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Characterization of *Spongospora subterranea* f. sp. *subterranea*, the Cause of Powdery Scab of Potato in Pakistan

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Abstract: Powdery scab of potato is caused by *Spongospora subterranea* f. sp. *subterranea*. Current study differentiated the powdery scab from deep-pitted scab, which is closely related to common scab (*Streptomyces scabies*). Pathogen was characterized by light and electron microscopy. Light microscopy (LM) of spores of raised pustules and galls showed a characteristic spongy and honeycomb-like structure. Whereas, spore like bodies of deep-pitted lesions were rounded to oval compact and dark colored bodies. Scanning electron microscopy (SEM) of spore balls of local isolate of *S. subterranea* revealed no difference in structure on comparison with spore balls of European isolate. Whereas, spore like bodies of deep-pitted scab showed difference in structure. Bioassay and ELISA tests did not show any correlation of spores of powdery and deep-pitted scab.

Key words: Powdery scab, *Spongospora subterranea*, potato, cystosori, deep-pitted scab

Introduction

Powdery scab is one of the cosmetic diseases of potato caused by *Spongospora subterranea* (wallr.) Lagerheim. Pathogen produces scab or blister on the tuber surface (Butler and Jones, 1949). It causes severe tuber distortion and reduces the market value of the crop. Whereas, on roots produces wart like galls (Melhus *et al.*, 1916). *Spongospora subterranea* forms long lived cystosori cluster (spore balls) in lesions and can be both tuber-borne and soil-borne (Adams *et al.*, 1987). The cystosori are reservoir of potato mop-top virus (Avery, 1983). Each cystosorus is 19-85 μm in diameter, irregular sphere or ovoid in shape and usually has a hollow center often with small pores between adjacent sori (Jones, 1978). These spaces contribute to the spongy nature of the spore balls, from which the generic name, Spongospora was derived. Each cystosorus is an aggregate of cysts or resting spores of about 3.4–4.5 μm in diameter (Lawrence and Makenzie, 1981) and 500–1000 in number. Each can release a biflagellated primary zoospore (Kole, 1954) that can infect their host.

In Pakistan, previously no work has been done on morphology and characterization of cystosori (spore balls) of *S. subterranea*. There was confusion about the structure of the spores of deep-pitted scab (common scab) and powdery scab. Therefore, it was essential to characterize the fungus before studying its biology, epidemiology and management.

Materials and Methods

Light microscopy (LM): Slides of spore balls or cystosori were prepared by cutting transverse sections of scabbed lesion or by scrapping the brown powdery mass from raised and deep-pitted lesions of scabbed tubers. Mounted in cotton blue and observed under light microscope at 10 and 40x magnification. Spore size was measured with calibrated microscope.

Electron microscopy (EM)

Preparation of spore material: Scabbed lesions were cut from heavily infected tubers of cv. Cardinal, collected from Sharan Potato Research Station of Kaghan valley and from deep–pitted scabbed tubers collected from Sahiwal in Punjab area. Homogenized in tap water at 9,000 rev. pm. The suspension was passed through a column of sieves (500, 100, 80 and 40 μm mesh size). Then 40-80 μm fractions were collected on filter paper, air-dried and stored at 4 °C for further use. Inoculum of European isolate of *S. subterranea* was obtained from Dr. Merz, Switzerland.

Scanning electron microscopy (SEM): Cystosori or spore balls were fixed in 3 % glutaraldehyde in 0.1 M Caccodylate buffer (pH 7.4) for 12 h and were dehydrated in an ethanol series (30/50/70/90/99% v/v; each for 5 min.) followed by 100 % acetone. The spore material was then embedded in Epon-Araldite and dried probes were then coated with gold palladium (3-30nm) and examined under scanning electron microscope (JSM-35 CF).

Bioassay test

Preparation of bait plants: Tomato cv. Montfavet H 36-5 (Swiss variety) were prepared as bait plants by sowing seeds in sterilized sand. After germination, seedlings were irrigated with 3 fold dilution of stock nutrient solution (NS) contained 0.710 g Ca $(NO_3)_2$. $4H_2O$, 0.505 g KNO_3, 492 g $MgSO_4$. $7H_2O$ and 0.272 g KH_2PO_4, 0.20 g FE-EDTA and 1ml of Hoagland's A solution per litre (Merz, 1989) and kept in growth room at 15 h of light (12,000 lux, cool white fluorescent tubes) at 18 °C and 9h dark period at 16 °C. Then three weeks old tomato seedlings were transferred to plastic tray (7.5x 9.5 cm^2) containing nutrient solution. In each container 12 plantlets were held in slit of sponge plugs by inserting into holes of the tray cover and kept under same conditions of the growth room as described above. After one week the roots were washed before use in the bioassay.

Solution culture test: After one week, plantlets were transferred to another tray containing nutrient solution with homogenized spores inoculum of suspected powdery scab, deep-pitted scab and Swiss isolate of *S. suberranea*. Plants were baited with the inoculum for 10 days in growth room under same conditions as described above. After 10 days of baiting period roots of tomato plants were washed and cultivated in fresh nutrient solution. After 7 days roots were harvested, cleared and stained for microscopy.

Zoosporangium staining procedure: Whole roots were cleaned in absolute ethanol/chloral hydrate/water (1:1:1, w/w/w) for 10 minutes at 60 °C and then stained for 5 minutes at 60 °C in a staining solution contained 3 % formaldehyde, 6 % lactic acid, 3.5 % phenol, 87.2 % ethanol/water (1:1, v/v), and 0.3 % water blue (w/w) (sol. heated once at 80 °C before use). The roots were then fixed in lactic acid for 5 minutes and stored in sterilized distilled water at 4 °C before evaluation of root infection (Merz, 1989).

Evaluation of root infection: The roots of 6 plants per tray (one treatment) were taken for evaluation. The whole roots were suspended in sterilized distilled water in a large size petri plate (16 cm diameter) and observed the blue stained zoosporangia in

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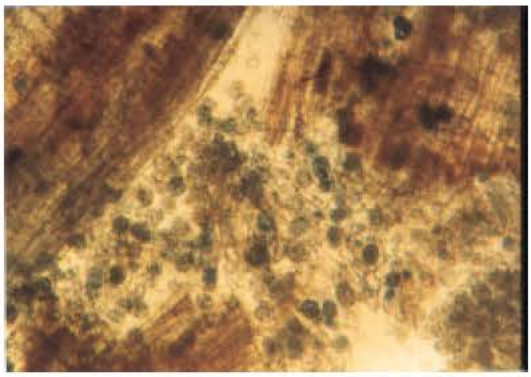
root hair and epidermal cells of roots under a stereomicroscope with 20-fold magnification. Rating of root infection was done on 0-4 scale of Merz (1989), where 0= no sporangia, 1= only a few sporangia, 2= several roots with sporangia, 3= sporangia regularly present or moderate infection, 4= sporangia regularly present or heavy infection.

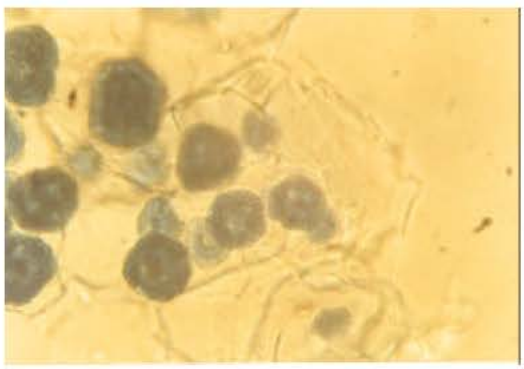
Serological test (ELISA): Plate-trapped antigen of Enzyme Linked Imunosorbant Assay (PTA-ELISA) technique was used for detection of *Spongospora subterranea* as described by Harrison *et al.* (1993). Samples of spore balls and spore like bodies of Pakistani scabbed potatoes and Swiss spore balls of *Spongospora subterranea* were prepared by grinding in 0.05 mol L^{-1} sodium carbonate buffer (pH 9.6) with pestle and mortar and left for at least one hour at room temperature. Supernatant (100 µl/well) was pipetted into micro-titre plate and incubated over night at 4 °C. Next day plates were washed (three times) with 0.05% phosphate buffer saline containing Tween-20 (PBS-T) and bovin serum albumin (BSA) (0.5%wt/vol) (100µl/well) was added to wells and incubated for three h at room temperature. The washing step was repeated as above and then anti-rabbit gamma- globulin conjugating alkaline phosphate with dilution of 1 in 1000 PBS- T containing BSA (0.5% wt/ vol. (150ml/ well) was added to the wells and incubated for 3 hours at room temperature. Finally, the plates were incubated with substrate (2-nitrophenyl phosphate) (substrate tablets, 0.75 mg/ml) in 10% diethanolamine (PH 9.8) (100 µl/well) at room temperature for one hour and their optical absorbance was measured with micro-plate reader at 405 nm (A 405).

Results

Microscopy: Out of two types (powdery and deep-pitted) of scab lesions the light microscopic observation of spore of suspected powdery scab at 10 and 40x magnification, showed the typical structure of spore balls of *S. subterranea*. Their size was 40-80 µm.



a) at 10x magnification



b) at 40x magnification

Fig. 1: Spore balls or cystosori of *Spongospora subterranean*

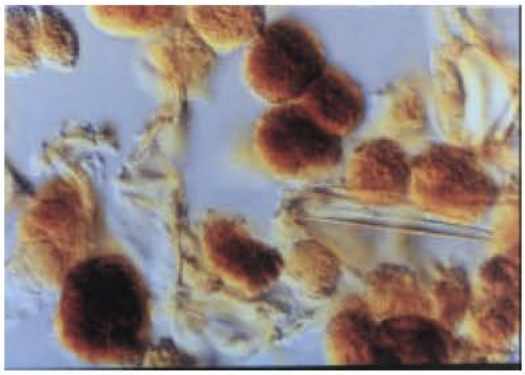
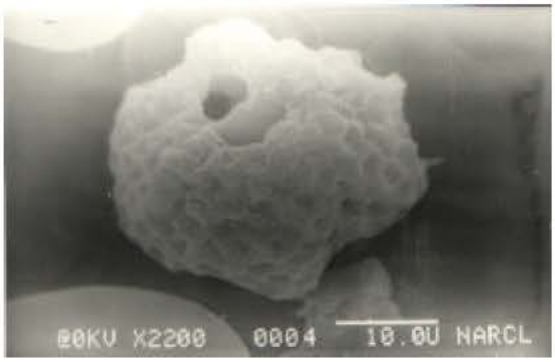
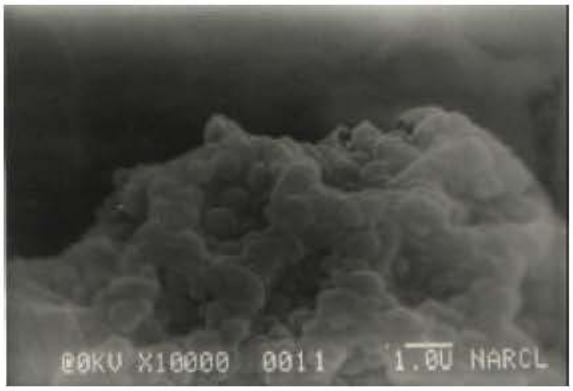


Fig. 2: Light micrograph of spore like bodies of deep-pitted scab



a) at 2200 X magnification



b) at 10000 X magnification

Fig. 3: Scanning electron micrograph of cystosorus of *Spongospora subterranean*

Each spore consists of numerous spores and form the characteristic honeycomb-like structure with hollows (Fig. 1a, b). On the other hand the spores obtained from second type of scab or deep-pitted type showed circular to oval shape, light to dark brown colour, their size was between 10-60 µm and their structure was not of typical honey comb spongy nature as spores of *S. subterranea* have (Fig. 2).

Scanning electron microscopy (SEM) of spore balls of two different types (powdery and deep-pitted) of scab revealed that the spores (cystosori) of first type consist of numerous cysts or resting spores, which stuck together to form irregular channeled spore ball (Fig. 3 a, b). On the other hand the spores of second type of lesions (deep-pitted) showed dark colour with hard texture and

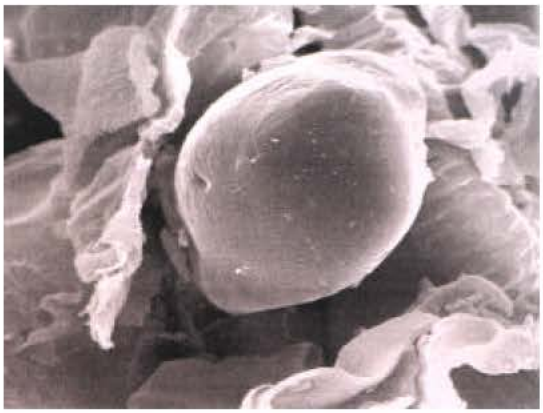


Fig. 4: Scanning electron micrograph of spore like body of deep-pitted scab

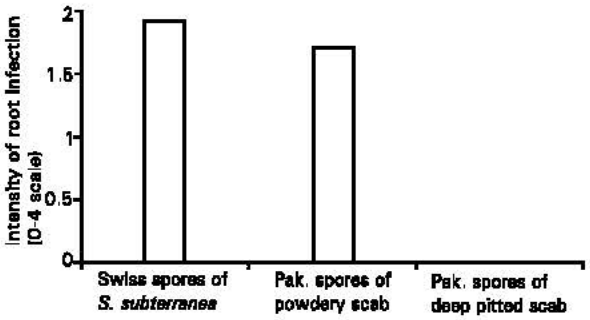


Fig. 5: Root infection of tomato bait plants to Swiss spores of *Spongospora subterranea*, Pakistan spores of powdery and deep-pitted scab on 0-4 scale

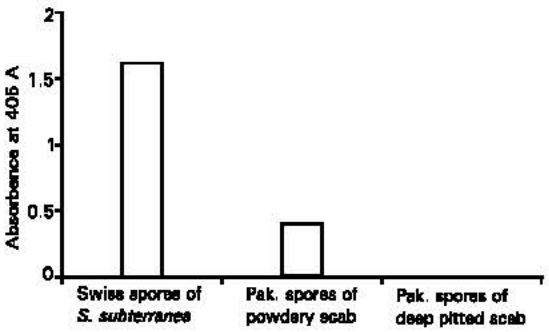


Fig. 6: ELISA reaction of Swiss spores, of *S. subterranea* Pakistani powdery scab and deep-pitted spores against *Spongospora subterranea* antisear

smooth surface (Fig. 4).

Bioassay: The root infectivity level on 0-4 scale was 1.9 and 1.7 of spores of Swiss isolate and spores of suspected Pakistani powdery scab respectively after the microscopic observation of epidermal cells of roots and root hairs of tomato bait plants. On the other hand, spore like bodies of deep-pitted scab did not give any indication of root infection in the form of zoosporangial formation (Fig. 5).

ELISA: Plate-trapped antigen of ELISA gave A_{405} value 2 of Swiss spores and 0.5 of spores of Pakistani isolate of *S. subterranea* against antiserum of *S. subterranea*. Spores of deep-pitted lesions did not give any reaction against that antiserum (Fig. 6).

Discussion

Identity of *Spongospora subterranea* was ambiguous since its first report of Turkensteen (1987). Current study characterized the pathogen as *Spongospora subterranea* by using all tools of identification including light and electron microscopy, bioassay and serology. The light microscopy of spore structure of suspected powdery scab showed honeycomb like structure resemble to spore balls of *S. subterranea* as reported by Osborne (1911), Kole (1854) and Lahert and Kavanagh (1985). While the spores of deep-pitted scab showed dark colored structure with smooth surface. Scanning electron microscopy (SEM) showed that spore ball or cystosorus consist of an outer shell of cyst in which cavities are present with an inner network of channels lined with cysts as reported by Lahert and Kavanagh (1985). The honeycomb like structure of spore ball was quite different from smooth walled spores of deep-pitted scab by scanning electron microscopy, which conclude that deep-pitted type of lesions are not related to powdery scab as previously described by Turkensteen (1987). The morphological resemblance of spore ball of Pakistani origin with spore ball (*S. subterranea*) of Swiss origin also confirms the spores of Pakistani origin as *S. subterranea*. Which is further supported by the results of bioassay and serological tests. The spores of *S. subterranea* of Pakistani origin gave root infection in tomato plants during bioassay test and also gave reaction to antiserum of *S. subterranea* in serological test. While the spore like bodies of deep-pitted lesions did not give any indication for its relation to *S. subterranea*. This type of deep-pitted lesions on tuber surface is related to common scab as described in literature and is generally assumed that the causal agent is related to species of *Streptomyces*. So current study confirms the presence of two types of scabs; powdery and deep-pitted scab, which apparently resemble to each other but have different causal organisms.

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