Crop Protection 89 (2016) 178–183

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Dry heat treatment of Andean lupin seed to reduce anthracnose infection

César E. Falconí [a, *], Viviana Yánez–Mendizábal [b]

[a] Universidad de las Fuerzas Armadas ESPE, Departamento de Ciencias de la Vida, Carrera de Ciencias Agropecuarias, Sangolquí, Ecuador
[b] Universidad de las Américas, Centro de Investigación, Estudios y Desarrollo de Ingeniería, Facultad de Ingeniería y Ciencias Agropecuarias (CIEDI–FICA), Quito, Ecuador

ARTICLE INFO

Article history:
Received 21 March 2016
Received in revised form
15 July 2016
Accepted 16 July 2016
Available online 26 July 2016

Keywords:
Dry heat
Lupinus mutabilis
Anthracnose
Colletotrichum acutatum

ABSTRACT

The potential of dry heat treatment of Andean lupin seed to reduce seed–borne infection of the anthracnose pathogen, *Colletotrichum acutatum,* was investigated. First, the effect of dry heat (65 °C) over duration times of 0–96 h on germination and disease incidence after germination was evaluated for artificially– and naturally–infected seed. Dry heat treatment from 8 to 96 h reduced disease incidence after germination to undetectable levels in four cultivars compared with 7.5% disease incidence after germination in seed maintained at room temperature. Moreover, heat treatments of 4–12 h showed seed germination rates that were equivalent to the non–treated control. Under greenhouse conditions, dry heat treatments for 8 or 12 h reduced transmission of the pathogen from seed by 75 or 85%, respectively and dry heat treatment increased emergence of seedlings in comparison the non-treated control. Dry heat treatment is an environmentally friendly alternative for reducing anthracnose infections in Andean lupin seed.

1. Introduction

Andean lupin (*Lupinus mutabilis* Sweet) is a native legume domesticated by indigenous people of South America for its food value (Jacobsen and Sherwood, 2002). In Ecuador, this legume is considered an agro–industrial food source to produce nutritional dishes for children and young people of the National Educational System (Acuerdo Interministerial 1–10, 2010). Recently interest in growing *L. mutabilis* has extended worldwide due to its value as a low–fat protein source in foods such as soups, breads and snacks (Jacobsen and Sherwood, 2002).

Despite the agro–industrial importance of Andean lupin, there are significant problems that limit its production, especially fungal diseases similar to those reported for other lupin species (Talhinhas et al., 2002; Thomas, 2003). In Ecuador, anthracnose caused by the seed–borne pathogen, *Colletotrichum acutatum,* is the most devastating fungal disease (Falconi et al., 2013; Peralta et al., 2012). Planting infected seed usually results in poor emergence and infected seedlings. Most aerial parts of the plant, especially pods, seeds, and stems are infected (Falconi et al., 2013).

In Ecuador, anthracnose control in Andean lupin is based on a combination of genetic resistance and fungicide seed–treatments. Recently L. *mutabilis* cvs. 'ECU–2658' and 'I–450 Andino' were released based on their adaptation to different environmental conditions and good agronomic qualities such as white grain color and early date of maturity (Peralta et al., 2012, 2004). Both cultivars, however, are susceptible to anthracnose and their yield in presence of disease is not high enough to satisfy market demand. For this reason, farmers more frequently grow 'Criolla' seed, an anthracnose–tolerant cultivar locally adapted to Cotopaxi and Chimborazo provinces.

The production of disease–free seeds with strict seed certification programs minimizes yield reduction from fungal seed–borne diseases (Mancini and Romanazzi, 2014); however this is difficult to perform for lupin under Andean conditions. The Instituto Nacional Autonomo de Investigaciones Agropecuarias (INIAP) is an institution that deals with the production of disease–free seeds; however, seed production is used mainly for research purposes. Under INIAP guidance, smallholder farmers attempt to select apparently healthy seed but are not always successful. Farmers sell phenotypically healthy seed in nearby towns

* Corresponding author. Plant Pathology Research Group, ESPE, P.O. Box 171–5–231B, Sangolquí, Pichincha, Ecuador.
E-mail addresses: cefalconi@espe.edu.ec (C.E. Falconí), viviana.yanez@udla.edu.ec (V. Yánez–Mendizábal).

http://dx.doi.org/10.1016/j.cropro.2016.07.021
0261-2194/

but retain their own low–quality seed for sowing. This seed is exchanged within local networks, which increases risk of pathogen dissemination (Falconi, 2012).

Seed infection is the main source of primary inoculum and further epidemics in the field. The pathogen is located on the seed coat and embryo (Agarwal and Sinclair, 1997). During germination the pathogen is transferred from seed coat by colonizing the cotyledons, radicle and plumule of the emerging seeds (Yesuf and Sangchote, 2005). Primary infections as low as 1 in 10,000 seeds are predicted to produce over 15% yield loss when lupin–susceptible cultivars are grown in high rainfall areas (Thomas and Sweetingham, 2000). Fungicide seed treatments have been reported to reduce transmission to emerging plants, but they are not completely effective (Thomas and Sweetingham, 2003). In this context, methods to improve disease management that support and sustain local communities are needed to suppress anthracnose of Andean lupin.

Several studies have shown that dry heat treatment is an alternative to reduce fungal seed infections. Clear et al. (2002) and Gilbert et al. (2005) demonstrated that dry heat treatment at 70 and 80 °C for different periods of time eradicated seed infections caused by *Fusarium graminearum* in wheat and barley. However, temperatures over 70 °C or 80 °C reduced significantly the viability of the seed. Thomas and Adcock (2004) reported that dry heat exposure of *Lupinus angustifolius* seed for 4 days at 70 °C or 8 days at 65 °C reduced anthracnose infection caused by *Colletotrichum gloeosporioides* to an undetectable level without greatly affecting seed germination. Dry heat treatment of 65 °C could be an efficient treatment to reduce or eradicate *Colletotrichum acutatum* seed infection in Andean lupin.

This study reports laboratory and greenhouse experiments to determine the potential performance of dry heat treatment at 65 °C for reducing or eradicating infection of *C. acutatum* in Andean lupin seed. We also evaluated the effects of the most promising seed treatments on germination of seed, seed moisture content, emergence of seedlings, and transmission of infection from seed to the seedlings.

2. Methods

2.1. Seed and fungal pathogen

The seed used in this study was from Andean lupin (*Lupinus mutabilis* Sweet). Anthracnose–infected seed was harvested from greenhouse experiments where lupin plants (cvs. ECU–2658 and I–450 Andino) were artificially infected. For inoculations, 2 ml of *Colletotrichum acutatum* isolate Lup 18 (EMBL ITS accession number JN543063) suspensions of 10^6 spores/ml were sprayed on the apical parts of plants in pod fill, by using a small hand–held Venturi atomizer (aerograph) with an air pump. The inoculated area of each plant was completely covered with a black plastic bag for 72 h. A piece of cotton drenched in sterile distilled water was added to the bags before sealing to maintain high relative humidity and to promote infection (Falconi et al., 2015). Naturally infected seed (cv. Criolla) was obtained from Andean lupin farmers of Cotopaxi and Chimborazo provinces. Seed lots were stored at 4 ± 1 °C and low humidity to maintain seed infection levels until experiments commenced.

2.2. Dry heat treatment

Dry heat treatments of seed lots were conducted in fan–assisted convection ovens (Memmert, Schwabach, Germany) in which temperatures were monitored with a data logger (IFC 400, MadgeTech, Inc, Warner, New Hampshire, USA). Temperatures in the ovens were maintained at 65 ± 2 °C. Seed aliquots of 27±1 g were placed in open, individual glass Petri dishes (9 × 1.5 cm) and were grouped within the oven to avoid spatial variability. Seed exposure time treatments in the ovens were 0, 1, 2, 4, 8, 12, 24, 48, 72, and 96 h. Seed lots in similar dishes at room temperature (25 ± 2 °C) were used as control. When removed from ovens, seed lots were placed in plastic bags and stored at 4 °C until all heat–duration treatments within that experiment had been completed. For each seed lot, four replicates of 100 seeds were treated at each exposure time. The experiment was repeated twice.

2.3. Laboratory assessment of dry heat efficacy against C. acutatum

For determination of the pathogen incidence on seed, four 100–seed replicates from each seed lot and exposure time were superficially disinfected with a 95% ethanol rinse, followed by 0.5% sodium hypochlorite for 5 min and rinsed twice in sterile distilled water. Seeds were plated onto Petri plates with potato dextrose agar (PDA) amended with chloramphenicol (500 mg L^{-1} Chloromycetin; Parke Davis Co., Detroit, Michigan, USA). Plates were incubated for 9 days at 25 °C under a 14 h photoperiod provided by fluorescent lights. After incubation, the presence or absence of *C. acutatum* was determined by microscopic observation (50 × magnification for colony morphology and 400 × magnification for conidia identification).

For determination of seed germination, four 100–seed replicates from each seed lot and exposure time were placed systematically between two thoroughly wetted sheets of Grade S50 seed germination paper (Sartorius, Madrid, Spain). The sheets holding the seed were then rolled to form a cylinder. One end of the roll was constricted with an elastic band and rolls were placed together, constricted end down within plastic bags. After 7 days' incubation at 20 ± 2 °C (12–h light dark cycle), rolls were opened and the proportion of germinated seed determined. The percentage of infection and germination from different dry heat treatments were determined.

2.4. Effect of dry heat on seed moisture content

Quadruplicate samples of 10-g non–treated and dry–heat treated seeds as described above were placed in aluminum–weighing boats and dried in a convection oven at 130 ± 2 °C for 3 h. The moisture content (MC) of seed was calculated based on the weight loss after drying and expressed as percentage based on methods of ISTA (1999) and Booth and Bai (1998):

$$\%MC = [M2 - M3] \times [100/(M2 - M1)] \quad (1)$$

where, *M1* represents the weight of container, *M2* is weight of container and seeds before drying and *M3* is weight of container and seeds after drying.

2.5. Effect of dry heat seed treatment on seed anthracnose infection, emergence of seedlings and pathogen transmission from seed to the seedlings

Artificially–infected (cvs. ECU–2658 and I–450 Andino) and naturally–infected seeds (cv. Criolla Cotopaxi and Chimborazo) were randomly sub–sampled into approximately 1.25 kg seed lots and dry–heat treated for 8 and 12 h as described above. Control treatments were non–heated seed with or without fungicide. The fungicide treatment was Vitavax 300® WP (Carboxin 200 g $kg^{\rightarrow 1}$ + Captan 200 g $kg^{\rightarrow 1}$, Chemtura Agrosolutions) at 2 g kg^{-1} of seed.

For planting, seed was randomly subsampled from the dry