Application of Biotechnologies to Wheat Breeding

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The Application of Molecular Markers in Wheat Improvement at CIMMYT

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Abstract

Molecular markers have many applications to crop improvement. When tightly linked to genes of interest, they can be used to indirectly select for the desirable alleles. In addition, molecular markers can be used for dissecting polygenic traits into their Mendelian components or quantitative trait loci (QTL), thus increasing our understanding of the inheritance of such traits. Scientists in the Applied Biotechnology Center at CIMMYT have been collaborating with the wheat program in the development and use of molecular markers for wheat improvement. Development of cultivars with durable leaf rust and yellow rust resistance is an important breeding objective of CIMMYT. Resistance to leaf and yellow rust is controlled by a number of minor genes and is referred to as adult plant resistance (APR). Linkage mapping and bulked segregant analysis are being used with several recombinant inbred line populations segregating for leaf rust and yellow rust resistance in order to find molecular markers associated with APR genes. hree markers with significant association with durable leaf rust resistance have been found in one population. In a second population, five and two quantitative trait loci (QTL) were detected for resistance to leaf and yellow rust, respectively. In addition, molecular markers are being used to transfer Thinopyrum intermedium *derived resistance to barley yellow dwarf virus into different bread wheats. Markers are also being used to detect wide cross derivatives with enhanced meiotic pairing that would facilitate the detection of introgression of chromosomal segments from wild relatives of wheat that carry important biotic and abiotic attributes into cultivated wheat.*

Introduction

Molecular markers (DNA markers), reveal sites of variation at the DNA level. These markers have the advantage of being numerous in nature and not affected by the environment as in the case of morphological markers. Molecular markers reveal neutral sites of variation and therefore, unlike morphological markers, do not show phenotypic effects. The expression of most genes is quantitative in segregating populations and is confounded by the environment.

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Progress of identifying molecular markers in wheat has been comparatively slow due to its large genome size and low levels of polymorphism at the molecular level. However, there have been considerable successes in developing molecular markers to tag leaf rust resistance and other genes derived from the wild species (reviewed in Hoisington *et al.*, 1998). This is often possible due to the presence of large segments of introgressed chromatin from the wild species in wheat background and high frequency of polymorphism between the introgressed segments and the corresponding counterparts of the wheat genome. Further, once molecular polymorphisms are identified and genes derived from wild species are tagged, these markers often become stable enough to be used in practical breeding programs due to lack of pairing between the introgressed chromosomal segment and the wheat homoelogs. The presence or absence of genes and the number of genes for traits such as adult plant resistance (APR) to leaf rust and yellow rust can be suggested or hypothesized in different breeding populations. However, identification of these genes and combining them together in cultivars can be facilitated when markers are available for the genes of interest.

In the Applied Biotechnology Centre at CIMMYT, efforts are underway to develop molecular markers associated with various biotic parameters in wheat such as durable leaf and yellow rust resistance, barley yellow dwarf virus (BYDV) resistance/tolerance and resistance to head scab. Considerable success has also been made in identifying molecular markers with tight linkage to genes controlling aluminum tolerance in rye (*Secale sereale)* as well as studying genetic diversity in a set of historically important wheat cultivars. Efforts are also being made to apply molecular markers developed in other laboratories in facilitating breeding efforts. The work underway in the identification of markers associated with APR, BYDV and the utilization of markers for the detection of wide hybrid derivatives of wheat with enhanced meiotic pairing will be presented in detail.

Mapping durable leaf rust resistance

Rusts are the most common diseases of cultivated wheat and the use of resistant cultivars offers the most effective form of control of the disease. Over 40 mainly race-specific leaf rust genes identified from the wheat gene pool or derived from the wild relatives of cultivated wheat are known (Knott, 1989; Roelfs *et al.*, 1992). Most of these genes have been used either singly or in combinations to develop cultivars with rust resistance. The slow rusting, durable type of resistance, is effective in adult plants and is known to be complex in inheritance (Knott and Yadav, 1993). Gavinlertvatana and Wilcoxson (1978) reported that between 3 to 21 genes could be involved in slow rusting type of resistance. The gene *Lr34* has been reported to increase the latent period, decrease the infection frequency and uridial size (Drijepondt and Pretorius, 1989). Although *Lr34* itself may not confer adequate protection against high disease pressure (Singh and Gupta, 1992), the *Lr34* complex, defined as the result of additive interaction of *Lr34* with several other slow rusting genes, would confer adequate adult

plant resistance (Singh and Rajaram, 1992; Roelfs, 1988). *Lr34* is known to be either pleotrophic or closely linked to the phenological trait leaf tip necrosis (LTN) of adult plants (Singh, 1992).

Several recombinant inbred line (RILs) populations are being used at CIMMYT with the objective of developing molecular markers associated with *Lr34* and other durable leaf rust/yellow rust resistance genes. The availability of such markers would facilitate the breeding efforts where considerable efforts are being made to combine several slow rusting genes in CIMMYT wheat germplasm for obtaining cultivars with durable resistance under high disease pressure. One such population is composed of 77 RILs derived from the cross 'Parula' (resistant) X 'Siete Cerros' (partially susceptible). Leaf rust data were available for two years from replicated field trials conducted at Ciudad Obregón (Sonora, Mexico). Initial studies with the population were conducted with restriction fragment length polymorphism (RFLP) analysis with parental screening and mapping with approximately 250 RFLP probes. Subsequently, bulked segregant analysis was conducted with 500 Operon decamer primers (Operon Technologies, Alameda, Calif.). Two bulks made from the 10 most resistant entries and 10 most susceptible entries were analyzed. However, no significant association between molecular markers and leaf rust resistance could be observed in these studies further emphasizing the difficulty associated with wheat at the molecular marker level.

A subsequent study was conducted with genomic DNA enriched for low copy sequences (William *et al.*, 1997). Screening the two bulks with 400 Operon decamer primers identified three polymorphisms between the two bulks. The three amplification products were cloned and were used as probes in Southern analysis with the 77 RIL populations. The three clones derived from Operon decamer primers OPG-05, OPI–16 and OPR–03 were designated CMTG05–500, CMTI16–1500 and CMTR03–500 respectively. Linkage analysis using MAPMAKER indicated that the two loci *Xcmtg05-500* and *Xcmti16-1500* were tightly linked with 2% recombination (LOD score of 17.1). There was no significant linkage between these two loci and the third locus *Xcmtr03-500*. There was also no linkage between the three molecular markers and leaf tip necrosis (*Ltn*).

One way analysis of variance showed significant association of the three molecular markers as well as the phenological marker, LTN, with factors controlling leaf rust resistance. Leaf tip necrosis, confirming its tight linkage with *Lr34* explained 20–30% of the total phenotypic variation. The two tightly linked loci *Xcmtg05-500* and *Xcmti16-1500,* also explained a similar proportion of the total phenotypic variation (22 – 30%). The alleles detected by *Xcmtg05-500* and *Xcmti16-1500* were present in the susceptible parent 'Siete Cerros' and were associated with susceptibility. Using these two markers, homozygous resistant parents could be detected (absence of band). The third locus *Xcmtr03-500,* the allele identified in the resistant parent 'Parula' explained approximately 10% of the total phenotypic variation. Chromosomal locations

for the three clones were determined using cytogenetic stocks of 'Chinese Spring'. The two loci *Xcmtg05-50* and *Xcmti16-1500* could be located on chromosome 7BL.Clone CMTR03–500 revealed two loci, one of that could be located on chromosome 1BS. This study has revealed two loci associated with leaf rust resistance on chromosome 7BL that seem to be as effective as *Lr34 (Ltn)* in conferring resistance as well as another component of slow rusting resistance located on chromosome 1B (William *et al.*, 1997).

Another population being used for mapping APR genes for leaf rust and yellow rust is a recombinant inbred line population (220 RILs) derived from 'Frontana' X 'INIA66'. A full molecular map is being developed using this population. Currently, the map consists of 451 markers on 33 linkage groups. The markers were derived from 118 RFLPs, 19 microsatellites (SSRs), 312 amplified fragment length polymorphisms (AFLPs) and two morphological markers (LTN, and pseudo black chaff, PBC). Phenotypic data have been collected for leaf rust for 1991/1992, 1994/1995 (Ciudad Obregón, Sonora, Mexico) and yellow rust (data collected in Toluca, Mexico, 1993). Quantitative trait loci (QTL) have been identified for both leaf rust and yellow rust resistance. Five QTLs were associated with leaf rust and two with yellow rust resistance. As in the previous study, *Ltn* explained 51% and 37% of the phenotypic variation for leaf rust and yellow rust, respectively, further indicating its strong linkage with *Lr34* and *Yr18* (Table 1). The second QTL for yellow rust resistance coincided with the gene *Sr2*, identified by its association with the morphological marker pseudo black chaff (PBC), where a QTL with minor effects was identified for leaf rust as well. Genotyping the population with new microsatellite markers and sequence tagged sites (STS) is currently underway (Khairallah *et al.*, 1998).

Other populations that are segregating for leaf rust and yellow rust and are currently being utilized in the molecular marker work include, 'Avocet' X 'Parula', 'Avocet' X 'Tonichi' and 'Avocet' X 'Pavon'. Each population has approximately 150 F_5 lines, which are being used in bulked segregant analysis and development of molecular maps.

Markers for barley yellow dwarf resistance

Barley yellow dwarf is caused by a group of phloem limited luteoviruses collectively known as the barley yellow dwarf viruses (BYDVs), and is transmitted by aphids. Significant economic losses have been reported in most cultivated cereals including wheat (Pike, 1990). Although some tolerance to BYDV has been identified in wheat, it is known to be only partially effective and is affected by environmental influence (Zhou *et al.*, 1990). Resistance to BYDV has been incorporated into cultivated wheat by tissue culture derived chromosomal translocation lines from *Thinopyrum (Agropyrum) intermedium* (Banks *et al.*, 1995; Larkin *et al.*, 1995).

Table 1. *Quantitative trait loci detected for leaf rust and yellow rust resistance in 'Frontana' x 'Inia-66' population.*

Linkage Group	Position (cM)	Likelihood ratio[a]	Phenotypic Variation (R^2%)
Leaf rust			
3B	76	15.7	5.0
4B	29	14.0	5.0
Group 6-2	12	14.4	2.0
7D	54	88.4	51.1
Group 7-2	159	15.4	12.2
Yellow rust			
3B	77	10.4	18.6
7D	54	44.9	36.6

[a] Likelihood ratio = 11.5 is equivalent to LOD score of 2.5.

Efforts are underway to develop molecular markers and utilize them to transfer the introgressed chromosomal segment of *Th. intermedium* into different wheat backgrounds and to study its effectiveness and inheritance against the background of the tolerant gene *Bdv1* identified in CIMMYT wheats (Singh *et al.*, 1993). Currently, four RFLP markers and two microsatellite markers have been identified and a microsatellite marker is routinely used to identify the presence of the translocated chromosomal segment on 7DL in different bread wheat backgrounds (Ayala *et al.*, unpublished). Efforts are also underway to convert the RFLP markers into more convenient STS markers. In addition, phenotypic data have been collected on BYDV tolerance for the RIL populations of 'Frontana' X 'INIA 66' and 'Opata' X 'Synthetic'. Molecular marker analysis is underway to identify the loci controlling tolerance to BYDV in these populations.

Application of molecular markers in wide hybrids in wheat

Intergeneric hybridization of wheat with its wild relatives is an attractive method of transferring certain desirable traits from wild relatives into cultivated wheat. However, the limitations associated with the procedure include lack of interchromosomal associations even when the hybrids are successfully made, mainly due to the presence of several genes controlling pairing (*Ph* genes) between homoelogous chromosomes. The strongest effect has been observed for the *Ph1* locus (located on 5BL) which effectively suppresses pairing between homoelogous chromosomes (Sears and Okomato, 1958; Riley and Chapman, 1958). Wheat lines carrying deletions of the

Ph1 locus (*ph1b*) have been identified (Sears, 1977). Molecular markers based on the polymerase chain reaction (PCR) that facilitate identification of the *ph1b* deletion stocks have been developed (Qu *et al.*, 1998; Gill and Gill, 1996).

At CIMMYT, we have successfully applied one of the markers (Qu *et al.*, 1998) to facilitate the identification of wide hybrid derivatives, that carry alien chromosomes from species such as *Thinopyrum bessarabicum* in the selfed BC_1 progenies of the F_1 intergeneric hybrids with 'Chinese Spring' *ph1b* deletion stocks. Over 300 lines were analyzed with the PCR marker and lines that carry the *ph1b* deletion identified and later confirmed by meiotic analysis to have multivalent pairing involving wheat and *Th. bessarabicum* chromosomes. This PCR marker is being used in the identification of wide hybrid derivatives involving several other wild relatives of wheat that carry important biotic and abiotic attributes.

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References

Banks, P.M., P.J. Larkin, H.S. Bariana, E.S. Lagudah, R. Appels, P.M. Waterhouse, R.I.S. Brettell, X. Chen, H.J. Xu, Z.Y. Xin, Y.T. Qian, X.M. Zhou, Z.M. Cheng, and G.H. Zhou. 1994. The use of cell culture for subchromosomal introgressions of barley yellow dwarf virus resistance from *Thinopyrum intermedium* to wheat. Genome 38: 395-405.

Drijepondt, S.C., and Z. Pretorius. 1989. Greenhouse evaluation of adult-plant resistance conferred by the gene *Lr34* to leaf rust of wheat. Plant Dis. 73: 669-671.

Gavinlertvatana, S. and R.D. Wilcoxson. 1978. Inheritance of slow rusting of spring wheat by *Puccinia recondita* f. sp. *tritici* and host parasite relationships. Trans. Br. Mycol. Soc. 71: 413-418.

Gill, K.S., and B.S. Gill. 1996. A PCR-based screening assay of *Ph1*, the chromosome pairing regulator gene in wheat. Crop Sci. 36: 719-722.

Hoisington, D., N. Bohorova, S. Fennell, M. Khairallah, A. Pellegrineschi and J. M. Ribaut. 1998. The application of biotechnology to wheat improvement: New tools to improve wheat productivity. FAO Book Chapter.

Khairallah. M., H. Guillén-Andrade, J. Alarcón, C. Rodríguez, L. Ayala, M. Henry, R.P. Singh, C. Jiang, P.J. Sharp and D. Hoisington. 1998. Mapping of durable

resistance to leaf rust and tolerance to BYDV in wheat. *In:* Proceedings of the 9th Intl. Wheat Genetics Symp. Vol 2 (3): 282-284.

Knott, D.R. 1989. The wheat rusts – breeding for resistance. *In:* Monographs on Theoretical and Applied Genetics, Vol 12. Springer-Verlag, Berlin, New York. Pp.201.

Knott, D.R. and B. Yadav. 1993. The mechanism and inheritance of adult plant leaf rust resistance in 12 wheat lines. Genome 36: 877-883.

Larkin, P. J., P.M. Banks, E.S. Lagudah, R. Appels, C. Xiao, X. Zhiyong, H. Ohm, R.A. W. McIntosh. 1994. Disomic *Thinopyrum intermedium* addition lines in wheat with barley yellow dwarf virus resistance and with rust resistance. Genome 38: 385-394.

Pike, K. S. 1990. Review of barley yellow dwarf virus crop losses. *In:* World perspectives on barley yellow dwarf. *Edited by* P. A. Burnett. Int. Wheat and Maize Improvement Centre (CIMMYT), Mexico. pp 368-382.

Qu, L.J., T.N. Foote, M.A. Roberts, T.A. Money, L. Aragon-Alcaide, J.W. Snape, and G. Moore. 1998. A simple PCR-based method for scoring the *ph1b* deletion in wheat. Theor. Appl. Genet. 96: 371-375.

Riley, R. and V. Chapman. 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182: 713-715.

Roelfs, A.P. 1988. Resistance to leaf and stem rust in wheat. *In:* N.W. Simmonds, and S. Rajaram (eds.), Breeding strategies for resistance to the rusts of wheat. Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Mexico D.F., Mexico. pp10-22.

Roelfs, A.P., R.P. Singh and E.E Saari. 1992. Rust diseases of wheat: concepts and methods of disease management. Mexico, D.F.: CIMMYT.

Sears, E.R. 1977. An induced mutant with homoeologous pairing in common wheat. Can. J. Genet. Cytol. 19: 585-593.

Sears, E.R. and M. Okomoto. 1958. Intergenomic chromosome relationships in hexaploid wheat. Proc. Xth Int. Congr. 2: 258-259.

Singh, R.P. 1992. Association between *Lr34* for leaf rust resistance and leaf tip necrosis in wheat. Crop Sci. 32: 874-878.

Singh, R. P. and A.K. Gupta. 1992. Expression of wheat leaf rust resistance gene *Lr34* in seedlings and adult plants. Plant Dis. 76: 489-491.

Singh, R.P. and S. Rajaram. 1992. Genetics of adult-plant resistance of leaf rust in 'Frontana' and three CIMMYT wheats. Genome 35: 24-31.

Singh, R. P., P.A. Burnett, M. Albarran and S. Rajaram. 1993. *Bdv1* a gene for tolerance to barley yellow dwarf virus in bread wheats. Crop Sci. 33: 231-234.

William, H.M., D. Hoisington, R.P. Singh and D. Gonzalez-de-Leon. 1997. Detection of quantitative trait loci associated with leaf rust resistance in bread wheat. Genome 40: 253-260.

Zhou, G.H., Y.T. Qian, Z.M. Cheng and L.Y. Wang. 1990. A cereal germplasm 'Zhong 4' for resistance to barley yellow dwarf virus in China. *In:* P. A. Burnett. (ed.) World perspectives on barley yellow dwarf. Int. Wheat and Maize Improvement Centre (CIMMYT), Mexico. pp 394-395.

Resumen

La Aplicación de Marcadores Moleculares en el Mejoramiento de Trigo en CIMMYT

Los marcadores moleculares tienen varias aplicaciones en los programas de mejoramiento de cultivos. Estos, cuando están estrechamente vinculados a genes de interés, pueden ser utilizados indirectamente para seleccionar los alelos deseables. Además, los marcadores moleculares pueden ser usados para diseccionar características poligénicas en sus componentes Mendelianos o loci para caracteres cuantitativos (QTL), aumentando así nuestro conocimiento sobre la herencia de estos caracteres. Los científicos en el Centro de Biotecnología Aplicada del CIMMYT están colaborando con el Programa de Trigo en el desarrollo y uso de marcadores moleculares para el mejoramiento de trigo. El desarrollo de cultivares con resistencia duradera a la roya de la hoja y roya estriada es un objetivo importante para el CIMMYT. La resistencia a estas royas esta controlada por un gran numero de genes menores y es referida como resistencia en estado de planta adulta (RPA). El mapeo de ligamientos y el análisis de masales segregantes esta siendo utilizado con varias poblaciones de líneas recombinantes endocriadas que están segregando para genes de resistencia a la roya de la hoja y roya estriada, con el propósito de encontrar marcadores moleculares asociados con los genes para RPA. Tres marcadores que muestran asociación significativa con la resistencia duradera a la roya de la hoja han sido encontrados en una población. En una segunda poblacion, 5 y 2 QTLs fueron detectados para resistencia a la roya de la hoja y roya estriada respectivamente. Además, los marcadores moleculares están siendo utilizados para transferir resistencia al virus del enanismo amarillo de la cebada derivado del Thinopyrum intermedium *en diferentes trigos harineros. Los marcadores también están siendo utilizados para detectar los derivados de las cruzas amplias con una mayor frecuencia de apareamiento meiótico que facilitaran la detección de la introgresión de los segmentos cromosomales de las especies aliadas del trigo que poseen características bióticas y abióticas importantes para su transferencia al trigo cultivado.*