The genetics of wheat harvest quality

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Searching for answers

Location
Roseworthy
Angas Valley
Winulta

Key messages

- **The digital protocol for rapid black point assessment has been validated.**
- **The digital protocol for threshability assessment has been established.**
- **Validation of pre-harvest sprouting (PHS) markers has occurred which will assist in early generation selection.**

Why do the trial?

Wheat harvest quality is an important component of gross-return to grain growers. Accumulators will downgrade deliveries which contain sprouted grains, low falling number (minimum of 300 seconds), excessive black pointed grains (5% tolerance), unmillable material above the screen (0.6% tolerance) or a low test weight (minimum 76 kg/hL). Previous studies have relied upon labour-intensive manual assessment methods for black point and threshability when studying the genetic background of these traits in wheat. This limits the amount of germplasm that can be assessed, thus limiting early stage selection in breeding programmes. This is further disrupted by the strong influence of maturity and environmental conditions on the expression of black point, threshability and PHS. The aim of this research is to validate the high-throughput digital protocol for black point, establish a digital protocol for threshability and to validate the effects of recently published PHS resistance DNA markers using Australian Grain Technologies Pty. Ltd. (AGT) breeding data.

How was it done?

Gladius, Scout and Mace were crossed to produce three breeding populations. These three varieties have a diverse phenotypic response for the three target traits. Scout is susceptible to black point, has poor threshability, and is resistant to sprouting. Mace, on the other hand, is moderately resistant to black point, has good threshability, and a moderate level of sprouting resistance. Gladius is susceptible to sprouting, moderately susceptible to black point and has moderately good threshability.

Three breeding populations consisting of crosses between Gladius/Scout (124 lines), Scout/Mace (171 lines) and Gladius/Mace (234 lines) were sown in single, 2.5 m long rows at Roseworthy (on 3 June 2016) and were also sown in plots of 1.3 x 3.2 m at three sites: Angas Valley (1 June 2016), Roseworthy (15 May 2016) and Winulta (18 May 2016). The plots were harvested at harvest maturity using a Wintersteiger Classic plot harvester.

The plot and row samples were analysed for black point incidence using a pre-existing digital imaging protocol. To validate the digital protocol, a 300-grain sample of a subset of the row experiment samples was manually counted for black point incidence. Grains were considered as being affected by black point when staining affected more than half of the germ.

The grain samples from the row experiment were analysed for pre-harvest sprouting and threshability. Threshability was measured by manually separating unthreshed material from clean grain, and calculating the percentage by weight. A digital imaging protocol was also developed, to enable rapid digital assessment.

For pre-harvest sprouting assessment, three heads from 650 breeding lines in the row experiment were plucked at physiological maturity, which coincides with the grain being at approximately 20% moisture. The grains from these heads were sprouted in a laboratory experiment, where the grains were germinated in the dark at a room temperature of 20°C. Grains were classified as being germinated once the germ had visibly split. Germinated grains were counted and removed every 24 hours for seven days, and sound, ungerminated grains also counted on the seventh day. A germination index (GI) (range of 0 to 1) was then calculated. Additional PHS resistance data was provided by AGT on 1463 lines from advanced breeding trials between 2011-2016. All of the lines from the breeding populations and the 1463 lines provided by AGT were characterised using DNA markers for the presence or absence of three genes known to contribute to improved PHS resistance. The AGT breeding lines were used to validate how useful these DNA markers will be as tools to breed future varieties with improved PHS resistance.

What happened?

There was a strong (r^2=0.83, P<0.001) positive correlation between manual and digital assessment of black point incidence using the AGT imaging protocol (Figure 1). This correlation illustrates that the digital assessment protocol is an accurate and very fast method to measure black point compared to other assessment methods.

Digital image analysis generated a strong correlation (r^2=0.63, P<0.001) (Figure 2a) and (r^2=0.53, P<0.001) (Figure 2b) between a digital count (TCP) and a manual count of unthreshed material, and digital area (TAP) and a manual area measurement of unthreshed material, respectively. The strong correlation for each of these traits offers an accurate and direct assessment of threshability.

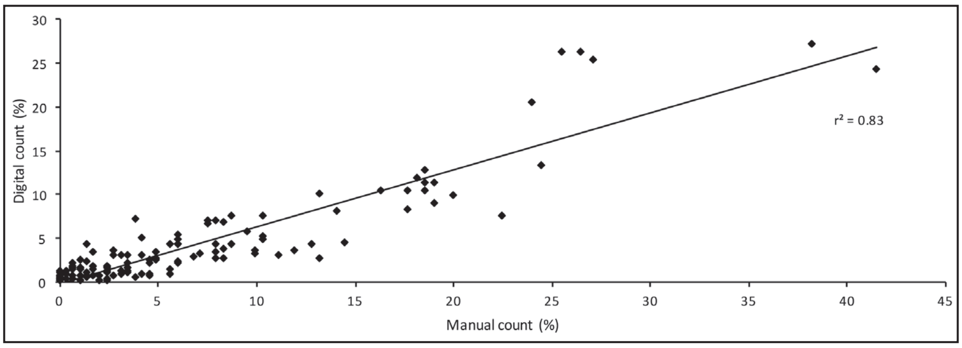

Figure 1. Comparison of manually counted black point incidence and black point incidence determined by the AGT imaging protocol in clean row experiment samples. Line of best fit shown, (P<0.001).

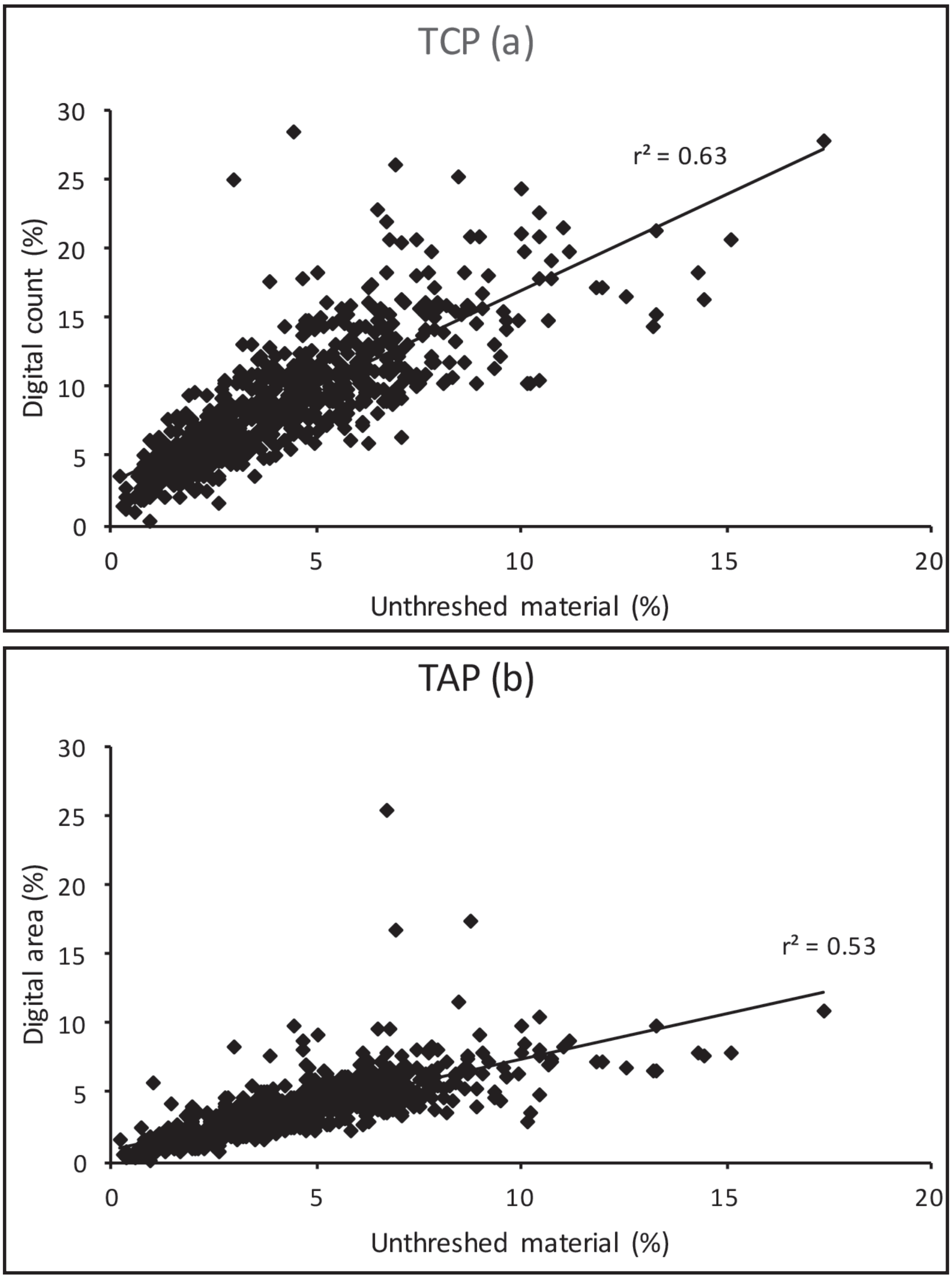

Figure 2. a) digital count percentage of unthreshed material (TCP) and b) digital area percentage of unthreshed material (TAP), P<0.001, plotted against a manual measurement of unthreshed material in 648 grain samples.

All four of the DNA markers that were tested improved the PHS resistance of varieties. Three of these four markers appear to be selecting for a significant improvement in sprouting resistance within the AGT germplasm. The presence of each of these three markers generated an improvement of 0.039 to 0.042 on the GI scale. This is a measurable difference in the field which would differentiate between a variety particularly susceptible to sprouting (Gladius) and a moderately susceptible (Mace) variety.

What does this mean?

The high-throughput imaging protocols developed for black point and threshability assessment have been demonstrated to accurately assess black point and threshability of grain samples. The high-throughput digital protocol will allow much faster phenotyping of germplasm. Previously, assessment was limited to only several hundred breeding lines, as opposed to the tens of thousands that can be assessed using the digital protocol. This will allow phenotypic selection in earlier generations, as opposed to late stage breeding lines.

Pre-harvest sprouting resistance selection based solely on the four DNA PHS markers can occur immediately since these markers have now been validated in a large breeding dataset. These markers can be used in early generations to enhance the level of PHS resistance in breeding germplasm, where pyramiding of at least two markers appears to select for an adequate base level of genetic PHS resistance. This base level of sprouting resistance will mean breeders can quickly and easily eliminate varieties with a genetic background that is susceptible to PHS, such as Gladius.

The more direct, high throughput assessment of black point and threshability, in addition to the validation of developed PHS DNA markers will enable breeders to increase early generation selection, with enhanced performance incorporated into new varieties, benefiting growers. Improved varieties will result in higher returns to growers through less downgrading associated with unmillable material above the sieve, test weight, black point and sprouted grains.

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