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Wheat Research Station, Wellington – An Overview

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IARI, Regional station, Wellington played a historically pivotal role in bringing the food self sufficiency in India as high yielding wheat varieties namely Lerma Rojo 64A, Sonora 63, Sonora 64, Mayo 64 alongwith 630 selected samples of superior semidwarf wheat lines sent to India from Mexico by noble laureate Dr. N.E. Borlaug as harbingers of Indian green revolution were first multiplied at this station during summer season of 1964. The seeds thus harvested during wheat off-season (summer) of 1964 were distributed countrywide for revelation to farmers through multilocational trials and further spread of these varieties in the entire country. After cultivation of these Mexican wheats, India became food self sufficient country. In due course of time, this station has blossomed into an excellent off – season nursery center mainly for wheat and also for several other winter crops for research organizations of the entire country. By doing generation advance during off-season of the crop year, it becomes possible for the Indian wheat breeders of all ICAR institutes and SAUs in cutting short the varietal development period to half i.e. from eight years to four years. This important research activity can not be undertaken elsewhere in the country as reliably as at Wellington.

Breeding for rust resistant varieties can be done more efficiently at IARI, Regional Station, Wellington as compared to other parts of the country owing to "year around" availability of rust inoculum through the agency of self sown plants and off season grown wheats needed to screen breeding progenies effectively for rust resistance. Almost all Indian wheat breeders grow their breeding populations obligatorily at Wellington so as to screen for three rusts simultaneously alongwith other diseases such as foliar blights, powdery mildews, head scab etc. Such natural facility for screening for rust resistance is not available anywhere in the country except at Wellington. A very strong backcross programme started at this station three decades ago and has established very strongly now. As a result, several genetic stocks to be used as donors for yield traits have been generated here and registered with NBPGR. Also small seed quantities are distributed to all the Indian wheat breeders from time to time for usage in future wheat breeding programmes of the country. Being unique location in the country where wheat can be grown during any time in the year (generally 2-3 crops are taken in one year), such pre breeding activities especially in wheat crop can be undertaken with a convincing success rate in our country only at Wellington.

IARI Regional Station, Wellington ideally located in south Indian Nilgiri hills is gifted with natural environment for conducting research on Wheat – rust system for the entire year and hence is thus effectively employed to identify rust pathotypes in the surrounding areas which act as primary source of inoculum for rabi crop grown in central and western India. The field isolates are maintained here as pathotypes more efficiently due to congeniality of weather remaining in favour of wheat rusts survival *in vitro*. Intensive surveying is accomplished here for race mapping and detection of any new variations. Indian pathotypes of wheat rust pathogens collected in this way are thus employed to postulate rust resistance genes in the breeding progenies in pipeline to become future wheat cultivars in India. Infrastructure needed for such types of works is already existing in a well maintained manner at this station. Rust pathology work is undertaken here very much parallel to other wheat rust laboratories in the country for supplementary/confirmatory conclusions. Moreso, this station is an excellent standby wheat rust laboratory in the country.

Further, challenges of wheat stem rust race Ug99, and current situation of the spread of this disease poses a serious threat to food security, especially in the developing countries including those in the Indian sub continent. Country like India which is at the direct risk, should be pro active to conduct Ug99 specific surveillance. The gradual spread of the *Yr9*-virulent wheat stripe rust from eastern Africa to the South Asia suggests that the entire wheat area in Asia (except China) is a common epidemiologic zone that is connected to Eastern Africa. Therefore, if a new race like Ug99 arises anywhere in the African continent, given time, it could spread throughout the above said Asian epidemiologic region. Also there is possibility of the long distance travel of rust spores, in the jet streams and on the clothing of world travelers. It is only a matter of time until Ug99 reaches across the Saudi Arabian peninsula and into the Middle East, South Asia, and eventually, East Asia. Keeping above facts in consideration, the Nilgiri hills in south India are one of the prospective targets of Ug 99 virulence. Continuous vigil is thus imperative for introduction of this dreadly variant of black rust pathogen in Indian locations and Wellington station is an ideal place in the country to pursue this national activity since black rust survives *in vivo* here throughout the year.

IARI, Regional Station, Wellington

IARI regional Station, Wellington fulfils the nationally important Wheat research needs through the mandatory activities listed below:

- Organization of national off – season nursery mainly for wheat, barley and triticale. Facilities are also extended to other crops namely mustard, pea, lentil, safflower, sugarcane etc.
- Breeding disease resistant and high yielding wheat cultivars suitable for cultivation under agro – ecological conditions of southern hills in particular and the whole country in general.
- Wheat rust pathotyping and rust resistance gene postulation in India – supplementary/standby national facility (parallel ICAR facilities exist with DWR, Regional Station, Flowerdale, Shimla).
- Pyramiding of genes for durable rust resistance in popular high yielding Indian wheat cultivars.
- Popularisation of rust resistant cultivars in southern hill ranges with a purpose to curtail reservoir of primary rust inoculum responsible for rust epiphytotics in peninsular and central India and popularizing wheat cultivation in non traditional wheat growing areas in India with an objective to enhance the national wheat production for ensuring food security in the country.
- Discerning genetics of rust resistance in Indian wheat germplasm through phenotyping rust response using pathotypes prevailing in southern hills, central and peninsular India.
- Co-ordination of All India Wheat & Barley Improvement trials in southern hill zone.

New avenues of wheat research at Wellington – highlights of achievements

i) Development of molecular marker for Lr32 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.

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

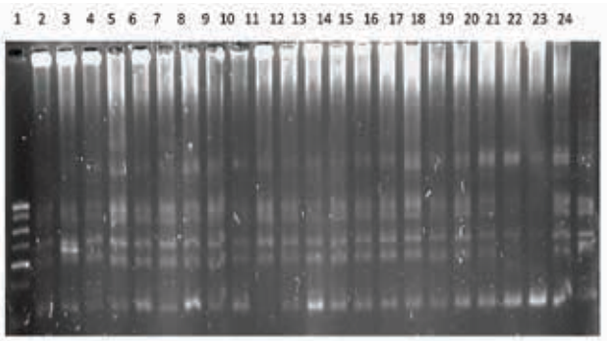


Figure: 1A (1)100bp Ladder, (2)Agralocal, (3).Tc+ *Lr32*, LANE 4 to 24:Genotypes, (4) *Lr32*, (5) Vidisha, (6) Vaishali, (7) HD 2189, (8) PBW 343, (9) Kanchan, (10) Kite, (11) FLW2, (12) FL W6, (13) FLW8, (14) Kalyansona (15) Sonalika, (16) NIAW 34, (17)C 306, (18) Eagle, (19) Chinese spring, (20) NW 1012, (21) King, (22) Harrier, (23) Takari, (24)

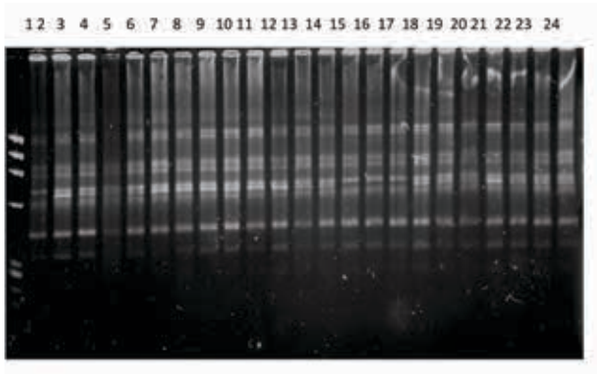


Figure: 1B (1)100bp Ladder, (2)Agralocal, (3).Tc+Lr32, LANE 4 to 24:F_2 population of AgralocalX Tc+Lr32, (4) 77A, (5) 77B, (6) 77C, (7) 78A, (8) 78B, (9) 78C, (10) 78D, (11) 79A, (12) 79B, (13) 79C, (14) 79D (15) 80A, (16) 80B, (17) 80C, (18) 80D, (19) 81A, (20) 81B, (21) 81C, (22) 81D, (23) 82A,

Fig.1 PCR screening of F_2 population using AP-PCR $SS9L_{700}$

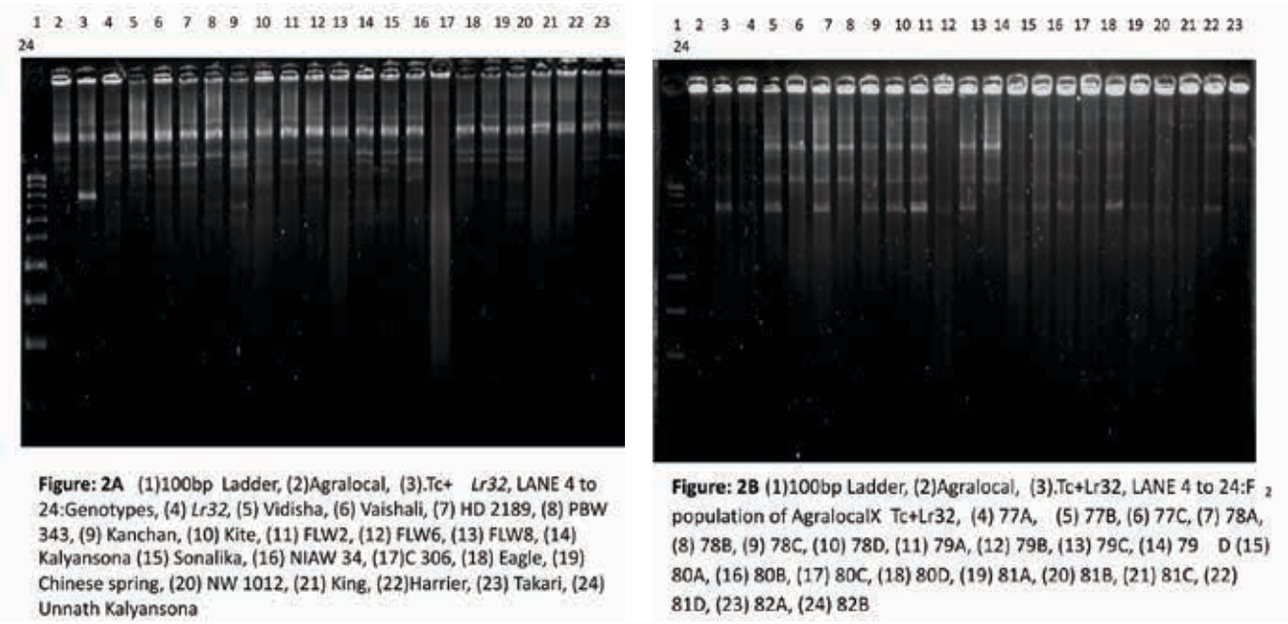


Figure: 2A (1)100bp Ladder, (2)Agralocal, (3).Tc+ *Lr32*, LANE 4 to 24:Genotypes, (4) *Lr32*, (5) Vidisha, (6) Vaishali, (7) HD 2189, (8) PBW 343, (9) Kanchan, (10) Kite, (11) FLW2, (12) FLW6, (13) FLW8, (14) Kalyansona (15) Sonalika, (16) NIAW 34, (17)C 306, (18) Eagle, (19) Chinese spring, (20) NW 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) 79 D (15) 80A, (16) 80B, (17) 80C, (18) 80D, (19) 81A, (20) 81B, (21) 81C, (22) 81D, (23) 82A, (24) 82B

Fig. 2 PCR screening of F_2 population using ISSR marker $UBC801_{800}$

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.

iii) Protein marker to detect 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

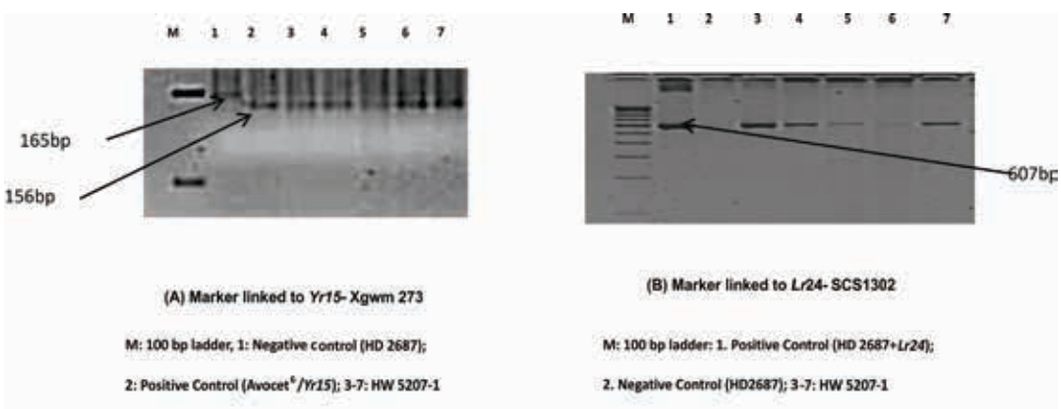


Fig.3 Validation of presence of genes *Lr24* and *Yr 15* in HW 5207-1

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

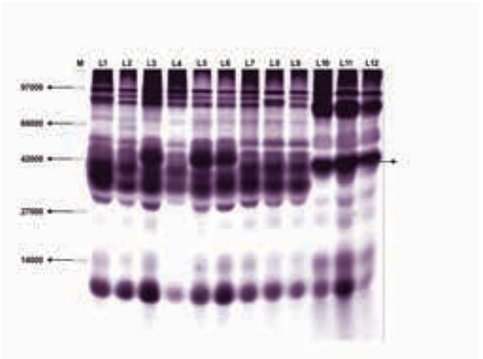


Fig. 4 Banding patterns of seed protein extracts from wheat stocks and various controls subjected to SDS-PAGE electrophoresis

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.

Therapeutic wheat variety HW 1095 released

A semi – dwarf, disease resistant, nutritionally rich, economically viable and high yielding *dicoccum* (Samba wheat) wheat developed at Indian Agricultural Research Institute, Regional Station, Wellington using mutation technique is released for parts of Tamil Nadu and Southern hill zone, including non traditional areas in collaboration with Tamil Nadu Agricultural University, Coimbatore as state release. This variety is the outcome of a meticulously planned dicoccum improvement programme which was undertaken at IARI, Regional Station, Wellington during 2002 for developing semi dwarf dicoccum varieties without altering the quality of the earlier dicoccum varieties NP200, NP 201 and NP 202, employing mutation breeding. The gamma irradiation of 10(100 Gy), 20(200Gy), 30 (300Gy), 40(400Gy) Kr-rays was given at optimal seed moisture level (Gray: The Gray (Gy) is the unit of absorbed dose and is 1Joul per kg). The irradiated seed were sown as M1 and desirable plants were picked at M2 in 200Gy dose. The stable population was fixed at M4 and during 2005 it was entered in All India co-ordinated Wheat trials as HW 1095. The variety has also been named alternatively as TNAU Wheat COW 2.

Dicoccum whole wheat flour in the regular diet of diabetic patients significantly reduces total lipids ($p \leq .01$), triglycerides ($p \leq .01$) and LDL Cholesterol ($p \leq .05$) (Yenagi *et al.,* 2001). HW 1095, the dicoccum wheat may thus posses therapeutic properties that can effectively reduce the cardiovascular risk factors. Managing diabetes, a life long ailment, with medicines is very expensive and *dicoccum* diet plays a crucial role in reducing the levels of plasma cholesterol and lowering glycemic responses. Hulled wheat grain of *dicoccum* is used mainly as alternative of expensive medicines in the health food market. Most of the suggested beneficial effects of these cereal are due to specific characteristics of their fibre. The pyrolysis fragments derived from the polysaccharide fraction were significantly more abundant in emmers (*dicoccum*) than in the other genotypes, whereas the highest percentage of lignin-derived pyrolysis fragments was detected in *durum* wheat. Many results suggests that the emmer (*dicoccum*) genetic material may represent a source of high-value dietary fiber and it has much higher fiber contents than common wheat.

Salient features of HW 1095

- It is a NP200 - Mutant through Gamma irradiation (200 Gray) and it matures in 110 days which belongs to early duration group.
- The culture HW 1095 recorded a mean grain yield of 4040 kg/ha, which is an increase of 26 % grain yield over the NP 200 in a total of 98 trials for the past five years. The culture NP 200 was used as check. The yield of NP 200 was 3190 kg/ha.
- The culture HW 1095 has 10-12 productive tillers with long and slightly tapering ears. The special attribute of this culture is the broader and waxy green foliage, drooping leaves, non-lodging habit and non shattering of grains. In respect of quality, it is rich in protein (13.2%) and posseses high sedimentation value (25). The reddish colour grain provides good grain appearance score of 8.
- The culture exhibited resistance to black (stem), yellow (stripe) and brown (leaf) rusts. There is no major incidence of pests in this samba wheat culture.
- HW 1095 occurred 11/ 18 times in first non-significant group indicating wider adaptability and stability in performance across the zones.

Wheat rust monitoring – emphasis on Ug99 race of stem rust

Wheat rust pathotyping was accomplished in 85 field samples of brown rust and 52 of black rust collected from Wellington area in Nilgiri hills during 2010-11. In brown rust, races of 77 group were identified with field dominance of race 77 - 5 followed by 77 - 8, 77A and 77-7. In black rust, two races 40A and 40-1 were found prevailing in equal proportions.

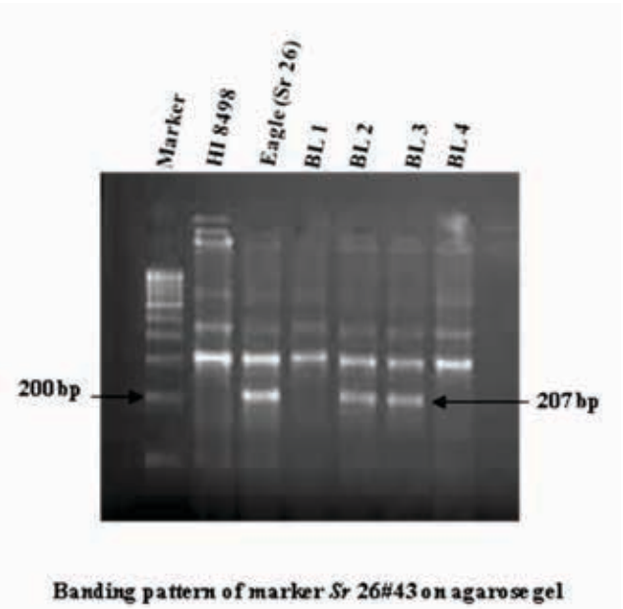
Ug 99 nursery comprising wheat lines named as Morocco (no Sr gene), LMPG (no Sr gene), Seri - MACS 2496, Bacanora - WH 542, Attila - PBW 343, Sr31/LMPG, Sr24 (Tr380-14),Sr36(Cook-2), Sr36(Cook), Sr36(LMPG) has been planted at Shimla, Delhi, Indore and Wellington in rabi, 2010 for the purpose of monitoring expected incidence of race Ug 99 in India. This nursery is grown at Wellington throughout the year by doing sowings in such a manner that green foliages are always available to trap the Ug 99 or its variants in case , they reach Nilgiri hills. Critical observations made in the nursery for last one year indicated that race Ug 99 or its variants are not present in Nilgiri hills.

A new pathotype of *Puccinia striiformis tritici* recorded at Wellington

Wellington area in Nilgiri hills of Tamil Nadu is traditionally known to harbour pathotype I (38S102) of wheat yellow rust pathogen since mid seventies. No further evolution of this pathogen could be noticed even after diverse germplasm sources having major resistance genes were planted here by various wheat breeders of the country and were available throughout the year (continued host – pathogen contact) for serving as mutational ground for pathogen to throw new variants. Why mutation for new variation in *P.striiformis* has been so poor in Nilgiris is a matter of further investigation. But incidently, during the rabi season of crop year 2010-11, a variant has been monitored in several field samples showing wider virulence than pathotype I (38S102) with a remarkable feature that alongwith several resistant genes it can infect *Yr* 9, a gene present in most of the present day Indian wheat cultivars. Samples have also been sent to DWR, Regional Station, Flowerdale for further confirmation of the novel variation observed in *P. striiformis* at Wellington.

Introgression of novel alien genes into popular Indian Durum cultivars – A new initiative

In India, durum wheat (*Triticum durum*) occupies second place after bread wheat in production and area of cultivation.



Banding pattern of marker *Sr* 26#43 on agarose gel

It is cultivated primarily in Central, Peninsular and Western Plain zones of India. As in Bread wheat (*Triticum aestivum*), durum wheat is also affected by three rust pathogens viz., stem, leaf and yellow rust. Incorporation of major / minor rust resistance genes is found to be an effective strategy to control the rust in bread wheat in India and worldwide. Currently majority of these rust resistance genes are effectively exploited only in the *Triticum aestivum* background and not in the durum background. Hence an attempt was made at I.A.R.I. - Regional Station, Wellington to transfer the alien stem rust resistance gene into durum background employing conventional Back Cross (BC) and Marker Aided Selection (MAS) approach. The effective stem rust resistance gene *Sr* 26 which was transferred from the long arm of a group 6 *Agropyron elongatum* chromosome to wheat chromosome 6AL (Knott, 1968) is used. Gene *Sr* 26 is one among the few major genes effective against the Ug 99 lineage. Initial crosses were performed in Rabi 2009 and the F_1's were critically evaluated. Leaf samples were collected from BC_1F_1 lines three weeks after sowing and genomic DNA was extracted from leaf samples according to Cetyl tri methyl ammonium bromide (CTAB) based procedure with minor modifications. Polymerase chain reaction (PCR) was performed using AFLP marker for *Sr* 26#43 (Mago *et al*., 2005). The PCR products were electrophoresed at 3% agarose gel for about one hour, visualised under UV light and photographed. Among the four BC_1F_1 lines evaluated for the presence or absence of *Sr* 26 gene, two lines (BL2 & BL3) showed polymorphism at 207 bp, whereas the other two lines (BL1 & BL4) did not show polymorphism for *Sr* 26 gene. It is now in the BC_1F_1 stage, further critical evaluation of the backcross lines is required to know whether alien chromosome in durum background have yield or quality defects.

Breeding for multiple resistance in wheat using wild species/relatives

In any wheat breeding program the goal is developing varieties with stable and high yield over wide range of environmental conditions alongwith resistance to all the rusts. Variability in primary gene pool has nearly been exhausted in the case of diseases' resistance which necessitates the transfer of genes from wild species of secondary and tertiary gene pool. In the past, such transfer has contributed genes of leaf and stem resistance to cultivated hexploid wheat. An attempt was made at IARI, R.S., Wellington, to transfer *Thinopyron ponticum* derived linked genes *Lr*19 + *Sr*25, *Triticum timopheevi* derived linked genes *Sr*36 + *Pm*6 both present in Australian cultivar 'Cook' and *Ae. squarrosa* derived leaf rust gene *Lr* 32 in different combination in the backround of popular bread wheat HD 2402 and HS 240. The multiple disease resistant wheat lines HW 4013 (HS 240 carrying *Lr*32) and HW 4205 (HD 2402 carrying *Lr*19 + *Sr*25 and *Sr*36+ Pm6) were constituted at BC2F2 stage. The line HW 4205 is resistant to the leaf rust, stem rust and powdery mildew which is attributed to the presence of resistance genes *Lr*19 + *Sr*25 and *Sr*36 + Pm6 and these genes together confers high degree of resistance

at Wellington although a virulent race has been reported from peninsular zone. The recurrent parent HD 2402 is already conferring resistance to yellow rust. The gene *Lr*32 offers high degree of resistance to leaf rust in the variety HW 4013 which in addition has stem rust gene *Sr*31 and stripe rust resistance gene *Yr*9. These constituted varieties are being tested under common variety trial of IARI.The multiple disease resistant wheat lines HW 4013 carries *Lr* 32 , *Sr*31 and *Yr* 9 (Donor: Thatcher, Recurrent parent: HS 240). The line HW 4205 is resistant to leaf rust, stem rust and powdery mildew which is attributed to the presence of resistance genes *Lr*19 + *Sr*25 and *Sr*36+ *Pm*6 (Donor: Cook, Recurrent parent: HD 2402)

Strengthening genetic base of rust resistance in Indian Wheat germplasm

Superior back cross derivatives of 20 popular cultivars carrying *Lr*45, *Lr*45+*Sr*31, *Lr*45+*Lr*19+*Sr*25, *Lr*35, *Lr*39, *Lr*47 along with *Yr*10 and stem rust genes *Sr*2, *Sr*14, *Sr*22, *Sr*24, *Sr*25, *Sr*26, *Sr*27, *Sr*29, *Sr*30, *Sr*33, *Sr*35, *Sr*36, *Sr*44 have been advanced to BC_3F_4 stage. Populations having been introgressed with *Lr*35, *Lr*39, *Lr*47 along with *Yr*10 advanced to BC_3F_5.

Populations availed after introgression of targeted stem rust genes (*Sr*2, *Sr*14, *Sr*22, *Sr*24, *Sr*25, *Sr*26, *Sr*27, *Sr*29, *Sr*30, *Sr*33, *Sr*35, *Sr*36, *Sr*44) in order to pyramiding them in at least 20 popular cultivars advanced to BC_3F_3 stage (pre-emptive breeding for Ug99 race resistance). BC_3 generation of populations bred for durable resistance involving APR genes *Lr*34, *Lr*46, *Lr*67, *Sr*2, *Sr*22 etc. raised successfully. Also raised BC1 of F1 population carrying new rust resistance genes *Lr*44, *Lr*32, *Lr*19, *Lr*53, *Lr*57 etc. Work on molecular marker development and validation for *Lr45* is in progress. World known head scab resistant stock Sumai – 3 has been crossed with popular Indian wheat cultivars. F_1 will be harvested in April, 2011. As many as 20 offsprings of cultivated x wild relatives F_1 cross were evaluated for the traits of yield and rust resistance. As many as 60 popular Indian wheat cultivars have been subjected to genetic analysis of necrotic (Ne genes) behaviour.

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