Proceedings of the 10th Australian Barley Technical Symposium, 2001

Field screening using a controlled environment for black point resistance in barley

Maria Sulman[1], Glen Fox[1], Kerry Bell[2], Kevin Young[3], Andy Inkerman[1]

[1] Barley Quality Laboratory QDPI, AFFS, Farming Systems Institute, Toowoomba.
[2] Biometry Section, QDPI, AFFS, Toowoomba
[3] Agriculture WA, Esperence.

Abstract

A field screening technique has been developed for black point. It reliably produces high levels of black point to (i) assist in selecting for resistance, and (ii) provide samples that could be used in biochemical studies of black point. Ninety genotypes (including resistant wheat varieties) were grown in a replicated trial with two planting dates in three environments. The environments consisted of (i) a high humidity tunnel with ground irrigation and overhead mist sprays, (ii) a dry tunnel environment with ground irrigation, and (iii) an uncovered environment with ground irrigation. Results to date indicate that the high humidity environment produce the highest level of black point with the greatest range between susceptible and resistant genotypes. The resistant genotypes included Harrington, Chevron, WA5034 and Kaputar. The more susceptible genotypes included Grimmett, Tallon, Skiff, Schooner, Blenheim, Manley and Caminant. Two crosses, Nasu Nijo/85S:1851//85S:1851/TR118 and TR232/Skiff//NasuNijo/Fitzgerald demonstrated considerable variation in resistance. The system developed in this study clearly demonstrates a strong relationship between high humidity and the development of black point and would provide a reliable screening technique for selecting breeding lines with resistance to black point

Introduction

Black point is a brown-black discolouration at the germ end of otherwise healthy barley or wheat caryopsis. Up to 10% of the Australian malting barley is downgraded each year due to black point. Black point is the internationally recognised term to describe this discolouration, terms like "black tip", "kernel smudge" and "black end" should be avoided. In barley, black point appears mainly in the lemma and palea tissue (glume) and in severe cases discolouration can also occur in the testa. In wheat, the discolouration occurs in the outer pericarp and inner seed coat tissue and in some cases may extend along the groove on the ventral side of the grain (Williamson 1997).

Environmental factors such as heavy morning dews, rainfall and high humidity are known to cause kernel discolouration and in more severe cases of weathering, the germ end of some kernels may be stained dark brown (Brinkman and Luk 1979, Young 1997).

Field observations of black point have shown that this kernel discolouration occurs randomly in a head, as not all the kernels in the same head develop black point. Early symptoms of black point were seen in barley kernels between anthesis and pre-turning. The intensity of black point discolouration varies according with environmental conditions. The black point discolouration has been observed to go from light brown to black (simulating different stages of black point development) in some barley cultivars grown under a high humidity environment (unpublished data).

The objective of this work was to develop a reliable technique to determine resistance levels of black point in barley cultivars and to provide samples that could be used in biochemical studies.

Materials and Methods

Preliminary field screening was conducted to assess the environmental conditions that would trigger high levels of black point in barley cultivars. In 1999, nine barley cultivars (TR118, TR 328, Tallon, Kaputar, WA5034, Schooner, Namoi, Kino-Nijo 7, WB328) were grown in two environments at Wellcamp (Toowoomba). Environment one consisted of a rain out shelter with ground irrigation. The second environment was uncovered. The rain out shelter trial was conducted under the same environmental conditions that trigger high levels of black point in wheat.

In 2000, ninety barley varieties from overseas and Australian barley breeding programs were screened for resistance to black point in a hill plot experiment at Hermitage Research Station, Queensland. The barley cultivars were grown in a replicated trial with two different planting times (2 weeks apart) under three different environmental conditions using a completely randomised design. The different planting times and reps were separated by buffer rows in each tunnel. A buffer row was also cultivated at the edges of the tunnel. The three environments consist of i) a high humidity tunnel with ground and overhead mist irrigation, ii) a dry tunnel with ground irrigation and iii) an uncovered environment with ground irrigation. The tunnel frames were covered with Agtuff gold clear polythene of 2 mm thickness. The high humidity tunnel had the plastic buried in a trench along each side, and in the dry tunnel the polythene was placed 50 cm above the ground level along each side.

Heads were harvested at physiological maturity, threshed and scored for black point. A sub-sample of 300 kernels was taken and the number of grains exhibiting black point was counted in three sets of 100 using the Australian Wheat Board inspection tray. The level of black point was reported as percentage.

Results and discussion

The morning temperatures during grain filling and ripening were very similar for all the environments; they varied from 12^{o}C to a maximum of 20^{o}C during the hottest days. The main differences in temperature between the environments were recorded at noon. The high humidity environment reached temperatures around 50^{o}C -57^{o}C during the hottest days. Whereas the temperatures registered in the dry tunnel and the uncovered site were around 40^{o}C -51^{o}C and 28^{o}C - 38^{o}C respectively. The relative humidity values registered from 12 a.m. to 6 a.m. were around 87%-98%, 80%-99% and 75% to 98% for the high humidity, dry and uncovered environment respectively.

The percentages of black point are taken from the back transformed means table (tables 1 and 2). The levels of black point in the cultivars located at the front ends of the tunnels were slightly lower than those varieties grown at the middle. Therefore, an angular transformation was required for the statistical analysis to adjust the black point straight means for the edge effect.

The levels of black point encountered in the three environments were significantly different ($p = 0.007$). The high humidity environment was the one that triggered the highest level of black point followed by the uncovered and the dry tunnel environment for the barley varieties screened.

The levels of black point encountered in the barley cultivars grown at Wellcamp under the rain out shelter with ground irrigation and the uncovered environment were from 0% to 10%. Whereas there was a significant ($p < 0.001$) and broad variation in response to black point between genotypes in the high humidity environment at Hermitage Research Station. Some barley cultivars displayed very high levels of black point (WI2976, 57%; WADH14648, 52%; WADH14486, 50%). Whereas some genotypes achieved black point levels lower than 10 % (Chevron, 9%; Harrington, 7%; WADH14697, 4%, WADH14700, 5%; and WADH14613, 2%).

The black point levels exhibited by the cultivars analysed may be higher than the values encountered in the field by farmers. This may be the result of severe environmental conditions (high humidity, high temperature, and moisture) that this material was exposed to. For instance the levels of black point reached by Lang (16% to 28%), a resistant wheat variety, are extremely high and never reported before (Peter Williamson pers comm).

All the commercial varieties screened for black point exhibited very low levels of resistance to black point under our experimental conditions. All of them have the potential to develop black point levels outside of the standard specifications (> 10%). Grimmet, Schooner and Tallon were found to be the most susceptible commercial malting cultivars. Kaputar, a feed variety, developed the most tolerance among the commercial feed cultivars.

The levels of black point in most of the susceptible cultivars analysed varied broadly across environments. Whereas, genotypes that exhibited very good levels of resistance to black point had consistent low levels of black point across the three environments tested. The level of black point was shown to depend on the environment. For instance, Grimmett, a susceptible cultivar, exhibited black point levels of 42%, 9% and 21% in the high humidity, dry, and uncovered environment respectively. Whereas, Harrington had black point scores of 7%, 6% and 5% for the high humidity, dry and uncovered environment respectively. Similar results were obtained in wheat trials at Leslie Research Centre.

The low levels of black point encountered in the hulless barley varieties screened may be due to the way black point levels are counted. For the hulled cultivars, the visual assessment is conducted on the husk and for the hulless genotypes the count is performed in the naked kernel. Black point discolouration is known to be present sometimes in the glume tissue only. Therefore some hulless kernels that have black point may not be counted as the glume tissue was discarded after threshing.

Results obtained at the Wellcamp and Hermitage trial showed that barley requires different growing conditions or maybe more stressful conditions for the development of high levels of black point than wheat cultivars. The levels of black point exhibited by some susceptible barley cultivars (Schooner and Tallon) were around 10% in the Wellcamp trial, whereas, the levels of black point achieved by Sun239V, a very susceptible wheat variety, were around 50%. The high humidity environment at Hermitage triggered the highest level of black point in the barley cultivars screened. This was not the case for the wheat cultivars, where the lowest levels of black point were achieved in the high humidity environment.

Black point is only a consistent problem when barley and wheat are grown outside of their natural Mediterranean environment. Normally the grain ripen and dries rapidly in an almost moisture free atmosphere. Under conditions of high humidity ripening and drying is prolonged. These conditions are likely to interfere with many of the mechanisms, which prepare mature grain for the long wait until the next growing season. Cochrane (1994) found that under similar environmental conditions peroxidases and phenol could combine to form discolouration at the embryo end of barley grain.

Table 1. Percentage of black point in some barley and wheat cultivars

Cultivar	Black point levels (%)		
	High Humidity Tunnel	Dry Tunnel	Uncovered
Barley			
ANT87%353	32	13	26
B*1302	29	12	22
Bearpaw	14	6	7
BHM/SKF//SKF-35	26	7	17
Blenheim	25	14	15
Cameo	30	11	11
Caminnat	25	9	13

Chevron	9	1	1
CMO*KORU 123	17	10	11
CMO*KORU 85	19	10	13
Fitzgerald	17	14	23
Gairdner	13	14	11
Grimmett	42	9	21
Harrington	7	6	5
Kaputar	11	4	2
Koru (Gilbert)	21	8	14
KTR/TLN-DH-83	23	12	19
Lindwall	21	8	18
Manley	26	6	8
Namoi	3	2	2
SB85744	1	1	4
Schooner	24	25	48
Skiff	27	8	7
Stander	36	20	15
Stirling	24	13	22
Tallon	39	13	26
TLN/KTR-DH 32	22	3	1
TLN/KTR-DH 51	39	9	21
TLN/KTR-DH 63	20	5	6
TLN/KTR-DH 73	36	11	14
TLN/KTR-DH 76	12	4	4
TLN/KTR-DH 1-132	21	3	6
TR118	18	4	9
WA5034	16	2	2
WI2976	57	27	47
WI3102	19	3	10
Yerong	27	19	45
Wheat			
Hartog	9	17	26
Lang	16	28	17
Sun239V	43	50	61
Tasman	15	21	16

Table 2. Levels of black point (%) of the Western Australia Doubled Haploid population.

Cultivar	Black point levels (%)		
	High Humidity Tunnel	Dry Tunnel	Uncovered
WADH14462	39	13	10
WADH14469	31	13	19
WADH14470	31	9	17
WADH14479	39	5	9
WADH14486	50	6	8
WADH14493	9	2	2
WADH14494	22	3	4
WADH14497	11	4	4
WADH14503	18	4	4
WADH14504	19	6	7
WADH14505	7	1	4
WADH14530	13	4	16
WADH14531	13	8	9
WADH14533	10	5	5
WADH14539	18	6	4
WADH14543	14	5	14
WADH14547	15	6	8
WADH14548	40	5	12
WADH14550	27	14	18
WADH14552	32	6	12
WADH14582	12	4	8
WADH14583	19	4	6
WADH14592	15	8	12
WADH14595	6	6	5
WADH14613	2	1	3
WADH14614	10	3	4
WADH14616	30	4	10
WADH14618	10	4	2
WADH14638	34	16	22
WADH14648	52	37	50
WADH14649	41	17	32

WADH14650	42	21	32
WADH14651	20	8	15
WADH14653	36	20	28
WADH14665	14	7	12
WADH14678	14	5	12
WADH14679	10	3	4
WADH14681	6	3	5
WADH14688	15	2	3
WADH14690	10	1	2
WADH14695	7	1	6
WADH14697	4	3	2
WADH14698	10	3	5
WADH14700	5	2	1
WADH14702	10	1	3
WADH14706	7	3	3
WADH14717	14	3	5
WADH14793	35	7	4

Table 3. Statistical analysis across environments

Variable name	d.f	Wald / d.f.	Chi-sq prob
Environment	2	4.95	0.007
Planting time	1	0.00	0.954
Cultivars	89	11.24	< 0.001

Conclusions

The high humidity environment proved to provide the ideal conditions for developing high levels of black point in barley cultivars. This environment triggered a wide range of levels of black point (2% - 56%) that allowed us to distinguish between resistant and susceptible varieties. This high humidity tunnel is a very reliable technique and easy to duplicate. It does not rely on a consistent weather pattern from year to year for triggering high levels of black point. However, there is limitation in the number of lines that can be screened and for large-scale resistance breeding a rapid laboratory test is desirable.

All the Australian commercial varieties were very susceptible to black point under the environmental conditions tested. However, there are some lines from overseas

(Harrington, and Chevron) as well as from Australian breeding program (WADH14505, WADH14595, WADH14613, WADH14681, WADH14695, WADH14697, WADH14700 and WADH14706) that showed levels of black point much below the cut off standard (10%).

Wheat seems to require different environmental conditions to barley for the development of high levels of black point.

Acknowledgments

The authors wish to thank the Grains Research and Development Corporation for their financial support. Peter Williamson and Miriam Michalowitz are acknowledged for their encourage and advice. Robert Amos and Andrew Skerman are also acknowledged for their contributions to agronomic aspects.

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