

DETERMINATION OF THE FUNGI RESPONSIBLE FOR BLACK POINT IN BREAD WHEAT AND EFFECTS OF THE DISEASE ON EMERGENCE AND SEEDLING VIGOUR

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Abstract: The kernels of the bread wheat (*Triticum aestivum* L.) cultivars Flamura 85, Katia, Pehlivan and Sana from farmer fields of Tekirdağ province in 2000 and 2001 were analyzed to identify the fungi associated with the black point disease. Fungi in the embryos, endosperms and seed coats of the kernels (healthy and black pointed) separately, were determined by plating them on agar medium. The same cultivars were used to test the effect of black point on emergence at day five and ten, and seedling vigour (length of shoot, dry weight of roots and shoots) in pot experiments. *Alternaria alternata* (Fr.) Keisler was the dominant fungus in black pointed kernels for both years and isolated from the endosperm and seed coat especially, but present at low level. None of the fungi from black pointed kernels were isolated from healthy kernels. Black point delayed seedling emergence from the kernels of 2000. However the reduction in seedling vigour was significantly higher in the affected kernels of 2001. High rainfall during the anthesis and milk stages in 2000 and 2001, respectively contributed to delayed maturity of the crop and had probably an impact on black point infection.

Key words: *Alternaria alternata*, cultivar, *Triticum aestivum*

Ekmeklik buğdayda embriyo kararmasına neden olan fungusların tespiti, ve hastalığın çimlenme ve fide çıkış gücü üzerine etkisi

Özet: Bu çalışmada ekmeklik buğday (*Triticum aestivum* L.) çeşitlerine (Flamura 85, Katia, Pehlivan ve Sana) ait taneler 2000 ve 2001 yıllarında Tekirdağ'da bulunan çiftçi tarlalarından toplanmış, sağlıklı ve embriyo kararması hastalığı belirtisi gösteren tanelerin embriyo, endosperm ve tane kabukları ayrılarak agar ortamına alınmış, gelişen funguslar tanımlanmıştır. Aynı çeşitler saksı denemelerinde kullanılarak hastalığın çimlenme ve fide çıkış gücü (sürgün uzunluğu, sürgün ve kök kuru ağırlığı) üzerine etkisi belirlenmiştir. Her iki yılda da *Alternaria alternata* (Fr.) Keissler, embriyo kararması gösteren tanelerin özellikle endosperm ve tane kabuğu kısımlarından en yaygın tür olarak ancak düşük oranlarda izole edilmiştir. Sağlıklı görünen tanelerde ise hasta tanelerden izole edilen fungusların hiçbirisine rastlanmamıştır. Embriyo kararması belirtisi gösteren, 2000 yılına ait tanelerde çimlenmenin geciktiği, 2001 yılına ait tanelerde ise önemli derecede fide çıkış gücünün azaldığı belirlenmiştir. 2000 ve 2001 yıllarında tanelerin sırasıyla süt olum ve çiçeklenme dönemlerinde yüksek miktarda yağın yağmurlar olgunlaşmayı geciktirerek hastalığın ortaya çıkmasında muhtemelen etkili olmuştur.

Anahtar kelimeler: *Alternaria alternata*, çeşit, *Triticum aestivum*

Introduction

Black point disease is characterized by a brown to black discoloration of the embryos of the wheat (*Triticum aestivum* L.) kernels. The disease can be a problem in wheat in areas receiving heavy rainfall during the early stages of kernel development (Machacek and Greaney, 1938; Greaney and Wallace, 1943; Kilpatrick, 1968). The disease reduces the commercial grade of wheat causing economic losses to producers. Black point incidence exceeding 10% results in downgrading of the grain (Canadian Grain Commission, 1983). Black

pointed kernels had also an adverse effect on the quality of the flour (Rees et al., 1984; Lorenz, 1986). Resistance to this disease is under different genetic control in certain cultivars (Conner and Davidson, 1988).

Alternaria alternata (Fr.) Keisler (most frequently), *A. triticina* Prasada & Prabhu, *Bipolaris sorokiniana* (Sacc.) Shoem., *Cladosporium cladosporioides* (Fres.) de Vries, *Curvularia lunata* (Wakker) Boedijn, *C. pallescens* Boedijn, *Drechslera halodes* (Drechsler) Subram. & Jain, *D. spicifera* (Bain.) v. Arx, *Fusarium graminearum* Schwabe, *F. moniliforme* Sheldon, *F. proliferatum* (Matsushima) Nirenberg, *F. semitectum* Berk.&Rav. and *Trichothecium roseum* (Pers.) Link ex Gray have been reported to be associated with black point in wheat grown in different countries (Machacek and Greaney, 1938; Greaney and Wallace, 1943; Adlaka and Joshi, 1974; Rana and Gupta, 1982; Conner, 1987; Khanum et al., 1987; Conner and Kuzyk, 1988; Fakir et al., 1989; Zhang et al., 1990; Agarwal et al., 1993; Dhruj and Siddiqui, 1994; Fernandez et al., 1994; Conner et al., 1996). *Alternaria* sp., *Cladosporium* sp., *Drechslera* sp., *Epicoccum* sp., *Fusarium* sp. and *Phoma* sp. were isolated from black pointed kernels of wheat cultivars grown in Central Anatolia, Southern Anatolia and Çukurova regions of Turkey (Biçici and Çınar, 1988; Tunalı and Aktaş, 1999; Sağır and Akıncı, 2001). In all these studies, the associated fungi were determined by plating the intact kernels on agar medium or on moistened filter paper. The presence of fungal mycelium in different parts of black pointed kernels has been determined by microscopical observations (Bhowmink, 1969; Lorenz, 1986; Agrawal et al., 1987). Several studies examined the relationships between black point and germination or emergence, 1000 kernel weight or yield for common wheat in the presence of *A. alternata* as the dominant fungus (Rees et al., 1984; Khanum et al., 1987; Cromey and Mulholland, 1988; Mellado et al., 1990), of *B. sorokiniana* (Machacek and Greaney, 1938; Zhang et al., 1990), *Drechslera* spp. and *Fusarium* spp. (Maloy and Specht, 1988) and *F. proliferatum* (Conner et al., 1996). Toklu et al. (1999) noted that the germination rate, radicle length, coleoptil length and wet root weight of some bread wheat genotypes such as 84 ÇZT 04, Ka“S”/Nac, Van“S”Bb/Kal, Tow“S”/Pew“S” and Vee#10/F 78183 were not negatively affected by black point. However, no detailed study has been reported on the incidence of fungi in different parts of wheat kernels with black point and the effect of the disease on emergence and seedling vigour of bread wheat cultivars from Trakya region.

The present study was undertaken to screen the presence of fungi in the embryo, endosperm and seed coat of black pointed kernels of four bread wheat cultivars collected from farmer fields in Tekirdağ province exposed to natural weather conditions and, to determine the effect of black point on emergence and seedling vigour.

Materials and Methods

'Flamura 85', 'Katia', 'Pehlivan' and 'Sana' are the most widely used bread wheat cultivars in Trakya region. Kernels of these wheat cultivars were obtained from the fields of various farmers in the province of Tekirdağ after harvest in 2000 and 2001. The fields were not treated with any fungicide controlling black point whereas standard cultural practices and plant protection measurements for other diseases were normally applied. Rainfall and temperature were recorded daily at the meteorological station in the city during anthesis (Growth Stage, 10.5.1-10.5.4, Feekes scale) (Large, 1954), milk (G.S. 11.1), mid-dough (G.S. 11.2) and ripe (G.S. 11.4) stages for both years. First, the naturally infected kernels were sterilized by dipping them in 1% sodium hypochlorite solution for 3 min, followed by several rinses in sterile distilled water. They were then soaked in sterile distilled water for 1 h, filtered and dried on sterile filter paper. The embryos, endosperms and seed coats were separated with a sterile scalpel under a stereomicroscope. The same procedures were applied to healthy (symptomless) kernels. The kernel parts were placed individually on potato dextrose agar (PDA-Oxoid) in a glass Petri dish of 10 cm diameter. The experimental unit was a Petri dish for each type of kernel parts (embryo, endosperm and seed coat) and ten fragments were put in a Petri dish. Ten replicates were considered for each type of kernel parts and for each cultivar. All Petri dishes were incubated at 20 °C for ten days and then fungi growing from the samples were identified under a microscope.

Emergence and seedling vigour were investigated in kernels naturally infected with black point in all cultivars obtained from fields in 2000 and 2001. The kernels were surface sterilized as mentioned above. Ten replicates of twenty kernels per cultivar were sown at depth of 2.5 cm in plastic pots (18 cm X 9 cm X 7 cm) containing perlite and the pots were placed in a controlled room set to a temperature and relative humidity of 18.8 °C and 70.4 %, respectively, monitored by thermohygrograph. Photoperiod was around 12-14 hours daylight.

Healthy kernels (symptomless) of each cultivar were used as control for the same procedures. Emergence was counted on the 5th and 10th days. Seedling vigour, based on the length of the shoot, and dry weight of roots and shoots, was determined after 15 days, using ten randomly selected seedlings from each replicate per cultivar for both years. The primary shoot was measured to assess the length of shoot. The roots and shoots were oven-dried at 30 °C for 24 h, as described by Gilbert et al. (1995) and weighed immediately after cooling, to determine dry weight. Percent reductions for emergence and seedling vigour were calculated for each sample based on the formula: $100 \times (a-b)/a$, where a is; the data of the healthy kernel, b is; the data of the naturally infected kernel.

All the experiments were arranged in a completely randomized design. Data on the incidence of fungi and, the percent reductions for emergence and seedling vigour were subjected to an angle (arcsin) transformation prior to analysis of variance (ANOVA) procedures of SPSS (Statistical Package for Social Sciences, Inc., 2001, Model 11.0. Chicago). Means were compared at the level of 5 % for Duncan's Multiple Range Test (Steel and Torrie, 1960).

Results

Rainfall was high during the milk and anthesis stages in 2000 and 2001, respectively (Table 1). In 2001, average maximum temperature was also lower at the anthesis stage than at the other stages.

Table 1. Total rainfall (mm) and average of daily minimum and maximum temperatures for Tekirdağ during the growth stages of kernels in 2000 and 2001.

| Growth stages of kernels * | 2000 | | | | 2001 | |
|----------------------------|---------------|------------------|------|---------------|------------------|------|
| | Rainfall (mm) | Temperature (°C) | | Rainfall (mm) | Temperature (°C) | |
| | | Min. | Max. | | Min. | Max. |
| Anthesis | 0 | 9.9 | 20.0 | 52.8 (4) | 11.9 | 17.1 |
| Milk | 43.6 (5) ** | 14.6 | 21.2 | 2.6 (3) | 11.1 | 24.6 |
| Mid dough | 11.8 (2) | 16.3 | 22.6 | 4.4 (1) | 17.0 | 25.1 |
| Ripe | 0.2 (1) | 15.7 | 23.9 | 6.4 (4) | 19.5 | 22.7 |

* Feekes scales; Anthesis: 10.5.1-10.5.4, Milk: 11.1, Mid-dough: 11.2, Ripe: 11.4. ** Number of days of rain in parenthesis.

The most dominant organism isolated from black pointed kernels of four cultivars belonging to 2000, was *A. alternata*, but it was present at low levels (Table 2). This species was isolated at higher rates from the endosperm and seed coat for all cultivars than from the embryo, and there were significant differences ($P=0.05$) between the kernel parts of cvs. Flamura 85 and Sana. The cv. Sana had the highest rate of incidence of this pathogen from the seed coat. *F. culmorum* was isolated from the endosperm of cv. Flamura 85 only at 4% in 2000. Other fungi, such as *A. niger*, *C. cladosporioides* and *Drechslera* sp. were isolated from kernel parts of one or two cultivars at low rates.

The frequency of isolation of *A. alternata* from kernel parts was low in 2001. This fungus was isolated at higher rates from the endosperm and seed coat of all cultivars, except for cv. Flamura 85, than from the embryo (Table 2). The incidence of endosperms infected by *A. alternata* in black pointed kernels of cv. Pehlivan was significantly higher than that of the embryo and there were no significant differences among kernel parts of the other cultivars. The other fungi, except for *F. culmorum*, were isolated at low levels from most of the cultivars. *S. botryosum* was found in the endosperm and embryo of the kernels of cvs. Flamura 85 and Katia, respectively, in very low levels. None of fungi from black pointed kernels were isolated from healthy kernels for both years.

Black pointed kernels sown in the pots were a factor in the reduction of emergence for all cultivars from 2000 (Table 3), by day 5 and 10, the percent reduction was significantly higher ($P= 0.05$) in the cv. Pehlivan than in other cultivars. The length of shoot and dry shoot weight were reduced at low level in all cultivars. However, the differences among the cultivars were not significant. The dry root weight decreased significantly with black point infection in cvs. Flamura 85 and Katia.

Table 2. The incidence of fungi detected in parts of black pointed kernels of four bread wheat cultivars harvested in 2000 and 2001.

| Cultivar | Kernel parts * | Percentage of the parts of wheat kernels contaminated with | | | | | |
|-------------|----------------|--|--------------------------|-------------------------------------|-----------------------|--------------------------|------------------------------|
| | | <i>Alternaria alternata</i> | <i>Aspergillus-niger</i> | <i>Cladosporium cladosporioides</i> | <i>Drechslera sp.</i> | <i>Fusarium culmorum</i> | <i>Stemphylium botryosum</i> |
| 2000 | | | | | | | |
| Flamura 85 | Emb. | 3.0 c ** | 0 | 0 b | 0 | 0 b | 0 |
| | End. | 15.0 ab | 0 | 0 b | 0 | 4.0 a | 0 |
| | SC | 6.0 c | 0 | 0 b | 0 | 0 b | 0 |
| Katia | Emb. | 1.0 c | 0 | 0 b | 0 | 0 b | 0 |
| | End. | 7.0 bc | 0 | 0 b | 0 | 0 b | 0 |
| | SC | 7.0 bc | 0 | 0 b | 0 | 0 b | 0 |
| Pehlivan | Emb. | 5.0 c | 0 | 0 b | 0 | 0 b | 0 |
| | End. | 7.0 bc | 0 | 0 b | 1.0 | 0 b | 0 |
| | SC | 8.0 bc | 0 | 1.0 ab | 0 | 0 b | 0 |
| Sana | Emb. | 8.0 bc | 1.0 | 3.0 a | 0 | 0 b | 0 |
| | End. | 17.0 a | 0 | 0 b | 0 | 0 b | 0 |
| | SC | 23.0 a | 0 | 3.0 a | 0 | 0 b | 0 |
| 2001 | | | | | | | |
| Flamura 85 | Emb. | 2.0 ab | 0 b | 1.0 | 0 b | 0 | 0 b |
| | End. | 0 b | 0 b | 0 | 0 b | 0 | 2.0 a |
| | SC | 0 b | 0 b | 1.0 | 0 b | 0 | 0 b |
| Katia | Emb. | 2.0 ab | 0 b | 1.0 | 0 b | 0 | 1.0 ab |
| | End. | 5.0 ab | 0 b | 0 | 2.0 a | 0 | 0 b |
| | SC | 6.0 a | 0 b | 0 | 2.0 a | 0 | 0 b |
| Pehlivan | Emb. | 0 b | 0 b | 0 | 0 b | 0 | 0 b |
| | End. | 6.0 a | 2.0 a | 1.0 | 0 b | 0 | 0 b |
| | SC | 2.0 ab | 0 b | 0 | 0 b | 0 | 0 b |
| Sana | Emb. | 1.0 ab | 0 b | 0 | 0 b | 0 | 0 b |
| | End. | 6.0 a | 0 b | 1.0 | 0 b | 0 | 0 b |
| | SC | 3.0 ab | 0 b | 0 | 0 b | 0 | 0 b |

*Emb., embryo; End., endosperm; SC., seed coat. ** Means followed by different letters within a column for each year indicate statistically significant differences at the level of 5 % for Duncan 's Multiple Range Test.

In the kernels of 2001, the emergence of only cv. Katia was reduced at day 5, and this reduction was significant compared with other cultivars. The percent reductions in the length of shoot, and dry shoot weight were significantly higher in the seedlings of Flamura 85 and Katia than those of other cultivars. However, dry root weight of seedlings from affected kernels of all cultivars, except for cv. Pehlivan was reduced significantly.

Table 3. Reduction in emergence and seedling vigour from black pointed kernels of four bread wheat cultivars harvested in 2000 and 2001

| Cultivar | Percent reduction in emergence | | Percent reduction in seedling vigour based on | | |
|-------------|--------------------------------|--------|---|-----------------|------------------|
| | Day 5 | Day 10 | Shoot length | Dry root weight | Dry shoot weight |
| 2000 | | | | | |
| Flamura 85 | 45.0 b * | 2.2 c | 2.6 | 6.6 b | 3.4 |
| Katia | 24.1 b | 1.1 c | 1.2 | 11.6 a | 1.7 |
| Pehlivan | 84.2 a | 23.5 a | 2.4 | 2.6 bc | 1.3 |
| Sana | 44.8 b | 13.3 b | 3.7 | 0 c | 3.0 |
| 2001 | | | | | |
| Flamura 85 | 0 b | 0 | 13.2 a | 19.6 a | 13.4 a |
| Katia | 50.0 a | 1.0 | 11.1 a | 23.5 a | 17.5 a |
| Pehlivan | 10.8 b | 2.0 | 0 b | 5.8 b | 0.3 c |
| Sana | 0 b | 1.1 | 4.5 b | 18.6 a | 5.9 b |

*Means followed by different letters within a column for each year indicate statistically significant differences at the level of 5 % for Duncan 's Multiple Range Test.

Discussion

Fungi isolated from the endosperm and seed coat of black pointed bread wheat kernels were at higher rates than from the embryo. Previous studies reported that the black point symptoms were due to a dense mycelial mat at the embryo (Bhowmink, 1969) and the fungi did not invade the endosperm (Lorenz, 1986). Agrawal et al. (1987) noted that inter- and intracellular mycelium of *A. alternata* occurred in all the components of infected kernels including the embryo. In contrast, Williamson (1997) observed that the discoloration at the embryo end of black pointed kernel was associated with peroxidases produced by this tissue, but not with density of fungal hyphae. This is the first report to determine the incidence of fungi, causing black point on bread wheat, in different parts of the kernels.

The occurrence of *A. alternata* as a dominant species on black pointed kernels and other fungi, except for *Stemphylium botryosum* and *F. culmorum*, agrees with other reports (Greaney and Wallace, 1943; Rana and Gupta, 1982; Khanum et al., 1987; Conner and Kuzyk, 1988; Fakir et al., 1989; Zhang et al., 1990; Agarwal et al., 1993; Dhruj and Siddiqui, 1994; Fernandez et al., 1994). *A. alternata* was isolated at higher frequency from infected kernel parts of all cultivars (mostly endosperm) in 2000 than in 2001. Several studies reported that there were differences among years in the frequencies of fungi isolated from black pointed kernels, but in that the infection was caused almost entirely by *A. alternata* (Machacek and Greaney, 1938; Greaney and Wallace, 1943; Conner and Kuzyk, 1988; Conner and Davidson, 1988; Cromey and Mulholland, 1988; Fakir et al., 1989; Agarwal et al., 1993; Fernandez et al., 1994). In this study, the parts of the black pointed kernels did not appear to have high frequency of *A. alternata* and other fungi, but the environmental factors, especially high rainfall at anthesis or milk development stages were probably responsible for black point as previously reported (Kilpatrick, 1968; Adlaka and Joshi, 1974; Conner, 1987; Conner et al., 1990).

The seedling emergence of all cultivars in 2000 was delayed by black point infection but seedling vigour was not affected at high levels. However, in the kernels of 2001, a significant reduction in the seedling vigour was determined for the cvs. Flamura 85, Katia and Sana. In a few cases, a negative effect of black point on germination and seedling emergence was observed in bread wheat cultivars (Rees et al., 1984; Zhang et al., 1990; Conner et al., 1996; Toklu et al., 1999).

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