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Biotechnology Applications for Wheat Improvement at CIMMYT

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Abstract: Despite the tremendous advances made by plant breeders in increasing the global food production, there is still a significant need for increased quantity and quality of food items in various regions of the world. Although this can be partially solved by upgrading the storage and distribution networks, crop performance and yield potentials are constantly challenged by various biotic and abiotic stress factors. As additional tools to facilitate the global wheat breeding efforts at CIMMYT, we have used molecular markers for characterizing loci that confer adult plant resistance to leaf rust and yellow (stripe) rust, which are globally important diseases in wheat. We have also established a biotechnology laboratory that is charged with acquiring, validating and applying markers for certain traits that are important to CIMMYT wheat breeders. Use of PCR based markers coupled with rapid DNA extraction procedures have enabled application of markers on a wide range of material. Genetic engineering procedures have also been used to establish procedures as well as for experimenting with genes that confer resistance to various biotic and abiotic stresses in wheat.

Key Words: molecular markers, leaf rust, yellow rust, genetic engineering

Introduction

Traditional plant breeding activities have resulted in tremendous yield gains in most cultivated crop species. Global wheat breeding efforts over the past 40 years have made significant contributions in enhancing the yield potential and stability, as well as developing cultivars with more durable levels of resistances to a diverse array of biotic and abiotic stresses. Average developing country yields for most crop species have more than doubled in this time span, avoiding major famines. However, efforts of wheat breeders are constantly challenged by various biotic and abiotic stress factors that threaten yield stability in many wheat growing regions. In biotic stresses, this is due to the ability of the pathogens to evolve and mutate to more virulent forms. Among the biotic stress factors, diseases such as leaf (brown) rust, stripe (yellow) rust, and head scab are considered as globally important diseases in wheat. The challenges due to abiotic stress factors such as limited availability of water, soil acidity and alkalinity continue to pose significant challenges for the wheat breeding community. It is estimated that by 2020, the global wheat demand would be about 40% greater than its current levels of 552 million tons (Rosegrant et al. 1997).

While plant breeders have made significant contributions, the last few decades also have seen major advances in science, specifically in the area of molecular biology and biotechnology. Recent advances in biotechnology have resulted in understanding the genetic basis of living organisms as well as products and processes useful for the well being of humanity. These scientific advances have resulted in increased understanding and characterization of various genes at the molecular level that are associated with traits important to plant breeders. Tools based on DNA markers that have been made available by countless researchers around the world have begun to significantly contribute to further increase in effectiveness of crop breeding.

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In wheat, where alien transfers in the form of chromosomal translocations are common, many genes of race specific nature for a wide range of biotic stresses have been introgressed into wheat from wild relatives and markers have been used for tagging such genes (Ayala et al. 2001). A large number of publicly available microsetellites (Roder et al. 1999) as well as techniques such as amplified fragment length polymorphisms (AFLPs) (Vos et al. 1995) have also enabled identification of markers associated with genes that confer durable resistance for diseases such as leaf and yellow rust (Messmer et al. 2000; Suenaga et al. 2003;), powdery mildew (Liu et al. 2001) and fusarium head blight (Anderson et al. 2001).

This paper reports the activities underway at CIMMYT's Applied Biotechnology Center to utilize biotechnology applications to facilitate the efforts of wheat breeders in meeting the challenges by using tools of biotechnology to complement the plant improvement efforts.

Biotechnology Applications

Biotechnology research efforts are based on applied research activities that would benefit the two mainstream crop improvement activities at CIMMYT as well as providing training for personnel in the national programs in biotechnology applications. Specific to wheat related activities, current research efforts are focused on the development of molecular markers for traits of importance in the wheat improvement activities, adoption and application of markers that have resulted from other global research efforts, genetic diversity studies aimed at characterization of wheat germplasm collections to identify useful alleles for genes of interest and genetic engineering activities aimed at developing genetically modified spring wheats with value added traits.

Durable Resistance to Leaf and Yellow Rust

CIMMYT's breeding strategy to develop wheats with improved levels of resistance to a range of biotic stresses encompasses the utilization of genes conferring "durable resistance". Among the diseases that are of global significance, rusts diseases, mainly leaf rust and yellow rust are more prominent. More than 40 leaf rust resistance genes (*Lr*) and 30 yellow rust resistance genes (*Yr*) have been identified in wheat and related species. Most of these confer a hypersensitive reaction at the seedling stage and have been overcome by new races of *Puccinia recondita* or *Puccinia striiformis* for leaf rust and yellow rust respectively. Cultivars containing "slow rusting" genes or "adult plant resistance" (Caldwell, 1968), that confer durable resistance (Johnson, 1978) or race non-specific resistance, have maintained their levels of resistance in wide range of agro-climatic conditions and over long durations. The components that cause slow rusting response include, longer latency period, smaller uredial size, and low infection frequency. It is well known that the wide adaptation of semi-dwarf CIMMYT wheats is partially due to the presence of durable stem rust resistance that was incorporated into early CIMMYT wheat germplasm from the wheat cultivar 'Hope' which still remains effective in many wheat growing regions of the world.

Breeding wheat cultivars with durable rust resistance based on genes conferring race non-specific form of resistance has proven to be an effective strategy. However, this strategy poses significant challenges to the wheat breeders compared to incorporating single genes with race specific form of resistance. In order to obtain acceptable levels of resistance under high disease pressure, cultivars have to be developed which contain 4/5 of the slow rusting genes. Using the classical breeding approaches, it is not possible to reliably estimate the number or the effects of such genes that are present in breeding material. CIMMYT's breeding strategy for durable resistance to biotic stresses, more specifically rust diseases includes three parameters; a) identification and combine diverse sources of resistance/tolerance, b) epidemiology evaluations by monitoring the pathogen distribution and evolution, and c) utilization of molecular markers to characterize the genes conferring slow rusting resistance and tag such genes with molecular markers to enable breeders to manipulate them in breeding material using marker assisted selection strategies.

Table 1 lists the populations and strategies currently being used with the objective of identification, characterization and marker development for genes that confer durable leaf and yellow rust resistance in CIMMYT spring wheats. As opposed to developing full linkage maps to achieve the above objectives, which can be time consuming and resource intensive in wheat, we have combined bulked segregant analysis and partial linkage mapping to meet the objectives mentioned above. Bulked segregant analysis (BSA), which involves pooling of

entries at the two extremes for a segregating trait (Michelmore et al. 1991), has been effectively used for identifying molecular markers associated with disease resistance genes in a number of species (Eastwood et al. 1994; Williams et al. 2001). In Avocet x Pavon76 population (Table 1), we have been able to identify three loci that have significant effects on leaf rust resistance and five loci that have effects on yellow rust resistance. Bulked segregant analysis enabled identification of markers associated with *Lr46* (Sing et al. 1998) as well as to establish the association of *Lr46* with *Yr29* (William et al. 2003). Linkage mapping of the markers associated with *Lr46/Yr29* using the International Triticeae Mapping Initiative (ITMI) population established the precise genomic location of these genes on the long arm of chromosome 1B (William et al. 2003). In Avocet x Pavon76 and other populations listed in Table 1, some of the loci identified have common effects on both leaf rust and yellow rust whereas some other loci have individual effects on only one of the two diseases (Table 2 a). In Avocet x Parula and Avocet x Tonichi populations also we have been able to characterize several loci associated with resistance to the two rust diseases (Table 2b & 2c). Molecular markers have proven to be advantageous and rapid in the identification of these slow rusting loci as well as establishing their genomic locations. In some cases, the loci characterized with molecular markers have enabled identification of gene designations for several slow rusting loci (Table 2).

In addition to using BSA for the characterization of loci that confer slow rusting resistance, we have developed a full linkage map of Frontana X Inia66 using a recombinant inbred line population. The linkage map of Frontana x Inia 66 currently has approximately 600 markers although only about 330 markers were used for the linkage map construction due to various reasons such as clustering. Frontana is a Brazilian cultivar with proven durable leaf and yellow rust resistance. The linkage map contains 33 linkage groups and the D-genome does not yet have a full coverage with markers. More markers are being used to complete the map. The genotyping of the population was done using restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), sequence-tagged sites (STSs) and amplified fragment

Table 1. Populations used for mapping adult plant resistance (APR) to leaf and yellow rust.

Population	Estimated APR genes for leaf rust in the R parent	No. RILs[a]	Strategy used for mapping
Frontana (R) x INIA66	Lr34 + 2-3 genes	223	Full linkage map
Avocet x Pavon 76 (R)	Lr46 + 2 genes	148	BSA[b] + partial mapping
Avocet x Parula (R)	Lr34 + 2-3 genes	141	BSA[b] + partial mapping
Avocet x Tonichi (R)	Lr34 + 2-3 genes	144	BSA[b] + partial mapping
Avocet x Pastor	2/3	40 F5 families	BSA[b] + partial mapping

a – Recombinant inbred lines
b– Bulked segregant analysis

Table 2a. Loci associated with slow rusting resistance in Avocet X Pavon76 poulation.

Chromosome	Closest marker	% reduction in mean disease severity for the two marker classes		
		Leaf rust	Stripe rust	Named genes
1BL	gwm259	35	27	Lr46, Yr29
4B	gwm495	18	15	
6A	gwm356	14	18	
6B	PstAggMseCAA	-	18	
3BS	PstACgMseCgT	-	11	Yr30, Sr2

Table 2b. Loci associated with slow rusting resistance in Avocet X Parula poulation.

Chromosome	Closest marker	% reduction in mean disease severity for the two marker classes		
		Leaf rust	Stripe rust	Named genes
1BL	gwm259	15	16	Lr46, Yr29
7DS	gwm295	56	46	Lr34, Yr18
7B	PCR105	29	-	
3BS	Glk2	-	12	Yr30, Sr2
Unknown	PstAAgMseCTA	22	14	

Table 2c. Loci associated with slow rusting resistance in Avocet X Tonichi poulation

Chromosome/ Region	Closest marker	% reduction in mean disease severity for the two marker classes		
		Leaf rust	Stripe rust	Named genes
7DS	Gwm295	56	24	Lr34, Yr18
7D	Gwm885	26	13	
Gp7	Wmc405	14	10	
3BS	AFLP	-	12	
6B	gwm219	-	12	Yr30, Sr2
Unknown	AFLP	-	16	

length polymorphisms (AFLPs). The characterized loci conferring resistance to leaf rust and yellow rust are presented in Table 3. In addition to the QTLs detected in Frontana, the susceptible parent Inia66, contributed one QTL of resistance to each disease. The locus identified on chromosome 3BS for yellow rust resistance, derived from Inia66 corresponds to *Yr30* (Singh et al. unpublished), which is also linked to the *Sr2* complex (Table 3). This population has the advantage of segregating for other characters including resistance to *Fusarium* head scab, *Septoria tritici*, barley yellow dwarf virus (tolerance), and sprouting, allowing us to map these traits as well in future.

Marker Implementation

When a molecular marker is considered for use in the breeding program, several criteria are taken in to account. Among the important criteria are: a). linkage between the marker and the gene of interest, in-order to avoid false positives. b). repeatability and reliability of the marker/s and c). cost and reliability of field screening. If the marker is located within the gene of interest, such markers can be used as perfect markers since there is no recombination between the marker and the gene of interest. When markers with some recombination with the genes of interest are used, such markers may be used to increase allele frequency for the gene of interest in breeding populations based on the recombination frequency. Repeatability and reliability of markers ensure the robustness of the assays. Markers should ideally be used in scenarios where field screening is expensive and / or when such screening is laborious or unreliable due to environmental influences. A laboratory that is capable of providing these tools for the benefit of plant breeders should have the capacity to conduct high throughput, high quality DNA extractions and high throughput marker assays. At the Applied Biotechnology Center, we are using a set of PCR based markers for key traits that are difficult to screen reliably in the field.

Following markers are currently being used on a routine basis in wheat improvement activities at CIMMYT.

1. Cereal cyst nematode resistance gene, designated as *Cre*1 – Developed by CSIRO – Plant Industry group in Canberra, Australia. The gene was

Table 3. Summary of QTL for durable leaf and yellow rust resistance in the RIL population of Frontana x Inia66.

Leaf rust			Yellow rust		
Chrom. Location	Closest marker(s)	Phenotypic variance (%R^2)	Chrom. Location	Closest marker(s)	Phenotypic variance (%R^2)
1B	AFLP	5	1B	AFLP	7
3DL	AFLP	5 [a]	2BL	KsuF11	4
5B	glk165	7	3BS	Glk683	19 [a]
7DS	gwm 295/Ltn	46	7DS	gwm 295/Ltn	25
	Total	61		Total	44

[a] - effect from Inia66

identified in an Australian cultivar and is located on chromosome 2BL. The marker is diagnostic for *Cre1.*

2. Cereal cyst nematode resistance gene, designated as *Cre*3 – Developed by CSIRO – Plant Industry group in Canberra, Australia. The gene was identified in *Triticum tauschii,* is located on chromosome 2DL (Lagudah et al. 1997).
3. A marker for barley yellow dwarf virus (BYDV) resistance, derived from an introgressed chromosome segment from *Thinopyrum intermedium,* is located on chromosme 7DL. The marker was developed at CIMMYT (Ayala et al. 2001).
4. Marker for Chinese Spring *ph1b* mutant – Developed at John Innes Center and is diagnostic for the deletion that involves the *Ph1* gene on chromosome 5BL, a suppressor of homoeologous chromosome pairing (Qu et al. 1998).
5. Marker for *Aegilops ventricosa* derived resistance to stripe rust (*Yr17*), Leaf rust (*Lr37*) and stem rust (*Sr38*) (Oliver Robert, pers. comm.). The translocation from *Ae. ventricosa* is present on chromosome 2AS.

The sources containing *Cre1* and *Cre3* genes have been extensively used in crosses with improved CIMMYT wheats with the aim of introgressing these genes into CIMMYT wheats targeted mainly for marginal environments but also being used in wheats targeted for high rainfall and irrigated areas. Better root health is critical in marginal environments where poor water uptake is often related to poor root health. In addition, these sources also have been used in durum x bread wheat crosses. We are routinely applying these markers in order to identify material in segregating populations to enable the breeders to selectively advance the lines containing the genes of interest. Crosses have also been made with the aim of combining *Cre1* and *Cre3* genes utilizing markers in high yielding backgrounds.

The microsatellite marker derived from *Thinopyrum intermedium* (*gwm* 37) is being effectively used to transfer the alien chromosome segment from donor line carrying the introgression into different bread wheats with the ultimate aim of combining the alien derived resistance with tolerance available in wheat for BYDV. The STS marker derived from *Ae. ventricosa* is used in a limited capacity, mainly in bread wheat x durum wheat crosses, to identify the durum derivatives carrying the translocation.

Genetic Engineering

The Applied Biotechnology Center has been successful in developing techniques for mass production of fertile transgenic wheat (*Triticum aestivum* L.) through biolistic methods using immature embryos (Pellegrineschi et al. 2002). CIMMYT's elite cultivars are co-bombarded with marker gene and a gene of interest with co-transformation efficiencies of around 25-30%. The reliability of this method opens the possibility for the routine introduction of novel genes that may induce resistance to biotic and abiotic stresses as well as have applications in various quality and nutritional parameters. We are conscious about the public perception of genetically modified organisms and are of the opinion that through means of education and communication, the general public opinion will favor the eventual deployment

of transgenics in a broad scale. CIMMYT would continue to work on establishing stable transgenics with genes of economic importance in both wheat and maize.

The first group of genes being evaluated are the pathogenesis related (PR) proteins, such as the thaumatin-like protein (TLP) from barley, chitinase, and 1-3 b-glucanase. Stable integration of the genes in the genome and inheritance in the progeny were determined by phenotypic analyses that challenged the plants against a wide range of pathogens. The anti-fungal activity of the endogenous thaumatin-like proteins were analyzed in T_1 and T_2 progeny plants. The transgenic wheats were challenged by a host of pathogens including *Alternaria*, *Fusarium*, *Helminthosporium*, *Pitium* and *Rhizoctonia*. The preliminary results from the in-vitro and in-vivo assays have indicated that for *Alternaria*, the plants containing tahumatin-like constructs had shown positive responses in the form of disease reaction including immunity in some cases. Current efforts of wheat transformation activities include use of constructs with receptor-like kinase protein isolated from rice, anti-secalins, low molecular weight glutenins and certain constructs containing genes of interest in tolerance to drought and other abiotic streses (DREB genes). We have not been able to conduct field experiments with wheat transformed with various gene constructs in Mexico to ascertain their effectiveness under field conditions.

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