FINAL REPORT

GRDC Grains Research & Development Corporation

SIP4 Defect Elimination in Wheat - Black Point

DAQ00045

Project Details

- **Project Code:** DAQ00045
- **Project Title:** SIP4 Defect Elimination in Wheat - Black Point
- **Start Date:** 01.07.2002 **End Date:** 30.06.2007
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Summary

Black point (BP) of wheat is a dark discolouration of the germ end of otherwise healthy grain. It is commonly a problem when grain ripening occurs under humid conditions, and affects the marketability of the grain if present at levels greater than 5%. A five-year project (2002-2007) was funded by GRDC to conduct a national phenotypic screening service, supply independent information on the BP status of wheat lines (released or nearing release), and identify different sources of resistance. This report concludes this project and reports on the major achievements.

Conclusions

Apart from the unusually dry grain-filling season of 2002, all subsequent years of this project produced a good range of BP scores which allowed for sufficient discrimination between tolerant and susceptible lines. Field screening still appears to be the most reliable method of determining the BP status of wheat lines and is an important component of molecular marker work through the provision of phenotyping data.
The interpretation of data by breeders is dependent on a number of factors including what the initial cross was aiming to achieve and the targeted growing area for the particular variety. Some growers experience problems with BP on a regular basis, while others rarely or never have a problem. For these latter growers, BP resistance is probably not a feature they are concerned with in a new variety. This may cause breeders to dismiss the importance of breeding for BP resistance for some target regions.
There are four major potential sources of variation in the BP screening method. These are:

1. achieving the correct environmental conditions conducive to BP development;
2. overcoming vast differences in maturity of the various lines;
3. the uncertainty about the integrity of seed sources; and
4. inconsistency of grain assessment. Smaller sources of variability include cross-contamination of grain during cassette-cell filling and threshing; mixed plots during planting; and mislabelling of plots or failure to include label in harvest bag. A discussion of these potential sources of variation was prepared following the GRDC mid-term review report recommendation, and was sent to the defect elimination project leader (Dr Mares) for wider distribution. A copy is available on request.

Recommendations

Breeding will overcome the problem of BP if selection for resistance is a priority. This will depend on the attitude of individual breeders to the problem and the particular cross in question (what it was aiming to achieve). Lines which regularly achieve high BP scores (such as SUN239V) should be eliminated from breeding programs as early as possible.

Research into the development of screening for BP under controlled environment conditions may be useful, and there is some funding for this in the new suite of defect elimination projects.

Until controlled environment screening can be successfully achieved, field screening is vital and should continue; currently it is the only large-scale method of determining the BP phenotype and is more reliable than marker assisted selection. Field screening is essential for the provision of independent assessment of current and future varieties, providing feedback to breeding programs and vital information to growers.

It is important to keep all current trial sites as backup and verification of results. The Leslie Research Centre (LRC) is a very important site for extreme years when the higher humidity sites may give excessive scores or be destroyed or damaged by inclement weather (sprouting, weathering, physical destruction of the trial). Due to funding reductions in the new project, the Bundaberg Research Station (BRS) has been dropped as a BP screening site (due to the higher costs involved with travel and only 50% of research officer's time attributed).

The adoption of an objective measurement of BP should continue in future screening work, and may result in improving the rapidity of the process.

Outcomes

1. A reduction in grain downgraded at silo receival due to BP.
2. A measurable reduction and eventual elimination of BP in the germplasm used by the breeding programs and in released varieties.

Achievement/Benefit

Screening Nurseries (Queensland):

The project began ahead of schedule and prior to funding to enable screening to begin in the first year. Approximately 1000 lines were screened in 2002 incorporating elite lines and parental introductions from most of the Australian breeding programs. One single seed descent and two doubled haploid populations with potential for molecular marker development and pyramiding resistance to BP were also included.

2002 was an exceptionally dry season for disease nurseries in general in South East Queensland (SEQ) conditions between anthesis and harvest were not ideal for development of BP symptoms, with hot dry winds keeping humidity at very low levels. Even with regular irrigation, levels of BP in susceptible standards were lower than expected. However, some lines developed levels of BP higher than the susceptible controls. The levels of BP in the susceptible controls were only slightly above the industry standard (5%) and many of the moderately susceptible lines may have escaped detection. Results were distributed to breeders in time for the 2003 season.

National screening of BP continued in 2003 with modifications to the proposal due to the discovery of wheat streak mosaic virus (WSMV) at LRC. The trial size was reduced slightly to allow for a large wheat curl mite buffer and a complete repeat trial established at BRS, Queensland counts from Bundaberg showed that levels of BP in susceptible standards were very high with good discrimination between resistant and susceptible lines and it was decided to add BRS as an additional screening site for subsequent years. Results demonstrate the BRS provides more rigorous screening for BP, with a lower percentage of plots achieving less than 6% BP in most years. This was most pronounced in 2003, with 77% of plots at LRC and 34% of plots at BRS achieving less than 6% BP. LRC is still very important in years when late rain causes excessive weathering or sprouting at the other sites. Therefore, the three sites used were BRS, LRC and Millicent, South Australia (SA).

In 2004, modification of a mechanical planter allowed the BP trial to be planted by machine rather than hand-planted as previously necessary. This saved considerable labour costs, with most of the trial preparation and planting being done by the research officer. The 2004 screening nursery consisted of two planting dates at LRC, one at BRS, with two replicates per planting date (i.e. six replicates in total). Results for the second planting date at LRC demonstrated that later planting resulted in a similar range of BP scores and a higher proportion of plots achieving less than 6% BP. It was decided that the added information gained from two planting dates did not warrant the increased resource consumption and the increased time required for assessment. BP counts from Bundaberg in susceptible standards were again very high with good discrimination between resistant and susceptible lines.

The 2005 and 2006 screening nurseries consisted of one planting date at both LRC and Bundaberg, with two replicates per site (i.e. four replicates in total). Results for 2006 were similar for both BRS and LRC in terms of percentage of plots achieving less than 6% black point (64% and 63%, respectively).

Simultaneous trials of advanced lines were grown at Millicent in each year of the project, which provided verification of the results.

Diallel trial:

A full diallel set of crosses was made in 2002, with F2s of some crosses produced during the 20022003 summer increase at LRC. Crosses were made using resistant parents with possible different sources of BP resistance (Lang[A], Genaro, Cascades, SW95-50213), for possible pyramiding and also one susceptible parent, Cunningham. Twenty-one F1s and seven F2s were grown alongside the BP screening nursery in Bundaberg in 2003 due to their higher WSMV risk (having been produced in the 200203 summer nursery alongside infected plants). Segregating F2 plants were harvested and assessed as individual plants. Initial results from the diallel (2003) indicated that resistance is not dominant and that there are several different resistances involved. Pyramiding of these resistances into future varieties is possible with very high levels of resistance found in some RxR doubled haploids and Hartog[A] x Genaro SSD populations. The trial was to be repeated in 2004, however, due to unforeseen circumstances, it was instead repeated in the 2005 season.

In 2005, a technician was appointed to work on the diallel trial, which was repeated at both Bundaberg and LRC. This trial consisted of 25 lines (five parents plus F1 progeny including reciprocals), by two sites, by five replicates and was considerably expanded to include black pointed and clean phenotypes (two seed types). A variation request to extend this milestone was accepted. DNA was collected from both sites to support a complementary PhD project at the

University of Southern Queensland (USQ) which aims to use micro satellite markers (simple sequence repeats [SSR]) to generate a genetic linkage map of the full diallel population. The fine mapping of these potential genomic regions will maximise chances of identifying any markers which are closely linked to the BP resistance genes. Synteny studies are being undertaken to compare the genetic similarities of BP resistance in wheat and barley.

In 2006, the diallel trial was completed. This experiment provided valuable information on resistance sources, inheritance of resistance and pyramiding of resistance genes. The main finding from the diallel was that while most of the variation in the F2 populations could be explained by additive effects, there was some non-additivity. This implies that the genes display some dominance; however, since progeny and parents have the same overall values, it can be inferred that resistance may be either dominant or recessive. The analysis also indicated that, while variety scores give a good indication of their value as parents, additional information could be obtained from the behaviour of their progenies in crosses. The amount of F2 variation within crosses between resistant parents was greater than could be accounted for by phenological effects, suggesting that they had different resistance genotypes. This could suggest that greater BP resistance could be achieved by combining resistance genes from different sources. A manuscript on the results of the original diallel trial is being prepared.

To overcome a number of limitations with the original diallel trial (e.g. the use of only a single susceptible parent which gave no information on the behaviour of crosses between susceptible parents), a new set of diallel crosses was produced in 2006. A full diallel was produced, with three resistant and three susceptible parents, yielding 297 individual crosses and 7200 F1 seeds, most of which (284 crosses, about 6500 seeds) have corresponding seed of the single plant parents. The crosses also have varying levels of yellow spot resistance, and could also be used for studies of inheritance of this disease. Further work on this diallel was proposed for the new project beginning in 2007, however, funding limitations have prevented this.

Grain assessment:

The purchase of specialised software (AxioVisionRel 4.4) and a digital camera from Carl Zeiss during 2005, has enabled more objective and consistent assessment of black pointed grain. Efforts to fine-tune the programming (including extensive correlation tests) ensured the software was accurate enough to use for assessing the 2005 trial data. Further fine-tuning of the software was conducted in 2006. This was achieved by using an algorithm which calculates the area of BP discolouration and converts this to a percentage of black pointed grains. The same program can also simultaneously be used to count the number of black pointed grains in the sample, from which the percentage BP can be calculated. Both methods provide good correlations with manual counts.

All other hardware/software combinations attempt only to count the number of black pointed grain. These are much slower than manual counting as no other software to date can differentiate grains which are touching. AxioVision has the advantage over other digital scanning systems in that it can assess the BP level in a randomly placed sample. There is no need for grain to be spaced in a matrix tray. The digital camera also has the advantage of rapid digital capture and the software has the ability to analyse all the images automatically from a folder of stored images. While the combined process of image capture and software assessment may not be much different to manual counting in terms of time efficiency, it should provide a more objective and consistent method of grain analysis. We believe that this is the first record of this type of image analysis being applied to black pointed grain.

Other Research

Further funding for the completion of the new diallel trial (started in 2006) would achieve further genetic understanding of the various resistances and possible incorporation with yellow spot resistance. In particular, it would be of interest in breeding for both yellow spot and BP to determine the potential for obtaining resistant lines from crosses between susceptibles, in addition to the potential for getting greater resistance from crosses between resistants.

The BP component of the new defect elimination in wheat projects includes funding for improved screening methods (controlled environment phenotyping) and further molecular marker work (genotyping, quantitative trait loci (QTL) pyramiding) which will be useful for further improving the assessment of the BP resistance of breeding lines or lines close to release.

Intellectual Property Summary

Any screening of germplasm will not entitle DPI&F to equity in that germplasm except in the case of germplasm that DPI&F already has an equity share in.
Information gathered from screening trials is to be made available to other wheat breeding programs as applicable.

Additional Information

Standards used for the screening nursery were expanded in 2005 to cover a broader range of maturities. Standards were mostly chosen from the list of recommended varieties for Queensland in 2005, apart from SUN239V and Cascades, which were retained for their high levels of respective susceptibility and tolerance.

The project supervisor (Dr. Peter Williamson), resigned from the DPI&F effective 07 August 2007, and was unavailable for the completion of this final report. The final report was prepared by Miriam Michalowitz (research officer) under the supervision of Dr. Emma Colson, Science Leader.

We would like to thank the GRDC for funding this work, and Dr. Tony Done for valuable technical and scientific assistance with the diallel trial, including all the genetic analysis.