6. SUMMARY

6.1. Transfer of rust resistance genes to Indian wheat cultivars

6.1.1. Constitution of near-isogenic lines

1. Specific genes for rust (leaf and stem) resistance were transferred from alien hexaploid wheat stocks into three Indian hexaploid wheat cultivars viz. MACS 2496, HD 2687 and PBW 343. The donor parents include 6 hexaploid wheat stocks carrying a total of 5 leaf rust resistance genes (Lr19, Lr24, Lr28, Lr32, Lr37), and four stem rust resistance genes (Sr24, Sr25, Sr36, Sr38) present either individually or in combination (linked). Transfers were made using simple backcross breeding method followed by selection.

2. Selection was exercised either in BC2 and/or BC5 generations and finally one near-isogenic line each in BC2F5 and BC3F5 was constituted from all 18 cross combinations. All the constituted lines were screened against individual rust races at seedling stage in the glasshouse and with a mixture of rust races at adult plant stage in natural/artificial conditions in the field. Immune to moderately resistant reaction at seedling stage and highly resistant reaction at adult plants stage provided by the above incorporated genes strongly advocate the use of specific rust resistance genes for durable resistance.

3. All the rust resistance genes used provided moderate to high degree of resistance to respective rusts in the field condition. Specific rust resistance genes like Lr19, Lr24, Lr28, Lr32, Lr37 and Sr38 provided their resistance individually, while other rust resistance genes like Sr24 and Sr25 provided their resistance in combination with other resistance genes already present in the genetic background of recurrent parents.

4. Yield performance of the constituted near-isogenic lines was tested under artificially created rust-free condition created by using the chemical 'Contaf'. Many of the constituted near-isogenic lines gave significantly higher grain yield than the chemical
treated control plants. In general, the agronomic performance of the plants constituted at BC$_2$F$_5$ was superior to BC$_3$F$_5$ selected plants for plant height, tiller number per plant, spike length, number of spikelets per spike, 1000-grain weight and grain yield. However, the various agronomical characters recorded in BC$_3$F$_5$ were also comparatively superior to those of the untreated recurrent parents. Number of tillers/plant, number of spikelets/spike and 1000-grain weight are the most important yield determining characters in the constituted near-isogenic lines. The near-isogenic lines constituted at BC$_2$F$_5$ had very good agronomic characteristics, but the seed quality (plumpness, seed weight, seed size, seed colour) was poor in many lines. Based on seed quality coupled with good agronomic characters and yield, 13 lines were finally selected for commercial purpose and the remaining 5 lines were grouped as genetic stocks for use in further breeding programmes.

6.1.2. Confirmation of transfer of rust resistance genes to Indian wheat cultivars

1. Transfer of rust resistance genes into Indian wheats was confirmed through morphological, genetical and biochemical markers.

2. The presence of morphological markers of the donor parents such as awnless spike (Darf/3 Ag*6/Kite - Lr24+Sr24), lax spike (Thatcher*8/VPM 1 - Sr38+Lr37+Yr17) and reduced yellow pigment in the seed flour (Cook*6/C 80-1 - Lr19+Sr25) in the F$_1$ hybrid derivatives of different crosses between Indian wheats X donor parents suggests the successful transfer of these morphological characters along with rust resistance genes from the donor parents to recipient Indian wheats.

3. Inheritance studies in near-isogenic lines for Lr19, Lr37 and Lr24 involving crossing of each of the near-isogenic lines with universal susceptible wheat cultivar Agra Local showed that the rust resistance in the near-isogenic lines each was due to a single dominant gene. The F$_1$ hybrids exhibited complete rust resistance, while the F$_2$ plants segregated into 3 resistant : 1 susceptible ratio to respective rusts. Similarly,
the BC1 hybrids segregated into a ratio of 1 resistant: 1 susceptible to respective rusts. These results confirm the presence of rust resistance gene.

4. The F2 segregation data of the monosomic and disomic F1 hybrids of the cross between the complete set of monosomics of the wheat variety Chinese Spring and the constituted near-isogenic lines for Lr19 (HD 2687), Lr37 (PBW 343) and Lr24 (MACS 2496) were studied for respective rust resistance. It showed that segregation of 3:1 ratio (resistant and susceptible) except for lines 7D, 2A and 3D respectively, thereby confirming the successful incorporation of these genes on to the respective chromosomes of the recipient wheat parents.

5. Studies on changes in the enzymatic activities of peroxidase, polyphenol oxidase, catalase, lipoxygenase in the leaves of 25 day old plants of rust susceptible wheat parents and rust resistant near-isogenic lines inoculated with respective rust pathogens showed altered activity. Constituted lines showed higher peroxidase activity compared to healthy controls during 2-7 days after post inoculation. The activity of polyphenol oxidase showed an increase in all near-isogenic lines during 3-7 days of post inoculation, while it showed declined activity in the susceptible parents. Catalase activity was higher in susceptible wheat parents than resistant near-isogenic lines. The lipoxygenase activity was increased both in susceptible wheat parents and their near-isogenic lines after two days of inoculation, but subsequently declined towards 7 days after inoculation in resistant plants, whereas consistent increase was noticed in the susceptible parents. The total lipid content of the leaves showed an increase in both susceptible and rust resistant near-isogenic lines after two days of inoculation but subsequently decreased with increase in post inoculation time. The percent decrease was more in susceptible parents than the resistant near-isogenic lines. The soluble protein content increased in resistant near-isogenic lines after 24 hours of inoculation but subsequently decreased towards later stages of infection.
The percent decrease was more in susceptible than resistant lines towards 7 days after inoculation. The specific activities of Ribonuclease-I and combined Ribonuclease-II and Nuclease-I were high at 15th day stage compared to 10th day in susceptible as well as resistant lines. Resistant near-isogenic lines had relatively higher chlorophyll content than the susceptible wheat parents. The contents of total free amino acids were increased up to 8th day of inoculation both in susceptible and in resistant wheat lines, after that there was slight reduction in both the cases. Respiration rate increased to a greater extent in resistant near-isogenic lines compared to susceptible wheat parents. After 3rd day of inoculation the reduction in respiration rate was drastic in susceptible parents, while in resistant near-isogenic lines the respiration rate level was more or less constant. Significant increase in total free phenols, tannin and nuclear DNA contents was observed in the constituted near-isogenic lines over their respective recurrent wheat parents.

6.2. Genetic divergence and character association in hexaploid wheat

1. The $D^2$ values corresponding to the paired comparisons between 120 hexaploid wheat varieties were grouped into 11 clusters.

2. The cluster I being the largest included 62 genotypes followed by cluster IV with 20, cluster VI with 13 and cluster XI with 11 genotypes. Remaining genotypes were distributed in 7 clusters with 2 genotypes in each cluster.

3. Analysis of variance for each individual character showed highly significant differences among the genotypes for all the sixteen characters.

4. The mean performance of each cluster for the 16 characters under study showed appreciable differences for all the characters.

5. The characters viz. harvest index, protein content, flag leaf area and straw strength contributed maximum to genetic divergence. The characters like days to heading, days to maturity, plant height and threshability contributed minimum to the genetic divergence.
6. The inter cluster distances between the members of the clusters II and X exhibited maximum followed in descending order by the members of clusters II and IX, II and IV, IV and VI, III and IV, II and VIII, VI and IX, II and III, III and V. The members in the cluster IX and X exhibited minimum inter cluster distances.

7. The intra cluster distance between the members of cluster VI was maximum followed in descending order by clusters IV, I and XI. The intra cluster distance between the members of cluster II exhibited minimum distance.

8. The correlation studies revealed that the trait plant height showed positive correlation with all the traits except 1000-grain weight and texture. The trait tillers / plant showed positive correlation with plant height, spike length, spikelets per ear, grain yield / plant, 1000-grain weight, grain yield, straw strength and harvest index, and showed negative correlation with other traits. The trait spikelets / ear showed positive correlation with all the traits except texture and biological yield. The trait harvest index showed negative correlation with biological yield, texture and days to maturity, and showed positive correlation with other traits. The trait biological yield showed negative correlation with tillers / plant and spike length, and positive correlation with other traits. Grain yield showed positive correlation with plant height, tillers / plant, spike length, spikelets per ear, grain yield / plant, 1000-grain weight, grain yield, straw strength, texture, flag leaf area, protein content and harvest index.

9. In the present study, six genetically diverse and superior genotypes were selected mainly from cluster IV, II and XI. As these six divergent genotypes were exceptionally superior to all the remaining genotypes for one or more traits. It is proposed that these genotypes may be involved in a multiple crossing programme to recover transgressive segregants with high genetic yield potential.
6.3. Hybrid weakness in hexaploid and tetraploid wheats

6.3.1. Hexaploid wheat (Triticum aestivum)

1. A total of eighty-five hexaploid wheat varieties were crossed with three hexaploid tester varieties viz. Triticum macha var. subietschchunicum (Ne₁ ne₂ Ch₁ ch₂), Triticum aestivum cv. C 306 (Ne₁ ne₂ ch₁ Ch₂) and Triticum aestivum cv. Sonalika (ne₁ Ne₂ ch₁ Ch₂).

2. Out of eighty-five aestivum wheat varieties, twenty-one of them produced normal F₁ hybrids with all the three testers, indicating the non-carriers of necrosis (Ne₁, Ne₂) and chlorosis genes (Ch₁, Ch₂).

3. Fifty-two varieties showed both necrotic and chlorotic symptoms with T. macha, but only necrotic symptoms with the second tester C 306, and normal with Sonalika indicating that these varieties are carriers of the genes Ne₂ and Ch₂ (ne₁Ne₂ch₁Ch₂).

4. The remaining twelve varieties showed only chlorotic symptoms with tester T. macha, normal with tester C 306 and necrotic with tester Sonalika indicating that these varieties are carriers of Ne₁ and Ch₂ (Ne₁ ne₂ ch₁ Ch₂).

5. From the above crosses it is concluded that out of 85 hexaploid wheat varieties, fifty-two varieties are carrying the Ne₂ and Ch₂, (ne₁ Ne₂ ch₁ Ch₂) and twelve varieties are carrying both the Ne₁ and Ch₂ (Ne₁ ne₂ ch₁ Ch₂). The remaining twenty-one (25%) varieties are non-carriers (ne₁ne₂ch₁ch₂) for both these genes.

6.3.2. Tetraploid wheat (Triticum dicoccum)

1. Eleven Triticum dicoccum varieties were crossed with three hexaploid wheat testers. Three hybrids showed normal symptoms with all the three testers indicating that the varieties are non-carriers for both necrosis and chlorosis genes (ne₁ ne₂ ch₁ ch₂).

2. Six varieties produced normal hybrids with T. macha, chlorotic symptoms with C 306 and both necrotic and chlorotic symptoms with Sonalika thus indicating that these varieties are carriers of Ne₁ and Ch₁ genes (Ne₁ ne₂ Ch₁ ch₂).
3. Two varieties produced normal hybrids with *T. macha* and C 306, and hybrids with necrotic symptoms with Sonalika, suggesting that these testing varieties are carry only the *Ne₁* gene (*Ne₁ ne₂ ch₁ ch₂*).

4. Results obtained from the above crosses revealed that out of eleven varieties, six of them are carriers of *Ne₁* and *Ch₁* genes (*Ne₁ ne₂ Ch₁ ch₂*), two were carriers of *Ne₁* gene (*Ne₁ ne₂ ch₁ ch₂*) and remaining three were non-carriers for both the genes (*ne₁ ne₂ ch₁ ch₂*).

6.3.3. Tetraploid wheat (*Triticum durum*)

1. Of the twenty-three durum varieties, four of them produced normal hybrids with all the three testers indicating that these genotypes are non-carriers for both necrosis and chlorosis genes (*ne₁ ne₂ ch₁ ch₂*).

2. Nineteen varieties produced normal hybrids with *T. macha*, chlorotic symptoms with C 306 and both necrotic and chlorotic symptoms with Sonalika thus indicating that these varieties are carriers of *Ne₁* and *Ch₁* genes (*Ne₁ ne₂ Ch₁ ch₂*).

3. From the results it is evident that out of twenty-three durum varieties, nineteen of them are carriers of *Ne₁* and *Ch₁* (*Ne₁ ne₂ Ch₁ ch₂*) and the remaining four are non-carriers (*ne₁ ne₂ ch₁ ch₂*) for both the genes.

6.4. Allelic variation of HMW glutenin subunits in Indian hexaploid wheat cultivars

1. Thirty-two cultivars of *Triticum aestivum* were analysed for their allelic variations of High Molecular Weight Glutelin Subunits (HMW GS) by Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE).

2. A total of 10 alleles were identified, three (a, b, c) at the *Glu-Al* locus, four (a, b, c, d) at the *Glu-BI* locus and three (a, b, d) at the *Glu-DI* locus.

3. The most frequent HWM glutenin subunits were 2 at *Glu-Al* locus, 7 at *Glu-BI* locus and 5+10 at *Glu-DI* locus.
4. The most frequent protein combinations are $2^*, 7+8, 2+12$ and $2^*, 7, 5+10$. The \textit{Glu-1} quality score ranged from 5–10. The \textit{Glu-1} quality score 8 is present in large number of cultivars.

5. It is predicted that the cultivars posses high \textit{Glu-1} score are having good bread making quality i.e. above 8, and the cultivars having \textit{Glu-1} score below 7 are posses very poor bread making quality.