Turk J Agric For
32 (2008) 267-272

The Relationship between Black Point and Fungi Species and Effects of Black Point on Seed Germination Properties in Bread Wheat

Faruk TOKLU[1,*], Davut Soner AKGÜL[2], Mehmet BİÇİCİ[2], Tolga KARAKÖY[3]

[1]Çukurova University, Vocational School of Kozan, Department of Technical Programmes, Seed Science Programme, 01500 Kozan, Adana - TURKEY

[2]Çukurova University, Faculty of Agriculture, Department of Plant Protection, 01330 Adana - TURKEY

[3]Çukurova Agricultural Research Institute, Doğankent, Adana - TURKEY

Received: 26.11.2007

Abstract: This study was undertaken to investigate the relationship between some fungi species and black point incidence and the effect of black point on seed weight, germination percentage, seedling emergence, seedling establishment, number of embryonic roots, and coleoptile length under field conditions in bread wheat. In this research, black-pointed and black point-free kernel samples of 5 bread wheat cultivars, namely Ceyhan-99, Doğankent-1, Yüreğir-89, Seyhan-95, and Adana-99 - commonly grown under the agroclimatic conditions of Çukurova Region, were used. Isolations from the black point-affected and black point-free kernels indicated that *Alternaria* spp. are the predominant fungi, followed by *Chaetomium* sp and *Aspergillus niger*. *Epicoccum* sp and *Sclerotium* sp were of minor importance. Discoloured kernels were heavier than normal ones. Germination percentage, seedling emergence, and seedling establishment under field conditions were reduced by the black pointed seeds, whereas number of embryonic roots and coleoptile length were not affected by black point.

Key Words: Bread wheat, black point, fungi, germination

Ekmeklik Buğdayda Embriyo Kararması ile Bazı Fungus Türleri Arasındaki İlişki ve Embriyo Kararmasının Tohumun Çimlenme Özelliklerine Olan Etkisi

Özet: Bu araştırma, ekmeklik buğdayda ortaya çıkan embriyo kararması ile bazı fungus türleri arasındaki ilişkilerin belirlenmesi ve embriyo kararmasının tohum ağırlığı, çimlenme oranı, sürme gücü, tarla koşullarında çıkış oranı, çim kökü sayısı ve koleoptil uzunluğuna etkisinin belirlenmesi amacıyla yürütülmüştür. Araştırmada; Çukurova koşullarında yaygın olarak yetiştirilen Ceyhan-99, Doğankent-1, Yüreğir-89, Seyhan-95 ve Adana-99 ekmeklik buğday çeşitlerinin embriyo kararmalı ve embriyo kararmasız tohumları kullanılmıştır. Embriyo kararmalı ve kararmasız tohum numunelerinden yapılan izolasyonlar; en yüksek fungal koloni sayısının *Alternaria* spp.'ye ait olduğunu, bunu *Chaetomium* sp. ve *Aspergillus niger* fungus türlerinin izlediğini ve en düşük fungal koloni sayısının *Epicoccum* sp. ve *Sclerotium* sp.'de bulunduğunu göstermiştir. Embriyo kararmalı tohumların ağırlığı, kararmasızlara göre daha yüksek saptanmıştır. Embriyo kararması, çimlenme oranı, sürme gücü ve tarla koşullarında çıkış oranında düşüşe neden olurken, embriyonal kök sayısı ve koleoptil uzunluğu üzerinde herhangi bir etkisi saptanmamıştır.

Anahtar Sözcükler: Ekmeklik buğday, embriyo kararması, fungus, çimlenme

Introduction

Black point is common in all wheat growing regions of the world (Lorenz, 1986) and characterized by a dark discoloration of the embryo sides of the wheat and barley grains (Mak et al., 2006). Diseased kernels are discolored, and are black at the ends of the seed. Embryos are often shrivelled and brown to black in color. When seed moisture content exceeds 20%, coupled with the relative humidity above 90%, the amount of black point increases dramatically. Diseased plants are often uniform in the fields or are associated with wetter, more humid areas of the field. Seed with black point is more likely to have seedling blight and root rot problems. In most countries where cereals are grown commonly, black point can result

* Correspondence to: fapet@cu.edu.tr

in reduced grain quality and value (Wang et al., 2003). The disease can affect grain quality since food products made from infected kernels have displeasing odor and color. Some fungi, such as *Alternaria* spp., *Aspergillus*, *Fusarium* spp., and *Penicillium,* which cause black point on wheat kernels, may produce toxic substance. Grains with black point symptoms are more difficult to market than clean grains. Marketing authorities have determined the upper limit as 5% for seeds in silos in Australia (Rees et al., 1984; Lehmensiek et al., 2004).

In previous studies, although more than 100 fungi species could have been obtained from wheat seeds, it has been reported that *Alternaria, Aspergillus, Chaetomium, Fusarium, Helminthosporium, Myrothecium, Nigrospora, Penicillium, Phoma, Rhizopus,* and *Stemphylium* species have usually been isolated from black pointed seeds (Hosford, 1975; Martin and Gilman, 1976; Agarwal et al., 1983; Rees et al., 1984; Conner and Kuzyk, 1988; Conner and Whelan, 1989; Sisterna and Sarandon, 2005). Biçici and Çınar (1988) stated that high rates of black point symptoms in the embriyo and endosperm of kernel samples were determined and *Phoma, Alternaria, Fusarium,* and *Epicoccum* were isolated from the wheat kernels and *Phoma* comprised 90% of these genera in Çukurova Region. Fungal infection severity is associated cultivar characteristics and environmental conditions (Gooding et al., 1993). The environment had a major impact on the incidence of black point. Low temperature and high rainfall during the period of grain filling resulted in severe kernel discoloration (Wang et al., 2002).

Numerous other studies indicated that black point is not associated with any particular fungus but may be a result of abiotic stress conditions such as heavy rain, high humidity, and extremes of temperature during the grain filling period (Conner, 1989; Kumar et al., 2002; Clarke et al., 2004; Sadasivaiah et al., 2004). Black point discoloration may cause the degradation of seed coat, which could be explained by increased levels of active enzymes and compounds in the seed coat triggered by humidity during the grain filling period. Peroxidase catalyses the oxidation of phenolic compounds (Williamson, 1997).

Previous studies showed that black point reduced the germination rate, number of embryonic roots, and coleoptile length (Toklu et al., 1999) and delayed seedling emergence and reduced seedling vigour (Özer, 2005).

The objective of the current study was to investigate the relationship between fungi species and black point incidence and to determine the effect of black point on seed weight, germination percentage, seedling emergence, number of embryonic roots, coleoptile length and seedling establishment under field conditions for some commercial bread wheat cultivars grown in Çukurova Region.

Materials and Methods

This study was carried out in laboratory and the field conditions in the Vocational School of Kozan and Plant Protection Department in the Faculty of Agriculture at Çukurova University during the growing season of 2005-2006. The kernels of 5 bread wheat cultivars, Adana-99, Ceyhan-99, Doğankent-1, Seyhan-95, and Yüreğir-89 - commonly grown in the Çukurova Region, were used as research material in this study.

The wheat samples, harvested from regional yield trial in 2004-2005 growing season, were provided from the Çukurova Agricultural Research Institute. Harvested seed samples were stored at room temperature with 13%-15% humidity until the research period. Meteorological data during the grain filling period at Adana presented in Table 1. Black-pointed and black point-free kernel

Table 1. Monthly minimum, maximum, and mean temperatures (°C), precipitation (mm), and relative humidity (%) for grain filling period in Adana.

Location/Year	Temperature (°C)			Precipitation (mm)	Humidity (%)
	Min.	Max.	Mean		
Adana/2005					
April	12.6	23.3	17.5	53.0	68.7
May	15.8	27.3	22.0	41.1	67.8
June	19.3	29.6	25.7	16.1	72.7

samples of each cultivar were used in the laboratory and field studies. All kernels were surface sterilized with 1% sodium hypochloride (NaOCl) solution for 3 min, followed by several rinses in sterile distilled water and dried on filter paper. Agar-plate method was used to isolate the fungi species from the seed samples. Surface sterilized kernel samples were placed into the petri plates (9 cm diameter) containing potato dextrose agar (PDA). Five petri plates containing 7 kernels in each petri were repeated 3 times. All petri plates were incubated at 25 °C for 21 days in darkness and then the growing fungal colonies on the PDA plates were examined under dissecting and compound microscope. The fungi were subcultured on PDA petri plates, and identified based on cultural characteristics and colony morphologies.

Standard germination procedure was applied to the black-pointed and black point-free kernel samples according to ISTA rules. For the germination tests, kernel samples were placed into the petri dishes with 25 seeds per each with 4 replications in the germination cabinet at 20 °C temperature. Germination percentage was calculated 8 days after sowing (DAS).

Seedling establishment tests were conducted in the growth chamber, which is light, humidity, and temperature controlled. The sand used for seedling establishment was sieved using 2 mm openings. The kernels were surface sterilized in 1% NaOCl solution for once and black-pointed and black point-free 20 kernels from each cultivars were sown to plastic bags (20 cm × 10 cm × 15 cm) containing sand. Then they were placed into the growth chamber at 20 °C with 60% humidity and daylight of 13 h. Percentage of seedling emergence was calculated 14 DAS, by counting the established seedlings. Germination and seedling establishment tests were arranged according to completely randomized design with 4 replications.

Black-pointed and black point-free kernels of 5 bread wheat cultivars were also grown to obtain seedling establishment under field conditions. Field trial was laid out in a randomized completely block design with 3 replications. Each plot consisted of 5 cm apart 4 rows containing 18 plants with 5 cm plant to plant distance. All other standard practices were kept same during the period of germination. After the germination, seedling emergence rate was calculated by counting the number of seedling from both black-pointed and black point-free plots in the field conditions.

Means of black-pointed and black point-free kernels of each cultivar were compared by Student's t test ($P = 0.05$).

Results

Alternaria, Aspergillus, Chaetomium, Epicoccum, and *Sclerotium* species were isolated from discoloured and normal wheat seeds plated on PDA (Table 2). *Alternaria* sp. was the most predominant fungus species in both black-pointed and black point-free kernel samples. *Aspergillus, Chaetomium Epicoccum,* and *Sclerotium* were observed at a low rate.

Table 2. Mean numbers of fungal colonies in black-pointed and black point-free kernel samples.

Cultivars	Alternaria spp.		Aspergillus niger		Chaetomium sp.		Epicoccum sp.		Sclerotium sp.	
	BP	BPF	BP	BPF	BP	BPF	BP	BPF	BP	BPF
Adana-99	17.8 a*	13.6 b	0.4	1.2	1.8	2.4	0.0	0.0	0.0	0.0
Ceyhan-99	16.2	14.0	0.0	1.8	3.2	2.6	0.2	0.6	0.2	0.0
Doğankent-1	16.0 a	10.6 b	0.6	1.0	3.0	3.4	0.0	0.4	0.0	0.0
Seyhan-95	15.2	11.6	0.6	1.8	3.4	2.8	0.2	0.2	0.0	0.2
Yüreğir-89	14.6	12.0	3.6	2.6	2.0	2.2	0.2	0.0	0.0	0.0
Mean	16.0 a	12.4 b	1.0	1.7	2.7	2.7	0.1	0.2	0.0	0.0

BP: Black pointed kernels, BPF: Black point-free kernels

*: Values followed by the same letter do not differ significantly between discoloured and normal kernel samples of each cultivar at 0.05 probability level.

Numbers of fungal colonies were not significantly different isolated from both black-pointed and black point-free kernel samples except *Alternaria* spp. A higher level of *Alternaria* spp. was observed in discoloured kernels, compared to the normal ones.

Mean temperature, precipitation and humidity values are convenient to the development of seed-borne fungus species for the effective grain filling period in April and May (Table 1). Black-pointed seed percentage for the Adana-99, Ceyhan-99, Doğankent-1, Seyhan-95, and Yüreğir-89 cultivars were found to be 10%, 6%, 7%, 3% and 5%, respectively.

Significant differences were found between discoloured and healthy kernel samples based on the means of all cultivars for 1000-kernel weight, germination percentage, and seedling emergence, while there was no significant difference based on the number of embryonic roots and coleoptile length (Table 3). Black point reduced the seedling establishment under the field conditions in some cultivars (Figure 1).

In all cultivars, discoloured grains were heavier than the normal ones (Table 3). Germination percentage was affected negatively by black point in all cultivars. Reduced seedling emergence was detected for the discoloured kernels in all bread wheat cultivars except Yüreğir-89. Seedling establishment in the field conditions was not significant according to the means of all cultivars; however, on the cultivar basis, a negative effect of black point was observed. Numbers of embryonic root and coleoptile length were not affected by black point.

Discussion

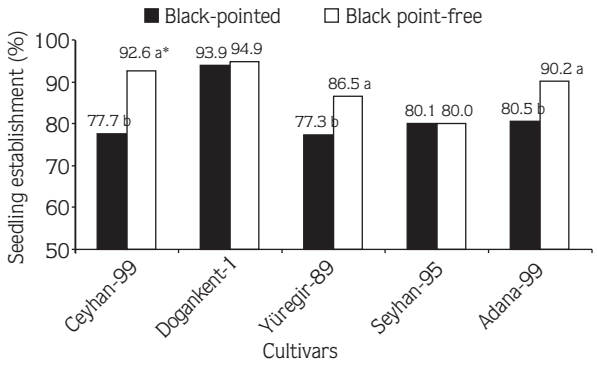
From the results obtained, it can be inferred there is no clear evidence showing an association between fungi species and black point. *Alternaria* spp. was observed to be the most frequently isolated fungi within all fungi species for discoloured and healthy kernel samples. Occurence of *Aspergillus niger* and *Chaetomium* sp. was thought to be not associated with black point because of few isolations from both discoloured and normal kernels for all cultivars. Higher isolation frquency of *Alternaria* in the discoloured kernels might be correlated with black point. Monthly precipitation, mean temperature, and relative humidity for grain filling period were favourable for some fungus species. In addition, relative humidity was somewhat high at the grain maturity period in June. The previous findings on black point are also unclear and contradictory. In a range of studies, black point have been associated with some fungi species such as *Alternaria alternata*, *Bipolaris, Cochliobulus, Epicoccum, Fusarium, Cladosporium, Chaetomium,* and *Sclerotium* (Rees et al., 1984; Jacobs and Rabbie, 1987; Agarwall and Sinclair, 1987; Conner and Kuzyk, 1988; Özer, 2005; Sisterna and Sarandon, 2005); however, in several studies the cause of black point has been associated with extreme environmental conditions such as heavy rain, high humidity, and extreme temperature during the grain filling duration (Jacobs and Rabbie, 1987; Basson et al., 1990; Ellis et al., 1996; Williamson, 1997; Kumar et al., 2002; Clarke et al., 2004; Sadasivaiah et al., 2004; Mak et al., 2006).

Table 3. Laboratory observations of researched plant characters in black point-affected and black point-free kernel samples of 5 bread wheat cultivars.

Cultivars	1000-kernel weight (g)		Germination percentage (%)		Seedling emergence (%)		No. of embriyonic roots		Coleoptile length (cm)	
	BP	BPF	BP	BPF	BP	BPF	BP	BPF	BP	BPF
Ceyhan-99	51.8 a*	46.6 b	88.3 b	100.0 a	90.0	97.5	4.33	4.33	5.21	5.15
Doğankent-1	54.3 a	43.2 b	95.0	100.0	98.8	100.0	4.40	4.47	5.87	5.42
Yüreğir-89	50.9	47.3	85.0 b	96.7 a	85.0	80.0	4.47	4.37	5.62	5.31
Seyhan-95	49.4 a	39.4 b	81.7 b	98.3 a	80.0 b	98.8 a	4.87	5.07	5.55	5.69
Adana-99	51.0 a	44.5 b	91.7	100.0	80.0 b	97.5 a	4.83	4.33	5.37	5.35
Mean	51.5 a	44.2 b	88.3 b	99.0 a	86.8 b	94.8 a	4.58	4.51	5.52	5.38

BP: Black pointed kernels, BPF: Black point free kernels

*: Values followed by the same letter do not differ significantly between discoloured and normal kernel samples of each cultivar at 0.05 probability level



*: Values followed by the same letter do not differ significantly between discoloured and normal kernel samples of each cultivar at 0.05 probability level.

Figure 1. Seedling establishment percentage for black-pointed and black point-free kernel samples in field conditions.

Moschini et al. (2006) reported that a positive relationship was found between disease incidence and sample moisture variables such as total amount and frequency of precipitation.

Black point incidence differences among bread wheat cultivars (ranged 3% to 10%, depending on the cultivars) could be explained by the genotypic response of different cultivars. In the wheat breeding studies, genotypic effect of the black point should be considered carefully. Black point natural incidence levels were mainly influenced by cultivars and nitrogen fertilizer application, but not by tillage system (Sisterna and Sarandon, 2005).

This study showed that black point affected kernels were heavier than the black point-free kernel samples for all cultivars. This might be explained by the outside kernels on the spikelets' being more affected compared to the inner ones from the environmental conditions, such as rain, humidity, and temperature, during the grain filling period. These results are in agreement with the findings of Rees et al. (1984) and Sisterna and Sarandon (2005) who stated that discoloured grains were heavier than normal ones. For further information, detailed investigation should be conducted on the seeds that have different positions on a spikelet.

Germination rate, seedling emergence and seedling establishment under the field conditions were reduced by black pointed seeds. This result is in accordance with Hudec (2007) who reported that fungal species of *Alternaria, Penicillium, Aspergillus* and *Fusarium* genera and *Cochliobolus sativus* were able to kill or reduce embryo vigor. According to the results of our study; sowing rate should be increased for black-pointed seed lots to obtain optimum seedling rate in field conditions. Numbers of embryonic root and coleoptile length were not affected from the black point.

Acknowledgements

We would like to thank Dr. Nazım Dinçer and Dr. Sadiye Yaktubay from Çukurova Agricultural Research Institute for providing kernels of bread wheat cultivars and Faheem Shahzad Baloch for rewieving the manuscript.

References

Agarwal, G.P., B. Awastly and M.K. Thakur. 1983. Studies on the wheat grain storage in Madhya Pradash: efficacy of certain fungicides against black point disease of wheat. Rev. of Plant Pathology. 62: 137.

Agarwall, V.K. and J.B. Sinclair. 1987. Principles of Seed Pathology. 1. CRC Press, Boca Raton, Florida.

Basson, A.B.K., O.T. de Villiers and C.J. Rabie. 1990. Effect of black ends on quality characteristics of Clipper barley and malt. American Society of Brewing Chemists. 48: 8-13.

Biçici, M. and A. Çınar. 1988. Çukurova Bölgesi'nde yetiştirilen buğday tanelerinde görülen siyah leke hastalığının etiyolojisi ve önemi. V. Türkiye Fitopatoloji Kongresi, 18-21 Ekim, ANTALYA.

Clarke, M.P., M.J. Gooding and S.A. Jones. 2004. The effects of irrigation, nitrogen fertilizer and grain size on Hagberg falling number, spesific weight and black point of winter wheat. Journal of the Science of Food and Agriculture. 84: 227-236.

Conner, R.L. 1989. Influence of irrigation and precipitation on incidence of black point in soft white spring wheat. Canadian Journal of Plant Pathology. 11: 388-392.

Conner, R.L. and A.D. Kuzyk. 1988. Black point incidence in soft white spring wheat in Southern Alberta and Saskatchewan between 1982 and 1987. Canadian Plant Disease Survey. 68: 27-31.

Conner, R.L. and E.D.P. Whelan. 1989. Role of chromosome 5B in controlling black point incidence in hard red spring wheat. Canadian Journal of Plant Sciences. 69: 675-679.

Ellis, S.A., M.J. Gooding and A.J. Thompson. 1996. Factors influencing the relative susceptibility of wheat cultivars (*Triticum aestivum* L.) to black point. Crop Protection. 15: 69-76.

Gooding, M.J., A.J. Thompson, F.M.B. Collingborn, S.P. Smith and W.P. Davies. 1993. Black point on wheat grain: Influences of cultivar, management and season on symptom severity. Aspects of Applied Biology. 36: 391-396 Cereal Quality III.

Hosford, R.M. Jr., 1975. *Phoma glomerata*, a new pathogen of wheat and triticale, cultivar resistance related to wet period. Phytopathology. 65: 1236-1239.

Hudec, K. 2007. Influence of harvest date and geographical location on kernel symptoms, fungal infestation and embryo viability of malting barley. International Journal of Food Microbiology. 113: 125-132.

Jacobs, B. and C.J. Rabie. 1987. The correlation between mycelial presence and black point in barley. Phytophylactica. 19: 77-81.

Kumar, J., P. Schafer, R. Huckelhoven, G. Langen, H. Baltruschat, E. Stein, S. Nagarajan and K.H. Kogel. 2002. Bipolaris sorokiniana, a cereal pathogen of global concern: Cytological and molecular approaches towards better control. Molecular Plant Pathology. 3: 185-195.

Lehmensiek, A., A.W. Campbell, P.M. Williamson, M. Michalowitz, M.W. Sutherland and G.E. Daggard 2004. QTL's for black point resistance in wheat and the identification of potential markers for use in breeding programs. Plant Breeding. 123: 410-416.

Lorenz, K. 1986. Effects of black point on grain composition and baking qualityof New Zeland wheat. N. Z. J. Agric. Res. 29: 711-718.

Mak, Y., R.D. Willowa, T.H. Roberts, C.V. Wrigley, P.J. Sharp and L. Copeland. 2006. Black point is associated with reduced levels of stress, disease- and defence-related proteins in wheat grain. Molecular Plant Pathology. 7: 177-189.

Martin, P.M.D. and G.A. Gilman. 1976. A consideration of mycotoxin hypothesis with special reference to the mycoflora of maize, sorghum, wheat and groundnuts. Tropical product institute, 6-105, London, VIII + 112pp.

Moschini, R.C., M.N. Sisterna and M.A. Carmona. 2006. Modelling of wheat black point incidence based on meteorological variables in the southern Argentinean Pampas region. Australian Journal of Agricultural Research. 57: 1151-1156.

Özer, N. 2005. Determination of the fungi responsible for black point in bread wheat and effects of disease on emergence and seedling vigour. Trakya Univ. J. Sci. 6: 35-40.

Rees, R.G., D.J. Martin and D.P. Law. 1984. Black point in bread wheat. Effects on quality and germination and fungal associations. Australian Journal of Experimental Agriculture and Animal Husbandry. 127: 601-605.

Sadasivaiah, R.S., S.M. Perkovic, D.C. Pearson, B. Postman and B.L. Beres. 2004. Registration of 'AC Andrew' wheat. Crop Sci. 44: 696-697.

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

Toklu, F., H. Özkan and T. Yağbasanlar. 1999. Bazı ekmeklik ve makarnalık buğday genotiplerinde dane kararmasının çimlenme ve fide gelişimine etkisi. Türkiye 3. Tarla Bitkileri Kongresi. Cilt I, Genel ve Tahıllar. Adana. 378-383.

Wang, H., M.R. Fernandez, F.R. Clarke, R.M. DePauw and J.M. Clarke. 2002. Effects of foliar fungicides on kernel black point of wheat in southern Saskatchevan. Canadian Journal of Plant Pathology. 24:287-293.

Wang, H., M.R. Fernandez, T.N. McCaig, Y.T. Gan, R.M. DePauw and J.M. Clarke. 2003. Kernel discoloration and downgrading in spring wheat varieties in western Canada. Canadian Journal of Plant Pathology. 25:350-361.

Williamson, P.M. 1997. Black point of wheat. In vitro production of symptoms, enzymes involved, and association with *Alternaria alternata*. Australian Journal of Agricultural Research. 48: 13-19.