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

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 and Gujarat districts 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 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.

Key Words: Black point, *A. alternata*, incidence, wheat.

Introduction

Wheat (*Triticum aestivum* L.) belongs to the family Gramineae. It ~~is~~ originated in South West Asia. Wheat has been described 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 (Anon., 2019a). 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 about 29.8 million hectares ~~area~~ with a total production of 103.32 million tons (Anon., 2021). Banaskantha, Sabarkantha and Gandhinagar districts of

Gujarat, 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 (Anon., 2021a). 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 (Fakir, 1999).

Black Point was first described in the United States in 1913 (Watkins and Prentise, 1997). 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 (Watkin and Prentise, 1997). 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 (Watkin and Prentise, 1997). Black point of wheat is commonly encountered with bold wheat grain varieties under Uttarakhand conditions (Solanki et al., 2006).

Materials and Method

Present investigation on studies on black point of wheat (*Triticum aestivum* 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 form 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.

1. Collection of wheat seed samples, incidence of black point disease, symptomatology, assessment of fungi characterization and seed health status

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 form 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.

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. 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.

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 (Sahu and Agrawal, 2003). 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 250℃ 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 400seeds 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 (Thippeswamy and Lokesh, 1997).

Vigour index (VI) = (Mean root length + Mean shoot length) × % of seed germination

4. Biochemical Analysis

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 (Dubes *et al*., 1956). 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 H_2SO_4 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

Total soluble sugar = Sample OD × Graph factor × Dilution factor

4.2 Estimation of total protein by Micro-kjeldahl method

The standard procedure did total protein estimation from black point infected wheat seeds (Anon., 1965). Forty mg. of wheat flour was taken in the glass digestion tube. Two ml. of concentrated H_2SO_4 and the digestion mixture (1:3 of $CuSO_4$ and K_2SO_4) 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 to15 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 H_2SO_4. The percentage of nitrogen and protein were calculated using the following formula.

Calculation

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

% Protein= %N 6.25

5.0 Statistical analysis

Data obtained from various aspects of present investigation were subjected to statistical analysis ~~by~~ using the analysis of variance technique (Steel and Torrie, 1980). 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. ±) 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.

Result 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:

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.

Table 1: Incidence of black point disease in wheat varieties

	Disease incidence (%)*		
District		Varieties	
	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

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 (1986) 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 (1987) reported higher incidence of black point disease in humid environment due to rainfall at the time of seed formation, seed maturation and premature seed sensense. Conner *et al*. (1992) observed higher incidence of black point disease on wheat due to excessive application of nitrogenous fertilizers and frequent irrigation. Ellis et al. (1996) found the positive relationship between black point incidence and kernel mass. Fernandez *et al.* (2000) reported higher black point severity on the wheat kernels due to cool, wet weather conditions and frost during seed development which delayed ripening.

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 (1987), Monaco *et al*. (2004) and Sisterna and Sarandon (2005).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 (1997) 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*. (2002) correlated severe kernel discoloration to low temperature and high rainfall during the period of grain filling.

References:-

Anonymous (1965): A.O.A.C. Official methods of analysis of the association of official agricultural chemists. 10th Ed. Washington D.C. pp.744-745

Anonymous (2019a): Wheat: Post- harvest operations (FAO).www.fao.org/es/ess/top/country.html

Anonymous (2021): Wheat production data 2021. *www.dnaindia.com/india/report_wheat-productioexpected-toreach-all-time-high-in-2021*

Anonymous (2021a): JAU experts advise to wheat growers to hold produce. *www.blackseagrain.net/photo/india.-jau-experts-advise-wheat-growers- to-hold-produce*

Conner, R.L., Carefoot, J.M., Bole, J.M. and Kozub, G.C. (1992). The effect of nitrogen fertilizer and irrigation on black point incidence in soft white spring wheat. *Plant Soil*, **140**: 41-47.

Dubes M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Colurimetric methods of determination of sugars and related substances. *Anal. Chem.,* **28**: 350

Ellis, S.A., Gooding, M.J. and Thompson, A.J. (1996). Factors influencing the relative susceptibility of wheat cultivars toblack point. *Crop Prot*., **15**: 69-76.

Fakir G.A. (1999). Seed health: an indespensible agro-techonlogy for crop production. Lecture note for course on Agro-technology and Environment Management for the CARITAS officers at GTI, BAU, Mymensingh, June 21-30, pp.1-4.

Fernandez, M.R., Clarke, J.M., DePauw, R.M., Irvine, R.B. and Knox, R.E. (2000). Black point reaction of duram and common wheat cultivars grown under irrigation in southern Saskatchewan. *Plant Dis.*, **84**: 892-894.

Gaur, A. (1986). Black point of wheat. In: *Problems and progress of wheat pathology in South Asia*. (Eds. Joshi, L.M., Singh, D.V. and Srivastava, K.D.), Malhotra publishing house, New Delhi. pp. 230-241.

Monaco, C., Sistema, M., Perella, A. and Bello, G.D. (2004). Preliminary studies on biological control of the black point complex of wheat in Argentina. *World J. Micro. Biotechn.*,**20**: 285-290

Sahu, R.K and Agrawal, V.K. (2003). Effcect of fungicidal seed treatment on seed borne fungi germination and seedling vigour of rusty shield barear. (*Peltophorum terruineum*Dence benth). *J. Mycol. Pl. Pathol.,* **33**: 84-88.

Sisterna, M.N. and Sarandon, S.J. (2005). Preliminary studies on the natural incidence of wheat black point under different fertilization levels and tillage systems in Argentina. *PlantPathology Journal*, **4**: 26-28

Solanki, V.A., Augustine, N. and Patel, A.A. (2006). The impact of black point on wheat trade and its management. *Indian Phytopath.*, **59**: 44-47.

Steel, R.G.D. and Torrie, J. H. (1980). Principles and procedures of statistics. 2nd Ed. MacGraw Hill Book Co., New Delhi.

Thippeswamy, T. and Lokesh, S. (1997). Effects of leaf extracts on seed mycoflora germination and seedling vigour ofsunflower. *Int. J. Tropical Plant Diseases*, **15**: 53-58. Tisserat, N., M. Burrow and R. Koski. (2007). Black point diseaseof wheat. *Can. J. of Plant Pathol*., **29**: 284-289.

Wang, H., Fernandez, M.R., McCaig, T.N., Gan, Y.T., DePauw, R.M. and Clarke, J.M. (2003). Kernel discoloration and downgrading in spring wheat varieties in western Canada.*Can. J. Plant Pathol.,* **25**: 350-361.

Watkins, J.E. and L.J. Prentise. (1997). Black point disease of wheat. Neb Guide. Publ. Institute of Agriculture and Natural Resources, Lincoln Extn., Univ of Nebraska, U.S.A.

Weise, M.V. (1987). Compendium of wheat diseases, 2nd Ed., APS Press, St. Paul, MN, USA. p.112.

Williamson, P.M. (1997). Black point of wheat: *in vitro* production of symptoms, enzymes involved, and association with *Alternaria alternata. Aust. J. Agric. Res.*, **48**: 13-19.

Yadav, P., Kumar, D., Patni, V., Arora, D.K. and Kant, U. (2004). Localization of metabolites in blight and wilt affected seeds of cumin (*Cuminum cyminum*). *J.Mycol. Pl. Pathol.,* **34**:188-193.