

Marker Assisted Selection of Soft Red Winter Wheat for Pest Resistance

Jerry Johnson^{1*}, Zhenbang Chen¹, Lilian Miranda¹ and Yong Weon Seo²

¹Crop and Soil Sciences, University of Georgia, Griffin Campus, Griffin, GA 30223

²Div. of Biotechnology, Korea University, Seoul 136-713, Republic of Korea

* Corresponding author: jjohnson@griffin.uga.edu

Abstract

Soft red winter wheat, accounting for 15-20 percent of total wheat production, is primarily grown in the east region of the United States. The majority of the wheat cultivars grown in the U.S. are developed from public breeding programs. Marker-Assisted Selection (MAS) is suited for introgression of major genes and QTLs in wheat breeding programs for cultivar development. The USDA-ARS has established four wheat regional genotyping laboratories (GL) to assist programs in the use of MAS. The Wheat Coordinated Agricultural Project (Wheat CAP) has also been funded that includes 20 public breeding programs and the four GL's to identify molecular markers linked to quantitative disease and insect resistance and also quality traits and to use MAS to incorporate useful traits into high yielding cultivars. As part of wheat CAP, our lab is mapping a QTL for adult stripe rust resistance in two populations. To improve insect and disease resistance, MAS has been used in our lab to introduce resistant genes for Hessian Fly (*H13*), leaf rust (*Lr19*, *Lr37*, *Lr46*), powdery mildew (*Pm1*, *Pm21*), stripe rust (*Yr5*, *Yr15*, *Yr17*, *Yr26*, *Yr29*), Fusarium head blight (QTLs 3BS and 5AS), and barley yellow dwarf (*BYV 2* and *3*). A sequence characterized (SCAR) marker for curl mite and powdery mildew resistance, a SNP marker for *Yr5*, and a SCAR marker for Hessian fly resistance have also been developed. An overview of the MAS strategies of our program will be presented.

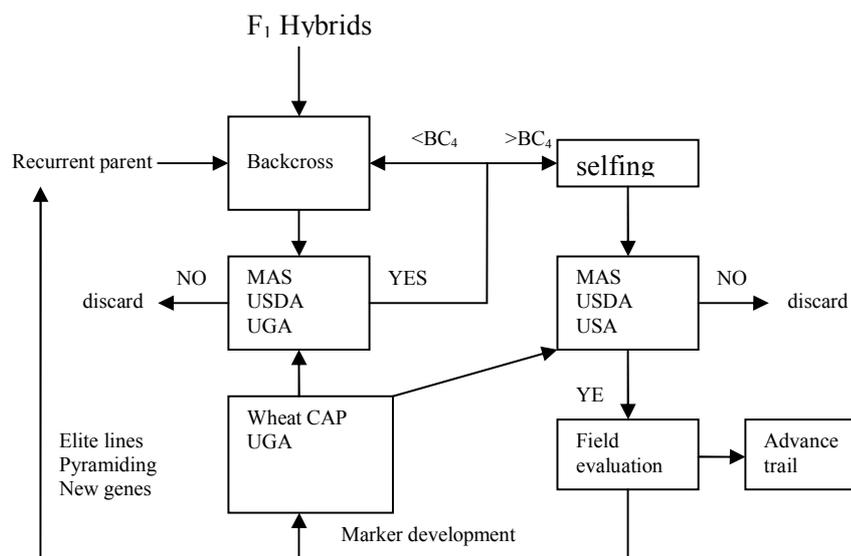
Introduction

Soft red winter wheat (SRWW) (*Triticum aestivum* L. em Thell) is widely grown in the southeastern region of the United States. SRWW accounts for 15-20% of the total wheat production in the US. Average yields of SRWW have doubled from 1300 to 5000 kg/ha during 40 years before 1990. From 1990 and 2000, grain yields have increased 50% (Snyder 2000). Breeders are continuously seeking ways to use new technology for grain yield improvement and to incorporate pest resistance. Major pests in the US include leaf rust, stripe rust, powdery mildew, and Hessian fly. Pyramiding resistant genes and releasing new cultivars resistant to multi-pests and multi-races can effectively control the incidences of diseases and insects and reduce the cost of production. The introduction of some traits into plants can be very difficult and expensive. Molecular marker assisted selection (MAS) has made it easier or even possible in some cases to tract resistant genes during cultivar development and prevent the loss of original favorable genes. Several MAS projects in the USDA in partnership with universities, Initiative for the Future of Agriculture and Food Systems (IFAFS) and a Wheat Coordinated Agricultural Project, have nationally supported the molecular marker development and application in public breeding programs. These projects have resulted in the development of DNA markers closely linked to resistant genes to fungi, insects, and viruses (Anderson, 2000; Dubcovsky, 2000). Thus, these projects have allowed breeders to implement and expand MAS within their breeding programs. Information and protocols are publicly available from these MAS projects

(maswheat.ucdavis.edu). Since the majority of commercial cultivars grown in the US are developed from public wheat breeding programs, the USDA has established Regional Genotyping Laboratories to assist programs in the use of MAS by high-throughput genotyping equipment for extraction and marker screening procedures.

Methods and strategies

Backcross breeding has been employed in our program to incorporate exotic and native resistant genes into our locally adapted genetic backgrounds, AGS2000 and its derivatives. Among 1200 crosses that our program makes annually, about 250 are backcrosses. Backcrossed progenies (BC_1) are selected by either molecular markers or traditional screening when correspondent markers are not available. Resistant plants identified by DNA markers or phenotyping are used for further backcrossing or selfing when the donor genetic background is very close to the recurrent parents. For the crosses with target genes that do not have molecular markers, these crosses will be progressed to BC_4 or BC_5 to generate iso-genic lines, which would be excellent materials for marker development or gene discoveries. A diagram of our Marker Assisted Backcross Breeding (MABB) is shown in Fig. 1.



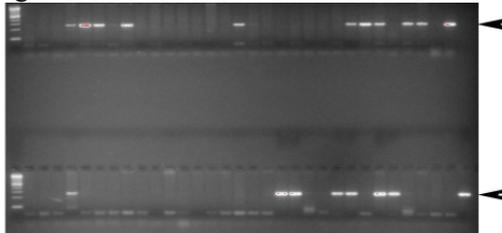
Breeding materials for recurrent parents are mainly our local adaptive cultivars and their derivatives. Donor parents for resistant genes are selected from both native and exotic sources. Breeding materials are also shared from five public breeding programs (SunGrain) in the southeastern region of the US. Our objective is to improve the milling quality and resistance to leaf and stripe rust, powdery mildew, Fusarium head blight (FHB), and Hessian fly, and maintain high grain yield.

Results and Discussions

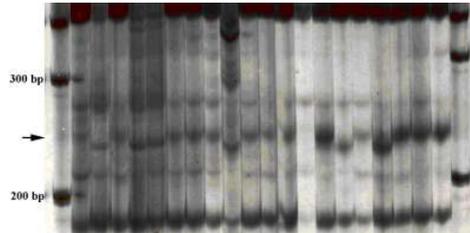
Efficiency and Effectiveness: MAS can be applied at the early seedling stage. Fifty percent of the seedlings in BC_1 can be eliminated after screening when one marker is employed. 75% discarded if two independent markers are used, which improves the reliability of selection and also increases the efficiency of backcross breeding. However, the effect of enhancing the

recurrent genetic background recovery with MAS can be very limited for wheat, which has a genome size of over 6 times that genome of maize (Frisch and Melchinger 2005).

Fig.2



A. Screening using Lr37, PCR products, stained with ethidium bromides and displayed on 2% agarose gel.



B. Screening for Lr34 with SSR marker XGWM259, PCR products stained with silver and displayed on 6% acrylamids gel.

The effectiveness of a marker is not only depends on its relationship (the genetic distance between the target gene and markers) to the gene but also on how friendly the marker can be used. A user-friendly marker can be easily applied for screening and provides unambiguous result for selection (Fig.2A) while markers like XGWM259 for Lr34 are relatively more time consuming and often not easy to differentiate polymorphisms (Fig.2B).

Dissecting QTL: MAS are increasingly being used for dissecting and tagging QTLs. QTLs are getting lot of attentions because they are often underlying economically very important traits, such as yield (Diab, *et al.*2007), quality (Kuchel, *et al.*2006), and some disease resistance (Jia, *et al.* 2005). In our backcross breeding program, QTLs are employed for FHB resistance. SSR markers on 3BS, 5AS and 2DL were used to assist the selections for QTLs of FHB resistance from 'Sumai3'. Large chromosome fragments were involved on 3B and 5AS. Yield dragging has been associated with QTL on 3BS. Multiple QTLs spread over the genome for one trait and multiple markers for one QTL have hindered their applications in breeding programs.

Secondly, average ratio of physical distance and genetic distance is about 5.5 Mb/cM, the ratio can be as high as 12Mb/cM because crossovers on some regions are limited (Faris, *et al.* 2000; Gill, *et al.* 1996; Stein, *et al.* 2000). A fragment of 20 cM can be a large portion of a chromosome arm. Selection with multiple markers for several QTLs for one trait may introduce a significant portion of the donor's genome, which can have undesirable traits. Dissecting QTLs will reveal the nature of each QTL and can develop markers closer to the gene and increase the efficiency of MAS (Harjes, *et al.* 2008).

Molecular markers are being developed in Wheat CAP, the UGA molecular lab., and other available sources. Five of our recurrent parents were genotyped with 2000 SSRs at the USDA Genotyping Lab. Molecular markers for *Lr37*, *Lr21*, *H13*, *BYV2*, *BYV3*, *Yr15*, *Yr5*, and *FHB* QTLs on 3BS, 5AS and 2DL had been used for screening progenies. MAS is applied from first generation of backcross until homozygous plants are identified. About 5000 data point are annually generated from the USDA Genotyping lab and the UGA molecular lab. Populations are being mapped for milling quality traits and for stripe rust, barley yellow dwarf, and Hessian fly resistance. Our program has developed a sequence characterized (SCAR) marker for curl mite

resistance and powdery mildew resistance, a SNP marker for *Yr5* resistance, and a SCAR marker for Hessian fly resistance. MAS is a powerful tool for breeders in the development of cultivars. New technology for wheat such as SNPs (single nucleotide polymorphisms) and DART (Diversity Array) will play an important role in the future.

References

Anderson, J.A. 2000. Marker-assisted selection of disease resistance genes in wheat. p. 71-84. In M.M. Kohli and M. Francis. Application of biotechnologies to wheat breeding . CIMMYT

Diab AA, Fahmy AH Hassan OS, Nachit MM, and Momtaz OA. (2007) Identification of Chromosomal Regions and Genetic Contributions of Genes Controlling Yield and Other Agronomic Traits in Durum Wheat Grown under Different Egyptian Environmental Conditions. World J. Agric. Sci.. 3:401-422.

Doubcovshy, J. 2004. Marker-assisted selection in public breeding programs. Crop Sci. 44:1895-1898.

Faris J D, Haen K, Gill B S. Saturation mapping of a gene-rich recombination hot spot region in wheat. Genetics. 2000;154:823–835.

Frisch M and Melchinger AE. (2005) Selection Theory for Marker-Assisted Backcrossing. Genetics, Vol. 170, 909-917, June 2005, Copyright © 2005

Gill K S, Gill B S, Endo T R, Taylor T. Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics. 1996;144:1883–1891

Harjes CE, Rocheford TR, Bai L, Brutnell TP, Kandianis CB, Sowinski SG, Stapleton AE, Vallabhaneni R, Williams M, Wurtzel ET, Yan J, and Bukler ES. (2008) Natural Genetic Variation in Lycopene Epsilon Cyclase Tapped for Maize Biofortification. Science. 319:330-333.

Jia G, Chen P, Qin G, Bai G, Wang X, Wang S, Zhou B, Zhang S, Liu D. (2005) QTLs for Fusarium head blight response in a wheat DH population of Wangshuibai/Alondra's'. Euphytica, 146:183-191.

Kuchel H, Langridge P, Mosionek, L, Williams, K, Jefferies S.P. (2006) The genetic control of milling yield, dough rheology and baking quality of wheat. Theor. Appl. Genet..112. 1487-1495.

Snyder, CS. (2000) Soft Red Winter Wheat –High Yields Achieved with Intensive Management. Better Crop 84:23-25.

Stein N, Feuillet C, Wicker T, Schlagenhauf E, and Keller B. (2000) Subgenome chromosome walking in wheat: A 450-kb physical contig in *Triticum monococcum* L. spans the *Lr10* resistance locus in hexaploid wheat (*Triticum aestivum* L.). Proc Natl Acad Sci.. 97: 13436–13441.