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TILLING for low phytic acid (lpa) seed mutants in wheat

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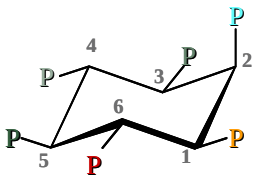
Background.

Phytic acid (PA) is the main storage form of phosphorus in cereal seeds. Phytic acid is recognized as an anti-nutritional factor as neither humans nor swine and poultry are able to digest and utilize PA. Phytic acid is also a strong chelator of important minerals such as Fe, Zn, Mn and Ca, which may contribute to lack of micronutrients in populations where cereals are the primary source of nutrition. Furthermore, in husbandry, excretion of PA can lead to accumulation of P in soil and water, and subsequently to eutrophication.



Phytic acid stores grain phosphorus

We are using two strategies to identify and characterise low phytic acid (lpa) mutants as a possible solution to these problems.

- Phenotypic screens for lpa mutants and development of mapping populations.
- TILLING, Targeting Induced Local Lessions IN Genomes, to screen for mutations in key genes controlling biosynthesis and transport of phytic acid.

Development of gene specific primers

Myo-inositol 3-phosphate synthase (MIPS) catalyses the first committed step in the biosynthesis of phytic acid. Three potential wheat homoeologues of the rice MIPS01 gene were identified using the WhETS transcription server (http://www4.rothamsted.bbsrc.ac.uk/whets/cgi-bin/whets1.3/whets_home.pl.)

Primer combinations expected to amplify PCR products of approximately 1000 bp were designed for each of the wheat homoeologues and tested on DNA from three wheat ancestors to identify primer pairs specific for each of the three wheat genomes (Figure 2). Subsequently, the three homoeologues were mapped to bins on wheat chromosomes 4A, 4B and 4D, respectively using nulli-tetrasomic (Figure 2), ditelosomic and deletion lines.

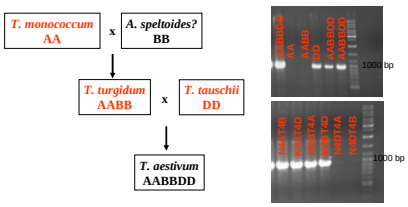


Figure 2. Left: Origin of wheat. Species highlighted in red were used to test specificity of primers for single genomes (top right), prior to mapping on nulli-tetrasomic lines (bottom right).

Wheat TILLING populations.

Seeds of six spring wheat varieties were imbibed in water for 20-22 hours before treatment with three doses of EMS in three replications (Figure 1). After one round of selfing, part of the seeds were given a second round of EMS treatment to increase the mutation frequency in the populations.

At present six populations are available as M_2 seeds. From one of these populations (Amaretto), DNA has been extracted and quantified from 1000 seedlings.

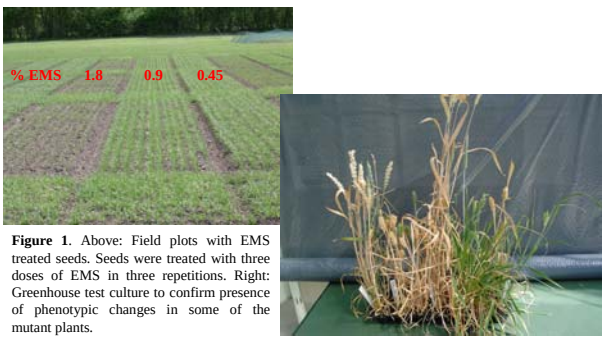


Figure 1. Above: Field plots with EMS treated seeds. Seeds were treated with three doses of EMS in three repetitions. Right: Greenhouse test culture to confirm presence of phenotypic changes in some of the mutant plants.

TILLING in wheat

Primer pairs screened so far have displayed an average mutation frequency of 3.8% corresponding to 1 mutation/26.2 kb. A tendency for a higher mutation frequency for the MIPS-4A homoeologue compared to the others have been observed, however, this needs to be confirmed in further screenings.

Mutations causing amino acid changes have been identified in each of the three wheat MIPS homoeologues. Plants carrying mutations in homozygous state have been identified and are currently multiplied for phenotypic evaluation.

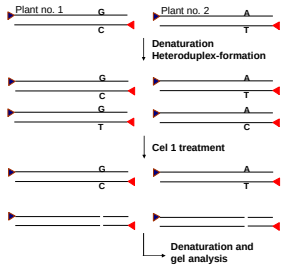
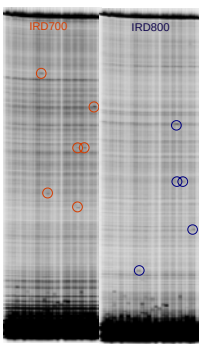


Figure 3. DNA from two plants were mixed prior to PCR with labelled specific primers. If one of the plants carry a mutation in the amplified fragment, the Cel 1 enzyme cuts the mismatches between the two sequences. Labelled fragments were detected on LICOR NEN 4300.



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Conclusion

Several wheat TILLING populations have been established in our lab. The screenings performed so far indicate that at least one of these populations have a high mutation frequency making it suitable for TILLING for a number of key traits.