Nilgiri Wheat News

(May – August, 2010)
IARI, Regional Station, Wellington
Vol 2 (2)

Wheat research turns molecular at Wellington

i) Development of molecular marker for *Lr 32* gene

Leaf rust caused by *Puccinia recondita* f.sp. *tritici* Rob.ex.Desm., surviving in nature in ample range of climatic conditions is considered to be the most important pathogen of wheat in the country as well as in the world . One of the ways to overcome the losses due to this disease is developing resistant cultivars. Even though many leaf rust resistance genes have been reported world wide but molecular markers linked to these genes have been discovered for only few of them. In this context, *Lr32* gene, which is not yet explored in agricultural application, is considered for molecular marker development because of its effective resistance against leaf rust. In order to develop molecular marker for *Lr32* gene, F_2 population of Agra Local X Tc+*Lr32* was taken as mapping population. Two markers AP-PCR $SS9L_{700}$ and ISSR marker $UBC801_{800}$ have been found to be associated with *Lr32* gene (Figure 1A and Figure 2A), since they were polymorphic to the gene. About 119 F_2 population of Agra Local X Tc+*Lr32* were screened using PCR technique using both the primers for the segregation of *Lr32* gene and it was found that the gene followed 3:1 Mendelian ratio for both of the primers (Figure 1B and Figure 2B). It was also found that two molecular markers AP-PCR $SS9L_{700}$ and ISSR marker $UBC801_{800}$ were not tightly linked since they followed the 9:3:3:1 Ratio of independent assortment. Future studies will be continued for the confirmation of the reported molecular markers in other *Lr32* careers and their linkage analysis.

Fig.1: PCR screening of F2 population using AP-PCR $SS9L_{700}$

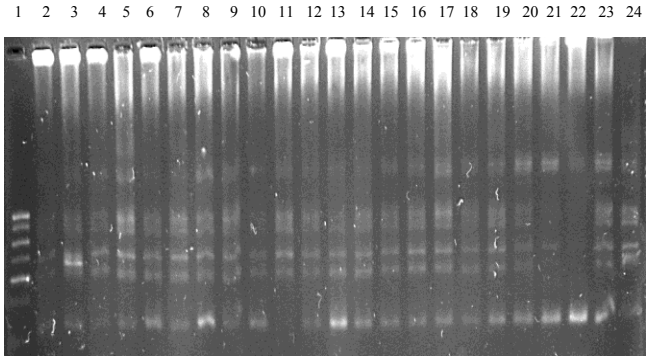


Figure: 1A (1)100bp Ladder, (2)Agralocal, (3).Tc+*Lr32*, LANE 4 to 24:Genotypes, (4) *Lr32*, (5) Vidisha, (6) Vaishali, (7) HD 2189, (8) PBW 343, (9) Kanchan, (10) Kite, (11) FLW2, (12) FLW6, (13) FLW8, (14) Kalyansona (15) Sonalika, (16) NIAW 34, (17)C 306, (18) Eagle, (19) Chinese spring, (20) NW 1012, (21) King, (22) Harrier, (23) Takari, (24) Unnath Kalyansona

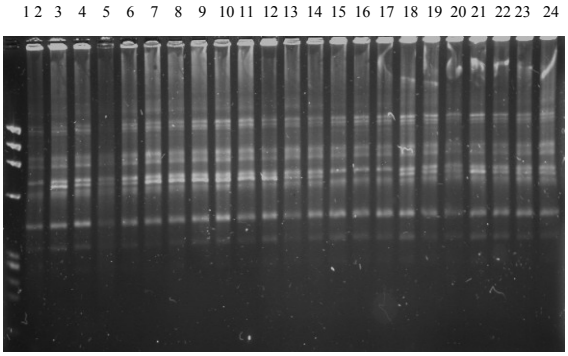


Figure: 1B (1)100bp Ladder, (2)Agralocal, (3).Tc+Lr32, LANE 4 to 24:F_2 population of AgralocalX Tc+Lr32, (4) 77A, (5) 77B, (6) 77C, (7) 78A, (8) 78B, (9) 78C, (10) 78D, (11) 79A, (12) 79B, (13) 79C, (14) 79D (15) 80A, (16) 80B, (17) 80C, (18) 80D, (19) 81A, (20) 81B, (21) 81C, (22) 81D,

Fig.2: PCR screening of F2 population using ISSR marker UBC801$_{800}$

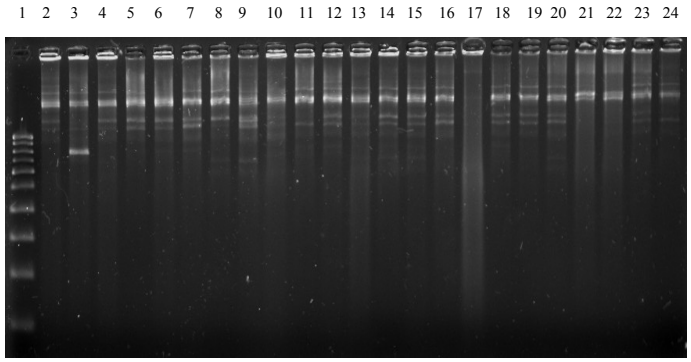


Figure: 2A (1)100bp Ladder, (2)Agralocal, (3).Tc+*Lr32*, LANE 4 to 24:Genotypes, (4) *Lr32*, (5) Vidisha, (6) Vaishali, (7) HD 2189, (8) PBW 343, (9) Kanchan, (10) Kite, (11) FLW2, (12) FLW6, (13) FLW8, (14) Kalyansona (15) Sonalika, (16) NIAW 34, (17)C 306, (18) Eagle, (19) Chinese spring, (20) NW 1012, (21) King, (22) Harrier, (23) Takari, (24) Unnath Kalyansona

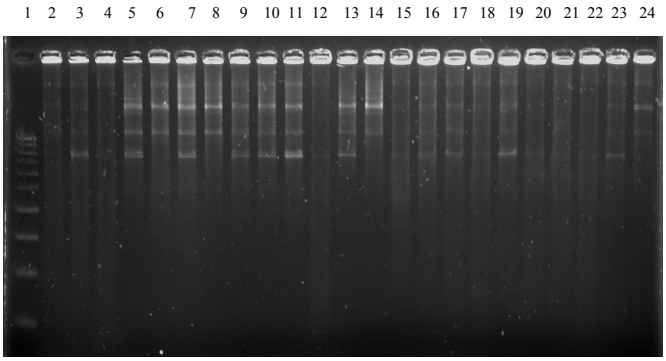
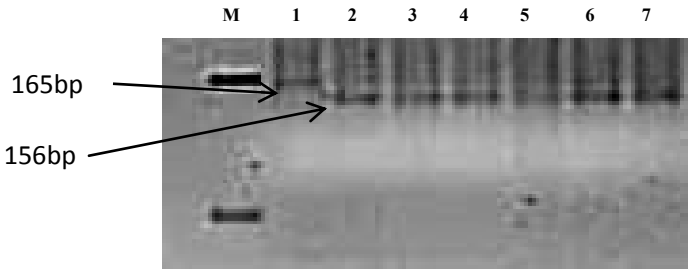


Figure: 2B (1)100bp Ladder, (2)Agralocal, (3).Tc+Lr32, LANE 4 to 24:F_2 population of AgralocalX Tc+Lr32, (4) 77A, (5) 77B, (6) 77C, (7) 78A, (8) 78B, (9) 78C, (10) 78D, (11) 79A, (12) 79B, (13) 79C, (14) 79D (15) 80A, (16) 80B, (17) 80C, (18) 80D, (19) 81A, (20) 81B, (21) 81C, (22) 81D, (23) 82A, (24) 82B

ii) A wheat variety developed for central India applying marker assisted selection (MAS)

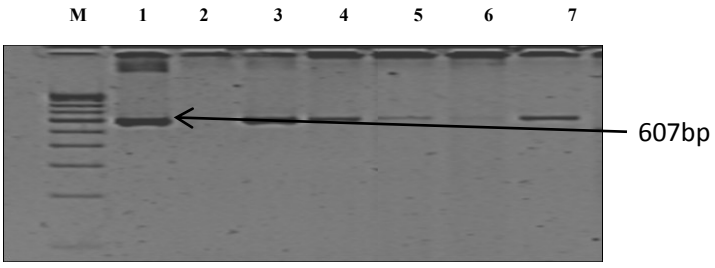
The Central Zone is the migratory route of stem and leaf rust uredospores to main wheat growing areas in Northern plains. Since the rain-fed wheat crop is sown early followed by timely sown irrigated wheat, the rust inoculum built up on any susceptible rain-fed variety will be of serious threat to the timely sown wheat crop in central India and Northern plains. Hence, the development of high yielding, rust resistant wheat varieties and their deployment in Central zone with an objective of diversifying the genetic basis of rust resistance is of paramount importance in order to contain the dissemination of uredospores to the Northern plains of the country. Keeping this in view, a bread wheat variety HW 5207-1 has been developed using MAS (Fig. 3) at this station carrying a high degree of stem and leaf rust resistance imparted by genes such as *Sr2*, *Sr24/Lr24*, hence it will act as an effective genetic barrier. Although, yellow rust is not a serious problem in this zone but it has been sporadically observed in areas of Rajasthan and U.P. adjoining to Madhya Pradesh. Thus, the presence of *Yr*15 gene in HW 5207-1 is also a desirable and added advantage.

Fig.3: Validation of presence of genes *Lr24* and *Yr 15* in HW 5207-1



(A) Marker linked to *Yr15*- Xgwm 273

M: 100 bp ladder, 1: Negative control (HD 2687); 2: Positive Control (Avocet^{6}/*Yr15*); 3-7: HW 5207-1



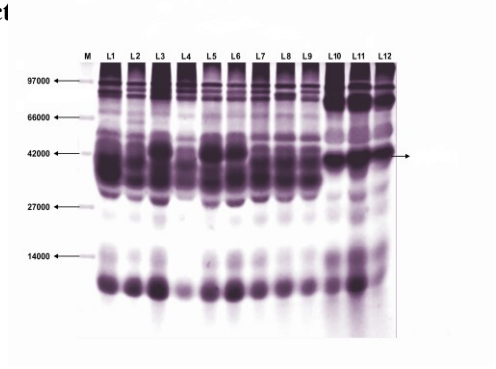
(B) Marker linked to *Lr*24- SCS1302

M: 100 bp ladder: 1. Positive Control (HD 2687+*Lr24*); 2. Negative Control (HD2687); 3-7: HW 5207-1

iii) Protein marker as a tool to detect *Secale cereale* – derived linked genes *Sr31, Lr26, Yr9* and *Pm8* in wheat

The six Indian popular wheat cultivars HD 2329, HD 2285, HP 1205, WH 147, J 24 and Lok-1 already carrying *Sr24+Lr24* which were introgressed with *Sr31* gene complex through conventional backcross method at IARI, Wellington were confirmed for the presence of *Sr31*.The molecular level analysis was carried out in CPMB & BT, TNAU, Coimbatore. The protein was extracted using the protein extraction buffer. The extracted protein was electrophoresed in a vertical dual gel unit (Sigma-Aldrich). Electrophoresis was carried out at a constant of 30mA until the bromophenol blue dye migrated to 1.5-2cm above the gel base. SDS-PAGE was carried out applying standard procedure.The gel was then rinsed with distilled water and destained in 10% (v/v) acetic acid and 30% (v/v) methanol for 20 minutes, followed by washing in distilled water for 50 minutes with gentle shaking. Finally the gel was documented on a digital gel documentation unit. The data on phenotyping of the constituted lines were also obtained at IARI, Regional Station, Wellington. The SDS-PAGE procedure revealed patterns of water soluble proteins to detect the 1BL/1RS translocation in wheat cultivars.The SDS-PAGE results showed that all the wheat stocks introgressed with *Secale cereale*-derived linked genes *Sr31, Lr26, Yr9* and *Pm8* viz., HW 4042(HD 2329 with *Lr28*),HW 4044 (Lok-1 with *Lr28*),HW 4047(WH 147 with *Lr28*),HW 4049(HD 2285 with *Lr28*),HW 4062 (J 24 with *Lr28*) carried the *Sec-1* band and the presence of the linked genes *Sr31, Lr26, Yr9* and *Pm8* thus confirming the 1BL.1RS translocation (Fig. 4). The recurrent parent HP 1205 already carrying *Sr31* gene complex shows the *Sec-1* band. The obtained protein bands corresponded to the secalins of the rye parent which were present in the wheat cultivars carrying 1B/1R translocation. The *Sec-1* band was not found in the recurrent parents HD 2329, HD 2285, WH 147, J 24 and Lok-1 that do not posses *Sr31* gene thus suggesting the absence of the 1B/1R translocation.

Fig. 4. Banding patterns of seed protein extracts from wheat stocks and various controls subjected to SDS-PAGE elect



For the cultivars listed below the presence and absence of *Sec-1* band therefore the presence or absence of 1BL.1RS, is indicated by (+) and (-), respectively. From left to right: M-Marker (14-97Kda), 1.HW 4444(+), 2. WH 542(D)(+), 3.HW 4049(+), 4..HW4042(+), 5.HW 2038(RP)(-),.6.HW 2037(RP)(-),.7.HW 4062(+),.8.HW 4044(+),.9.HW 4047(+),.10.R-1(+),.11.R-2(+),.12.R-4(+); D-Donor, RP-Recurrent parent, R-Rye parent.

Genetic stocks for rust resistance

Final constitutions of lines carrying *Lr*45+*Lr*19, *Lr*45+*Sr*31, *Lr*45+*Yr*10 and their combinations in 30 popular Indian bread wheat cultivars are complete. BC3F3 generation has been raised carrying resistance genes *Lr*35*, Lr39, Lr*47 along with *Yr*10 and the seed has been harvested successfully.

Rust pathotyping in Nilgiris

As many as 167 samples of wheat brown rust and 40 of wheat black rust collected from Wellington area in Nilgiri hills were analysed between May to August, 2010. Brown rust race 77-5 was found dominant followed by 77A, 77-8, 17 and

77-7. In black rust, two races 40A and 40-1 prevailed in equal proportions.

Distinguished Visitors

Dr. C.D. Mayee, Chairman, ASRB honoured the station by his kind visit in July, 2010.

Dr. S. Nagarajan, Chairman, PPVFRA graced the station by his kind visit in May, 2010.

Dr. H. Bariana, University of Sydney visited in July, 2010 for undertaking a collaborative research programme on wheat rust resistance breeding.

News

Dr. M. Sivasamy has been on deputation of two months w.e.f. 29th July, 2010 to CIMMYT, Mexico for carrying out a visiting scientist assignment on wheat rust resistance breeding.

Joining

Sh. Bhadra Kumar, Assistant joined station in June, 2010.

Retirement

Mr. A. Hallan (SSS) retired on 31st May, 2010 after serving the station for 32 years. Best wishes for his healthy and prosperous retired life.

Notice for summer nursery users

Black and brown rust epiphytotics are in full bloom these days. Please visit Wellington for selections and rust scoring as soon as possible since disease symptoms will remain so maximum for two weeks from now and may not be visible as crop is drying fast due to maturity.