APPROVED SUBCONTRACTOR PROJECT:

Non-destructive screening of WGIN Paragon mutants for grain NUE traits - Richard Weightman, ADAS

Background:

The EMS mutated Paragon population (EMPP) is a major resource but its trait data are sparse (1) because screening of the full EMPP is a real challenge: the number of lines is large (~6500), seed samples are valuable and quantities for testing or field phenotyping are small (<50g). However, scanners are now available to characterise a wide range of traits on small quantities of grain, quickly and non-destructively.

Image analysers (e.g. the Marvin grain analyzer) provide rapid assessments of individual seed weight and size (length & width). Further, standard Near Infra-Red (NIR) calibrations predict hardness and contents of grain protein, moisture and starch. In addition, successful NIR calibrations have been developed in the GREEN grain project (LK0959) for protein components (gliadins, HMW glutenins, LMW glutenins and Albumins & Globulins) as well as for alcohol processing yield (litres per tonne). An opportunity therefore exists to screen existing seed of all EMPP lines for grain quality traits, allowing lines with altered expression to be identified for future investigation. Any variants for protein traits could facilitate development of N efficient ideotypes such as those proposed by the GREEN grain project or the WGIN.

In the GREEN grain project it was predicted that wheat with genetically reduced nitrogen (N) storage in both the canopy and the grain would give reduced N fertiliser requirements. Gliadins are responsible for most grain N storage in wheat, so NIR calibrations for gliadin were developed and lines with low gliadin were sought. Variation in gliadin contents amongst elite wheats was slight. A wider search amongst candidate UK elite germplasm (and mapping populations derived from them) also showed disappointing variation. However, based on previous mutant studies (2,3,4) there is a good chance that within the Paragon mutants lines will exist with contrasting levels of gliadin. If mutants could be identified with much reduced gliadin content (and reduced protein content per se, but without major disruption of grain formation or starch per grain), this could enable further investigation of genetic control of gliadin synthesis and deposition, and tests of whether low grain N storage improves N use.

This proposal is therefore to provide as much grain trait information as possible for the EMPP lines using non-destructive scanning techniques, to identify potentially interesting lines and to conclude on the potential for future investigation. The NIR calibrations and approach developed here could subsequently be used with other WGIN germplasm, as well as in commercial breeding programs.

References

(1) See http://www.wgin.org.uk/wgin_2003-2008/ResearchAct/ParagonMutant2006PhenotypeData.xls

(2) Mertz, E.T., L.S. Bates, and O.E. Nelson. 1964. Mutant Gene that changes protein composition and increases lysine content of maize endosperm. Science. 145:279-280. Purdue University, Lafayette, Indiana.

(3) Doll, H., Koie, B. & Eggum, B.O. (1974). Radiation Botany 14, 73.

(4) Bhatia, C.R., Jagannath, D.R. & Gopal-Ayengar, A.R. (1970). Induced micro-mutations for grain protein fractions in wheat. p99 in Improving plant proteins by nuclear techniques. IAEA, Vianne

Workplan:

This work will be led by ADAS in collaboration with John Innes Centre (JIC), Scottish Crop Research Institute (SCRI), FOSS and NIR specialists, Aunir.

The calibrations developed in the GREEN grain project are currently on FOSS 'Infratec' machines - the standard machine for commercial use by the grain industry. The Infratec has a narrow spectral range and cannot be used with small samples, so the utility of these new NIR calibrations could be enhanced by their transfer to a more advanced research platforms such as the FOSS 6500, 5000 or XDS. These have a wider spectral range so can predict traits such as starch content more accurately, as well as being capable of analysing small sample sizes (~10g). Transfer of the calibrations will entail scanning of existing GREEN grain samples which have known reference data provided by ADAS for protein composition and by Scotch Whisky Research Institute for alcohol yield.

An NIR platform will be provided for the project by Aunir. Around 400 reference samples will be transferred from SCRI to Aunir, along with associated reference data from ADAS & SWRI. Aunir will scan the reference samples on the 6500 and develop calibrations for:

. Alcohol yield (litres/tonne)
. Residue viscosity (mPA)
. Gliadin content (% DM)
. LMW Glutenin content (%DM)
. HMW Glutenin content (%DM)
. Albumin & Globulin content (%DM)
. Gliadin fraction (proportion of protein)
. LMW fraction (proportion of protein)
. HMW fraction (proportion of protein)
. Albumin & Globumin fraction (proportion of protein)

Usable NIR calibrations have been developed for each of the above for the FOSS Infratec, with alcohol yield and gliadin content giving the best predictions. It is expected that calibrations with the greater scanning range will be even more robust than those developed so far. In addition, standard calibrations will be available for grain protein, grain moisture, starch content and hardness.

The 6500 machine will then be transferred to JIC where Aunir will train JIC staff to use the machine with a small sample cell. JIC staff will then scan samples from the EMPP with the 6500, and with the Marvin seed analyzer (already available at JIC) to provide additional estimates of mean weight per grain, grain length and grain width. It is estimated that about 100 samples will be scanned per day; the total number of samples scanned will be at least 3000 and, depending on the scanning rate achieved, it may include all 6500 lines.

Predictions for all traits will be transferred to ADAS where data will be analysed, the relationships between traits studied and compared with previous data from elite germplasm. Lines of interest will be identified, including outliers from the normal relationships between traits (e.g. lines with both high protein and high starch; low gliadin content and high protein content, etc).

Findings, conclusions and recommendations for future research will be drawn up in collaboration with JIC and SCRI and will be presented in a final report, available on the WGIN website. Calibrations developed in the project will be made available for use on other genetic resources, subject to commercial arrangements of FOSS, Aunir and the GREEN grain project consortium.

Relevance to WGIN:

This project directly supports and is complementary to a number of WGIN's objectives:

A. Resource development: Paragon gamma and EMS mutant lines (Objective 4)

The NIR technology proposed in this project allows phenotyping to be quick, cheap and non-destructive, without risk to the samples. Once scan data have been collected it is possible to make predictions from new variables or updated calibrations retrospectively without having to rescan the samples.

Rapid screening of grain traits by NIR could potentially add considerable value to all of the genetic resources available to WGIN and elsewhere for modest cost, including the mapping populations, diversity sets & the Watkins collection (WGIN 2 Objectives 3, 4 & 5).

This project will deliver phenotype data for at least a large subset of the Paragon mutant population, which will be available for reference and relation to other measured traits into the future. Interesting lines from the Paragon mutant population will be identified for future investigations.

B. Targeted traits: Improvements of nitrogen use efficiency and quality QTLs linked to NUE (Objective 8)

The traits assessed in this project are important both for grain quality and for nitrogen use efficiency. Reducing protein content, for example via reduced gliadin content, could be associated with reduced N fertiliser requirement. Low protein and high alcohol yield are target traits for distilling and bioethanol markets. Protein composition is important for breadmaking quality, especially the content of gliadin and LMW glutenins.

This project will assess possible sources of these grain quality traits in the EMPP and set up the potential for genetic control of these traits to be studied further.