

INFECTION OF *ALBUGO CANDIDA* (PERS. EX LEV.) KUNZE IN MUSTARD SEEDS

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Seeds of mustard (*Brassica juncea* Coss.) naturally infected with *Albugo candida* (Pers. ex Lev.) Kunze and carrying an oospore load of 8700 spores/g were categorised and studied. Cleared wholemount preparations and microtome sections revealed the fungal mycelium in seed coat of 4 and 10 per cent bold-symptomless seeds whereas in 100 and 100 per cent symptomatic seeds, respectively. In bold white-crusteds seeds, the mycelium was observed in seed coat and endosperm. Bold-discoloured seeds with white mycelium and oospores and shrivelled-discoloured seeds carried the mycelium in seed coat, endosperm and cotyledons but the oospores in seed coat only.

Keywords: *Albugo candida*; Histopathology; Mustard seed.

Introduction

White rust caused by *Albugo candida* (Pers. ex Lev.) Kunze is an important disease of crucifers including rape and mustard occurring throughout India¹⁻³. The oospores of *A. candida* have been detected in seed washings in *Brassica campestris* and *B. juncea*⁴⁻⁶. Although the histopathology of hypertrophied inflorescence and stem has been investigated¹, there is no study on *A. candida* infected seeds.

Materials and Methods

Seed sample (no: 2918, Cv. RL-18) of mustard (*Brassica juncea* Coss) naturally infected with *Albugo candida* carrying a heavy oospore load of 8700 oospores/g in seed washing test was selected. The seeds were categorised on the basis of dry seed examination. Each category was handled separately using clearing and wholemount preparations⁷ and microtome sectioning⁸. In the former, 50 seeds per category and in the latter 10 seeds per category were used. Microtome sections were stained with safranin - fast green combination and some with cotton blue.

Results and Discussion

Dry Seed Examination:- The percentage of seeds of different categories, namely (I) bold-symptomless, (II) bold white-crusteds (Fig. 1A), (III) bold-discoloured with white mycelium and oospores and (IV) shrivelled - discoloured seeds was 88, 5, 5 and 2 respectively. Symptomless and white-crusteds seeds did not differ in size but the crusted region was raised, looking like blisters. The seeds in other two categories were smaller than symptomless seeds.

Structure of Seed :- Seeds of mustard are spherical, reddish brown to black, with marked reticulations and minute stipples. Anatomically, the seed comprises seed coat, endosperm and a curved embryo with two conduplicate cotyledons and an embryal axis (Figs. 1F, 2A). The seed coat consists of flattened epidermis, subepidermis, palisade layer of thick-walled pigmented cells of unequal height and layer of compressed parenchyma. The outer two layers are not very distinct at maturity. Endosperm is 1-layered⁹.

Cleared Wholemout Preparations :-

Epidermis and sub-epidermis were isolated as one unit. Bold - symptomless seeds revealed broad, coenocytic, branched and intercellular mycelium of *Albugo candida* in seed epidermis and subepidermis in 4% seeds only. But, similar mycelium as well as oospores of the pathogen were observed in this zone in 100% seeds of all the categories of symptomatic seeds (Fig. 1B, C). Different stages of oospore development were also observed in these preparations. In symptomatic seeds, fungal mycelium also invaded palisade layer (Fig. 1D) in 26, 44 and 56 per cent and endosperm in 10, 18 and 42 per cent seeds of II, III and IV categories respectively. 8 and 18 per cent embryonal infection (Fig. 1E) was observed in the last two categories respectively.

Microtome Section :- In bold - symptomless seeds only 1 out of 10 seeds revealed mycelium in seed coat whereas in other categories, it was universally present (Figs. 1G-I, K, 2B-D). Infection caused slight (Figs. 1G, 2B) to conspicuous increase in parenchyma layers (Fig. 1H). The increase was maximum in seeds with white mycelium and oospores (category III) becoming upto 8 layers while in uninfected seeds, there are only 2 layers. The cells also showed hypertrophy (Figs. 1H, I, 2C).

Different stages from unfertilized oogonia, antheridia (Fig. 1J) to mature oospores were observed in inter-cellular spaces. The palisade layer had weak thickening particularly of the radial walls in sections of symptomatic seeds of all categories (Figs. 1G-1, 2B-D). No infection could be observed in the compressed parenchyma layer in any category.

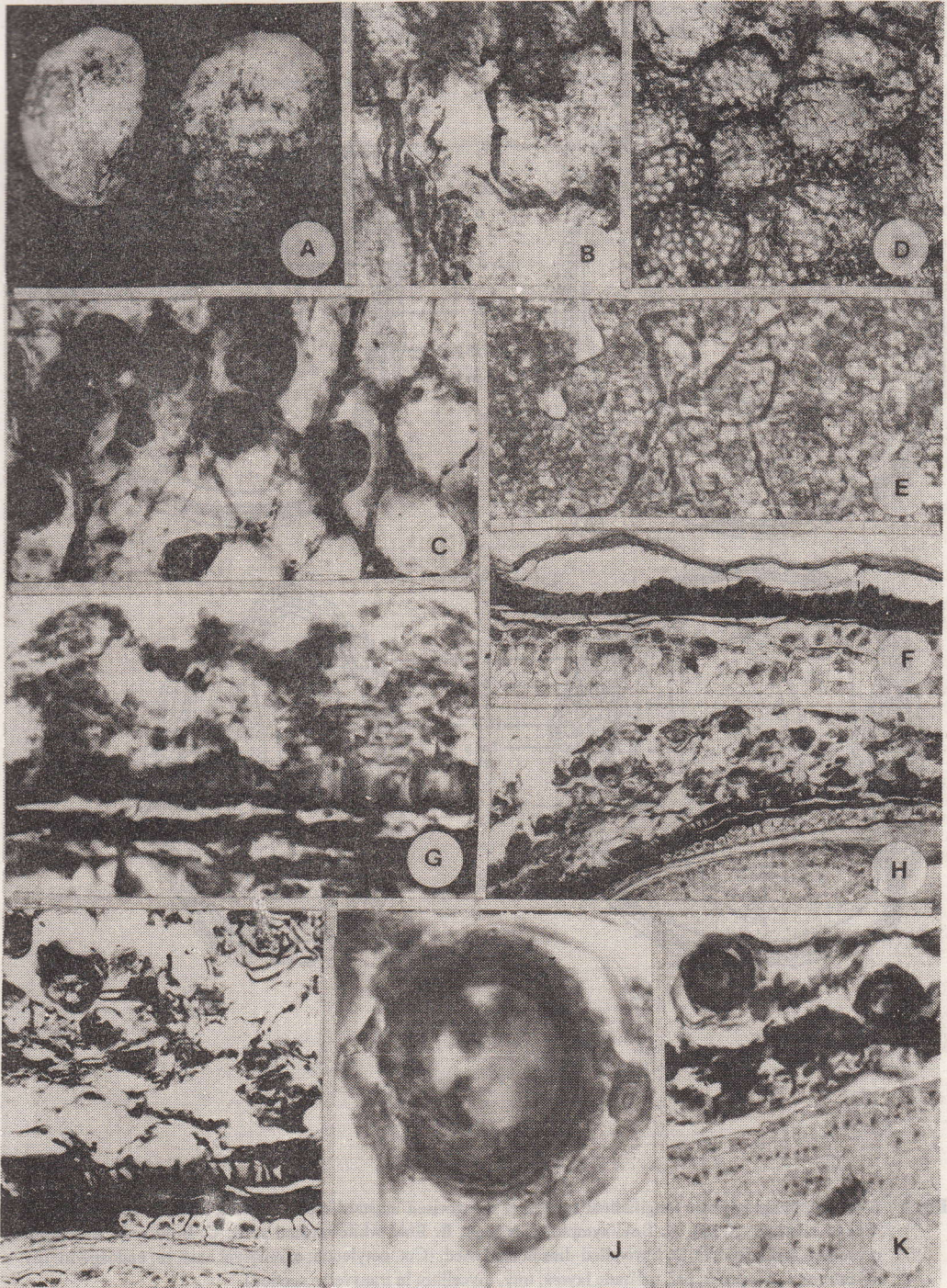
The fungus occasionally invaded endosperm and embryo. In endosperm, bits of mycelium could be observed in 1, 3 and 4 seeds in II, III and IV categories respectively. Embryonal infection was recorded in 1 and 2 seeds in the III and IV categories only in the peripheral layers.

This report regarding mycelial and oospore infection of *A. candida* in mustard seeds is interesting. Similar infection of *Albugo bliti* was observed in seeds coat of *Amaranthus retroflexus*¹⁰. It will be of interest to determine the period of survival of this infection in mustard seeds and its role in transmission of the disease, particularly in light of recent reports⁵ about short survival period of soil-borne oospores of *Albugo candida*.

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Fig.1A-K: Histopathology of *A. candida* infected mustard seeds. **A.** White-crusted seeds (X 15). **B-E.** Cleared wholemout preparations of seed components showing mycelium and oospores of *A. candida*. **B, C.** Epidermis (X 125), **D.** Palisade layer (X 125), **E** Cotyledon (X 250). **F-K.** Microtome longitudinal sections of categorised seeds showing mycelium and oospores. **F.** Normal seed coat, endosperm and embryo of bold-symptomless seed (X 125); **G.** White-crusted seed showing mycelium in seed coat, enlarged cells of epidermis and subepidermis and weakened radial thickening of palisade cells (X 125); **H, I.** Seeds with white mycelium and oospores showing increased number of parenchyma layers of seed coat (X 50 and 125 respectively); **J.** Mature seed to show oogonium and antheridium in seed coat (X 500); **K.** Shrivelled-discoloured seed showing oospores and mycelium in seed coat (X 125).



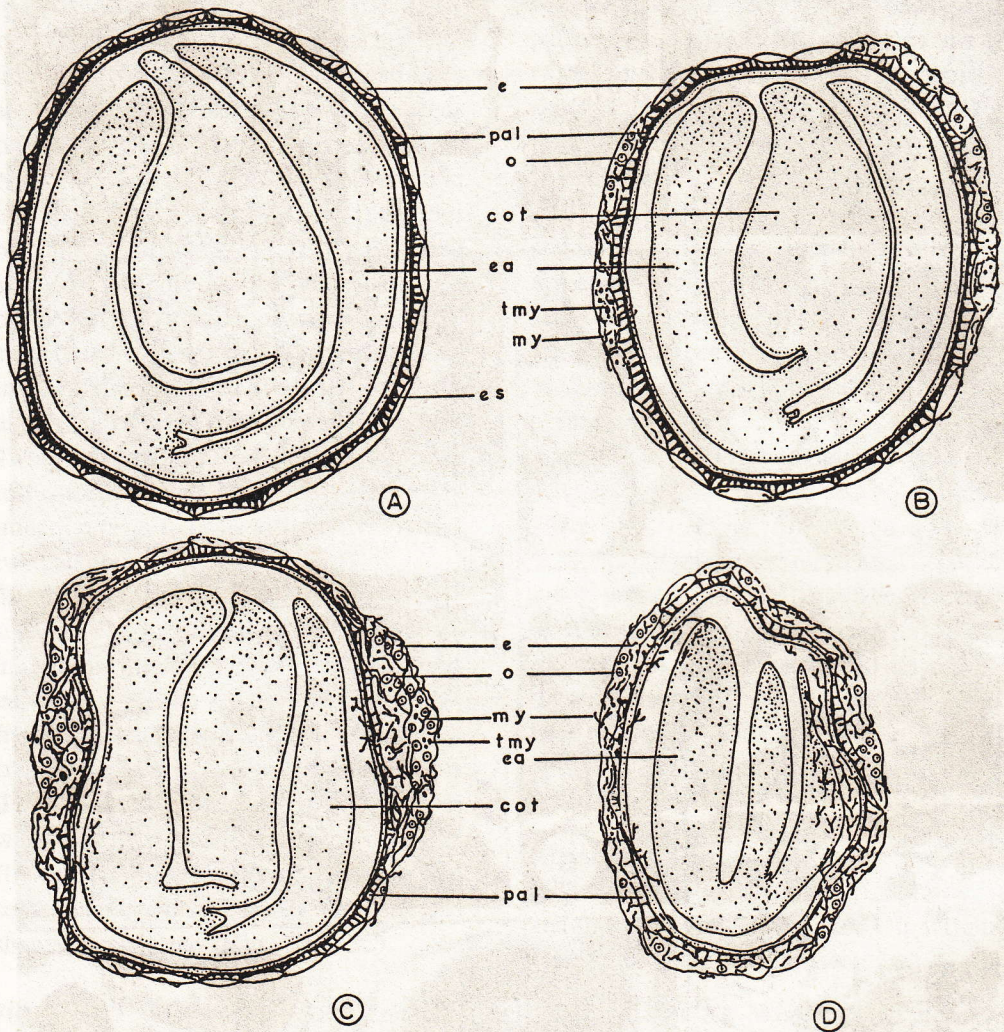


Fig. 2 A-D: Semidiagrammatic longitudinal section of categorised mustard seeds showing location and expanse of *Albugo candida* (X 23). A. Bold-symptomless seed; B. Bold white-crusted seed; C. Bold seed with white mycelium and oospores; D. Shrivelled-discoloured seed. (Cot, cotyledon; e, embryal axis; es, endosperm; my, mycelium; o, oospores; pal, palisade layers; tmy, mycelium in transverse section).

References

1. Vasudeva R S 1958, *Rapeseed and Mustard*; 77-81 Ed. D P Singh (Hyderabad, India Oilseed Committee).
2. Bilgrami K S, Jamaluddin and Rizwi M 1979, *Fungi of India*; (New Delhi, India : Today & Tomorrow's Printers).
3. Anonymous 1988, *Technology for maximising production of rapeseed - mustard in Rajasthan*. (Hyderabad, India : Ex. Bull. V : ICAR Publication, Directorate of Oilseeds Research).
4. Petrie G A 1975, *Can. Plant Dis. Surv.* 55 19
5. Verma U and Bhowmik T P 1988, *Int. Tropical Plant Dis.* 6 265
6. Sharma J, Agrawal K and Singh D 1990, *J. Indian Bot. Soc.* 69 197
7. Singh D, Mathur S B and Neergaard P 1977, *Seed Sci. & Technol.* 8 85
8. Johansen DA 1970, *Plant Microtechnique*; (New York, U. S. A. : McGraw Hill).
9. Vaughan J G 1970, *The structure and utilization of oil seeds*; (London, UK : Chapman & Hall Ltd.)
10. Melhus I E 1931, *Iowa State Coll. J. Sci.* 5 185

Introduction

Presence of cadmium and mercury in the environment during germination of the seeds is considered as toxic conditions and germination is related with the concentration of heavy metal. Cadmium and mercury have been thought as an extremely hazardous to the environmental protection agency. Seedling growth was retarded by heavy metals. Heavy metals decreased the metabolic processes of the cell including respiratory and protein content. Cadmium and mercury were selected as a source of heavy metals and their effects on growth and development of cowpea seedlings were studied. Cowpea is one of the extensively cultivated pulse crops in India.

Materials and Methods

Cowpea (*Vigna sepium* L. var. Pusa Bahamra) seeds were germinated in the petriplates lined with filter paper in laboratory conditions being distilled water (D.W), CdCl_2 (5×10^{-6} M) and HgCl_2 (5×10^{-6} M) as media upto 96h. Seedlings were analyzed for their growth and biochemical changes at the intervals of 24h. Growth parameters studied were elongation of root, hypocotyl, cotyledon

and total seedling length. Fresh weight, dry weight and moisture percent of embryo and cotyledon were also recorded. Protein metabolism was studied from the embryo and cotyledon of seedlings at intervals of the interval of 24h. Parameters studied include protein activity, protein content, glutamic acid content and proline content.

Results and Discussion

Table - 1 represents the data on seedling growth of cowpea seedlings grown in different media. The elongation of root, hypocotyl and epicotyl was increased with increase in growth period. The growth rate of root and hypocotyl in metal treated seedlings was low indicating adverse effect of heavy metals on seedling growth. This supports the earlier findings (1,2). The adverse effect of heavy metals was more on root as short indicating root is more sensitive to heavy metals than shoot. Sivakki³ reviewed the effects of heavy metal toxicity on root growth. Heavy metal accumulation is more in root than in aerial parts of the plant.

Data on fresh weight, dry weight and percent moisture of embryo and cotyledon was represented in Table 1. The fresh weight