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Wheat black point: Role of environment and genotype

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ABSTRACT

Wheat black point is an important quality defect in many areas of the world. The aims of this study were to identify grain tissues involved and to develop a better understanding of the genetic and environmental mechanisms that control black point. Results (of microscopic and genetic investigations) were consistent with control of pigment synthesis being resident in the tissues comprising the grain coat. Analysis of data for a black point susceptible variety revealed a strong correlation between black point and rainfall 20–30 days after anthesis. Application of overhead misting to wheat plots during grain ripening in field experiments appeared to validate this relationship and black point symptoms preceded the appearance of any fungal infestation. A 3-years trials at a high risk field site involving commercial varieties and a doubled haploid population derived from a resistant x susceptible cross indicated significant contributions from genotype, environment and genotype x environment. Germination rate of black pointed developing and ripe grains increased, with no apparent impact on seed viability. The results suggest that black point development can be independent of fungal presence. Furthermore, dark pigment synthesis in susceptible genotypes appears to occur if specific environmental conditions coincide with a sensitive period in grain development.

1. Introduction

Wheat black point refers to the presence of black or brown pigmentation in the grain coat overlying the embryo and scutellum tissues. The pigment fragments during milling causing speckiness in the flour which are then obvious in end-products such as durum pasta and Asian style noodles and as a consequence, reduces the quality of grains and therefore its marketability around the world (Williamson, 1997). Grain with black point symptoms at a level of more than 5% in bread wheat or 3% in durum wheat is downgraded on receival at Australian grain silos (Lehmensiek et al., 2004) resulting in significant financial losses to growers. Whilst there is substantial genetic variation in black point susceptibility/resistance (Christopher et al., 2007; Fernandez et al., 2011; Lehmensiek et al., 2004 and Sissons et al., 2010), whether symptoms develop is dependent on the environmental conditions, and possibly biotic factors, present during grain development and ripening. Whilst black point has been the subject of much investigation, unfortunately the mechanisms involved in the appearance of the dark pigment are still unresolved. The high degree of variation in occurrence of black point coupled with the poor understanding of the factors that trigger this defect significantly hinder genetic and biochemical studies as well as efforts by breeders to develop resistant varieties.

Early studies, for example King et al. (1981) and Rees et al. (1984) reported that black point occurs due to infection of grains with fungi. Whilst it seems clear that the conditions that favour black point also favour fungal infestation, these early studies invariably looks at grain at some considerable time after the initial triggering event and demonstrate a possible association but not necessarily a causal relationship. In contrast, Fernandez et al. (2011) conducted more rigorous experiments in which they inoculated spikes with 2 different fungal organisms by dipping them in spore solutions. Their results indicated that kernel discolouration was greatest in inoculated samples.

Williamson (1997) also used an inoculation approach, treating spikes shortly after flowering, but was unable to demonstrate any association between infection with *Alternaria alternata* and incidence of black point symptoms. Moreover, Williamson (1997) proposed that the appearance of dark pigment might be linked to damage of the grain coat and be the result of peroxidase acting on

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phenolic compounds and hydrogen peroxide in the outer tissue layers of grains. Walker et al. (2008) also concluded that stress or wounding of the embryo of barley by environmental factors during grain development might lead to black point formation. In their study, occurrence of low vapour pressure deficit, high humidity, and low temperatures was associated with the formation of black point in susceptible varieties. The critical period for black point incidence, in presence of *Alternaria alternata* and some other fungi in Argentina, has been reported to be Zadoks growth stage 71–87 or 30 days after heading and to persist for 14–20 days (Moschini et al., 2006). During this period of time, warm weather (>17 °C) and daily relative humidity of 60–85% reportedly increased black point incidence.

Interestingly, Mak et al. (2006) did not find any fungal or bacterial proteins in black point samples sourced from Queensland, Australia, while they detected high levels of stress-proteins in healthy grains. These researchers concluded that abiotic factors are responsible for black point initiation and that fungi are secondary.

The aims of this investigation were to determine the timing of appearance of symptoms on grains in a field environment, and to conduct a thorough microscopic examination of developing and ripe grains to identify the tissues involved as well as any presence of fungal mycelia and seed coat modification. In addition, to examine the role of genetic and environmental factors in black point incidence, grain germination, seedling establishment and growth, and finally the distribution of affected grain within spikes.

2. Materials and methods

2.1. Plant material

Due to the unpredictable and often localised occurrence of black point, samples with black point symptoms used for grain appearance, microscopy and germinability in this study were opportunistically collected from field trials where high levels of black point were observed. Black or grey discoloration on the surface of ripe wheat grain may be due to black point and or general fungal staining although these can usually be differentiated based on the spatial distribution of the pigmentation, black point being restricted to the area covering the embryo and in some cases the crease whereas fungal staining may affect all parts of the grain coat. In this study which is focused on black point, only grain free from fungal staining was used for microscopic investigation and germination in order to avoid confounding effects. Whilst not examined as part of the current study, the presence of fungi on the grain may be anticipated to adversely affect germination and seedling establishment. Wheat varieties, Hume, Tasman and Petrie as well as breeding lines SUN325B (dormant genotype) and SUN239V have previously been shown to be very prone to black point whereas Kennedy has consistently shown good resistance. AUS1408 and AUS1490 are black point susceptible but dormant genotypes previously described in Mares et al. (2005). Seed of these lines together with the varieties included in the varietal survey were from stocks held at the University of Adelaide, from the originating wheat breeding company or from the National Variety Trial network.

The doubled haploid populations, Tasman x Kennedy and Hume x Kennedy, were developed from F_1's produced at the University of Adelaide using the wheat x maize (*Zea mays*) system (Kammholz et al., 2001).

Field trials were conducted at the Waite campus of the University of Adelaide (34.9670° S, 138.6355° E), in a farmer's field located in a high black point risk area near Millicent (37.6050° S, 140.3593° E) in the south-east of South Australia and in one season, 2006, at the Leslie Research Centre, Toowoomba, Queensland (27.5598° S, 151.9507° E) Australia. Field trials at Millicent were replicated, randomised 5 m twin rows in a rectangular block, 10 plots in width, located within a farmer's commercial wheat field. Toowoomba trial as described in Lehmensiek et al. (2004).

2.2. Grain appearance and microscopy

Samples of grain of several genotypes including Hume, SUN325B and SUN239V, showing significant levels of black point (BP score > 20%) were collected from field trials and separated into black point (BP)-affected and non-affected sub-samples. Grain from these sub-samples were subsequently examined to compare grain appearance, evidence of cracks in the grain coat overlying the embryo, distribution of black point pigment within the tissues comprising the grain coat, presence of fungal mycelia, and the micro-structure of the grain tissues overlying the embryo.

2.2.1. Grain appearance and distribution of pigment

Grains were examined visually as well as with a stereo-microscope for evidence of gross physical changes and presence of cracks in the grain coat. BP-affected and non-affected grains with no cracks in the grain coat visible under a stereo microscope were examined by Scanning Electron Microscopy (SEM) for any micro-cracks. Eight different samples of six grains with or without pericarp were prepared for SEM. All grains were hand-threshed from a sample of SUN325B (20.3% black point). Three grains of each sample were cut adjacent to and parallel to the scutellum then mounted on stubs cut surface down to allow a better view of the proximal ends of the grains. In addition, some grains were incubated on moist filter paper in petri dishes for 18 h to facilitate dissection of the outer pericarp overlying the embryo section of the grains. The grains and pericarp tissues were examined under the stereo-microscope and fixed on the stubs using glue or a carbon tab, respectively, and then coated with platinum for SEM examination.

2.2.2. Presence of fungal mycelia

Affected and non-affected grains from wetted Hume heads in the field in 2014 (refer to details of wetting treatment later in the Materials and Methods, section 2.5) as well as black point grains of SUN325B and SUN239V sourced from non-wetted control were stained to detect any presence of fungal mycelia. Grains were imbibed on filter paper in petri dishes overnight and the seed coat at embryo side were stripped off. These seed coats were photographed under stereo-microscope and then stained in Trypan Blue and finally examined under a compound microscope.

2.2.3. Internal structure of BP-affected and non-affected grains

The pericarp tissues were removed from the embryo end and the surface of the underlying testa tissues examined using SEM. In order to examine the continuity of the modified aleurone layer overlying the embryo, BP-affected and non-affected grains of Hume and SUN325B were cut parallel to the crease and after coating were observed using SEM. In addition, the proximal end of BP-affected and non-affected grains of SUN325B were sectioned at four positions with a razor blade, and fixed on the stubs for SEM. Thin sections of fresh grains, harvested soon after appearance of black point symptoms, were cut parallel with the grain crease and stained in aqueous solutions of Calcofluor white (Sigma–Aldrich, St. Louis, USA). The aleurone layers in these sections were examined using a Nikon A1R Laser Scanning Confocal microscope.

2.3. Timing of appearance of black point symptoms and fungal mycelia and the distribution of affected grain within spikes

Grain samples from wetted heads from the field experiment