Marker implementation in wheat breeding programs: status and prospects

Gandon B., Crépieux S.

in

Molina-Cano J.L. (ed.), Christou P. (ed.), Graner A. (ed.), Hammer K. (ed.), Jouve N. (ed.), Keller B. (ed.), Lasa J.M. (ed.), Powell W. (ed.), Royo C. (ed.), Shewry P. (ed.), Stanca A.M. (ed.).
Cereal science and technology for feeding ten billion people: genomics era and beyond

http://www.ciheam.org/
http://om.ciheam.org/

Marker implementation in wheat breeding programs: Status and prospects

B. Gandon and S. Crépieux
Limagrain, Site Ulice, Av. G. Gerschwin, ZAC Les Portes de Riom, RIOM 63204, France

SUMMARY – There has been a large and rapid accumulation of genomics tools in wheat during the last decade, which has seen the development of both genetic and physical maps, as well as large amounts of EST sequence information. These developments have been coupled with the emergence of high throughput technologies and new biometric tools, which have allowed advances in molecular marker technology and implementation. Wheat breeders now have powerful tools with which to select traits that are difficult to improve by conventional means (e.g. laborious field scorings, low heritability, destructive or expensive assays). DNA markers have had a major impact on the identification, introgression and combination of agronomic traits controlled by major genes, but have had little impact on the selection of quantitative trait loci (QTL). Implementation of these DNA based technologies in conventional breeding programs through Marker Assisted Selection (MAS) is now gaining considerable importance as the unit cost per assay decreases. This report will present an overview of the possible marker applications within wheat breeding programs and some considerations about their future expansion.

Introduction

Wheat is the second largest crop in the world with 624 millions tons produced in 2005, 22% of which was grown in Europe (FAO, 2006). In the next twenty years, cereal demand is estimated to grow by 50% worldwide, with a substantial increase in animal feed requirements (Reynolds and Borlaug, 2006). In Europe, since the 1950's, wheat yields have continually increased by an average of 0.1 tonne/ha, which is due to both to improvements in intensive agricultural practices and the release of new varieties. Biotechnology is expected to play a major role in increasing wheat productivity by improving the control of both biotic and abiotic stress, increasing water and nitrogen use efficiencies and providing special varieties for different industrial end-uses.

The rapid development of molecular tools for wheat

The first pre-requisite for genome analysis is the development of molecular markers and a linkage map. Since the first wheat RFLP maps were published in 1995, the major contribution to mapping efforts has been the development of 2,200 of SSR markers (http://wheat.pw.usda.gov/GG2/), many through public-private consortia. Mapping information have been improved by the development of composite maps (http://www.grs.nig.ac.jp/wheat/komugi/maps) and these maps are now aligned physical map via the bin mapping of selected SSRs on deletion lines (http://wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical/).

More recently, BAC libraries, offering both genome-wide coverage as well as chromosome-specificity (Safar *et al.*, 2004) have been developed, which constitute a powerful tool for map-based cloning. A recent example of map-based cloning is the isolation of the *Ph1* locus (Griffiths *et al.*, 2006), which prevents homoeologous chromosome pairing during meiosis in both durum and bread wheat.

The International Triticeae EST Cooperative (ITEC: http:/wheat.pw.usda.gov/genome/) has released 1,350,395 ESTs for the *Triticeae* (853,401 for wheat) which are all publicly available (http://www.ncbi.nlm.nih.gov/). A chromosome bin map of 16,000 EST loci has also been published (Qi et al., 2004) giving the opportunity to develop SNP markers for high density mapping. However, the task of developing SNPs in wheat is much harder compared to other crop species due to the hexaploid nature of the genome, which leads to difficulties in designing genome-specific PCR primers.

However the NSF plant genome program has made the first attempt to use EST sequences for SNP development and has so far delivered 17,174 primers, of which 1102 are reported as being polymorphic (http://wheat.pw.usda.gov/SNP/).

These genomic tools have been used in a wide range of applications resulting in the mapping of many agronomically important genes and QTL, as well as in map-based gene cloning of some key loci (Table 1). In wheat, more than 100 genes have been mapped mainly for disease, nematode or insect resistance (for review see Feuillet & Keller, 2005). Published markers are now available for an increasing number of key traits prevalent in European germplasm, as well as for traits from exotic material enabling their rapid introgression via MAS:

(i) Brown rust and yellow rust: *Lr9*, *Lr10*, *Lr19*, *Lr24*, *Lr37/Yr17/Sr38*, *Yr15*, *Lr34/Yr18*, *Lr46/Yr29*

(ii) Eyespot: *Pch1*

(iii) Powdery mildew: *Pm3*, *Pm4b*, *Pm8/Pm17*, *MlRe*

(iv) *Septoria tritici*: *Stb6*

(v) *Fusarium* resistance: QTLs coming from Sumai3 (*Qfhs.ndsu-3BS*), Frontana, European sources

(vi) Genes involved in plant development: *RhtB1b* (*Rht1*), *RhtD1b* (*Rht2*), *Rht8*, *Vrn1*

(vii) Quality: high molecular weight glutenin PCR specific alleles, puroindolins, and waxy wheats.

Markers located up to 5 cM from the gene of interest can be used to increase the frequency of the linked trait in the breeding program. Then, the accuracy of MAS can be improved by the use of two flanking markers. Nevertheless, the ideal scenario is the development of markers from the target gene sequence itself. This approach avoids the problem of recombination between the marker and the trait and allows the selection for or against particular alleles at that locus. These so-called "perfect markers" have been developed for the waxy gene (*Wx-B1* allele; McLaughan *et al.*, 2001), the *RhtB1b* and *RhtD1b* loci (Ellis *et al.*, 2002), the *Vrn1* and *Vrn2* loci (Yan *et al.*, 2003; 2004) and several resistance genes. In the case of disease resistance loci, resistance genes analogs (RGA's) provide a valuable resource for marker development (eg Cre1, Lr10) and several genes of interest have now been cloned (Table 1). The development of more chromosome specific libraries, in combination with the high-density mapping and the high level of synteny between grass species should accelerate the isolation of more genes over the next few years.

Table 1. Important agronomic genes isolated in wheat

Gene	Trait	Reference
Cre1, Cre3	Nematode resistance	De Majnik *et al.* (2003)
Lr10	Leaf rust	Feuillet *et al.* (2003)
Lr21	Leaf rust	Huang *et al.* (2003)
Lr1	Leaf rust	Tyrka *et al.* (2004)
Pm3b	Powdery mildew	Yahiaoui *et al.* (2005)
Tsn1	Tan spot	Haen *et al.* (2004)
Vrn1, Vrn2	Vernalization	Yan *et al.* (2003) (2004)
PinA, PinB	Hardness	Beecher *et al.* (2002)
Q	Domestication trait	Faris *et al.* (2003)
Ph1	Chromosome pairing	Griffiths *et al.* (2006)
HMWG	Gluten strength	De Bustos *et al.* (2001)
Wx-B1	Waxy gene	McLaughan *et al.* (2001)

The major revolution for wheat breeding in the last decade has been the access to information at the DNA level. These first results have given crucial information for MAS of simply inherited traits and the possibility to approach more quantitative ones.

On technological front, recent improvements in automated DNA sequencing would allow the whole wheat genome to be sequenced (http://www.wheatgenome.org/), which was inconceivable even just a few years ago due to its large size (i.e. the wheat genome is 5X larger than the human genome). It is also now possible to envisage the use of sequencing as a direct genotyping method. Plant genotyping has also reaped the benefits from developments made in human research in the area of new high throughput technologies based on DNA chips and SNPs (for review see Syvanen, 2005). However for applied MAS, some technologies may not be appropriate for all applications (e.g. genome-wide genotyping chips would be an expensive way to follow a few key loci in a segregating population). In addition, new techniques such as TILLING (Target Induced Local Lesions In Genomes; Till *et al.*, 2003) will enable the rapid identification of new alleles in candidate genes in either EMS-mutagenised populations or by surveying genetic resources. For example, Slade *et al.*, (2005) have recently used of this technique to identify allelic series of variants for the waxy gene on the A and D genomes.

The current impact of DNA tools on wheat breeding

Despite the huge investment in genomic and molecular tools, we have not yet seen their impact on wheat breeding through cultivar release for several reasons. Firstly, there were not enough "perfect markers" available in wheat during these last years. Breeders are still relying on phenotypic information for critical traits when there is a possibility of recombination between the gene and the marker. Secondly, although genotyping costs were rapidly decreasing the costs of marker deployment on a large-scale were still relatively high. The situation is now changing as more markers of real interest for the breeders are available and MAS can be applied to wheat breeding in a variety of ways:

The use of informative markers

(i) Characterisation of the parental lines with markers (plus phenotypic data) in order to select the best parental combinations. For example, markers can give information on the origin of disease resistance in breeding germplasm, the number of genes involved, and the resistance mechanisms (e.g. adult plant resistance or race specific resistance). Identifying the number of major genes segregating in any particular cross can also help to define the optimal size of the breeding population.

(ii) Pyramiding gene combinations in segregating progenies by selecting for or against both dominant and recessive alleles, which can be done in very early generations e.g. F2 or F3 enrichment (Bonett *et al.*, 2005). This application of MAS is particularly important when the trait is difficult/time-consuming to select for in the field as for instance the lowly heritable traits, for which selection can be done only in late generations or when there are favourable conditions of scoring.

(iii) Introgression of genes via a backcross (BC) strategy. Compared to conventional backcross programs, marker-assisted backcrossing allows the rapid introgression of a target trait, which can be recessive, in conjunction with recovery of the recurrent parent background. The use of markers is particularly important when introgressing genes from exotic germplasm in order to reduce linkage drag.

Overall MAS efficiency can be increased by using half seed analysis in F2 enrichment , back cross or DH procedures. The kernels are cut in half and DNA is then extracted from the endosperm, which provides enough template for 100 marker amplifications. After marker selection, the half kernels, carrying the embryo, can be sown for further inbreeding or backcrossing. The ability to analyse half grains saves space in the field or glasshouse and avoids the tedious identification of single plants. A practical example will be shown for the introgression of disease resistance to develop pre-breeding material.

Marker assisted recurrent selection

If Marker Assisted Selection (MAS) works well for "simple traits" in strategies aiming to introgress, backcross or follow a few genes, the situation is less clear for quantitative traits. In the past, the identification of marker-trait linkages has relied on the development of experimental bi-parental crosses; however it is difficult to extrapolate such QTL results to other populations, and this in turn limits their utility in conventional wheat breeding schemes. The other drawback is that such experimental populations represent a large drain on resources, both in terms of phenotyping and marker screening. Thus, there are no publications on the efficient use of markers for the selection of quantitative traits such as yield and end-use quality, which are main breeding targets in any wheat breeding program. Nevertheless, the reduction in the cost per data-point and a better availability of markers across the whole genome, has allowed QTL detection and MAS to move, within the private companies, from "pilot experiments" (one or two bi-parental population screened, used for QTL detection then for MAS) to numerous populations, increasing the chance of MAS success (enhanced numbers game). The best example, and probably the first demonstration of marker efficiency for quantitative traits, is shown in Johnson's 2004 publication on his maize MARS programs (the R of MARS for Recurrent, meaning numerous cycles of re-crossing in early generations). It was shown that a large-scale use of markers in numerous bi-parental maize populations, first for QTL detection then for MARS on yield (rapid cycles of recombination and selection on associated markers for yield) allowed increased efficiency in long-term selection by increasing the frequency of favourable alleles.

The MARS strategy starting in wheat is based on the combination of multiple breeders' populations, taking into account pedigrees and optimised linkage disequilibrium to facilitate QTL detection. The ultimate goal of this kind of project is to provide breeders indices on all types of traits in order to help the decision process and target early re-crossing and selection. Nevertheless, the next real challenge behind the use of this genome-wide genotypic information is to gain a better understanding of complex traits, whose expression is also governed by pleiotropy, epistasis, hybrid vigour or genome stability and G x E interactions.

Genetic resources

To complement a better exploitation of the variability existing in elite cultivars, access to new gene diversity is needed for successful long-term breeding strategy (i.e. better management of wheat disease resistance). For example, the simultaneous introgression and detection of QTL using advanced backcross populations involving exotic donors (Tanksley and Nelson, 1996) was first used in tomato. This approach allows the detection and transfer beneficial alleles from un-adapted germplasm into elite material and has also recently been used in barley (Pillen *et al.,* 2003) and for introgressions from synthetic wheats (Huang *et al*., 2003; 2004). This kind of activity remains expensive for single initiatives so that collaborations would be welcome.

Conclusion

Markers have gradually been integrated into breeding programs, not as a big revolution replacing conventional breeding, but as an additional tool. This integration is only possible through a close interaction between breeders and molecular labs so that there is a mutual understanding of what is required for an optimised use of markers within the breeding schemes. The question of the use of markers is now behind. It is more a question of allocation of means, keeping an appropriate balance between phenotyping and molecular technology. Undoubtedly the use of MAS will go on increasing in the future as costs continue to decrease and more markers for traits of interest become available.

References

Beecher, B., Bettge, A., Smidansky, E. and Giroux, M.J. (2002). Expression of wild-type PinB sequence in transgenic wheat complements a hard phenotype. *Theoretical and Applied Genetics,* 105: 870-877.
Bonnett, D.G., Rebetzke, G.J. and Spielmeyer, W. (2005). Strategies for efficient implementation of molecular markers in wheat breeding. *Molecular Breeding,* 15: 75-85.

De Bustos, A., Rubio, P., Soler, C., Garcia, P. and Jouve, N. (2001). Marker assisted selection to improve HMW-glutenins in wheat. *Euphytica,* 119: 69-73.

De Majnik, J., Ogbonnaya, F.C., Moullet, O. and Lagudah, S. (2003). The Cre1 and Cre3 nematode resistance genes are located at homeologous loci in the wheat genome. *MPMI,* Vol 16, 12: 1129-1134.

Ellis, M.H., Spielmeyer, W., Rebetzke, G.J. and Richards, R.A. (2002). "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. *Theor. Appl. Genet.,* 105: 1038-1042.

Faris, J.D., Fellers, J.P., Brooks, S.A. and Gill, B.S. (2003). A Bacterial Artificial Chromosome Contig Spanning the Major Domestication Locus Q in Wheat and Identification of a Candidate Gene. G*enetics,* 164: 311-321.

Feuillet, C., Travella, S., Stein, N., Albar, A., Nublat, A. and Keller, B. (2003). Map based isolation of the leaf rust disease resistance gene Lr10 from the hexaploid wheat *(Triticum aestivum* L.) genome. *PNAS* vol 100, 25: 15253-15258.

Feuillet, C. and Keller, B. (2005). Molecular markers for disease resistance, The example wheat. In: "Molecular Marker System in Plant Breeding and Crop Improvement". Eds Lorzh and Wenzel *Biotechnology in Agriculture and Forestry*, 55: 353-370.

Griffiths, S., Sharp, R., Foote, T.N., Bertin, I., Wanous, M., Reader, S., Colas, I. and Moore, G. (2006). Molecular charachterization of *Ph1* as a major chromosome pairing locus in polyploid wheat. *Nature,* 439, 9: 749-752.

Haen, K.M., Lu, H.J., Friesen, T.L. and Faris, J.D. (2004). Genomic targeting and high-resolution mapping of the *Tsn1* gene in wheat. *Crop Science,* 44: 951-962.

Huang, X.Q., Kempf, H., Ganal, M.W. and Röder, M.S. (2004). Advanced back-cross QTL analysis in progenies derived from a cross between a German elite wheat variety and a synthetic wheat. *Theor Appl Genet,* 109: 933-943.

Huang, X.Q., Cöster, H., Ganal, M.W. and Röder, M.S. (2003a). Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat *(Triticum aestivum* L*.). Theor Appl Genet.,* 106: 1379-1389.

Johnson, R. (2004). MARS, association genetics, importance of accurate phenotypic data. *Plant Breeding Reviews*, (J. Janick, ed.), 24: 293-309.

McLauchlan, A., Ogbonnaya, F.C., Hollingsworth, B., Carter, M., Gale, K.R., Henry, R.J., Holton, T.A., Morell, M.K., Rampling, L.R., Sharp, P.J., Shariflou, M.R., Jones, M.G.K. and Appels, R. (2001). Development of robust PCR-based DNA markers for each homoeo-allele of granule-bound starch synthase and their application in wheat breeding programs. *Australian Journal of Agricultural Research,* 52(11-12): 1409-1416.

Pillen, K., Zacharias, A. and Léon, J. (2003). Advanced backcross QTL analysis in barley (*Hordeum vulgare* L.). *Theor Appl Genet.,* 107: 340-352.

Qi , L.L. (2004). A chromosome bin map of 16,000 Expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. *Genetics,* 168: 701-712.

Reynolds, M.P and Borlaug, N.E. (2006). Centenary review. Applying innovations and new technologies for international collaborative wheat improvement. *Journal of Agricultural Science,* 144: 95-110.

Safar, J., Bartos, J., Janda, J., Bellec, A., Kubalakova, M., Valarik, M., Pateyron, S., Weiserova, J., Tuskova, R., Cihalikova, J., Vrana, J., Simkova, H., Faivre-Rampant, P., Sourdille, P., Caboche, M., Bernard, M., Dolezel, J. and Chaloub, B. (2004). Dissecting large and complex genomes, flow sorting and BAC cloning of individual chromosomes from bread wheat. *Plant Journal,* 39: 960-968.

Slade, A.J., Fuerstenberg, S.I., Loeffler, D., Steine, M.N. and Faccioti, D. (2005). A reverse genetic, non transgenic approach to wheat crop improvement by TILLING. *Nature Biotechnology,* 23: 75-81.

Syvanen, A-C. (2005). Toward genome-wide SNP genotyping. *Nat. Genet.*, 37: 5-10.

Tanksley, S.D., Grandillo, S., Fulton, T.M., Zamir, D., Eshed, Y., Petiard, V., Lopez, J. and Beck-Bunn, T. (1996). Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium. Theor Appl Genet.,* 92: 213-224.

Till, B.J., Reynolds, S.H., Greene, E.A., Codomo, C.A., Enns, L.C., Johnsonn, J.E., Burtner, C., Odden, A.R., Young, K., Taylor N.E., Henikoff, J.G., Comai, L. and Henikoff, S. (2003). Large-Scale Discovery of Induced Point Mutations With High-Throughput TILLING (2003). *Genome Research,* Vol. 13, 3: 524-530.

Tyrka, M., Blaszczyk, L., Chelkowski, J., Lind, V., Kramer, L., Weilepp, M., Wisniewska, H. and Ordon, F. (2004). Development of the single nucleotide polymorphism marker of the wheat Lr1 leaf rust resistance gene. *Cell Mol Biol Lett.,* 9: 879-889.

Yahiaoui, N., Srichumpa, P., Dudler, R. and Keller, B. (2005). Genome analysis at different ploidy

levels allows cloning of the powdery mildew resistance gene Pm3b from hexaploid wheat. *Plant J.,* 37: 528-538 .

Yan, L., Loukoianov, A., Tranquili, G., Helguera, M., Fahima, T. and Dubcovsky, J. (2003). Positional cloning of the wheat vernalization gene *VRN1. PNAS,* vol. 100, 10: 6263-6268.

Yan, L., Loukoianov, A., Blechl, A., Tranquilli, G., Ramakrishna, W., SanMiguel, P., Bennetzen, J.L., Viviana Echenique, V. and Dubcovsky, J. (2004). The Wheat *VRN2* Gene Is a Flowering Repressor Down-Regulated by Vernalization. *Science,* Vol. 303, 5664: 1640-1644.