



Incidence of Black Point on Wheat Seeds in Different Location of Uttarakhand, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

An experiment was conducted to determine the incidence of black point disease in wheat varieties with different locations caused by *A. alternata* fungal pathogens of wheat grain. Fresh seeds of popularly cultivated wheat varieties, preferably viz., GW 366, GW 496, Lok 1, and GW 1, were collected after harvesting season from the farmers' fields of wheat growing areas of middle Uttarakhand viz., Sahaspur, Roorkee, Haridwar, and Dehradun. At least 50 samples were collected from each of the districts. Five primary samples were collected and mixed from the harvested seed lot to have composite samples. Working samples were prepared from composite samples by repeated halving method. The black point disease incidence might be due to the inherent boldness of the seeds. Florets remain more open in bold-seeded varieties, enhancing the chances of more seed infection during seed development. Moreover, low night temperatures during the flowering period, severe dew at the seed maturation stage, frequent and heavy crop irrigation, and excessive application of nitrogenous fertilizers may lead to a higher incidence of the

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black point disease. Besides, infected seeds reveal seed rot, necrosis, and shriveling. High humidity due to prolonged rainfall or heavy dew deposition during seed maturation favors the black point. As our research findings varied, GW366 is significantly the highest incidence of black point disease.

Keywords: Black point; *A. alternate*; incidence; wheat.

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) belongs to the family Gramineae. It originated in South West Asia. Wheat has been known as “King of Cereals”. It is a staple food of humans. Its value in the human diet is as a source of carbohydrates and protein. It is cultivated in almost all the subtropical and temperate regions of the world. The major growing species of wheat in India is bread wheat (*Triticum aestivum*), accounting for more than 85 percent of the total wheat area [1]. India is the second-highest producer of wheat in the world. Punjab, Haryana, Rajasthan, Uttarakhand, Uttar Pradesh and Madhya Pradesh are major wheat-producing states. It covers area about 29.8 million hectares with a total production of 103.32 million tons [2]. Haridwar, Sahaspur, and Roorkee are important wheat-growing districts of Uttarakhand. The area under wheat crop in Uttarakhand is about 0.4 million hectares with a production of 0.8 million tons [3]. Wheat plants at all growth stages are prone to the attack of numerous diseases such as black stem rust, orange rust, yellow rust, powdery mildew, yellow ear rot, ear cockle, loose smut and black point of wheat [4].

Black Point was first described in the United States in 1913 [5]. Several fungi are infected to black point disease in wheat, but in my study, the pathogen *alternaria alternate* is the main cause of black point disease. The black point disease adversely affects grain and seed quality. High humidity due to prolonged rainfall or heavy dew deposition during seed maturation favors the black point. Wet weather promotes sporulation and infection by black point-causing fungi [5]. Infection of *A. alternata* affects milling quality because it reduces grain's nitrogen, gluten, fatty acid, potassium, calcium, zinc and manganese content. Discolored seed is discounted in value. Besides, infected seeds reveal seed rot, necrosis and shriveling. High humidity due to prolonged rainfall or heavy dew deposition during seed maturation favors the black point. Wet weather promotes sporulation and infection by black point causing fungi [5]. Black point of wheat is

commonly encountered with bold wheat grain varieties under Uttarakhand conditions [6].

2. MATERIALS AND METHOD

Present investigation on studies on black point of wheat (*Triticuma estivum*L.) under middle Uttarakhand condition was under taken details of the materials and methods followed are described hereunder. The experiment was conducted at the Department of Plant Pathology, RCP College, Kishanpur, Roorkee during 2021-22 crop season. Seeds of a popular wheat varieties GW 366, GW 496, Lok 1 and GW 1 were collected after harvesting season from the farmers' fields of wheat growing areas of middle Uttarakhand and Gujarat districts viz., Sahaspur, Roorkee, Haridwar and Dehradun. Atleast 50 samples were collected from each of the districts.

2.1 Collection of Wheat Seed Samples, Incidence of Black Point Disease, Symptomatology, Assessment of Fungi Characterization and Seed Health Status

2.1.1 Collection of wheat seed samples

Fresh seeds of popularly cultivated wheat varieties preferably viz., GW 366, GW 496, Lok 1 and GW 1 were collected after harvesting season from the farmers' fields of wheat growing areas of middle Uttarakhand districts viz., Sahaspur, Roorkee, Haridwar and Dehradun. Atleast 50 samples were collected from each of the districts. Five primary samples were collected and mixed from the harvested seed lot to have composite samples. Working samples were prepared from composite samples by repeated halving method.

2.1.2 Assessment of incidence of black point disease

Fifty gram seeds from each of the working samples were critically assessed visually and magnifying hand lens, wherever required, for the incidence of black point disease by counting black point infected seeds.

2.2 Symptomatology

Infected wheat seeds collected from each of the wheat growing selected pockets were examined visually by means of magnifying lens under artificial light as well as under stereoscopic microscope. Then, symptoms observed on seeds were described accordingly.

2.3 Assessment of Infected Seed Samples in Respect of Seed Health i.e. Seed Weight, Seed Germination and Seedling Vigour

1000 wheat seed were weighted after being picked at random. The paper towel method was used to examine the germination of seeds [7]. The paper towel method was used to examine the germination of seeds. The distilled water was used to soak one sheet of germination paper. On the first sheet, fifty seeds of the appropriate treatment were distributed uniformly. After placing a second sheet of germination paper on top of the first, the sheet was carefully moistened. Both sheets were rolled along with wax coated paper. The rolled papers were incubated in seed germinator at 25°C for seven days. At the end of incubation, rolled towel papers were carefully opened. Germinated and un-germinated seeds were counted from each of the treatments. Successful germination was defined as the seed sprouting a seedling. For each of the treatments, four replications of 100 seeds were kept. Total 400 seeds each of the samples were assessed. Seedling vigour was assessed on the basis of shoot and root length and per cent seed germination using following formula [8].

$$\text{Vigour index (VI)} = (\text{Mean root length} + \text{Mean shoot length}) \times \% \text{ of seed germination}$$

2.4 Biochemical Analysis

2.4.1 Estimation of total soluble sugar by phenol-sulphuric acid method

Total soluble sugar estimation from the black point infected wheat seeds were determined by phenol-sulphuric acid method [9]. Two hundred mg of flour sample was extracted for soluble sugar in 80 per cent ethanol. One mg of extract was evaporated to dryness and dissolved with 10 ml of distilled water in 30 ml volume test tube. In a similar way, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standard glucose solution (0-100 μ) was pipette into a series of tubes.

The volume of each tube was adjusted to 1 ml with distilled water. One ml of 5 per cent phenol solution and 5 ml of H₂SO₄ were added to each tube and shaken well. Again after 10 min, the contents of the tubes were shaken and placed in an ice bath for 20 min. The absorbance was read at 490 nm. The amount of total sugar was calculated using the standard graph as per following formula.

Calculation:

$$\text{Total soluble sugar} = \text{Sample OD} \times \text{Graph factor} \times \text{Dilution factor}$$

2.4.2 Estimation of total protein by Microkjeldahl method

The standard procedure did total protein estimation from black point infected wheat seeds [10]. Forty mg. of wheat flour was taken in the glass digestion tube. Two ml. of concentrated H₂SO₄ and the digestion mixture (1:3 of CuSO₄ and K₂SO₄) were added to the digestion flask tube. Then, the tubes were transferred on electric burner for 2 hrs. to carry out the digestion at 370°C. The content of the tube became colorless after digestion. This was the indication of the completion of digestion. Then, 10 to 15 ml. of double distilled water was added to the digestion tube, followed by ten ml. of 40 percent NaOH.

As a result of which the whole solution turned black. Ten ml. of 4 per cent boric acid was taken into 250 ml. The conical flask and two drops of mixed indicator were added. Then, the digestion tube containing the solution was steam distilled in KEMPLUS machine, and ammonia was trapped in the boric acid so that the solution in the conical flask turned blue. This solution was titrated with 0.02N H₂SO₄. The percentage of nitrogen and protein were calculated using the following formula.

Calculation:

$$(\%N) = (\text{Titration reading of sample} - \text{blank}) \times \text{Normality} \times 100 \times 14.007 / \text{Sample taken (mg.)}$$

$$\% \text{ Protein} = \%N \times 6.25$$

2.5 Statistical Analysis

Data obtained from various aspects of present investigation were subjected to statistical analysis using the analysis of variance

technique [11]. The standard methods of analysis of variance for complete randomized design and transformations were used in the experiments. The test of significance among the treatments was worked out by 'F' test. The appropriate standard error (S.Em. \pm) was computed in each case. For the treatment effects, which were found significant, the critical difference (CD) at a 5 percent probability level was worked out to compare two treatment means.

3. RESULTS AND DISCUSSION

The present investigation on black point disease of wheat was undertaken at the Department of Plant Pathology, College of Agriculture, RCP College, Kishanpur, Roorkee. The results and discussion of present investigation on various aspects are presented hereunder:

3.1 Incidence of Black Point in Wheat Seeds

The results of the incidence of black point are presented in Table 1. Results revealed significant differences in the incidence of black point of wheat for each GW 496, GW 366 and Lok 1 varieties regarding area. Variety GW 496 revealed significantly the highest incidence (8.00%) of black point in Sahaspur district which was at par to Dehradun district (7.75%). However, Dehradun (7.75%) remained at par with Roorkee (7.00%) and Haridwar (6.25%) districts areas in respect to black point incidence in variety GW 496.

For variety GW 366, significantly the highest incidence of black point was in Haridwar (16.25%) followed by Sahaspur (15.25%) and Roorkee (14.50%). Variety GW 366 showed the lowest incidence (11.25%) in Dehradun district area.

With respect to variety Lok 1, the highest incidence of black point was observed in Dehradun district (13.00%). Roorkee district was next in incidence (10.75%), whereas minimum incidence was observed in Haridwar (7.00%) and Sahaspur (7.25%) district area.

Among the varieties, overall GW 366 revealed the highest mean incidence (14.31%) followed by Lok 1 (9.50%) and GW 496 (7.25%). None of the eight seed samples of variety GW 1 collected from Roorkee district revealed incidence of black point.

Relatively higher incidence of black point disease in GW 366 and Lok 1 varieties might be due the inherent boldness of the seeds. Florets remain more open in bold seeded varieties thereby enhance chances of more infection to seed during development of seeds. Moreover, low night temperature during the flowering period coupled with severe dew at the time of seed maturation stage, frequent and heavy irrigation to crop as well as excessive application of nitrogenous fertilizers may lead to the higher disease incidence of the black point disease.

Gaur [12] noted the higher infection of black point disease in the larger grains of the spike, while shrunken and small seeds remained free from infection. Weise [13] reported higher incidence of black point disease in humid environment due to rainfall at the time of seed formation, seed maturation and premature seed sense. Conner et al. [14] observed higher incidence of black point disease on wheat due to excessive application of nitrogenous fertilizers and frequent irrigation. Ellis et al. [15] found the positive relationship between black point incidence and kernel mass. Fernandez et al. [16] reported higher black point severity on the wheat kernels due to cool, wet weather conditions and frost during seed development which delayed ripening.

Table 1. Incidence of black point disease in wheat varieties

District	Disease incidence (%)*		
	GW 496	GW 366	Lok 1
Sahaspur	8.00	15.25	7.25
Roorkee	7.00	14.50	10.75
Haridwar	6.25	16.25	7.00
Dehradun	7.75	11.25	13.00
Mean	7.25	14.31	9.50
Em.	0.27	0.81	0.45
C. D. 0.05	0.83	2.49	1.37
C.V. %	7.44	11.27	9.36

* Average of four replications

3.2 Symptomatology

Critical observations of black point infected seeds of variety GW 496, GW 366 and Lok 1 collected from the farmers' fields of four districts of middle Uttarakhand and Gujarat viz., Sahaspur, Roorkee, Dehradun and Haridwar revealed following symptoms. Infected seeds revealed brownish-black discoloration in scattered manner on various parts of the seed. Seeds showed brownish-black discoloration prominently restricted to embryonic end of seed. The embryo of the seeds appeared quite shriveled. The floury endosperm of the severely infected seeds appeared brownish-black. Conspicuous brownish-black patches could be seen on the pericarp of infected seeds. Moreover, similar types of discoloration appeared on ventral crease and brush end of seeds. Observations of pericarp of severely infected seeds under the microscope revealed the presence of conspicuous fungal growth in some seeds. Flour colour of infected seeds was dull white in contrast to creamish white of healthy seeds. Earlier, similar symptoms such as discoloration on embryo, pericarp, endosperm, brush end, ventral crease tissues and shriveled embryo etc. have been described by Weise [13], Monaco et al. [17] and Sisterna and Sarandon [18]. Such visible abnormalities of wheat seeds might have been produced partly due to one or more mycotoxins produced by pathogens during the infection. Moreover, congenial temperature and humidity during flowering and seed maturation period as well as faulty application of nitrogenous fertilizers might have favoured the growth of pathogen and thereby aggravated symptomatology. Williamson [19] noted black point discoloration due to degradation of the seed coat. Further, he noted that this may be caused by increased levels of active enzymes and compounds in the seed coat that are triggered by humidity during the later stages of grain filling. Wang et al. [20] correlated severe kernel discoloration to low temperature and high rainfall during the period of grain filling.

4. CONCLUSION

The study determined the incidence of black point disease in wheat varieties with different locations caused by *A. alternata* fungal pathogens of wheat grain. The findings revealed that High humidity due to prolonged rainfall or heavy dew deposition during seed maturation favors the black point. GW366 is significantly the highest incidence of black point disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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