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# Transfer of Rust Resistance from *Triticum aestivum* L. Cultivar Chinese Spring to Cultivar WL 711

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***ABSTRACT.*** *Durable rust resistance can be obtained in wheat cultivars by deployment of slow rusting and adult plant resistance genes as against the race specific resistance genes effectiveness of which is short lived. Triticum aestivum cultivar Chinese Spring possess slow rusting genes. Chinese Spring with $Ph^1$ was used to induce homoeologous pairing for precise transfer of rust resistance for 5M chromosome of Aegilops ovata to 5D of wheat in WL711 background. The advance back cross generation derivatives and their progenies were screened, at seedling stage against two pathotypes of leaf rust and one pathotype of yellow rust and, under field conditions with an aim to identify the resistant introgression lines carrying rust resistant genes transferred from Aegilops ovata. The introgression lines were analysed with 5D specific microsatellite markers to identify resistant plants carrying minimum alien chromatin. However, a number of plants with susceptible infection type at seedling stage like Chinese Spring and without any 5D specific Aegilops ovata alleles showed low incidence of both the rusts indicating that the slow rusting genes from Chinese Spring have been transferred to WL711.*

## INTRODUCTION

Leaf rust, caused by *Puccinia recondita* Rob Ex. Desm. f. sp. *tritici* Eriks. Henn and yellow rust caused by *Puccinia striiformis* Westend. are the major fungal diseases of bread wheat (*Triticum aestivum* L.). Genetic base for rust resistance among wheat cultivars is very narrow. Disease resistance contributed by race specific genes breaks down soon after their deployment due to ever evolving pathogens. There is an urgent need to search for sources of non-race specific durable resistance.

Durable resistance operating at adult plant stage confers a non-hypersensitive response (Johnson and Law 1975). Slow rusting resistance has a longer latent period (Kolmer 1996).Therefore, the identification and use of potential sources of slow rusting genes would be a useful strategy for durable rust resistance. *Triticum aestivum* cultivar Chinese Spring is known to possess adult plant resistance. Unaru (1950) reported that it is due to a single gene. McIntosh and Baker (1966) identified *Lr* 12 on its chromosome 4A. Dyck and Kerber (1971) further showed that Chinese Spring has one or more additional genes for its superior adult plant resistance. Piech and Sypryn (1978) located the gene for adult plant resistance to leaf rust on chromosome 7D of Chinese Spring. Dyck (1987) identified it as *Lr* 34. Later on Singh and McIntosh (1984) located another gene *Lr* 31 in Chinese Spring.

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During the present investigation a leaf and stripe rust resistant substitution line carrying 5M chromosome of *Aegilops ovata* substituted for 5D chromosome of wheat was crossed with a Chinese Spring stock having a suppressor for *Ph* locus ($Ph^I$) for the induction of homoeologous pairing with an aim to transfer rust resistance genes from 5M to 5D with minimum linkage drag. Rust resistance genes from *Aegilops ovata* impart seedling as well as field resistance. But in addition to the plants having seedling as well as field resistance, a number of plants have been identified which had seedling susceptibility but had adult plant resistance for leaf rust.

This situation could be attributed to the simultaneous transfer of adult plant resistance genes from one of the above wheat parent such as Chinese Spring. Therefore, the present report deals with screening of back cross derivatives for transfer of durable rust resistance from Chinese Spring to rust susceptible *T. aestvum* cv. WL711.

## MATERIALS AND METHODS

A wheat 5M-5D substitution line carrying genes for leaf and yellow rust resistance was developed by crossing *Triticum aestivum* cv. WL711 (AABBDD) with *Aegilops ovata* Acc. 3547 (UUMM) and subsequent back crossing to WL711(NN-non necrotic). The 5M-5D substitution was confirmed by C-banding and microsatellite markers (Dhaliwal *et al* 2002). Chinese Spring with $Ph^I$ gene (Chen *et al* 1994) was used for inducing homoeologous pairing between 5M and 5D. Selected 22 alien introgression derivatives, derived from the cross *Triticum aestivum* cv WL711 (5M-5D substitution)/Chinese Spring ($Ph^I$) $//^{*2}$ WL 711 (NN) and their selfed progenies were screened for rust resistance and characterized by molecular analysis.

During 2000-2001 wheat season, introgression lines in $BC_1$ and $BC_2$ were screened for adult plant reaction to both yellow and leaf rusts under field conditions. It was recorded as a percentage of leaf area covered by rust according to modified Cobb's scale as described by Petersen *et al* (1948). Adults plants with infection types classified as, F (free from the disease), R (resistance), TR (trace resistant), MR (moderate resistant) and S (susceptibility).

In the following 2001-2002 season selfed progenies of these lines were screened for rust resistance using two leaf rust pathotypes (77-5 and 104-2) and one yellow rust pathotypes (46S119) individually at seedling stage under artificial inoculation condition (Table 1). 10 plants from each progeny were tested for each pathotypes. Fourteen days after inoculation the infection types were recorded according to the modified scale of Stakman *et al* (1962). Seedling with infection type (IT) 0; , ; , 1 and 2 were classified as resistant and with IT 3, 4 as susceptible. All these plants were transplanted in field and screened for resistance to both the rusts under field condition (Table 1).

Parental material was screened for polymorphism by using the 5D chromosome specific microsatellite markers already mapped by Röder *et al* (1998) and Pestosova *et al* (2000). For this molecular characterization only the 5D anchored STMS markers which were polymorphic between *Ae. ovata* and wheat were used. STMS Xgwm 190 marker was used to detect the transfer of *Ae. ovata* chromatin to short arm of 5D chromosome (5DS) and Xgwm 583 and Xgdm 138 markers helped to monitor long

arm of 5D chromosome (5DL), of selected introgression lines and progenies of introgression lines 2, 18, 19 and 21.

Then all the derivatives and 3 selected segregating progenies were characterized for *Aegilops ovata* chromosome 5M translocations in wheat background. *In vitro* PCR amplification was performed as described by Röder *et al* (1998) with some modifications. PCR products were resolved by electrophoresis in 2.5% high resolution Metaphor gel (Amersco). Gels were visualized by staining with ethidium bromide using UVP Gel Documentation system model GDS 7600.

## RESULTS AND DISCUSSION

Donor species *Aegilops ovata,* Acc.3547 and *ovata* 5M-5D substitution line as well as some derivatives and their progeny plants were totally resistant to leaf and yellow rusts at both seedling and adult stages while the recipient parent WL 711 showed IT '3' at seedling stage and 90 S susceptibility at adult plant stage to both the rusts. Chinese Spring showed IT '3' at seedling stage but had comparatively less severity of 20 S and 30 S for leaf rust and yellow rust, respectively under field conditions (Table 1). On the basis of rust reaction, resistant introgression lines can be classified into three major categories. Namely (1) Resistant at seedling stage as well as at adult stage to both the rusts, (2) Susceptible at seedling stage but resistant at adult plant stage under field condition to both the rusts and (3) Resistant to leaf rust but susceptible to yellow rust.

Marker Xgwm 190 generated a high molecular weight single allele in WL 711, WL 711 (NN) and CS (S). Whereas it generated low molecular weight single allele in *Aegilops ovata* as well as in 5M-5D substitution line and some of progeny plants of derivative No.2 (Fig 1).

In wheat the marker Xgwm 583 amplified only a single region but two different loci in *Aegilops ovata* ($O_1$ $O_2$ ). However, out of these two loci, low molecular weight $O_2$ locus was present in the substitution line as well as some of progeny plants of derivative No.2 (Fig 1).

Most of the plants which showed resistant reaction similar to *Ae. ovata* and wheat 5M-5D substitution lines to both the rusts at both stages had the resistance gene(s) transferred from 5M of *Ae. ovata* to 5D chromosome. This was indicated by the presence of *Aegilops ovata* specific allele of Xgwm 190 marker on 5DS.

These plants, therefore, might possess slow rusting genes from sources other than *Ae. ovata.* Such genes being absent in WL711 might have been transferred from Chinese Spring used for induction of homoeologous pairing. Many workers have shown the slow rusting gene(s) in Chinese Spring (Unrau, 1950, Piech and Sypryn 1978, McIntosh and Baker 1966, Dyck and Kerber 1971, Dyck 1987, 1991). Normally "Chinese Spring" shows around 20 S to 30 S susceptibility to leaf rust and yellow rust respectively. But in this case incidence of rusts ranged between resistant to 40 S rust severity. Samborski and Dyck (1982) showed the ability of *Lr* 34 to interact with other genes to increase the limited resistance. Roelfs (1988) suggested that *Lr* 12 or *Lr* 13 combined with *Lr* 34 could have contributed to the durable leaf rust resistance of several wheat cultivars. Singh and Rajaram (1992) have shown the presence of highly effective slow rusting resistance to leaf rust in several CIMMYT wheat lines. Lower rust severity

of some of the lines could be attributed to the interaction of *Lr*34of Chinese Spring with *Lr*13 of WL711.

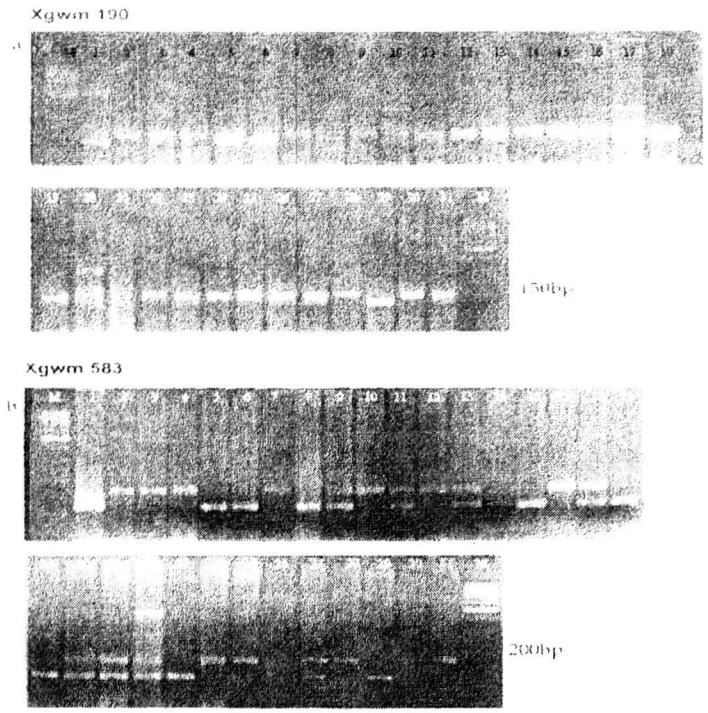


**Fig.1. Amplification pattern of microsatellite markers used for molecular characterization of progeny of derivative number 2 along with parents. (a). Xgwm 190 on chromosome 5DS (b). Xgwm 583 on chromosome 5DL, M- Marker( 50 bp Lader), Lane No. 1- *Aegilops ovata Acc* 3547, No.2- *Triticum aestivum* CS(S), No.3- *T. aestivum* cv. WL711(NN), No.4-*T. aestivum* cv.WL 711, No. 5- 5M-5D substitution line (Dwarf), No. 6- 5M-5D substitution line (Tall) and No. 7 to 31 progeny plants 2-1 to 2-25 respectively.**

**Table 1. Rust reaction of selfed progenies of some derivatives under artificial inoculation condition at seedling stage and at adult plant stage under field condition in 2001- 2002.**

| I | Parent/ Progeny | II | Adult Plant | | P.N. | III | Adult Plant | | P.N. | IV | Adult Plant | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | LR | YR | | | LR | YR | | | LR | YR |
| | *Ae.ovata* | 0; | F | R | | 0; | R | R | | 0; | R | R |
| | CS(S) | 3 | 20S | 30S | | 3 | 20S | 30S | | 3 | 20S | 30S |
| | WL711 | 3 | 90S | 90S | | 3 | 90S | 90S | | 3 | 90S | 90S |
| | WL711(NN) | 3 | 90S | 90S | | 3 | 90S | 90S | | 3 | 90S | 90S |
| | 5M-5D(D) | 0; | R | R | | 0; | R | R | | ; | R | R |
| | 5M-5D(T) | 0; | R | R | | 0; | R | R | | ; | R | R |
| 2 | 2-1 | e | - | - | 2-9 | e | R | R | 2-19 | 3/x | TR | R |
| | 2-2 | 1 | F | F | 2-10 | 3 | R | R | 2-20 | ; | R | R |
| | 2-3 | 3 | R | R | 2-11 | 1 | R | R | 2-21 | xi | R | R |
| | 2-4 | 3 | 20S | 10S | 2-12 | ; | R | R | 2-22 | x | <5S | <5S |
| | 2-5 | 2 | 35S | 10S | 2-13 | 1 | R | 10S | 2-23 | ; | R | R |
| | 2-6 | ; | E | e | 2-14 | 3 | R | R | 2-24 | x | 80S | 80S |
| | 2-7 | 1 | R | 15S | 2-15 | 3 | R | R | 2-25 | ; | R | R |
| | 2-8 | 3 | 40S | 40S | 2-16 | ; | R | R | | | | |
| | | | | | 2-17 | 1 | R | R | | | | |
| | | | | | 2-18 | 3 | R | R | | | | |
| 18 | 18-1 | 3 | 70S | 70S | 18-11 | 3 | 70S | 60S | 18-21 | 3 | 10S | 80S |
| | 18-2 | 3 | 60S | 50S | 18-12 | 3 | <5S | <5S | 18-22 | ; | R | R |
| | 18-3 | 3 | 30S | - | 18-13 | 3 | 40S | 40S | 18-23 | ;1+ | R | 20S |
| | 18-4 | 1- | F | F | 18-14 | 2 | R | TR | 18-24 | ; | R | 5S |
| | 18-5 | 1 | R | R | 18-15 | e | R | R | 18-25 | ; | R | 20S |
| | 18-6 | 2 | MR | MR | 18-16 | 3 | 50S | 50S | 18-26 | 3 | R | 10MR |
| | 18-7 | 3 | 30S | 30S | 18-17 | 3 | 70S | R | 18-27 | ; | R | - |
| | 18-8 | 1 | R | R | 18-18 | 1 | R | R | | | | |
| | 18-9 | ; | R | R | 18-19 | ; | R | | | | | |
| | 18-10 | 1 | R | R | 18-20 | 3 | 60S | | | | | |
| 19 | 19-1 | 3 | <5S | <5S | 19-9 | 3 | 5S | 10S | 19-17 | 3 | TR | 30S |
| | 19-2 | 3 | R | 20S | 1910 | 0; | F | F | 19-18 | ; | R | TR |
| | 19-3 | 3 | R | R | 19-11 | 1 | R | R | 19-19 | 3 | 10S | 50S |
| | 19-4 | 1 | F | F | 19-12 | 0; | - | - | 19-20 | ; | R | R |
| | 19-5 | 1 | R | R | 19-13 | e | 20S | 20S | 19-21 | 3 | - | 90S |
| | 19-6 | 1 | F | F | 19-14 | e | R | R | 19-22 | 3 | R | R |
| | 19-7 | 3 | 70S | 10S | 19-15 | e | R | R | 19-23 | x | R | R |
| | 19-8 | 1 | R | <5S | 19-16 | e | <5S | <5S | 19-24 | x | R | R |
| | | | | | | | | | 19-25 | ; | R | R |
| | | | | | | | | | 19-26 | ; | R | TR |
| 21 | 21-1 | 1 | F | F | 21-11 | e | F | F | 21-19 | ; | TR | TR |
| | 21-2 | 1 | F | <5S | 21-12 | e | F | F | 21-20 | ;- | R | R |
| | 21-3 | 3 | 40S | 60S | 21-13 | e | F | F | 21-21 | ;- | R | R |
| | 21-4 | 1 | F | F | 21-14 | e | F | F | 21-22 | ;- | TR | TR |
| | 21-5 | 3 | 15S | 10S | 21-15 | 1 | R | R | 21-23 | ; | TR | TR |
| | 21-6 | 2 | F | F | 21-16 | e | F | F | 21-24 | ;- | R | R |
| | 21-7 | 2 | R | R | 21-17 | 1 | 30S | 5S | 21-25 | ;- | R | R |
| | 21-8 | 3 | 30S | 30S | 21-18 | 3 | 80S | - | 21-26 | ;- | - | - |
| | 21-9 | 1 | R | R | | | | | 21-27 | ;- | - | - |
| | 21-10 | 1 | F | F | | | | | | | | |

e-Probably escaped inoculations

5M-5D(D)-Dwarf line/ (T)- Tall line, LR-leaf rust, YR-yellow rust,

I-Derivative No. , II-Seedling Stage (77-5), III-Seedling Stage (104-2) , IV-Seedling Stage (YR-46S 119), P. No.- Progeny No.

F-Free From the Disease, R Resistance, TR- Trace Resistance, MR- Moderate Resistance S-Suscestibility %

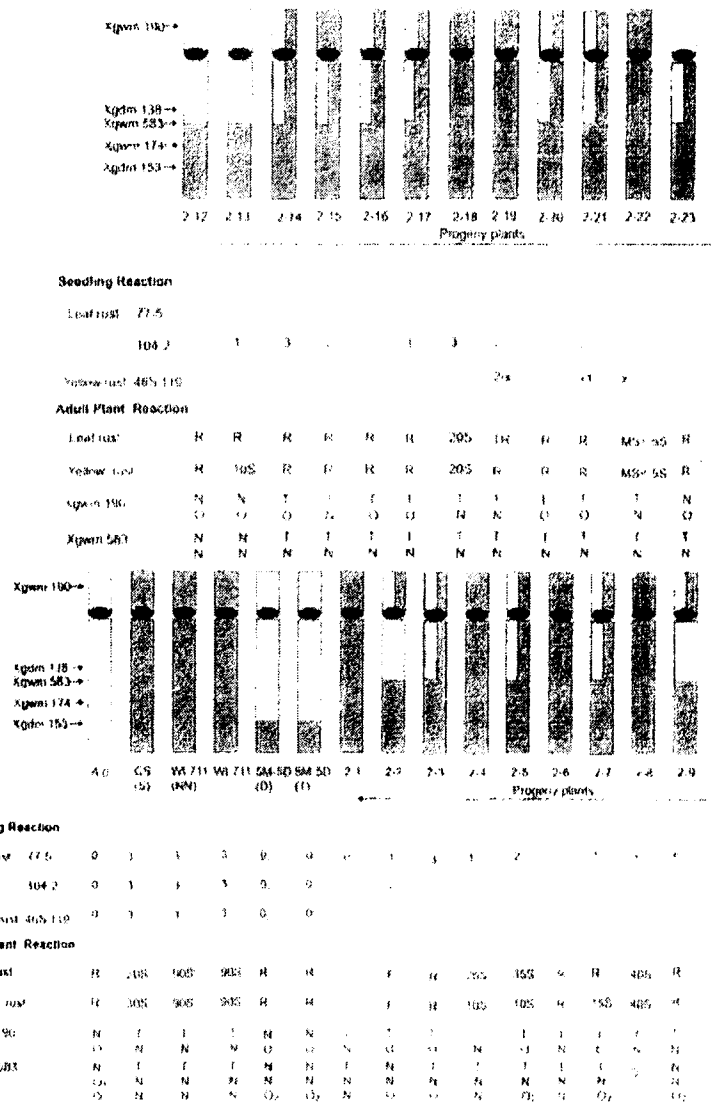


**Fig. 2. Graphical representation of structural changes inchromosome 5D in the progeny ($BC_2F_3$) of derivative number 2 along with rust reaction T, *T. aestivum* allele: $O_1$ / $O_2$, *Aegilops ovata* alleles: N, Null allele**

Most of these plants had similar infection types for leaf rust and yellow rust at seedling and adult plant stage. McIntosh (1992) reported that the adult plant leaf rust resistance gene *Lr* 34 displays adult plant resistance to yellow rust also due to a tightly linked gene. Singh (1992) and McIntosh (1992) have shown that moderate level of durable adult plant resistance to stripe rust of wheat cultivar *Anga* and Winter wheat (*Bezostaya*) is controlled by in part by the *Yr* 18 which is completely linked with *Lr* 34.

It is, therefore, concluded that the two closely linked slow rust resistance genes *Lr* 34 and *Yr* 18 have been transferred to high yielding wheat cultivar WL711.

A number of plants in some progenies with IT 3 at seedling stage gave complete resistance to one or both the rusts at adult plant stage (Table 1.) This resistance may have been due to the transfer of adult plant resistance also from 5M chromosome of *Ae. ovata* or due to interaction of various slow rusting and APR genes in the parents. The presence of APR genes, however, needs to be confirmed by appropriate rust screening.

## CONCLUSIONS

A number of derivatives and plants from their progenies were resistant to both the rusts, at seedling stage as well as adult stage. They showed similar IT as that of *Aegilops ovata*. Graphical presentation of these plants showed induced translocations from *Aegilops ovata*. Some of progeny plants with susceptible seedling reaction to either of the rusts, but very low incidence at adult plant stage under field conditions, without showing any of *Aegilops ovata* specific alleles, might have slow rusting gene(s) in one of the wheat parent with $Ph^I$ used in the crossed for induced homoeologous chromosome pairing. In the progeny plants which showed susceptible IT at seedling stage, but resistance at adult plant stage to both rusts, without any *Aegilops ovata* specific STMS 190 allele, the genes capable of giving adult plant resistance in combination with other genes also might have been transferred from "Chinese Spring".

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