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Short Communication

Survival of *Colletotrichum capsici* in decade-long cryopreserved Chilli (*Capsicum annuum*) seeds

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Cryopreservation of orthodox seed germplasm from most common annual agronomic and horticultural species having desiccation and LN-tolerant has been carried out for more than 24 years at NBPGR [1, 2, 3]. The cryopreserved material is regularly monitored for seed viability and other parameters. Seed-health testing for pest-free conservation of germplasm at NBPGR was first initiated in 1998 for the material collected under National Agricultural Technology Project (NATP). In addition to quarantine of PGR under exchange, the Plant Quarantine Division of NBPGR also undertakes seed-health testing of germplasm conserved in the National Gene Bank (NGB) including cryogenebank material for pest-free conservation and further distribution. The present study reports observations recorded during seed health testing of cryopreserved chilli (*Capsicum annuum* L.) accessions.

A consignment comprising eight accessions of chilli seed was received by PQ Division in the month of September 2011 from the cryogenebank for seed-health testing prior to its release to the indenter. These accessions of chilli seeds were received at cryogenebank from NBPGR Regional Station, Hyderabad, India in 2001. The seed lots were desiccated to between 5 to 7% moisture contents before packing in 2 ml polypropylene cryovials and suspending at temperatures between -170^0 to -180^0C. The viability of fresh seed samples, as tested by Petri plate germination method [4], ranged from 84 to 92%. None of the samples were given any physico-chemical treatments before storage. After 11 years of cryostorage the samples were thawed at room temperature and tested for viability and seed health.

During seed-health testing, all the chilli seed samples comprising 15-20 seeds, were first examined visually and then under stereo-binocular microscope for discolouration, deformation, malformation, fungal growth and fructifications, etc. Abnormal and unhealthy-looking seeds showing black spots were subjected to blotter test and examined on eighth day for presence of fungal pathogen(s) [5]. Fungi were identified on the basis of colony characters, fruiting bodies and conidia under stereo-binocular microscope and compound microscope. A single acervulus of the fungus on seed was picked up and transferred on potato dextrose agar (PDA) to isolate pure fungal cultures for ascertaining the identification [6].

During the dry seed examination of eight samples, only one sample, IC 214991 comprising 15 seeds, two seeds showed tiny black spots on seed surface. These spots in the blotter test developed into fungal colony showing dark acervuli (Fig.1a). Acervuli were sub-epidermal emerging by disrupting outer epidermal cell walls of host. Setae were dark brown, rigid, swollen at the base, slightly tapered to the paler acute apex, 1- to 5-septate, 250 x 6 µm (Fig.1b). Conidia were

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hyaline, falcate with acute apex and narrow truncate base, aseptate, uninucleate, 18-23 × 3-5 µm (Fig.1c). Fungal colony on PDA was initially white which later became grey-dark brown. Mycelium formed light to dark grey cottony growth and acervuli with abundant dark setae. Based on the morphological characters of the acervuli, conidia and mycelial culture growing on the seed and PDA, the fungus was identified as *Colletotrichum capsici* (Syd.) Butler and Bisby [7].

The anthracnose fungus is reported to be seed-borne [8] and is known to survive in and on seed as acervulus and micro-sclerotia [9]. Survival of mycelia and stromata in colonized chilli seed had been reported by Manandhar *et al.* [10]. Mycelium is present in the outer and inner layers of the testa and in the endosperm which emerges to the surface after disrupting the seed coat [11]. Siddiqui *et al.* [12] reported the anthracnose fungus to be viable for over eight years in chilli seeds stored at 5°C. The pathogen can persist in soil, infected crop residues and weed [3]. The infected seeds give rise to weak seedlings which then become the primary source of inoculum in the field. During warm and wet season conidia from the acervuli get dispersed with wind or splashed by rain or irrigation water from diseased to healthy plants. All the samples were given fungicidal treatment with a mixture of mancozeb (0.2%) and bavistin (0.1%) as curative/prophylactic measure.

C. capsici which causes anthracnose disease in a wide range of hosts including cereals, legumes, vegetables, perennial crops and tree fruits, is one of the most important plant pathogens worldwide [3] and there has been a recent record of the fungus on *Jatropha curcus* [13]. Chilli is an important economic vegetable crop world wide, which gets severely infected by anthracnose causing yield losses up to 50% [14]. The disease is widely present in India and other chilli producing regions of the world [3]. Anthracnose disease complex of chilli is caused by more than one *Colletotrichum* species. Than *et al.* [15] have reported occurrence of different *Colletotrichum* species such as *C. acutatum* (Simmonds), *C. capsici*, *C. coccodes* (Wallr.) Hughes, *C. dematium* (Pers.) Grove, *C. gloeosporioides* (Penz.) Penz. and Sacc. and *C. nigrum* (Wallr.) Hughes to be associated with anthracnose in chilli in various parts of the world. In India the disease is caused by *C. capsici* [16]. In addition to its wide distribution, there are reports of existence of variability in the pathogen [3]. Sharma *et al.* [17] reported existence of 15 pathotypes of *C. capsici*, from Himachal Pradesh in northern India based on the symptoms developed, whereas Montri *et al.* [18] reported three pathotypes in Thailand on the basis of quantitative infection on chilli. During last 3 decades, during processing 6398 samples of chilli germplasm imported from various countries, *C. capsici* was intercepted in seed samples from Nigeria, Taiwan, USA and Zambia [19].

The PGR are stored for utilization and posterity but full benefits of any storage system are realized only when the seeds intended for storage have high initial quality. Detection of *C. capsici* in the chilli seed conserved for more than ten years at temperature as low as –180°C shows that the fungus can survive even at this low temperature. These diseased seeds can act as a source of infection and can spread disease to new areas. Similar studies done on rice have proved that white tip nematode also survived in cryopreservation [20]. In view of India being the major chilli producing country, the disease has a great impact on chilli production, as the current commercial varieties are considered to be susceptible [21]. This further highlights the importance of seed-health testing in conserving disease-free material for quality assurance and minimizing the risk of spreading disease thus protecting Indian agriculture.

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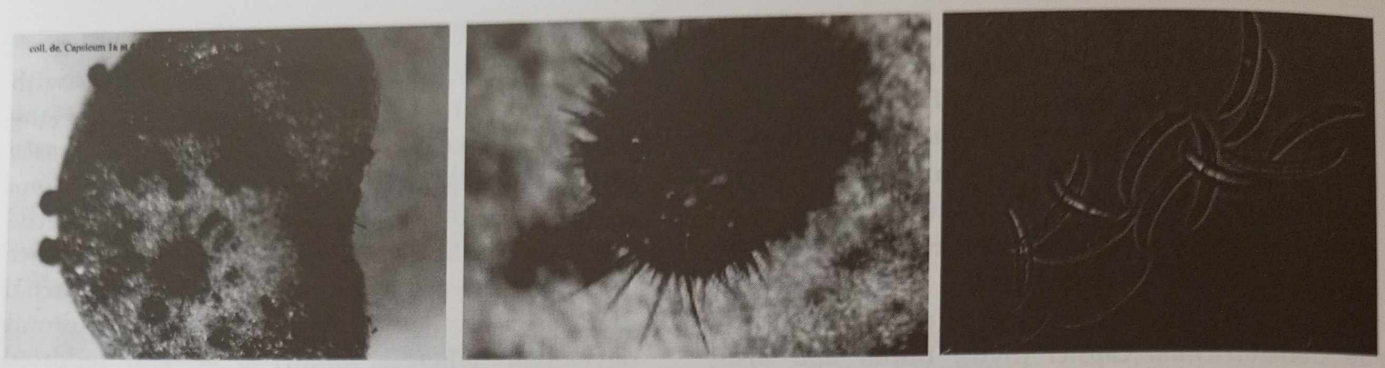

(a) (b) (c)

Fig. 1. (a) Chilli seed showing acervulus of *Colletotrichum capsici*, (b) acervulus with setae, (c) conidia of *C. capsici*

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