Bot. Bull. Academia Sinica 28: 123-130 (1987)

HISTOPATHOLOGY OF *ALTERNARIA TENUIS* INFECTED BLACK-POINTED KERNELS OF WHEAT

KAILASH AGRAWAL, JYOTSNA SHARMA,
TRIBHUWAN SINGH and DALBIR SINGH

Department of Botany, University of Rajasthan
Jaipur-302004, INDIA

(Received September 26, 1986; Accepted March 11, 1987)

Abstract

Two wheat seed samples which contained 41 and 23 per cent black-pointed seeds and revealed 70 and 52 per cent natural infection of *Alternaria tenuis* respectively have been studied. Seeds were categorised into 4 types on the basis of degree of discolouration. The mycelium of *A. tenuis* was confined to the pericarp of bold normal looking seeds and seeds with brown discolouration at the germinal end. In seeds with dark brown to black discolouration at the germinal end and with the discolouration extending on seed surface, the inter- and intracellular mycelium occurred in all the components of seed including embryo. The expanse of the fungal mycelium was positively correlated with the intensity of symptoms of black point. The host cells showed depletion of contents, necrosis and browning.

Key Words: wheat; black-pointed kernels; *Alternaria tenuis;* Histopathology.

Introduction

Black point disease of wheat has been reported from many parts of the world (Korobeinikova, 1975; Neergaard, 1977). In India the disease is widespread (Agarwal *et al.*, 1968; Gill and Tyagi, 1970; Dharam Vir, 1974; Kulkarni and Hegde, 1980). Dastur (1932) has reported *Helminthosporium sativum* as the causal fungus of black point. *H. sativum* and *Alternaria tenuis* have been reported to be associated with the disease by many workers (see Neergaard, 1977). However, Dharam Vir *et al.* (1968) showed that the epidemic in 1967-68 wheat crop in north-west India was mainly due to *A. tenuis* and *H. sativum*. Adam (1950), Agarwal *et al.* (1968) and Gill and Tyagi (1970) have also reported *A. tenuis* as the main cause of this disease. The fungal hyphae have been observed in the pericarp of discoloured seeds (Bose, 1923; Curzi, 1926; Bhowmik, 1969; Kulkarni and Hegde, 1980). However, no detailed account is available. A study on the seed-borne mycoflora of wheat in

Rajasthan has revealed the wide-spread occurrence of black point in all its cultivars. Isolation and identification of the causal organisms revealed that *A. tenuis* is predominant besides *Cladosporium oxysporum, Curvularia lunata, Drechslera sorokiniana* and *D. tetramera* (Agrawal, 1984). Histopathology of black-pointed seeds due to *A. tenuis* was studied and the results are described herein.

Materials and Methods

Two seed samples (accession nos. 586 and 610) which contained high incidence of black-pointed seeds and showed heavy infection of *A. tenuis* were used for the study. The samples were subjected to dry seed examination. 400 seeds per sample were examined with unaided eyes as well as under stereoscopic binocular microscope for recording the incidence of black-pointed kernels. To study the mycoflora, 200 black-pointed seeds, 100 untreated and 100 pretreated with an aqueous solution of sodium hypochlorite (2% available chlorine) for five min were tested by blotter method. 25 seeds were sown per Petri plate, incubated for seven days at 22±2°C in alternating cycles of 12 h fluorescent day light and darkness. Data on fungi associated with the seeds was made on the 8th day of sowing.

To study the histopathology of seeds, the following methods were used.

Component plating—10 seeds of each category for each of the samples made in dry seed inspection were washed thoroughly with distilled water in test tubes individually (one seed/tube), soaked separately for 2-3 h in water, dissected aseptically to separate the various seed components namely pericarp, aleurone layer, endosperm and embryo. Each component of the seed was surface sterilized for 5 min with aqueous solution of sodium hypochlorite and incubated by using standard blotter method. The per cent incidence of the pathogen on various seed components was recorded on the 8th day of incubation.

Clearing and wholemount preparations—10 seeds from each category in both the samples, were boiled individually in 5% aqueous KOH solution for 3-4 min, the pericarp was peeled-off and the embryo was dissected out. The remaining part was again boiled for 5-7 min to separate the aleurone layer from the endosperm. The components thus separated, were washed thoroughly with distilled water to remove the alkali. Pericarp and aleurone layer were cleared in lactophenol for 5-10 min; the endosperm and embryo required 12 h for clearing at 80°C. All the components were stained in trypan blue prepared in lactophenol and mounted in poly venyl alcohol (PVA).

Microtome sectioning—10 seeds from each category of both the samples were used. Seeds were first softened in distilled water at 80°C for 2 h and then fixed in 70% ethanol for 48 h. They were dehydrated through tertiary butyl alcohol (TBA) series, infiltrated and embedded in paraffin wax. The embedded material was cut

into blocks; some blocks immersed in 1% aqueous solution of sodium lauryl sulphate for 24 h. Serial microtome sections 12-15 μm thick were cut, stained with safranin 0 in methyl cellosolve and light green combination, cleared in clove oil and mounted in D.P.X. (Johansen, 1940). Some slides were also stained in trypan blue, prepared in anhydrous lactophenol and mounted in PVA.

Results

Dry Seed Examination

The incidence of black-pointed seeds in sample nos. 586 and 610 was 41 and 23 per cent, respectively. On the basis of discolouration, the seeds were categorised into (I) bold normal looking, (II) brown discolouration at the germinal end, (III) dark brown to black discolouration at the germinal end and (IV) seeds with the discolouration extended to other regions (Fig. 1). The incidence of seeds of different categories was 59.0, 17.0, 20.5 and 3.5 per cent, respectively, in sample no. 585 and 77.0, 9.5, 12.0 and 1.5 per cent in sample no. 610.

Incubation Test

The infection of *A. tenuis* in sample nos. 586 and 610 was found to be 70% and 52%, respectively. Besides *A. tenuis*, *Aspergillus flavus* (6%), *A. niger* (1%) and *Penicillium* sp. (2%) in the former and *A. flavus* (2%) and *A. niger* (5%) in the latter samples were recorded. All other fungi except *A. tenuis* were eliminated by chlorine pretreatment indicating their superficial association. *A. tenuis* occurred in 57 and 47 per cent seeds in sample 586 and 610 respectively after the pretreatment. Usually, better growth of *A. tenuis* was seen at the brush end in the bold normal looking seeds but in seeds of other categories it was conspicuous in discoloured areas.

Location and Histopathology

Component plating—Seed components on incubation yielded a high frequency of *A. tenuis* but the growth and sporulation varied. In bold normal looking seeds (category I) and seeds with brown discolouration (category II), it was recorded only on pericarp, while seeds of III and IV categories revealed infection in all the seed components (Figs. 2-5). Growth was relatively slow and sparse on pericarp and embryo but fast and dense on aleurone and endosperm. In seeds of I and II categories infection was observed only in pericarp (Table 1). In III and IV categories, the pathogen was isolated from all the components in both the samples. Aleurone, endosperm and embryo in seeds of III category had 70, 40, 30 and 40, 30, 30 per cent infection in sample nos. 586 and 610, respectively, while in VI category

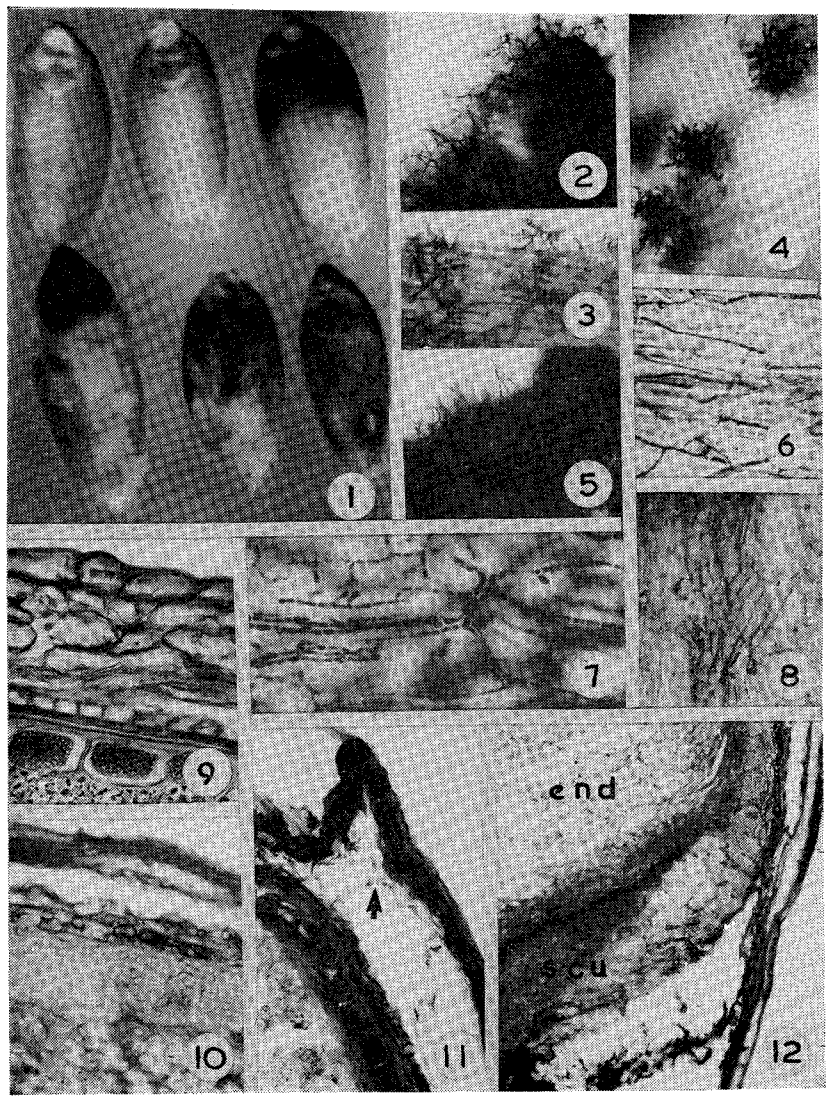

Fig. 1. Normal (upper row extreme left) and black-pointed wheat kernels. Note the variation in degree of pigmentation at the germinal end and its extension to other regions (×6).

Figs. 2-5. Growth of *Alternaria tenuis* on wheat seed components tested by standard blotter method. Fig. 2. Pericarp (×80), Fig. 3. Aleurone layer (×65), Fig. 4. Endosperm (×80) and Fig. 5. Embryo (×16).

Figs. 6-8. Wholemount preparations of the components of black-pointed wheat seeds showing septate and branched mycelium of *A. tenuis*. Fig. 6. Hypodermis of pericarp (×200), Fig. 7. Cross cell layer of pericarp (×200) and Fig. 8. Parts of embryonal tissue (×200).

Figs. 9-12. Microtome sections of black-pointed wheat seeds. Fig. 9. Part of longitudinal section of bold healthy seed showing normal pericarp, aleurone layer and endosperm (×400). Fig. 10. As above from black discoloured seed showing mycelium in epicarp, hypoderm and cross cell layer of pericarp (×200). Figs. 11, 12. As above from heavily discoloured seed. Note the mycelium in pericarp, aleurone layer, endosperm and embryo. Also note the depletion of cell contents (×400). end: endosperm; scu: scutellum.

Table 1. *Frequency of* ***Alternaria tenuis*** *in seed components of categorised black-pointed seeds (component plating) using the blotter method (10 seeds/category per sample)*

Seed categories	Seed components							
	Sample ac. no. 586				Sample ac. no. 610			
	Peri-carp	Aleu-rone	Endo-sperm	Embryo	Peri-carp	Aleu-rone	Endo-sperm	Embryo
Bold normal looking	4	—	—	—	6	—	—	—
Black-pointed:								
Brown	10	—	—	—	6	—	—	—
Dark brown to black	10	7	4	3	10	4	3	3
Discolouration extended to other regions	10	10	10	10	10	6	6	6

the infection in all these components was 100 and 60 per cent each in the two samples (Table 1).

Clearing and wholemount preparations—Out of 10 seeds, bold normal looking of each sample examined, 5 of 585 and 3 of 610 had septate, branched mycelium in the pericarp mainly confined to the brush region. In seeds of II category, the hyphae were seen in parenchymatous cells (Fig. 6) and cross cells (Fig. 7) of the pericarp of all the seeds but these could not be located in aleurone, endosperm and embryo. In seeds of III and IV categories, the hyphae frequently occurred in pericarp, aleurone layer and endosperm. Necrosis of cells and brown pigmented material along with the inter- and intracellular mycelium were observed in the discoloured pericarp. The mycelium in embryo (Fig. 8) was observed in 3 and 2 seeds out of 10 in sample no. 585 and 610, respectively.

Microtomed sections—Sections of seeds of different categories revealed the precise distribution of fungal hyphae in various seed components. Histologically, a wheat kernel consists of pericarp, seed coat, nucellar layer, endosperm and embryo (see Agrawal *et al.*, 1985). The account of distribution of mycelium in seeds belonging to four categories is given below separately for clarity:

Bold normal looking seeds: The longitudinal sections of such seeds consisted of besides pericarp a well differentiated embryo, copious endosperm and single layered aleurone (Figs. 9, 13 I). Out of 10 seeds, only in 3, a weak infection was observed in parenchymatous cells of pericarp restricted to brush end (Fig. 13 I).

Seeds with brown discolouration: Infection was common in pericarp and was mainly confined to the embryal end of the seed. Inter- and intracellular mycelium was seen in epicarp, hypoderm and cross cells (Fig. 13 II). No hyphae were located in aleurone layer, endosperm and embryo.

Seeds with black discolouration: A heavy infection was observed in the

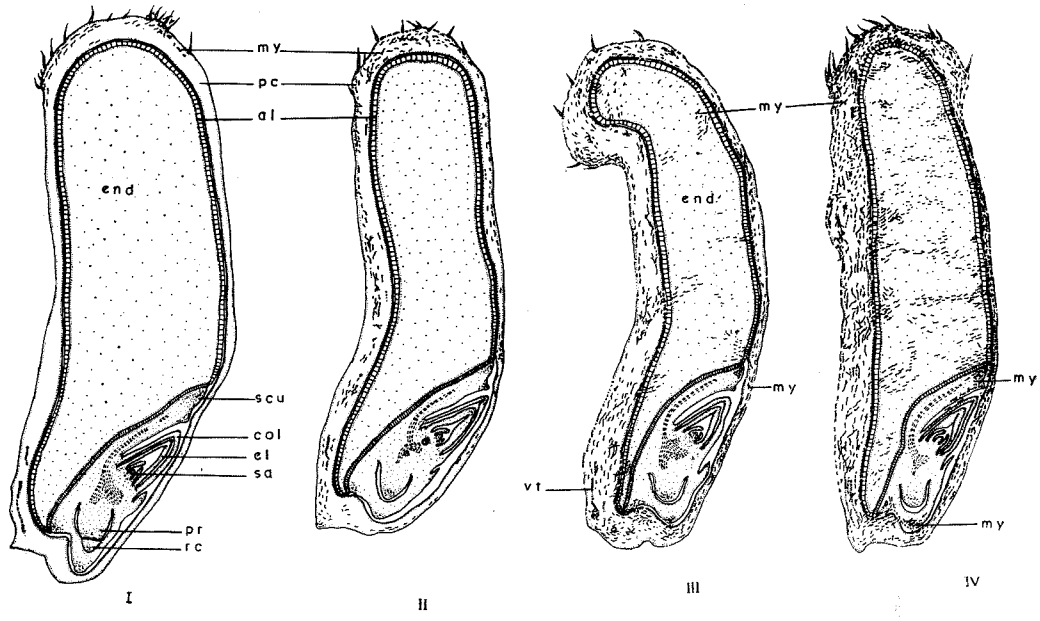

Fig. 13. Longitudinal sections (semidiagrammatic) of black-pointed wheat seeds (categories I-IV) infected with *Alternaria tenuis*. Note the increase in spread of mycelium with increased degree of infection (*al*, aleurone; *col*, coleoptile; *el*, first embryonic leaf; *end*, endosperm; *my*, mycelium; *pc*, pericarp; *pr*, primary root; *rc*, root cap; *sa*, shoot apex; *scu*, scutellum; *vt*, vascular trace). ×10.5.

pericarp (Fig. 11). Its components viz. epicarp, hypoderm, parenchyma and cross cells carried heavy aggregation of hyphae at the embryal end and showed necrosis of host cells. A weak to moderate infection was observed in aleurone layer, endosperm and embryo (Fig. 13 III). Necrosis of cells was also observed in the scutellum.

Seeds with discolouration extended to other regions: Sections of such seeds revealed the presence of fungal hyphae in all the seed components (Figs. 11, 12, 13 IV). Dense aggregation of hyphae was observed in pericarp of embryal as well as beard regions. The cells were distorted and showed severe necrosis and browning. The testa was frequently penetrated by the mycelium which occurred in abundance in the aleurone layer and starchy endosperm (Figs. 11, 12). The cells of aleurone showed depletion in aleurone grains and were filled with profusely branched hyphae. The embryo was also infected in majority of seeds. Its cells showed loss of contents and necrosis particularly in the scutellum and the coleoptile (Fig. 12).

Discussion

The histopathological investigations of the categorised *A. tenuis* infected seeds

in the present study have shown that normal looking bold seeds and seeds with brown discolouration had the mycelium confined to various layers of the pericarp. But, those with dark brown to black discolouration at the germinal end or extending on seed surface carried abundant mycelium in all components of seed. The amount of mycelium was certainly more in discoloured regions than the non-discoloured parts. These observations are partly in agreement with the studies of of Bose (1923), Curzi (1926), Rosella (1930), Prabhu and Prasad (1967), Bhowmik (1969) and Kulkarni and Hegde (1980) who observed thick septate and brown mycelium in the pericarp of black-pointed seeds. However, Bhowmik (1969) stated that the mycelium neither invaded nor discoloured the embryo. Contrary to this, Kulkarni and Hegde (1980) although failed to observe the mycelium in the embryo, did record its discolouration. The present study has very well demonstrated that the expanse of mycelium is positively correlated with the intensity of symptoms of black point. Heavily infected seeds carried the mycelium in all its components including embryo.

Acknowledgements

University Grants Commission, New Delhi, India is duly acknowledged for providing the senior researh fellowship to the senior author (K.A.).

Literature Cited

Adam, D.B. 1950. Black point and smudge in wheat. J. Dep. Agric. Aust. **54**: 76-77.

Agarwal, N.S., S.P. Duggal, and A. S. Yadav. 1968. Black tipped wheat kernels in the indigenously grown mexican varieties of wheat. Bull. Grain Technol. **6**: 113-116.

Agrawal, K. 1984. Studies on seed-borne mycoflora and some important seed-borne diseases in wheat cultivars grown in Rajasthan. Ph. D. Thesis, University of Rajasthan, Jaipur, India, 288 pp.

Agrawal, K., T. Singh, D. Singh, and S.B. Mathur. 1985. Studies on glume blotch disease of wheat I. Location of *Septoria nodorum* in seed. Phytomorphology **35**: 87-94.

Bhowmik, T.P. 1969. Alternaria seed infection of wheat. Pl. Dis. Reptr. **53**: 77-80.

Bose, S.R. 1923. *Alternaria* within the pericarp of the wheat seed. Nature **178**: 640-641.

Curzi, M. 1926. La "Puntatura" della cariossidi di frumento e Una nuova specie di Alternaria. Riv. Pat. Veg. **16**: 125-136.

Dastur, J.F. 1932. Foot rot and black point disease of wheat in the Central Provinces. Agri. Livestk., India **2**: 275-282.

Dharam Vir. 1974. Study of some problems associated with post harvest fungal spoilage of seeds and grains. Current trends in Plant Pathology, Lucknow Univ., Botany Dept., India, pp. 221-226.

Dharam Vir, K. L. Adalkha, L.M. Joshi, and F.D. Pathak. 1968. Preliminary note on the occurrence of black point disease of wheat in India. Indian Phytopath. **21**: 234-235.

Gill, K.S. and P.D. Tyagi. 1970. Studies on some aspects of black point disease in wheat. J. Res. Ludhiana **7**: 610-617.

Johansen, D. A. 1940. Plant Microtechnique. McGraw Hill Co., New York.

Korobeinikova, A.V. 1975. Vredonoschost al'ternarioza semyan pschenitsy. Zashchita Rastenii **5**: 23.

Kulkarni, S. and R.K. Hegde. 1980. Black point disease of wheat in karnataka. Current Research 9: 33.

Neergaard, P. 1977. Seed Pathology. The MacMillan Press Ltd., London.

Prabhu, A.S. and R. Prasad. 1967. Evaluation of seed infection caused by *Alternaria triticina* in wheat. Proc. Int. Seed Test. Assoc. 32: 647-654.

Rosella, E. 1930. Surune moucheture de l'Orge Rev. Path. Veğ. et Ent. Agr. 17: 345-348.

Alternaria tenuis 感染黑斑小麥穀粒的組織病理學

K. AGRAWAL, J. SHARMA, T. SINGH and D. SINGH

Department of Botany, University of Rajasthan, India

以兩種小麥種子爲樣本，分別觀察到，有41和23%爲具有黑斑的種子，且顯示出70和52%的種子有自然感染 *Alternaria tenuis* 的情形。以種子色變的程度，將其區分爲四種類型。正常厚皮外觀的種子可將 *A. tenuis* 的菌絲局限在果皮的部分，且僅在其有胚芽的一端有褐變的現象。如果種子胚芽端出現暗褐到黑的色變現象，且色變範圍擴展到種子表面，則細胞間與細胞內的菌絲將發生在種子的所有部位，包括胚。眞菌菌絲的擴展與黑斑的密度呈正相關，寄主細胞將出現內容物的消耗，壞疽和褐化。