Validation of Black Point QTL in wheat

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ABSTRACT

Black Point (BP) is a dark discolouration of the embryo end of wheat and barley grains. It can result in reduced grain quality and value and is a significant problem in most Australian wheat growing areas. Estimated losses through downgrading have been as high as $50 million annually. Quantitative trait loci (QTL) for BP resistance have previously been identified in Sunco and Cascades. The aim of this study was to use a full diallele to validate these QTL and also to determine if two other varieties with increased resistance mirror existing sources of resistance or are potentially novel sources of resistance for BP. The BP resistant lines Lang, Cascades, SW95-50213, and Genaro, and one BP susceptible line, Cunningham were used as parental lines in the full diallele. As both Lang and Sunco are Cook derivatives, Lang was used to validate the Sunco QTL. Marker regression analysis confirmed the QTL on chromosome 2BS in Lang crosses. Genaro and SW95-50213, which are more resistant to BP than Sunco or Cascades, indicated an association between BP resistance and chromosomes 2B and 2A, respectively. The identification of markers for BP resistance in a number of different resistant sources will facilitate the pyramiding of genes for BP resistance in wheat.

INTRODUCTION

BP is a significant problem in most Australian cereal growing areas. In wheat, the BP discoloration takes place 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[i,ii]. Several researchers have linked the appearance of BP to the presence of fungi on the grain[iii,iv,v,vi]. However,[2] found no evidence proving a direct causative association between fungal infections and BP. There is an urgent need to identify novel sources of BP resistance that can be used in Northern region breeding programs of Australia. The current project focuses on germplasm of particular relevance to the Northern breeding region of Australia using a diallel between a range of resistant varieties and one susceptible variety to validate or identify new markers for BP resistance. QTL for BP resistance in wheat have previously been identified from two Australian mapping populations segregating for resistance to BP: Sunco/Tasman and Cascades/AUS1408[vii]. QTL were identified on 2B, 1D, 3D, 4A, 5A, and 7A (Sunco/Tasman) and 2A, 2D and 7A (Cascades/AUS1408). The current project aims to:

1. Validate the markers in the region of the QTL previously identified from Sunco, Tasman and Cascades within a full diallel of material suitable for the Northern Region breeding programme of Australia.
2. Determine if two new sources of resistance for BP (SW95-50213, Genaro) reflect new QTL.

MATERIALS AND METHODS

Plant Material

Given the critical role of the environment in the development of BP, wheat trials conducted in 2005 were carried out in two different Queensland locations, namely at the Leslie Research Centre (LRC) in Toowoomba and the Bundaberg Research Station (BRS). The validation studies were based on a diallel made up of significant sources of previously identified resistance [Lang (La), Cascades (Ca)], susceptibility [Cunningham (Cu)] and two new potential sources of resistance [SW95-50213 (SW) and Genaro (Ge)].Variety Lang (QT3765/SUNCO) carries the Cook derived 2B translocation from *Triticum.timopheevii*[viii]. This translocation has previously been associated with BP resistance[7]. Non-Cook derivatives which do not have the 2B translocation but display BP resistance and were included in the present study were Genaro (CGSS95Y00047S), Cascades (AROONA*2//TADORNA/INIA F 66) and SW95-50213 (Land race). Cunningham (3AG3/4*CONDOR//COOK) was used as the susceptible parent. A full diallel consisting of 5 parents and 20 crosses and 2500 F_2 plants was designed and split into two half diallel of 1250 individuals (250 parental plants and 1000 F_2 lines) planted at LRC and BRS respectively.

DNA extraction, microsatellite amplification and validation

DNA from leaf samples was extracted using a mixer mill (Qiagen) and the Wizard® Genomic DNA Purification Kit (Promega). PCR was conducted using microsatellite markers according to[ix]. Amplifications were carried out in a gradiant thermocycler (Biometra) and the products were separated using a Corbett Robotics Gel-Scan 2000 (Corbett Robotics Australia) using Ethidium bromide staining. Diallel investigation was primarily aimed at confirmation of previously identified BP QTL from[7]. MapManager QTXb20[x] was used for linkage analysis of SSR markers.

RESULTS AND DISCUSSION

The distribution of black point scores of the 2005 trials is shown in Figure 1. The mean BP scores in BRS trial were lowest in Ge/SW (7.0%) and ranged between 9.0 - 17.0% BP in other crosses. The parental genotypes displayed significant differences in BP [mean; Cu = 31.3%, SW =1.7%, Ca =14.1%, Ge = 6.3% and Lang = 9.5%. In the LRC trial, the mean BP scores were lowest in Ca/SW (2.3%) and ranged between 2.3 - 6.1% BP in other crosses. The parental genotypes displayed significant differences in BP [mean; Cu = 17.8%, SW =0.2%, Ca =1.8%, Ge =2.0 % and La = 6.2%].

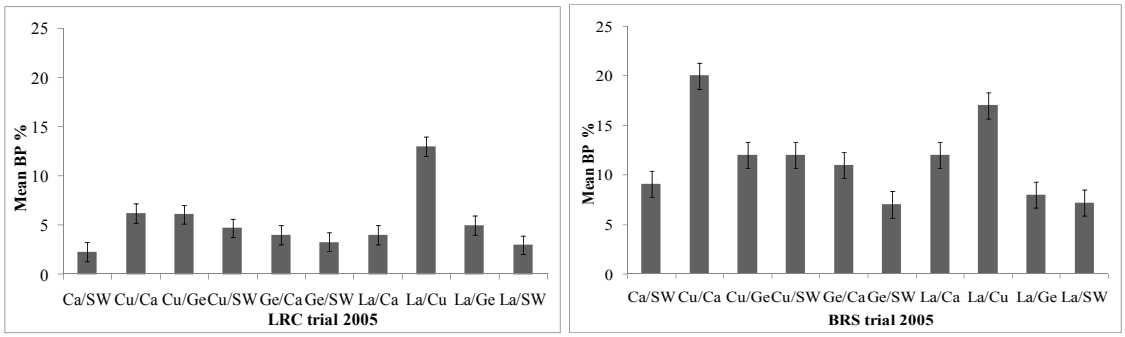

Figure 1. Distribution of mean black point scores (mean ± SE) of ten crosses of diallel at LRC and BRS 2005.

Table 1. Marker analysis results of half diallel trial planted at BRS and LRC in 2005 (Legends: La- Lang, Cu- Cunningham, Ca-Cascades, SW-SW95-50213, Ge- Genaro; R^2- phenotypic variance)

Cross	Site	Parent	Chr	Locus	LRS	R^2	P value
Ge/Ca	BRS	Ge	2B	gwm374	13	19	0.0014
La/Cu	BRS	La	2B	wmc35	9.8	10	0.0017
La/Cu	BRS	La	2B	wmc154	10.8	11	0.0045
La/Cu	BRS	La	2B	gwm501	18.5	22	0.0001
SW/Ge	LRC	SW	2A	wmc170	12.2	22	0.0004

Preliminary validation of QTL for BP resistance in wheat was undertaken using the 2005 BRS trial. Parents and populations of 100 of each of 10 crosses were screened with microsatellites for collection of genotypic data. Based on a preliminary analysis of the phenotypic data, no reciprocal effect on BP was identified. Given that no reciprocal phenotypic effect was identified at either site (LRC, BRS), a site specific pooled analysis was undertaken (Table 1). A suggestive QTL on chromosome 2A linked with the marker gwm294 (LOD 2.3 and phenotypic variance (PV) of 13%) in the Ca/AUS1408 doubled haploid population was identified by[7]. It was not possible to validate this marker in either the LRC or BRS trials in this study. However, the markers on 2A showed an association with BP resistance contributed by SW in the SW/Ge (2A) cross. Chromosome 2B has previously been associated with BP resistance and this was confirmed in the present study in the La/Cu cross. In this cross, wmc35, wmc154 and gwm501 had an indicative LRS scores of 9.8, 10.8 and 18.5 and explained a PV of 10, 11and 22%, respectively. A QTL on 2B was also identified in the Ge/Ca cross (LRS score of 13 and PV of 19%).

Not surprisingly environment had a significant influence on the occurence of BP and the identification of associated QTLs. This is most clearly demonstrated by site specific QTL identified at either LRC or BRS. The 2A QTL associated with SW95-50213 was only present at the LRC site whilst the Genaro associated QTL on 2B and all Lang associated QTL on 2B were only identified at BRS. Interestingly the 2A QTL contributed by SW95-50213 identified at LRC and the 2B QTL contributed by both Lang and Genaro identified at BRS were significant (LRS = 12.2, 18.5, 13.0 respectively). The presence of environment specific QTL associated with BP resistance raises issues with the reliability of using a more extreme environment such as BRS to screen material for suitability in other regions in which wheat is normally grown. This may be mitigated by the complex nature of BP as ranking across environments remained fairly consistent.

REFERENCES

[i] McIntosh R, Williamson P, Colin W (2004) CEREALS | Grain Diseases. In 'Encyclopedia of Grain Science' pp. 228-238. (Elsevier: Oxford).

[ii] Williamson P (1997) Black point of wheat: in vitro production of symptoms, enzymes involved, and association with *Alternaria alternata*. *Australian Journal of Agricultural Research* **48**, 13 -20

[iii] Ellis S, Gooding M, Thompson A (1996) Factors influencing the relative susceptibility of wheat cultivars (*Triticum aestivum* L.) to black point. *Crop protection* **15**, 69-76.

[iv] Hudec K (2007) Pathogenicity of fungi associated with wheat and barley seedling emergence and fungicide efficacy of seed treatment. *Biologia* **62**, 287-291.

[v] Monaco C, Sisterna M, Perello A, Dal Bello G (2004) Preliminary studies on biological control of the blackpoint complex of wheat in Argentina. *World journal of Microbiology and Biotechnology* **20**, 285-290.

[vi] Morejon K, Moraes M, Bach E (2006) Identification of Bipolaris bicolor and Bipolaris sorokiniana on wheat seeds (*Triticum aestivum* L.) in Brazil. *Braz. J. Microbiology* **37**, 247-250.

[vii] Lehmensiek A, Campbell A, Williamson P, Michalowitz M, Sutherland M, Daggard G (2004) QTLs for black-point resistance in wheat and the identification of potential markers for use in breeding programmes. *Plant Breeding* **123**, 410-416.

[viii] Friebe B, Jiang J, Raupp W, McIntosh R, Gill B (1996) Characterization of wheat-alien translocations conferring resistance to disease and pests: current status. *Euphytica* **91**, 59-87.

[ix] Roder M, Korzun V, Wendehake K, Plaschke J, Tixier M, Leroy P, Ganal M (1998) A Microsatellite map of wheat. *Genetics* **149**, 2007-2023.

[x] Manly K, Cudmore R, Meer J (2001) MapManager QTX, cross- platform software for genetic mapping. *Mammalian genome* **12**, 930-932.