



SHORT COMMUNICATION

Survival of *Alternaria brassicicola* in cryo-preserved *Brassica* spp. seeds

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ABSTRACT: Seed health testing of seven accessions of *Brassica* spp. conserved in the year 2001 at -180°C in liquid nitrogen at National GeneBank, ICAR-NBPGR, New Delhi resulted in detection of *Alternaria brassicicola* in three accessions of *B. juncea*, IC-113148, Pusa Bold and Prakash in the year 2015. Detection of *A. brassicicola* in cryopreserved *Brassica* seeds shows that the fungus can survive even at ultra-low temperature for a long duration.

Keywords: *Alternaria* blight, *Brassica* spp., cryo-preservation, seed health testing

Cryo-preservation of orthodox seed germplasm from most common annual agricultural and horticultural crop species having desiccation and liquid nitrogen (LN)-tolerance has been carried out for more than 25 years at ICAR-NBPGR (Mandal and Chaudhury, 2004; Chaudhury, 2003). The storage of viable biological material at ultra-low temperatures of -180°C and below using LN is one of the most efficient cryo-preservation methods. The cryo-preserved material is regularly monitored for seed viability and other parameters. The Division of Plant Quarantine, ICAR-NBPGR, New Delhi undertakes seed health testing (SHT) of germplasm conserved in the National Gene Bank including cryo-genebank material. The present study was undertaken to detect the presence of seed-borne fungal pathogen(s) associated with *Brassica* seeds already cryo-preserved for about 14 years during SHT.

Seven accessions of *Brassica* viz., four accessions of *Brassica juncea* (IC-113148, Prakash, Pusa Bold, Rajat), two accessions of *B. rapa* (IC-113122 and IC-113149) and one accession of *B. campestris* (IC-73188) from ICAR-NBPGR Regional Station, Hyderabad, India conserved in the cryo-genebank since 2001, were received from the SHT prior to its release to the indenter in the year 2015. As per standard practice for cryo-preservation, the seed lots were desiccated to 5-7 percent moisture content and tested viability of seed by Petri plate method (Chaudhury *et al.*, 1989) which ranged from 84.0 to 100.0 percent before packing in 2 ml polypropylene cryo-vials prior to storage in LN. The viability of fresh seed samples tested by Petri plate germination method (Chaudhury *et al.*, 1989), ranged from 84.0 to 100.0 percent prior to storage. None of the

samples were given any physico-chemical treatments before storage. The samples conserved for 14 years of cryo-storage were thawed at room temperature and tested for viability and seed health. In general, the seed viability of the seven samples ranged from 80.0 to 100.0%, similar to that observed in samples before cryo-storage.

During SHT, all the seed samples were first examined visually and then were subjected to blotter test. Depending on the availability of the seeds, 10-15 seeds of each accession after surface sterilization with NaClO (4%) were placed on 3 layers of moist blotters in sterile plastic Petri plates (110 mm) and incubated for 7 days at 22±1°C under alternate cycles of 12 h light and darkness. Plates were examined for the presence of associated fungal growth on the 8th day under stereo-binocular microscope (Mathur and Kongsdal, 2003) for fungal growth with typical morphological characters. Further, single spore of the fungus grown on seeds was picked up under stereo-binocular microscope using hand-made needle (Akhtar *et al.*, 2014) and transferred on potato dextrose agar (PDA) plate. The inoculated plates were incubated as mentioned above and the fungal characters were studied in detail making temporary mounts under compound microscope for ascertaining the identity of associated fungus.

During SHT using blotter test, fungal infection on seeds of three accessions of *B. juncea*, namely IC-113148, Pusa Bold and Prakash was observed ranging from 10-20% which affected seed germination. The fungus developed black, shiny fungal growth consisting of conidia in long, narrow chains showing typical characteristics of *Alternaria*. Conidia produced on the

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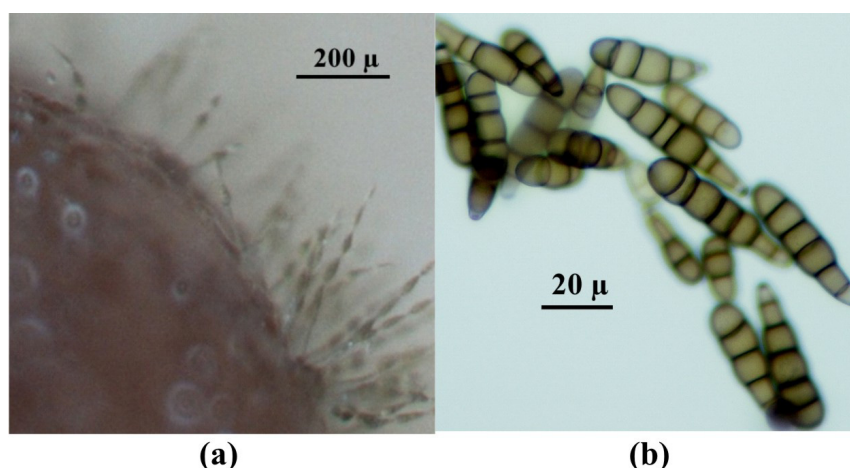


Fig. 1. *Brassica* seed showing fungal growth under stereo-binocular microscope at 100X (a) and conidia of *A. brassicicola* under compound microscope at 400x (b)

seeds were straight, obclavate with 11-15 transverse and 0-8 longitudinal or oblique septa, pale to greyish olive and smooth measuring the dimension of 75-350 x 20-30 μm (Fig. 1a). Conidia were straight, cylindrical usually tapering slightly towards the apex. The basal cell was mostly rounded with transverse septa (1-11), pale to dark olivaceous brown measuring 18-130 x 8-10 μm (Fig. 1b). Fungal colonies developed on PDA were initially white later became grey to blackish and mycelium formed light to dark grey cottony growth. Based on the morphological characters developed on the seed as well as on PDA, the fungus was identified as *Alternaria brassicicola* (Schwein.) Wiltshire (Mathur and Kongsdal, 2003). All the infected samples were given fungicidal treatment with a mixture of mancozeb (0.2%) and carbendazim (0.1%) as a curative measure before their release.

The PGR are stored for utilization and posterity but full benefits of any storage system are realized only when the seeds intended for storage are of high quality including freedom from seed-borne pathogens. Detection of *A. brassicicola* in *Brassica* seeds conserved for about fourteen years at temperature -180°C in LN shows that the fungus can survive even at ultra-low temperature. Similar observations have been reported for *Colletotrichum capsici* from chilli (Dev *et al.*, 2012) and *Dendryphion penicillatum* in opium poppy seeds (Akhtar *et al.*, 2016) under cryo-preservation.

However, Kumar and Gupta (1994) reported survival of *A. brassicicola* and *A. brassicicola* in Indian mustard seeds up to 6 months at the temperature ranging between 4.0 to 46.1°C with 4.0-99.0% relative humidity (RH) and more than 6 months at lower temperature and higher RH. In view of India being the major rapeseed-mustard producing country, the disease has a great impact on its production, as the current commercial varieties are

considered to be susceptible to *Alternaria* blights. Therefore, seed health testing is essential before conserving seed material for quality assurance and minimizing the risk of spread of disease.

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