

# Effect of Seed Thermotherapy to Reduce the Viral Diseases of Faba Bean (*Vicia Faba* L.) in the Interandean Valley of Cochabamba, Bolivia

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## Abstract

In Bolivia, the faba bean (*Vicia faba* L.) is cultivated in the Andean region, Altiplano and Interandean valleys. In these valleys medium-sized and early varieties are cultivated. Viral diseases are a limiting factor in faba bean production. The aim of the present work was to study the effect of viral diseases on faba bean crops in the Interandean Valleys region, and how different treatments might be protective. Experimental plots were established in 2009 and 2014 in the Central Valley of Cochabamba (altitude 2550 m). In 2009, the seeds underwent one of three pre-sowing treatments: no treatment, pre-moistening with water for 1 h, or disinfection with Arconis fungicide. In 2014, the seeds underwent one of three other pre-sowing treatments: no thermotherapy, thermotherapy at 44-46 °C for 20 min, or thermotherapy at 44-46 °C for 10 min. The 2014 treatments were performed using both certified and uncertified seed. After sowing, the incidence of viral diseases - based on symptomatology - was recorded over plant growth, and disease progress curves (DPCs) constructed. Green pod and dry grain yields were also recorded in experimental plot year 2014. In 2009, disease incidence reached 26%, and in 2014 it reached 90%, with no significant differences between treatments in either year. Linearizing the DPCs using logit functions showed the 2009 DPC data best fitted the Gompertzian growth model, while that for 2014 best fitted the logistic model. The slopes of the linearized DPCs showed the 2009 treatments to have no effect on the disease progression rate. In 2014, and for both certified and uncertified seeds, thermotherapy had no effect on the rate of disease progression, nor on green pod or dry grain yield. In summary, Bolivian faba bean crops are affected by viral disease to different extents in different years. Pre-sowing seed thermotherapy or Arconis treatments provide little protection from the appearance and progression of viral disease.

**Keywords:** New Diseases; Polycyclic Disease; Infection Rate; Disease Intensity

## Introduction

In Bolivia, faba beans (*Vicia faba* L.) are cultivated on the Altiplano (altitude 3800 m), in the Puna region (3000-4000 m), and in the Interandean Valleys (2000-3000 m). Varieties are grown that produce grains ranging from small to large in size (Piérola, 1997) [1]. The small grain-size varieties grown in the Interandean Valleys are early varieties (green pods appear within 3-4 months of germination) being the most common is the variety Pairumani 1 and is used in this work (released by the *Centro de Investigaciones Fitocogenéticas de Pairumani, Cochabamba*). In the High Andes areas of the Altiplano, and in the Puna, however, large-grain varieties are grown, such as Gigante de Copacabana, Usnayo and others. These are late varieties, with green pods and dry grains appearing 6-8 months after germination.

Viral diseases limit faba bean production (Waterworth and Hadidi, 1998; Kumari and Makkouk, 2007), but in Bolivia little work has been done [2,3]. Otazu *et al.* reported bean mosaic disease in the Depts [4]. of Cochabamba and Tarija. Much later, Zambrana and de Quitón (1995) examined plant samples collected in highland and valley areas that were apparently affected by viral disease [5]. Using indicator plants and electron microscopy they were able to identify bean yellow mosaic virus (BYMV) and alfalfa mosaic virus (AMV). Finally, work performed by Céspedes (2007) indicated that, in the Interandean Valleys of Cochabamba, faba bean crops were affected by BYMV, bean leafroll virus (BLRV) and AMV [6].

In recent years, the Interandean Valleys have seen the intensive cultivation of a reduced number of faba bean varieties (Piérola, 1997) [1], and viral disease has become generalized, reducing the yields obtained, including in the Cochabamba Valleys. According to Subramanya Sastry (2013), the viruses BYMV and AMV, are transmitted through seeds although in low per cents [7]. The use of seed quality, certified and non-certified could play a role in the spread of viruses. The certified seed comes from the inspection by a State institution (Seed certification office in Bolivia) and the non-certified seed, usually, is the farmer's seed. These factors together with the climatic variations may be modifying traditional patterns of disease incidence and driving the emergence of other fungal and viral diseases. Garrett *et al.* (2006), Disease progress has been described by nonlinear growth functions, for a variety of comparative reasons, with rate of disease increase, source and amount of initial disease, and final level of disease estimated (Jeger 2004) [8,9].

There are several methods of combating seed-transmitted virus diseases. According to Subramanya Sastry (2013) [7], seed disinfection by heat is one of avoidance of virus inoculum from infected seeds, most attempts to eliminate virus by heat treatment have been done with high temperatures for relatively short periods or at low temperatures for longer periods by means of hot water or day heat treatments. On the other hand, it also indicates that elimination of some seed-transmitted viruses in certain crops was also achieved by soaking them in chemical solutions for varying periods. The present work examines the effects of viral disease on faba beans grown under Interandean valley conditions in the Dept. of Cochabamba, and whether certain treatments might be protective.

## Materials and Methods

### Study Area

Experimental plots were established during the winter sowing season (March-August) of 2009 and 2014 in the Central Valley of Cochabamba at La Tamborada, in the municipal area of Cercado (Dept. Cochabamba), on land owned by the *Facultad de Ciencias Agrícolas y Pecuarias de la Universidad Mayor de San Simón* (altitude 2550 m; 17° 26' 35" S, 66° 06' 52" W, and 17° 30' 34" S, 66° 09' 25" W; mean rainfall 450 mm; mean temperature May-November 26 °C). The climate here is semi-arid. Spring and autumn are dry, and during winter the days can be warm even though the nights can be cold.

### Experimental Plot, Year 2009

The soil was prepared using agricultural machinery. Sowing and cultivation practices up to harvest were performed in the traditional manual form of the area. The variety planted was Pairumani 1, a medium-grain variety with a short reproductive cycle. Sowing was performed on 27th March 2009, following a random block design with three treatments and four repetitions. These treatments were: T0 - Control (no treatment); T1 - pre-moistening of the seed with water 1 h before sowing; and T2 - pre-sowing disinfection of the seed using Acronis (thiophanate-methyl 36.9% + pyraclostrobin 4.1%). Each experimental unit was 10 m long with 0.7 m between rows (n=6) and 0.3 m between plants. During their vegetative growth cycle the plants received three applications of fungicide and insecticide at commercial doses, i.e., 1) with Fastac (alpha cypermethrin 10%) at emergence; 2) with Fastac (alpha cypermethrin 10%) + Cabriotop (pyraclostrobin 50 g/kg+methyram 550 g/kg) at the start of first flowering; and 3) with Amistar Top (azoxystrobin 20 g + difeconazole 12.5 g) at the start of second flowering. During growth the plants were banked up twice and irrigation provided from emergence to harvest as required.

### Experimental Plot, Year 2014

Soil preparation, sowing and cultivation practices were carried out as the 2009 year. The variety plated was Pairumani 1, but of two types: 1) Certified seed (theoretically virus free) obtained from the *Centro de Investigación Pairumani*, and 2) non-certified seed obtained from a grower. Sowing was performed on 23rd March 2014, following a random block design with three treatments, i.e., T0 - Control (no seed thermotherapy); T1 - seed thermotherapy at 44-46 °C for 20 min; and T2 - seed thermotherapy at 44-46 °C for 10 min, and two subtreatments, i.e., certified seed and non-certified seed. Each experimental unit was 10 m long with 0.7 m between rows (n=6) and 0.3 m between plants.

During their vegetative growth cycle the plants received three applications of fungicide and insecticide at commercial doses, i.e., 1) with Fastac (alpha cypermethrin 10%) at emergence; 2) with Fastac (alpha cypermethrin 10%) + Cabriotop (pyraclostrobin 50 g/kg+methyram 550 g/kg) at the start of first flowering; and 3) with Opera (pyraclostrobin 13.3% + epoxiconazole 5%) at the start of second flowering. During growth the plants were again banked up twice and irrigation provided from emergence to harvest as required.

### Disease Incidence and Analysis of Disease Progress Curves

The viral disease incidence was recorded based on symptomatology described by Counti *et al.* (2000) in both years, to avoid errors of edge effect was examined the two central rows in each experimental unit, in agreement with Campbell and Madden (1990) [10]:

$$\text{Incidence (\%)} = \frac{\text{N}^\circ \text{ of sick plants with symptoms of viral disease}}{\text{Total no}^\circ \text{ of plants}} \times 100$$

Disease progress curves (DPCs) were constructed from the incidence data, and linearised using the logit functions of the logistic =  $\ln(y/(1-y)) = \ln\{y_0/(1-y_0) + r_L t\}$ , Gompertzian =  $-\ln(-\ln y) = -\ln(-\ln y_0) + r_G t$  and exponential =  $\ln(y) = \ln(y_0) + r_E t$  growth models (Campbell and Madden, 1990; Jeger, 2004) [9,10]. The models to which the collected data best fitted were determined via the R<sup>2</sup> value.

### Yield (t. ha<sup>-1</sup>)

Yield (t.h<sup>-1</sup>) - green pod and dry grain - was estimated only in 2014 using data for the central two rows of plants in each experimental unit (one row for each yield variable). Green pod yield was determined by collecting the pods at maturity for all three flowerings. The dry grain yield was determined by leaving the collected plants in a vertical position on a threshing floor until the grains were completely ripe. This was followed by manual separation of the beans, winnowing, and recording of the dry grain yield in t/ha.

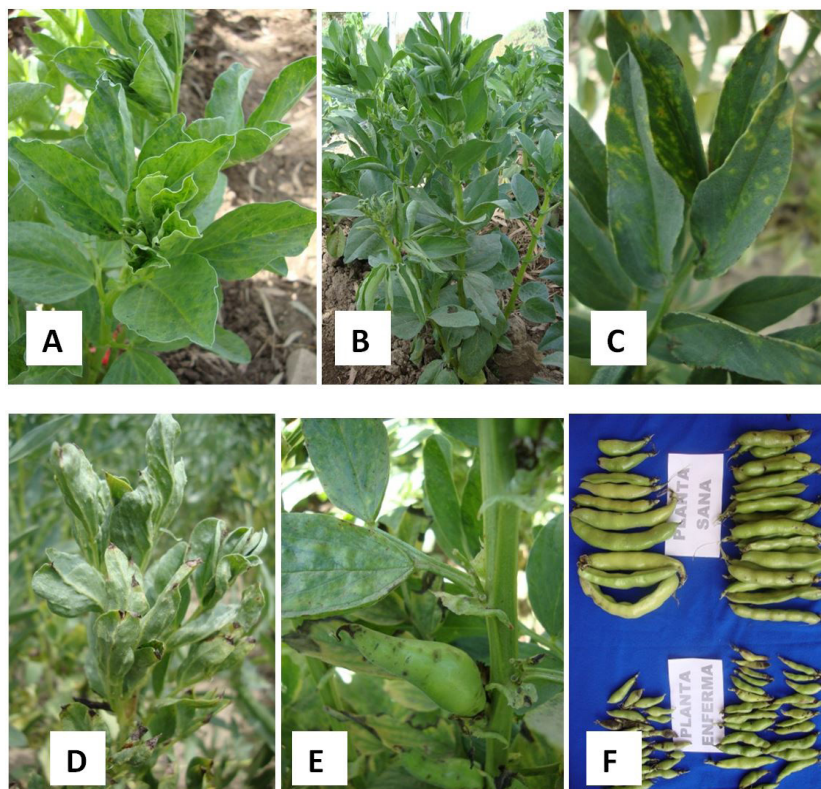
### Statistical Analysis

The fit of the DPCs to the models, and the differences in green pod and dry grain yield, were analysed using SAS software.

## Results

### Leaf and Pod Symptoms Caused by Viral Disease

Viral disease was recorded in the form of symptom complexes affecting to leaf and pods. Leaf symptoms complexes included mosaic (Figure 1A), leaf curling (Figure 1B), chlorotic rings (Figure 1C) and leaf deformations patterning (Figure 1D). Mosaic patterning was the most common symptom, while problems of leaf curling and chlorotic rings were isolated. The most common pod symptoms were deformation, size reduction and isolated necrosis (Figure 1E and F).

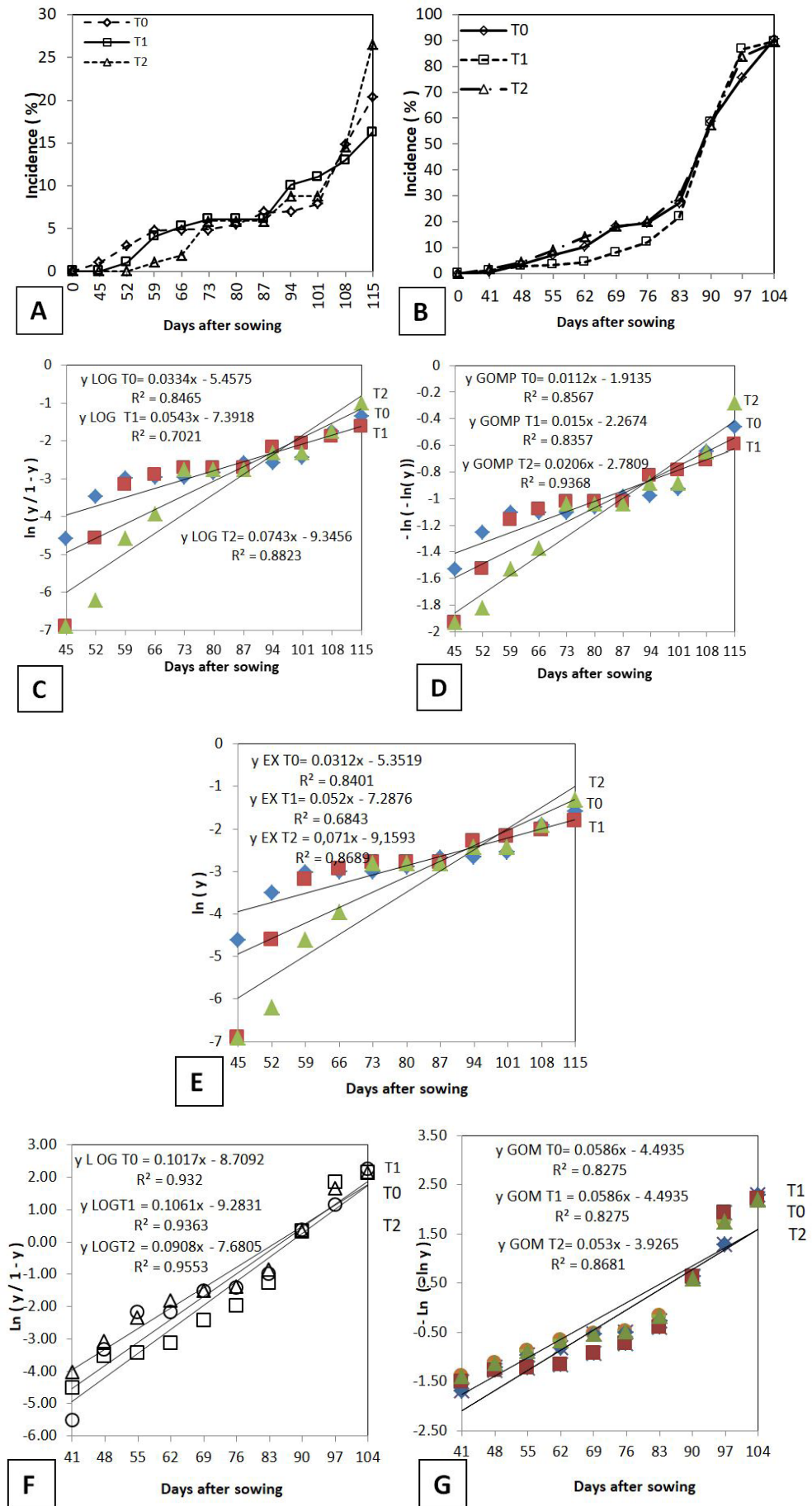


**Figure 1:** Viral disease symptoms in faba bean **A:** Mosaics (likely BYMV); **B:** Apical curling (likely BLRV); **C:** Chlorotic rings; **D:** Leaf deformation; **E:** Pod mosaics and deformation; **F:** Reduced pod size (Planta sana=Pods size Healthy plant and Planta enferma=Diseased plant)

### Analysis of the Diseases Progress Curves

The DPCs for the two study seasons were different. The curve for 2009 showed only a growth phase (Figure 2A), while that for 2014 reached a point of inflection (Figure 2B). In 2009, the incidence of viral disease reached 15-26% with no significant difference between treatments (Figure 2A), while in 2014 incidence reached around 90% for all treatments (Figure 2B). In both years viral disease symptoms began to appear from emergence until 41 days after sowing (Figure 2A and B).



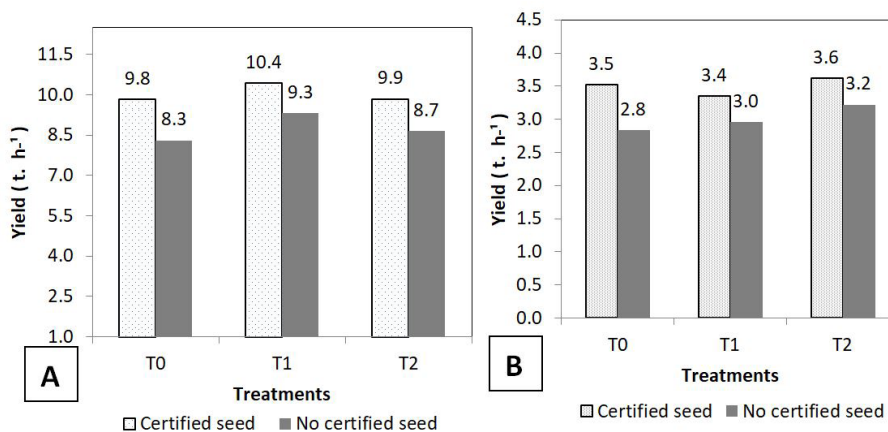


**Figure 2:** Disease progress curves **A:** 2009; **B:** 2014. Linearization of the latter curves; **C:** 2009, logistic model; **D:** 2009 Gompertzian model; **E:** Exponential model; **F:** 2014 logistic model; **G:** 2014, Gompertzian model ( $r$ =red numbers)

The growth models analysis of DPCs for the two study seasons were different. The 2009 curves were best described (according to the associated regression coefficients [ $R^2$ ]) by the Logistic and Gompertzian models (Figure 2D; see Figure 2C for less well fitting logistic model), while the 2014 the curves were best described by the logistic model (Figure 2E; see Figure 2F for less well fitting Gompertzian model). For 2009, the disease progression rates ( $r$ ), i.e., the slope of the linearized curves, as per the Gompertzian model, were similar for the different treatments:  $r_{T0}=0.0112/\text{day}$ ,  $r_{T1}=0.0118/\text{day}$  and  $r_{T2}=0.0181/\text{day}$  (Figure 2C and D). For 2014, the figures for the logistic model were again similar:  $r_{T0}=0.1017/\text{day}$ ,  $r_{T1}=0.1061/\text{day}$  and  $r_{T2}=0.0908/\text{day}$  (Figure 2E).

## Yield

For 2014, no differences were seen in the green pod yields between the seed thermotherapy treatments and controls, either for the certified or non-certified seeds (T1=9.3 and 10.4 t.ha<sup>-1</sup> and T2=9.9 and 8.7 t.ha<sup>-1</sup> compared to T0=8.3 and 9.8 t.ha<sup>-1</sup> respectively) (Figure 3A). Neither were any differences seen with respect to dry grain yield, again irrespective of seed thermotherapy treatment or seed origin (T1=3.0 and 3.4 t.ha<sup>-1</sup>, and T2=3.2 and 3.6 t.ha<sup>-1</sup> compared to T0=2.8 and 3.5 t.ha<sup>-1</sup> respectively) (Figure 3B).



**Figure 3:** Yield (t.ha<sup>-1</sup>) under different thermotherapy treatments A: Green pod; B: Dry grain. Year: 2014. No significant differences were detected (F001 and F005)

## Discussion

Viral disease was here recorded in the form of symptom complexes; the plants could therefore have been simultaneously infected with different viruses. Other authors have used this symptom complex technique and also detected the causal viruses. For example, Zambrana and Quitón (1995) [5], who examined field samples from the valleys and highlands of Cochabamba, managed to identify BYMV and AMV in faba bean. Vargas *et al.* (1997), using the DAS ELISA technique, identified BYMV, AMV and BLRV, while Céspedes (2007), who examined crops in the Central Valley of Cochabamba, detected BYMV, BLRV and AMV (the viruses with the largest distribution to affect faba bean in Bolivia) [6,11]. Kumari and Makkouk (2007) indicate that among the viruses that cause yellowing and dwarfing, BLRV is the most widely distributed in several parts of the world, while among those that cause mosaic patterning and mottling, BYMV and AMV are important in several African and Asian countries [3]. The pod symptoms recorded (deformation, reduced size, isolated necrotic areas) match those described by Vargas *et al.* (1997) for different viral diseases [11].

The DPCs for 2009 and 2014 were different. In 2009, viral disease incidence reached a maintained 15-26% across the different treatments (no significant difference) (Figure 2A). In 2014, viral disease incidence reached around 90% for all treatments, falling thereafter (Figure 2B). The 2009 curves agree with that described by Nutter (1997), with a “J” shape and no inflection point. However, they better fit the Gompertzian model [12]. In 2014, the sigmoid form of the curves also agrees with that reported by Nutter (1997), who indicates a sigmoid shaped DPC to commonly describe viral epidemics, but the data are best described by the logistic model [12]. Madden *et al.* (2007) indicate the logistic model to describe polycyclic epidemics well [13]. The difference between the DPCs for the two years might be due to variations in environmental conditions such as temperature and humidity, to biological factors such as differences in the size of the aphid population (viral vectors) or the size of other leguminous crops that might act as viral reservoirs (Conti *et al.*, 2000) [14]. The use of differently contaminated seed (Subramanya Sastry, 2013), or even climate change altering traditional patterns of incidence and promoting the emergence of new viral and fungal diseases (Garrett *et al.*, 2006), might also help explain these observations [7,8]. In summary, it might be said that the incidence of viral disease in the study area is variable.

In 2009, the disease progress rates (“ $r$ ”) obtained with the Gompertzian linearization of the DPCs was similar for all treatments (T0, T1 and T2). This suggests that the disinfection treatment did little to reduce disease development. For the 2009 treatments, the yields of green pod or dry grain were not determined. That was just for 2014.

In 2014, the  $r$  values obtained with the logistic model were higher than those obtained in 2009 with the Gompertzian model. Indeed, the results for all the 2014 treatments suggest that the seed used (certified and non-certified) probably was contaminated with viruses. According to Subramanya Sastry (2013), the viruses BYMV and AMV are transmitted in low per cent by seed of faba

bean [7]. In the latter year, no differences were seen between the seed thermotherapy treatments with respect to the final green pod or dry grain yields, either within the certified or non-certified seed groups. But according to Subramanya Sastry (2013), the seed-transmitted infection of ULCV was completely eliminated by treating the urd (*Vigna mungo*) bean seeds in water bath for 30 min at 55 °C without affecting the seed germination [7]. The yields of both are similar to those recorded by Herbas and Waaijenberg (2000) for certified seed under experimental conditions at San Benito in the High Valley of Cochabamba [15]. Quitón 2000 and Moreira and Milan 1995, who investigated the use of thermal treatments (45-50 °C for 20 min), also reported little difference with respect to controls in terms of protection against viral disease [16,17].

## Conclusion

In conclusion, Bolivian faba bean crops are affected by viral disease to different extents in different years, perhaps due to different environmental and or/other conditions. Pre-sowing seed thermotherapy or Arconis treatments provide little protection from the appearance and progression of viral disease [18].

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