black in color. Most ecotypes of Royal Quinoa are suitable for pearled quinoa (Bonifacio *et al.*, 2012). The research on industrial quality of these varieties is in progress.

Adapting varieties implies an analysis of options in terms of producing them in different ecoregions such as the Southern, Central, and Northern Altiplano, Valleys and Sub Tropics.

The precocity is a character that enables the management of adverse environmental factors through the following mechanisms:

- 1) avoid frost through a shorter production cycle.
- 2) possibility of delayed planting caused by lack of moisture in the planting season.
- 3) prevent losses and enable replanting in case of: seedlings buried by winds in sandy soils, clay soils crusting, seedling loss by frost or drought.

Mildew resistance is a key component to produce organic quinoa in areas outside the Southern Altiplano. Quinoa is susceptible to disease under conditions of high relative humidity and soil moisture (Bonifacio, 2006) and susceptible varieties (Utusaya) can register a severity of 100% (Danielsen and Ames, 2000). Therefore resistance to mildew is essential to produce quinoa in areas of high relative humidity, to reduce the use of fungicides for disease control, reduce production costs and promote grain filling.

The bitter and sweet varieties have not received differentiation until now. Bitter quinoa is produced, processed and sold in greater proportion. However, given the volumes of water that are used (not recycled) to remove the saponin, the difficulties of eliminating foamy water and, the increased energy expenditure

(scarifying, washing and drying); the interest in sweet quinoa is reemerging.

This sweet quinoa, although it has its limitations due to the preferences of a couple of bird species, requires less time for scarifying and/or washing; therefore it produces less losses during processing. It does not generate foamy water and pearled grain does not have the strong, pungent odor that bitter varieties have. Due to these qualities of sweet quinoa, it becomes an alternative for production areas in the Central and Northern Altiplano, as well as for lines of grain processing and transformation. Therefore sweet varieties generated three decades ago are currently subject of differentiated demand.



Variety affected by mildew

Industrial quality refers to the physical and chemical properties of specific grain varieties. In that sense, the varieties for flour, flakes, and for pasta among other uses and varietal differentiation, require the appropriate quality for each process. Varieties with industrial quality give more

relevance to the criteria of varietal purity and homogeneity, so often promoted by instances of variety registration and seed certification.

According to Bodoín (2009), demand and use of quinoa certified seed is very low compared to the volume of local seed used for commercial production, and for cultivar expansion.

Table 1 lists the varieties generated by PROINPA, their adaptation, production

cycle, grain characteristics and potential uses, and shows the varieties' recommendation domains. This information can be used in the context of a risk management quinoa production strategy, taking into account the effects of climate change, disease prevalence (mildew) and, the demands of producers and the market (with and without saponin, industrial quality, etc.).

Table 1. General characteristics of quinoa varieties generated by PROINPA Foundation

| Variety | Adaptation areas | Production cycle | Saponin | Grain color and size | Quality or use |
|-----------------------|--|------------------|---------|----------------------|-------------------------|
| <i>Jach'a Grano</i> | Central and Northern Altiplano. Open valleys and highlands | Early | Bitter | White, large | Pearled, flaked |
| <i>Kurmi</i> | Central and Northern Altiplano. Open valleys and highlands | Semi-late | Sweet | White, large | Pearled, flaked |
| <i>Aynuqa</i> | Central Altiplano | Semi-early | Sweet | White, large | Pearled, flaked |
| Horizontes | Central and Southern Altiplano | Semi-early | Bitter | White, large | Pearled, flaked |
| <i>Qosuña</i> | Southern and Central Altiplano | Semi-early | Sweet | White, large | Pearled, flaked, milled |
| Blanquita | Northern Altiplano, valleys and highlands | Semi-late | Sweet | White, medium | Milled, flaked |
| <i>Qanchis Blanco</i> | Southern and Central Altiplano | Early | Bitter | White, large | Pearled |
| <i>Maniqueña</i> | Southern and Central Altiplano | Early | Bitter | White, large | Pearled |
| <i>Kariquimeña</i> | Southern and Central Altiplano | Early | Bitter | White, large | Pearled |

Source: Own elaboration based on Bonifacio, Vargas y Aroni (2003); Bonifacio y Vargas (2005); Bonifacio, Aroni y Vargas (n/y); Bonifacio, Vargas y Rojas (n/y); Bonifacio, Vargas, Aroni y Quispe (n/a).



Kurmi Variety



Blanquita Variety



Jach'a Grano Variety



Qusuna Variety



Royal Quinoa in flowering phase
(variation by plant color)



Royal Quinoa in maturing phase

Challenges

Today, consumers are looking for information about the quality of the products they consume (protein content, protein quality, gluten-free); it is for that reason that quinoa has a high international demand.

The challenge, for the Bolivian quinoa to be more competitive in the international market, is to research its nutraceutical properties such as antioxidant content, iron and zinc, etc., and incorporate such features in new varieties.

The growing quinoa production areas, lead to a search for technology of easy application over large areas. This is the case of cropping areas that are progressively using more combined harvesters. Therefore, the demand for genetic improvement seeks the development of new varieties with plant architecture suitable for mechanization (uniform size and uniform maturity).

The actors in the quinoa value chain that are in the processing link, need to be informed about the qualities of the new varieties, in terms of protein content, amylopectin, antioxidants, etc., in order to specialize the use of varieties. This would give way to processed products with better quality and identity, since they would not be processed with varietal mixtures, as it is currently done.

Finally, to achieve extensive use of varieties, it is necessary that actors specialized in the industry become involved in the production of high quality seed. This would allow producers, associations and companies in the quinoa value chain to have constant availability

and access to these varieties, meeting the standards required by the corresponding authorities. Currently, the vast majority of producers, uses farmers' own and local seed, even for use on cultivar expansion (Bodoin, 2009).

Participating institutions

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- UMSA (Universidad Mayor de San Andrés).
- Benson Institute.
- PREDUZA-WU, (Proyecto de Resistencia Duradera para la Zona Andina - Universidad de Wageningen). Project of Lasting Resistance for the Andean Region - Wageningen University.

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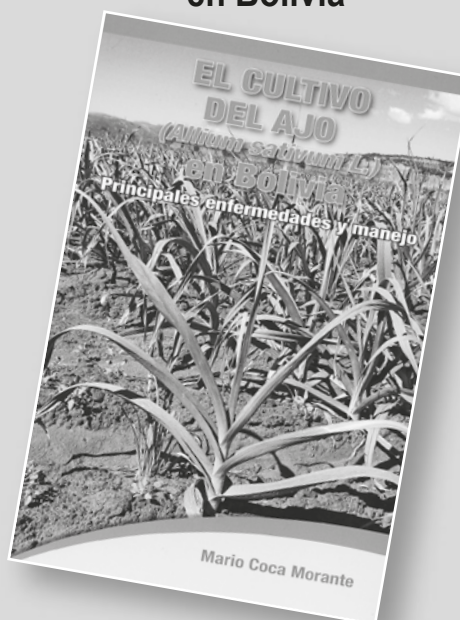
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Libro (94 páginas) que en su contenido proporciona información resumida sobre la importancia del ajo en Bolivia, tomando en cuenta la potencialidad del cultivo, señalando algunas de las causas que están afectando la producción. Esta publicación es una herramienta práctica que ayudará a los productores, técnicos y estudiantes a realizar una correcta identificación de las enfermedades del ajo, realizando una adecuada aplicación de medidas para el manejo y control de las mismas.

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**El cultivo del ajo
(*Allium sativum* L.)
en Bolivia**



Parasitoid complex associated with the quinoa moth - key crop pest in the Bolivian Altiplano

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Summary. The quinoa moth (*Eurysacca quinoa*) causes losses of over 30% in the cultivation of quinoa, and significantly reduces grain quality. One of the alternative control methods in organic production, is based in natural enemies; particularly parasitoids. The study was implemented in communities in the Northern, Central and Southern Altiplano of Bolivia, seeking to update information on associated parasitoids for *E. quinoa*. The collection of moth larvae was conducted in 2013, using the method of entomological mantle. The larvae were placed in plastic containers of 200 ml capacity containing quinoa branches. These containers were transferred to the laboratory for rearing until adults and/or parasitoids were obtained. Identification was completed through morphological comparison (adults and cocoons) and through the use of taxonomic keys. It was evidenced that the larval population of *E. quinoa* is regulated by a complex of six parasitoids: five wasp species (Hymenoptera) and one fly species (Diptera). Average Natural parasitism of the twelve communities was 28%, with a maximum of 44.7%. *Cotesia* sp. and *Meteorus* sp. were the species that recorded the highest levels of parasitism in the Northern, Central and Southern Altiplano. Parasitoids hold great potential as an alternative for the integrated management of *E. quinoa*.

Keywords: Entomology; Organic Production; Natural Enemies, Parasitoids

Resumen. Complejo parasitoide asociado a la polilla de la quinua – plaga clave en el Altiplano Boliviano. La polilla de la quinua (*Eurysacca quinoa*) causa pérdidas mayores al 30% en el cultivo de quinua y disminuye significativamente la calidad del grano. Una de las alternativas de control, dentro de la producción orgánica, la constituyen sus enemigos naturales, particularmente los parasitoides. Buscando actualizar información sobre los parasitoides asociados a *E. quinoa*, se implementó el estudio en comunidades del Altiplano Norte, Centro y Sur de Bolivia. La colecta de larvas de polilla se realizó el año 2013, utilizando el método del manto entomológico. Las larvas se colocaron en recipientes de plástico de 200 cc de capacidad que contenían ramas de quinua. Estos recipientes fueron trasladados a laboratorio para su cría hasta la obtención de adultos y/o parasitoides, su identificación fue mediante comparaciones morfológicas (adultas y cocones) y con ayuda de claves taxonómicas. Se evidencia que la población larval de *E. quinoa* es regulada por un complejo de seis parasitoides: cinco especies de avispas (Hymenoptera) y una especie de mosca (Diptera). El parasitismo natural promedio, de las doce comunidades, fue de 28%, con un máximo de 44.7%. Las especies de *Cotesia* sp. y *Meteorus* sp. fueron las que registraron mayores niveles de parasitismo en el Altiplano Norte, Centro y Sur. Los parasitoides tienen un importante potencial como alternativa para el manejo integrado de *E. quinoa*.

Palabras clave: Entomología; Producción Orgánica; Enemigos Naturales, Parasitoides

Introduction

The quinoa (*Chenopodium quinoa* Willd) crop in the Bolivian Altiplano is attacked by several species of pest insects. One of these species is the quinoa moth or "*qhona qhona*" *Eurysacca quinoae* Povolny 1997 (Lepidoptera: Gelechiidae), listed as a key pest of the crop (Avalos 1996) because it causes losses of over 30% and significantly reduces grain quality (Saravia and Quispe, 2003; PROINPA, 2013).

The first reports on parasitoid insects of quinoa moths were registered in Patacamaya in 1997, by the *Quinoa Programme* from the ex - IBTA (IBTA, 1997). Later, specific pieces of work were performed in some communities of the Central and Southern Altiplano (Mamani, 1998, PROINPA 2003; PROINPA 2013).

As a result of these studies it is known that the percentage of natural parasitism in larvae of *Eurysacca quinoae* reaches up to 40% and, that the parasitoid complex consists of nine species. Out of these nine species, seven belong to the order Hymenoptera from the families Braconidae (*Meteorus* sp., *Apanteles* sp., *Microplitis* sp.), Ichneumonidae (*Deleboea* sp., *Venturia* sp., *Diadegma* sp.) and Encyrtidae (*Copidosoma* sp.), and two belong to the order Diptera from the families Tachinidae (*Phytomyia* sp. and *Dolichostoma* sp.) (IBTA 1997; Mamani 1998, and Saravia Quispe 2006; Saravia *et al.*, 2008; PROINPA 2013).

A parasitoid is understood as every insect, which in the larval stage is parasitic of other arthropod (host), while in the adult phase it lives freely to breed and seek its host.

Unlike the parasite, the parasitoid in most cases ends up killing the host (Van Driessche *et al.*, 2007).

The diversity of predators and parasitoids of a given pest species is related to the diversity of flowering plants associated to a cultivar. The nectar and pollen of these flowers are important additional food sources necessary for multiplication (Altieri and Nicholls, 2010).

Areas of quinoa production in the country are located in three ecoregions: Northern, Central and Southern Altiplano, these differ mainly by the rainfall regime, the quality of soils and crops produced. The Southern Altiplano is the driest region (150-300 mm/year), with sandy soils and low fertility where the main crop is the Royal Quinoa. The Northern Altiplano has higher rainfall (500 mm/year), soils are more productive and crops produced include potatoes, quinoa, barley, broad beans among others. The Central Altiplano presents intermediate weather and soil conditions, relating to both Northern and Southern Altiplano.

Given the background mentioned and in order to update information on the parasitoids associated with the quinoa moth, the present study was carried out in three ecoregions of the Altiplano. The objective was to identify the parasitoids and determine natural parasitism on larvae of *E. quinoae* in quinoa plots of the Bolivian Altiplano.

Materials and methods

Location. The work was implemented in twelve quinoa producing communities of the Altiplano, one from the Northern Altiplano (Lacaya), eight from the Central

Altiplano (Contorno Centro, Contorno Arriba, Cañaviri, Villa Manquiri, Colquencha, Viscachani, Calpaya and Crucero Belen) and three in the Southern Altiplano (Colcha K, Vinto and Julaca) (Figure 1).

E. quinoa sampling and larval rearing.

The collection of larvae in the quinoa plots of different communities was conducted between February and March 2013, period of highest incidence of the pest.

This process was performed using the method of "Entomological mantle", which consists of a canvas of 1 m x 1 m stretched at the base of the plant, then a careful

shaking of the the panicle to produce the downfall of larvae on the canvas. Larvae obtained were placed in 200 ml sized plastic containers enclosing paper towels at the base, and quinoa leaves and branches as food for the larvae.

These containers were placed in Styrofoam carriers that were carried to the Entomology Laboratory of PROINPA Foundation in Quipaquipani Center (Viacha-La Paz). At the laboratory larvae were selected by size, and placed in specific quantities, in containers, to continue their rearing until they reached the phases of adult moths or parasitoids.

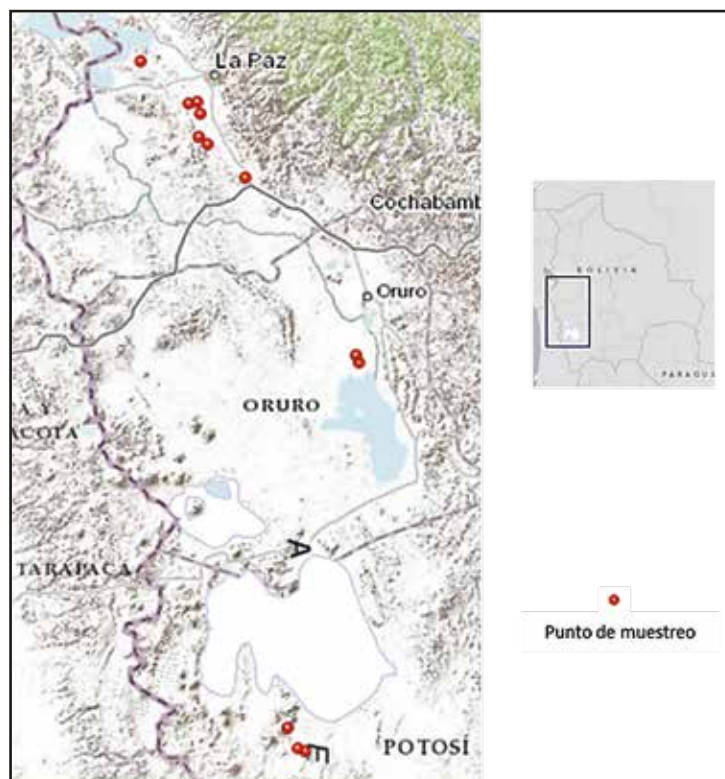


Figure 1. Location map of sampling points of *E. quinoa* larvae in the Bolivian Altiplano

Percentage of natural parasitism and taxonomic identification. To calculate the percentage of natural parasitism by species and community, the following formula was used:

$$\text{Parasitism (\%)} = \frac{\text{Parasitized larvae}}{\text{Collected larvae}} \times 100$$

The identification of parasitoids was made by comparison and using taxonomic keys. For identification by comparison, representative specimens of these biological controllers were mounted, and compared to specimens described by Mamani (1998), Saravia and Quispe (2006) and Costa *et al.* (2009).

In some cases the identification was confirmed using taxonomic keys provided by Sharkey (2006) for Braconidae, and Gauld (1991) for Ichneumonidae, both corresponding to the Neotropical region.

In parallel specimens of interest were sent to specialized centers (USDA) for identification.

Results and discussion

Parasitoid complex associated with larvae of E. quinoa

The parasitoid complex that is regulating the population of *E. quinoa* in quinoa plots located in the Bolivian Altiplano, is composed of six species (Table 1, Figure 2). Of these six species, five correspond to the order Hymenoptera and one to Diptera.

Among the wasps, all belong to the sub order Apocrita, characterized by having the first abdominal segment together with

the metathorax and separated from the rest of the abdominal segments with a narrow waist (Fernandez and Sharkey, 2006). These wasps are grouped into two superfamilies:

- Ichneumonoidea (medium to large wasps).
- Chalcidoidea (micro-wasp).

Cotesia sp. and *Meteorus* sp. correspond to the Braconidae family; *Venturia* sp. and *Deleboea* sp. to Ichneumonidae, and *Copidosoma* sp. to Encyrtidae (Chalcidoidea). The only Diptera species *Phytomyptera* sp. belongs to the Tachinid family, from the Oestroidae superfamily, and the sub order Brachycera.

Among the parasitoids, the micro-wasp *Copidosoma* sp. (Encyrtidae), is a polyembryonic egg parasitoid that mummifies moth larvae before they reach the pupal stage. From mummified larvae, between 28 to 33 micro-wasps can emerge.

The rest of the wasps are larvo-pupal endoparasitoids. They infect their host (quinoa moth larvae) in the early larval stages, but the effect can only be observed when they kill the host in the pupal stage. Biologically this parasitism strategy is known as *koinobiont*. There is another strategy called *idiobiont* parasitism that occurs in species that permanently paralyze their hosts, preventing them to continue their development after being parasitized. This is a desirable feature for parasitoids that are meant to be used in biological control programs. In this study no parasitoids with these characteristics were found.



a) Adult and cocoon of *Cotesia* sp.
(Braconidae)



b) Adult and cocoon of *Meteorus* sp.
(Braconidae)



c) Adult and cocoon of *Venturia* sp.
(Ichneumonidae)



d) Adult and cocoon of *Deleboea* sp.
(Ichneumonidae)



e) Adult and mummified larvae of
Copidosoma sp. (Encyrtidae)



f) Adult and puparium of *Phytomyia* sp.
(Tachinidae)

Table 1 shows the percentage of natural parasitism recorded in the different communities of the Northern, Central and Southern Altiplano. Overall, the average percentage of natural parasitism was higher than 28%, with the communities of the Central Altiplano (Viscachani,

Contorno Arriba and Cañaviri) recording the highest percentages of parasitism, with values above 40%, in comparison to Colcha K, Vinto and Julaca (Southern Altiplano), where the lowest percentages of parasitism were observed, with values below 5%. The rest of the communities:

Lacaya, Calpaya, Crucero Belén, Contorno Centro and Villa Manquiri (the first from the Northern Altiplano and the rest from the Central Altiplano), recorded natural parasitism of *E. quinoae* fluctuating between 27% and 38%. The average of 28% of parasitism is a value that comes close to the efficiency levels of control for this pest, which is obtained by using botanical extracts and/or some bioinsecticides available in local markets. This shows the important role that these parasitoids play in the natural regulation of *E. quinoae*.

Natural parasitism percentages recorded in each of the communities was the product of a joint action of a complex of two to six parasitoids, regulating the larval population of *E. quinoae* at different levels (Annex 1). According to Table 1, the parasitoids that contributed most to the regulation of quinoa moth larvae in the twelve communities are *Cotesia* sp. and *Meteorus* sp., recording average parasitism above 6%. In contrast, the micro-wasp *Copidosoma* sp. was the species that contributed least to the control of *E. quinoae* (1.2%).

The communities that are characterized by greater diversity of parasitoids are Viscachani, Contorno Centro and Lacaya, the first two located in the Central Altiplano and the last in the Northern Altiplano, recording a total of six parasitoids. The communities Colcha K, Vinto and Julaca in the Southern Altiplano, recorded two and three parasitoids (Table 1).

Depending on the abundance of parasitoids (Table 1) two groups can be clearly observed. The first formed by the communities of Central and Northern Altiplano, where Viscachani and

Contorno Centro stand out with the largest number of parasitoids. The second group consists of the communities in the Southern Altiplano with low amounts of these biological control agents. The differences can be explained by the characteristics of agricultural production in each of these areas, in addition to the floristic composition of weeds and native plants registered and distributed in these areas.

The distribution and relative importance of the six parasitoids identified was highly variable. Nevertheless, there clearly is one species found in all locations: the Tachinido *Phytomyptera* sp. which showed an average frequency of 5.3%, being the most abundant parasitoid in Cañaviri (14.3%) and the second most abundant in Colquencha (11%). In this regard, Stireman *et al.* (2009) state that flies belonging to the Tachinidae family are diverse and are important natural enemies of many pests. All known species of this family are parasitoids of arthropods, mainly of lepidopteran larvae, the taxonomic group to which *E. quinoae* belongs to.

The percentages of parasitism recorded in this work are lower than those reported by Mamani (1998) and PROINPA (2013), who indicate that populations of quinoa moth larvae in the Central Altiplano have natural parasitism ranging between 43% and 65%.

Conclusions

- The quinoa moth larval population in the sampled communities is being regulated by a complex of six parasitoids: five wasps (Hymenoptera) and one fly (Diptera). Among the

wasps, the species registered were: *Cotesia* sp. (Braconidae), *Meteorus* sp. (Braconidae), *Deleboea* sp. (Ichneumonidae), *Venturia* sp. (Ichneumonidae) and *Copidosoma* sp. (Encyrtidae), and the fly was identified as *Phytomyia* sp. (Tachinidae).

- The average percentage of natural parasitism was 28% in the twelve communities, standing out Viscachani with 44.7% of parasitism on average.
- Parasitoids *Cotesia* sp. and *Meteorus* sp. were the species with higher levels of parasitism recorded in communities of the Northern, Central and Southern Altiplano.
- Significant natural parasitism percentages recorded in *E. quinoa* larvae, in quinoa producing communities of the Bolivian Altiplano, show the need to implement integrated pest management practices that conserve and increase beneficial organisms in these agroecosystems.
- Undoubtedly, this work shows only a partial perspective of the natural parasitism on *E. quinoa*, as the sampling period covered a limited time span. Additionally, only parasitism on the larval stage of one agricultural campaign was studied. The possibility of sampling new parasitoids in the same places and at different times, with the same or other collection methods, is not ruled out.
- Within the activity of parasitoids elements that influence their control capability are the effects of climate change and environmental variability. Insect pest species are able to respond quickly to environmental changes. However, the effect that this response

has on the populations of natural enemies (parasitoids and predators) is still unknown. This is an aspect to be considered when thinking about implementing biological control programs.

- One of the limitations for the use of parasitoids (directed releases) in biological control programs is the maintenance of massive rearing in the laboratory. For this purpose, the first step is to develop mass host (*E. quinoa*) rearing and to generate information on the biology and reproduction of its parasitoids, to later have available a tool of natural biological control.

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Table 1. Ratio of parasitoids and percentage of natural parasitism in larvae of *E. quinoae*, in decreasing order of abundance, from communities in the Bolivian Altiplano (2013)

| Parasitoid | Type of parasitoid | % of natural parasitism / Community | | | | | | | | | | | | |
|----------------------------------|--------------------|-------------------------------------|---|------------------------|-------------------------|-----------------------|----------------------|---|---|-----------------------------|------------------------|---------------------|----------------------|---------|
| | | Viscachani ^{AC} | Contorno ^{AC} Arriba ^{AC} | Cañaviri ^{AC} | Colquecha ^{AC} | Calpaya ^{AC} | Lacaya ^{AN} | Crucero Be- ^{AC} Ién ^{AC} | Contorno ^{AC} Centro ^{AC} | Villa Manqui- ^{AC} | Colcha K ^{AS} | Vinto ^{AS} | Julaca ^{AS} | Average |
| HYMENÓPTERA | | | | | | | | | | | | | | |
| Apocrita: Ichneumonoidea | Endo | 11.6 | 13.4 | 8.6 | 8.0 | 7.8 | 14.7 | 5.8 | 8.6 | 0.0 | 0.0 | 1.5 | 0.6 | 6.7 |
| Braconidae: Microgastrinae | Larva-Pupal | | | | | | | | | | | | | |
| <i>Cotesia</i> sp. Cameron. | | | | | | | | | | | | | | |
| Braconidae: Meteorinae | Endo | 18.4 | 8.6 | 5.7 | 0.5 | 11.2 | 5.3 | 6.4 | 6.8 | 6.5 | 3.2 | 0.0 | 0.0 | 6.1 |
| <i>Meteorus</i> sp. Haliday. | Larva-Pupal | | | | | | | | | | | | | |
| Ichneumonidae: Campopleginae | Endo | 1.0 | 7.7 | 7.9 | 16.5 | 8.0 | 4.7 | 0.0 | 3.9 | 9.0 | 0.8 | 0.8 | 0.0 | 5.0 |
| <i>Venturia</i> sp. Schrottky. | Larva-Pupal | | | | | | | | | | | | | |
| Ichneumonidae: Banchinae | Endo | 10.4 | 0.6 | 0.0 | 0.0 | 7.0 | 5.5 | 17.9 | 6.2 | 1.0 | 0.0 | 1.5 | 0.0 | 3.3 |
| <i>Deleboea</i> sp. Cameron. | Larva-Pupal | | | | | | | | | | | | | |
| Apocrita: Chalcidoidea | Endo | 0.9 | 2.5 | 3.6 | 2.5 | 0.0 | 0.8 | 1.3 | 0.5 | 2.5 | 0.0 | 0.0 | 0.0 | 1.2 |
| Encyrtidae: Encyrtinae | Endo | | | | | | | | | | | | | |
| <i>Copidosoma</i> sp. Ratzeburg. | Endo | | | | | | | | | | | | | |
| DÍPTERA | | | | | | | | | | | | | | |
| Brachycera: Oestroidae | Ecto | 2.4 | 9.4 | 14.3 | 11.0 | 3.9 | 4.2 | 1.3 | 5.3 | 8.0 | 0.8 | 0.8 | 1.9 | 5.3 |
| Tachinidae: Tachininae | Larva pupario | | | | | | | | | | | | | |
| <i>Phytomyptera</i> sp. Rondani. | | | | | | | | | | | | | | |
| Total | | 44.7 | 42.2 | 40.1 | 38.5 | 37.9 | 35.2 | 32.7 | 31.3 | 27.0 | 4.8 | 4.6 | 2.5 | 28.5 |

References: NA = Northern Altiplano; CA = Central Altiplano; SA = Southern Altiplano; Endo = Endoparasitoide; Ecto = Ectoparasitoide

Annex 1: Percentage of natural parasitism of the quinoa moth parasitoid complex in twelve communities in the Bolivian Altiplano (2013)

| Localidad | Meteorus sp. | Cotesia sp. | Venturia sp. | Deleboea sp. | Copidosoma sp. | Phytomyptera sp. |
|-------------------------------|-----------------|----------------|-----------------|-----------------|-------------------|---------------------|
| Viscachani ^{CA} | 18.4 | 11.6 | 1.0 | 10.4 | 0.9 | 2.4 |
| Contorno Arriba ^{CA} | 8.7 | 13.5 | 7.7 | 0.6 | 2.5 | 9.4 |
| Cañaviri ^{CA} | 5.7 | 8.6 | 7.9 | 0.0 | 3.6 | 14.3 |
| Colquecha ^{CA} | 0.5 | 8.0 | 16.5 | 0.0 | 2.5 | 11.0 |
| Calpaya ^{CA} | 11.2 | 7.8 | 8.1 | 7.0 | 0.0 | 3.9 |
| Lacaya ^{NA} | 5.3 | 14.7 | 4.7 | 5.5 | 0.8 | 4.2 |
| Contorno Centro ^{CA} | 8.6 | 6.8 | 6.2 | 5.3 | 3.9 | 0.5 |
| Villa Manquiri ^{CA} | 6.5 | 0.0 | 9.0 | 1.0 | 2.5 | 8.0 |
| Crucero Belén ^{CA} | 6.4 | 5.8 | 0.0 | 17.9 | 1.3 | 1.3 |
| Colcha K ^{SA} | 3.2 | 0.0 | 0.8 | 0.0 | 0.0 | 0.8 |
| Julaca ^{SA} | 0.0 | 1.5 | 0.8 | 1.5 | 0.0 | 0.8 |
| Vinto ^{SA} | 0.0 | 0.6 | 0.0 | 0.0 | 0.0 | 1.9 |

References: NA = Northern Altiplano; CA = Central Altiplano; SA = Southern Altiplano;

Lepidoptera associated to quinoa in the Bolivian Altiplano: Taxonomic Update

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Summary. The objective of this work was to update the taxonomy of lepidopteran pests associated to quinoa in the Bolivian Altiplano; and to map their geographical distribution. To this end lepidopteran larvae were collected in quinoa fields, in the villages of Jalsuri, Konani (Central Altiplano), Salinas de Garci Mendoza and Chacala (Southern Altiplano), and reared to obtain adults at the Entomology Laboratory of PROINPA Foundation, located in the village of Quipaquipani (Viacha, La Paz). Adult insects were also collected using standard type light traps. Adults from larvae collected in the villages of Jalsuri and Konani, were identified as *Copitarsia incommoda* Walker, *Helicoverpa quinoa* Pogue and Harp, and *Dargida acanthus* Herrich - Schaffer. Identifications were led by Dr. Michael Pogue, a lepidopteran identification specialist from the Entomological Museum of the United States Department of Agriculture (USDA).

Keywords: Entomology; Pests; Taxonomic Identification

Resumen. Lepidópteros asociados al cultivo de quinua en el Altiplano Boliviano: Actualización Taxonómica. El objetivo del presente trabajo fue actualizar la taxonomía de las plagas de lepidópteros asociados al cultivo de la quinua, en el Altiplano Boliviano, y conocer su distribución geográfica. Con esta finalidad se recolectaron larvas de lepidópteros en campos de quinua, en las localidades de Jalsuri, Konani (Altiplano Central), Salinas de Garci Mendoza y Chacala (Altiplano Sur) y criados hasta la obtención de adultos en el Laboratorio de Entomología de la Fundación PROINPA, ubicado en la localidad de Quipaquipani (Viacha, La Paz). También se recolectaron insectos adultos utilizando trampas luz tipo estándar. Los adultos, provenientes de larvas colectadas en la localidad de Jalsuri y Konani, fueron identificados como *Copitarsia incommoda* Walker, *Helicoverpa quinoa* Pogue and Harp, *Dargida acanthus* Herrich – Schaffer. Las identificaciones fueron lideradas por el Dr. Michael Pogue, especialista en la identificación de lepidópteros del Museo Entomológico del Departamento de Agricultura de los Estados Unidos (USDA).

Palabras clave: Entomología; Plagas; Identificación Taxonómica

Introduction

Correct identification of species that damage crops is the basic tool for their management. In the Bolivian Altiplano, early work with sex pheromones for integrated pest management of the quinoa crop showed that there was inaccuracy in

the identification of major pests of this crop (Saravia *et al.*, 2013). Subsequent work to identify lepidopteran pests associated with the cultivation of quinoa in the Bolivian Altiplano showed that harmful species for quinoa in this area are: *Helicoverpa gelatopoeon*, *Copitarsia incommoda*, *Dargida acanthus* and

Tatochila mercedis (diurnal butterfly larva).

Recent work based on mitochondrial DNA analysis and insect genitalia dissection, by Michael Pogue from the US Department of Agriculture (USDA) in coordination with PROINPA entomologists, showed that the species *Helicoverpa gelotopoeon* corresponds to *Helicoverpa quinoa* (Pogue, 2014). This specialist also indicates that it would be difficult to differentiate the species *H. quinoa*, *H. gelotopoeon* and *H. titicacae* only by morphological characters.

Identification of insects and pests in general is a difficult task. In some cases there is great similarity between adults and immature stages of some species, which makes it difficult to identify them properly, often creating confusion and misidentification. Therefore it is necessary to use advanced methods such as dissection of genitalia or even molecular analysis of mitochondria to separate similar species. A tool of vital importance for species identification is the availability of specific taxonomic keys, also called dichotomous keys. These are guides that present a list of phrases that describe the particular characteristics of the organisms to classify or identify.

As a background we must say that according to Saravia and Quispe (2005), lepidoptera species associated with the quinoa crop are part of the Noctuidae complex denominated night pests or owl moths. Adults are moths commonly known by farmers as *rafaelitos* or *soul carriers*, considered unlucky. They have a short and robust body, covered by scales or medium sized dark brown hairs.

According to Ortiz and Zanabria (1979), the larvae of these moths are known as ticonas, ticuchis, sillwi kuro and earthworm, common names farmers give to larvae belonging to the Noctuidae family. The ticonas are a complex group, consisting of three genera. They feed cutting the newly emerged plants, destroying the apical leaves and panicles in formation. One larva per plant is enough to cause serious damage to the crop.

The ticonas complex (*Helicoverpa*, *Copitarsia* and *Dargida*) negatively influence production, causing significant economic losses (Saravia and Quispe, 2005).

It is important to highlight that collecting insects requires the application of a range of techniques due to the large number of species and diversity of life habits each holds. Most of the techniques used meet specific objectives depending on the type of study. However, techniques are generally divided into direct (active) and indirect (passive) collection techniques (Steyskal *et al.*, 1986; Borror *et al.* 1989).

Direct collection is one in which insects are actively sought in the ecosystems, using a variety of tools such as entomological nets, sieves, gardening shovels, vacuum aspirators, etc. This strategy is widely used by most collectors and is most suitable to capture insects in immature stages.

Indirect collection is one in which insects are caught using some kind of attractant and which does not imply direct search in the ecosystems. The type and number of traps and lure to be used also depends directly on the research objectives. Among the traps without attractants there

are "fall" traps, "Malaise" traps and "interception or window" traps. Among the traps with bait or attractants, the name is given by the type of bait used, the most important are dung bait traps (baited with excrement), fruit traps, carrion traps, light traps, Berlese funnel and pheromone traps (Marquez, 2005).

The objectives of this research were to update the taxonomy of lepidopteran pests associated with the quinoa crop and their geographical distribution, in quinoa production areas of the Bolivian Altiplano.

Methodology

The identification work in this study was developed in three stages:

In the **first** stage, immature specimens were collected in quinoa fields of the Bolivian Altiplano, during the period of greatest pest attack. The method used was direct collection. Larvae collected were transferred to PROINPA's Entomology Laboratory in Quipaquipani Center, where they were singled out to prevent cannibalism and were raised on artificial diet until they reached the pupal and adult stages. Specimens were then mounted following the technical recommendations suggested by Borror *et al.* (1989). In parallel, adult insects were captured using light traps. The light trap used was the standard, which contained inside a cloth bag with potassium cyanide to kill insects trapped. The next day, the insects were

placed in glass jars and transported to the laboratory to proceed with the mounting.

In the **second** stage material was selected, prepared and packed to be sent to the Entomology Laboratory of the US Department of Agriculture (USDA) for identification.

The **third** stage involved the identification of specimens sent to members of the Entomology Laboratory of the USDA, who using the morphological description, dissection of genitalia and mitochondrial analysis identified specimens submitted. Results from the analysis were presented during a visit of Dr. Michael Pogue from USDA to Bolivia in January 2014.

To understand and visualize the geographical distribution of species, the sampling points were georeferenced to develop distribution maps using the ArgGIS® application.

Results and discussion

Species identification

Table 1 shows the list of species that were managed from 2008 to 2013 and the list of species identified in 2014 by Dr. Pogue. The table shows that there are 10 species of Lepidoptera associated with the quinoa crop in the Bolivian Altiplano.

Table 1. Changes in the taxonomic classification of Lepidoptera pests of quinoa in Bolivia, in two time periods

| Nro. | Classification used during the period 2008 – 2013 | Species identified during the year 2014 |
|------|---|--|
| 1 | <i>Eurysacca melanocampta</i> Meyrick | <i>Eurysacca melanocampta</i> Meyrick |
| 2 | <i>Eurysacca quinoa</i> Povolný | <i>Eurysacca quinoa</i> Povolný |
| 3 | <i>Copitarsia incommoda</i> (Walker) | <i>Copitarsia incommoda</i> (Walker) |
| 4 | -- | <i>Copitarsia patagonica</i> Hampson |
| 5 | <i>Heliothis titicaquensis</i> | <i>Helicoverpa titicacae</i> Hardwick |
| 6 | <i>Helicoverpa gelotopoeon</i> (Dyar) | <i>Helicoverpa quinoa</i> Pogue and Harp.* |
| 7 | <i>Helicoverpa atacamae</i> Hardwick | <i>Helicoverpa atacamae</i> Hardwick |
| 8 | <i>Dargida acanthus</i> (Herrich-Schäffer) | <i>Dargida acanthus</i> (Herrich-Schäffer) |
| 9 | <i>Tatochila</i> sp. | <i>Tatochila mercedis</i> (Eschscholtz) |
| 10 | <i>Agrotis andina</i> Köhler | <i>Agrotis peruviana</i> (Hampson) |

Source: Own elaboration based on the results of Pogue (2014) and San Blas (2014).

* New specie.

In Table 1 it is important to note that the species identified as *H. gelotopoeon*, in 2008, was currently identified as *Helicoverpa quinoa* by the same author. This new species was reported as a quinoa pests based on morphological characters, genitalia dissection and mitochondrial analysis. This author suggests that it is difficult to distinguish between *H. quinoa* and *H. gelotopoeon* only by their morphological differences and that analysis of genitalia is required. Other species that have undergone changes in taxonomy are *Heliothis titicaquensis* and *Agrotis andina*, which were identified as *Helicoverpa titicacae* and *Agrotis peruviana* according to a morphological and phylogenetic analysis performed by Pogue (2014) and San Blas (2014). Another aspect that stands out is the inclusion of *Copitarsia patagonica* in the

2014 list. The other species listed in the Table maintained their identification.

Geographical distribution of the identified species

Table 2 shows the list of lepidopteran pests species associated with the quinoa crop in the Bolivian Altiplano, detailing the family to which they belong, the method of collection, the type of pest and its geographical distribution. This table shows that five species of Lepidoptera: *Eurysacca melanocampta*, *Eurysacca quinoa*, *Copitarsia incommoda*, *Helicoverpa titicacae* and *Helicoverpa quinoa*, were listed as key crop pests of quinoa in the Bolivian Altiplano, four as casual pests: *Copitarsia patagonica*, *Helicoverpa atacamae*, *Dargida acanthus*, *Tatochila mercedis* and one as potential pest: *Agrotis peruviana*.

Figure 1 describes, in detail, the geographical distribution and abundance of the species *Helicoverpa titicacae* and *H. quinoa* in the Bolivian Altiplano. It can be observed that *Helicoverpa titicacae* is found mainly in the Northern Altiplano and extends into the Central Altiplano. Conversely *H. quinoa* is distributed in quinoa production areas located between the salt flats of Uyuni and Coipasa (Southern Altiplano), extending into the Central Altiplano without reaching the Northern Altiplano.

Figure 2 shows the distribution and abundance of *Eurysacca melanocampta* and *E. quinoae* in different quinoa production areas of the Bolivian Altiplano.

According to this figure, *Eurysacca melanocampta* is abundant in the Northern Altiplano and extends into the Central Altiplano. In contrast, *E. quinoae* is abundant in the South and Central Altiplano where it is found in high populations.

Table 2. Geographic distribution of Lepidoptera species associated with the quinoa crop in the Bolivian Altiplano

| No. | Species | Sub family | Family | Collection method | Type of pest | Distribution |
|-----|--|-------------|-------------|-------------------|--------------|--------------|
| 1 | <i>Eurysacca melanocampta</i> Meyrick | -- | Gelechiidae | CL | C | NA, CA |
| 2 | <i>Eurysacca quinoae</i> Povolny | -- | Gelechiidae | CL, LT | C | NA, CA, SA |
| 3 | <i>Copitarsia incommoda</i> (Walker) | Cucullinae | Noctuidae | CL, LT | C | NA, CA |
| 4 | <i>Copitarsia patagonica</i> Hampson | Cucullinae | Noctuidae | CL, LT | O | CA, SA |
| 5 | <i>Helicoverpa titicacae</i> Hardwick | Heliothinae | Noctuidae | CL, LT | C | NA, CA |
| 6 | <i>Helicoverpa quinoa</i> Pogue and Harp.* | Heliothinae | Noctuidae | CL, LT | C | CA, SA |
| 7 | <i>Helicoverpa atacamae</i> Hardwick | Heliothinae | Noctuidae | CL | O | SA |
| 8 | <i>Dargida acanthus</i> (Herrich-Schäffer) | Hadeninae | Noctuidae | CL, LT | O | NA, CA, SA |
| 9 | <i>Tatochila mercedis</i> (Eschscholtz) | -- | Artidae | CL | O | NA, CA |
| 10 | <i>Agrotis peruviana</i> (Hampson) | -- | Noctuidae | LT | P | NA; CA; SA |

References: Collection Method: CL = Collection of larvae in quinoa plants, LT = Collection with light traps in quinoa plots. Type of Pest: K = Key Pest, O = Occasional Pest, P = Potential Pest. Distribution: NA = Northern Altiplano, CA = Central Altiplano, SA = Southern Altiplano.

* = First unpublished report (Pogue, 2014).

Source: Own elaboration based on the results of Pogue (2014) and San Blas (2014)

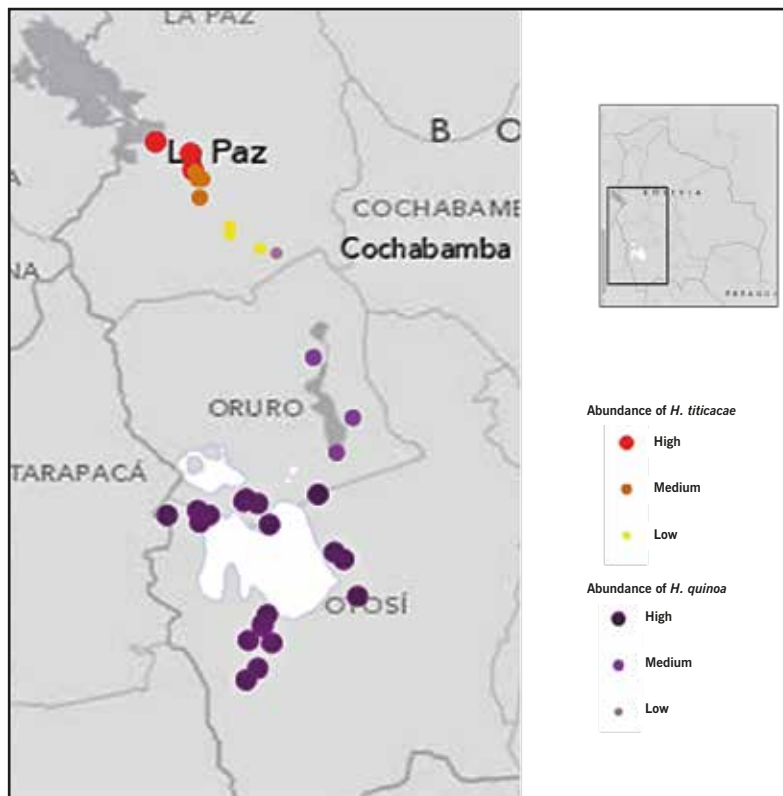


Figure 1. Distribution and abundance of *Helicoverpa titicacae* y *H. quinoa* in the Bolivian Altiplano (2013)

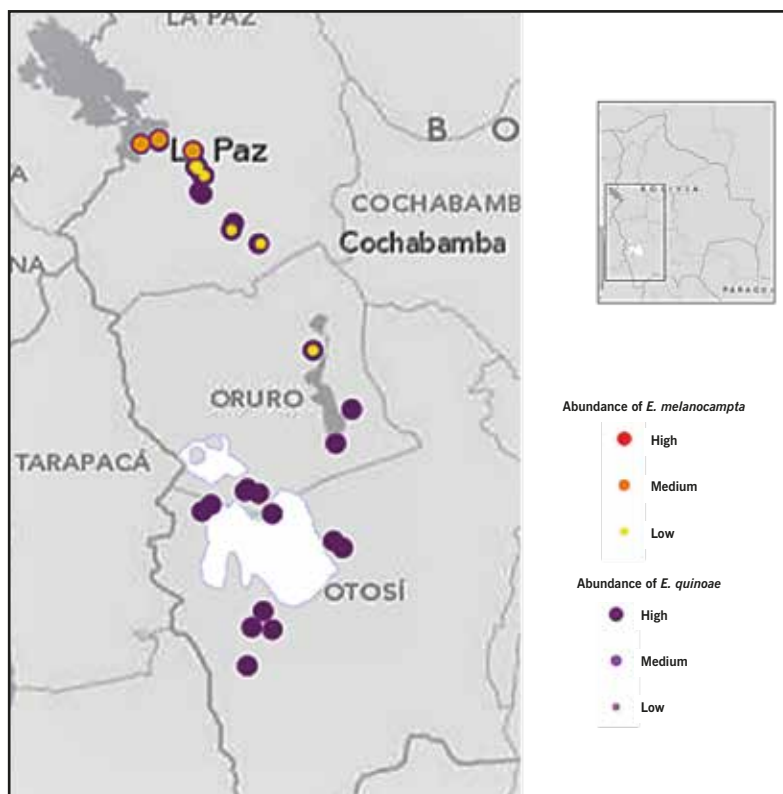


Figure 2. Distribution and abundance of *Eurysacca melanocampta* and *E. quinoae* in the Bolivian Altiplano (2013)

Conclusions

- In the Bolivian Altiplano there are ten Lepidoptera species associated with the quinoa crop, including five listed as key quinoa pests: *Eurysacca melanocampta*, *Eurysacca quinoae*, *Copitarsia incommoda*, *Helicoverpa titicacae* and *Helicoverpa quinoa*; four as casual: *Copitarsia patagonica*, *Helicoverpa atacamae*, *Dargida acanthus*, *Tatochila mercedis* and one as potential pest: *Agrotis peruviana*.
- *Helicoverpa titicacae* is abundant in the Northern Altiplano and extends into the Central Altiplano. In contrast, *H. quinoa*, an endemic Pest of the Bolivian Altiplano, abounds in the Southern Altiplano and extends into the Central Altiplano without reaching the Northern Altiplano.
- *Eurysacca melanocampta* is the dominant species in the Northern Altiplano and extends into the Central Altiplano, and *E. quinoae* is the dominant species in the Southern and Central Altiplano.

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Bacteria associated with the cultivation of quinoa in the Bolivian Altiplano and their biotechnological potential

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Summary. Quinoa grows in semi-deserted areas under extreme conditions of humidity and temperature. Therefore it is also possible that its symbiont microorganisms are also adapted to these extreme conditions. These organisms hold great potential for the development of biotechnology adapted to quinoa. For this purpose, quinoa plants and soil samples from the Southern Altiplano were collected and taken to the laboratory where different bacteria species were isolated and where their potential ecosystem services were examined (nitrogen fixation, phosphorus solubilization or IAA generation). Later, the bacteria were identified by molecular techniques: *Bacillus amyloliquefaciens*; *B. tequilensis*; *B. vallismortis*; *B. subtilis*; *B. pumilus*; *B. licheniformis* and *B. firmus* (isolated from leaves). *B. aryabhattai*; *B. horikoshii*; *B. megaterium*; *B. pumilus* and *Paenibacillus odorifer*; *Pseudomonas* sp.; *B. subtilis*; *Azotobacter* sp. (isolated from roots); *B. subtilis*; *B. pumilus*; *B. amilequefasciens* (isolated from grain). *B. cereus*, and *B. thuringiensis* were also isolated from the rhizosphere, and the latter species as endophyte. This great diversity of species and strains of bacteria associated with quinoa are a potential to develop biotechnology products to improve the sustainability and productivity of quinoa cultivation in the Southern Altiplano of Bolivia.

Keywords: Microorganisms; Symbionts; Environmental Services

Resumen. Bacterias asociadas al cultivo de la quinua en el Altiplano Boliviano y su potencial biotecnológico. La quinua crece en condiciones extremas de humedad y temperatura en una zona semidesértica, por tanto es posible que también sus microorganismos simbioses estén adaptados a esas condiciones extremas. Estos microorganismos representan un gran potencial para el desarrollo de biotecnología adaptada a la quinua. Para ello se colectaron plantas de quinua y muestras de suelo del Altiplano Sud, estas fueron llevadas al laboratorio donde se aislaron diferentes especies de bacterias y se examinó el servicio ambiental que pueden brindar (fijador de nitrógeno, solubilizador de fósforo o generador de AIA). Luego las bacterias fueron identificadas mediante técnicas moleculares: *Bacillus amyloliquefaciens*; *B. tequilensis*; *B. vallismortis*; *B. subtilis*; *B. pumilus*; *B. licheniformis* y *B. firmus* (aislados de las hojas). *B. aryabhattai*; *B. horikoshii*; *B. megaterium*; *B. pumilus* y *Paenibacillus odorifer*; *Pseudomonas* sp.; *B. subtilis*; *Azotobacter* sp. (aislados de las raíces); *B. subtilis*; *B. pumilus*; *B. amilequefasciens* (aislados del grano). También se aisló *B. cereus*, *B. thuringiensis* de la rizósfera, y esta última especie como endófito. Esta gran diversidad de especies y cepas de bacterias asociadas a la quinua representan un potencial para desarrollar productos biotecnológicos destinados a mejorar la sostenibilidad y productividad del cultivo de quinua en el Altiplano Sud de Bolivia.

Palabras clave: Microorganismos; Simbioses; Servicios Ambientales

Introduction

Most soils, where naturally propagated plants grow, are colonized by microbial communities covering a wide variety of genera and species. These obligate or facultative symbiont microbiota, is associated with the recycling of nutrients, increasing plant growth and speeding up development, and improving the resistance of plants to environmental stress. There is also a relation with the production of plant hormones, metabolites and enzymes to induce basal plant resistance; with soil pathogen suppression or action as entomopathogens (Sturz *et al.*, 2000 and Harrison, 2005).

There are microorganisms (fungi, bacteria, actinomycetes and others) living within plants and are called endophytes. These are located in intracellular or intercellular spaces in the vascular tissue (Reinhold-Hurek and Hurek, 1998). These, being in close association with plants, have beneficial effects of greater importance compared to those living in the area influenced by the root (rhizosphere). Endophytic microorganisms have less competition for nutrients than those located in the rhizosphere and therefore provide direct benefits to the host plant (Muñoz-Rojas and Caballero, 2003).

This paper seeks to explore the natural advantages of symbiosis that quinoa plants have in their natural habitat, in the Bolivian Southern Altiplano. This arid and semi-deserted region has soils with low fertility and high erosion, where processes of nutrient cycling, retention of soil moisture and microbial activity are limited. Due to these characteristics there is low diversity of native and cultivated species in the region.

Despite the rough climatic and edaphic conditions of the Altiplano, quinoa develops properly, namely it is a plant adapted to those ecological conditions. Although no experimental data are available, it can be assumed that the symbiotic microorganisms in quinoa should also be well adapted to this habitat. Therefore, exploring the microbiology of this plant is an opportunity to understand the phenomena of plant nutrition, fertility restoration, pathogen suppression and other mechanisms that are present in these extreme ecosystems. This knowledge holds great potential to restore soil fertility in the Altiplano in an attempt to mitigate the negative effects of the environment.

Materials and methods

A) Sampling: The plant samples were collected from organic quinoa production plots. Five plants were systematically collected from each production plot, 10 plots were considered from three communities in the areas of Salinas de Garci Mendoza, Challapata and Quillacas from the department of Oruro. Likewise, in the department of Potosí samples were collected from plots in the communities of Chacala, Mañica and Llica. Different organs (stem, leaves and grains) were used to isolate bacteria and endophytic fungi. Microorganisms from the rhizosphere and rhizoplane were also isolated. Samples were placed in plastic bags, kept cold in coolers and transported to the laboratory where they were stored at 10°C until processing. Samples destined to molecular analysis were stored at -20°C.

B) Isolation of endophytic bacteria: For the isolation of endophytic bacteria, the protocol “Microbiology Manual” of Dion and Magallon (2009) was used. Tissues were fragmented, superficially sterilized, then each piece was placed in Trypticase Soy Agar (TSA) and incubated at 28°C for 72 hours. Once colonies were grown, they were isolated in test tubes with TSA for further characterization (Figure 1).



Figure 1 Process of endophytic microorganisms isolation

C) Characterization of environmental services by microorganisms: Once isolated, the environmental service provided by each bacterial culture was researched. The following services were identified: nitrogen fixation, phosphorus solubilization, and production of indole acetic acid (IAA). The potential offered by these microorganisms, to develop future biotech products for recycling nutrients, promoting growth and protecting quinoa plants in their highly vulnerable habitat, was established. The procedure to determine each of the environmental services was as follows:

c.1. Nitrogen-fixation: Work was performed on the basis of the Dion and Magallon (2009) protocol. Bacterial strains were planted in Burk's culture medium. Plates were incubated 5 to 15 days at 28°C. The incubation time varied according to the kind of bacteria treated, which is why a daily monitoring of the test was performed.

c.2. Phosphorus-solubilization: Bacteria to be assessed were planted in a plate containing NBRIP with tricalcium phosphate as culture medium. Plates were incubated 5 to 15 days at 28 °C. Incubation time varied depending on the kind of bacteria treated, which is why a daily monitoring of the test was performed (CIP, 2008). Bacterial strains that showed a transparent halo around the planted colony were recorded as positive.

c.3. Indole acetic acid (IAA) generation: IAA is a secondary metabolite produced by bacteria in their stationary period of growth. Therefore, the culture broth incubation time depended on the bacterial growth curve. Incubation then was extended long enough to ensure that bacteria reached the stationary phase. For

this purpose the studied strains were reactivated in Soy Trypticase broth (TSB), seeding the colonies of each strain in a tube with TSB medium, supplemented with 5 μ M (micro mol) of L-tryptophan, and incubated for 7 days at 28°C (Gordon and Weber, 1950). Samples that revealed with tones of red were reported as positive. Once grown, bacteria colonies were isolated in tubes with TSA for further testing and characterization.

D) Molecular identification: Genome regions of each microbial isolation, were amplified through PCR, conserved and used as identity indicators; using primers whose sequences were obtained from the literature (Lane, 1991, Krüger *et al.*, 2009). These conserved regions correspond to genes encoding ribosomal RNA from the small and large ribosomal subunit. The primers used for these bacteria were: 27A or 27C (sense primer) and 1488 or 1492 (antisense primer), (Lane, 1991). The primers were synthesized by AlphaDNA, Quebec, Canada; and a DNA Polymerase (Phusion, Finnzymes, Finland) with high fidelity was used in order to minimize the introduction of mutations in the amplified genome regions. Each PCR product was purified with GeneJet (Fermentas, USA) columns prior to their shipment to the Sequencing Center at the University of Chicago, Chicago, IL, USA, where fragments were sequenced using the Sanger technique (Sanger *et al.*, 1977). The sequences obtained were edited with the BioEdit software (Hall, 1999) and then checked against databases using the BLAST software (Altschul *et al.*, 1990).

Results and discussion

Diversity by region

The results showed that the variability of microbial populations in the rhizosphere and rhizoplane are higher, where more symbiont or non-symbiont micro-organisms are concentrated (Figure 2). It was also determined that there are endophytic microorganisms in quinoa seeds or grains, being the dominant strains from the species *Bacillus subtilis*, *B. pumilus* and *B. amiloliquefasciens* and other unidentified. Endophytism is one of the most efficient forms of plant-microorganism symbiosis, mainly because microorganisms have the same dissemination system as the plant; this natural mechanism ensures that quinoa grains will be accompanied by their symbionts, wherever they go (Figure 3).

Isolation and characterization of environmental services

Some nitrogen-fixing bacteria are free-living, ie which do not require a host plant for carrying out the process of nitrogen fixation. 260 strains of endophytic bacteria where isolated from quinoa samples from the Southern Altiplano. 31 isolations were detected as potential for nitrogen fixation (Figure 4).

Out of 480 strains of quinoa endophytic bacteria from plants of the Southern Altiplano, 72 were detected as phosphorus solubilizers (Figure 5).

28 positive strains with the ability to generate the phytohormone IAA were isolated (Figure 6).

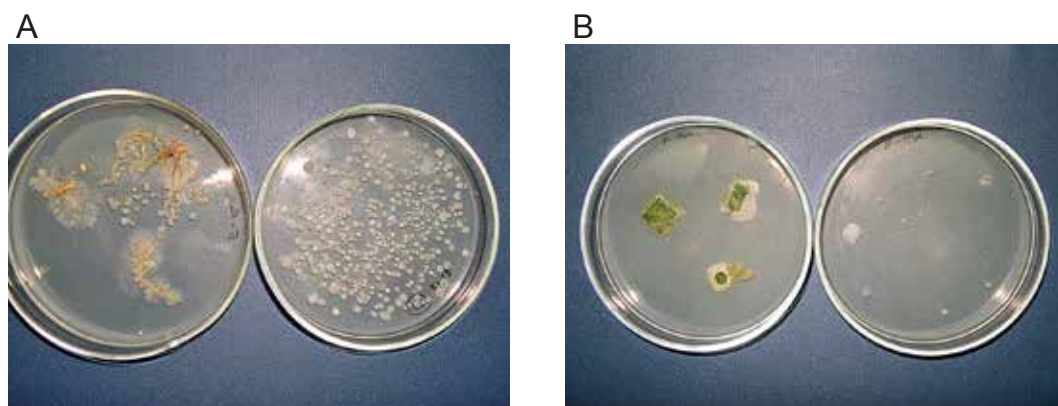


Figure 2. Endophytic bacterial colonies isolated from: A) roots B) leaves

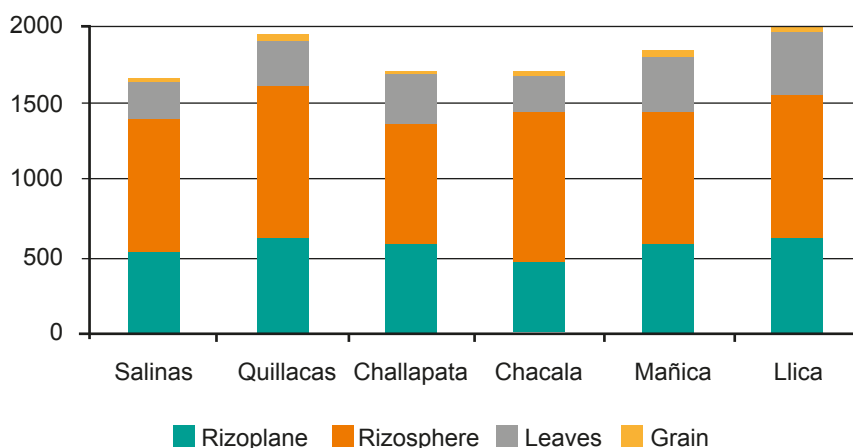


Figure 3. Isolation of microbial populations associated with quinoa plants from different communities in the Southern Altiplano

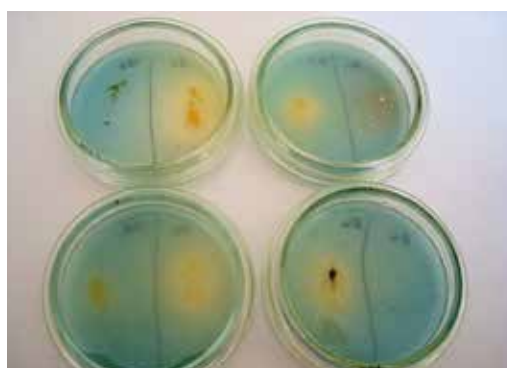


Figure 4. Reaction of nitrogen-fixing free life strains of quinoa bacteria in culture media



Figure 5. Evaluation of phosphorus solubilizing strains (The ones showing the halo around the colony are positive)

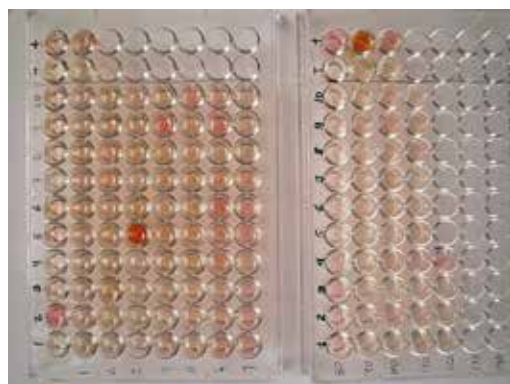


Figure 6. Revealing of the IAA
(Bacteria that produce phytohormones
have a dark red color)

Several strains of bacteria were identified based on their ability to provide environmental services to quinoa plants. The following distribution was obtained:

- 235 nitrogen-fixing strains
- 456 phosphorus solubilizing strains
- 789 IAA generating strains

This demonstrates the potential of microbial biodiversity for the development of technology that can help mitigate the effects of soil erosion in these arid regions of Bolivia.

Molecular identification of bacterial isolations

Molecular identification of a number of bacterial isolations that were selected by their functional characteristics and environmental services, was performed through sequencing of the gene encoding 16S ribosomal RNA and the use of the BLAST software. The most frequent bacteria with high agroindustrial potential were the following:

- *Bacillus amyloliquefaciens*; *B. tequilensis*; *B. vallismortis*; *B. subtilis*;

B. pumilus; *B. licheniformis* and *B. firmus* (isolated from leaves).

- *Bacillus aryabhattai*; *Bacillus horikoshii*; *B. megaterium*; *B. pumilus* and *Paenibacillus odorifer*; *Pseudomonas* sp.; *B. subtilis*; *Azotobacter* sp. (isolated from roots).
- *Bacillus subtilis*; *B. pumilus*; *B. amilequefasciens* (isolated from quinoa grain).

There is no observable relationship between species of bacteria, the environmental service, and collection areas (Table 1). There seems to be a random possibility of finding predominance of a strain in a given area of the Bolivian Altiplano.

The bacteria identified as *Azotobacter* sp., in addition to being nitrogen-fixing, is capable of solubilizing phosphorus. On the other hand, the genus *Rhizobium* isolated from the rhizosphere of quinoa plants belongs to a group of rhizobia that do not enter in symbiosis with plants, meaning they are free-living (Becquer *et al.*, 2013). A similar situation takes place with isolations from the genus *Flavobacterium*, which act freely fixing nitrogen to the soil.

The endophytic bacteria have advantages over rhizospheric bacteria, due to the wide variety of nutrients they can access, and the protection provided by the plant against adverse environmental conditions (Reinhold-Hurek & Hurek, 1998). The endophytic bacteria are important especially for plants growing under extreme conditions. An example of this is *B. subtilis* that, in addition to promoting growth in plants, is a systemic resistance inducer. It activates the jasmonic acid cycle, which is responsible for inducing plant resistance to pathogen attack.

Table 1. Collection areas, molecular identification and environmental services provided by bacteria associated with the quinoa crop

| Collection Area | Identity | Environmental service |
|---|--|---|
| Salinas de Garci Mendoza | <i>Bacillus pumilus</i> , <i>Bacillus liquefasciens</i> | Phosphorus solubilization |
| Chacala, Llica y Salinas de Garci Mendoza | <i>Bacillus subtilis</i> , <i>Bacillus vallismortis</i> , <i>Bacillus liquefasciens</i> | Indole Acetic Acid (IAA) |
| Chacala, Challapata, Llica | <i>Bacillus</i> sp., <i>Bacillus aryabhattai</i> , <i>Bacillus horikoshii</i> | Indole Acetic Acid (IAA) |
| Llica, Chacala, Quillacas | <i>Bacillus firmus</i> , <i>Bacillus tequilensis</i> | Phosphorus solubilization |
| Llica, Quillacas | <i>Bacillus pumilus</i> | Phosphorus solubilization |
| Quillacas, Mañica, Chacala | <i>Bacillus subtilis</i> | Indole Acetic Acid (IAA) |
| Salinas de Garci Mendoza | <i>Paenibacillus</i> | Nitrogen fixation |
| Chacala y Mañica | <i>Bacillus simplex</i> | Indole Acetic Acid (IAA) |
| Quillacas, Chacala | <i>Bacillus licheniformis</i> , <i>Bacillus subtilis</i> , <i>Bacillus liquefasciens</i> | Phosphorus and IAA solubilization |
| Challapata | <i>Azotobacter</i> sp. <i>Pseudomonas</i> sp. | Nitrogen fixation and Phosphorus solubilization |
| Challapata y Quillacas | <i>Rhizobium</i> sp., <i>Flavobacterium</i> sp. | Nitrogen fixation |

Likewise, the genus *Azotobacter*, which acts as nitrogen-fixing for non-legume species like quinoa, is an alternative to develop research programs to improve the production of organic quinoa in the Bolivian Altiplano.

Other genuses

Bacillus thuringiensis was obtained from 13 samples, eight soil samples, four from the rhizosphere and one quinoa endophyte

(Table 2). To understand the insecticidal spectrum activity of Cry toxins (δ -endotoxin crystal) held by the bacteria, 10 genes Cry were analyzed by PCR. Results indicate that most of the quinoa related *B. thuringiensis* isolates, have the Cry 1 and Cry 2 genes. These Cry 1 genes are active against lepidopteran insects (moths and butterflies) and Cry 2 toxins affect mainly Diptera (flies), but also Lepidoptera. Characterization must be continued to specify toxin subgroups and confirm their toxic activity through bioassays on insect larvae.

Table 2. Other genera of bacteria detected in different collection areas, molecularly identified, and the environmental services they provide

| Collection Area | Identity | Environmental service |
|-------------------------------------|-------------------------------|---|
| Llica, Chacala, Challapata | <i>Bacillus thuringiensis</i> | Entomopathogenic of Lepidoptera and other species |
| Llica, Mañica, Qillacas, Challapata | <i>Bacillus cereus</i> | Entomopathogenic of Coleoptera |

Particular emphasis should be placed on the isolation of *Bacillus thuringiensis* found as an endophyte, because after being sprayed it can stay protected from ultraviolet rays inside the plant, having a longer residual effect as entomopathogen. The species *Bacillus cereus*, an entomopathogen of Coleoptera, was also isolated from soil samples. This species is important for the development of bioinsecticides that can contribute to the management of this group of insect pests.

Conclusions

- Results show that there are a significant number of bacteria associated with quinoa plants, several of which are beneficial species that provide environmental services. These bacteria have great biotechnological and agroindustrial potential for organic and sustainable quinoa production.
- 31 nitrogen-fixing bacteria, 72 phosphorous solubilizing bacteria and 28 IAA generating strains were detected. The fact that some species are repeated does not necessarily mean that they are the same strain. There are isolates from the genera *Azotobacter*, *Pseudomonas*, *Rhizobium* and *Flavobacterium* that are free-living and nitrogen fixers, a fact that still

needs to be studied.

- Endophytes from the species *Bacillus subtilis*, *B. pumilus* and *B. amiloliquefasciens* and other non-identified were detected in quinoa grains.
- Detection of entomopathogens such as *Bacillus thuringiensis*, for the control of lepidopteran, is a resource that must be evaluated given its potential for the development of bioinsecticides for organic pest management.
- *Bacillus cereus*, as entomopathogen is representative of strains that can be used for the management of Coleopteran pests not only for quinoa production, but for other crops in the Altiplano region.

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Plagas y enfermedades en el cultivo de Quinua

Editores:

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La quinua en los últimos años ha tomado un interés global, se la cultiva en más de 50 países en todos los continentes y en diferentes pisos y zonas agroecológicas. Sin embargo, la mayor producción es en la zona Andina de Bolivia y Perú donde ha sido domesticada, cubriendo actualmente cerca del 80% de la demanda internacional. Es en esta zona donde se ha generado la mayor información sobre el manejo del cultivo, su diversidad genética y sus problemas. Por ello, el presente documento hace referencia principalmente a las plagas y enfermedades, sus ciclos, comportamientos y los efectos causados en la productividad.



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Synthesis and development of sex pheromones for two species of Noctuidae, key pests of the quinoa crop

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Summary. The work describes the synthesis and development process of sex pheromones for two species of Noctuidae (*Helicoverpa quinoa* and *Copitarsia incommoda*), considered key pests of the quinoa crop. It is a joint effort between researchers from PROINPA and the Pherobank Company of the Netherlands. In this context, PROINPA was responsible for mass rearing of the species under laboratory conditions, the permanent provision of pupae, field evaluations of protopheromones and the development of traps for their use. Pherobank was responsible for the identification of compounds, synthesis of protopheromones and pheromones. As a result of this coordinated effort, the specific synthesis of sex pheromones was achieved for *H. quinoa*, an important pest in the Southern Altiplano, and *C. incommoda*, key pest in the Central and Northern Altiplano.

Keywords: *Helicoverpa quinoa*; *Copitarsia incommoda*, Integrated Pests Management

Resumen. Síntesis y desarrollo de feromonas sexuales para dos noctuideos, plagas clave del cultivo de quinua. El trabajo describe el proceso de síntesis y desarrollo de feromonas sexuales para dos especies de noctuideos (*Helicoverpa quinoa* y *Copitarsia incommoda*), consideradas plagas clave del cultivo de la quinua, en un trabajo conjunto entre investigadores de la Fundación PROINPA y la Empresa Pherobank de Holanda. En este marco, PROINPA fue responsable de la cría masiva de las especies en condiciones de laboratorio, la provisión permanente de pupas, las evaluaciones de las protoferomas en campo y el desarrollo de trampas para su uso. Pherobank estuvo a cargo de la identificación de los compuestos, la síntesis de las protoferomonas y la síntesis de las feromonas. Producto de este trabajo coordinado se logró sintetizar feromonas sexuales específicas para *H. quinoa*, plaga importante en el Altiplano Sur y *C. incommoda*, plaga clave en el Altiplano Central y Altiplano Norte.

Palabras clave: *Helicoverpa quinoa*; *Copitarsia incommoda*, Manejo Integrado de Plagas

Introduction

Helicoverpa quinoa and *Copitarsia incommoda* are key pests of the quinoa crop in the Bolivian Altiplano, where the largest volumes of this Andean grain are produced for both domestic and export markets. The larvae of these species along with others that make up the Noctuidae complex (*Helicoverpa titicacae* and *Dargida acanthus*) cause considerable losses, reaching up to 30% of yield per year and an estimate of USD 60 million for the year 2014. The type of damage caused by these larvae depends largely on the species, the phenological state of the plant and the stage of the larvae. The newly hatched larvae feed on the quinoa plant inflorescence during its formation, and when the larvae are larger they behave as defoliators.

During the phenological stages of flowering and physiological maturity, larvae cause significant damage by eating the forming grain or by drilling the rachis of the panicle, causing its collapse.

The greatest damage from these Noctuidea species are caused during grain filling at the stage of milky ripening and starchy ripening, when larvae begin to eat the grains. Farmers fight them using light traps and through the application of eco-insecticides or chemical-pesticides, depending on the destination of production.

Five years ago, to provide farmers with organic easy to apply control alternatives, PROINPA posed the synthesis and development of sex pheromones. These sex pheromones are chemical signals emitted by females to attract males of the

same species. The artificially synthesized pheromones are installed in traps, which catch male insects, preventing the next generation, thus reducing the pest population. Currently sex pheromones of different species are successfully used as a component of Integrated Pest Management (IPM).

The objective of the work delivered was to synthesize and develop sex pheromones for *H. quinoa* and *C. incommode*, key pests of the quinoa crop in the Bolivian Altiplano.

Life cycle of *H. quinoa*. According to studies performed in the Quipaquipani Center (Viachan, La Paz), the life cycle of *H. quinoa* is very singular. Of a total of 400 larvae under observation in conditions of 25°C temperature and 65% relative humidity, 50% reported a lifespan of 223 ± 36 days from egg to adult (including adult longevity), 25% remained in the pupal stage until the next agricultural season, and 15 % died before reaching adulthood.

Life cycle of *C. incommoda*. According to Choquehuanca (2011) The life cycle of *C. incommoda* reared in laboratory at 25°C temperature and 65% relative humidity, has a duration of 99.91 days including adult longevity. The incubation period is 5.5 days, the duration of the larval stage is 29.04 days, prepupal status lasts 3.03 days and pupal 16.3 days; adults live an average of 19.85 days.

Damages and losses caused by larvae from the Noctuidae complex. Damages caused by larvae from the Noctuidae complex are multiple. Young larvae mine the inflorescence during formation, in

more advanced stages larvae defoliate plants, drill the stem at the base causing the collapse of the panicle and, consume the grains. Estimated losses due to the attack of larvae from the Noctuidae complex and of the quinoa moth are of approximately 30% of yield (Saravia and Quispe, 2005).

Geographical distribution of *H. quinoa* and *C. incommoda*. Studies on the distribution of these species in the quinoa producing regions have shown that *H. quinoa* is the dominant species in the Southern Altiplano Bolivia, and *C. incommoda* is the dominant species in the Central and Northern Altiplano. In these regions the species *H. titicacae* and *Dargida acanthus* can also be found but in lower population densities.

Pheromones. They are secretions that cause specific reactions in individuals of the same species that perceive them (Ramirez, 1996). Pedigo (1996) states that sex pheromones are chemical signals emitted by females to attract males of the same species. Currently sex pheromones of different species can be synthesized and used for Integrated Pest Management (IPM).

Types of pheromones. There are several types of pheromones, including sexual, aggregation, tracking and alarm pheromones (Owen, 2013).

Advantages and disadvantages of pheromones. Like any tool used for IPM, pheromones have advantages and disadvantages. The main advantages attributed to them are: they are specific and ensure the survival of natural enemies; they are not toxic and do not

leave chemical residue on the crops, that is to say it is an environmentally clean technology. They are biodegradable and environmentally friendly; they are easy to install and operate. For all the above mentioned benefits, pheromones are accepted in organic production. The identified disadvantages include the following: only attract males, may be affected by weather (winds) and do not provide information on the direct damage to the crop (Cisneros, 1997).

Pheromone traps. Pheromones should be used in traps. There are various types of traps designed to increase the efficiency of capture, that are currently offered by companies that produce pheromones. Traps are designed taking into account the flight behavior of different species of pests. To capture *H. quinoa*, the traps tested were: drum type, basin type, cone and several variants of the funnel, which can operate with or without water. Of these traps, the most widespread type in quinoa producing areas of the Altiplano is the dry funnel because it does not require water to catch insects.

Materials and methods

Noctuidae rearing for pheromone synthesis. The synthesis of the sex pheromone of *H. quinoa* and *C. incommoda*, began in 2007 with the mass rearing of these species and several deliveries of pupae to the Pherobank Company of the Netherlands. Noctuidae rearing was implemented through a protocol developed at PROINPA's Entomology Laboratory from March to December 2007. The protocol includes the steps listed below:

Collection of larvae. Larvae of Noctuidae were collected in quinoa plots, at different times and in different locations of the Southern and Central Altiplano.

Larvae adaptation to laboratory conditions. Larvae were separated individually in medium size plastic containers in order to prevent cannibalism. These larvae were fed with quinoa leaves until they pupated (Figure 1). Once they reached the pupal stage they were disinfected and separated into groups of ten, and placed in larger plastic containers until eclosion.



Figure 1. Larvae fed with quinoa leaves

Reproduction. The eclosed adults were installed in groups of 10-16 individuals, in plastic bottles of 3800 cc volume, as recommended by Quispe (2000). These vials were used as reproduction cameras and to obtain eggs (Figure 2). To facilitate the laying of eggs, blotter strips were hung on the side walls of the bottles. Oviposition was verified, collection took place through cutting lots of blotting paper containing the eggs. These lots of paper were later placed in medium size containers for maturation, eclosion and breeding of a new generation of larvae.



Figure 2. Reproduction camera

Rearing of the next generation. The new generation of larvae was reared on an artificial diet developed and adjusted in the Entomology Laboratory of PROINPA, through placing 30 neonate larvae per container with diet (Figure 3).



Figure 3. Rearing with artificial diet

After 10 days larvae were separated in containers with diet, where they concluded their larval stage and reached

the pupal stage. Subsequently pupae were-harvested (Figure 4), disinfected with a 10% concentration of sodium hypochlorite, separated into groups of ten individuals and placed in medium plastic jars until adult emergence.



Figure 4. Pupae harvest

Pheromone synthesis. For the synthesis of pheromones pupae of the species *H. quinoa* and *C. incommoda* were sent to Pherobank in the Netherlands where the pheromone components of these species were identified using the combined method of Gas Chromatography (GC) linked to Mass Spectrometry (MS) and Electroantennography (EAG).

Once protophheromones were developed by Pherobank, they were sent back to Bolivia for field trials and adjustments in the formulation of the pheromone.

These evaluations were performed in the community of Chacala, municipality of Uyuni, and in the Quipaquipani Center, located in the department of La Paz.

Results and discussion

Pupae shipments for identification and synthesis of specific pheromones

Several shipments of pupae to Pherobank were performed. Pupae came from the mass rearing of these species in the Entomology Laboratory of PROINPA. The samples were enclosed in plastic containers, in batches of 20 to 40 properly sexed pupae (Figure 5).

Synthesis of pheromones

In late April 2008, as a result of laboratory work delivered by Pherobank in the Netherlands, 17 protophheromones for the Noctuidae complex were formulated (Figure 6).



Figure 5. Shipping of pupae in plastic containers



Figure 6. Protopheromones sent by Pherobank for field evaluations

Based on the information generated in field trials carried out by PROINPA's technical staff in the Bolivian Altiplano, until June 2009 a pheromone for the species *H. quinoa* was synthesized; and in June 2013 another pheromone was synthesized for *C. incommoda*.

Conclusions

- There are protocols for mass rearing of *H. quinoa* and *C. incommoda*.
- There are sex pheromones for *H. quinoa*, key pest of the quinoa crop in the Southern Altiplano; *C. incommoda*, key pest of the quinoa crop in the Central and Northern Altiplano; and *Agrotis peruviana*, considered a potential pest of the quinoa crop in Bolivia.

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Mass dissemination of the Integrated Pest Management (IPM) strategy for quinoa

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Summary. Quinoa is one of the most important crops in Bolivia. 131,190 hectares are cultivated by 45,000 families producing 61,659 tons of grain for marketing. One of the limiting factors for quinoa production is the incidence of insect pests such as the cut-worms: *Helicoverpa*, *Copitarsia* and *Agrotis*; and the quinoa moth (*Eurysacca*), that altogether cause 30% of damage to the crop. The economic losses as a result of these pests are significant, particularly taking into account that the value of quinoa by 2013, reached US\$147,520,893. PROINPA Foundation developed an efficient management strategy to control quinoa pests, with emphasis on the use of pheromones and natural insecticides, commercialized in the Bolivian Altiplano through an alliance with BIOTOP SRL, ANAPQUI, CADEQUIR and enterprises associated to CABOLQUI. Elements of the strategy have managed to reach a total of 20,000 hectares and have reduced losses in US\$14,618,275, for the benefit of farming families and the country.

Keywords: Phytopathology; Pheromones; Natural Insecticides

Resumen. Difusión masiva de la estrategia de Manejo Integrado de Plagas (MIP) en quinua. La quinua es uno de los cultivos más importantes en Bolivia, 131.190 hectáreas son cultivadas por 45.000 familias que producen 61.659 toneladas de grano para su comercialización. Uno de los factores limitantes en la producción de la quinua es la incidencia de insectos plaga como las ticonas: *Helicoverpa*, *Copitarsia* y *Agrotis* y la polilla de la quinua (*Eurysacca*), que juntas ocasionan un 30% de daño al cultivo. Las pérdidas económicas, a consecuencia de estas plagas, son significativas, tomando en cuenta que el valor de comercialización de quinua al año 2013 alcanzó los 147.520.893 USD. La Fundación PROINPA desarrolló una estrategia de manejo eficiente en el control de plagas de la quinua, con énfasis en el uso de feromonas e insecticidas naturales, comercializados en el Altiplano Boliviano, en alianza con BIOTOP SRL, ANAPQUI, CADEQUIR y empresas asociadas a CABOLQUI. Elementos de la estrategia han logrado cubrir un total de 20.000 hectáreas y reducir las pérdidas en 14.618.275 USD, en beneficio de las familias de productores y del país.

Palabras clave: Fitopatología; Feromonas; Insecticidas Naturales

Problem description and Project actions

Quinoa (*Chenopodium quinoa* Willd.) Is one of the most important crops in Bolivia, acreage extends up to 131,190 hectares and production reached 61,659 tons in 2013. The families involved in its cultivation are around 45,000, from the North, South and Central Altiplano (PROINPA Foundation and AUTAPO Foundation, 2005).|

One of the factors that adversely affects crop productivity is the incidence of a variety of insect pests, such as the so-called ticona (cutworm) complex composed of several species of moths, *Copitarsia incommoda*, *Helicoverpa quinoa*, *Agrotis andina* and the quinoa moth *Eurysacca quinoae* (PROINPA, 2010). These pests in their larval state produce damage to the crop, affecting grain quality and causing production losses of up to 30%, which for 2013 correspond to 147.520.893.- USD per annum (based on the price of 1,800 BS / qq, prevailing at the Challapata market in June 2014).

To control these pests in organic production systems, products registered and accepted in organic regulations must be used. However, there is limited supply of these products to be able to use them in an extensive scale. For that reason PROINPA has developed a strategy for the efficient management of pest control, based on bio-registered products approved for organic production. With the purpose of expanding and disseminating the strategy, PROINPA has partnered with BIOTOP SRL, who coordinated outreach activities with independent

producers, organic quinoa associations and companies.

Methodology

The strategy proposed by PROINPA, for integrated pest management (IPM) in organic production has been implemented quite successfully in over 20,000 hectares in 2013. This strategy is based on monitoring of adults and larvae, preventive treatments, alternation of products (active ingredients and modes of action), timely applications and use of adjuvant products. The integrated management components are:

⇒ *Installation of pheromone traps (for noctuidae)*: The installation of four traps per hectare within the plot (with a distance of at least 25 m between traps), helps identify the first presence of adult insects and the beginning of the oviposition period. In areas where the population of noctuidae is still low, the use of the four traps/ha keeps larval populations at levels that do not cause significant damage ($\leq 5\%$ damage).

⇒ *Field Inspection*: Regular inspection visits should be made in at least four stages of crop development: six true leaves, beginning of panicle formation, grain formation and milky grain. At each inspection at least 10 plants per hectare should be sampled at random. If the presence of eggs and / or early stages of larvae are observed in 20% of the evaluated plants, preventive applications are recommended. When larvae of more advanced stages are observed or in case of increased incidence, the application of control treatments is recommended.

⇒ *Application of preventive treatments:* the application of lime sulfur plus chili is recommended because of its contact mode of action which affects the central nervous system of the insect, allowing good control of eggs and of larvae in early stages. Additionally, this product has a repellent effect for adult insects, protecting the crop for at least 15 days from new pest oviposition phases.

⇒ *Application of control treatments:* a control treatment at the beginning of panicle formation is recommended. During this phenological period protection against these pests is very important. Control treatments can be made when insect monitoring detects populations that justify applications. Damage to the crop causes proliferation of side branches resulting in handling difficulties and lower performance. When the presence of at least one larva per plant is observed, the application of Spinosad® is recommended.

Another moment of care is the growth stage of milky grain, because it is the time when the larvae begin to feed on the grain in formation, and can cause significant economic damage. At this point it is important to perform field inspections. For ticonas (cutworms), if five larvae per panicle are observed in ten plants, and in the case of moths, if five larvae are observed per panicle; the application of Spinosad® is recommended. Spinosad® is a natural high efficiency insecticide (> 93%) accepted in organic production, whose mode of action is through contact and ingestion, allowing an efficient larval control and minimal effect on existing beneficial insect fauna.

⇒ *Alternation of treatments:* Following IPM principles and in order to avoid the emergence of resistant populations, alternation of treatments is recommended; ie alternating the application of natural insecticides considering their active ingredients and different modes of action, avoiding more than two continuous applications of the same product per crop cycle. For example, lime sulfur and chili extract can be alternated with Spinosad®.

⇒ *Using adjuvants:* Because of the presence of large amounts of oxalates in the surfaces of leaves, stems and panicles of quinoa, bio-adhesion is difficult. Therefore, it is very important to apply a bonding agent such as Agricultural Vegetable Oil, which acts as a dispersing agent, improves application coverage and prevents the formation of large droplets. Application of Agricultural Oil ensures the efficiency of products.

Achievements

During the 2013-2014 crop year, the alliance PROINPA and BIOTOP SRL, has worked in partnership with various producer associations and companies:

In CADEQUIR, ANAPQUI, CADEPQUIOR, SINDAN, Jacha Inti, APQUISA, APQO Villa Florida, APQO-Toledo, APQO-Keluyo, FDTA Valles, IDEPRO, APROACH, APROCAL, Andean Valley, APROQUIROT, APROCOTES, APROACAY, APRA-Chacala, APROQUIOS, and APRACCUK, knowledge, attitudes and practices of approximately 2,800 associates (men and women) were strengthened. These

farmers were reached through capacity building events delivered by technicians and promoters, who personally spread the Integrated Pest Management strategy for organic quinoa production, reaching about 20,000 acres with this technology.

The implementation of the strategy reduced losses from 30% to 10% in the 20,000 acres that used Integrated Pest Management. In economic terms, with values of 1.800 Bs/qq (market price in June 2014), this means, 14,618,275 USD that producers have stopped losing, and represent a benefit to the country through improvement of crop productivity, enhanced yield, better product quality and environmental stewardship.

On the other hand, in quinoa production areas of the Bolivian Altiplano, a total of 70,926 units of pheromones for quinoa cutworms (ticonas) have been sold, covering more the 20,000 ha, representing 30% of the area cultivated with organic quinoa. In the case of lime sulfur and chili extract, in the past three crop years, a total of 20,684 liters have been sold, covering approximately 8300 hectares. In the case of Spinosad®, in the past three crop years, a total of 117,400 grams have been sold, covering approximately 4000 hectares.

Figure 1 shows the positive trend of the use of products in the last three years, particularly high for the case of lime sulfur and higher for Spinosad.

Conclusions and recommendations

- As a result of advocacy and training activities for technicians, promoters and farmers, the quinoa pest management strategy developed by

PROINPA and disseminated by BIOTOP SRL, has been accepted by organic quinoa producers, and in several cases has been adjusted with the implementation of local practices and inputs. This situation can be evidenced through sales data recorded by BIOTOP SRL.

- This initiative by PROINPA and BIOTOP SRL has been well received, especially by producer associations and companies dedicated to the production of organic quinoa. The process has resulted in agreements, higher volumes of bio-inputs sold and supported by training for adequate use; being all reflected in higher income for farmers.
- The implementation of the strategy for Integrated Management of insect pests used for organic quinoa production in 20,000 hectares, allowed producers to reduce losses in 14.618.275.- USD.
- The task ahead is a larger scale dissemination of the integrated management strategy, in order for most producers to have access to it and to bio-inputs for application in organic quinoa production. For example, many farmers still use spraying backpacks, resulting in an inefficient product application and control.

The implementation of community control campaigns should also be considered, because if one farmer controls and others don't, efficiency is lost and the damages continues. This will depend on the coordinated efforts of producers, associations, companies, state institutions (municipalities, governorates, etc.), organic certifiers and other actors.

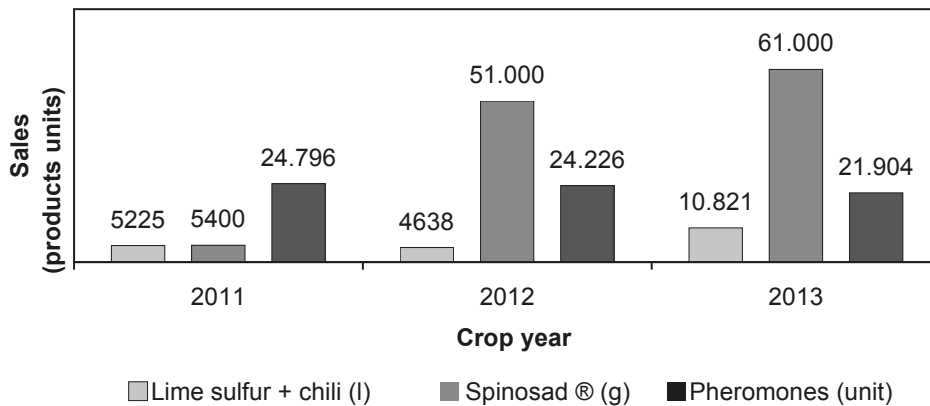


Figure 1. Units of Lime sulfur plus chilli extract, Spinosad ® and pheromones comercialized by BIOTOP SRL and used for quinoa production during the 2011 – 2013 crop years.

- The development of an integrated management strategy that responds to organic production demands is a continuous research work that should be directed to the development of new, more efficient bio-inputs (plant extracts, etc.) permitted for organic production and which can be easily accessed in local markets.

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Native shrubs and their prospective contribution to the sustainability of quinoa production

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Summary. Quinoa production statistics presented by the Ministry of Agriculture of Bolivia during the last 10 years indicate that average quinoa yields do not exceed 600 kg/ha, thus the increased production figures are determined by expansion of the agricultural frontier. When examining production patterns, in traditional quinoa producing areas there is evidence that yields are generally very low due to the following reasons: accelerated soil erosion; low fertility; proliferation of pests; frosts and poor management of the production process. In Bolivia, much has been said about the sustainability of quinoa production, but there isn't a policy decision to make this wish come true, mainly because there is no coordination between actors for this purpose. Within this framework, PROINPA Foundation has begun the implementation of a series of activities to exploit the biodiversity potential of shrub species in the region. These activities are based on a productive system focus, which can make the production of quinoa sustainable. Information was collected on similar experiences in the country, related to highland native shrubs and their management. Yet the most important element of this process was the voluntary participation of producers who offered their plots of land for seed collection and to implement the suggested practices. Seed collection through traditional methods was satisfactory and nursery multiplication of native species was very good, with results that exceed project expectations. However, there were difficulties in direct field sowing. Despite achieving some germination, problems associated with seedlings buried by strong winds were crucial for the loss of material.

Keywords: Agricultural Management; Repopulation; Seed Quality

Resumen. Los arbustos nativos y las perspectivas de su contribución a la sostenibilidad de la producción de quinua. Las cifras de producción de quinua que presenta el Ministerio de Agricultura en Bolivia, en los últimos 10 años, indican que los rendimientos promedio en el país no superan los 600 kg/ha y el incremento de la producción está determinado por la expansión de la frontera agrícola. Cuando se examina el comportamiento de la producción, en zonas tradicionales del cultivo, se tiene la evidencia que los rendimientos en general son muy bajos por las siguientes causas: erosión acelerada de suelos; baja fertilidad; proliferación de plagas; ocurrencia de heladas y manejo deficiente del proceso productivo. En este marco, la Fundación PROINPA, ha iniciado una serie de actividades para aprovechar las potencialidades de la biodiversidad de especies arbustivas de la región, con un enfoque de sistemas de producción que puede hacer sostenible la producción de este grano. Se recopiló información de experiencias similares en el país, relacionadas con los arbustos nativos del altiplano y las propuestas de su manejo. Sin embargo lo más importante en este proceso fue la participación voluntaria de productores, quienes ofrecieron sus terrenos para recolectar semilla e implementar las prácticas sugeridas. La

colecta de semilla, mediante métodos artesanales, fue satisfactoria y la multiplicación en vivero de las especies nativas fue muy buena, con resultados que superan las expectativas del Proyecto. Sin embargo se tuvo dificultades en las siembras directas en campo. A pesar de lograrse alguna germinación, los problemas relacionados con el enterramiento de plantines debido a fuertes vientos fueron determinantes para la pérdida del material.

Palabras clave: Manejo Agronómico; Repoblamiento; Calidad de Semilla

Description of the problem and the actions taken to solve it

In the Southern Altiplano three areas of quinoa production can be differentiated: mountains, hills or slopes and plains or pampa. The soils are generally sandy, loamy sand or sandy loam (Soraide, 2011; Orsag *et al.*, 2013.). In these types of soils the most serious problem is erosion, which results in low fertility and low quinoa yields.

In some traditional quinoa producing areas, abandoned plots can be observed, which have lost their productive capacity after being cultivated with quinoa for several years. It is common to see plots of 10, 20 or more hectares that have been turned into sandbanks. A preliminary estimation in the community of Chacala shows that sandbanks can reach up to 600 ha, being very susceptible to wind erosion. Due to this situation new plots and new areas are prepared for growing quinoa, expanding the agricultural frontier and falling into quinoa monoculture.

According to Altieri and Nichols (2000), monocultures that expand throughout the world are characterized by the cultivation of the same species on the same land year after year; where ecological complementarity between soil, crops and animals is nonexistent and where a

specific crop expands beyond its natural environment or supporting area.

This is the case of quinoa that expands from slopes towards the plains. (Aroni, 2008, Aroni and Bonifacio, s/a., Soraide, 2011). Therefore, several authors have addressed the problem of sustainability of quinoa from reflective and critical points of view, hence suggesting solutions (Soraide, 2011 Medrano and Torrico, 2009, Jaldín, 2010, Jacobsen, 2011 and 2012, Winkel *et al.*, 2012, Orsag *et al.*, 2013, Ormachea and Ramirez, 2013).

According to the Bolivian Institute of Foreign Trade (IBCE) (2013), during the 2001-2002 crop year Bolivia registered more than 37,000 hectares of quinoa cultivated land, after 10 years, during the 2011-2012 crop year, this area had increased to 96,544 hectares.

With this trend, a growth of 12% is expected for the period 2013-2014 in comparison to the previously mentioned area. The increase in area allows for increased production volumes, while unitary yields in the Southern Altiplano remain at about 500 kg/ha.

The consequence of the expansion of the agricultural frontier for quinoa production is the reduction of grazing area and the reduction in the population of llamas. Thus negatively affecting the availability

of manure for agriculture (Aroni, 2008, Jaldín, 2010, Vallejos *et al.*, 2011, Bonifacio Aroni and s/a).

The most serious environmental effects in recent years have been caused by the strong winds that carry large amounts of sand from surface layers of loose soils. This causes seedlings in the establishment phase to be buried, reduces the natural repopulation of native flora, causes the destruction of houses, etc. This transportation of soil can be easily perceived on plot edges and on access roads. Accumulation of soil can be observed in places where t'ula plants grow and rills can be observed by t'ula fields and roads.

This situation only confirms that the true vocation of the Southern Altiplano is livestock production, but because of the great market opportunity of quinoa, soils are extensive and intensively used for agriculture. The production and commercialization of quinoa brings new alternatives for a population that for centuries has had few income generation options, and for individual farmers it brings in the opportunity to exit poverty.

In the Altiplano agro-ecosystem and particularly in the Southern Altiplano, practices of deforestation of native species, to clear new land for quinoa production, are causing serious problems in soil conservation and the elimination of natural habitats. This in turn is causing low productivity of soils and proliferation of pests that attack the quinoa crop.

In the last five years, the sustainability of quinoa production emerged as a fundamental problem. To address this

problem, soil conservation practices are proposed, including the implementation of living barriers, physical barriers, soil repopulation with native species, use of manure, and crop rotation, among others (Puschiasis, 2009, VSF-CICDA 2009, Orsag *et al.*, 2013). Each of these practices has a theoretical foundation and holds its own contribution to reduce soil erosion. However, the feasibility of their implementation on a larger scale needs further consideration.

Among priority themes, Jaldín (2010) mentions: the ecological capacity of the ecosystem in relation to territorial dynamics, sustainable models for quinoa production, environmentally sustainable technologies among others; raising a series of questions that should be addressed through research.

In this context, PROINPA Foundation implements actions to contribute to the sustainability of quinoa based production systems, through research and the use of native species ancestrally adapted to these areas, particularly shrubs or t'ulas. For this purpose, the most efficient technology is sought, one that holds the possibility of large-scale implementation, easy replicability and economic feasibility. Based on these concerns, the technological alternatives considered important are: the revaluation of shrubs or t'ulas, the search for specific adaptation sites of the different species, methods of seed collection, germination tests, propagation methods, transplanting and establishment of demonstration plots and implementation of multi species living barriers.

Recognition and revaluation of shrubs

Native shrubs play various roles in quinoa centered production systems. The name *t'ula* is the generic name of evergreen woody and semi-woody native shrubs, growing in semi arid areas (Alzérreca *et al*, 2002, Zamora, 2008,. Pizarro, 2013, Bonifacio Aroni and s/a). However there are local names for each species as discussed below:

Parastrephia lepidophylla is known by the native names *sup'u tula*, *aymar T'ula*, *sip'u T'ula*, *khiruta*, *pacha-taya*, *taya t'ulalos*. These names hold reference to the dense growth habit (*sup'u*), attached resinous leaves (*sip'u*), adaptation to icy environments (*pacha-taya*, *taya T'ula*), hardness (*khiru T'ula*) and the possibility of its use as fuel (*t'ula* for bread, firewood).



Parastrephia lepidophylla (Well.) Cabrera

sup'u tula, *aymar t'ula*, *sip'u t'ula*, *khiruta*, *pacha-taya*, *taya*, *tuya*, *tola de pan*, *leña*

Parastrephia lucida (Meyen) Cabrera whose native names are *Uma T'ula*, *yacu t'ula*, *qulla t'ula*, water firewood, in reference to plants adapted to relatively moist soils, or plants used as water indicators (*uma t'ula*, *yacu t'ula*), and plants with medicinal uses related to fever reduction and as cure for dislocations (*qulla t'ula*).

Fabiana densa Remy with common names like *tara-tara*, *tara*, *tola*, *tolilla*, *pichana*, representing the fasciated stem shape irregularly up to the branching in some cases (*tara*, *tara/tara*), and thin branches (*tolilla*), suitable for the use as broom (*pichana*).

Parastrephia quadrangulare holds the native names *t'it'i t'ula*, *sunsu t'ula* in reference to its open - semi rosette-like, growth habit, with waywardly stem positions (*t'it'i*) or irregular flowering in different seasons (*sunsu*, a derivative of foolish).



Parastrephia lucida (Meyen) Cabrera

uma t'ula, *yacu t'ula*, *qulla t'ula*, *leña de agua*, *pacha-taya*, *taya*, *tola de pan*, *leña*



Fabiana densa Remy

tara-tara, tola, tolilla, tara, pichana

Lampaya medicinalis Moldenke: known by the native names *lamphaya* or *lamphayo*, which reflects its use as fuel in the kitchen (*lawá* and *phaya* or firewood for cooking).

Baccharis tricuneta, *B. tola*; a plant whose native names *ñak'a tula*, *ñak'a*, *orqu ñak'a*, *qachu ñak'a*, meaning *t'ula* female or *t'ula* male, represent the dioecious condition of plants where male is (*orqu*) and female (*qachu*).



Parastrephia quadrangulare Cabrera

t'iti'i t'ula, sunsus t'ula

Senecio clivicolus is known by the native names *qariwa* and *waych'a*, referring to its prolific and invasive growth condition (*aqariwa*) or to the open shape of the stringy foliage (*waych'a*).

Alcantholippia deserticola Phil., is known by the common name *rica-rica* in clear reference to the use of the leaves for the preparation of infusions and drinks that taste sweet.



Lampaya medicinalis Moldenke

lamphaya, lamphayo



Baccharis tricuneta

ñak'a tula, ñak'a, orqu ñak'a, qachu ñak'a, t'ula hembra, t'ula macho



Senecio clivicolus
qariwa, waych'a



Alcantholippia deserticola Phil.
rica-rica

Adaptation of species

The different native species have different performance and adaptation patterns in different Altiplano eco-regions. In a panoramic perspective different species were observed as part of pure and mixed vegetation patches, located on slopes, plains, sandbanks, wetlands, etc.

Bibliographical references do not mention or emphasize the adaptation of these species to ecological niches of the Altiplano (Zamora, 2008; Soraide, 2011; Román *et al.* 2011; Orsag *et al.* 2013), they are generally regarded as species from semi-arid areas.

While Alzérreca *et al.* (2002) offer alternatives for handling tola fields, it must be considered that for targeted utilization of species, it is necessary to understand their adaptation in specific and broad sense. This understanding needs also to be related to alternatives for seed production and plant development.

Thus, the *uma t'ula* species is well adapted to plains near wetlands, where soil moisture is abundant. Farmers associate it with underground aquifers, a fact reflected in the native name *uma t'ula*, that translated literally means water prone *t'ula*.

The *lampaya's* preferred habitat is in plains with sand accumulation, dunes or hillsides, but always in sandy soils with frequent occurrence of winds. The *lampaya* has the characteristic of forming abundant organic matter at the base of the plant where insect fauna proliferates. This proliferation of insects contributes to the decomposition of accumulated leaves. It is very common to find there coleopteran larvae (*laqatu*), the favorite food of quirchincho (*Chaetopractus nationi*).

Tara-tara, is a species that grows well on slopes and foothills, which are relatively sheltered from the strong winds and where soils are loam, sandy loam up to clay loams.

The sites where *tara-tara* grows are good for quinoa production (fertility indicator).

The *sup'u t'ula*, grows well both in plains and slopes, being the most widespread species in the Altiplano. It prefers loam to clay loam, deep soils with relatively acid reaction. The *sup'u t'ula* forms abundant leaf litter at the base of the plant which is a source of organic matter.

The *qariwa* is a semi woody species of multi-seasonal to biennial cycle. This is a very common species in the Northern Altiplano, which in recent years has moved to the Central Altiplano and lately can be found in the Southern Altiplano colonizing considerable areas.

This species is characterized by its high prolificity and frost tolerance. It grows in winter, blooms in summer and dies after forming seed during the rainy season. In some areas it is considered an invasive species. However, in semi-arid areas it could be an alternative source of plant soil cover and organic matter. In any case it must be accompanied by a management strategy.

The *rica-rica* is a semi thorny shrub that grows on hillsides. It has a deep root which enables its establishment even in compact soils. It is a highly drought tolerant plant. The thorny branches help it to withstand animal grazing.

Seed collection methods

The study of the reproductive biology of the species has revealed its vegetative and reproductive stages. Shrubs flower

between September and October. Fruiting takes place between November and December. In many cases there has been fruiting outside this period (January, February, March) probably because of the disruptions caused by climate change.

Seed collection was originally artisanal, using conditioned containers (cut jerrycan), locating the cut part against the wind to facilitate seed collection.

In some cases, wide mouth polypropylene bags have been used to collect seeds. The fruits and seeds of shrubs are very sensitive to wind scattering, even with very light winds. Their dissemination structures are formed by the thistledown which are organized in a sort of parachute.

To facilitate collection a portable vacuum was adapted to obtain power supply from a car battery. Although the management of the equipment in the field is somewhat difficult, it facilitates seed collection, especially from the ñaka (*Baccharis tricuneata*). Based on this experience, a more powerful electric vacuum is being adapted, in addition to entomological net type seed collectors.

Seed processing of shrub species includes the removal of dissemination structures (thistledown, bracts and residues of male flowers). This work requires the use of eye protection and masks to prevent inhalation of plant particles and other impurities adhered to the seed.



Manual collection of sup'u t'ula seeds



Seed collection with battery operated portable vacuum (12 v 76 A)



*Nak'a t'ula seeds
(seed processing)*



*Fruit/seed of lamphaya
(non-processed seed)*

Nursery multiplication of species

In the native species nurseries of Quipaquipani (La Paz), Rancho Grande (Oruro) and Chacala (Potosí), methods for mass multiplication of native species are being tested. These are the places where seedlings receive the necessary care to ensure the rearing of vigorous plants that can survive and develop in the field after the final transplant.

Multiplication practices include the use of seedbeds followed by replanting in

bag-pots, and direct planting in bag-pots, the latter method being cheaper and more efficient.



Sup'u t'ula multiplication stalls in the Chacala nursery

Direct seeding in the field (scattering) has had little success due to wind burial and trawling of seeds, and by fast dehydration of sprouting seeds. It is necessary to continue adjusting some direct planting techniques using pelleted seed (clay pellets, use of pregerminated seed, etc.).

The materials used were seeds of the following species: *ñak'a t'ula* (*Baccharis tricuneata*), *sup'u t'ula* (*Parastrephia lepidophylla*), *uma t'ula* (*Parastrephia lucida*) *tara tara* (*Fabiana densa*), *lam phaya* (*Lampaya castellani*), *qariwa* (*Senecio clivicolus*).

Final transplant in living barriers

In quinoa production areas, particularly those in the Southern Altiplano, plots of quinoa are distributed erratically without any management standard. In some cases you can see very wide plots in the direction of local winds, a condition that facilitates erosion processes.

To mitigate the effect of erosion, living barriers have been established transversal to the direction of local winds. The width of the plots is of 50 to 60 m, the length of the plots varies from 100 m to 300 m. This design should be applied to all new parcels being incorporated into commercial production.

It has been proven that the seedlings of native species developed in small bags, are easier to transport and handle in the field. Something to note is the time of transplantation, which must necessarily coincide with the onset of rains. Once rain arrives there is a period of one month to transplant shrubs successfully.

Given the magnitude of the problem that concerns the sustainability of quinoa production, the proposal of reforestation with native species is a contribution that in the medium and long term can help an ecological stabilization and help achieve sustainability. The millennial adaptation of native species and the perspective of their use in quinoa production systems must be emphasized. This process would help reduce soil erosion, generate organic matter locally and would provide food and shelter for beneficial insects and micro-organisms, enabling the achievement of a desired ecological balance.

Being a big task, reforestation with native and naturalized species, is a matter that concerns higher levels of public decision making such as municipalities, departmental governments and the national government.



Living barriers with *ñak'a tu'la*

Moreover, resources and time required for the establishment and management of native shrubs should be considered in the context of ecological and environmental services. Given its nature these services are eligible for the support of national public actors and international cooperation bodies.

In this context, the first experiences implemented by PROINPA Foundation have enabled the following achievements:

- Recognition and revaluation of the main shrubs of native species.
- Identification and description of the diversity of species and the genetic diversity within shrub species.
- Determination of the ecological adaptation of existing native species in the Altiplano.
- Development of mechanisms for native species seed collection.
- Development of methods for mass multiplication of shrubs.
- Understanding about the viability of seeds from shrub species.
- Evaluation of the multi species living barriers feasibility of implementation.
- Definition of methods and times of final transplant for living barriers.

In all previous work, it is important to note the involvement of producers, who through their local knowledge and experience have made a valuable contribution to this initiative that seeks to make sustainable use of local biodiversity in quinoa production systems in the Bolivian Altiplano.

Communities of farmers involved:

- Communities of Chacala and Chita, Potosí.
- Community of Rancho Grande, Oruro.
- Collaborating farmers from Buena Vista, Aroma, Oruro.

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Formation of the *Chenopodium quinoa* Willd. (quinoa) core collection in Bolivia with morphological and molecular data

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Summary. The core collection is representative of a crop's genetic diversity, aimed at promoting a more efficient use of material conserved in genebanks. The objective of this study was to form a core collection of quinoa with morphological and molecular data, and to evaluate its representativeness, so that the entity responsible for the custody of the Bolivian Quinoa Collection, can have the necessary information to facilitate decisions-making regarding the use and evaluation of the genetic diversity of quinoa germplasm. The *M or Maximization of Diversity Strategy* was used for the selection of representative materials that form the core collection, using the PowerCore ® software. The study was conducted with the *Bolivian Quinoa Collection* formed by materials from the Altiplano, the inter-Andean valleys and wild materials that had complete data on morphological and molecular characterization. Two core collection scenarios were identified: 1) 31% of the total collection representing 100% of the genetic diversity and, 2) 24% of the total collection representing 80% of the genetic diversity. By comparing the diversity indices between the total collection and the core collection, the representativeness of the latter genetic diversity was confirmed.

Keywords: Genetic Diversity; Microsatellites; Genebank

Resumen. Formación de la colección núcleo de *Chenopodium quinoa* Willd (quinua) en Bolivia con información morfológica y molecular. La colección núcleo es representativa de la diversidad genética de un cultivo, dirigida a promover un uso más eficiente de los materiales conservados en los Bancos de Germoplasma. El objetivo de este trabajo fue conformar una colección núcleo de quinua con datos morfológicos y moleculares y evaluar su representatividad, de forma que la entidad responsable de la custodia de la *Colección Boliviana de Quinua*, cuente con información necesaria para facilitar la toma de decisiones en cuanto al uso y evaluación de la diversidad genética del germoplasma de quinua. Se utilizó la *Estrategia M o de Maximización de la Diversidad* para la selección de los materiales representativos que conforman la colección núcleo, utilizando el programa informático PowerCore ®. El estudio se realizó en la Colección Boliviana de Quinua conformada por materiales provenientes del altiplano, los valles interandinos y materiales silvestres que contaban con datos completos de caracterización morfológica y molecular. Se identificaron dos escenarios de colección núcleo: 1) 31% de la colección total que representa el 100% de la diversidad genética y 2) 24% de la colección total representando el 80% de la diversidad genética. La comparación de índices de diversidad, entre la colección total y la colección núcleo, corroboró la representatividad de diversidad genética de ésta última.

Palabras clave: Diversidad genética; Microsatélites; Banco de Germoplasma

Problem description and Project actions

The Bolivian Quinoa Germplasm Collection has a wide genetic variability with over 3,000 accessions of cultivated and wild materials collected in different agroecological regions of the country, corresponding to the departments of La Paz, Oruro, Potosí, Cochabamba, Chuquisaca and Tarija. The collection also features germplasm from Peru, Ecuador, Colombia, Argentina, among others (Rojas *et al.*, 2010).

When germplasm collections are large in terms of number of accessions, the use of genetic material is difficult and therefore the implementation of core collections is recommended.

According to Jaramillo and Baena (2000), a core collection is a subset of germplasm accessions, representing 10% to 15% of the total number of accessions, while at the same time the subset must represent at least 70% to 80% of the genetic variability.

Therefore, core collections are important for users of genebanks, e.g. breeders, in order for them to use appropriately and more frequently the rich variability conserved in genebanks. They are important because they enable users to find valuable genes and sources of resistance to abiotic and biotic factors, and quality traits; in order to incorporate them into new varieties in the process of breeding for adaptation to climate change, and for the selection of materials of higher quality and/or suitability for agroindustrial uses.

To initiate this study the availability of the following elements was previously verified:

- Information on morphological and molecular data of quinoa germplasm accessions. Morphological information was generated since the nineties (Rojas 2003, Rojas *et al.*, 2001). Molecular information and part of the morphological information were generated in the first decade of the twenty first century, when PROINPA Foundation was in charge of the administration of the National High Andean Grains Genebank, by appointment of the Bolivian government (Rojas 2008).
- Bioinformatic tools that can be applied in the identification of the core collection, such as the PowerCore[®] program which uses the M Maximization Technique to search for representative accessions (van Hintum *et al.*, 2003; Kyu - Won *et al.*, 2007, PASW, 2009).

Methodology

Selection of accessions based on morphological and molecular data

Molecular information was obtained from the characterization of the Bolivian Quinoa Collection with eight microsatellite markers (Table 1). These markers are important because they have advantages such as: codominance, multiallelism and high heterozygosity. The markers were selected from a library of microsatellites (SSR) because of their high level of polymorphism (Maughan *et al.* 2004).

Table 1. Characteristics of microsatellites used for the molecular characterization of the *Bolivian Quinoa Collection*

| No. | Microsatellite | Repeat Unit | Sequence of forward primer (5'-3') | Sequence of reverse primer (5'-3') | HT * (°C) |
|-----|----------------|---------------|------------------------------------|------------------------------------|-----------|
| 1 | QCA006 | (CA)15CG(CA)4 | gctctattaaggaaatgaggttca | gccattcaattcagcaaagg | 51 |
| 2 | QATG019 | (ATC)12 | ccaaacaaagacaataag-gaaacc | cgagggtgaaggagattcca | 60 |
| 3 | QAAT051 | (AAT)14 | ccttcgacaaggtccatta | cgtccatagtggaggcattt | 53 |
| 4 | QCA058 | (GT)17 | ctcgaccagcagggtctg | ctagctaggcgtgcctgac | 60 |
| 5 | QAAT050 | (AAT)17 | ggcacgtgctgctactata | tggcgaatggttaatttgc | 51 |
| 6 | QAAT074 | (ATT)14 | atggaacacccatccgataa | atgcctatcctatcctcca | 55 |
| 7 | QAAT076 | (ATT)30 | gcttcatgtgtataaaatgccaat | tctcggtctccactaatttt | 55 |
| 8 | QAAT022 | (TTA)29 | tggtcgatatagatgaaccaa | ggagcccagattgtatctca | 53 |

*: HT: Hybridization Temperature

Molecular characterization yielded diversity indices as the Polymorphic Information Content (PIC) and heterozygosity (H), for each set of samples coming from the different geographic regions. Quinoas from the Central Altiplano (CA) and Southern Altiplano (SA) were the most abundant, and also the most diverse (tables 2 and 3).

For this study, the data of the alleles (in base pairs) of every microsatellite were transformed into binary data and arranged in a matrix representing the presence of an allele as 1 and the absence as 0. The morphological information was obtained from the evaluation of the germplasm collection. 48 variables were selected that included data on: stem coloring, plant architecture, stem, leaf, inflorescence and/or panicle, grain characteristics, saponin, yield, phenology and tolerance to abiotic (frost) and biotic (mildew) factors.

Quinoas from every region have an agromorphological pattern that distinguishes them from each other. In the Central Altiplano quinoas grow from 0.5

to 1.2 m, with short branches, the glomerulate panicle prevails and the phenological cycle lasts 168.40 ± 14.05 days.

Quinoas in the Southern Altiplano have larger grain (2.20 to 2.67 mm in diameter), high saponin content; with a prevailing amarant-like panicle.

In the Northern Altiplano, quinoas have a height of 0.8 to 1.5 m, the glomerulate panicle prevails, the grain is small to medium in size and white in color, the growing season is fairly late to late (175 days).

In inter-Andean valleys quinoas can reach up to 2.5 m or more in height, with branches reaching the second third of the plant and very toothed leaves. The phenological cycle is late (188-205 days), amarant-like panicles are dominant, medium grain size (2 ± 0.13 mm) and high saponin content (Rojas and Pinto 2013, Bonifacio *et al.* 2012; Rojas 2003, Rojas *et al.* 2001).

For the present study the above morphological data were arranged in a matrix where the names of the accessions and morphological descriptors are indicated with several levels of descriptors (3-11).

Based on available information about molecular and morphological characterization, out of all the accessions from the quinoa collection, 1672 Bolivian accessions from five regions were selected: Northern Altiplano, Central Altiplano, Southern Altiplano and inter-Andean valleys and natural habitats for the wild quinoas (ajaras) (Table 2).

Statistical Analysis: Forming the core collection of quinoa

To form the core collection, a matrix with molecular and morphological data was developed. This information was introduced in the PowerCore® program, which enables sampling through the Strategy M Maximization (van Hintum *et al.* 2003). This strategy involves the use of data indicating the magnitude of the diversity of markers used for the characterization of germplasm. It ensures the inclusion of high allelic richness for loci/markers used and directly takes into account, in those loci, the magnitude of the variation and deviation to the model or pattern. Importantly, the M strategy not only defines the number of accessions that should come from different groups, but also identifies the accessions that need to be included (Schoen and Brown, 1994; Schoen and Brown, 1995; Cortez 2011). Thus, with the accessions selected by the program, a dendrogram of the core collection was generated. Using the program NTSYSpc ® version 2.1.10 the

structure of this dendrogram was compared with that of the total collection. Diversity indices between the two collections were also compared to check the representativeness of the core collection (Rohlf 2000; Zambrano *et al.* 2003).

Achievements

The data from the analysis, with a level of 100% of representation of the genetic diversity, generated a subset of 486 accessions corresponding to 31% of the total collection. Applying a level of 80% of representation of the genetic diversity, as suggested by the literature (Frankel *et al.*, 1984; Jaramillo and Baena, 2000; Ozer *et al.*, 2004), a subset of 410 accessions was reached, which stands for 24% of the total collection (Table 2).

Table 2 shows the number of accessions in the core collection by region. The percentage of accessions from each region is variable; this indicates the degree of redundancy (duplicated or genetically very similar material) of accessions within each region.

This is the case of material from the Northern Altiplano, from where a higher percentage of accessions has been considered both at 80% and 100% of representation (46% and 57%, respectively), compared with the other regions. This is because in this region there is less redundancy, therefore most accessions are genetically different from one another.

A similar situation of low redundancy is also observed in the material from the inter-Andean valleys. It is noteworthy that

Table 2: Number of accessions of the core collection by region, selected through maximizing the total collection to 80% and 100% of representation

| Region | Total Acces-sions | 80% of genetic representation | | 100% of genetic representation | |
|----------------------|-------------------|-------------------------------|----|--------------------------------|----|
| | | Core collection | % | Core collection | % |
| Central Altiplano | 791 | 117 | 14 | 146 | 18 |
| Southern Altiplano | 372 | 108 | 29 | 135 | 36 |
| Northern Altiplano | 111 | 50 | 46 | 63 | 57 |
| Inter-Andean valleys | 269 | 114 | 42 | 142 | 53 |
| Natural Habitats | 129 | 22 | 17 | 27 | 21 |
| Total | 1.672 | 411 | 25 | 513 | 31 |

the number of accessions identified for the core collection of this region is greater than the number of accessions from the core collection of the Southern Altiplano and very close to that of the Central Altiplano. This implies that although the total number of accessions from the Central and from the Southern Altiplano is greater than that of the inter-Andean valleys, it is likely that in the first two regions of the Altiplano, several genetically similar materials were collected.

Comparison of the core collection with the total collection, by visualization of the dendrograms developed with the data from each collection, shows that the core collection conserves the structure of the original collection.

Figure 1 shows an example for material from the Northern Altiplano, where the genetic structure (arrangement of the accessions into groups and subgroups) of both collections (total and core) is the same.

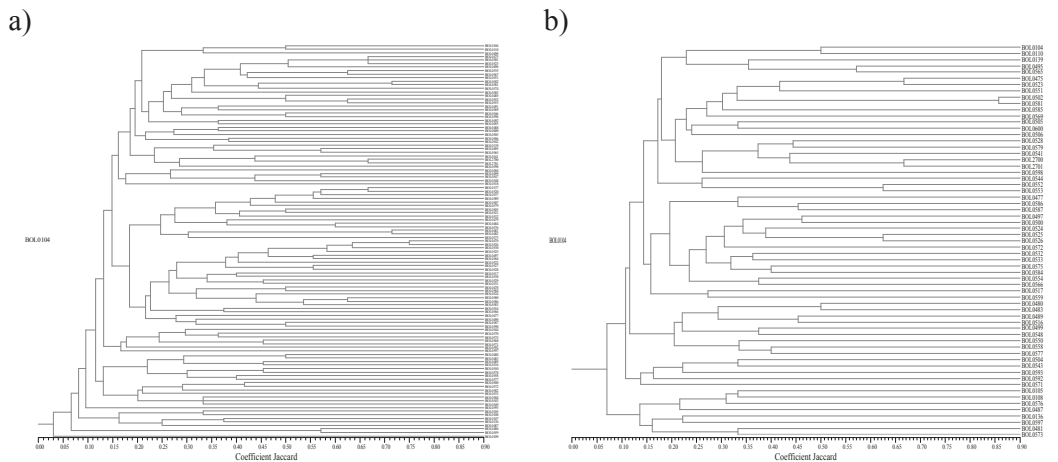


Figure 1: Comparison of dendrograms to visualize the genetic structure of:
a) Total Collection of the Northern Altiplano (111 accessions)
b) Core collection from the same region (63 accessions)

Table 3 compares PIC data from the total collection, with PIC data from the core collection, arranged by region. Diversity indices of the core collection are slightly higher than those of the original collection because redundancies are reduced. Therefore, the allele frequencies are higher.

The representativeness of the core collection is consistent, although the *Bolivian Quinoa Collection* has such a diverse and complex structure. Genetic diversity in the core collection is maintained and redundancy has been reduced to a minimum.

Conclusions

- The methodology used for this study enabled the identification of the most representative quinoa accessions, namely the group of accessions in which the amplitude of the genetic diversity found in the total collection

was maintained, based on microsatellite markers, agromorphological descriptors and assessments of mildew and frost tolerance. The quality and quantity of information used to form the core quinoa collection in this study, ensures the inclusion of a wide range of accessions that can be considered for the selection of genetic material in future assessments, either with particular agromorphological characteristics or with mildew and frost tolerance characteristics.

- It is important to mention that the formation of a core collection is dynamic. Future research could include variables related to the demands of the current context, such as climate change, which requires early and drought tolerant varieties; or the market, which demands varieties with nutritional and agroindustrial qualities.

Table 3. PIC values by region for the eight microsatellites used for the total collection and the core collection

| Locus | PIC value by region TC | | | | | PIC value by región CC | | | | |
|---------|------------------------|------|------|------|------|------------------------|------|------|------|------|
| | CA | SA | NA | IAV | NH | CA | SA | NA | IAV | NH |
| QCA006 | 0.72 | 0.69 | - | - | - | 0.74 | 0.71 | - | - | - |
| QATG019 | 0.69 | 0.66 | - | - | - | 0.70 | 0.67 | - | - | - |
| QAAT051 | 0.68 | 0.79 | 0.52 | 0.75 | 0.79 | 0.69 | 0.80 | 0.51 | 0.75 | 0.78 |
| QCA058 | 0.76 | 0.81 | - | - | - | 0.76 | 0.82 | - | - | - |
| QAAT050 | 0.88 | 0.83 | 0.75 | 0.79 | 0.88 | 0.89 | 0.83 | 0.76 | 0.79 | 0.87 |
| QAAT074 | 0.92 | 0.92 | 0.88 | 0.93 | 0.91 | 0.92 | 0.91 | 0.88 | 0.93 | 0.91 |
| QAAT076 | 0.88 | 0.90 | 0.90 | 0.86 | 0.89 | 0.88 | 0.90 | 0.90 | 0.87 | 0.89 |
| QAAT022 | 0.92 | 0.92 | 0.87 | 0.94 | 0.92 | 0.92 | 0.92 | 0.87 | 0.94 | 0.92 |
| Average | 0.81 | 0.82 | 0.78 | 0.85 | 0.88 | 0.81 | 0.82 | 0.78 | 0.86 | 0.87 |

References: CA: Central Altiplano; SA: Southern Altiplano; NA: Northern Altiplano; IAV: inter-Andean Valleys; NH: Natural Habitats; PIC: Polymorphic Information Content; TC: = Total Collection; CC=Core Collection

Participating agencies

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Memoria electrónica del Primer Encuentro Internacional de la Tuna para Forraje como una Medida de Adaptación al Cambio Climático en Bolivia. Documento que presenta todos los trabajos expuestos y enviados para la reunión realizada en Cochabamba, en el mes de mayo de 2014. Además de los textos académicos, presenta videos ilustrativos sobre la utilización de la tuna con fines forrajeros. También presenta libros completos de la FAO, SEBRAE e ICARDA, sobre la tuna en especial y las zonas áridas en general. La publicación de la Memoria fue realizada con el apoyo de GIZ PROAGRO y el auspicio del Ministerio de Desarrollo Rural y Tierras y el Vice Ministerio de Desarrollo Rural Agropecuario, a través del Instituto de Innovación Agropecuaria y Forestal.



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Potential uses of the genetic diversity of quinoa in agroindustry: Opportunities and Challenges

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*Work funded by: SINARGEAA; McKnight Foundation;
UNEP/GEF Project; NUS/IFAD Project; PROINPA Foundation*

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Summary. In addition to its nutritional and health benefits, quinoa has an extraordinary genetic diversity that is expressed in the variability of its plant colors, inflorescence and seed. These qualities make it a potential crop to produce quality food. Since the eighties, quinoa has experienced a significant growth in demand, and it is from 2005 on-wards that a real boom sets forth export volumes of this grain and its subproducts. Bolivia is the world leader in quinoa production and export. Companies and/or associations operating in this sector have turned their efforts to the export of raw material, which currently dominates sales outside Bolivia. Several others have also begun transforming products and byproducts based on quinoa. However, processed products are made with raw material from mixed quinoa (different varieties) and thus, at agroindustrial level, they do not reach the desired market quality. In this context, it is important to address the opportunities of "quinoa genetic diversity" for the development of better processed products, and in this way strengthen the quinoa agroindustry and the country's economy.

Keywords: Nutritional Value; Added Value; Export

Resumen. Potenciales usos de la diversidad genética de la quinua en la agroindustria: Oportunidades y desafíos. La quinua, además de sus beneficios para la nutrición y salud, posee una extraordinaria diversidad genética que se expresa en la variabilidad de colores de la planta, inflorescencia y semilla. Estas cualidades la convierten en un cultivo con potencial para producir alimentos de calidad. Desde los años ochenta, la quinua ha experimentado un notable incremento de la demanda y a partir del año 2005, se presenta un verdadero boom en los volúmenes de exportación del grano y sus productos derivados. Bolivia es líder a nivel mundial en la producción y exportación de quinua, las empresas y/o asociaciones que operan en el rubro, han volcado sus esfuerzos a la exportación de materia prima, que es lo que actualmente predomina en las ventas al exterior y varias de ellas han iniciado la transformación de productos y derivados a base de quinua. Sin embargo, estos productos transformados son elaborados con materia prima que viene de quinua mezclada (diferentes variedades) y por ello a nivel agroindustrial, no alcanzan la calidad requerida por el mercado. En este marco es importante direccionar las oportunidades que brinda la "diversidad genética de quinua", para la elaboración de productos transformados y de esta forma fortalecer la agroindustria de quinua y la economía del país.

Palabras clave: Valor Nutritivo; Valor Agregado; Exportación

Quinoa (*Chenopodium quinoa* Willd.) has outstanding intrinsic characteristics including its genetic variability and its functional properties. Its diversity forms an extremely valuable gene pool and is expressed in the variability of plant colors, inflorescence and seed, plant shape, nutritional value, productive performance and length of growing season. In the last twenty years, significant scientific information demonstrating the benefits of quinoa for health, beyond basic nutrition, was generated (Rojas *et al.* 2010a). The knowledge and information contained in the genetic diversity of quinoa, must be used to further leverage the benefits of quinoa for consumption, and particularly for the industry.

From a nutritional and dietary point of view, quinoa is a natural source of plant protein of high nutritional value, because of its higher proportion of essential amino acids; giving it a higher biological value than wheat, rice and maize; and comparable only with milk, meat and eggs. As a source of plant protein, quinoa helps the body develop and grow, conserves heat and energy, it is easy to digest and, combined with other foods, produces a complete and balanced diet that can replace animal foods (Rojas *et al.*, 2010a; Ayala *et al.*, 2004).

In Bolivia, the research on quinoa breeding began in the early sixties, focusing on achieving high performance varieties, with white large grain, as was the demand at that time. Since the eighties, due to export markets expectations, cultivation has expanded significantly, and demands have not only included white, but also red and black quinoa. Likewise, changes in climate have

generated new demands like earliness, to adjust planting to delayed rain cycles and complete harvest within a shorter crop cycle (Vargas *et al.*, 2013).

Traditionally companies and/or associations have focused their efforts on the export of raw materials, which is what currently prevails in terms of foreign sales. However, in the last decade companies have begun the processing and export of quinoa based products and subproducts. The difficulty to achieve optimal and competitive products for the international market is that the products are made with mixed quinoa (different varieties in different proportions) and consequently at agroindustrial level, it is not possible to obtain the same quality for the same preparation at different times.

Considering the above, it is important to study and take advantage of the genetic diversity for the manufacture of processed quinoa products, using in its true magnitude the existing genetic potential to obtain agroindustrial products of higher quality. It is possible to identify, select and develop varieties with high protein content, with small diameters of starch granule to process homogeneous popped grain, with stable percentages of amylose and amylopectin to make puddings, gelatinized porridge, instant creams, noodles, etc.

Nutritional value and agroindustrial capacity of quinoa's agromorphologic - genetic diversity

In Bolivia, the first initiatives to implement a quinoa germplasm collection

date back to the early sixties, under the initiative of Ing. Humberto Gandarillas (Rojas *et al.*, 2010b). Originally, the efforts focused on the registration of agromorphological information. In 1985 and 2001 the first and second quinoa catalogs were published (Espindola and Saravia, 1985, Rojas *et al.*, 2001).

The 2001 catalog describes the genetic variability of 2,701 quinoa accessions through 59 qualitative and quantitative agromorphologic variables. This document was prepared during the period of time when PROINPA Foundation was in charge of the administration of the quinoa germplasm collection, as custodian appointed by mandate from the Bolivian government (Rojas *et al.*, 2010b).

When quinoas reach physiological maturity they express a wide variety of plant and grain colors, including white, cream, yellow, orange, pink, red, purple,

light brown, brown and black. In the Bolivian collection 66 grain colors and four shapes, have been characterized, by the appearance of the endosperm. This gives quinoa, features that can be properly exploited for the manufacture of processed products.

In order to increase the use of diversity in the manufacture of quinoa processed products, interaction with exporters of this species was promoted. Until 2010, nutritional information of 555 accessions of the Bolivian Quinoa Collection was registered, along with information on agroindustrial characteristics of 260 accessions (Rojas and Pinto, 2013).

Table 1 presents a summary of the statistical parameters registered for each feature of the nutritional value and agroindustrial characteristics of quinoa, expressed on a dry basis (Rojas *et al.*, 2007; Rojas *et al.*, 2010a).

Table 1. Characteristics of nutritional - agroindustrial value of quinoa germplasm accessions from Bolivia, and simple statistical parameters (n = 555 accessions)

| Component | Minimum | Maximum | Average | SD |
|---------------------------|---------|---------|---------|-------|
| Protein (%) | 10.21 | 18.39 | 14.33 | 1.69 |
| Fat (%) | 2.05 | 10.88 | 6.46 | 1.05 |
| Fiber (%) | 3.46 | 9.68 | 7.01 | 1.19 |
| Ash (%) | 2.12 | 5.21 | 3.63 | 0.50 |
| Carbohydrates (%) | 52.31 | 72.98 | 58.96 | 3.40 |
| Energy (Kcal/100 g) | 312.92 | 401.27 | 353.36 | 13.11 |
| Starch granule (μ)* | 1 | 28 | 4.47 | 3.25 |
| Inverted sugar (%)* | 10 | 35 | 16.89 | 3.69 |
| Water filling (%)* | 16 | 66 | 28.92 | 7.34 |

Analysis delivered by LAYSAA, Cochabamba, Bolivia;

SD: Standard Deviation; *: n = 266 (Source: Rojas, Pinto *et al.*, 2010)

Quinoa accessions show a wide variability for most of the studied traits, which is an indicator of the genetic potential of this germplasm.

Figure 1 shows the variation in the frequency distribution of protein content for 555 accessions of the Bolivian quinoa collection. In the majority of accessions the protein content varies from 12.0% to 16.9%, while there are a small number of accessions (42) with contents that range from 17% to 18.9%. The latter group is an important source of genes to promote development of products with high protein content (Rojas *et al.*, 2010a). This information was corroborated by other similar studies by Reynaga *et al.*, 2013.

The fat content ranges from 2.05% to 10.88%, with an average of 6.39% (Table 1). β o (1991) and Moron (1999) cited by Jacobsen and Sherwood (2002), indicate that the fat content of quinoa has a high value, due to its high percentage of non-saturated fatty acids. Accessions with

these values can be used for the production of fine vegetable oils for culinary and cosmetic use.

Genetic variation on size of starch granule ranged from 1 μ to 28 μ (Table 1, Figure 2). It is very important that the starch granule be small, to facilitate the process of texturing and expansion, because spaces between granules allow more air to enter for exchange and formation of air bubbles (Rojas *et al.*, 2007). This feature is important for the agroindustry because it enables the use of various cereal and legume mixes, to take advantage of the functional characteristics of quinoa.

The content of inverted sugars varies from 10% to 35% (Table 1). This variable expresses the amount of sugar that begins fermentation by splitting or inversion. It is the parameter used to determine the quality of carbohydrates. It is also an important parameter by which quinoa can be classified as a food fit for diabetics.

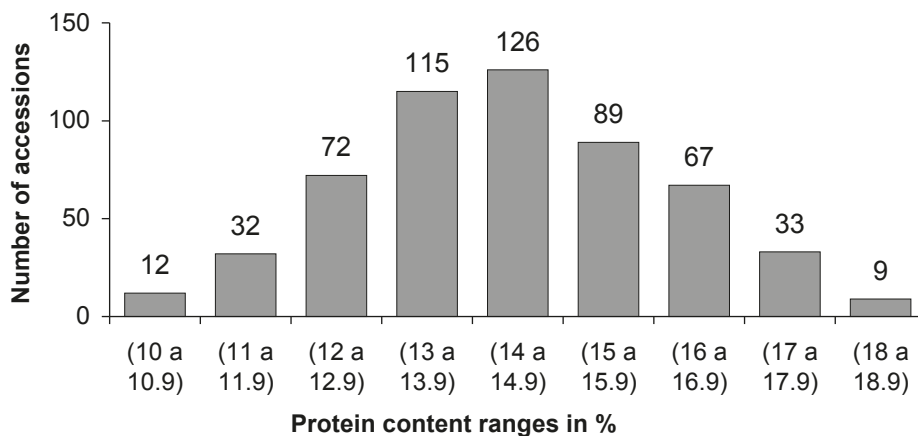


Figure 1. Protein content variation of 555 quinoa accessions

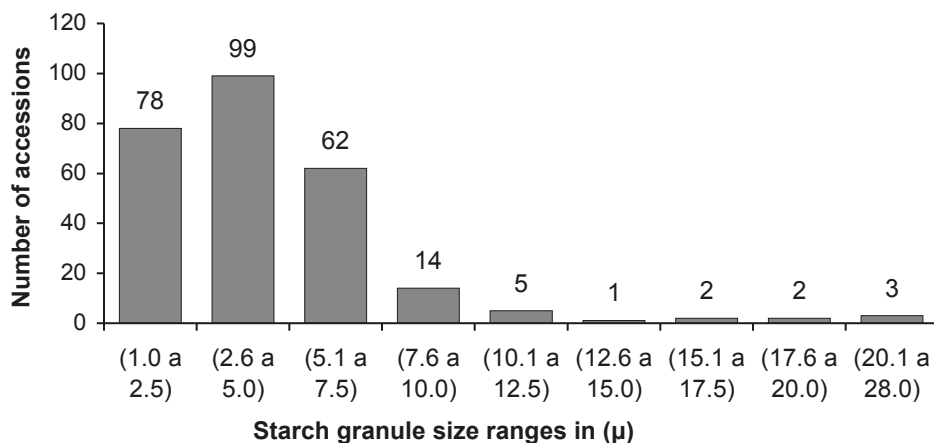


Figure 2. Starch granule size variation in 266 accessions of quinoa

The optimum content of inverted sugar is $\geq 25\%$. Quinoa accessions analyzed from the germplasm meet this condition and have potential to be used in mixtures with flour to process breads, cereals, etc.

The variable of “water filling” shows a range of variation from 16% to 66% (Table 1). This variable measures the water absorption capacity of starch, important parameter for processing of pasta, bread and pastries. The ideal value for this parameter, in industrial applications, is $\geq 50\%$. Considering the variability within quinoa in relation to this characteristic, there is an important gene pool that can be used for the development of such processed products.

Recently PROINPA Foundation, through its quinoa breeding program is prioritizing the inclusion of criteria related to nutritional value and agroindustrial characteristics for the development of quinoa varieties, while seeking at the same time that they comply with market parameters, productivity and adaptation to climate change.

Table 2 shows results of quinoa materials that are in the process of generation of new varieties.

According to Table 2, the Kurmi variety has 16.11% protein. Through selection techniques applied to deviants of this variety, the line K-Chullpi has been obtained. The K-Chullpi line has a protein content of 18.20% which is higher than that of Kurmi. It also has a starch granule diameter of 1.5 μ , being better than the Kurmi variety that has a diameter of 2.1 μ . However, both varieties have excellent aptitude for the development of expanded products and popped grain.

Similarly, the iron content increased significantly in the K-Chullpi line, reaching 4.8 mg/100 g of dry matter, in comparison to the Kurmi variety that has 1.2 mg/100 g of dry matter. Varieties with these characteristics may be an alternative for programs conducted by the Bolivian Government, that focus on maternal and child malnutrition and, breastfeeding.

Table 2. Agromorphologic characteristics, nutritional value and agroindustrial potential of quinoa varieties and lines

| Name of variety / line | Average Parameter | | | | | | | | | | |
|------------------------|-------------------|-----|------|------|-----|------|-----|----|----|------|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| Real Blanca | 180 | 125 | 650 | 14.5 | 3.9 | 60.3 | 5.2 | 23 | 31 | 12.2 | 2.1 |
| J'acha Grano | 135 | 120 | 1400 | 14.2 | 3.8 | 58.3 | 3.6 | 21 | 19 | 10.5 | 2.1 |
| Blanquita | 170 | 110 | 1500 | 13.8 | 4.2 | 39.2 | 1.1 | 19 | 33 | 16.6 | 1.8 |
| Kurmi | 155 | 120 | 1550 | 16.1 | 4.3 | 61.5 | 2.1 | 20 | 26 | 15.9 | 1.2 |
| Aynoka | 155 | 110 | 1200 | 13.6 | 4.3 | 59.3 | 2.8 | 21 | 21 | 15.1 | 4.5 |
| Kosuña | 155 | 100 | 1000 | 14.8 | 4.5 | 49.3 | 4.8 | 15 | 28 | 15.9 | 3.5 |
| Línea K-Chullpi | 160 | 115 | 1250 | 18.2 | 3.1 | 61.4 | 1.5 | 18 | 21 | 21.5 | 4.8 |
| Línea 118 Cf | 150 | 115 | 1250 | 16.8 | 6.1 | 42.1 | 2.8 | 15 | 31 | 16.5 | 2.7 |

References: 1: Physiological maturity (days); 2: Plant height (cm);
 3: Grain yield (kg/ha); 4: Protein (%); 5: Fiber (%);
 6: Starch content (%); 7: Starch granule (%); 8: Inverted sugar (%);
 9: Water filling (%); 10: Amylose (%); 11: Iron (mg/100)

Source: Own elaboration based on analysis by LAYSAA in 2012, Cochabamba

In addition, although the *11Cf* line comes from the variety *Aynoka*, there is an increase in protein content from 13.65% to 16.85%, and an increase in fiber from 4.25% to 6.10%. The *diameter of the starch granule* remains at 2.8 μ , which means that, both have potential characteristics for the elaboration of expanded products and popped grain. However, the iron content went down from 4.5 mg/100 to 2.7 mg/100 g of dry matter.

On the other hand, in the particular case of the “Real Blanca” variety, it has significant protein content (14.49%), but its value of starch granule diameter is of 5.2 μ , thus being not suitable for the production of expanded products and popped grain.

Challenges and opportunities

The strategic lines of research for quinoa cultivation need to consider the genetic diversity available in the country, that is also the most important worldwide. This genetic diversity, if properly used, offers enormous potential for diverse fields of application. Otherwise, it will continue to be underutilized, as it has been so far, with the export of raw material from mixed varieties.

There are 66 colors of quinoa grain, whose antioxidant properties, that can help develop nutraceutical products, have not yet been studied. Despite the wide diversity of shapes, sizes and colors of quinoa grain, the consumer at the time of purchasing the product in different markets and fairs, differentiates only three colors of quinoa: white, brown and black.

It is necessary to strengthen the development of agroindustrial products using the genetic diversity of quinoa properly, thus generating high quality and standardized products, which can offer the same quality over time. The genetic base available for quinoa also allows the country to take the lead in the development and export of processed products with standard quality.

It is important to take into account that the varieties with potential for agroindustrial processing should also reach the necessary standards for production and adaptation to climate change. Varieties should have early cultivation cycles to adapt to climate variability and to the specific characteristics of every productive region of the country.

Initiatives that promote the use of native and improved seed varieties are required, because quality standards need to be met from sowing to harvest. The production process needs to be improved and volumes demanded by the agroindustry need to be met. But overall, the process needs to contribute to the sustainability of the quinoa business in production areas.

In this challenge, each actor involved in the production and marketing of quinoa, plays an important role. However, this role will be best matched if worked through a country strategy that guides the actions of all actors involved in the quinoa sector.

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SEMILLA DE QUINUA

La Fundación PROINPA pone a su disposición una amplia gama de variedades de semilla de quinua certificada con pureza varietal superior a 95% y con alto poder germinativo que facilita la obtención de mejores rendimientos en el cultivo.

PROPIEDADES: La oferta de semilla de quinua tiene los siguientes atributos:

- Variedades mejoradas
- Precocidad (120 días)
- Grano de colores: blanca real, grano rojo y grano negro
- Tamaño del grano
- Calidad harinera

VARIEDADES

Se dispone de semilla para diferentes fines comerciales (harinas, grano de color, grano tamaño real, etc) en las siguientes variedades:

- Maniqueña
- Kariquimeña
- Kanchis Blanco
- Kurmi
- Rosa Blanca
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- Jacha Grano
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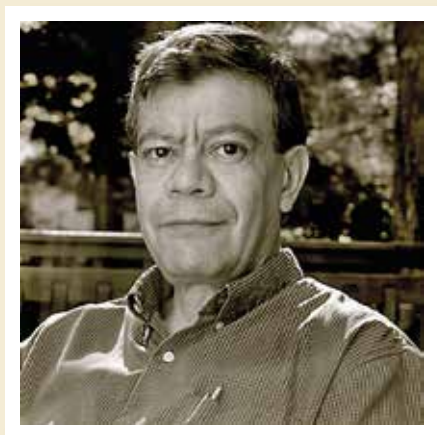
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Abreviaciones y siglas utilizadas en el presente número de la Revista de Agricultura:

| | |
|-----------|--|
| ADN | Ácido desoxirribonucleico |
| ADNr | Ácido desoxirribonucleico ribosómico |
| AIA | Ácido Indol Acético |
| ANAPQUI | Asociación Nacional de Productores de Quinua |
| APQUISA | Asociación de Productores de Quinua de Salinas |
| ARN | Ácido ribonucleico |
| BYU | <i>Brigham Young University</i> |
| CABOLQUI | Cámara Boliviana de Exportadores de Quinua y Productos Orgánicos |
| CG | Cromatografía de Gas |
| DANIDA | <i>Danish International Development Agency</i> |
| EAG | Electroantenografía |
| EM | Espectrometría de Masas |
| FDTA | Fundación para el Desarrollo Tecnológico y Agropecuario |
| FOB | <i>Free On Board</i> (Libre a Bordo) |
| FONTAGRO | Fondo Regional de Tecnología Agropecuaria |
| H | Heterocigosidad |
| IBCE | Instituto Boliviano de Comercio Exterior |
| IBTA | Instituto Boliviano de Tecnología Agropecuaria |
| IDEPRO | Instituto para el Desarrollo de la Pequeña Unidad Productiva |
| INE | Instituto Nacional de Estadística (Bolivia) |
| MIP | Manejo Integrado de Plagas |
| PCR | <i>Polymerase Chain Reaction</i> (Reacción en Cadena de la Polimerasa) |
| PIC | Contenido de Información Polimórfica |
| PROINPA | Fundación para la Promoción e Investigación de Productos Andinos |
| SD | Desviación estándar |
| SINARGEAA | Sistema Nacional de Recursos Genéticos para la Alimentación y la Agricultura |
| SINDAN | Sociedad Industrial de Alimentos Naturales y Orgánicos |
| sp | Especie |
| spp | Especies |
| ssp | Sub especie |
| SSR | Simple Sequence Repeats |
| TSA | Tripticasa de Soya |
| TSB | Caldo Tripticasa de Soya |
| UMSA | Universidad Mayor de San Andrés |
| USD | Dólar estadounidense |
| USDA | <i>United States Department of Agriculture</i> |
| VDRMT | Viceministerio de Desarrollo Rural y Tierras |

RECOGNITION TO DR. CARLOS PEREZ



Dr. Carlos Perez, born in La Paz, sociologist trained at the College of Oneonta from the State University of New York and a Ph.D. in Anthropology from the State University of New York at Binghamton, has a long and fruitful career as a researcher in and out of Bolivia.

For the last six years he has worked for the McKnight Foundation, an organization that invests and promotes technological innovation in the Andean region and in other parts of the world. Throughout his productive career, Dr. Perez has demonstrated a particular sensitivity and commitment with Bolivian ancient cultures, the highlands and quinoa.

His perception of the Andean worldview and his awareness of the demands and needs of small farmer families in the Andean region has been his personal identity seal and work motivation throughout his years of professional life.

Like very few professionals working in institutions that finance technology development, his first concern is to strengthen and improve the efficiency and competitiveness of researchers and research institutions.

As monitoring responsible for projects funded by the McKnight Foundation, he believes that context is variable and that projects must be in constant reflection, adjusting and amending plans, ensuring that benefits reach target groups in the fastest and most effective way.

Dr. Perez is a professional who combines strategic vision with pragmatism. He has the ability to strike balance between the ambition of reaching goals and the limitations of reality. He is a lifelong researcher of quinoa, convinced that it is one of the greatest legacies of the Bolivian culture, that is allowing thousands of farmer families to escape poverty, and at the same time, convinced that achieving sustainable production is feasible. His vision and approach to global issues such as climate change, are an inspiration to researchers, technicians and producers.

He is convinced that a key success factor is the complementation of capacities and efforts from producers, researchers, marketers and exporters, including entrepreneurs.

Finally, his human virtues are invaluable. He has the wisdom and the capacity to motivate, to generate passion and commitment with development, thus promoting better conditions for the poor. His actions speak of his profound commitment to service.

With these lines we wish to pay tribute and recognition to his important contribution to the well-being of families living in rural areas of Bolivia, Peru, Ecuador and many other places where the positive energy of Carlos changed attitudes and practices, benefiting the lives and opportunities of thousands of human beings.

Cochabamba, July 2014

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