

Key trait screening on global wheat accessions using KASP genotyping markers

A new open resource for the wheat breeding community.



D. Vyas¹, D.E. Mather², K.H.P. Khoo², M. Garcia³, S. Kirby¹, J. Curry¹ and J. Anthony¹.

¹ LGC - Genomics Division, Hoddesdon, UK. Darshna.vyas@lgcgroup.com / Jeffery.anthony@lgcgroup.com

² The University of Adelaide, SA 5005, AUSTRALIA

³ Hartley Grove, Urrbrae PMB 1 Glen Osmond, AUSTRALIA

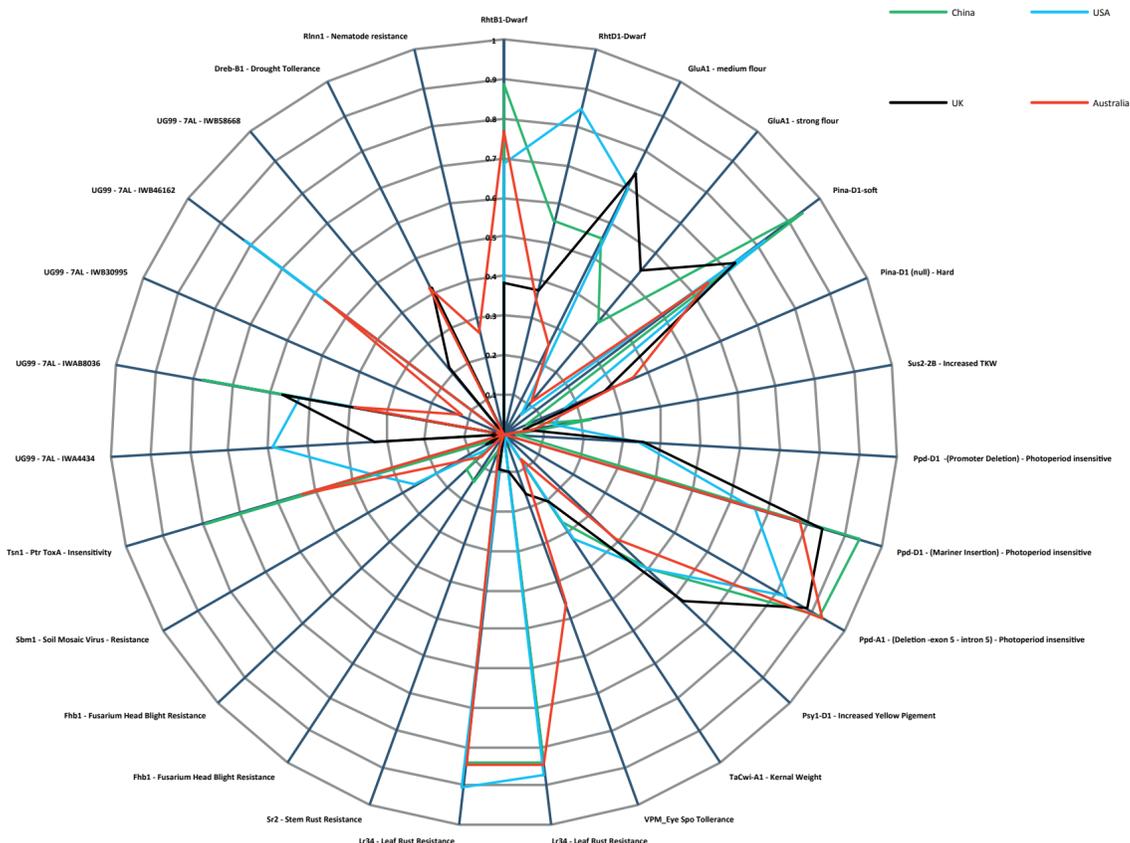
Introduction

Greater availability of high-throughput genotyping methods has led to an explosion of single nucleotide polymorphism (SNP) sequence data from plant genomes. This has enabled the discovery of large numbers of potential SNP markers for varietal improvement. Translation of potential markers from large-scale surveys into tools useful for breeding programs has been the goal. Over the past five years, a collection of quantitative trait loci (QTL) related to yield, quality and disease resistance, identified in wheat, have been converted into KASP™ SNP assays by the scientific community. The use of KASP genotyping as a cost-effective, efficient tool for introgression of important traits has now been widely published.

The current study is offered as an example of how KASP genotyping assays for QTL can be used to survey potential breed-lines for crop development using these publically available markers. We selected wheat accessions from around the world including varieties currently used for breeding programs in Australia, and the current UK recommended list. We included historical breeding accessions from both China and the USA. We sought to highlight the robustness of KASP genotyping chemistry by comparing crude (hotshot) and sbeadex™ extraction methods, both well established in agricultural biology.

We also aimed to highlight that KASP genotyping is a convenient, platform agnostic technology that is scalable. We performed experiments on both the high-throughput (1,000,000 data-point / day) SNPline™ from LGC and Douglas Scientific's Nexar™, as well as commonly used low-throughput qPCR machines (1,000 data-point / day). These genotyping results are available in full through our online Assay Search Tool (www.lgcgroup.com/assays/), which also holds a further 8,000 wheat assays. We hope that the results will be useful for the wheat breeding community to utilise and add to in the future.

QTL marker frequency by country



Methods

Wheat accessions

All wheat accessions except those from Australia were provided by Mick Ambrose and Adrian Turner of the Germplasm Resources Unit, John Innes Centre. Australian accessions were provided by Melissa Garcia of Hartley Grove, Urrbrae PMB 1 Glen Osmond, AUSTRALIA.

Sampling

Seed and leaf samples were collected using LGC's Plant Sample Collection Kit™.

DNA extraction

Total genomic DNA was isolated from plant tissue using either hotshot NaOH crude extraction method or LGC's sbeadex DNA extraction chemistry.

Genotyping

SNP genotyping was performed using LGC's KASP genotyping chemistry. The number of PCR cycles required to reach the reaction endpoint was compared, as well as the allele call-rate.

Platforms

We ran 154 samples across 50 KASP SNP assays. These were run in their entirety on the LGC SNPline to provide baseline data. Subsets of these samples and assays were also then run on the Nexar, plus two additional market standard qPCR instruments.

Assays

We selected a number of traits that have been identified as important to the wheat breeding community. We divided these into sub-categories of yield, quality and disease resistance. A full list of assays, along with the complete genotyping results and publication lists, can be found at: www.lgcgroup.com/assays/.

Results

Markers

Figure 1 shows positive trait allele frequencies for a collection of KASP SNP assays which screen for a range of yield, quality and disease resistance QTLs in global wheat accessions. The markers represent established traits with a long history of introgression as well as newly identified, disease-specific markers. The following results summary highlights some of the trends observed.

A clear global trend for alleles associated with both quality and yield was observed in both older accessions from the USA (n17) and China (n17) compared with current UK (n70) and Australian (n16) accessions. The Chinese and USA varieties predate the UK and Australian accession by 15 - 20 years.

Yield

The RhtB1 and RhtD1 reduced-height, higher-yield phenotype was originally identified in 1935. Further work and development of lines lead to the green revolution. The RhtB1 and RhtD1 SNP markers, identified in 2005, have been widely selected for with the exception of UK varieties. Varieties with either RhtB1 or RhtD1 exhibit the dwarfing characteristic; the presence of both alleles leads to a detrimental decrease in yield.

For the three photoperiod (Ppd1) alleles tested, we found a high frequency of 2/3 in all the accessions across all global regions, demonstrating pan-global relevance of this trait. Ppd1 alleles have a positive association with increased ear growth and have long been selected for.

Genes within the Dreb family are involved with osmotic regulation to temperature stress. The Dreb-B1 KASP assay provides an important marker for developing drought tolerance (1). USA accessions contained a three-fold lower incidence of drought tolerance allele Dreb-B1 whilst Australia, UK and China accessions were found to have around 41% incidence of the allele.

Quality

Starch quality is a major target for the development of all wheat accessions, and is often selected for differently across the world, and within countries, due to differences in consumer demand. Our data showed markers developed in the 1980s and early 1990s, such as two alleles for GluA1 and allele Pina-B1, have been incorporated widely in global accessions (2). More recently identified alleles such as Sus2-2B (3) and TaCwi-A1 (4) are new markers and are not widely introgressed in the newer varieties tested.

Disease resistance

Rust - Ug99 / Lr34 / Sr2

The identification of seedling resistance to Ug99 in 2014 provided a new group of alleles that could be used in pyramiding strategies for developing stem rust resistance (5). We tested KASP assays for eight Ug99 rust resistance alleles. Of the eight tested, only five gave positive frequency. IWAB46162 and IWAB8036 are the most important alleles for conferring resistance to Ug99 and produced the highest frequencies. IWAB8036 was present in accessions from all regions; WB46162 was present in all accessions except UK. IWAB58668 was only found in UK accessions. A number of the Chinese accessions tested carried 3 of the 5 Ug99 alleles.

The most widely adopted disease QTL in all samples tested was Lr34. This region is well known to be linked with broad spectrum leaf rust tolerance. The two resistant alleles for Lr34 were present in 84 - 90 % of all samples tested. Stem rust resistance QTL Sr2 was only found in Australian and Chinese accessions.

Fhb1

Fhb1 was first identified in Sumai-3 (variety included as a positive control). Fhb1 positive alleles occurred at a comparatively low level in all populations (2 - 10%). Only China contained accessions that were positive for the Fhb1 - UMN10 region marker.

Nematodes

Nematode lesions cause root damage that can lead to stunting and reduction in yield. The Rlnn1 nematode resistance marker was seen only in accessions from Australia. These were found as heterozygotes in 26% of accessions tested.

Methodology comparisons

- Both extraction methods gave informative call data for all the assays tested.
- With the sbeadex extracted samples, we were able to genotype 95% of all samples for all assays.
- Using a non-optimised hotshot crude extraction method, there was a reduction of ~10 - 15% on the genotyping call rate. This may have more to do with the differences in sample varieties and their ability to grind correctly.
- Genotyping data quality was comparable across all platforms.

Conclusions

We have successfully screened for valuable QTLs and associated trait markers in wheat using KASP genotyping.

Comprehensive genotyping data has been presented from global accessions with a wide range of trait markers. The markers include many established quality, yield and disease traits adopted in many commercial accessions, and also new traits that could be beneficial to new breeding programs. In particular, we have used newly available disease trait markers where identification has been made possible due to reduction in the cost of sequencing and the availability of public databases of wheat sequence information.

The result quality was effected to a degree by the extraction method but not the genotyping. Results from hotshot extracts could be improved with method optimisation. We were able to identify markers for a host of different traits for the first time in accessions that had not previously been tested. We were able to complete all the genotyping quickly, within a day for 154 samples against the 50 alleles tested. We have demonstrated a cost-effective and rapid way of screening for valuable traits.

Pricing for KASP™ genotyping

Key trait screening for global wheat accessions using KASP genotyping markers – “The complete solution”



Jeff Anthony and Darshna Vyas
 LGC - Genomics Division, Hoddesdon, UK
 Jeffery.anthony@lgcgroup.com

Introduction

LGC has developed a Complete Solution for genotyping “starting at the plant” and delivering data in the most cost effective manner.

- Plant tissue is collected directly into our plant sampling kit to overcome the requirement for phytosanitary regulations and standardise material ready for high throughput DNA extraction.
- DNA is extracted using sbeadex™ or KlearGene chemistry for high quality, high throughput purification.
- KASP genotyping in our dedicated labs, or on our SNPLine automation platform.
- Turnaround times 3 weeks*.

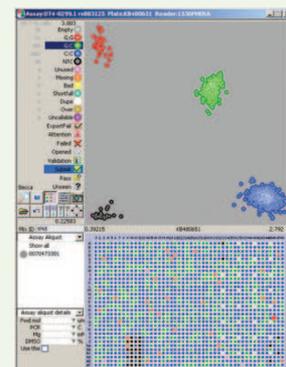


KASP™ - the complete genotyping solution

KASP markers	5	10	48	96	192
Price per sample from**	\$1.90 £1.20 €1.60	\$2.30 £1.50 €2.10	\$4.30 £2.75 €3.80	\$4.70 £3.00 €4.10	\$5.40 £3.50 €4.80

** USD, GBP, EUR.
 Including DNA extraction, new assay design and genotyping service.

KASP genotyping data



Conclusion

The ability to screen whole or partial populations for disease resistance or yield has historically been very cost constrained.

By using LGC’s genotyping services you can screen more samples on more SNP markers faster.

Or run in house using validated KASP assays.

* If need faster talk to us about fast turnaround.